Mechanisms of Development 76 (1998) 3–18

Review article

Molecular regulators involved in vertebrate eye development

Dominique Jean, Kenneth Ewan, Peter Gruss*

Department of Molecular Cell Biology, Max Planck Institute of Biophysical Chemistry, Am Fassberg, 37 077 Göttingen, Germany

Accepted 9 July 1998

Abstract

Development of the eye can be subdivided into three phases. The first phase is the formation of the major structures of the eye by the processes of induction and regional specification. The second is the maturation of these structures to form the functional eye, and the third phase is the formation of neuronal connections between retina and the optic tectum. These processes are tightly regulated by signalling cascades that direct axonal outgrowth, cellular proliferation and differentiation. Some members of these signalling cascades have been identified in recent studies. These include secreted factors which transmit signals extracellularly, and receptors and transcription factors which are members of intracellular signalling pathways that respond to extracellular signals. This review summarizes the recent research that has implicated these factors in playing a role in eye development on the basis of functional or expression criteria.

Keywords: Induction; Lens; Retina; Transcription factor; Secreted factor; Eye

1. Introduction

The development of the eye has long been studied using classical embryological methods in the chick and in amphibia. These experiments defined the interactions between different tissues during the formation of eye structures; however, they did not identify the molecules regulating these processes. By far the most information has come from the study of previously characterized molecules that were found to have a functional role in aspects of eye development, or which are suspected of having such a role because of expression in the developing eye. Mouse suppliers and a number of laboratories have identified more than 100 spontaneous mouse eye mutations (reviewed in Graw, 1996; see also Mouse Genome database 3.1 at URL: http://www.informatics.jax.org/). Recently, large scale screens of zebrafish have also led to a number of eye mutants (Heisenberg et al., 1996; Karlstrom et al., 1996; Malicki et al., 1996). The responsible molecules for almost all the zebrafish mutants and many of the mouse mutants are unknown; however, independently characterized genes have been correlated with other mutants on the basis of functional and chromosomal location data (e.g. the *Sey* mutant with the *Pax-6* gene). Another source of information are the human eye syndromes. Positional cloning of the genes at the mutated loci can confirm data from mouse models. From all of these sources, many regulatory molecules have been identified. These factors are described below.

2. Generation of early eye structures

A series of induction and regional specification events generate the lens vesicle, double layered optic cup, optic stalk and the precursor tissue of the cornea from an outpocketing of the forebrain neuroectoderm and the overlying head ectoderm (see Fig. 1). This phase of eye morphogenesis can be subdivided into (1) the formation of the optic vesicle, (2) the induction of the lens placode and subsequent formation of the optic cup and lens vesicle. During these developmental events, a third process restricts the retinal fate to the optic vesicle and the optic cup.

* Corresponding author. Tel.: +49 551 2011361; fax: +49 551 2011504; e-mail: pgruss@gwdg.de

0925-4773/98/$19.00
PII S0925-4773(98)00117-8

2.1. Formation of the optic vesicle

Regionalization of the neuroectoderm along the rostro-caudal and proximo-distal axes begins soon after its formation. One of the first regions to be defined is the eye field in the anterior neural plate. This region evaginates from the forebrain during the neural folding process to become first the optic pit then the optic vesicle. Evagination of the optic vesicle is absent in mouse embryos that are null mutants of the gene for the transcription factor Rx (Mathers et al., 1997). The phenotype suggests that the regionalized specification of the lateral wall of the forebrain to the optic vesicle fate requires the action of Rx protein. The expression of Rx in two lateral patches in the anterior neural plate and subsequently in the optic vesicles that develop from these patches is consistent with this proposed function. Two other transcription factors with a similar expression pattern to Rx are Rax, a rat paired-domain-containing protein (Furukawa et al., 1997a), and the T-box family member ET (Li et al., 1997) cloned in chick and *Xenopus*. These factors have yet to be functionally characterized.

2.2. Formation of the lens vesicle and optic cup

When the optic vesicle makes contact with the overlying ectoderm, it induces the contacted ectoderm to develop into lens tissue. The induced ectoderm thickens to form the lens placode, which then invaginates and is pinched off from the rest of the ectoderm to form the lens vesicle. Simultaneously, the medial part of the optic vesicle narrows to become a stalk-like structure and the lateral part is pushed in, possibly because of lens placode invagination, to form the bilayered optic cup (see Fig. 1). The three neural structures created by these processes, the inner layer of the optic cup, the outer layer of the optic cup, and the optic stalk develop into the neural retina, RPE and certain glial cell populations of the optic nerve respectively.

The process of lens placode induction by the optic vesicle is not fully understood in molecular terms, because the factor produced by the optic vesicle that triggers the signalling cascade resulting in the induction event has yet to be identified. It is known that this inducing factor is secreted because the insertion of a filter between the optic vesicle

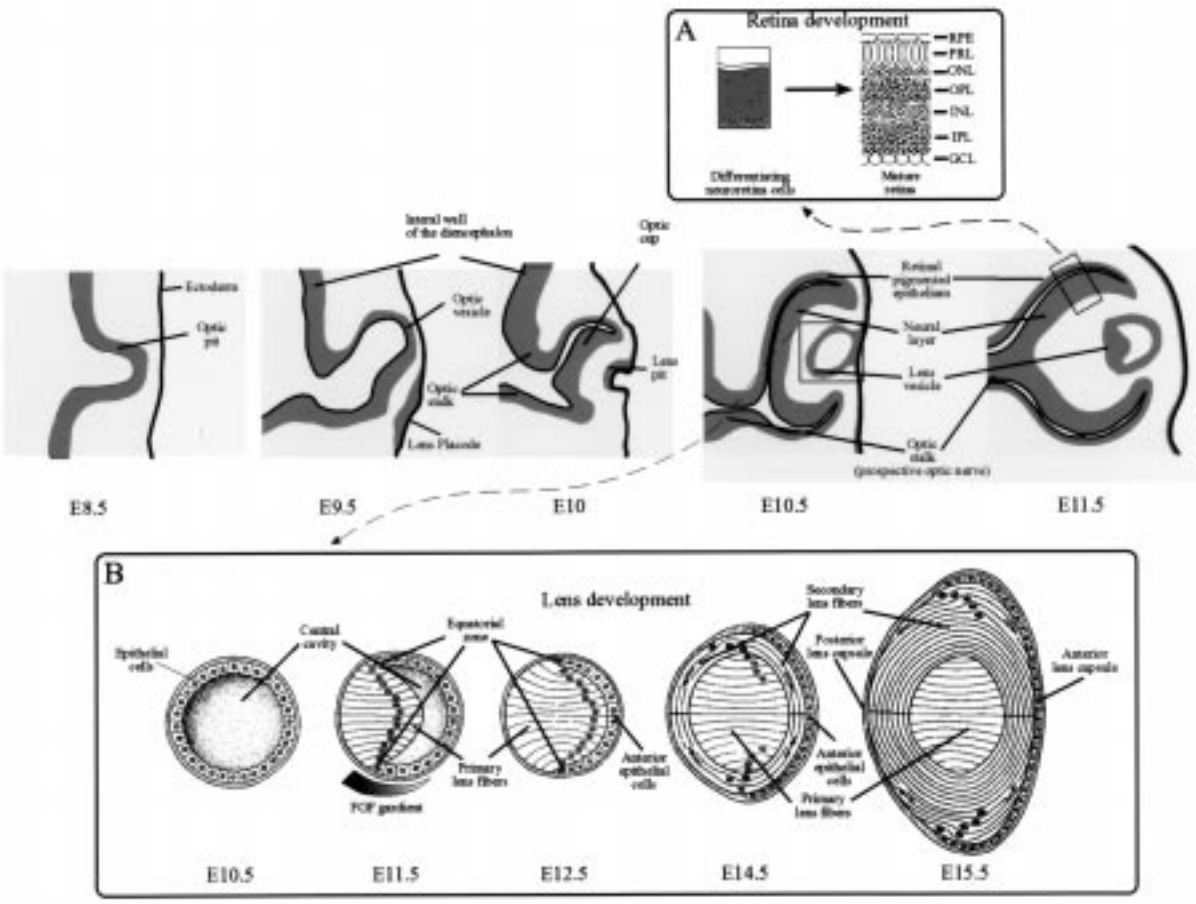

Fig. 1. Schematic representation of eye development. Representation of the main embryonic stages E8.5 to E11.5 of mouse eye development. The boxed area in the E10.5 eye diagram indicates the lens vesicle at the same stage as shown on the left in panel B. The boxed area shown in the retina of the E11.5 eye is the same stage as shown in the left of panel A. (A) Differentiation of the neuroretina. PRL, photoreceptor layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. (B) Differentiation of the lens. Embryonic days (E) 10.5 to 15.5 depicted. The arrow seen in the E11.5 lens vesicle shows the direction of the FGF concentration gradient from high (left) to low (right).

and the head ectoderm in explant assays still resulted in lens-like structures and crystallin expression (Karkinnen-Jaaskelainen, 1978). Null mutants of the gene for Lhx-2, a LIM homeobox transcription factor expressed at the optic vesicle stage, fail to develop the lens placode suggesting that the lens induction factor normally secreted by the optic vesicle is not made (Porter et al., 1997). Failure of lens placode induction is also a feature of the natural *Pax-6* mutants, *Sey* (Hogan et al., 1986, 1988; Hill et al., 1991), Sey^{Neu} (Hill et al., 1991) and the rat mutant *rSey* (Matsuo et al., 1993). *Pax-6*, however, is also expressed in the presumptive lens ectoderm and in the induced lens placode (Walther and Gruss, 1991; Li et al., 1994). This pattern of expression suggested that the Pax-6 protein is part of the mechanism that allows the ectoderm to respond to a lens induction signal from the optic vesicle. Explant recombination experiments using homozygous *rSey* tissue and wild-type tissue demonstrated that the homozygous ectoderm was unable to respond to the induction signal, but that optic vesicles of null mutant embryos could still induce the wild-type ectoderm to form lens tissue (Fujiwara et al., 1994). Moreover, in chimeras of wild-type and *Sey* homozygote embryonic cells, the *Sey* homozygote cells are excluded from the lens placode (Quinn et al., 1996). In conclusion, these null mutant studies suggest that Lhx-2 is part of the molecular circuitry that regulates secretion of the lens inducing molecule and that Pax-6 confers the ability to respond to the secreted molecule to the overlying ectoderm.

Although the lack of a developing lens placode is the major reason why optic cup development stops at the lens induction stage in null mutant *Lhx-2* and *Pax-6* mice and rats, the absence of these factors in the optic vesicle itself may be a contributing factor to the arrest of further development. Heterozygote Sey mice display microphthalmia (Hogan et al., 1986; Hill et al., 1991) as a result of haploinsufficiency. Another transcription factor which may play a role in both development of the optic vesicle into the optic cup and in lens induction is Gli-3 (Hui and Joyner, 1993; Hui et al., 1994), which is expressed in E9.5 mouse optic vesicles. Some homozygote embryos of the extra-toes natural mutant of Gli-3 fail to develop the lens placode and the optic vesicle does not involute to form the optic cup (Franz and Besecke, 1991).

2.3. Restriction of retinal fate to the optic cup

In normal development, the optic cup and the optic stalk differentiate into the retina and the optic nerve tract, respectively. The junction between the optic cup and the optic stalk narrows to form the optic disc, through which the retinal ganglion cell axons project. Recently, several factors have been shown to prevent the optic stalk differentiating into retinal tissue and establish the optic disc as the boundary between the two tissues.

Sonic hedgehog (Shh) has been shown to be a key regulator of midline development. In the *Shh* null mutant mouse embryo (Chiang et al., 1996), the development of midline structures was severely affected. This was also seen in the cyclops mutant zebrafish embryo (Macdonald et al., 1995), which lacks Shh expression. The consequence for eye morphogenesis is that retinal development extends so medially that the two retinas fuse to form one. Ectopic Shh expression led to smaller optic cups and enlarged optic stalks in the zebrafish (Macdonald et al., 1995) and actually prevented both optic cup formation and Pax-6 expression in the newt (Mizuno et al., 1997). Thus, high levels of Shh protein seem to predispose optic vesicle tissue to an optic stalk fate, and low or negligible levels allow optic vesicle tissue to follow the retinal fate. This suggests that Shh-mediated signalling represses optic vesicle cells from following retinal developmental pathways.

