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# Molecular Aspects of Vertebrate Retinal Development

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## Abstract

The formation of retina from neural plate has been mapped extensively by anatomical and molecular methods. The major cascades of transcription factor expression have been identified, and deficits resulting from transcription factor knockouts are well characterized. There is extensive cross-regulation, both positive and negative, at the transcriptional level between transcription factors and this is vital in the formation of neural compartments. Many transcription factors are important at both early stages of optic cup formation and later stages of terminal differentiation of retinal cell types. The transcription factor cascades can be regulated by extrinsic factors, and some of the intracellular signaling pathways whereby this is achieved have been identified. Defining the quantitative interactions between regulatory molecules will be the next step in understanding this excellent model of vertebrate central nervous system (CNS) development.

**Index Entries:** Transcription factor; optic vesicle; optic cup; photoreceptor; neurogenesis.

## Introduction

The retina has long been used as a model system for neural development. As an outgrowth of the forebrain vesicle, it is closely related to many of the most intensively studied areas of the CNS. It is accessible, and its relatively simple laminated structure with few long-range connections has allowed clear definition of cell position and cell differentiation. As a primary sensory structure the retina does not receive neural input that might influence development, and this may help account for the success of many studies of retinal development in vitro.

Since the last time this topic was reviewed in this journal *(1)* there has been an explosion of information about molecules expressed in retina and the ways in which they function during retinal development. Although this wealth of new information has refined our under-

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standing of retinal development, most of the hypotheses and models derived from earlier anatomical and experimental embryological studies remain valid. Perhaps the most striking finding in recent years has been the molecular conservation of aspects of retinal development across phyla. Molecules used by humans carry out very similar functions in files to such an extent that the human molecules can sometimes complement genetic deficiencies in flies.

Although much of the rapid progress in understanding retinal structure and development has come from studies of DNA and RNA molecules, retinal proteins and particularly antibodies against those proteins have played an important role. Antibodies provided a series of cell type specific markers that have allowed the identification and purification of retinal cell types and the assay of numerous factors that affect retinal development *(1).*

In the first issue of *Molecular Neurobiology,* it was stated that with the tools now available, the next few years would almost certainly bring about a revolution in our understanding, not only of individual cell types, but also of the mechanisms governing patterning in the nervous system, and perhaps biology in general *(1).* From the discussion that follows it is clear that this revolution has occurred.

## Anatomical Landmarks of Retinal Development

The following description, and most of this review, is specifically aimed at describing the retinal development of mammals—though many aspects are common to other species, including fish and birds. The first detectable anatomical structures that will give rise to the retina are the optic pits, depressions in the anterior neural plate. As the neural plate folds up, these pits form bulges from the ventrolateral aspect of each side of the forebrain at the border between the future diencephalon and telencephalon *(2).* This is a very early event, occuring even before the neural tube has closed at its anterior aspect (Fig. 1A). This outgrowth defines the optic vesicle stage and it continues to expand so that it becomes closely apposed to the surface ectoderm, and is separated from the forebrain by a distinct optic stalk. In concert with changes in the ectoderm that lead to formation of the lens, the neuroepithelium surrounding the optic vesicle expands ventrally and invaginates to form an optic cup (Figs. 1B–D). Within the outer layer of the optic cup cell determination events give rise to all the cell types of the mature retina, except the astrocytes of the optic nerve layer and the vascular cells that all migrate in through the optic stalk. The timing of these events varies between species. In the rat, optic vesicle outgrowth can be clearly seen by embryonic d 11 (E11) and the invagination to form the optic cup begins at E12. By E13 the optic cup is deep and the ventral lips are in contact across the optic fissure *(2).*

As the optic cup forms, the first retinal neurons—retinal ganglion cells—become postmitotic and migrate to the inner edge of the retina. The remainder of the retinal cell types are formed in a characteristic sequence, although there is extensive overlap between the various cell types. During the embryonic period cone photoreceptors, horizontal cells, and amacrine cells are formed and move to their correct layers. The amacrine and ganglion cells extend processes into the developing inner plexiform layer so that at birth—in rats and mice—the retina consists of a large outer neuroblast layer and an inner region of more mature cells. Within the outer neuroblast layer, however, are embedded cones and horizontal cells. In the first few postnatal days the rod photoreceptors, bipolar cells, and Müller glial cells are formed. By the end of the first postnatal week, the outer retina has clearly divided into the outer nuclear layer of photoreceptors and an inner nuclear layer of bipolar, horizontal, amacrine, and Müller cells. During the second postnatal week, most of the final retinal maturation occurs. The most obvious signs of this are synaptogenesis in the plexiform layers and outer-segment formation by the photoreceptors.

