REVIEW ARTICLE

Mouse models of cataract

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Abstract

Much of our knowledge about the function of genes in cataracts has been derived from the molecular analysis of spontaneous or induced mutations in the mouse. Mutations affecting the mouse lens can be identified easily by visual inspection, and a remarkable number of mutant lines have been characterized. In contrast to humans, most of the genetic mouse cataract models suffer from congenital cataracts, and only a few develop cataracts in old age. Therefore, the mouse cataract models contributed rather to the understanding of lens development than to the ageing process taking place in the lens. A prerequisite for molecular analysis is the chromosomal localization of the gene. In this review, several mouse models will be discussed with emphasis on the underlying genetic basis rather than the morphological features as exemplified by the following: (i) the most frequent mutations in congenital cataracts affect genes coding for γ-crystallins (gene symbol: *Cryg*); (ii) some postnatal, progressive cataracts have been characterized by mutations in the β-crystallin encoding genes (*Cryb*); (iii) mutations in genes coding for membrane proteins like MIP or connexins lead to congenital cataracts; (iv) mutations in genes coding for transcription factors such as *FoxE3, Maf, Sox1*, and *Six5* cause cataracts; (v) mouse models suffering from hereditary age-related cataracts (e.g. Emory cataract) have not yet been characterized genetically. In conclusion, a broad variety of hereditary congenital cataracts are well understood at the molecular level. Further, expression patterns of the affected genes in several other tissues and organs outside the eye, is making it increasingly clear that isolated cataracts are the exception rather than the rule. By further understanding the pleiotropic effects of these genes, we might recognize cataracts as an easily visible biomarker for a number of systemic syndromes.

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Introduction

There are an estimated 50 million blind people in the world, and cataracts (opacities of the lens in the eye) are responsible for half of these cases (Johnson and Foster 2004). In USA, over 1.2 million cataract operations are performed per year; the costs are over 3.4 billion $ (West 2000). For age-related cataracts, it is thought (based on twin studies) that the heritability for nuclear and cortical cataracts is around 50% (Hammond *et al*. 2000, 2001). However, there are only few genetic studies for age-related cataracts reported till date (Okano *et al*. 2001; Jun *et al*. 2009). Because of the small size of the families usually available for detailed genetic investigations, it is necessary to look for appropriate animal models to identify genes responsible for cataract formation and to analyse the mechanisms leading to the opacification of the lens. Mouse is one of the best model systems, because it is very well characterized among mammals from a genetic point of view, and observed molecular alterations are comparable to that in man. Moreover, a phenotype-driven approach in experimental genetics picks up similar clinical manifestations in the mouse lens as observed in humans. A systematic approach to collect mouse cataract mutants was initiated more than 30 years ago by Kratochvilova and Ehling (1979), when they used paternal treatment of germ cells by X-rays and screened for cataracts in the offspring. The method was extended to the use of ethylnitrosourea (ENU) as a mutagenic agent (Ehling *et al*. 1985) and is now continued world wide as a valuable tool for a broad variety of disorders (Hrabé de Angelis *et al*. 2000; Clark *et al*. 2004; Barbaric *et al*. 2007; Takahasi *et al*. 2007).

This review focuses on both induced mutations in the mouse leading to cataracts and also mutations that have

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arisen spontaneously. It is organized according to the genes that are involved with a brief section at the end to include those mutants not yet attributed to a particular gene. The main part deals with congenital cataracts, but at the end we will discuss briefly also such mutants whose mutations are not yet attributed to a particular gene, but there are also a few examples of hereditary senile cataracts. Cataracts in transgenic mice (overexpressing genes ectopically) will not be discussed here.

Congenital cataracts in mice

Congenital cataracts are present at birth indicating pathological changes during embryonic development of the lens. Lens development is the result of a series of inductive processes (Graw 2003), and one of the most important events is the interaction of the lens placode with the surface ectoderm. It was discussed by Spemann (1924) as the crucial point in lens induction of the frog referring to the optic cup as the 'organizer of the lens'. The lens cup forms subsequently the lens vesicle, which is filled by elongation of its posterior cells leading eventually to primary lens fibres. The cells at the anterior pole of the lens vesicle remain as epithelial cells with a germinative ring of mitotic active cells (stem cell niche) around the central region. Daughter cells from this germinative region move to the equatorial region where they rapidly elongate and differentiate into secondary lens fibres forming concentric layers around the primary fibres of the lens nucleus. With this arrangement, the lens fibres towards the periphery are successively younger in terms of development and differentiation. As long as the lens grows, new secondary fibres move in from the equator onto the outer cortex of the lens. The lens continues to develop throughout life (Graw 2004, and references therein) with new secondary fibres moving in from the equator to the outer cortex of the lens.

