Genetic Aspects of Embryonic Eye Development in Vertebrates

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ABSTRACT  The vertebrate eye comprises tissues from different embryonic origins, e.g., iris and ciliary body are derived from the wall of the diencephalon via optic vesicle and optic cup. Lens and cornea, on the other hand, come from the overlying surface ectoderm. The timely action of transcription factors and inductive signals ensure the correct development of the different eye components.

Establishing the genetic basis of eye defects has been an important tool for the detailed analysis of this complex process. One of the main control genes for eye development was discovered by the analysis of the allelic series of the Smafm eye mouse mutants and characterized as Pax6. It is involved in the interaction between the optic cup and the overlying ectoderm. The central role for Pax6 in eye development is conserved throughout the animal kingdom as the murine Pax6 gene induces ectopic eyes in transgenic Drosophila despite the obvious diverse organization of the eye in the fruit fly compared to vertebrates. In human, mutations in the PAX6 gene are responsible for aniridia and Peter's anomaly. In addition to Pax6, other mutations affecting the interaction of the optic cup and the lens placode have been documented in the mouse.

For the differentiation of the retina from the optic cup several genes are responsible: Mif leads to microphthalmia, if mutated, and encodes for a transcription factor, which is expressed in the melanocytes of the pigmented layer of the retina. In addition, further genes are implicated in the correct development of the retina, e.g., Chx10, Dlx1, GH6, Msx1 and -2, Otx1 and -2, or Wnt7b. Mutations within the retinoblastoma gene (Rbl) are responsible for retinal tumors. Knock-out mutants of Rbl exhibit a block of lens differentiation prior to the retinal defect. Besides the influence of Rbl, the lens differentiates under the influence of growth factors [e.g., FGF, IGF, PDGF, TGF], and specific genes become activated encoding cytoskeletal proteins (e.g., filensin, phakinin, vimentin), structural proteins (e.g., crystallins) or membrane proteins (e.g., Mip). The optic nerve originates from the neural retina; ganglion cells grow to the optic stalk, forming the optic nerve. Its retrograde walk to the brain through the rudiment of the optic stalk depends on the correct Pax2 expression.  © 1996 Wiley-Liss, Inc.

Key words: Eye, development, gene, induction, mutation

INTRODUCTION

One of the key events in the concepts of embryology evolved at the beginning of this century, when Hans Spemann made a careful analysis of eye development. Spemann established that lens induction from the surface ectoderm is dependent on the underlying optic cup, which led to the organizer concept. It became a prototype for tissue interactions in embryonic development. But Spemann's later experiments revealed that the optic cup can not be referred to as the only organizer of the lens [Spemann, 1924], because lens induction is a multistep process. Recent advances in molecular and genetic technology such as homologous recombination now allow this process to be examined in great detail. Ocular development involves two intertwined processes. One is an ongoing series of inductive signals, which determines the initial architecture of the major components of the eye. The other is the coordinated differentiation of these components. The present paper

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summarizes some aspects of eye development from a genetic point of view and outlines new perspectives of developmental eye research.

**MORPHOLOGY OF EYE DEVELOPMENT**

**Early Eye Development**

The eye is a very complex structure that originates from primordial tissues derived from a number of sources, including the wall of the diencephalon, the overlying surface ectoderm, and immigrating neural crest cells. The complex process is schematically outlined in Figure 1. Development of the eye becomes first evident, when the lateral walls of the diencephalon begin to bulge out as optic grooves or optic placodes, which appear during early neurulation (stage with 5–7 pairs of somites). The optic grooves are located on either side of the presumptive forebrain region. They enlarge to form the optic vesicles, which terminate very close to the overlying surface ectoderm. Direct contact between these two tissues is evident in mouse embryos with about 20–25 pairs of somites. A close contact is required for the induction of the lens placode by the adjacent neuroepithelium of the optic vesicles. There is experimental evidence that the optic vesicle provides a final signal by a diffusible, but not yet identified inducer for lens determination. In embryos with 25–30 pairs of somites, the outer surface of the optic vesicles retract inward to form the optic cups, while the surface ectoderm in the localized regions overlying the outer border of the optic cups is induced to form the lens placode [Carlson, 1994; Kaufman, 1992; Piatigorsky, 1981].

**Formation of Lens and Cornea**

The lens placode forms the lens cup (also referred to as lens pit) and subsequently the lens vesicle; this transition is almost completed, when the embryo has 35–40 pairs of somites (human Carnegie stage 14–15). The lens vesicle is nearly spherical with a large central cavity. The cavity is then filled by the elongation of the epithelial cells at the posterior lens vesicle. These cells are called primary lens fibers [Kaufman, 1992]. The cells at the anterior pole of the lens vesicle remain as epithelial cells; the cell number is controlled by apoptosis [Morgenbesser et al., 1994]. A germinative ring of mitotic active cells is found around the central region. Daughter cells from the germinative region move into the equatorial region (or bow of the lens), where they soon elongate tremendously and differentiate into secondary lens fibers. They form concentric layers around the primary fibers of the lens nucleus. The midline, where secondary lens fibers from opposite points of the equator join, is referred to as the anterior and posterior lens suture. With this arrangement, the lens fibers toward the periphery are successively younger in developmental and differentiation terms. As long as the lens grows, new secondary fibers move in from the equator onto the outer cortex of the lens. The lens continues to develop throughout life, albeit at a slower rate, with new fibers successively added and retained for the entire life of the organism.

Both, the primary and secondary fiber cells lose their mitochondria and cell nuclei during the final differentiation process; for the primary fibers, it takes place in mice at E17/E18 and is finalized 2 weeks after birth, when the mice open their eyelids [Vrensen et al., 1991]. The secondary fiber cells, which encircle the primary fiber cells, lose their organelles, when they move from the outer to the inner cortex [Kuwabara, 1974]. Formation of the cornea is the result of the last series of major inductive events in eye formation, with the lens vesicle interacting with the overlying surface ectoderm. This induction results in the transformation of a typical surface ectoderm to a transparent, multilayered structure with a complex extracellular matrix and cellular contributions from several sources. At first, the basal ectodermal cells are stimulated by the lens vesicle to form a multilayered structure. The basal ectodermal cells increase in high and begin to secrete various collagen types to form the primary stroma. Later, two waves of neural crest cells reach the optic cup. They further migrate centrally between the primary stroma and the lens capsule leading to the corneal endothelium as a continuous layer, which secretes hyaluronic acid into the matrix. The hyaluronic acid causes the matrix to swell and to become a good substrate for the migration of a second wave of mesenchymal cells derived from the neural crest. Once the mesenchymal cells of the second wave have settled, they secrete collagen type I and hyaluronidase, which causes the stroma to shrink. Under the influence of thyroxine from the developing thyroid gland, this secondary stroma is dehydrated, and the collagen-rich matrix of epithelial and mesenchymal tissues becomes the transparent cornea [Hay, 1980].