The paired domain transcription factor Pax-2 plays a major role in establishing the boundary between the optic vesicle and stalk. In both the *Pax-2* null mutant mouse (Keller et al., 1994; Torres et al., 1996) and the zebrafish mutant of the *Pax-2* equivalent, Noi (Macdonald et al., 1997), there was a medial extension of RPE cells into the optic stalk and a failure of the formation of the optic disc. This results in the condition known as optic nerve coloboma. A heritable human optic nerve coloboma syndrome, which is associated with renal anomalies and vesicouretral reflux, has also been linked to mutations in the *PAX-2* gene (Sanyanusin et al., 1995; Schimmenti et al., 1995). Pax-2 is likely to be a member of the Shh-activated signalling cascade regulating medial optic vesicle development. Strong support for this notion has come from the finding that there is no expression in optic tissue of Pax-2 in the Shh null mutant mouse embryo (Chiang et al., 1996). Moreover, ectopic expression of Shh in the zebrafish led to a lateral extension of the expression domain of the Pax-2 equivalent, Noi, that corresponded to the loss of the Pax-6-positive, retina-fated tissue in the eye (Macdonald et al., 1995). Together, these findings strongly suggest that Pax-2 is a major component of the Shh-mediated signalling pathway that restricts Pax-6 expression to the lateral portion of the optic vesicle and subsequently to the optic cup. Another transcription factor with a similar function to Pax-2 in them optic stalk is Gli-3, whose natural mutant, extra-toes, has optic nerve coloboma (Franz and Besecke, 1991).

Another influence on mediolateral specification of optic structures is retinoic acid. Retinoic acid is synthesized from the retinaldehyde by a dehydrogenase. One of these is expressed only in the ventral retina of the zebrafish (Marsh-Armstrong et al., 1994), resulting in an asymmetric distribution of retinoic acid. This retinoic acid gradient is also found in mice (Riddle et al., 1993). Inhibition of this ventrally located enzyme leads to production of eyes lacking ventral retinal structures such as the optic disc and also the optic stalk (Marsh-Armstrong et al., 1994). These defects were also observed in embryos whose mother was starved of the retinoic acid precursor, Vitamin A (Warkany and Schraffenberger, 1946). Application of exogenous reti-

noic acid to zebrafish optic cups causes increased cellular proliferation in the optic stalk and even induces ectopic optic discs (Hyatt et al., 1996b) and Noi expression is found in the entire retina.

3. Maturation of eye structures

After their formation, the primary structures differentiate into their mature form. Neuronal and glial cell types are generated from the cells of the inner optic cup layer. The outer layer of the optic cup forms the retinal pigmented epithelium (RPE). The lens vesicle matures into the lens and the ectoderm overlying the lens develops into the cornea. Mesenchymal cells of neural crest origin surrounding the developing eye migrate and differentiate into the iris, sclera, ciliary body and lachrymal gland.

3.1. The lens

Lens vesicle development differs regionally (reviewed in Piatigorsky, 1981; Wride, 1996; illustrated in Fig. 1B). Cells of the posterior side of the vesicle facing the retina differentiate into primary fibre cells. These cells elongate, synthesize crystallin and lose their nucleus. The anterior side of the lens vesicle adjacent to the developing cornea remains epithelial. While cells in the center of the epithelium become quiescent, the cells in the equatorial zone on the margins of the lens epithelium continue to proliferate and some of these cells differentiate into secondary fibre cells. The secondary fibre cells augment the primary fibre cells during development. This pattern of quiescence, proliferation and differentiation suggested that the vitreous humor between the retina and the developing lens could induce the lens epithelial cells to form secondary fibre cells. Reversing the lens so that the lens epithelium faced the vitreous humor confirmed this hypothesis (Coulombre and Coulombre, 1963). Since then, a number of secreted factors found in the vitreous humor have been implicated in lens development (see Table 1).

FGF-1 (aFGF) and FGF-2 (bFGF) are major regulators of lens differentiation. An elegant explant experiment (McAvoy and Chamberlain, 1989) found that lower concentrations of FGF stimulated proliferation, similar to the marginal cells phenotype and higher concentrations induced differentiation into secondary fibre cells. Thus, FGF affects lens epithelial cells in a concentration dependent way (illustrated in Fig. 1). Additionally, FGF reduces apoptosis in secondary fibre cells (Chow et al., 1995). Consistent with these findings was a study of a transgenic mouse line that expressed a dominant negative form of FGF receptor type I (FGFR1). Mice exhibited a reduction in the number of epithelial cells and characteristic signs of apoptotic degeneration of fibre cells (Robinson et al., 1995). PDGF, another secreted factor, is strongly expressed in the iris and ciliary body, close to the equatorial zone of the lens and in the corneal endothelium. It was shown to maintain rat lens transparency and growth in organ culture (Brewitt and Clark, 1988). Over-expression of the PDGF-A isoform under the control of the *αA-crystallin* promoter in transgenic mice leads to an increase of cellular proliferation, and also differentiation to form fibre cells (Reneker and Overbeek, 1996). Mice homozygous for the natural mutation of the PDGF-A receptor, Patch, have a decreased num-

Table 1
Regulators of lens development

Molecule	Lens type	Species	Expression	Function	Reference
Eya.1	TF	Mo	Lens placode, vesicle	ns	Xu et al., 1997
FGF-1	SL	Mo, rat	Lens placode, transitional zone	Proliferation at lower levels, differentiation at higher levels	McAvoy and Chamberlain, 1989; Robinson et al., 1995; deIongh and McAvoy, 1993; Lovicu et al., 1997
FGF-2	SL	Mo, rat	NR, cornea, ciliary body and iris	Proliferation at lower levels, differentiation at higher levels	McAvoy and Chamberlain, 1989; Robinson et al., 1995; Lovicu et al., 1997
IGF-1	SL	Ch, Mo		Promotes differentiation	Beebe et al., 1987
IGF-2	TF	Ch, Mo		Promotes differentiation	Beebe et al., 1987
Pax-6	TF	Ch, Mo	All lens cells	Implicated in induction of crystallin gene expression	Hogan et al., 1986; Hill et al., 1991; Walther and Gruss, 1991; Li et al., 1994; Grindley et al., 1995; Hirsch and Harris, 1997; Macdonald and Wilson, 1997
PDGF-A	SL	Mo	Iris and ciliary body	Proliferation, maintenance of cell transparency	Brewitt and Clark, 1988; Morrison-Graham et al., 1992
Prox-1	TF	Ch, Mo	Lens placode	ns	Oliver et al., 1993; Tomarev et al., 1996
Six-3	TF	Mo	Lens placode	Induces crystallin expression?	Oliver et al., 1995; Oliver et al., 1996
Sox-1	TF	Mo	Fibre cells	Required for g-crystallin expression	Nishiguchi et al., 1998
Sox-2	TF	Ch, Mo	Lens placode, all lens cells	Implicated in induction of a and d crystallin gene expression	Kamachi and Kondoh, 1993; Kamachi et al., 1995
Sw3-3	TF	Ch	Lens placode, all lens cells	ns	Wang and Adler, 1994

SL, secreted ligand; TF, transcription factor; Ch, chick; Mo, mouse; NR, neural retina; ns, not yet studied.

ber of lens fibre cells (Morrison-Graham et al., 1992), which is consistent with the opposite effect observed in the PDGF overexpression experiment. IGF-1 has been shown to stimulate lens fibre differentiation (Beebe et al., 1987). This factor is expressed in the neural retina (Hernandez-Sanchez et al., 1995). Null mutant BMP-7 mice impaired lens development, suggesting a trophic role for this molecule (Dudley et al., 1995; Luo et al., 1995). BMP-7 is expressed in both the lens vesicle and the retina, and earlier in the head ectoderm and the optic vesicle. So far, the reverse experiment (ectopic expression of BMP-7 or addition of exogenous BMP-7) has not been reported.

Pax-6 protein plays a role in the induction of the expression of crystallins in the developing lens at the stage of the placode and the lens vesicle. So far, Pax-6 has been shown to bind to the promoter sequences of the mouse *αA-crystallin* gene (Cvekl et al., 1994), the mouse *αB-crystallin* gene (Gopal-Srivastava et al., 1996), the *ζ-crystallin* gene in guinea-pig (Richardson et al., 1995) and of the *δ1-crystallin* gene in chicken (Cvekl et al., 1995). Moreover, ectopic expression of Pax-6 induced the expression of βB1-crystallin in the ectoderm of *Xenopus* embryos and also the formation of lens-like structures (Altman et al., 1997). These studies suggest that Pax-6 plays a major role in regulating crystallin gene expression. Pax-6 most likely acts in concert with other transcription factors such as Six-3 (Oliver et al., 1995), Sox-1 (Nishiguchi et al., 1998), Sox-2 (Kamachi and Kondoh, 1993; Kamachi et al., 1995) and Eya-1 (Xu et al., 1997) in lens competent ectoderm during lens induction. The expression of these factors is likely to be mediated by lens induction as well as Pax-6, because these factors are not expressed in the ectoderm during the earlier period of widespread Pax-6 expression in the head ectoderm. Ectopic expression of mouse Six-3 in the killifish medaka leads to formation of crystallin-expressing lens like structures exclusively within otic placodes which normally develop into the internal ear. Thus ectopic expression of Six-3 in the otic placode can change the fate of the cells to become the lens (Oliver et al., 1996). It is of interest that another member of the Pax family, Pax-2, is expressed in the otic placode during development (Nornes et al., 1990; Torres et al., 1996). This could suggest that the action of Six-3 could involve interactions with Pax-6 in the lens placode. Although two splice variants of *Six-3* are known (Kawakami et al., 1996), it is not known which one is expressed in the lens placode. Sox-1, a Sry-related transcription factor, has a later role in lens development. Mice that are null mutant for Sox-1 expressed no γ-crystallin protein (Nishiguchi et al., 1998) with consequent changes in fibre cell morphology. Moreover, Sox-1 binding sites were found on the upstream promoter and enhancer sequences of the *γ-crystallin* genes. Another Sox factor, Sox-2 (also known as δEF2a) has been shown to bind the *δ1-crystallin* enhancer region (Kamachi and Kondoh, 1993; Kamachi et al., 1995). Overexpression of this factor increases *δ1-crystallin* enhancer activity in lens cells, but not in fibroblast cells (Kamachi et al., 1995) supporting previous observations that this stimulation depends on a lens-specific group of factors (Kamachi and Kondoh, 1993). Moreover, overexpression of Sox-2 is also able to enhance the mouse αA-crystallin promoter (Kamachi et al., 1995). Thus, this gene may be part of the common regulatory pathway for crystallin genes. The third transcription factor which first appears soon after lens placode induction is Eya-1 (Xu et al., 1997), a member of the mouse Eya gene family which are homologues of the *Drosophila* eye absent gene.

The withdrawal of differentiating lens fibre cells from the cell cycle appears to be regulated in part by the Rb (retinoblastoma) gene (Morgenbesser et al., 1994). Mouse embryos deficient for Rb had deficiencies in lens fibre cell differentiation due to non-withdrawal from the cell cycle prior to differentiation. Moreover, lens fibre cells underwent the process of apoptosis, which is normally limited to the lens epithelial cell population (Ishizaki et al., 1993). This apoptosis is p53-mediated, because it was not seen in Rb/p53 double null mutant embryos. Thus, when the normal withdrawal from the cell cycle does not occur during lens cell differentiation, a p53-dependent pathway apoptoses these cells.