Both the primary and secondary fibre cells lose their mitochondria and cell nuclei during the final differentiation process. For the primary fibres, it takes place in mice at E17/18 and is finalized two weeks after birth, when the mice open their eyelids (Vrensen *et al*. 1991). The secondary fibre cells, which lie appositionally upon the primary fibre cells, lose subsequently their organelles during their terminal differentiation (Kuwabara and Imaizumi 1974). Any alterations to this process will lead to congenital cataracts with features dependant at least in part, on the mode and site of action of the mutated gene(s).

Mutation in the β/γ-crystallin encoding genes

The largest subgroup among the mouse cataracts affects the γ-crystallins (gene symbol *Cryg*). *Cryg* genes belong to the superfamily of β-crystallin and γ-crystallin encoding genes, and comprise six closely related genes (*Cryga-f*) on mouse chromosome 1. *Cryg* genes are expressed in the eye lens at a very high level and thought to encode structural proteins. The γ-crystallin proteins are characterized by four so-called Greek-key motifs. The first *Cryg* mutation was characterized by Cartier *et al*. (1992); to date, 23 mouse mutants affecting the *Cryg* gene cluster have been published, affecting all six genes of the *Cryg* gene cluster (for a recent summary, see Graw 2009).

As far as we know today, mutations in the *Cryg* genes affect only the lens; however, there is a striking diversity of the particular cataract phenotypes. Even if in all mice a *Cryg* gene is altered by the mutation, the consequences for the lenses are different and might be related to distinct functions of the individual γ-crystallin, to the affected domains within a particular γ-crystallin, to the time point, when its expression starts, and/or to its particular expression level. A comparison of eight different and independent *Cryg* mutations (figure 1) demonstrates that the characterization of the phenotype does not allow any prediction of the mutated gene. Unfortunately, there is no overwhelming common mechanism for all *Cryg*-dependent cataracts. It is worth, however, looking at one *Cryg* mutation in more detail: the dominant cataract mutation *Nop* (nuclear opacity) arose spontaneously (Graw *et al*. 1984) and was characterized finally by a deletion of 11 bp and an insertion of 4 bp in exon 3 of the *Crygb* gene (allele symbol: $Crygb^{nop}$). This mutation leads to a frame-shift and creates a new stop codon allowing the incorporation of only six new amino acids. Using an antibody specific for the six novel amino acids, it was shown by Western blot analysis that the corresponding γ-crystallin protein is truncated after 144 amino acids (Klopp *et al*. 1998).

The first morphological abnormality in the mutant lenses was observed as swelling of lens fibres at embryonic day 15.5 followed by progressive degeneration of the lens core leading to a nuclear opacity and revealed additionally polar cataracts with vacuolization; nuclei of the cortical lens cells could also be detected in the area of the lens nucleus of the $Crygb^{nop}$ lenses (Graw *et al*. 1990b).

Biochemical investigations demonstrated a 2.5–5-fold increase of the concentration of oxidized glutathione (GSSG) in the $Crygb^{nop}$ lenses and an increase in lens water content. The latter was shown to correspond with an increased Na^+/K^+-ATPase activity (Graw *et al*. 1990a). This elevated Na^+/K^+-ATPase activity is due to the increased amount of the α1-isoform of the Na^+/K^+-ATPase, but more interestingly also caused by a de novo synthesis of the Na^+/K^+-ATPase isoforms α2 and α3, which cannot be found in the lenses of wild-type mice or in other tissues of the mutant mice (Moseley *et al*. 2002). More related to the cellular differentiation processes during cataract formation might be the observation that in the $Crygb^{nop}$ mutants the activity of Mg^{2+}-dependent DNase was reduced. This finding is the first experimental evidence for the stopping of the lenticular differentiation process in these cataractous lenses, because the degradation of DNA is an essential step for the breakdown of the cell nuclei in the primary lens fibre cells and the secondary fibre cells in the deep cortex. It is characteristic for the terminal