**Iris, Ciliary Body, Retina, and Optic Nerve: Derivatives of the Optic Cup**

While the lens and the cornea are being formed, profound changes also occur in the optic cup. The two layers of the optic cup begin to differentiate in distinct directions. The cells of the outer layer produce pigment and eventually form the pigmented layer of the retina. At the same time, the outer lips of the optic cup, where the developing neural and pigmental retinas meet, undergo a different transformation into the iris and ciliary body. The cells of the inner layer proliferate rapidly and generate a variety of glia, ganglion neurons, interneurons, and light-sensitive photoreceptor neurons. All these cells together constitute the neural retina. In humans and rodents, the development of the neural retina is not completed at birth but progresses postnatally.

The axons from the ganglion cells of the inner layer of the retina meet at the base of the eye and travel
Fig. 1. Schematic representation of early eye development. The main developmental stages of early vertebrate eye development are schematically drawn and correspond to embryonic days 9.5–12 of the mouse. a: The optic vesicle (1) has been evaginated from the brain and has contact to the surface ectoderm (3); the lens placode (2) is formed (mouse E9.5). b: Surface ectoderm (3) differentiates at the place of the lens placode (2) to the lens pit (or lens cup, 5). The optic cup is formed consisting of the inner (neural) layer (7) and the outer (pigmented) layer (8). The developing eye is connected with the brain by the optic stalk (4) (mouse E10). c: The lens vesicle (6) is closed and the surface ectoderm, which is next to the lens, will differentiate to the cornea (9) (mouse E11). d: In the developing lens, the primary fiber cells (10) grow into the lumen from the posterior wall. Between the inner (neural, 7) and the outer (pigmented, 8) layer of the optic cup (the prospective retina), the intraretinal space (11) can be observed. [Modified according to Gilbert, 1994; the stages were categorized according to Kaufmann, 1992].

down the optic stalk. Initially, the optic stalk represents a narrow neck that connects the optic cup to the diencephalon. Once the axons reach the optic stalk, they grow into it and relay the eye with the visual centers of the brain. The optic stalk is now referred to as the optic nerve [Carlson, 1994; Gilbert, 1994].

MOUSE MUTANTS AS INDICATORS FOR GENES IMPORTANT FOR EARLY PROCESSES IN EYE DEVELOPMENT

General Remarks

Much of our knowledge on the function of genes in mammalian development has come from the molecular analysis of spontaneous or induced mutations. Mutations affecting the eye can be easily identified, and, therefore, a remarkable number of such mutants have been described. A systematic evaluation of large mouse populations for mutations affecting the eye lens was initiated in 1979, when Kratochvilova and Ehling described for the first time the systematic screening for murine dominant cataract mutations in the F1 generation after paternal radiation treatment. Some of the mutants were grouped into allelic series [Kratochvilova and Favor, 1992; Everett et al., 1994; Favor, 1995], however, the complex genetic organization in mouse and the reproductive time have restricted the genetical characterization of mouse eye mutants, even though a tremendous number of cataract mutants has been obtained by the Neuherberg cataract research group [Ehling, 1991]. A general overview for the genes, which are involved in eye development and mapped at a particular chromosome, is given in Figure 2.
Eyeless in Drosophila—Small Eye in Mouse: Is Pax6 the Ocular Archeo Gene?

One of the most important genes in eye development is the paired-box gene Pax6, which was recognized as being affected in the various allelic forms of the mouse and rat Small eye (Sey) mutants as well as in the human inherited diseases Aniridia [Hill et al., 1991; Glaser et al., 1992, 1994] and Peter's anomaly [Hanson et al., 1994]. It has been observed that individuals with Pax6 mutations have abnormal development of the eye. The Sey mutations are often accompanied by a delayed closure of the fissure of the optic cup. There is a folding at the margin of the optic cup resulting in a keyhole-shaped opening. The obvious defect in the heterozygotes are small eyes. In homozygotes, eyes and nasal cavities do not develop; they die soon after birth [Hill et al., 1991]. The histological analysis of murine homozygous Sey mutants demonstrated the presence of optic vesicles, but the ectoderm does not give rise to a lens. The failure in lens development is attributed to a defect in the inductive interaction between the optic vesicle and the overlying ectoderm, since these tissues fail to make discrete contacts [Hogan et al., 1986]. In addition, tissue transplantation experiments in a rat Sey mutant (rSey) have shown that homozygous rSey head ectoderm loses its lens-forming competence early in development. Ectoderm from homozygous Sey−/− differentiates into lens tissue, even if it is cultured with optic vesicles.
from wild-type embryos. The failure of head ectoderm of homozygous rSey embryos to differentiate into lens tissue results from a defect in the early differentiation signaling from the neural plate or underlying mesenchyme before the optic vesicle grows out [Fujiwara et al., 1994].

Sey/Pax6 maps at mouse chromosome 2. The molecular analysis of the Pax6 mutations revealed a large deletion in the radiation-induced Seyallele and a point mutation leading to a TGA stop codon in the Seyallele. Moreover, a reduced splicing because of a point mutation within an intron affecting Pax6 transcripts was observed in the Sey allele [Hill et al., 1991]. In rSey, a single base insertion in an exon of Pax6 generated an abnormal splice site leading to a large deletion of 600 bp within the mRNA [Matsuo et al., 1993].

The pattern of Pax6 expression is complex, both spatially and temporally. Pax6 transcripts are first detected in the presumptive fore- and hindbrain of 8-day-old mouse embryos. Furthermore, Pax6 is expressed at E8.5 in the optic sulcus, the lateral evagination at the basis of the forebrain. Later, at E9.5, Pax6 is present in the epithelial layer of optic vesicle, the optic stalk, and the surface ectoderm, which will give rise to the lens. Between E10 and E12, Pax6 is strongly expressed in the inner layer of the optic cup, in the lens and in the surface ectoderm, which is at this stage part of the future cornea. In the elongating primary fiber cells, Pax6 has a posterior localization. At E15.5, Pax6 is expressed in the two layers of the neural retina, the anterior epithelium of the cornea, and in the lens. Besides the eye, Pax6 occurs in specific regions of the brain, the ventral neural tube and in the olfactory epithelium. In general, these domains of expression correlate well with the phenotypes of homozygous Sey mutants [Walther and Gruss, 1991; Hanson and van Heyningen, 1995].

The expression pattern of Pax6 in early eye development suggests the assumption of a stepwise determination of the lens, however, most of the target genes remain to be elucidated as well as the regulation of Pax6 itself. Additionally, it appears that Pax6 is responsible for growth, differentiation, and maintenance of the retina, lens, and cornea. Obviously, Pax6 is not the only gene necessary for the initial induction of lens and optic vesicle during eye development, since displacement of the optic vesicle does not alter the position of the Pax6 expression in the ectoderm [Li et al., 1994]. First experiments to learn more about the regulation of Pax6 expression demonstrated that the transcription factor c-myb is involved in the regulation of Pax6 expression [Plaza et al., 1995]. Moreover, it is tempting to speculate that sonic hedgehog (Shh) might be involved in the regulation of Pax6, since Shh is expressed in the murine forebrain at the 10-somite stage [Echelard et al., 1993], where Pax6 could be found as well. Additionally, Shh is involved in the regulation of other Pax genes (Pax1 and Pax3; Johnson and Tabin, 1995), and the Drosophila analogon, hedgehog, participates in the development of the eye imaginal disc [Heberlein et al., 1995]. By contrast, Pax6 can be inhibited by activin A in an in vitro system using the isolated chicken neural plate. This extinction by activin A is specific for Pax6, since Pax3 and Pax7 remain expressed under these conditions [Pituello et al., 1995].