3.2. Specification of neural retinal and retinal pigmented epithelium (RPE) fates

The optic cup consists of an inner and outer layer which develop into the neural retina and retinal pigmented epithelium (RPE), respectively. A number of molecules have been implicated in the specification and survival of the neural retina and the retinal pigmented epithelium (see Table 2). FGF-1 (acidic FGF) and FGF-2 (basic FGF) have been shown in a number of studies to induce RPE to generate neural retinal tissue after the neural retina had previously been removed (Park and Hollenberg, 1989; Pittack et al., 1991; Guillemot and Cepko, 1992; Zhao et al., 1995). Implantation of FGF-2 soaked beads medially to the chick optic cup induced the presumptive RPE to undergo neuronal differentiation resulting in two neural retinal layers (Pittack et al., 1997). Removal of the surface ectoderm and developing lens structures results in severely impaired neural retina development but this effect could be rescued by the implantation of FGF-1 (acidic FGF) secreting cells over the optic cup (Hyer et al., 1998). Another molecule with a putative trophic role is BMP-7; the proper development of both retinal layers is impaired in null mutant mice (Dudley et al., 1995; Luo et al., 1995). Analysis of this null mutant phenotype is complicated by the fact that BMP-7 is expressed in the developing lens (lens placode and lens vesicle), the developing retina (optic vesicle, optic cup and the neural retina), and also in the embryonic ectoderm. Trophic support for neural retina development is also provided by the RPE; RPE ablation by targeted expression of diphtheria toxin arrested neural retina development and prevented formation of the vitreous humor (Raymond and Jackson,

Table 2
Regulators of retinal development

Molecule	Type	Species	Expression	Function	References
BDNF	SL	Ch, Mo	Optic tectum, RPE	Survival of RGC; represses amacrine AII fate; induces RPE formation	Cohen-Cory et al., 1996; Herzog et al., 1994; Frade et al., 1997; Liu et al., 1997; Johnson et al., 1986; Rodriguez-Tebar et al., 1989;
BMP-7	SL	Mo	All NR layers, lens	Survival	Dudley et al., 1995; Luo et al., 1995
Chx-10	TF	Mo	Germinative layer, bipolar cells	Promotes bipolar cell fate	Liu et al., 1994; Burmeister et al., 1996
CNTF	SL	Mo, Ch, Xe	Muller glia	Promotes rod photoreceptor fate in chick; represses rod photoreceptor fate in mouse	Fuhrmann et al., 1995; Kirsch et al., 1996; Ezzeddine et al., 1997
Crx	TF	Mo	Photoreceptors	Promotes rod photoreceptor fate, represses amacrine and Muller glial fates	Furukawa et al., 1997b
Clox1	TF	Mo	NR	ns	Andres et al., 1992
Delta-1	BL	Mo, Ch, Xe	NR germinative layer	Represses differentiation of mitotic cells	Dorsky et al., 1997; Henrique et al., 1997
Dlx-1	TF	Mo	Undifferentiated NR	ns	Dollé et al., 1992
Eya-1	TF	Mo	RPE	ns	Xu et al., 1997
Eya-2	TF	Mo	Germinative layer	ns	Xu et al., 1997
Eya-3	TF	Mo	Germinative layer?	ns	Xu et al., 1997
FGF-1	SL	Ch, Mo	Lens vesicle	Induces NR formation, promotes proliferation of mitotic cells	Hyer et al., 1998; Lillien and Cepko 1992; Pittack et al., 1997
FGF-2	SL	Ch, Mo	GCL	Promotes proliferation of mitotic cells	Lillien and Cepko 1992; Zhao et al., 1995
GH-6	TF	Ch	Undifferentiated NR, GCL	ns	Stadler and Solursh, 1994
Gli-3	TF	Mo	ONL	ns	Hui et al., 1994
HES-1	TF	Mo	NR germinative layer	Represses differentiation of mitotic cells	Tomita et al., 1996a
IGF-1	SL	Mo	NR, orbital mesenchyme	Promotes differentiation of mitotic cells	Frade et al., 1996; Danias and Stylianopoulou, 1990
Inhibin	SL	Mo	Undifferentiated NR	ns	Ying et al., 1997
Isl-1	TF	Mo, rat	INL, GCL.	ns, no eye phenotype reported in null mutant	Pfaff et al., 1996; Thor et al., 1991
LIF	SL	Ch, Mo	Muller glia cells, amacrine and horizontal cells	Represses rod receptor fates	Neophytou et al., 1997
Lim-3	TF	Xe	INL of NR	ns	Taira et al., 1993
Mash-1	TF	Mo, Xe	Differentiating cells	Promotes generation of later appearing cell types	Guillemot and Joyner 1993; Tomita et al., 1996b
MITF	TF	Mo	RPE	RPE survival	Hertwig, 1942; Hodgkinson et al., 1993; Tassabehji et al., 1994
NeuroD	TF	Xe	Differentiating neurons	Promotes differentiation	Lee et al., 1995
Notch	R	Xe, Ch,Mo	NR germinative layer	Represses differentiation of mitotic cells	Austin et al., 1995; Bao and Cepko, 1997; Dorsky et al., 1995
Nr-1	TF	Mo	Differentiated neurons	ns	Swaroop et al., 1992
NT-3	SL	Ch, Mo	RPE, all NR layers	Promotes differentiation; survival of differentiated cells	de la Rosa et al., 1994; Bovolenta et al., 1996
Otx-2	TF	Ch, Mo	RPE	Unknown due to earlier functions	Boncinelli et al., 1993; Bovolenta et al., 1997; Matsuo et al., 1995
Pax-6	TF	Ze, Xe, Ch, Mo	NR germinative layer, amacrine cells, GCL, RPE	Arrested development at optic cup stage, separate RPE layer does not form	Hogan et al., 1986; Hill et al., 1991; Walther and Gruss, 1991; Li et al., 1994; Grindley et al., 1995; Hirsch and Harris 1996; Macdonald and Wilson, 1997

PDGF	SL	Ch, Mo	GCL, blood vessels	Chemoattractant for astrocyte precursors from optic nerve	Fruttiger et al., 1996
PEDF	SL	Human	RPE	Promotes survival of differentiated cells particularly photoreceptors	Steele et al., 1993
Pou4f1 (Brn-3a)	TF	Mo	GCL	ns	Xiang et al., 1995
Pou4f2 (Brn-3b)	TF	Mo	GCL	Differentiation of large subset of ganglion cells	Gan et al., 1996; Xiang et al., 1995
Pou4f3 (Brn-3c)	TF	Mo	GCL	ns	Xiang et al., 1995
Prox-1	TF	Ch, Mo	INL of NR, horizontal, bipolar, some amacrine cells	ns	Oliver et al., 1993; Tomarev et al., 1996
retinoic acid	SL	Ze, Mo, rat	Ventral NR	Promotes rod photoreceptor fate	Kelley et al., 1994; Hyatt et al., 1996a,b;
RPF-1	TF	Mo	GCL, amacrine cells	ns	Zhou et al., 1996
Shh	SL	Ze, Xe, Ch, Mo	GCL	Promotes all photoreceptor fates	Levine et al., 1997
Six-2	TF	Mo	GCL, INL, OCL of NR	ns	Kawakami et al., 1996
Six-3	TF	Ch, Mo, Xe, Ze	NR	ns	Oliver et al., 1995; Kawakami et al., 1996; Seo et al., 1998
Six-4/AR-EC	TF	Mo	GCL, INL, OCL of NR	ns	Kawakami et al., 1996
Six-5	TF	Mo	GCL, INL, OCL of NR	ns	Kawakami et al., 1996
Six-6	TF	Ze	NR	ns	Seo et al., 1998
sw3-3	TF	Ch	NR	ns	Wang and Adler, 1994
TGFα	SL	Mo, rat	NR	Promotes Muller glial fate	Anchan et al., 1991; Lillien and Cepko, 1992; Lillien, 1995
TGFβ3	SL	Mo, rat	NR	Mitogenic, promotes amacrine cell fate	Anchan and Reh, 1995
Thyroxine	TF	Rat	Thyroid	Promotes cone photoreceptor fate	Kelley et al., 1995
TrkB	R	Mo, rat, Ch	INL of NR	Receptor for BDNF, survival of RGC, represses amacrine AII fate	Cellerino and Kohler, 1997; Rickman and Rickman, 1996
Xath5	TF	Xe	NR germinative layer, GCL	Promotes ganglion cell fate	Kanekar et al., 1997
Xrx-1	TF	Xe	NR, RPE	ns	Casarosa et al., 1997

BL, membrane bound ligand; SL, secreted ligand; TF, transcription factor; Ch, chick; Mo, mouse; Xe, *Xenopus*; Ze, zebrafish; GCL, ganglion cell layer; INL, inner cell layer; NR, neural retina; ns, not yet studied; OCL, outer cell layer; RPE, retinal pigmented epithelium.

1995). This trophic role of the RPE is mediated partly by the neurotrophins BDNF and NT-3. NT-3 is required for the survival of differentiated neural retinal cells (Bovolenta et al., 1997) and BDNF has a trophic role for retinal ganglion cells (Frade et al., 1997). Another neurotrophic factor expressed in the RPE is PEDF (Steele et al., 1993). Regulation of RPE differentiation is less known. Mice that are homozygous for mi, the natural mutation of the transcription factor gene MITF, have impaired RPE development and resultant eye defects (Hertwig, 1942; Hodgkinson et al., 1993). Moreover, a mutation in this gene causes Waardenburg syndrome type-2 in humans (Tassabehji et al., 1994). Expression of the dominant negative form of trkB, the receptor for BDNF, resulted in RPE degeneration in later development (Liu et al., 1997). This finding suggested an autocrine mechanism since BDNF is only expressed in the RPE, not in neighbouring tissues.

3.3. The neural retina

During neural retina development, postmitotic cells generated in the germinative layer migrate laterally to form the striated, laminar pattern of the retina (Fig. 1A). They differentiate into either neuronal or Müller glial cells. Neuronal cells are also subdivided into three different classes: light-sensitive photoreceptor neurons (cones and rods), interneurons (bipolar, horizontal and amacrine neuron cells) and the retinal ganglion cells (RGC). Like many neural tissues, the different cell types are generated at different stages of development. The RGC appear first and the photoreceptors appear last. The astrocytes present in the retina are not produced by this process, but are descendants of immigrant cells (Watanabe and Raff, 1988) which may be chemotactically attracted from the optic nerve by PDGF secreted from RGC and the retinal capillaries (Fruttiger et al., 1996). Analysis of clones derived from single progenitor cells that were labelled with a LacZ-transducing replication-defective retrovirus, showed that the progenitor cells gave rise to several types of retinal cells and could contain both neurons and glia (Turner and Cepko, 1987; reviewed in Cepko et al., 1996). Clones of retinal cells labelled at later stages had only the later arising cell types. Neural retina development is regulated by a number of signals that promote terminal differentiation of germinative layer cells and by mitogenic signals that inhibit this differentiation. This competition between the two functional types of signals is needed to retain enough progenitor cells to generate the full range of cell types seen in the neural retina.