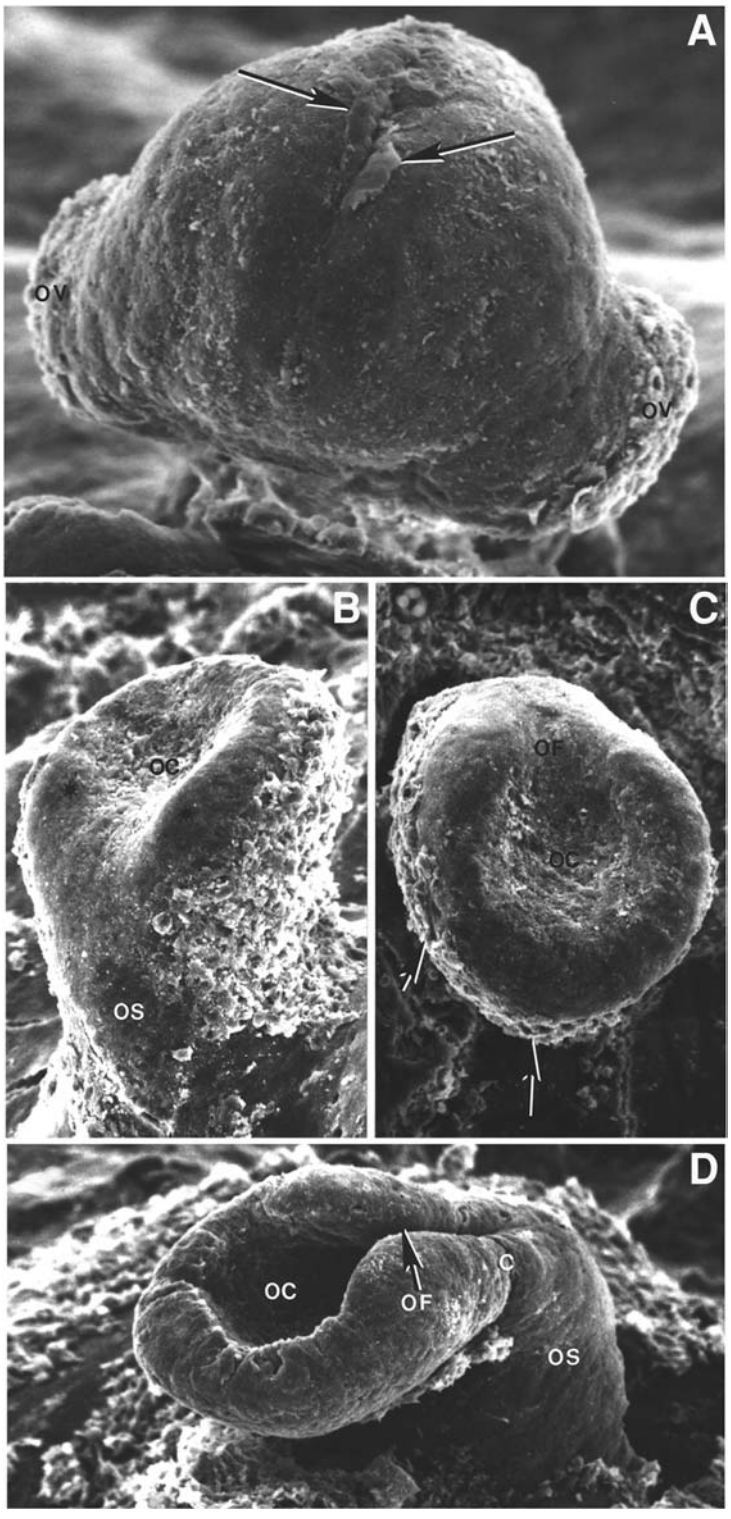


Fig. 1. Scanning electron micrographs of the developing rat optic cup. Adapted from ref. *(2)* **(A)** E 11 embryo showing two early optic vesicles (OV) with a broad attachment to the forebrain vesicle. The midline neural groove has not fully closed at this stage (arrows). Diameter of the optic vesicle is approx 100 m. **(B)** The optic cup (OC) and optic stalk (OS) at E12. The optic fissure is present on the ventral portion of the cup. **(C)** Lateral view of an E12 optic cup. Mesoectodermal cells remain attached at the outermost rim of the cup (arrows). **(D)** E13 optic cup (OC) showing that the optic fissure (OF) does not extend into the optic stalk (OS) but ends at the clear demarcation between the optic cup and the stalk (C). In **(B–D)** the diameter of the optic cup is approx 200 m.

Although many of the developmental events leading to formation of retinal cell types are regulated entirely within the neuroepithelial sheet, retinal development is influenced by the surface ectoderm and developing lens, the mesenchyme surrounding the optic cup, and the neural crest cells that migrate in to form pigment in the back of the eye, parts of the iris, and the corneal endothelium. The interplay between these extrinsic tissues and the retinal epithelium is similar to that seen in many other parts of the developing CNS.

## Fate Determination of Optic Cup from Neural Plate

Before any morphological signs of an optic vesicle can be observed, several molecular events have specified the eye field—that patch of neuroepithelium destined to give rise to the optic cup and its derivatives. In addition, pattern formation along major body axes, both the dorsal-ventral axis and the anterior-posterior axis, are incorporated into the folding and regional specification of the neuroepithelial sheet.

The formation of eye fields occurs as part of anterior neural plate development. A variety of transcription factors that serve essential roles in this event have been well documented, such as Pax6, Rx, Six3, Optx, and Otx2. Of these, Pax6 and Rx are the earliest genes specifying eye development detected, so far while the others are expressed in response to this initial specification.

Pax6 expression is restricted both anteroposteriorly and dorso-ventrally in the diencephalon of the mouse embryo. The disrupted normal development and regionalization of this region seen in small eye (Pax6Sey-1Neu) homozygous mice confirms its essential role *(3).* Mutations at various points in the pax6 gene give phenotypes of varying severity and heterozygous mutant animals show milder phenotypes, indicating that they are true hypomorphs. Gehring and his colleagues showed that expression of Pax6 in *Drosophila* imaginal discs changed the fate of cells such that ectopic eyes were formed on legs and antennae *(4)*

In vertebrates too, ectopic expression of Pax6 can lead to formation of eye tissue *(5).* The situation is clearly more complex however, since other cells in the neural plate express Pax6 and do not form eyes. It is likely that Pax6 can specify eye formation in the unique environment of a *Drosophila* imaginal disc but that in the more complex developing neural tissue of the vertebrate it is one of a series of molecules that are all essential for eye formation. It is interesting to note that the phenotype of Pax6 mutants varies across phyla. In *Drosophila* Pax6 mutants are eyeless. In mice they have rudimentary eyes whereas in humans eye formation is much more substantive and the defect is primarily in iris formation (aniridia). The reasons for these differences are not known but are probably relate to increased redundancy or interactions between regulatory factors in higher vertebrates that can partially compensate for the lack of Pax6.

Rx/rax is essential for normal eye development, and its misexpression has profound effects on eye morphology. In xenopus embryos, injected with synthetic Rx RNA develops ectopic retinal tissue and display hyperproliferation in the neuroretina *(6).* Mouse embryos carrying a null allele of this gene do not form optic cups and so do not develop eyes *(7).* In zebrafish, overexpression of either Rx1 or Rx2 results in the loss of forebrain tissue and the ectopic formation of retinal tissue *(8).* Both Pax6 and Rx/rax proteins are present in proliferating cells that give rise to both the neural retina and pigment epithelium *(6).* Whether these two proteins represent parallel developmental pathways is not yet known.

Six3 and Optx proteins are expressed early and their ectopic expression can induce formation of eye structures *(9–11).* They belong to a group of genes whose expression is controlled by Pax6 and have thus been defined as a second wave of gene expression necessary for retinal development.

Otx2 and Otx1, two of a family of transcription factors related to the *Drosophila* gene *ortho-*

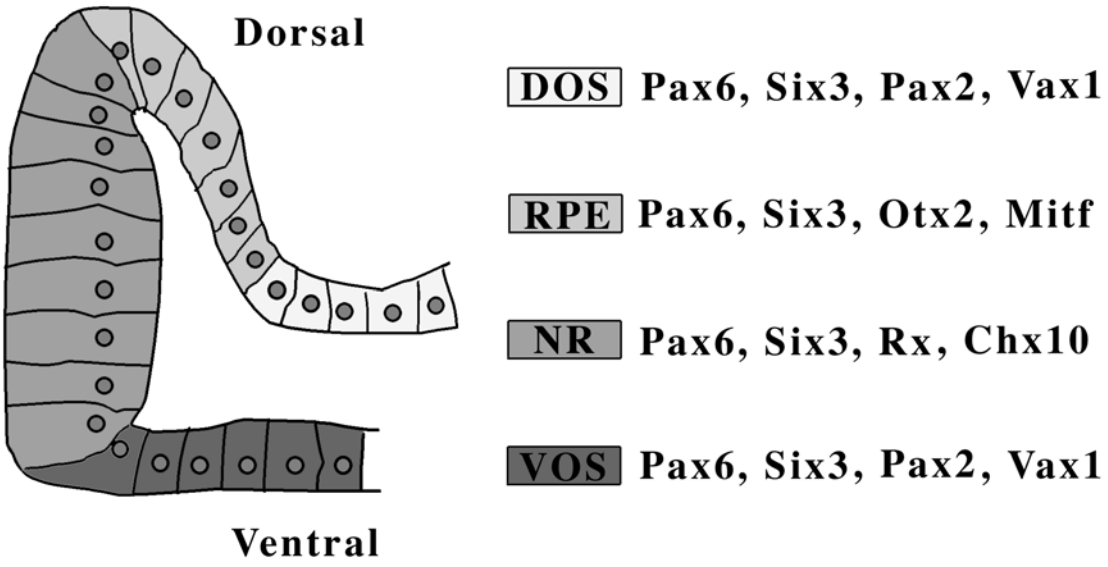


Fig. 2. Transcription factors delineating compartments in the developing optic cup. The neuroepithelium in the developing optic cup becomes divided into distinct compartments each expressing a unique array of transcription factors. DOS, dorsal optic stalk; RPE, retinal pigment epithelium; NR, neural retina; VOS, ventral optic stalk.