On the other side, looking for target genes of Pax6 in the eye, Pax6 protein seems to interact with binding sites for the transcription factors Ets [Plaza et al., 1994] and/or CREB [Cvekl et al., 1995a]. Recent papers demonstrated that Pax6 is involved during chicken lens development in the lens-specific transcription of the αA-crystallin gene [Cvekl et al., 1995a] and of the 81-crystallin gene [Cvekl et al., 1995b]. Additionally, it is essential for lens-specific expression of ζ-crystallin in guinea pigs [Richardson et al., 1995].

**Inductive Problems in Early Eye Development**

Developmental biologists obtained many informations from transplantation experiments during embryogenesis looking, if and when the transplanted tissue responds to the cellular environment. Similar experiments have been undertaken to investigate early eye and lens development. In *Xenopus*, the ectoderm, which forms the animal cap, becomes competent for lens formation for a very short period of time during gastrulation. At this stage of mid-gastrula, a planar signal from the presumptive neural plate is thought to provide the initial lens determining stimulus. An additional contribution to this early inductive signal may arise from the first involving tissue to underlie the lens area, a region of endoderm that will give rise to the foregut. At the neural plate stage the early lens-inducing signals have caused a lens forming bias in head ectoderm. At this stage it is thought that a signal from the neural plate continues to be important, enhanced by an inductive stimulus from mesoderm that underlies the lens ectoderm at this stage. At the neural tube stage, the optic vesicle has come into contact with the presumptive lens area and may provide the final signal for lens determination; presumptive lens ectoderm is specified [summarized according to Grainger, 1992].

From this short summary of early frog eye development, it becomes evident that it requires a number of inductive events. The available data on Pax6 mutations in humans, mouse, and rat, including the ectopic expression in *Drosophila* give some evidence that Pax6 is a master gene in eye development leading to the appropriate lens competence. However, other factors are necessary to get the entire network active. To dissect the unknown number of inductive events in early eye development, it is helpful to analyze further eye mutants, which exhibit defects in this early phase of gestation. Therefore, in the following section, some mouse mutants will be described, in which early steps in eye development are affected.
Eyeless (ey1, ey2) leads to anophthalmia, the absence of eyes [Chase, 1944]; its chromosomal localization is unknown. As in the Pax6/Sey mutants, the apposition of optic vesicles and surface ectoderm is affected in the mutants, and they lack lens induction. In contrast to Sey, no nasal abnormalities have been observed. In homozygous mutants, lens invagination at E10 is abnormal, with the lens smaller than normal and often lying off-center in the optic cup of ey1 mutants. The optic vesicle has an abnormal contact to the presumptive lens ectoderm. A reduced concentration of sulfated glycosaminoglycan (GAG) was observed between these structures [Webster et al., 1984]. GAGs have been shown to be important in mediating the connections between adjacent tissues of an organ [Massagué, 1991]. However, it remains unclear whether the reduced concentration of the GAGs between the optic cup and the ectoderm is a direct or indirect consequence of the mutation.

In mouse mutants referred to as myelencephalic blebs (my), the first morphological alteration becomes visible at E9.5. A large area of extracellular matrix is formed between the optic vesicle and the overlying presumptive lens ectoderm, whereas in the wild type almost no extracellular matrix can be observed. The close contact is accompanied by an increased concentration of (acidic) GAG in this extracellular matrix. At E12, the lens capsule is ruptured, accompanied by changes in the level and distribution of GAGs in the lenticular capsule. Additionally, the inner limiting membrane of the presumptive neural retina is affected and exhibit a greater deposition of laminin. At E14, the cornea and other structures of the eye cannot be identified. In addition to the eye defects, the my mice have also kidney anomalies [Center and Polizotto, 1992]. The mutation is located on mouse chromosome 3 [Davisson et al., 1976]. Phenotypically similar to my, but mapped to mouse chromosome 4, is the head blebs mutation (heb). Heb mice produce abnormal or absence of eyes due to prenatal blebs, usually on the head. Additionally, some fetal death, open eyelids, and folded retinas at birth can be observed [Varnum and Fox, 1981].

A further autosomal recessive murine mutant strain with disturbances in the early eye development exhibits aphakia (ak); this mutation is localized at chromosome 19. The extracellular matrix material that forms a firm attachment between the lens cup and the optic cup during invagination of the lens cup is abnormal in affected embryos, and there are abnormal deposits of the extracellular matrix material between the cells of the lens epithelium. The epithelium of the lens rudiment becomes disorganized and the lumen of the vesicle fills up with rounded cells [Zwaan and Webster, 1984].

Mice homozygous for the dominant mutation Extra toes die perinatally with multiple malformations involving the eye and other organs. Concerning the ocular development, three classes of the homozygotes can be identified in early mid-gestation: some form an apparently normal optic cup and are associated with an apparently normal lens vesicle, but others have distorted optic cups with small lens vesicles. This latter group goes on to develop small eyes and coloboma because the optic fissure of the optic nerve does not close. In the third category the optic cup and lens placode are not formed. This has prompted some to conclude that a defective optic vesicle causes these eye malformations [Franz and Besecke, 1991]. The mutation has been mapped to chromosome 13 [Peters et al., 1995] and has been shown to be a deletion within the gene Gli3 [Hui and Joyner, 1993]. Gli3, a zinc-finger transcription factor and oncogene, is interrupted by translocation in Greig syndrome families [Vortkamp et al., 1991]; it is also expressed during normal mouse development in various tissues outside the eye [Walterhouse et al., 1993]. The mutation is now referred to as Gli3<sup>xx</sup>.

Besides this naturally occurring mutations mice became deficient for the bone morphogenetic protein 7 (BMP7) by a gene-targeting approach. They revealed abnormalities at the very early eye development. The mutants die shortly after birth due to poor kidney development. Concerning eye development, at E11, when the optic cup has developed and the invagination of the lens vesicle is visible, the lens vesicle of the mutants was smaller or even absent. In addition, the developmental process of the entire eye was delayed, leaving the lens vesicle connected with the surface ectoderm [Luo et al., 1995]. This is consistent with the observation that BMP7 is present in the optic vesicle and the surrounding head ectoderm as early as E9.5 [Lyons et al., 1995].

The precise interaction between optic cup and overlying ectoderm is clearly a very important step in eye development because it is this interaction which is affected in all these mutations (Gli3<sup>xx</sup>, ak, ey1, heb, and my), in the BMP7 transgene, as well as in the Pax6/Sey mutation. The availability of such a broad range of mutants should lead to a more refined genetic dissection of early eye development, provided the identity of the mutated genes for ak, ey1, heb, and my can be deduced.