The signals that promote differentiation are secreted ligands (reviewed in Cepko et al., 1996; Harris, 1997; see Table 2 for description of expression and function). All these ligands, except for IGF-1 and NT-3, promote specific cell type fates. IGF-1 and NT-3 induce unbiased differentiation of mitotic cells into all cell types (Bovolenta et al., 1996; Frade et al., 1997). Interestingly, some ligands are expressed in previously generated neuronal cell types. CNTF and LIF, which repress the rod photoreceptor fate, are expressed in Müller glia, amacrine and horizontal cells (Fuhrmann et al., 1995; Neophytou et al., 1997), and Shh is expressed in RGC (Levine et al., 1997). Thus, the appearance of later generated cell types could well depend on the presence of earlier differentiated cell types. Ligand functions were investigated using in vitro explant and dissociated culture assays, except for a NT-3 study which involved the application of antibodies in vivo (Bovolenta et al., 1996). At the transcriptional level, the function of Crx, Chx-10, NeuroD and Pou4f-has been studied. Retrovirally-mediated overexpression of Crx, normally expressed only in photoreceptors, in clonal descendants derived from single infected mitotic cells in mouse retinas resulted in an increase in clones containing only rod photoreceptors and reduced the frequency of clones containing amacrine cells, interneurons and Müller glial cells (Furukawa et al., 1997b). Additionally, presumptive photoreceptor cells expressing a dominant-negative form of Crx failed to fully mature (Furukawa et al., 1997b). Mutations in this gene have been associated with cone-rod dystrophy in humans (Freund et al., 1997). In cone-rod dystrophy, the natural mutant of Chx-10, bipolar cells do not differentiate (Truslove, 1962; Burmeister et al., 1996). Thus, Chx-10 activity mediates the specification of the bipolar cell fate. In null mutants of the gene for Pou4f-2, a large proportion of retinal ganglion cells are missing, suggesting that the protein is required for differentiation of some RGCs (Gan et al., 1996). Forced expression of NeuroD promotes differentiation of all neuroectoderm cells, including neural retina cells (Lee et al., 1995). Like NeuroD, Mash-1 is expressed only in postmitotic neural retina cells (Guillemot and Joyner, 1993). Retinal explants of Mash-1 null mutant newborn mice exhibited lower numbers of later-generated neural retinal cell types than in wild-type explants (Tomita et al., 1996b). A number of other transcription factors are expressed in either mitotic or differentiated cell types (see Table 2), but have yet to be functionally analysed.

The inhibitory mechanism that preserves a pool of mitotic progenitor cells is mediated, at least in part, by the Delta-Notch-1 signalling molecules. Ectopic expression of Notch-1 receptor in *Xenopus* retinas impaired retinal differentiation (Dorsky et al., 1995). Similarly, retrovirally-mediated expression of constitutively-active Notch-1 receptor in isolated cells of embryonic chick and postnatal rat retinas led to clones of undifferentiated cells (Austin et al., 1995; Bao and Cepko, 1997). However, progenitor cells infected with the dominant-negative form of Notch-1 gave rise to a normal number of descendants with the normal range of cell types (Bao and Cepko, 1997). When chick retinas were infected with Delta-transducing retrovirus before generation of the first neurons, infected cells remained mitotic and did not differentiate if they were surrounded by other infected cells (Henrique et al., 1997). Similar findings were made when cells expressing Delta membrane-bound ligand were added to dissociated cultures of chick retinas (Austin et al.,

1995) and when *Xenopus* retinal cells constitutively expressed Delta (Dorsky et al., 1997). However, isolated cells misexpressing Delta differentiate into cell types that arise earlier in development (Dorsky et al., 1997; Henrique et al., 1997). Germinative layer retina cells infected with retroviruses transducing the dominant-negative form of Delta all differentiated (Henrique et al., 1997). Thus, Delta-Notch-1 signalling controls the ability of cells to respond to inductive signals, by instituting a degree of lateral inhibition in the germinative cell layer. At the transcriptional level, HES-1, a bHLH factor, has been shown to repress differentiation of neural retinal cells (Tomita et al., 1996a). Retrovirally-mediated overexpression of HES-1 in organ cultures of mouse and rat retinas prevented differentiation into the normal cell types. In null-mutant mice, rosette structures formed instead of laminae containing photoreceptors, and horizontal cells, ganglion cells and amacrine cells, but no bipolar cells.

3.4. Other eye structures

The other structures of the eye include the cornea, iris, ciliary body and the sclera. The cornea results from the induction of the ectoderm overlying the developing lens to secrete extracellular matrix molecules which allow the immigration of neural crest-derived mesenchymal cells. These cells form the corneal endothelium (reviewed in Hay, 1979). The iris and the ciliary body develop from the region on the outer lip of the optic cup where the prospective neural and pigmented retinal layers meet. Neural crest-derived mesenchymal cells also populate the developing iris. The sclera develops from the mesenchymal tissue surrounding the developing eye. Phenotypic analysis of some mouse mutants have found abnormal development of these structures. Pax-6 has a crucial role in both iris and cornea development which is sensitive to gene dosage (see below).

Hypoplasic irises (aniridia) are found in heterozygous Sey (Pax-6 mutant) mice (Hogan et al., 1986; Hill et al., 1991). It is significant that the posterior layer of the iris is formed by cells from the rim of the optic cup, which expresses Pax-6. The failure of these cells to migrate and differentiate is possibly the cause of the defect. The aniridia syndrome also occurs in humans as a result of mutations at the PAX-6 locus (Ton et al., 1991; Glaser et al., 1992; Jordan et al., 1992). After the migration of the cells, Otx1 is expressed in the developing iris and the ciliary body. (Boncinelli et al., 1993; Simeone et al., 1993). *Otx1* null mutants have impaired development of the iris and agenesis of the ciliary process (Acampora et al., 1996). Proper iris development requires the action of transforming growth factor α (TGFα). Loss of function of this secreted factor or of its receptor resulted in the arrest of iris development (Luetteke et al., 1993, 1994).

The cornea develops from ectodermal tissue that forms over the lens after its formation and which expresses Pax-6 (Walther and Gruss, 1991). This is thought to be the result of an induction event (Hay, 1979). Normal cornea development requires the correct dosage of *Pax-6*; opaque corneas are found in heterozygous Sey mice (Hogan et al., 1986; Hill et al., 1991). Mutations in one PAX-6 gene are also associated with the human syndrome for opaque corneas, Peteŕs anomaly (Hanson et al., 1994). TGF has been shown to have a role in formation of the corneal endothelium and stroma. In null targeted and natural (Waved-1) mutants, the cornea failed to completely separate from the anterior of the lens accompanied by the arrest of iris development (Luetteke et al., 1993). This is consistent with the phenotype observed in mice homozygous for *Waved-2*, the natural mutant of the gene for TGFα's receptor EGFR (Luetteke et al., 1994). Overexpression TGFα using the αA-crystallin promoter (Decsi et al., 1994; Reneker et al., 1995) resulted in mesenchymal cells accumulating perilenticularly. Thus, TGFα may be a chemoattractant for those mesenchymal cells that form the corneal endothelium and elements of the iris. PDGF may play a similar role. Loss of function mutants for the PDGF receptor failed to develop the neural crest derived structures in the eye, such as the corneal endothelium and the sclera (Morrison-Graham et al., 1992).

4. Outgrowth of retinal ganglion cell axons from the retina to the optic tectum

Light is detected by rod and cone photoreceptor cells and the resultant visual information is ultimately conveyed by the axons of the retinal ganglion cells from the retina to the optic tectum. The guidance of these axons during their outgrowth from the retina and the spatially-related matching of these axons to their neuronal targets in the tectum has long been studied in cellular terms. Molecules that influence the two processes have been identified in the last decade and these are listed in Table 3.

4.1. Formation of the optic nerve

The optic nerve contains, as well as RGC axons, three cell types: oligodendrocytes, Type I and Type II astrocytes (reviewed in Barres and Raff, 1994). These cells play a supportive role for the optic nerve axons. The oligodendrocytes and the Type II astrocytes both differentiate from the O-2A cell type (Temple and Raff, 1985, 1986), which migrates into the optic stalk from the optic chiasm (Small et al., 1987). The Type 1 astrocytes, which may be descended from optic stalk cells (Small et al., 1987), promote mitosis and motility of O-2A cells in vitro (Raff et al., 1985). Moreover, addition of antibodies to Type I astrocyte-conditioned medium prevents rat O-2A cell division in vitro (Raff et al., 1988). These processes are influenced by a number of secreted factors (see Table 3). Type 1 astrocytes secrete PDGF which is a mitogen and chemoattractant for O-2A cells (Noble et al., 1988; Raff et al., 1988). CNTF promotes the differentiation of O-2A cells into oligodendro-

Table 3
Regulators of optic nerve formation and retino-tectal projection

Molecule	Retino-tectal type	Species	Expression	Function	Reference
BF-1	TF	Ch, rat	N → T gradient in retina	Regulates RGC naso-temporal positional identity	Hatini et al., 1994; Yuasa et al., 1996
BF-2	TF	Ch, rat	T → N gradient in retina	Regulates RGC naso-temporal positional identity	Hatini et al., 1994; Yuasa et al., 1996
CNTF	SL	Mo, rat	Type 1 astrocytes	Induces O-2A cells into oligodendrocytes and Type II astrocytes	Hughes et al., 1988
DCC	R	Mo	RGC axons	Receptor for Netrin, axonal outgrowth, guidance in optic disc and nerve	Deiner et al., 1997
En-1	TF	Ch, Mo	P → A gradient in optic tectum	Regulates optic tectum antero-posterior positional identity	Friedman and O'Leary, 1996; Itasaki and Nakamura, 1996
En-2	TF	Ch, Mo	P → A gradient in optic tectum	Regulates optic tectum antero-posterior positional identity	Friedman and O'Leary, 1996; Itasaki and Nakamura, 1996
Eph-A3	R	Ch, Mo	T → N gradient in retina	RGC axon guidance in optic tectum	Cheng et al., 1995
Eph-B2	R	Ch	V → D gradient in optic tectum	ns	Braisted et al., 1997
Ephin A2	BL	Ch, Mo	P → A gradient in optic tectum	RGC axon guidance in optic tectum	Cheng et al., 1995; Nakamoto et al., 1996
Ephrin A5	BL	Ch, Mo	P → A gradient, not in extreme posterior tectum	RGC axon guidance in optic tectum	Frisen et al., 1998
Ephrin B1	BL	Ch, Mo	D → V gradient in retina	ns	Braisted et al., 1997
FGF-2	SL*	Xe	Optic nerve and chiasm	Axonal outgrowth, guidance in optic nerve	McFarlane et al., 1995; Walz et al., 1997
LIF	SL	rat	Type 1 astrocytes	Induces O-2A cells into oligodendrocytes and Type II astrocytes	Mayer et al., 1994
Netrin	SL	Mo	Optic disc, optic nerve	Axonal outgrowth, guidance in optic disc and nerve	Deiner et al., 1997
NT-3	SL	Mo	Type 1 astrocytes	Induces O-2A cells into oligodendrocytes and Type II astrocytes	Barres et al., 1993, 1994.
Pax-2	TF	Mo	Optic stalk, optic chiasm	Specification of optic nerve tract and optic chiasm	Torres et al., 1996
PDGF	SL	Mo, rat	Optic stalk	Chemoattractant and mitogen for O-2A cells	Raff et al., 1988; Noble et al., 1988
Radar	SL	Ze	D → V gradient in retina	ns	Rissi et al., 1995
Wnt-7b	SL	Mo	D → V gradient in retina	ns	Parr et al., 1993

BL, membrane bound ligand; SL, secreted ligand; TF transcription factor; Ch, chick; Mo, mouse; Xe, *Xenopus*; Ze, zebrafish; GCL, ganglion cell layer; INL, inner cell layer; NR, neural retina; ns, not yet studied; RGC, retinal ganglion cell; A, anterior; D, dorsal; N, nasal; T, temporal; V, ventral.

cytes and Type II astrocytes (Hughes et al., 1988). Other factors which have been shown to influence the differentiation pathway are LIF (Mayer et al., 1994) and NT-3 (Barres et al., 1993; Barres et al., 1994). Once these cells are differentiated, axons from the retina project down the optic nerve.