*denticle*, also have roles in the establishment of different eye territories *(12).* Mice with different numbers of functional copies of Otx1 and Otx2 show graded defects indicating that Otx proteins are required in a dose-dependent manner for normal eye development. During embryonic development, Otx mutants showed severely altered optic vesicle folding and the expression of pigment epithelium-specific genes was lost.

## Compartment Formation in the Developing Optic Cup

At the optic vesicle stage in mammals, the neuroepithelial sheet can be thought of as three domains (Fig. 2). One domain at the distal tip of the optic vesicle will give rise to the neural retina, another at its proximal base will give rise to the pigmented epithelium, and the third, consisting of cells connecting the optic vesicle to the rest of the brain, forms the optic stalk that will later give rise to the glia of the optic nerve. The optic stalk can be differentiated into separate dorsal and ventral portions, though the functional significance of these is not entirely clear. These domains are initially bipotential, capable of giving rise to either neural retina or pigmented epithelium but then become restricted in their developmental fate. There is increasing evidence that formation of these domains involves an interplay between groups of transcription factors and extrinsic soluble factors.

In the optic vesicle Pax6 and the basic-helix-loop-helix transcription factor Mitf are initially expressed in the whole neuroepithelial sheet. With maturation of the optic cup this distribution changes such that Pax6 is restricted to the front (retinal) layer of the optic cup and Mitf restricted to the back (RPE) layer. Rx/rax and Otx2 also show an initial broad distribution and then preferential expression in the neural retina and pigmented epithelium domains respectively. One model for this transition suggests that Pax6 and Mitf can inhibit each other at the transcriptional level. In support of this is

the finding that in mice homozygous for a nonfunctional Mitf mutant, the pigment epithelial layer formed, remained poorly differentiated and portions of it transformed into retina *(13,14).* Extensive evidence suggests that Mitf expression is regulated by members of the FGF gene family. For example, implantation of fibroblast growth factor-coated beads close to the base of the optic vesicle leads to a rapid down-regulation of Mitf and the development of an epithelium that, by morphology, gene expression, and lack of pigmentation, resembles the future neural retina *(15).* Similarly, removal of the surface ectoderm results in the maintenance of Mitf in the distal optic epithelium. This is accompanied by lack of expression of the neuroretina-specific transcription factor Chx10, whose transcript is expressed throughout the anterior optic vesicle and all neuroblasts of the optic cup *(16),* and conversion of this epithelium into a pigmented monolayer *(15).* In the chick, Fgf8 transcripts are first detected in the distal optic vesicle when it contacts the head ectoderm *(17).* Eventually Fgf8 expression increases and becomes localized to the central area of the presumptive neural retine. Treatment with exogenous FGFs leads to conversion of presumptive retinal pigment epithelium into neural retina *(17,18).* Ectopic expression of Fgf9 in the proximal region of the optic vesicle extends neural differentiation into the presumptive retinal pigment epithelium, resulting in a duplicate neural retina in transgenic mice *(19).* What is not clear from many of these studies is whether FGFs themselves are the active agents or whether they work indirectly. It has been suggested that FGFs can upregulate Pax6 expression and that it is Pax6 that inhibits expression of Mitf, suggesting that FGFs might determine optic cup cell layer fate directly. On the other hand, it has been shown that FGFs can act by influencing the expression of BMP proteins and that these might influence the transition of presumptive RPE into retina *(20).* There is still an extensive amount of work needed to fully elucidate these pathways.

Two transcription factors, Vax1 and Pax2 delineate the optic stalk compartment. The absence of Vax1 or Pax2 results in a proximal expansion of the activity of Pax6 and Rx. This, and other data, suggests that Vax1 and pax2 may interfere negatively with the expression of Pax6 and Rx in Xenopus *(21).* Pax2 expression was increased by the secreted factor sonic hedgehog (Shh), or a closely related signaling molecule, emanating from ventral midline tissue and, following Shh overexpression, embryos developed hypertrophied optic stalk-like structures *(22).* Thus, this combination of negative interactions between genes and positive actions of exogenous factors from proximal or distal poles is probably sufficient to allow formation of various compartments in the optic vesicle.

Other transcription factors, such as Lhx2, Msx1, Msx2 and Vax2, have been documented as essential factors for further eye development. In mouse, Lhx2 mutant embryos are anophthalmic, have malformations of the cerebral cortex, and die in utero due to severe anemia. In Lhx2–/– embryos specification of the optic vesicle occurs; however, development of the eye arrests prior to formation of an optic cup *(23).* Antisense attenuation of Msx-1, a homeobox-containing transcription factor, during early stages of neurulation produced hypoplasia of the maxillary, mandibular, and frontonasal prominences, eye anomalies, and somite and neural tube abnormalities. Eye defects consisted of enlarged optic vesicles, which may ultimately result in microphthalmia similar to that observed in Small eye mice homozygous for mutations in the Pax6 gene *(24).* In the mouse embryo, Msx2, a transcription factor structurally related to Msx-1, is expressed in cells of the optic vesicle that will form the neural retina, whilst the developing retinal pigment epithelium does not express this gene. Compared with cultures transfected with a control construct, Msx2-transfected cultures contained fewer cells expressing the RPE marker, Mitf, and more cells expressing class III beta-tubulin, a neuronal marker, suggesting that Msx2 can suppress the differentiated state of RPE cells and promote their differentiation into neural cell types *(25).*

Proper dorsal—ventral pattern formation of the optic cup is essential for vertebrate eye morphogenesis and topographic mapping of retinal projections. A number of exogenous factors have been implicated in this process and the ways in which these factors influence gene expression in the target cells is under active study *(26,27).* One of the key factors in dorsal ventral patterning is Shh. Both misexpressing Shh by virus and blocking Shh activity by antibodies resulted in disruption of ventral ocular tissues in chick *(28).* Decreasing endogenous Shh signals unexpectedly revealed a sharp morphological boundary subdividing dorsal and ventral portions of the optic cup. Shh signals differentially also influenced expression patterns of genes involved in ocular tissue specification and dorsal—ventral patterning within the ventral but not dorsal optic cup. This effect of Shh may be indirect and be mediated through BMP4 *(28).*

The emx-like transcription factor Vax2 shows considerable sequence homology to the related protein Vax1, particularly in their homeodmains. Vax2, however, is only expressed in the ventral retina. The overexpression of either the frog Xvax2 or the human Vax2 in xenopus embryos leads to an aberrant eye phenotype and, in particular, has a ventralizing effect on the developing eye *(29).* Vax2 overexpression also induces a striking expansion of the optic stalk, a structure deriving from the ventral most region of the eye vesicle, although it is possible that this represents cross reactive binding to targets of Vax1. Homozygous Vax2 mutant mice display incomplete closure of the optic fissure that leads to eye coloboma *(29).* At present we do not know whether there is any direct link between the ventralizing effect of exogenous factors like Shh and the expression of ventralizing genes like Vax2.