GENETIC CONTROL OF THE DEVELOPMENT OF LENS, RETINA, AND OPTIC NERVE

Lens Development

Cataracts: inherited anomalies of the lens. Cataracts as inherited lens opacities reflect developmental disturbances during lenticular differentiation in the already formed lens vesicle. As examples, the mutation of the gene coding for the membrane intrinsic protein (M, 26 kDa; Mip; Cat at mouse chromosome 10) is discussed together with two further mutant lines with lens-specific phenotypes (Cat3 at mouse chromosome 10, and Xcat at the X-chromosome).

The two Cat alleles, Cat<sup>−/−</sup> and Cat<sup>+</sup>, were mapped
20 cM distal to Sl at chromosome 10 [Muggleton-Harris et al., 1987]. A candidate gene for the Cat locus encodes Mip, since Mip was demonstrated to be localized at mouse chromosome 10 [Griffin and Shiels, 1992]. Recent data demonstrated a truncated form of Mip transcripts in Northern blots from CatPr lenses [Shiels and Griffin, 1993]. Sequence analysis of RT-PCR products derived from CatPr lenses revealed an aberrant 3' end [Shiels, 1995]. Mip forms specialized junctions between the fiber cells and can be first detected in the primary fiber cells of the early lens vesicle. In the CatPr mutant, beginning at E14, the cell nuclei in the deep cortex become abnormally pycnotic; degeneration of cytoplasm and destruction of the lenticular nucleus follow [Zwaan and Williams, 1969]. However, as obvious from Figure 2, the positions of the corresponding genes, Mip and Cat, at the chromosome 10 remain conflicting.

Two radiation-induced dominant mutations are allelic and designated CatI [Kratochvilova and Favor, 1992]. The mutants exhibit microphthalmia and total cataract. First identifiable morphological changes were observed at E14.5 as amorphous primary fibers in the anterior part of the lens [Löster J, Schäffer E, and Graw J: unpublished observations]. Linkage tests with visible markers revealed linkage with the marker Steel (Sl) on chromosome 10 [Löster et al., 1994a], making allelism with the CatPr mutation unlikely. A positional cloning approach is in progress to identify the affected gene and to understand the pathogenesis of this particular type of cataract.

The dominant X-linked cataract mutation Xcat was recovered after parental radiation [Favor and Pretsch, 1990]. Histological analysis of the embryonic development revealed that, in affected animals, the primary fiber cells are irregularly arranged and show small foci of cellular disintegration. Progressive degeneration of fibers occurs. However, the lens epithelium and the newly differentiated fibers show no clear abnormality, indicating that the mutation affects the differentiation of lens fiber cells at some point after their initial elongation. Analysis of crystallin and cytoskeleton proteins of postnatal cataractous lenses revealed no significant abnormalities when compared to the normal lens [Grimes et al., 1993]. Detailed genetical analysis placed the Xcat mutation in the distal end of the mouse X-chromosome. It suggests that this locus should map to a conserved block at Xp22.1–p22.3 in human. To this region, the Nance-Horan syndrome has been mapped [Stambolian et al., 1994].

The three examples on murine dominant cataract mutations described above demonstrate the variety of affected genes leading to the formation of lens opacity, i.e., cataract. As exemplified above, the mutants are characterized to distinct stages, and often the precise molecular genetic characterization is not yet completed. Because this approach from phenotype back to the genotype is often very laborious, the function of interesting genes or gene products have been tested by the use of transgenic mice creating new phenotypes by a defined gene.

**Use of transgenic mice for studies of lens development.** To analyze the effect of particular gene products to the development of the lens, a rapidly increasing number of transgenic animals were obtained by fusing the gene of interest either to the promoter of the αA- or the γF-crystallin gene. Both genes are commonly accepted to be expressed in a lens-specific manner. If the αA-crystallin promoter was fused to the highly cytotoxic Diphteria toxin gene, no lens is formed. This is combined with a marked reduction in eye size, increased retinal cell density, and extensive whirling of the retinal fiber layers [Kaur, 1989]. Morphological alterations can be observed only from E12.5 onwards. The cells in the central posterior embryonic lens appeared to be vacuolated and undergo necrosis [Key et al., 1992]. If the Diphteria toxin gene is fused to the γF-crystallin promoter, considerable heterogeneity varying from the reduced size of the eye to deficiency in nuclear fiber cells was observed [Breitman et al., 1987, 1989].

In contrast to the total loss of the lens, fusion of the γ-interferon gene to the αA-crystallin promoter leads in the transgenic mice to a normal development of the optic cup and lens vesicle at E12. Anomalies started at E14, when the transgene lens fails to form a well-defined lens bow because of the disorganization of lens cells at the posterior pole. At E18, lens and retinal differentiation program are completely disrupted. Molecular analysis revealed that in the transgenic lenses, the expression of fiber cell specific markers is dramatically reduced [Egwuagu et al., 1994].

When the SV40 large T antigen is targeted to the lens using either the αA- or the γF-crystallin promoter then the transgenic animals develop lens tumors. At E13, no elongation of primary lens fiber cells takes place in these transgenic mice but the lumen of the lens vesicle is filled by rounded cells. Elongated cells are no longer observed at the posterior pole. In juvenile transgenic mice, the lens was replaced by a disorganized mass that had ruptured the lens capsule and become infiltrated by blood vessels [Mahon et al., 1987; Bryce et al., 1993], demonstrating the importance of an intact lens capsule to confine tumor progression [Pichel et al., 1993].

All cases involving transgenes driven by the crystallin promoters affect the steps at E12–13. This corresponds to the developmental time when the αA- or γF-crystallin is activated. No effects outside the eye have been reported, demonstrating that the promoter fragment used is sufficient for lens-specific expression. The response of the lens to these various transgenes is mainly a stop of the secondary fiber cell differentiation program, and only few differences between "classical" cataract mutations and the transgene mutations can be observed. This might be due to a general alteration of the lenticular architecture by the transgene gene product. However, there are exceptions: one is the forma-
tion of lens tumors by the SV40 T antigen, and the genes encoding growth factors. Both aspects are discussed in detail below.

**Hormones and growth factors in lens development.** A growing body of evidence points to the essential role of hormones and growth factors in triggering key processes during organ development and differentiation. In the eye, several hormones and growth factors are known to be involved, e.g., cortisone, thyroxine, platelet-derived growth factor (PDGF), transforming growth factors (TGF-\(\alpha\) and \(\beta\)), insulin-like growth factor (IGF), as well as the acidic and basic fibroblast growth factors (a- and b-FGF).

Homozygous carriers of the lid gap (\(lg\)) mutation exhibit an extensive vacuolization of the lenses, which is first seen at E14. Later in development, defects of the cornea and retina occur, and at birth, the eye lids are open. Administration of thyroxine or cortisone at E10-11 or E14, respectively, prevents the phenotype, suggesting an essential function for these two hormones at particular stages of ocular development [Stein et al., 1967; Harris et al., 1984; Juriloff et al., 1985].

PDGF plays an important role in the developing lens, since its receptor PDGFR\(\alpha\) (gene symbol \(Pdgfr\alpha\)) can be detected in the mouse lens at E11.5, but not in the optic cup or the optic stalk. Mutants, defective in the expression of PDGFR\(\alpha\) (\(Patch\), at mouse chromosome 5) [Peters et al., 1995], demonstrate a malformed lens at E13 containing fewer fiber cells, distributed in a narrower band than normal littermates. Additionally, empty spaces in the lens matrix are seen in the Patch mutants [Schatteman et al., 1992].