Netrin-1 and its receptor Dcc have been shown to direct retinal ganglion cell axons through the optic disc (Deiner et al., 1997). RGC axons fail to grow through the optic disc of both *Netrin-1* and *DCC* null mutant mice. Moreover, Netrin-1 promotes neurite extension of RGCs in a dose-dependent manner. This effect is abolished by application of antibodies to DCC, suggesting that DCC is Netrin-1's only receptor. At the level of transcriptional regulation, the transcription factor, Chx-10 has also been shown to have a role in retinal axonal projections into the optic stalk. In the natural mutant, *or*, ganglion cell axons fail to project through the optic disc into the optic stalk and instead stay between the neural retina and the RPE (Truslove, 1962; Burmeister et al., 1996). This suggests that, in mutants, retinal ganglion cells have no way of recognizing cues in the optic stalk. FGF-2 (basic FGF) has been shown to play a chemotactic role in the outgrowth of RGC axons, at least in *Xenopus*. The protein is expressed along the optic nerve (McFarlane et al., 1995). Application of FGF-2 promotes neurite outgrowth and application of exogenous FGF-2 can divert RGC axons from the pathway (McFarlane et al., 1995). Expression of dominant-negative FGF receptors led to slower rates of axonal extension and also caused the axons to miss the tectum (McFarlane et al., 1996). The FGF-2 molecules could found to be bound to heparan sulfate side-chains of a particular form of perlecan variant that specifically binds FGF-2. Exogenous application of the heparan sulfates disrupt the axons entering the tectum (Walz et al., 1997). Digestion of these heparan sulfates with heparitinase impaired axonal elongation, and causes the axons to miss the tectum (Walz et al., 1997).

Outgrowth of optic nerve axons proceeds to the optic chiasm, where axons either project along an ipsilateral

tract to the optic tectum or cross the midline and project along the contralateral tract (reviewed in Mason and Sretavan, 1997). The percentage of RGC axons that cross the midline and those that do not vary between the vertebral classes. Cells of the chiasm are able to distinguish between axons fated to cross the midline and those that do not. A study of the axonal outgrowth from retinal explants found that axons from the ventrotemporal region of the retina, which stay ipsilateral in vivo, were repulsed by chiasm midline cells, while axons from other regions could extend through this line of cells (Wang et al., 1995). A critical role in optic chiasm development has been found to be played by Pax-2. Null mutant Pax-2 mice (Torres et al., 1996) and Noi mutant zebrafish (Macdonald et al., 1995) have abnormal chiasm formation in which the optic nerve axons are totally prevented (mouse) or mostly prevented (zebrafish) from projecting across the midline into the contralateral optic tract. It is significant that the region fated to become the chiasm does not express Shh in normal embryos but does in Pax-2 null mutants. This suggests that Shh may induce the expression of a surface molecule that is a barrier to optic axon projection and that the suppression of Shh expression in the chiasm region by Pax-2 prevents expression of this barrier molecule, thus permitting contralateral projections of optic axons.

5. Regulation of spatial relationships between retinal ganglion cell axons and their targets in the optic tectum

Visual information is spatially related, so the connections between the retinal ganglion cells and the cells of the optic tectum are similarly spatially-related. In the chick, topographical maps of neuronal connectivity have been well documented by heterotopic grafting experiments (reviewed in Sanes, 1993; Kaprielian and Patterson, 1994). For example, retinal ganglion cell axons of the temporal part of the retina project to the anterior part of the optic tectum and those from the nasal region of the retina innervate the caudal tectum. Matching of axons to their targets is postulated to be mediated by distributions of surface molecules in the retina, retinal axons and in the tectum that confer positional 'addresses' to cells in both the retina and the optic tectum (Sperry, 1963). In recent years, a number of molecules whose expression and/or function are consistent with Sperry's model have been identified (see Table 3).

The ephrin class of membrane-linked ligands and their Eph-class receptors have expression gradients and regulate the direction of axonal outgrowth in a number of tissues (reviewed in Gale and Yancopoulos, 1997; Pasquale, 1997; Sefton and Nieto, 1997). Ephrin-A2 (ELF1) is expressed in a high posterior to low anterior gradient in the tectum (Cheng et al., 1995) and one of its receptors, EphA3 (Mek4) is expressed in a high temporal to low nasal gradient (Cheng et al., 1995). When ephrin-A2 is ectopically expressed using RCAS expression vectors in the anterior tectum of the chick, the temporal RGC axons avoided the ephrin-A2 positive patches (Nakamoto et al., 1996). Moreover, axons from cultured temporal RGCs avoided stripes of membranes prepared from ephrin-A2, keeping to membrane stripes prepared from control cells (Nakamoto et al., 1996). Ephrin-A5 (Al-1/RAGS) has a similar gradient of expression, but in this case the higher concentration penetrates further anteriorly in the tectum and is absent from the most posterior edge of the tectum (Drescher et al., 1995; Frisen et al., 1998). In ephrin-A5 null mutant mice, nasal RGC axons projected to the posterior margin in the optic tectum and also to the inferior colliculus, which expresses ephrin-A5 but not ephrin-A2 (Frisen et al., 1998). At the level of transcription, anteroposterior tectal cell identity is regulated at a transcriptional level by the En factors (Friedman and O'Leary, 1996; Itasaki and Nakamura, 1996). Ectopic expression in the chick tectum using RCAS retroviral vectors resulted in innervation of the tectum by nasal RGC axons at inappropriately-anterior locations with ectopic *En* gene expression, while temporal axons often did not innervate the tectum at all. Similarly, the winged helix transcription factors, BF-1 and BF-2, display complementary expression gradients in the retina along the naso-temporal axis (Hatini et al., 1994; Yuasa et al., 1996). Temporal expression (by using a retroviral vector), of chick BF-1, which is normally expressed in the nasal retina, resulted in the axons of the temporal ganglion cells projecting to the caudal tectum instead of the rostral tectum (Yuasa et al., 1996). Nasal expression of chick BF-2, which is normally expressed in the temporal retina, resulted in the axons of the nasal ganglion cells projecting to the rostral tectum instead of the caudal tectum (Yuasa et al., 1996). Thus, the level of expression of these two factors gives the individual retinal ganglion cells positional identity along the naso-temporal axis.

Similar molecules exhibit dorso-ventral expression gradients in the retino-tectal system. EphB2 (Cek5/Sek3) is found in a high ventral to low dorsal expression gradient in RGC and one of its ligands, ephrin-B1 (LERK2), is expressed in a high dorsal to low ventral concentration gradient in the chick optic tectum (Braisted et al., 1997). The secreted factors Wnt-7b (Parr et al., 1993) and radar, a novel TGFβ family factor (Rissi et al., 1995), are expressed in a high dorsal to low ventral gradient in the mouse and the zebrafish, respectively. No functional studies have yet been published for these molecules, and transcription factors with these patterns of expression have yet to be identified.

6. Conclusions and perspectives

The mechanisms that regulate eye development vary according to developmental stage and tissue. Early development of eye structures is characterized by mediolateral interactions that generate the neural structures and by the induction of the lens placode. Three transcription factors

Rx, Lhx-2 and Pax-6 are known to be crucial to this phase. Later development is characterized by reciprocal interactions between the different tissues. Normal development of the retina requires normal development of the lens and vice versa. The same is true for the development of the neural retina and the retinal pigmented epithelium. These interactions are mediated by secreted ligands such as FGF-1, FGF-2, PDGF and the neurotrophins. Lens development is primarily regulated by secreted ligand gradients between the anterior and posterior of the lens. Higher concentrations of the ligands result in proliferation of the normally quiescent lens epithelial cells and even higher concentrations result in the induction of differentiation into lens fibres. Correct generation of retinal cell types is ensured by competition between secreted ligands that induce differentiation and Delta-Notch-1 signalling that mediates the inhibition of differentiation. Thus, enough progenitor cells are retained by this competition for the generation of later cell types. The formation of neuronal connections is characterized firstly by the formation of nerve tracts with cell types that support axonal extension, secondly, the outgrowth of RGC axons from the retina to the tectum, and thirdly, the positionally-based matching of RGC axons to their tectal cell targets. Formation of neuronal connections is regulated by concentration gradients of secreted, transcriptional regulator and surface molecules.

Many members of the signalling cascades which direct eye development have yet to be identified. The challenge in future research is to identify these members and dissect the signalling cascades involved. A significant step in the identification of novel regulatory elements has come from the recent zebrafish genetic screens, which employed chemical mutation of the genome. These have resulted in one optic vesicle formation mutant (Heisenberg et al., 1996); three new cyclopia mutants (Malicki et al., 1996); three mutants with pigmentation, defects (Malicki et al., 1996), seven neural retina mutants (Malicki et al., 1996), 23 mutants with either growth retardation or retinal degeneration (Malicki et al., 1996), one optic coloboma mutant (Karlstrom et al., 1996) and 19 retinal ganglion cell axon pathfinding mutants (Karlstrom et al., 1996). While the mutated genes have yet to be, or are being in the process of being positionally cloned, the generation of the mutants themselves suggest the later identification of many more members of regulatory pathways that control eye development. Moreover, the generation of null mouse mutants for novel genes expressed in the developing eye will identify other novel molecules that control eye development. Thus, research using the different vertebrate model organisms, each with their own strengths, will contribute to a greater understanding of vertebrate eye morphogenesis.

Acknowledgements

We would like to thank Dr. Naihe Jing for critical reading of the manuscript. We also wish to thank Ralf Altschäffel for photographic work. This work was supported by DFG (Leibniz) and SFB Grant 271.A9.

References

Acampora, D., Mazan, S., Avantaggiato, V., Barone, P., Tuorto, F., Lallemand, Y., Brulet, P., Simeone, A., 1996. Epilepsy and brain abnormalities in mice lacking the Otx1 gene. Nat. Genet. 14, 218–222.

Altman, C.R., Chow, R.L., Lang, R.A., Hemmati-Brivanlou, A., 1997. Lens induction by Pax-6 in *Xenopus laevis*. Dev. Biol. 185, 119–123.

Anchan, R.M., Reh, T.A., 1995. Transforming growth factor-beta-3 is mitogenic for rat retinal progenitor cells in vitro. J. Neurobiol. 28, 133–145.

Anchan, R.M., Reh, T.A., Angello, J., Baillet, A., Walker, M., 1991. EGF and TGF alpha stimulate retinal neuroepithelial cell proliferation in vitro. Neuron 6, 923–936.

Andres, V., Nadal-Ginard, B., Mahdavi, V., 1992. Clox, a mammalian homeobox gene related to Drosophila cut, encodes DNA-binding regulatory elements differentially expressed during development. Development 116, 321–334.

Austin, C.P., Feldman, D.E., Ida, J.A. Jr., Cepko, C.L., 1995. Vertebrate retinal ganglion cells are selected from competent progenitors by the action of Notch. Development 121, 3637–3650.

Bao, Z.Z., Cepko, C.L., 1997. The expression and function of Notch pathway genes in the developing rat eye. J. Neurosci. 17, 1425–1434.

Barres, B.A., Raff, M.C., 1994. Control of oligodendrocyte number in the developing rat optic nerve. Neuron 12, 935–942.

Barres, B.A., Raff, M.C., Gaese, F., Bartke, I., Dechant, G., Barde, Y.A., 1994. A crucial role for neurotrophin-3 in oligodendrocyte development. Nature 367, 371–375.

Barres, B.A., Schmid, R., Sendnter, M., Raff, M.C., 1993. Multiple extracellular signals are required for long-term oligodendrocyte survival. Development 118, 283–295.

Beebe, D.C., Silver, M.H., Belcher, K.S., Van Wyk, J.J., Svoboda, M.E., Zelenka, P.S., 1987. Lentropin, a protein that controls lens fiber formation, is related functionally and immunologically to the insulin-like growth factors. Proc. Natl. Acad. Sci. USA 84, 2327–2330.

Boncinelli, E., Gulisano, M., Broccoli, V., 1993. Emx and Otx homeobox genes in the developing mouse brain. J. Neurobiol. 24, 1356–1366.

Bovolenta, P., Frade, J.M., Marti, E., Rodriguezpena, M.A., Barde, Y.A., Rodrigueztebar, A., 1996. Neurotrophin-3 antibodies disrupt the normal development of the chick retina. J. Neurosci. 16, 4402–4410.

Bovolenta, P., Mallamaci, A., Briata, P., Corte, G., Boncinelli, E., 1997. Implication of OTX2 in pigment epithelium determination and neural retina differentiation. J. Neurosci. 17, 4243–4252.