The other major groups of molecules responsible for retinal axial patterning and topographic projections are the ephrins and their receptors *(30,31).* Recent studies have suggested that graded expression of some of these molecules in the retina is regulated by Vax2 *(30).* Although the regulation of expression of Vax2 is not fully worked out, we are close to understanding how the cascade of transcription factors results in expression of molecules used to regulate axon growth and topographic patterns of synaptic connections.

## Intrinsic Regulation of Rod Photoreceptor Development

Members of the Helix-Loop-Helix (bHLH) gene family play essential roles in many steps of retina development, and are particularly important in the formation of photoreceptors *(32,33).* The bHLH transcription factor NeuroD is important for the formation of several retinal cell types and is essential for the survival of a subset of rod photoreceptors in mice *(34).* Misexpression of neuroD in retinal neuroepithelium through retroviruses in chick produced a retina with three, instead of two, layers of photoreceptor cells *(35).* Expression of NeuroD, in turn, is regulated directly or indirectly by several other bHLH gene products. For example, the bHLH protein Hes1 seems to be a negative regulator of NeuroD and in Hes1 deficient mice differentiation was accelerated, such that rod and horizontal cells appeared prematurely and formed abnormal rosette-like structures *(36).* Recently, it was shown that misexpression of another bHLH gene, Hes6, in developing retina promoted rod photoreceptor differentiation, through suppression of Hes1 function *(37).* Retinal explants from mice deficient in another bHLH gene, Mash1 *(38),* showed that differentiation of rod, horizontal, and bipolar cells was delayed, though the site of action of this gene is not known. Thus, this diverse family of transcription factors has members that exert positive or negative effects on retinal differentiation, often by altering the expression of other members of the bHLH family.

We do not fully understand the sequence of events occurring at the time of rod photoreceptor generation that result in the most common measure of differentiation, the expression of the visual pigment protein opsin *(39,40).* The

action of NeuroD on rod photoreceptor development is not directly on opsin expression but is more likely on factors used to control opsin transcription. A detailed mapping of the opsin promoter has identified a series of regulatory sites in the 150 bp upstream of the transcription start site, as well as other sites further upstream *(41).* As well as the transcription complex binding to the TATAA box there are three clear regulatory sites in the proximal promoter. A homeodomain protein Crx, a member of the Otx gene family, can transactivate rhodopsin promoter-reporter constructs in vitro *(42).* Crx is expressed from E12.5 (mouse) to adult stages and is most prominent in the photoreceptor layer. Crx can also bind to and transactivate the genes for several other photoreceptor cell-specific proteins (interphotoreceptor retinoid-binding protein, rod phosphodiesterase beta subunit, and arrestin). Further studies demonstrated that in presumptive photoreceptor cells expressing a dominant-negative form of Crx, photoreceptor outer segments and terminals did not develop normally *(43).* Mutations in Crx are associated with an autosomal dominant form of cone-rod dystrophy (adCRD) at the CORD2 locus on chromosome 19q13 *(44),* further suggesting that Crx plays essential roles in both differentiation and maturation of photoreceptor cells.

A leucine zipper transcription factor, Vrl, acts synergistically with Crx to regulate rhodopsin transcription *(45).* Deletion of Vrl in mice results in the complete loss of rod function and super-normal cone function, mediated by S cones *(45).* Rod photoreceptors appeared to be functionally transformed into S cones in the Vrl–/– retina, though since these cell types are thought to come from different progenitor pools, a simple fate switch is unlikely. Mutations in human NR2E3, a photoreceptor nuclear receptor transcription factor, also lead to production of excess S cones at the expense of other photoreceptor subtypes in the enhanced S-cone syndrome *(46,47).* It has been proposed that Vrl acts as a 'molecular switch' during rod-cell development by directly modulating rod-specific genes while simultaneously inhibiting the S-cone pathway through the activation of Nr2e3 *(45).*

The third important regulator of opsin expression appears has the binding properties of a member of the Emx family of homeodomain transcription factors. Such proteins can interact with the Ret1 element in electrophoretic mobility shift assays and stimulate transcription from test constructs containing a Ret1/PCEI site *(48).* Since several of these proteins have been identified in developing retina, the identity of the protein essential for opsin expression is still unclear.

## Extrinsic Regulation of Rod Photoreceptor Development

In addition to the transcription factor cascades outlined above, the formation of rod photoreceptors is also regulated by extrinsic factors. Comparison of birth dates and time of opsin expression has shown a variable interval and mixed reaggregate cultures of different ages can influence the timing of opsin expression *(49,50).* These factors are likely to be produced within the retina itself since explant cultures of pure retina develop normally *(51).* The nature of positive factors stimulating rod development is still unknown. Factors such as FGF and taurine can increase opsin expression but probably increase cell survival or general health rather than serve in any instructive capacity. Retinoic acid can also enhance the production of rod photoreceptors *(52,53).* Since computer analysis of most mammalian opsin promoters reveals no clear sites for retinoid receptor binding, any effect of retinoic acid is likely to be indirect, perhaps on an earlier stage of rod photoreceptor development. Retinoic acid has a strong influence on the dorse-ventral axis during early retinal development, as well as having many other effects in the developing visual system, so its actions on opsin expression and rod development may be due to actions on precursor populations *(54).*

Other factors may act at earlier stages and regulate proliferating cell pools. For example,

activin A, a TGF beta-like protein, caused the progenitor cells in E18 rat retinal cultures to exit the cell cycle and differentiate into rod photoreceptors *(55).* Conversely, mice with homozygous deletion of the activin beta A gene showed a specific decrease in the number of rod photoreceptors compared to wild-type or heterozygous littermates. Proteins of the hedgehog family may have similar effects since recombinant Shh protein caused a transient increase in the number of retinal progenitor cells, and a 2- to 10-fold increase in the number of photoreceptors in retina culture *(56).* Injection of a cocktail of antisense oligonucleotides reduced expression of Shh and tiggy-winkle hedgehog (twhh) genes in the RPE and slowed or arrested the progression of rod and cone photoreceptor differentiation in Zebrafish *(57).*

There is much clearer evidence for negative factors that can suppress rod photoreceptor formation. Exogenous CNTF reduced the formation of rods in rat retinal cultures by 82–99% *(58).* Similar results have been obtained with chick retina *(59,60)* and mouse retina *(61).* The related cytokine LIF has been shown to have a similar effect *(62).* The mechanism of action of these cytokines is not completely understood but involves a Stat3 intracellular messenger and is accompanied by changes in expression of transcription factors including Hes1 and Otx2 that are important for rod generation from multipotential progenitors (Zhang et al., submitted).

If the mechanisms governing rod photoreceptor formation are similar to those of compartment formation discussed earlier, we might expect to find progenitor cells expressing molecules characteristic of several daughter cell types and the stabilization of one or more of these to be influenced by exogenous cytokines or other factors.

## Conclusions

We now have an extensive, though far from complete, molecular catalog that can describe retinal development from the initial formation of an eye field to final retinal maturation. Portions of this catalog mentioned in this article are listed in Table 1. Many examples of de novo expression of specific molecules correlate with previously defined morphological changes, though in some cases these are preceded by molecular changes.