A direct method for demonstrating the involvement of growth factors in ocular development is via homologous recombination methodologies to produce knockout mice. In the case of the TGF, homozygous “knockout” mice displayed ocular abnormalities including open eyelids at birth, reduced size of the eye ball, superficial opacity, corneal inflammation, dysgenesis of the anterior iris, and defects of lens and retina. The phenotype of TGF-\(\alpha\) knock-out mice (\(Tgf\alpha^{-}\)) is similar to that observed in the recessive mutation waved-1 (\(wa1\)). Allelism has been demonstrated between \(wa1\) on chromosome 6 and the \(Tgf\alpha^{-}\) mutants [Luetkje et al., 1993]. Additionally, all three isoforms of TGF-\(\beta\) (gene symbols \(Tgfb1\), \(Tgfb2\), \(Tgfb3\)) can be observed in embryonic mouse lens fiber cells, but not in the epithelial cells [Pelton et al., 1991]. In vitro studies using rat lens explants demonstrated the ability of TGF-\(\beta1\) to induce extensive and rapid elongation of lens epithelial cells and the accumulation of extracellular matrix, which led to capsule wrinkling. It also included lens cell apoptosis [Liu et al., 1994b]. Taken together, these studies clearly demonstrate a role for TGF in eye development.

**Insulin-like growth factors** (IGFs) could also stimulate lens cell elongation [Beebe et al., 1980, 1987]. High levels of IGF-I (\(Igfl\)) and IGF-II (\(Igfb2\)) were found in the aqueous humor and in the vitreous of the bovine eye, and the ciliary body, the cornea, the lens, and the retina all have distinctive expression patterns for the IGF-binding proteins 2 and 3 (\(Igfbp2\), \(Igfbp3\)) and the IGF-I receptor (\(Igfr1\)) [Alemany et al., 1990; Arnold et al., 1993]. The IGFs remain present in the eye, even in the adult stages, indicating a requirement throughout development and at all stages of lens differentiation.

The most detailed aspects on the role of growth factors during lens development, however, have been elaborated analyzing the **fibroblast growth factors** (FGF). Acidic or basic FGF are able to induce lens fibers, cell differentiation, proliferation, and migration, when added to lens epithelial cells. Moreover, DNA synthesis and fiber differentiation were achieved in a progressive concentration-dependent manner. FGF determines lens polarity and growth pattern; first evidence came from the anteroposterior differences in the availability of FGF in the ocular media and the lens capsule, which contains bFGF: only the posterior capsule has mitogenic activities, which could be neutralized by the use of an antibody against FGF [Lovicou and McAvoy, 1993]. Additional evidence for the participation of bFGF in lens development comes from observations concerning the transdifferentiation of embryonic chicken retinal pigmented epithelial cells into lens cells. The effect of transdifferentiation of cornea, retina, or iris into lens tissue is well known from Xenopus, chick, or newt [for review, see Ramaekers and Bloemendal, 1981], and bFGF is able to enhance the transdifferentiation of pigmented epithelial cells into lens cells in the chick embryo [Hyuga et al., 1993].

Acidic FGF (FGF-1; gene symbol \(Fgfl\)) was detected in the capsule of neonatal rat lenses, although highest levels are found in the nuclei of those cells which have started to differentiate. It is lost completely, when cells lose their nuclei. Transgenic mice, which are able to express and secrete FGF-1 into the extracellular space between the anterior epithelial cells, revealed marked abnormalities of these cells including their elongation. The epithelial cells in the newborn transgenic eyes have lost the normal cuboidal morphology and have become columnar or have totally elongated and became vacuolated. The morphological and additional biochemical parameters in these transgenic eyes reflect the onset of fiber cell differentiation. Finally, the secreted FGF-1 led to microphakia and associated microphthalmia [Robinson et al., 1995]. Moreover, recent studies analyzing the function of a truncated FGF receptor (\(Fgfr1\)) in transgenic mice exhibited defective lens development characterized by cataracts and microphthalmia, whereas other ocular structures remained normal [Robinson and Overbeek, 1995]. The experiments cited above support the hypothesis that FGF molecules and their receptors are necessary for lens fiber cell differentiation.

This summary concerning the influence of hormones and growth factors on lenticular development demon-
strates their important function. However, the target
genes of the hormones and growth factors in the eye are
not yet elaborated. For the understanding of ocular de-
velopment, the description of the complete signal cas-
cade is required.

Function of tumor suppressor genes in lens de-
development. One of the first hints for the involvement of tum-
sor suppressor genes in lens development and dif-
ferentiation came from results on transgenic mice car-
rying the SV40 T-antigen driven by the αA-crystallin
promotor. These transgenic mice developed lens tu-
ners. However, if the gene coding for the large T an-
tigen of the polyoma virus is fused to the αA-crystallin
promotor. These transgenic mice developed lens tu-
erm. The differential affinity for the Rbl gene product. The
function of the transgene SV40 T-anti-
gen was demonstrated to be due to the interaction with
Rb1, since a modified version of the SV40 T antigen,
mutated in the binding domain for the Rb family, did
not lead to any alterations in the lens (Fromm et al.,
1994).

The function of the Rb gene product in lens develop-
ment was analyzed in more detail using gene targeting
to inactivate Rb in the mouse. Homozygous Rb−/− mice
are lethal and die at E13−15. The loss of Rb function is
associated with unchecked proliferation of epithelial
cells, impaired expression of differentiation markers,
and inappropriate apoptosis in lens fiber cells. The in-
creased apoptosis in the Rb-deficient lenses is depend-
ent on the action of another tumor suppressor gene,
p53. This effect was demonstrated in mice embryos de-
ficient for both genes (Morgenbesser et al., 1994). Sim-
ilar results were obtained by inactivation of the Rb
gene product by the viral protein E7, which binds effi-
ciently to the phosphorylated Rb protein (Pan and
Griep, 1994). The system is much more refined, if the
bc12 oncogene is targeted to the lens in transgenic mice
by the αA-crystallin promoter. Expression of bc12 in the
lens induces microphthalmia and cataracts. The ter-
ninal differentiation of lens fiber cells appears to be
inhibited. Furthermore, bc12 transgenic mice are
mated to transgenic mice expressing a truncated SV40
T antigen in the lens, which inactivates Rb and acti-
vates thereby p53-dependent apoptosis. In those lenses,
where both transgenes (bc12 and truncated SV40 T an-
tigen) are expressed, apoptosis was found to be sub-
stantially reduced. It is concluded that bc12 can protect
lens fiber cells from the p53-dependent apoptosis, which
occurs after Rb inactivation (Overbeek and Fromm, 1995).