Braisted, J.E., Mclaughlin, T., Wang, H.U., Friedman, G.C., Anderson, D.J., O'Leary, D.D.M., 1997. Graded and lamina-specific distributions of ligands of EphB receptor tyrosine kinases in the developing retinotectal system. Dev. Biol. 191, 14–28.

Brewitt, B., Clark, J.I., 1988. Growth and transparency in the lens, an epithelial tissue, stimulated by pulses of PDGF. Science 242, 777–779.

Burmeister, M., Novak, T., Liang, M.Y., Basu, S., Ploder, L., Hawes, N.L., Vidgen, D., Hoover, F., Goldman, D., Kalnins, V.I., Roderick, T.H., Taylor, B.A., Hankin, M.H., McInnes, R.R., 1996. Ocular retardation mouse caused by Chx-10 homeobox null allele – impaired retinal progenitor proliferation and bipolar cell differentiation. Nat. Genet. 12, 376–384.

Casarosa, S., Andreazzoli, M., Simeone, M., Barsacchi, G., 1997. Xrx-1, a novel Xenopus homeobox gene expressed during eye and pineal gland development. Mech. Dev. 61, 187–198.

Cellerino, A., Kohler, K., 1997. Brain-derived neurotrophic factor/neurotrophin-4 receptor trkB is localized on ganglion cells. J. Comp. Neurol. 386, 149–160.

Cepko, C.L., Austin, C.P., Yang, X., Alexiades, M., Ezzedine, D., 1996. Cell fate determination in the retina. Proc. Natl. Acad. Sci USA 93, 589–595.

Cheng, H.-J., Nakamoto, M., Bergemann, A.D., Flanagan, J.G., 1995. Complementary gradients in expression and binding of ELF-1 and Mek4 in development of the topographical retinotectal projection map. Cell 82, 371–381.

Chiang, C., Litingtung, Y., Lee, E., Young, K.E., Corden, J.L., Westphal, H., Beachy, P.A., 1996. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383, 407–413.

Chow, R.L., Roux, G.D., Roghani, M., Palmer, M.A., Rifkin, D.B., Moscatelli, D.A., Lang, R.A., 1995. FGF suppresses apoptosis and induces differentiation of fibre cells in the mouse lens. Development 121, 4383–4393.

Cohen-Cory, S., Escandon, E., Fraser, S.E., 1996. The cellular patterns of BDNF and trkB expression suggest multiple roles for BDNF during Xenopus visual system development. Dev. Biol. 179, 102–115.

Coulombre, J.L., Coulombre, A.J., 1963. Lens development: fiber elongation and lens orientation. Science 142, 1489–1490.

Cvekl, A., Sax, C.M., Bresnick, E.H., Piatigorsky, J., 1994. A complex array of positive and negative elements regulates the chicken alpha A-crystallin gene: involvement of Pax-6, USF, CREB and/or CREM, and AP-1 proteins. Mol. Cell. Biol. 14, 7363–7376.

Cvekl, A., Sax, C.M., Li, X., McDermott, J.B., Piatigorsky, J., 1995. Pax-6 and lens-specific transcription of the chicken delta 1-crystallin gene. Proc. Natl. Acad. Sci. USA 92, 4681–4685.

Danias, J., Stylianopoulou, F., 1990. Expression of IGF-I and IGF-II genes in the adult rat eye. Curr. Eye Res. 9, 79–86.

Decsi, A., Peiffer, R.L., Qiu, T., Lee, D.C., Friday, J.T., Bautch, V.L., 1994. Lens expression of TGF alpha in transgenic mice produces two distinct eye pathologies in the absence of tumors. Oncogene 9, 1965–1975.

Deiner, M.S., Kennedy, T.E., Fazeli, A., Serafini, T., Tessier-Lavigne, M., Sretavan, D.W., 1997. Netrin-1 and DCC mediate axon guidance locally at the optic disc, loss of function leads to optic nerve hypoplasia. Neuron 19, 575–589.

de la Rosa, E.J., Arribas, A., Frade, J.M., Rodriguez-Tebar, A., 1994. Role of neurotrophins in the control of neural development: neurotrophin-3 promotes both neuron differentiation and survival of cultured chick retinal cells. Neuroscience 58, 347–352.

deIongh, R., McAvoy, J.W., 1993. Spatio-temporal distribution of acidic and basic FGF indicates a role for FGF in rat lens morphogenesis. Dev. Dyn. 198, 190–202.

Dollé, P., Price, M., Duboule, D., 1992. Expression of the murine Dlx-1 homeobox gene during facial, ocular and limb development. Differentiation 49, 93–99.

Dorsky, R.I., Rapaport, D.H., Harris, W.A., 1995. Xotch inhibits cell differentiation in the Xenopus retina. Neuron 14, 487–496.

Dorsky, R.I., Chang, W.S., Rapaport, D.H., Harris, W.A., 1997. Regulation of neuronal diversity in the Xenopus retina by Delta signalling. Nature 385, 67–70.

Drescher, U., Kremoser, C., Handwerker, C., Loschinger, J., Noda, M., Bonhoeffer, F., 1995. In vitro guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal protein related to ligands for Eph receptor tyrosine kinases. Cell 82, 359–370.

Dudley, A.T., Lyons, K.M., Robertson, E.J., 1995. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev. 9, 2795–2807.

Ezzeddine, Z.D., Yang, X., DeChiara, T., Yancopoulos, G., Cepko, C.L., 1997. Postmitotic cells fated to become rod photoreceptors can be respecified by CNTF treatment of the retina. Development 124, 1055–1067.

Frade, J.M., Marti, E., Bovolenta, P., Rodriguez-Pena, M.A., Perez-Garcia, D., Rohrer, H., Edgar, D., Rodriguez-Tebar, A., 1996. Insulin-like growth factor-I stimulates neurogenesis in chick retina by regulating expression of the alpha 6 integrin subunit. Development 122, 2497–2506.

Frade, J.M., Bovolenta, P., Martinez-Morales, J.R., Arribas, J.A., Barbas, J.A., Rodriguez-Tebar, A., 1997. Control of early cell death by BDNF in the chick retina. Development 124, 3313–3320.

Franz, T., Besecke, A., 1991. The development of the eye in homozygotes of the mouse mutant Exta-toes. Anat. Embryol. 184, 355–361.

Freund, C.L., Gregory-Evans, C.Y., Furukawa, T., Papaioannou, N., Looser, J., Ploder, L., Bellingham, J., Ng, D., Herbrick, J.A., Duncan, A., Scherer, S.W., Tsui, L.C., Loutradis-Anagnostou, A., Jacobson, S.G., Cepko, C.L., Bhattacharya, S.S., McInnes, R.R., 1997. Cone-rod dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (CRX) essential for maintenance of the photoreceptor. Cell 91, 543–553.

Friedman, G.C., O'Leary, D.D., 1996. Retroviral misexpression of engrailed genes in the chick optic tectum perturbs the topographical targeting of retinal axons. J. Neurosci. 16, 5498–5509.

Frisen, J., Yates, P.A., McLaughlin, T., Friedman, G.C., O'Leary, D.D., Barbacid, M., 1998. Ephrin-A5 (Al-1/RAGS) is essential for proper retinal axon guidance and topographic mapping in the mammalian visual system. Neuron 20, 235–243.

Fruttiger, M., Calver, A.R., Kruger, W.H., Mudhar, H.S., Michalovich, D., Takakura, N., Nishikawa, S., Richardson, W.D., 1996. PDGF mediates a neuron-astrocyte interaction in the developing retina. Neuron 17, 1117–1131.

Fuhrmann, S., Kirsch, M., Hofmann, H.D., 1995. Ciliary neurotrophic factor promotes chick photoreceptor development in vitro. Development 121, 2695–2706.

Fujiwara, M., Uchida, T., Osumi-Yamashita, N., Eto, K., 1994. Uchida rat (rSey): a new mutant rat with craniofacial abnormalities resembling those of the mouse Sey mutant. Differentiation 57, 31–38.

Furukawa, T., Kozak, C.A., Cepko, C.L., 1997a. Rax, a novel paired-type homeobox gene, shows expression in the anterior neural fold and developing retina. Proc. Natl. Acad. Sci USA 94, 3088–3093.

Furukawa, T., Morrow, E.M., Cepko, C.L., 1997b. Crx, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. Cell 91, 531–541.

Gale, N.W., Yancopoulos, G.D., 1997. Ephrins and their receptors – a repulsive topic. Cell Tissue Res. 290, 227–241.

Gan, L., Xiang, M., Zhou, L., Wagner, D.S., Klein, W.H., Nathans, J., 1996. POU domain factor Brn-3b is required for the development of a large set of retinal ganglion cells. Proc. Natl. Acad. Sci. USA 93, 3920–3925.

Glaser, T., Walton, D.S., Maas, R.L., 1992. Genomic structure, evolutionary conservation and aniridia mutations in the human PAX-6 gene. Nat. Genet. 2, 232–239.

Gopal-Srivastava, R., Cvekl, A., Piatigorsky, J., 1996. Pax-6 and alphaB-crystallin/small heat shock protein gene regulation in the murine lens. Interaction with the lens-specific regions, LSR1 and LSR2. J. Biol. Chem. 271, 23029–23036.

Graw, J., 1996. Genetic aspects of embryonic eye development in vertebrates. Dev. Genet. 18, 181–197.

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

Guillemot, F., Cepko, C.L., 1992. Retinal fate and ganglion cell differentiation are potentiated by acidic FGF in an in vitro assay of early retinal development. Development 114, 743–754.

Guillemot, F., Joyner, A.L., 1993. Dynamic expression of the murine Achaete-Scute homologue Mash-1 in the developing nervous system. Mech. Dev. 42, 171–185.

Hanson, I.M., Fletcher, J.M., Jordan, T., Brown, A., Taylor, D., Adams, R.J., Punnett, H.H., van Heyningen, V., 1994. Mutations at the PAX6 locus are found in heterogeneous anterior segment malformations including Peters' anomaly. Nat. Genet. 6, 168–173.

Harris, W.A., 1997. Cellular diversification in the vertebrate retina. Curr. Opin. Genet. Dev. 7, 651–658.

Hatini, V., Tao, W., Lai, E., 1994. Expression of winged helix genes, BF-1 and BF-2, define adjacent domains within the developing forebrain and retina. J. Neurobiol. 25, 1293–1309.

Hay, E.D., 1979. Development of the vertebrate cornea. Int. Rev. Cytol. 63, 263–322.

Heisenberg, C.P., Brand, M., Jiang, Y.J., Warga, R.M., Beuchle, D., van Eeden, F.J., Furutani-Seiki, M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D.A., Kelsh, R.N., Mullins, M.C., Odenthal, J., Nusslein-Volhard, C., 1996. Genes involved in forebrain development in the zebrafish *Danio rerio*. Development 123, 191–203.

Henrique, D., Hirsinger, E., Adam, J., Le Roux, I., Pourquie, O., Ishhorowicz, D., Lewis, J., 1997. Maintenance of neuroepithelial progenitor cells by Delta-Notch signalling in the embryonic chick retina. Curr. Biol. 7, 661–670.

Hernandez-Sanchez, C., Lopez-Carranza, A., Alarcon, C., de La Rosa, E.J., de Pablo, F., 1995. Autocrine/paracrine role of insulin-related growth factors in neurogenesis: local expression and effects on cell proliferation and differentiation in retina. Proc. Natl. Acad. Sci. USA 92, 9834–9838.

Hertwig, P., 1942. Neue Mutationen und Koppelungsgruppen bei der Hausmaus. Z. Indukt. Abstammungs Vererbungsl. 80, 220–246.

Herzog, K.H., Bailey, K., Barde, Y.A., 1994. Expression of the BDNF gene in the developing visual system of the chick. Development 120, 1643–1649.

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

Hirsch, N., Harris, W.A., 1997. Xenopus Pax-6 and retinal development. J. Neurobiol. 32, 45–61.

Hodgkinson, C.A., Moore, K.J., Nakayama, A., Steingrimsson, E., Copeland, N.G., Jenkins, N.A., Arnheiter, H., 1993. Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. Cell 74, 395–404.