Only a few stages of retinal development were discussed in the previous sections. A large amount of information about other stages and other aspects of development has also been derived in recent years. For example, the expression of a number of genes responsible for regulation of the cell cycle and cell proliferation have been studied and various hypotheses for the controlled generation of progenitor cell pools have been put forward. From even the limited data discussed in this article, however, a number of general conclusions can be drawn.

First, many molecules are used repetitively during development. Otx2 is an excellent example of this *(63).* Otx2 is expressed in the neuroepithelium during early eye field and optic vesicle formation. Its expression then becomes restricted to the proximal portion of the developing optic cup and is one of the first clear markers of the RPE. Otx2 is then also expressed transiently in developing ganglion cells and some other inner retinal neurons. Finally, it is expressed in the outer retina where it shows a maintained expression in bipolar cells. Another transcription factor, Pax6, is vital in very early development and continues to be expressed in mature ganglion cells.

This re-use of molecules during development is not confined to transcription factors. The intracellular signaling molecule Stat3 shows a pattern of expression during development consistent with a role in generation of neuronal cell types *(64).* In the adult it is primarily expressed in Müller glial cells where it is involved in stress responses *(66).* The signaling molecule Shh appears to regulate eye development from the earliest steps of compartment formation to terminal differentiation of retinal cell types. Within the necleus it is probable that the arrangement and availability of the cis-acting regulatory elements of the target genes controls the specificity of response to

Table 1
Signal Pathways in Retina Development

| Stages | Genes | Description | Genetic modification in mice | References |
|---|---|---|---|---|
| Anterior neural plate to optic vesicle | Pax6 | TF | y | *(66)* |
| | Otx2 | TF | y | *(12)* |
| | Rx | TF | y | *(6, 7, 8)* |
| | Six3 | TF | y | *(9, 10, 11)* |
| | Six6 (Optx2) | TF | – | *(67)* |
| | Hesx1 | TF | y | *(68)* |
| | TGIF | TF | – | *(69)* |
| | FGF8 | GF | – | *(17)* |
| Formation of optic cup | Pax2 | TF | y | *(70)* |
| | Vax1 | TF | y | *(21)* |
| | Otx1 | TF | y | *(12)* |
| | Otx2 | TF | y | *(12)* |
| | Pax6 | TF | y | *(3, 4)* |
| | Lhx2 | TF | y | *(23)* |
| | BF-1 | TF | y | *(71)* |
| | Msx1 | TF | y | *(24)* |
| | Msx2 | TF | y | *(25)* |
| | Tlx | TF | y | *(72)* |
| | Shh | SP | y | *(22, 28)* |
| | Fgf1 | GF | y | *(18)* |
| | Fgf8 | GF | – | *(17)* |
| | Fgf9 | GF | y | *(19)* |
| Position specification | Vax2 | TF | y | *(29, 31)* |
| | Pax2 | TF | y | *(22)* |
| | Tbx5 | TF | y | *(73)* |
| | Mitf | TF | y | *(14)* |
| | EphA3 | MP | – | *(30)* |
| | Ephrin-A5 | MP | – | *(31)* |
| | Ventroptin | SP | – | *(74)* |
| Neural retina and RPE | Chx10 | TF | y | *(16)* |
| | Mitf | TF | y | *(13, 14, 15)* |
| | Foxc1 | TF | y | *(75)* |
| | Foxc2 | TF | y | *(75)* |
| | Numb | TF | – | *(76)* |
| | BMP2 | SP | y | *(20)* |
| | BMP4 | SP | – | *(20, 26)* |
| Retinal neurogenesis | Math5 | TF | y | *(77)* |
| | Mash1 | TF | y | *(38)* |
| | Math3 | TF | – | *(78)* |
| | Chx10 | TF | y | *(16)* |
| | Otx2 | TF | y | *(63)* |
| | NeuroD | TF | y | *(34, 35, 78)* |
| | Hes1 | TF | y | *(36)* |
| | Hes5 | TF | y | *(79)* |
| | Hes6 | TF | – | *(37)* |

*(continues)*

Table 1 *(Continued)*

| Stages | Genes | Description | Genetic modification in mice | References |
|---|---|---|---|---|
| Retinal neurogenesis | Hesr2 | TF | – | *(80)* |
| | TRbeta2 | TF | y | *(81)* |
| | Pou4f2 (Brn3b) | TF | y | *(82)* |
| | Pou4f (Brn3) | TF | y | *(83)* |
| | CNTF | Cytokine | y | *(58–61)* |
| | LIF | Cytokine | y | *(62)* |
| | Notch | MP | y | *(84)* |
| | Shh | SP | y | *(56, 57)* |
| Formation of photoreceptor | Rax | TF | y | *(85)* |
| | Crx | TF | y | *(42–44)* |
| | Nrl | TF | y | *(45–47)* |
| | NeuroD | TF | y | *(34,35)* |
| | Erx | TF | – | *(48)* |
| | NR2E3 | NR | y | *(46, 47)* |
| | CNTF | Cytokine | y | *(58–61)* |
| | LIF | Cytokine | y | *(62)* |
| | Activin betaA | SP | y | *(55)* |

TF: Transcription Factor, GF: Growth Factor, SP: Secreted Protein, MP: Membrane Protein.
NR: Nuclear Receptor.

these molecules. Binding sites can vary in their availability during development. More importantly, it is clear that many genes require multiple transcription factors to initiate efficient expression. Thus, while individual factors can be used at different stages of development, combinations of factors many show a much more unique distribution.

Second, we are beginning to understand the networks of interactions that regulate expression of transcription factors. There are an increasing number of cases where cells express two transcription factors that can mutually repress each other (such as Pax2 and Pax6 or Mitf and Pax6 as discussed earlier). In a field of such cells it would be expected that one or other factor would become predominant and a mosaic pattern of expression would ensue. What actually happens is that more homogeneous compartments of expression of each factor are produced. In part this occurs because the expression of one or other transcription factor is enhanced by the actions of exogenous factors, and these factors act on only one portion or edge of the cell sheet. The actions of FGFs on Pax6 and Shh on Vax1 and Pax2 were described earlier. Similar interactions between sets of transcription factors and exogenous factors may also act to determine cell fate decisions by multipotential precursors. At present we do not know all the exogenous factors that regulate retinal development, nor their intracellular transduction pathways.

Third, there are a growing number of molecules for which expression and activation are regulated separately. This has been appreciated for a long time for molecules such as receptors and signal transduction molecules but more recently has also been found for transcription factors. For example, Otx2 can be found in the cytoplasm or the nucleus at different stages of development. Movement into the nucleus seems to be a regulated process and probably denotes active transcriptional regulation.

Finally, many studies of the retina have used it as a general model for the brain. Many of the molecules used during early retinal development are also used in other regions of the developing neuroepithelium. It appears that the basic mechanisms of compartment formation and neurogenesis are common. Determining when retina-specific molecules, or combinations of molecules, begin to play a role in retinal development will be an important next step in our overall understanding of the development of this important organ.