Taken together, all these results point to specific
roles in lens morphogenesis for Rb and p53 in regulat-
ing the exit from the normal cell division cycle in dif-
ferentiating lens fiber cells. This point of view is sup-
ported by experiments analyzing the expression patterns of cell cycle regulatory genes in developing
lenses. In normal lenses, expression of cyclin A, B, D1,
D2, and E, as well as the cyclin-dependent kinases
cdc2, cdk2, and cdk4 were detected only in the epithe-
lial cells. However, expression of all these genes, except
cyclins D1 and D2, was detected in fiber cells after Rb
inactivation. It is suggested that Rb blocks cell cycle
progression in the fiber cells by negatively regulating
expression of multiple cyclin and cyclin-dependent ki-
nase genes (Fromm and Overbeek, 1995).

Role of cytoskeleton in lens development and
differentiation. The cytoskeleton of the lens cells ex-
hibits a particular structure, the beaded filaments,
which are believed to be specific for the lens. Addition-
ally, the composition of the entire cytoskeleton varies
with age and state of differentiation. From a develop-
mental point of view vimentin, filensin, and phakinin
are of main interest, because these genes show partic-
ular changes in the expression patterns during embry-
onic development.

Vimentin is one of the main cytoskeletal proteins
synthesized in the lens; the corresponding gene (Vim)
is located on mouse chromosome 2 (Lyon and Kirby,
1995). During embryonic development in the chick lens
vimentin mRNA could be observed through 14 days of
embryonic development. However, at E16, vimentin-
specific mRNA cannot longer be detected. It is ex-
plained most likely by time-dependent activation of the
various inhibitory elements, which could be demon-
strated to be present in the vimentin promoter. The
reduction in the amount of vimentin-specific trans-
scripts is correlated to the onset of degradation of fiber
cell nuclei. Since vimentin can act as an anchor for cell
nuclei, reduced need for vimentin might be due to the
loss of fiber cell nuclei during development. This ratio-
nale is supported by the comparison of erythrocytes
from various species: vimentin expression ceases, if the
cell nuclei are extruded, and vice versa (Sax et al.,
1990). Additionally, overexpression of vimentin in the
lenses of the transgenic mice interfered with the nor-
mal differentiation of lens fibers: cell denucleation and
elongation processes were impaired and the animals
developed cataracts at 6−12 weeks of age (Capetanaki
et al., 1989).

During differentiation, vimentin is able to form com-
plexes with other proteins, in particular with α-crysts-
talin (Nicholl and Quinlan, 1994) and filensin (Merdes
et al., 1991). Filensin is expressed in a lens-specific
manner. The protein is also referred to as beaded fila-
ment structural protein 1; the encoding gene (Bfsp1)
maps to mouse chromosome 2 (Masaki and Watanabe,
1994). Since it is 11 cM apart from the cataract gene
Lop4 (Lyon and Kirby, 1995), it is unlikely a candidate
gene for Lop4 (Fig. 2). Together with α-crystallin and
phakinin, filensin forms the so-called beaded fila-
ments, which are unique for differentiated fiber cells [Carter et al., 1995]. However, in the early stages of human lens development, when the primary fiber cells are formed, all cells in the lens vesicle, including the epithelial cells, express phakinin and filensin. This expression pattern changes, when the lens epithelial cells begin to transform into adult pattern, and only filensin is expressed [Quinlaü R, unpublished observations].

The function of the different gene products forming the lens cytoskeleton are not yet exactly known. However, answers to these question might be expected from the discovery of mutations in these genes, from transgenic animals and/or knockout mice, which have lost the corresponding gene function.

**Differentiation processes in the developing lens—the crystallin connection.** Up to 90% of the soluble protein in the postmitotic lens cells consists of crystallins proteins. The mammalian lens contains three major classes, α-, β-, and γ-crystallins. α-Crystallins form high-molecular-mass aggregates. Recent findings on the structure and function of α-crystallin demonstrated that they have chaperone activity and that at least αB-crystallin belongs to the family of the small heat shock proteins [for recent reviews, see Groenen et al., 1994; Piatigorsky, 1992]. αA-crystallin expression (gene symbol CryA1) is observed even in the lens pit of the rat [van Leen et al., 1987], but not in the mouse. It is present in both species in the posterior half of the lens vesicle [Tréton et al., 1991]. By contrast, αB-crystallin can be detected first at the end of embryonic development [Aarts et al., 1988]. A different pattern between αA- and αB-crystallin expression cannot longer be observed in newborn rat lenses [van Leen et al., 1987].

The β/γ-crystallin superfamily exhibits a characteristic protein motif, the so-called Greek Key Motif, in a quadruple organization. It is considered essential for the extreme high protein concentration within the lens [Moormann et al., 1983]. The γ-crystallin transcription begins, when the lens vesicle has formed. Synthesis of β-crystallins (gene symbol Cryb) is seen when the secondary lens fibers begin to elongate, whereas in the secondary fiber cells the expression of γ-crystallins (gene symbol Cryg) is restricted to terminally differentiated fiber cells [van Leen et al., 1987]. Interestingly, the time schedule for crystallin expression varies within the animal kingdom: in contrast to mammals, in amphibians γ-crystallins appear first, probably simultaneously with β-crystallins. In particular, in X. laevis the γ-crystallins can be observed in the lens pla-code. Recent data demonstrated that in X. laevis γ-crystallin genes were expressed even at the gastrula stage [Smolich et al., 1994].

The examples cited above demonstrate that the expression of the crystallins is strongly regulated with respect to the spatial and temporal pattern. Such a specific pattern of gene expression must be regulated by a network of cell-type specific transcription factors. The first one involved in this network interacts with the αA-crystallin promoter and was referred to as αA-CryBP1 [Nakamura et al., 1990]; it was established as a zinc-finger protein. At least five factors including Pax6 are required for the expression of αA-crystallin in the lens [Cvekl et al., 1994, 1995a].

The γ-crystallins are expressed in mammals after the α-crystallins. The γ-crystallin genes contain in their promoter regions elements responsible for activators and repressors. In particular, one of them was recognized in the γE/F-crystallin promoters as a retinoic acid response element [Tini et al., 1993]. To test the function of RARE, the gene encoding the cellular retinoic binding protein I (CRAP I) was ectopically expressed in transgenic mice under the control of the αA-crystallin promoter. It was expected that it leads to a decrease in the intracellular concentration of retinoic acid and, subsequently, one of the γF-crystallin concentration. As expected, the transgenic animals formed cataracts, but the amount of γF-crystallin specific transcripts was not influenced [Perez-Castro et al., 1993]. An element from the γ-F-crystallin promotor suggested to be involved in regulation of transcription in lens cells, interacts with a zinc-finger protein referred to as γFBP. It was detected in chicken and is expressed besides the lens in presumotic mesoderm and then over the entire epithelial somite [Liu et al., 1994c].

Moreover, also α-crystallins are involved in specific interactions with the γD/E/F-crystallin promoter sequences [Pietrowski et al., 1994]. The interaction of α-crystallins with the γ-crystallin promoters enhances the expression of corresponding reporter gene constructs [Krausz E and Graw J: unpublished observations]. The available data on γ-crystallin expression revealed that several factors are involved in the lens-specific expression of this gene family demonstrating the complexity of the regulation of temporal and spatial expression of the γ-crystallins. However, the complete network of γ-crystallin regulators remains to be worked out.