Hogan, B.L., Hirst, E.M., Horsburgh, G., Hetherington, C.M., 1988. Small eye (Sey): a mouse model for the genetic analysis of craniofacial abnormalities. Development 103, 115–119.

Hogan, B.L., Horsburgh, G., Cohen, J., Hetherington, C.M., Fisher, G., Lyon, M.F., 1986. 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. Morphol. 97, 95–110.

Hughes, S.M., Lillien, L.E., Raff, M.C., Rohrer, H., Sendtner, M., 1988. Ciliary neurotrophic factor induces type-2 astrocyte differentiation in culture. Nature 335, 70–73.

Hui, C.C., Joyner, A.L., 1993. A mouse model of Grieg cephalopolysydactylly syndrome: the extra-toes^{J} mutation contains an intragenic deletion of the Gli-3 gene. Nat. Genet. 3, 241–246.

Hui, C.C., Slusarki, D., Platt, K.A., Holmgren, R., Joyner, A.L., 1994. Expression of three mouse homologs of the drosophila segment polarity gene cubitus interruptus, Gli-1, Gli-2 and Gli-3, in ectoderm- and mesoderm-derived tissues suggests multiple roles during postimplantation development. Dev. Biol. 162, 402–413.

Hyatt, G.A., Schmitt, E.A., Fadool, J.M., Dowling, J.C., 1996. Retinoic acid alters photoreceptor development in vivo. Proc. Natl. Acad. Sci. USA 93, 13298–13303.

Hyatt, G.A., Schmitt, E.A., Marsh-Armstrong, N., McCaffery, P., Drager, U.C., Dowling, J.C., 1996. Retinoic acid establishes ventral retinal characteristics. Development 122, 195–204.

Hyer, J., Mima, T., Mikawa, T., 1998. FGF1 patterns the optic vesicle by directing the placement of the neural retina domain. Development 125, 869–877.

Ishizaki, Y., Voyvodic, J.T., Burne, J.F., Raff, M.C., 1993. Control of lens epithelial cell survival. J. Cell. Biol. 121, 899–908.

Itasaki, N., Nakamura, H., 1996. A role for gradient En expression in positional specification on the optic tectum. Neuron 16, 55–62.

Johnson, J.E., Barde, Y.A., Schwab, M., Thoenen, H., 1986. Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. J. Neurosci. 6, 3031–3038.

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

Kamachi, Y., Kondoh, H., 1993. Overlapping positive and negative regulatory elements determine lens-specific activity of the delta 1-crystallin enhancer. Mol. Cell. Biol. 13, 5206–5215.

Kamachi, Y., Sockanathan, S., Liu, Q., Breitman, M., Lovell-Badge, R., Kondoh, H., 1995. Involvement of SOX proteins in lens-specific activation of crystallin genes. EMBO J. 14, 3510–3519.

Kanekar, S., Perron, M., Dorsky, R., Harris, W.A., Jan, L.Y., Jan, Y.N., Vetter, M.L., 1997. Xath5 participates in a network of bHLH genes in the developing Xenopus retina. Neuron 19, 981–994.

Kaprielian, Z., Patterson, P.H., 1994. The molecular basis of retinotectal topography. Bioessays 16, 1–11.

Karkinnen-Jaaskelainen, M., 1978. Transfilter lens induction in avian embryo. Differentiation 12, 31–37.

Karlstrom, R.O., Towe, T., Klosterman, S., Baier, H., Brand, M., Crawford, A.D., Grunewald, B., Haffter, P., Hoffman, H., Meyer, S.U., Muller, B.K., Richter, S., van Eeden, F.J.M., Nuesslein-Volhard, C., Bonhoefferm, F., 1996. Zebrafish mutations affecting retinotectal axon pathfinding. Development 123, 427–438.

Kawakami, K., Ohto, H., Takizawa, T., Saito, T., 1996. Identification and expression of Six family genes in mouse retina. FEBS Lett. 393, 259–263.

Keller, S.A., Jones, J.M., Boyle, A., Barrow, L.L., Killen, P.D., Green, D.G., Kapousta, N.V., Hitchcock, P.F., Swank, R.T., Meisler, M.H., 1994. Kidney and retinal defects (Krd), a transgene-induced mutation with a deletion of mouse chromosome 19 that includes the Pax-2 locus. Genomics 23, 309–320.

Kelley, M.W., Turner, J.K., Reh, T.A., 1994. Retinoic acid promotes the differentiation of photoreceptors in vitro. Development 120, 2091–2102.

Kelley, M.W., Turner, J.K., Reh, T.A., 1995. Ligands of steroid/thyroid receptors induce cone photoreceptors in vertebrate retina. Development 121, 3777–3785.

Kirsch, M., Fuhrmann, S., Wiese, A., Hofmann, H.D., 1996. CNTF exerts opposite effects on in vitro development of rat and chick photoreceptors. Neuroreport 7, 697–700.

Lee, J.E., Hollenberg, S.M., Snider, L., Turner, D.L., Lipnick, N., Weintraub, H., 1995. Conversion of Xenopus ectoderm into neurons by NeuroD, a basic helix-loop-helix protein. Science 268, 836–844.

Levine, E.M., Roelink, H., Turner, J., Reh, T.A., 1997. Sonic hedgehog promotes rod photoreceptor differentiation in mammalian retinal cells in vitro. J. Neurosci. 17, 6277–6288.

Li, H.S., Tierney, C., Wen, L., Wu, J.Y., Rao, Y., 1997. A single morphogenetic filed gives rise to two retina primordia under the influence of the prechordal plate. Development 124, 603–615.

Li, H.S., Yang, J.M., Jacobson, R.D., Pasko, D., Sundin, O., 1994. 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. 162, 181–194.

Lillien, L., 1995. Changes in retinal cell fate induced by overexpression of EGF receptor. Nature 377, 158–162.

Lillien, L., Cepko, C., 1992. Control of proliferation in the retina: temporal changes in responsiveness to FGF and TGF alpha. Development 115, 253–266.

Liu, I.S., Chen, J.D., Ploder, L., Vidgen, D., van der Kooy, D., Kalnins, V.I., McInnes, R.R., 1994. Developmental expression of a novel murine homeobox gene (Chx10): evidence for roles in determination of the neural retina and inner nuclear layer. Neuron 13, 377–393.

Liu, Z.Z., Zhu, L.Q., Eide, F.F., 1997. Critical role of TrkB and brain-derived neurotrophic factor in the differentiation and survival of retinal pigment epithelium. J. Neurosci. 17, 8749–8755.

Lovicu, F.J., deIongh, R.U., McAvoy, J.W., 1997. Expression of FGF-1 and FGF-2 mRNA during lens morphogenesis, differentiation and growth. Curr. Eye Res. 16, 222–230.

Luetteke, N.C., Qiu, J.H., Peiffer, R.L., Oliver, P., Smithies, O., Lee, D.C.,

1993. TGF alpha deficiency results in hair follicle and eye deformities in targeted and Waved-1 mice. Cell 73, 263–278.

Luetteke, N.C., Phillips, H.K., Qiu, T.H., Copeland, N.G., Earp, H.S., Jenkins, N.A., Lee, D.C., 1994. The mouse Waved-2 phenotype results from a point mutation in the EGF receptor tyrosine kinase. Genes Dev. 8, 399–413.

Luo, G., Hofmann, C., Bronckers, A.L., Sohocki, M., Bradley, A., Karsenty, G., 1995. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. Genes Dev. 9, 2808–2820.

Macdonald, R., Barth, K.A., Xu, Q., Holder, N., Mikkola, I., Wilson, S.W., 1995. Midline signalling is required for Pax gene regulation and patterning of the eyes. Development 121, 3267–3278.

Macdonald, R., Scholes, J., Strahle, U., Brennan, C., Holder, N., Brand, M., Wilson, S.W., 1997. The Pax protein Noi is required for commissural axon pathway formation in the rostral forebrain. Development 124, 2397–2408.

Macdonald, R., Wilson, S.W., 1997. Distribution of Pax 6 protein during eye development suggests discrete roles in proliferative and differentiated visual cells. Dev. Genes Evol. 206, 363–369.

Malicki, J., Neuhass, S.C., Schier, A.F., Solnica-Krezel, L., Stemple, D.L., Stainler, D.Y., Abdelilah, S., Zwartkruis, F., Rangini, Z., Driever, W., 1996. Mutations affecting development in the zebrafish retina. Development 123, 263–273.

Marsh-Armstrong, N., McCaffery, P., Gilbert, W., Dowling, J.E., Drager, U.C., 1994. Retinoic acid is necessary for development of the ventral retina in zebrafish. Proc. Natl. Acad. Sci. USA 91, 7286–7290.

Mason, C.A., Sretavan, D.W., 1997. Glia, neurons, and axon pathfinding during optic chiasm development. Curr. Opin. Neurobiol. 7, 647–653.

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

Matsuo, I., Kuratani, S., Kimura, C., Takeda, N., Aizawa, S., 1995. Mouse Otx2 functions in the formation and patterning of the rostral head. Genes Dev. 9, 2646–2658.

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

Mayer, M., Bhakoo, K., Noble, M., 1994. Ciliary neurotrophic factor and leukemia inhibitory factor promote the generation, maturation and survival of oligodendrocytes in vitro. Development 120, 143–153.

McAvoy, J.W., Chamberlain, C.G., 1989. Fibroblast growth factor (FGF) induces different responses in lens epithelial cells depending on its concentration. Development 107, 221–228.

McFarlane, S., Cornel, E., Amaya, E., Holt, C.E., 1996. Inhibition of FGF receptor activity in retinal ganglion cell axons causes errors in target recognition. Neuron 17, 245–254.

McFarlane, S., McNiell, L., Holt, C.E., 1995. FGF signalling and target recognition in the developing Xenopus visual system. Neuron 15, 1017–1028.

Mizuno, M., Takabatake, T., Takahashi, T.C., Takeshima, K., 1997. Pax-6 Gene Expression in Newt Eye Development. Dev. Genes Evol. 207, 167–176.

Morgenbesser, S.D., Williams, B.O., Jacks, T., DePinho, R.A., 1994. p53-dependent apoptosis produced by Rb-deficiency in the developing mouse lens. Nature 371, 72–74.

Morrison-Graham, K., Schatteman, G.C., Bork, T., Bowen-Pope, D.F., Weston, J.A., 1992. A PDGF receptor mutation in the mouse (Patch) perturbs the development of a non-neuronal subset of neural crest-derived cells. Development 115, 133–142.

Nakamoto, M., Cheng, H.-J., Friedman, G.C., McLaughlin, T., Hansen, M.J., Yoon, C.H., O'Leary, D.D., Flanagan, J.G., 1996. Topographically specific effects of ELF-1 on retinal axon guidance in vitro and retinal axon mapping in vivo. Cell 86, 755–766.

Neophytou, C., Vernallis, A.B., Smith, A., Raff, M.C., 1997. Muller-cell-derived leukaemia inhibitory factor arrests rod photoreceptor differentiation at a postmitotic pre-rod stage of development. Development 124, 2345–2354.

Nishiguchi, S., Wood, H., Kondoh, H., Lovell-Badge, R., Episkopou, V., 1998. Sox-1 directly regulates the γ-crystallin genes and is essential for lens development in mice. Genes Dev. 12, 776–781.

Noble, M., Murray, K., Stroobant, P., Waterfield, M.D., Riddle, P., 1988. Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. Nature 333, 560–562.

Nornes, H.O., Dressler, G.R., Knapik, E.W., Deutsch, U., Gruss, P., 1990. Spatially and temporally restricted expression of Pax2 during murine neurogenesis. Development 109, 797–809.

Oliver, G., Loosli, F., Koster, R., Wittbrodt, J., Gruss, P., 1996. Ectopic lens induction in fish in response to the murine homeobox gene Six-3. Mech. Dev. 60, 233–239.