We are beginning to move beyond cataloging the array of retinal molecules expressed during development. The next stage requires us to explain how they work. For any given molecule we need to know what other molecules interact with it, in what other ways its activity is regulated and the quantitative description of its effects on downstream targets. The tools for such analysis are available and so we can expect another revolution in our understanding. The large increase in the number of groups using retina as a model system suggests that this revolution will occur at an accelerating pace.

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## References

1. Barnstable, C. J. (1987) A molecular view of vertebrate retinal development. *Mol. Neurobiol.* **1,** 9–46.
2. Morse, D. E., and P. S. McCann. (1984) Neuroectoderm of the early embryonic rat eye. Scanning electron microscopy. *Invest. Ophthalmol. Vis. Sci.* **25,** 899–907.
3. Grindley, J. C., D. R. Davidson, and R. E. Hill. (1995) The role of Pax-6 in eye and nasal development. *Development* **121,** 1433–1442.
4. Halder, G., P. Callaerts, and W. J. Gehring. (1995) New perspectives on eye evolution. *Curr Opin. Genet. Dev.* **5,** 602–609.
5. Altmann, C. R., R. L. Chow, R. A. Lang, and A. Hemmati-Brivanlou. (1997) Lens induction by Pax-6 in Xenopus laevis. *Dev. Biol.* **185,** 119–123.
6. Mathers, P. H., and M. Jamrich. (2000) Regulation of eye formation by the Rx and pax6 homeobox genes. *Cell Mol. Life Sci.* **57,** 186–194.
7. Zhang, L., P. H. Mathers, and M. Jamrich. (2000) Function of Rx, but not Pax6, is essential for the formation of retinal progenitor cells in mice. *Genesis* **28,** 135–142.
8. Chuang, J. C., and P. A. Raymond. (2001) Zebrafish genes rx1 and rx2 help define the region of forebrain that gives rise to retina. *Dev. Biol.* **231,** 13–30.
9. Bernier, G., F. Panitz, X. Zhou, T. Hollemann, P. Gruss, and T. Pieler. (2000) Expanded retina territory by midbrain transformation upon overexpression of Six6 (Optx2) in Xenopus embryos. *Mech. Dev.* **93,** 59–69.
10. Lagutin, O., C. C. Zhu, Y. Furuta, D. H. Rowitch, A. P. McMahon, and G. Oliver. (2001) Six3 promotes the formation of ectopic optic vesicle-like structures in mouse embryos. *Dev. Dyn.* **221,** 342–349.
11. Loosli, F., S. Winkler, and J. Wittbrodt. (1999) Six3 overexpression initiates the formation of ectopic retina. *Genes Dev.* **13,** 649–654.
12. Martinez-Morales, J. R., M. Signore, D. Acampora, A. Simeone, and P. Bovolenta. (2001) Otx genes are required for tissue specification in the developing eye. *Development* **128,** 2019–2030.
13. Bumsted, K. M., and C. J. Barnstable. (2000) Dorsal retinal pigment epithelium differentiates as neural retina in the microphthalmia (mi/mi) mouse. *Invest. Ophthalmol. Vis. Sci.* **41,** 903–908.
14. Bumsted, K. M., L. J. Rizzolo, and C. J. Barnstable. (2001) Defects in the MITF(mi/mi) apical surface are associated with a failure of outer segment elongation. *Exp. Eye. Res.* **73,** 383–392.
15. Nguyen, M., and H. Arnheiter. (2000) Signaling and transcriptional regulation in early mammalian eye development, a link between FGF and MITF. *Development* **127,** 3581–3591.
16. Liu, I. S., J. D. Chen, L. Ploder, D. Vidgen, D. van der Kooy, V. I. Kalnins, and R. R. McInnes. (1994) Developmental expression of a novel murine homeobox gene (Chx10), evidence for roles in determination of the neuroretina and inner nuclear layer. *Neuron* **13,** 377–393.

17. Vogel-Hopker, A., T. Momose, H. Rohrer, K. Yasuda, L. Ishihara, and D. H. Rapaport. (2000) Multiple functions of fibroblast growth factor-8 (FGF-8) in chick eye development. *Mech. Dev.* **94,** 25–36.
18. Zhao, S., S. C. Thornquist, and C. J. Barnstable. (1995) In vitro transdifferentiation of embryonic rat retinal pigment epithelium to neural retina. *Brain Res.* **677,** 300–310.
19. Zhao, S., F. C. Hung, J. S. Colvin, A. White, W. Dai, F. J. Lovicu, D. M. Ornitz, and P. A. Overbeek. (2001) Patterning the optic neuroepithelium by FGF signaling and Ras activation. *Development* **128,** 5051–5060.
20. Ohkubo, Y., C. Chiang, and J. L. Rubenstein. (2002) Coordinate regulation and synergistic actions of BMP4, SHH and FGF8 in the rostral prosencephalon regulate morphogenesis of the telencephalic and optic vesicles. *Neuroscience* **111,** 1–17.
21. Hallonet, M., T. Hollemann, T. Pieler, and P. Gruss. (1999) Vax1, a novel homeobox-containing gene, directs development of the basal forebrain and visual system. *Genes Dev.* **13,** 3106–3114.
22. Macdonald, R., K. A. Barth, Q. Xu, N. Holder, I. Mikkola, and S. W. Wilson. (1995) Midline signalling is required for Pax gene regulation and patterning of the eyes. *Development* **121,** 3267–3278.
23. Porter, F. D., J. Drago, Y. Xu, S. S. Cheema, C. Wassif, S. P. Huang, E. Lee, A. Grinberg, J. S. Massalas, D. Bodine, F. Alt, and H. Westphal. (1997) Lhx2, a LIM homeobox gene, is required for eye, forebrain, and definitive erythrocyte development. *Development* **124,** 2935–2944.
24. Foerst-Potts, L., and T. W. Sadler. (1997) Disruption of Msx-1 and Msx-2 reveals roles for these genes in craniofacial, eye, and axial development. *Dev. Dyn.* **209,** 70–84.
25. Holme, R. H., S. J. Thomson, and D. R. Davidson. (2000) Ectopic expression of Msx2 in chick retinal pigmented epithelium cultures suggests a role in patterning the optic vesicle. *Mech. Dev.* **91,** 175–187.
26. Belecky-Adams, T. and R. Adler. (2001) Developmental expression patterns of bone morphogenetic proteins, receptors, and binding proteins in the chick retina. *J. Comp. Neurol.* **430,** 562–572.
27. Fuhrmann, S., E. M. Levine, and T. A. Reh. (2000) Extraocular mesenchyme patterns the optic vesicle during early eye development in the embryonic chick. *Development – Supplement.* **127,** 4599–4609.
28. Zhang, X. M., and X. J. Yang. (2001) Temporal and spatial effects of Sonic hedgehog signaling in chick eye morphogenesis. *Dev. Biol.* **233,** 271–290.
29. Barbieri, A. M., G. Lupo, A. Bulfone, M. Andreazzoli, M. Mariani, F. Fougerousse, G. G. Consalez, G. Borsani, J. S. Beckmann, G. Barsacchi, A. Ballabio, and S. Banfi. (1999) A homeobox gene, vax2, controls the patterning of the eye dorsoventral axis. *Proc. Natl. Acad. Sci. USA* **96,** 10729–10734.
30. Schulte, D., and C. L. Cepko. (2000) Two homeobox genes define the domain of EphA3 expression in the developing chick retina. *Development* **127,** 5033–5045.
31. Schulte, D., T. Furukawa, M. A. Peters, C. A. Kozak, and C. L. Cepko. (1999) Misexpression of the Emx-related homeobox genes cVax and mVax2 ventralizes the retina and perturbs the retinotectal map. *Neuron* **24,** 541–553.
32. Cepko, C. L. (1999) The roles of intrinsic and extrinsic cues and bHLH genes in the determination of retinal cell fates. *Curr. Opin. Neurobiol.* **9,** 37–46.
33. Kageyama, R., M. Ishibashi, K. Takebayashi, and K. Tomita. (1997) bHLH transcription factors and mammalian neuronal differentiation. *Int. J. Biochem. Cell Biol.* **29,** 1389–1399.
34. Morrow, E. M., T. Furukawa, J. E. Lee, and C. L. Cepko. (1999) NeuroD regulates multiple functions in the developing neural retina in rodent. *Development* **126,** 23–36.
35. Yan, R. T., and S. Z. Wang. (1998) neuroD induces photoreceptor cell overproduction in vivo and de novo generation in vitro. *J. Neurobiol.* **36,** 485–496.
36. Tomita, K., M. Ishibashi, K. Nakahara, S. L. Ang, S. Nakanishi, F. Guillemot, and R. Kageyama. (1996) Mammalian hairy and Enhancer of split homolog 1 regulates differentiation of retinal neurons and is essential for eye morphogenesis. *Neuron* **16,** 723–734.
37. Bae, S., Y. Bessho, M. Hojo, and R. Kageyama. (2000) The bHLH gene Hes6, an inhibitor of Hes1, promotes neuronal differentiation. *Development* **127,** 2933–2943.
38. Tomita, K., S. Nakanishi, F. Guillemot, and R. Kageyama. (1996) Mash1 promotes neuronal differentiation in the retina. *Genes Cells* **1,** 765–774.
39. Hicks, D., and C. J. Barnstable. (1987) Different rhodopsin monoclonal antibodies reveal different binding patterns on developing and adult rat retina. *J. Histochem. Cytochem.* **35,** 1317–1328.