**Are the γ-crystallin genes a “hot spot” for mutations?** The γ-crystallin encoding genes (Cryg) are organized as a cluster of six genes (γA->γF-crystallin) within approximately 50 kb on mouse chromosome 1; in human, the corresponding region is located at chromosome 2. The six genes are very similar and, in particular, the proteins sequences, which are deduced from the murine γE- and γF-crystallin genes, are identical [Graw et al., 1991]. Also between the different mammalian species analyzed so far, the variation of the γ-crystallin sequences are very small [den Dunnen et al., 1989; Graw et al., 1993]. The γE- and γF-crystallin genes, which are very strongly expressed in rodents during the late phase of gestation and in the juvenile phase, are not expressed in humans and, therefore, referred to as pseudogenes [Brakenhoff et al., 1990].

In mice, up to now, 8 independent mutations are defined to be associated with the γ-crystallin cluster: first of all, the Elo mutant (Eye lens obsolescence) was char-
acterized to carry a single nucleotide deletion in the γE-crystallin gene. The mutation destroys the reading frame of the gene, and at the protein level one of the Greek Key Motifs is affected [Cartier et al., 1992]. Furthermore, the allelic series of the Neuherberg Cat2 mutants [Kratochvilova and Favor, 1992; Everett et al., 1994] is closely linked to the γ-crystallin cluster [Löster et al., 1994b]. In one of these mutants, Cat2\(^{\alpha}\), the 3'-end of the γE-crystallin gene is affected [Graw, unpublished observations]. In human, a mutation within the promoter region reactivates the γE-crystallin gene and leads to the so-called "Coppock-like cataract" by expression of a truncated γE-crystallin of 8 kDa. The Coppock-like cataract affects only the embryonic nucleus of the lens, leading to a mild phenotype [Brakenhoff et al., 1994].

Since all effects observed in the E\(\alpha\)o and the Cat2 mutant eyes reflect the effects of at least one missing γ-crystallin, the various phenotypes in these mutants can now be described as functions of the affected genes. The decreased content of γ-crystallin specific transcripts precedes morphological alterations [Santhiya et al., 1995]. The mutants have a block in the maturation of primary lens fibers and in the differentiation of secondary lens fibers as demonstrated by the incomplete degradation of the nuclei in the primary and secondary fiber cells. Lens fiber elongation is also impaired, as they do not reach the poles of the lens leading to the phenotype of a suture cataract in the heterozygous state [Graw et al., 1989, 1990]. These results demonstrate that the γ-crystallins—like the α-crystallins—are involved in the pathway of lenticular differentiation. Since lenticular differentiation is driven by growth factors like FGF and proteins regulating the exit from the normal cell division cycle, it is expected that these mutants opens the possibility to establish a biochemical link between these two processes.

**Genes Affecting the Embryonic Development of the Retina**

Since in human and rodents retinal development is not completed at birth, important steps in this process occur postnatally. Also, a remarkable number of mutations are well documented affecting this late phase of retinal development. As examples, only the various forms of retinal degeneration (rd1, Rd2, rd3) and the important human disease retinitis pigmentosa are mentioned. Since this paper focuses at the embryonic development, all these late events are not discussed here. The main aspects of this section are the function of the microphthalmia and retinoblastoma gene, as well as an short overview on signaling genes involved in early retinal development.

**Microphthalmia (M\(\alpha\)).** Since the first discovery of mouse microphthalmia (M\(\alpha\)) mutation more than 50 years ago [Hertwig, 1942], at least 17 mutant alleles have been identified and characterized at chromosome 6 [overview in Steingrimsson et al., 1994].

The eyes of the mutants develop poorly because of the affected retinal pigmented epithelium. In addition, other cell types are also affected like the neural-crest-derived melanocytes, which in severe cases leads to deafness, owing to the lack of inner ear melanocytes. The phenotype of these mouse mutants may be either dominant or recessive. The affected gene could be identified as a member of the basic-helix-loop-helix leucine zipper family of transcription factors.

Recently, a human homolog was identified to the mouse Mi\(\alpha\) gene, referred to as MITF. Mutations within MITF were found in two patients suffering from Waardenburg-syndrome type 2; the mutations affect splice sites in the MITF gene leading to a short peptide of 11 amino acids. The other mutation lacks the motif, which is responsible for dimerization leading to an inactivation of the MITF gene as a transcription factor [Tassabehji et al., 1994].

**Function of the retinoblastoma gene during retinal development.** Retinoblastoma, a tumor of the retina, serves as a paradigm for tumorigenesis through a loss of function. The inactivation of both alleles of the retinoblastoma gene (R\(b1\)) in the developing retina is the rate-limiting step in its neoplastic transformation leading to the characterization of the wild-type allele of R\(b1\) as a tumor-suppressor gene [for overview, see Helen and Harlow, 1993]. Increasing evidence suggests that R\(b1\) functions in vivo by interacting with transcription factors such as E2F and MyoD. Complexes with a number of other factors (e.g., the SV40 large T antigen) have also been demonstrated in vitro and are discussed above. Ample evidence shows that R\(b1\) can repress transcription of cellular promoters regulating cell cycle progression. On the other hand, R\(b1\) can activate genes, which downregulate cell proliferation [Kouzarides, 1993].

In mouse, R\(b1\) maps to chromosome 14 [Peters et al., 1995]. During embryonic development, R\(b1\) expression was observed as early as E9.5 with the highest level found in brain, spinal cord and liver. Concerning ocular development, the retina of homozygous R\(b1\)^{−/−} mutants is normal until the death of the embryos at E12–15, whereas a highly disorganized structure of the lens was observed in homozygous R\(b1\)^{−/−} mutants around E14.5. These observation shows that the effect of R\(b1\) to the retina occurs later as compared to the lens [Maandag et al., 1994].

**Expression of cell signaling genes in the optic cup and in retinal development.** The retina comprises various morphologically distinct cell types, which have to be well organized. Therefore, genes will be described in the following section, which are involved in signal chains or pathways, where cells have to communicate with each other to built-up complex retinal structures. A very successful approach was to look for genes, which have similar functions in *Drosophila*. Because of their similarity to the *Drosophila* gene *Msh*, two related homebox containing genes
were discovered in mice. They are referred to as Msx1 (formerly Hox7.1) and Msx2 (formerly Hox8.1) and map to chromosomes 5 and 13, respectively [Peters et al., 1995]. Both are expressed in the mouse embryo during the early stages of eye development in a distinct spatial and temporal manner. Msx2 is expressed in the surface ectoderm and in the optic vesicle before invagination occurs in regions corresponding to the prospective corneal epithelium and neural retina, while Msx1 is expressed after formation of the optic cup, marking the domain for the prospective ciliary body. The expression pattern indicates that the inner layer of the optic cup is differentiated into three distinct compartments even before cellular differentiation becomes obvious. It is suggested that both, Msx1 and Msx2 are involved in regulation of the development of the iris, ciliary body, and retina [Monaghan et al., 1991].