Oliver, G., Mailhos, A., Wehr, R., Copeland, N.G., Jenkins, N.A., Gruss, P., 1995. Six-3, a murine homologue of the sine oculis gene, demarcates the most anterior border of the developing neural plate and is expressed during eye development. Development 121, 4045–4055.

Oliver, G., Sosa-Pineda, B., Geisendorf, S., Spana, E.P., Doe, C.Q., Gruss, P., 1993. Prox-1, a prospero-related homeobox gene expressed during mouse development. Mech. Dev. 44, 3–16.

Park, C.M., Hollenberg, M.J., 1989. Basic fibroblast growth factor induces retinal regeneration in vivo. Dev. Biol. 134, 201–205.

Parr, B.A., Shea, M.J., Vassileva, G., McMahon, A.P., 1993. Mouse Wnt genes exhibit discrete domains of expression in the early embryonic CNS and limb buds. Development 119, 247–261.

Pasquale, E.B., 1997. The Eph family of receptors. Curr. Opin. Cell Biol. 9, 608–615.

Pfaff, S.L., Mendelsohn, M., Stewart, C.L., Edlund, T., Jessell, T.M., 1996. Requirement for LIM homeobox gene Isl-1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. Cell 84, 309–320.

Piatigorsky, J., 1981. Lens differentiation in vertebrates: a review of cellular and molecular features. Differentiation 19, 134–153.

Pittack, C., Grunwald, G.B., Reh, T.A., 1997. Fibroblast growth factors are necessary for neural retina but not pigmented epithelium differentiation in chick embryos. Development 124, 805–816.

Pittack, C., Jones, M., Reh, T.A., 1991. Basic fibroblast growth factor induces retinal pigment epithelium to generate neural retina in vitro. Development 113, 577–588.

Porter, F.D., Drago, J., Xu, Y., Cheema, S.S., Wassif, C., Huang, S.P., Lee, E., Grinberg, A., Massala, J.S., Bodine, D., Alt, F., Westphal, H., 1997. Lhx-2, a LIM homeobox gene, is required for eye, forebrain, and definitive erythrocyte development. Development 124, 2935–2944.

Quinn, J.C., West, J.D., Hill, R.E., 1996. Multiple functions for Pax6 in mouse eye and nasal development. Genes Dev. 10, 435–446.

Raff, M.C., Abney, E.R., Fok-Seang, J., 1985. Reconstitution of a developmental clock in vitro: a critical role for astrocytes in the timing of oligodendrocyte differentiation. Cell 42, 61–69.

Raff, M.C., Lillien, L.E., Richardson, W.D., Burne, J.F., Noble, M.D., 1988. Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. Nature 333, 562–565.

Raymond, S.M., Jackson, I.J., 1995. The retinal pigmented epithelium is required for development and maintenance of the mouse neural retina. Curr. Biol. 5, 1286–1295.

Reneker, L.W., Overbeek, P.A., 1996. Lens-specific expression of PDGF-A alters lens growth and development. Dev. Biol. 180, 554–565.

Reneker, L.W., Silversides, D.W., Patel, K., Overbeek, P.A., 1995. TGF alpha can act as a chemoattractant to perioptic mesenchymal cells in developing mouse eyes. Development 121, 1669–1680.

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

Rickman, D.W., Rickman, C.B., 1996. Suppression of trkB expression by antisense oligonucleotides alters a neuronal phenotype in the rod pathway of the developing rat retina. Proc. Natl. Acad. Sci. USA 93, 12564–12569.

Riddle, R.D., Johnson, R.L., Laufer, E., Tabin, C., 1993. Sonic hedgehog mediates the polarizing activity of the ZPA. Cell 75, 1401–1416.

Rissi, M., Wittbrodt, J., Delot, E., Naegeli, M., Rosa, F.M., 1995. Zebrafish Radar: a new member of the TGF-beta superfamily defines dorsal regions of the neural plate and the embryonic retina. Mech. Dev. 49, 223–234.

Robinson, M.L., Overbeek, P.A., Verran, D.J., Grizzle, W.E., Stockard, C.R., Friesel, R., Maciag, T., Thompson, J.A., 1995. Extracellular FGF-1 acts as a lens differentiation factor in transgenic mice. Development 121, 505–514.

Rodriguez-Tebar, A., Jeffrey, P.L., Thoenen, H., Barde, Y.A., 1989. The survival of chick retinal ganglion cells in response to brain-derived neurotrophic factor depends on their embryonic age. Dev. Biol. 136, 296–303.

Sanes, J.R., 1993. Topographic maps and molecular gradients. Curr. Opin. Neurobiol. 3, 67–74.

Sanyanusin, P., Schimmenti, L.A., McNoe, L.A., Ward, T.A., Pierpont, M.E., Sullivan, M.J., Dobyns, W.B., Eccles, M.R., 1995. Mutation of the PAX-2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. Nat. Genet. 9, 358–363.

Schimmenti, L.A., Pierpont, M.E., Carpenter, B.L., Kashtan, C.E., Johnston, M.R., Dobyns, W.B., 1995. Autosomal dominant optic nerve colobomas, vesicouretral reflux and renal anomalies. Am. J. Med. Gen. 59, 204–208.

Sefton, M., Nieto, M.A., 1997. Multiple roles of Eph-like kinases and their ligands during development. Cell Tissue Res. 290, 243–250.

Seo, H.C., Drivenes, O., Ellingsen, S., Fjose, A., 1998. Expression of two zebrafish homologues of the murine Six3 gene demarcates the initial eye primordia. Mech. Dev. 73, 45–57.

Simeone, A., Acampora, D., Mallamaci, A., Stornaiuolo, A., D'Apice, M.R., Nigro, V., Boncinelli, E., 1993. A vertebrate gene related to orthodenticle contains a homeodomain of the bicoid class and demarcates anterior neuroectoderm in the gastrulating mouse embryo. EMBO J. 12, 2735–2747.

Small, R.K., Riddle, P., Noble, M., 1987. Evidence for migration of oligodendrocyte-type-2 astrocyte progenitor cells into the developing rat optic nerve. Nature 328, 155–157.

Sperry, R.W., 1963. Chemoaffinity in the orderly growth of nerve fiber patterns and connections. Proc. Natl. Acad. Sci. USA 50, 703–710.

Stadler, H.S., Solursh, M., 1994. Characterization of the homeobox-containing gene GH6 identifies novel regions of homeobox gene expression in the developing chick embryo. Dev. Biol. 161, 251–262.

Steele, F.R., Chader, G.J., Johnson, L.V., Tombran-Tink, J., 1993. Pigment epithelium-derived factor: neurotrophic activity and identification as a unique member of the serine protease inhibitor gene family. Proc. Natl. Acad. Sci. USA 90, 1526–1530.

Swaroop, A., Xu, J., Pawar, H., Jackson, A., Skolnick, C., Agarwal, N., 1992. A conserved retina-specific gene encodes a basic motif/leucine zipper domain. Proc. Natl. Acad. Sci. USA 89, 266–270.

Taira, M., Hayes, W.P., Otani, H., Dawid, I.B., 1993. Expression of LIM class homeobox gene Xlim-3 in Xenopus development is limited to neural and neuroendocrine tissues. Dev. Biol. 159, 245–256.

Tassabehji, M., Newton, V.E., Read, A.P., 1994. Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (MITF) gene. Nat. Genet. 8, 251–255.

Temple, S., Raff, M.C., 1985. Differentiation of a bipotential glial progenitor cell in a single cell microculture. Nature 313, 223–225.

Temple, S., Raff, M.C., 1986. Clonal analysis of oligodendrocyte development in culture: evidence for a developmental clock that counts cell divisions. Cell 44, 773–779.

Thor, S., Ericson, J., Brannstrom, T., Edlund, T., 1991. The homeodomain LIM protein Isl-1 is expressed in subsets of neurons and endocrine cells in the adult rat. Neuron 7, 881–889.

Tomarev, S.I., Sundin, O., Banerjeebasu, S., Duncan, M.K., Yang, J.M., Piatigorsky, J., 1996. Chicken homeobox gene Prox-1 related to *Drosophila prospero* is expressed in the developing lens and retina. Dev. Dyn. 206, 354–367.

Tomita, K., Ishibashi, M., Nakahara, K., Ang, S.L., Nakanishi, S., Guillemot, F., Kageyama, R., 1996. Mammalian hairy and Enhancer of split homolog 1 regulates differentiation of retinal neurons and is essential for eye morphogenesis. Neuron 16, 723–734.

Tomita, K., Nakamishi, S., Guillemot, F., Kageyama, R., 1996. Mash-1 promotes neuronal differentiation in the retina. Genes Cells 1, 765–774.

Ton, C.C., Hirvonen, H., Miwa, H., Weil, M.M., Monaghan, P., Jordan, T., van Heyningen, V., Hastie, N.D., Meijers-Heijboer, H., Drechsler, M., Royer-Pokora, B., Collins, F., Swaroop, A., Strong, L.S., Saunders, G.F., 1991. Positional cloning and characterizaztion of a paired box- and homeobox-containing gene from the Aniridia region. Cell 67, 1059–1074.

Torres, M., Gomez-Pardo, E., Gruss, P., 1996. Pax-2 contributes to inner ear patterning and optic nerve trajectory. Development 122, 3381–3391.

Truslove, G.M., 1962. A gene causing ocular retardation (or) in the mouse. J. Embryol. Exp. Morphol. 10, 652–660.

Turner, D.L., Cepko, C.L., 1987. A common progenitor for neurons and glia persists in rat retina late in development. Nature 328, 131–136.

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

Walz, A., McFarlane, S., Briekman, Y.G., Nurcombe, V., Bartlett, P.F., Holt, C.E., 1997. Essential role of heparan sulfates in axon navigation and targeting in the developing visual system. Development 124, 2421–2430.

Wang, L.C., Dani, J., Godement, P., Marcus, R.C., Mason, C.A., 1995. Crossed and uncrossed retinal axons respond differently to cells of the optic chiasm midline in vitro. Neuron 15, 1349–1364.

Wang, S.Z., Adler, R., 1994. A developmentally regulated basic-leucine zipper-like gene and its expression in embryonic retina and lens. Proc. Natl. Acad. Sci. USA 91, 1351–1355.

Warkany, J., Schraffenberger, S., 1946. Congenital malformations induced in rats by maternal vitamin A deficiency: I. Defects of the eye. Arch. Ophthalmol. 35, 150–169.

Watanabe, T., Raff, M.C., 1988. Retinal astrocytes are immigrants from the optic nerve. Nature 332, 834–847.

Wride, M.A., 1996. Cellular and molecular features of lens differentiation: a review of recent advances. Differentiation 61, 77–93.

Xiang, M., Zhou, L., Macke, J.P., Yoshioka, T., Hendry, S.H., Eddy, R.L., Shows, T.B., Nathans, J., 1995. The mBrn-3 family of POU-domain factors: primary structure, binding specificity, and expression in subsets of retinal ganglion cells and somatosensory neurons. J. Neurosci. 15, 4762–4785.

Xu, P.X., Woo, I., Her, H., Beier, D.R., Maas, R.L., 1997. Mouse Eya homologues of the Drosophila eyes absent gene require Pax6 for expression in lens and nasal placode. Development 124, 219–231.

Ying, S.Y., Zhan, Y., Zhang, Z., 1997. Changes in the immunoreactivity for inhibin in mouse retina during development of photoreceptor cells. Dev. Neurosci. 19, 184–188.

Yuasa, J., Hirano, S., Yamagata, M., Noda, M., 1996. Visual projection map specified by topographic expression of transcription factors in the retina. Nature 382, 632–635.

Zhao, S., Thornquist, S.C., Barnstable, C.J., 1995. In vitro transdifferentiation of embryonic rat retinal pigment epithelium to neural retina. Brain Res. 677, 300–310.

Zhou, H., Yoshioka, T., Nathans, J., 1996. Retina-derived POU-domain factor-1: a complex POU-domain gene implicated in the development of retinal ganglion and amacrine cells. J. Neurosci. 16, 2261–2274.