40. Treisman, J. E., M. A. Morabito, and C. J. Barnstable. (1988) Opsin expression in the rat retina is developmentally regulated by transcriptional activation. *Mol. Cell Biol.* **8,** 1570–1579.
41. Morabito, M. A., X. Yu, and C. J. Barnstable. (1991) Characterization of developmentally regulated and retina-specific nuclear protein binding to a site in the upstream region of the rat opsin gene. *J. Biol. Chem.* **266,** 9667–9672.
42. Chen, S., Q. L. Wang, Z. Nie, H. Sun, G. Lennon, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, and D. J. Zack. (1997) Crx, a novel Otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. *Neuron* **19,** 1017–1030.
43. Furukawa, T., E. M. Morrow, and C. L. Cepko. (1997) Crx, a novel otx-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. *Cell* **91,** 531–541.
44. Freund, C. L., C. Y. Gregory-Evans, T. Furukawa, M. Papaioannou, J. Looser, L. Ploder, J. Bellingham, D. Ng, J. A. Herbrick, A. Duncan, S. W. Scherer, L. C. Tsui, A. Loutradis-Anagnostou, S. G. Jacobson, C. L. Cepko, S. S. Bhattacharya, and R. R. McInnes. (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.
45. Mears, A. J., M. Kondo, P. K. Swain, Y. Takada, R. A. Bush, T. L. Saunders, P. A. Sieving, and A. Swaroop. (2001) Nrl is required for rod photoreceptor development. *Nat. Genet.* **29,** 447–452.
46. Haider, N. B., S. G. Jacobson, A. V. Cideciyan, R. Swiderski, L. M. Streb, C. Searby, G. Beck, R. Hockey, D. B. Hanna, S. Gorman, D. Duhl, R. Carmi, J. Bennett, R. G. Weleber, G. A. Fishman, A. F. Wright, E. M. Stone, and V. C. Sheffield. (2000) Mutation of a nuclear receptor gene, NR2E3, causes enhanced S cone syndrome, a disorder of retinal cell fate. *Nat. Genet.* **24,** 127–131.
47. Milam, A. H., L. Rose, A. V. Cideciyan, M. R. Barakat, W. X. Tang, N. Gupta, T. S. Aleman, A. F. Wright, E. M. Stone, V. C. Sheffield, and S. G. Jacobson. (2002) The nuclear receptor NR2E3 plays a role in human retinal photoreceptor differentiation and degeneration. *Proc. Natl. Acad. Sci. USA* **99,** 473–478.
48. Martinez, J. A., and C. J. Barnstable. (1998) Erx, a novel retina-specific homeodomain transcription factor, can interact with Ret 1/PCEI sites. *Biochem. Biophys. Res. Commun.* **250,** 175–180.
49. Watanabe, T., and M. C. Raff. (1990) Rod photoreceptor development in vitro: intrinsic properties of proliferating neuroepithelial cells change as development proceeds in the rat retina. *Neuron* **4,** 461–467.
50. Cepko, C. L., C. P. Austin, X. Yang, M. Alexiades, and D. Ezzeddine. (1996) Cell fate determination in the vertebrate retina. *Proc. Natl. Acad. Sci. USA* **93,** 589–595.
51. Sparrow, J. R., D. Hicks, and C. J. Barnstable. (1990) Cell commitment and differentiation in explants of embryonic rat neural retina. Comparison with the developmental potential of dissociated retina. *Brain. Res. Dev. Brain. Res.* **51,** 69–84.
52. Levine, E. M., S. Fuhrmann and T. A. Reh. (2000) Soluble factors and the development of rod photoreceptors. *Cell. Mol. Life. Sci.* **57,** 224–234.
53. Hyatt, G. A., E. A. Schmitt, J. M. Fadool and J. E. Dowling. (1996) Retinoic acid alters photoreceptor development in vivo. *Proc. Nat. Acad. Sci. (USA)* **93,** 13,298–13,303.
54. Wagner, E., P. McCaffery, and U. C. Drager. (2000) Retinoic acid in the formation of the dorsoventral retina and its central projections. *Dev. Biol.* **222,** 460–470.
55. Davis, A. A., M. M. Matzuk, and T. A. Reh. (2000) Activin A promotes progenitor differentiation into photoreceptors in rodent retina. *Mol. Cell. Neurosci.* **15,** 11–21.
56. Levine, E. M., H. Roelink, J. Turner, and T. A. Reh. (1997) Sonic hedgehog promotes rod photoreceptor differentiation in mammalian retinal cells in vitro. *J. Neurosci.* **17,** 6277–6288.
57. Stenkamp, D. L., R. A. Frey, S. N. Prabhudesai, and P. A. Raymond. (2000) Function for Hedgehog genes in zebrafish retinal development. *Dev. Biol.* **220,** 238–252.
58. Kirsch, M., S. Fuhrmann, A. Wiese, and H. D. Hofmann. (1996) CNTF exerts opposite effects on in vitro development of rat and chick photoreceptors. *Neuroreport* **7,** 697–700.
59. Fuhrmann, S., S. Heller, H. Rohrer, and H. D. Hofmann. (1998) A transient role for ciliary neurotrophic factor in chick photoreceptor development. *J. Neurobiol.* **37,** 672–683.
60. Xie, H. Q., and R. Adler. (2000) Green cone opsin and rhodopsin regulation by CNTF and staurosporine in cultured chick photoreceptors. *Invest. Ophthalmol. Vis. Sci.* **41,** 4317–4323.
61. Ezzeddine, Z. D., X. Yang, T. DeChiara, G. Yancopoulos, and C. L. Cepko. (1997) Postmitotic