Recently, another group of cell signaling molecules have been detected and characterized as homologues to wingless, a segment polarity gene in Drosophila. In the developing eye, at least one member of the Wnt family, Wnt7b (mouse chromosome 15) [Peters et al., 1995], is expressed at E9.5 in the optic stalk and in the dorsal portion of the optic vesicle, as well as in the diencephalon. The expression pattern is consistent with the hypothesis that Wnt7b is involved in establishing the dorsal-ventral axis of the optic vesicle [Parr et al., 1993].

The murine homolog to the Drosophila homeobox-containing gene distal-less (Dlx1, mapped to mouse chromosome 2) [Peters et al., 1995] is expressed in the prospective retina at E11.5 to E14.5. In situ hybridization has revealed strong signals, which are restricted to the neural retina layer, while more ventral parts of the eye epithelium, the prospective ciliary body and iris, are not labelled. Within the differentiating neural retina, labeling seems to be stronger in the deep layer (i.e., in more compacted cells). Hence, Dlx1 can be denoted as a molecular marker of the prospective neural retina prior to its morphological differentiation. However, the expression of Dlx1 is not restricted to the eye, as it can also be observed in brain tissues involved in the transmission of the optic signals [Dollé et al., 1992].

Two other homeobox containing genes, called orthodenticle in Drosophila and Otx1 and Otx2 in mice, can be observed during retinal development. Both are expressed in the mouse at E10 throughout the entire optic stalk, but at E12.5 Otx1 is limited to the prospective iris and ciliary epithelium. At E17, Otx1 can be found only in the iris. By contrast, Otx2 can be observed at E12.5 in the retinal pigment epithelium, the sclera and tissues surrounding the optic nerve. At E17, Otx2 expression is limited to the pigmented epithelium of retina and the neurosensory retina [Simeone et al., 1993].

Recently, a novel homeobox gene, Chxl0, has been identified to play a role in the determination of the murine neuroretina and the inner layer of the retina. In the developing mouse, the Chxl0 transcript is expressed throughout the anterior optic vesicle and all neuroblasts of the optic cup. In the mature retina, the Chxl0 protein is restricted to the inner nuclear layer. Besides the eye, Chxl0 transcripts can also be detected in the regions of developing thalamus, hindbrain, and ventral spinal cord [Liu et al., 1994a].

The chicken homeobox-containing gene GH6 has a broader spectrum of expression in the eye; besides the neural retina it can be found in the lens epithelium and the optic nerve. GH6, which shows remarkable homology to the human homeobox gene H6, is expressed relatively higher in the dorsal portion of the neural retina, but rather uniformly in the lens (chick stage 23 embryo) [Stadler and Solursh, 1994].

The examples described above demonstrate that a broad spectrum of genes is involved in early retinal development, however, there are no mutants described for any of these genes. Therefore the data can interpreted only with respect to the expression pattern; the exact function has to be elaborated.

**Optic Nerve Development**

Is Pax2 a master control gene for optic nerve development? Pax2 is the second Pax gene besides Pax6, which is expressed in the eye. According to Nornes et al. [1990], Pax2 expression during optic nerve development can be divided into two phases: (1) the morphogenesis of the optic cup and stalk, prior to axon growth; and (2) the period of axogenesis. Pax2 transcripts are first detected in the most distal region of the optic vesicle (opposed to the surface ectoderm), when it is making contact with the surface ectoderm. Later on, Pax2 is expressed over the ventral two-thirds of the invaginating epithelium and the ventral region of the optic stalk. The expression ends abruptly at the border of the diencephalon. This remains true, even when the axons enters the optic stalk. During axogenesis, Pax2 is absent from the neuroblastic layer of the retina; however, there is strong expression in the optic disc and extending along the entire optic nerve. From subsequent analysis of kidney development, it appears that Pax2 might be required for conversion of mesenchyme to epithelium [Rotthenspieler and Dressler, 1993].

In humans, a mutational analysis of Pax2 in a family with optic nerve colobomas, renal hypoplasia, mild proteinuria, and vesicoureteral reflux was conducted leading to the detection of a single nucleotide deletion in exon 5. It causes a frameshift in the Pax2 coding region for the octapeptide domain, which might be important for protein–protein interactions, and the whole C-terminal portion of the protein. It is predicted that the truncated protein could still be able to bind DNA, because the paired-box remains intact, but it may lack the ability to transactivate the expression of target genes. The human phenotype resulting from the Pax2 mutation was very similar to abnormalities, which have been reported in Krd mutant mice (kidney and retinal defects) [Sanyanusin et al., 1995]. The Krd mutation is a transgene-induced deletion at mouse chro-
Fig. 3. Flow chart of eye development. A simplified flow chart illustrates the major inductive events and tissue differentiation during eye development. Inductive events are indicated by arrows. Genes and chromosome 19, which includes the Pax2 locus [Keller et al., 1994], indicating that Pax2 is required for normal eye and kidney development.

**Ocular retardation by optic nerve aplasia.** In mouse, the first axons from the retinal ganglion cells enter the optic stalk on E12.5. The axons further extend to the ventral wall of the optic stalk and reach the primary optic center of the brain. Based on gene expression studies, it is obvious that at least the first axonal growth might be influenced by cell adhesion molecules (CAM). In particular, the NgCAM (neuro-glia-CAM) may act as a stabilizing factor for axon–axon and axon–glia contacts. Additionally, from expression pattern, it may be deduced that the cadherin family might play a role in the adhesion of the retinal tract and the axons from the retinal ganglion cells. Laminin is expressed in the ventral portion of the optic stalk, in which the first retinal axons grow [summarized according to Yamamoto and Schwarting, 1991].

Optic nerve development also needs the system of the central artery and vein to establish a pathway along the choroid fissure. The formation of the optic fissure is essential for the proper development of the optic nerve. A network is built up consisting of intercellular channels between neuroepithelial cell processes within the marginal layer of the embryonic retina and continuing without interruption to the optic stalk. In marked contrast to wild-type mice, there is a absence of these channels in a strain of mice (or, ocular retardation) with an inherited aplasia. In these mutants, the optic nerve fails to exit from the eye [Silver and Robb, 1979]. The mutation is mapped to chromosome 12 of the mouse [Hawes and Roderick, 1990]. Up to now, no further molecular analysis of this mutation has been reported.

As discussed for the initial phase of eye development, also for the optic nerve formation a multistep process has to be proposed, and one of the initial events might be mediated by Pax2. However, the or mutants demonstrate that additional functions are essential for proper optic nerve development.

**CONCLUSION AND PERSPECTIVES**

A large number of murine mutants involved in eye development have been discussed. As summarized in Figure 3, some of the corresponding genes might be understood as control genes for major inductive processes (e.g., Mi, Pax2, Pax6, Rb1), whereas some others seem to be responsible for the fine-tuning of tissue differentiation. However, learning from the expression of murine Pax6 in Drosophila leading to ectopic formation of ommatidial eyes in Drosophila antennae [Halder et al., 1995], we can obviously use the genetic system of Drosophila eye development as a paradigm for mammalian eye development, even if is organized in a quite different manner. Therefore, precise molecular analysis of Drosophila eye development will bring important informations to mouse geneticists. Combining advances from the Drosophila and mouse with modern concepts of developmental biology holds great promise for making rapid advance in our understanding of eye development and its unique properties of transparency and signal transduction.
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