cells fated to become rod photoreceptors can be respecified by CNTF treatment of the retina. *Development* **124,** 1055–1067.
62. Neophytou, C., A. B. Vernallis, A. Smith, and M. C. Raff. (1997) Muller-cell-derived leukaemia inhibitory factor arrests rod photoreceptor differentiation at a postmitotic pre-rod stage of development. *Development* **124,** 2345–2354.
63. Baas, D., K. M. Bumsted, J. A. Martinez, F. M. Vaccarino, K. C. Wikler, and C. J. Barnstable. (2000) The subcellular localization of Otx2 is cell-type specific and developmentally regulated in the mouse retina. *Brain. Res. Mol. Brain. Res.* **78,** 26–37.
64. Zhang, S.S-M., Wei, JY., Li, CJ., Barstable, CJ., Fu, XY. (2001) The Temporal and spatial expression pattern of signal transducer and activator of transcription factors in mouse eye development. *IOVS* **42,** S354.
65. Peterson, W. M., Q. Wang, R. Tzekova, and S. J. Wiegand. (2000) Ciliary neurotrophic factor and stress stimuli activate the Jak-STAT pathway in retinal neurons and glia. *J. Neurosci.* **20,** 4081–4090.
66. Li, H. S., J. M. Yang, R. D. Jacobson, D. Pasko, and O. Sundin. (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.
67. Jean, D., G. Bernier, and P. Gruss. (1999) Six6 (Optx2) is a novel murine Six3-related homeobox gene that demarcates the presumptive pituitary/hypothalamic axis and the ventral optic stalk. *Mech. Dev.* **84,** 31–40.
68. Dattani, M. T., J. P. Martinez-Barbera, P. Q. Thomas, J. M. Brickman, R. Gupta, I. L. Martensson, H. Toresson, M. Fox, J. K. Wales, P. C. Hindmarsh, S. Krauss, R. S. Beddington, and I. C. Robinson. (1998) Mutations in the homeobox gene HESX1/Hesx1 associated with septo-optic dysplasia in human and mouse. *Nat. Genet.* **19,** 125–133.
69. Gripp, K. W., D. Wotton, M. C. Edwards, E. Roessler, L. Ades, P. Meinecke, A. Richieri-Costa, E. H. Zackai, J. Massague, M. Muenke, and S. J. Elledge. (2000) Mutations in TGIF cause holoprosencephaly and link NODAL signalling to human neural axis determination. *Nat. Genet.* **25,** 205–208.
70. Nornes, H. O., G. R. Dressler, E. W. Knapik, U. Deutsch, and P. Gruss. (1990) Spatially and temporally restricted expression of Pax2 during murine neurogenesis. *Development* **109,** 797–809.
71. Huh, S., V. Hatini, R. C. Marcus, S. C. Li, and E. Lai. (1999) Dorsal-ventral patterning defects in the eye of BF-1-deficient mice associated with a restricted loss of shh expression. *Dev. Biol.* **211,** 53–63.
72. Yu, R. T., M. Y. Chiang, T. Tanabe, M. Kobayashi, K. Yasuda, R. M. Evans, and K. Umesono. (2000) The orphan nuclear receptor Tlx regulates Pax2 and is essential for vision. *Proc. Natl. Acad. Sci. USA* **97,** 2621–2625.
73. Koshiba-Takeuchi, K., J. K. Takeuchi, K. Matsumoto, T. Momose, K. Uno, V. Hoepker, K. Ogura, N. Takahashi, H. Nakamura, K. Yasuda, and T. Ogura. (2000) Tbx5 and the retinotectum projection. *Science* **287,** 134–137.
74. Sakuta, H., R. Suzuki, H. Takahashi, A. Kato, T. Shintani, S. Iemura, T. S. Yamamoto, N. Ueno, and M. Noda. (2001) Ventroptin a BMP-4 antagonist expressed in a double-gradient pattern in the retina. *Science* **293,** 111–115.
75. Smith, R. S., A. Zabaleta, T. Kume, O. V. Savinova, S. H. Kidson, J. E. Martin, D. Y. Nishimura, W. L. Alward, B. L. Hogan, and S. W. John. (2000) Haploinsufficiency of the transcription factors FOXC1 and FOXC2 results in aberrant ocular development. *Hum. Mol. Genet.* **9,** 1021–1032.
76. Cayouette, M., A. V. Whitmore, G. Jeffery, and M. Raff. (2001) Asymmetric segregation of Numb in retinal development and the influence of the pigmented epithelium. *J. Neurosci.* **21,** 5643–5651.
77. Brown, N. L., S. Patel, J. Brzezinski, and T. Glaser. (2001) Math5 is required for retinal ganglion cell and optic nerve formation. *Development* **128,** 2497–2508.
78. Inoue, T., M. Hojo, Y. Bessho, Y. Tano, J. E. Lee, and R. Kageyama. (2002) Math3 and NeuroD regulate amacrine cell fate specification in the retina. *Development* **129,** 831–842.
79. Hojo, M., T. Ohtsuka, N. Hashimoto, G. Gradwohl, F. Guillemot, and R. Kageyama. (2000) Glial cell fate specification modulated by the bHLH gene Hes5 in mouse retina. *Development* **127,** 2515–2522.
80. Satow, T., S. K. Bae, T. Inoue, C. Inoue, G. Miyoshi, K. Tomita, Y. Bessho, N. Hashimoto, and R. Kageyama. (2001) The basic helix-loop-helix gene hesr2 promotes gliogenesis in mouse retina. *J. Neurosci.* **21,** 1265–1273.
81. Ng, L., J. B. Hurley, B. Dierks, M. Srinivas, C. Salto, B. Vennstrom, T. A. Reh, and D. Forrest. (2001) A thyroid hormone receptor that is

required for the development of green cone photoreceptors. *Nat. Genet.* **27,** 94–98.
82. Xiang, M., L. Zhou, Y. W. Peng, R. L. Eddy, T. B. Shows, and J. Nathans. (1993) Brn-3b: a POU domain gene expressed in a subset of retinal ganglion cells. *Neuron* **11,** 689–701.
83. Liu, W., S. L. Khare, X. Liang, M. A. Peters, X. Liu, C. L. Cepko, and M. Xiang. (2000) All Brn3 genes can promote retinal ganglion cell differentiation in the chick. *Development* **127,** 3237–3247.
84. Bao, Z. Z., and C. L. Cepko. (1997) The expression and function of Notch pathway genes in the developing rat eye. *J. Neurosci.* **17,** 1425–1434.
85. Furukawa, T., C. A. Kozak, and C. L. Cepko. (1997) 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.