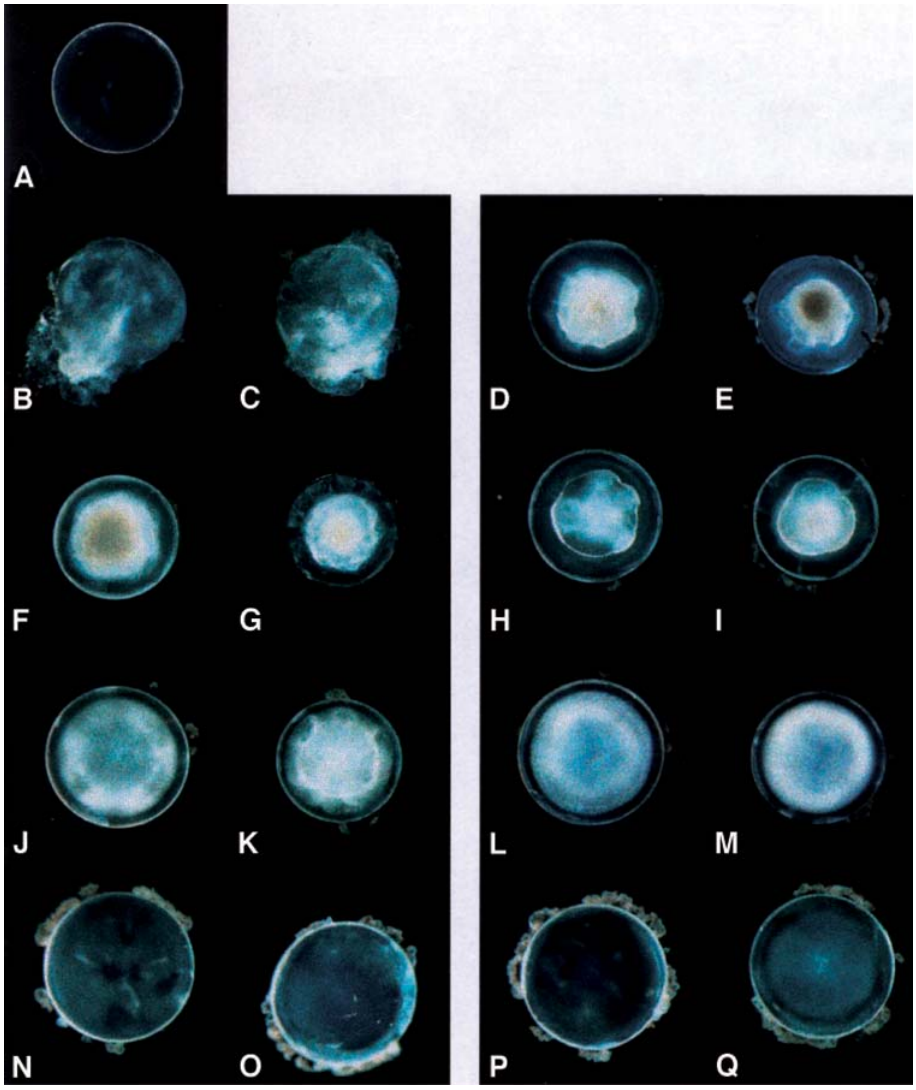

Figure 1. Lens opacities in different *Cryg* mutants. The lens is a transparent tissue which has to focus incoming light for the retina. The lens is mainly composed of fibre cells and is surrounded by a collagen-containing capsule. The lens fibre cells from either side meet each other forming a Y-shaped suture. Opacities (cataracts) can affect different parts of the lens, and classification systems follow frequently such regional characteristics. Therefore, we can distinguish, for example, nuclear cataracts (affecting the lens nucleus), cortical cataracts (affecting the lens cortex), suture cataracts (affecting the lens sutures), or total cataracts, which are frequently accompanied by smaller lenses (and even smaller eyes). Here we give examples for different types of cataracts of heterozygous and homozygous *Cryg* mutants at the age of three-weeks: (A) C3H wild type; $Cryga^{ENU369}$ (B) heterozygote and (C) homozygote; $Crygd^{K10}$ (D) heterozygote and (E) homozygote; $Crygc^{MNU8}$ (F) heterozygote and (G) homozygote; $Cryge^{Z2}$ (H) heterozygote and (I) homozygote; $Crygd^{ENU4011}$ (J) heterozygote and (K) homozygote; $Cryge^{ADD15306}$ (L) heterozygote and (M) homozygote; $Crygd^{ENU910}$ (N) heterozygote and (O) homozygote; and $Cryge^{ENU449}$ (P) heterozygote and (Q) homozygote (with permission from *Invest. Ophthalmol. Vis. Sci*. (Graw 2004)).

differentiation of normal lens fibre cells. In contrast, in the mutants the cell nuclei are present throughout the lens even at the age of three weeks (Graw *et al*. 1990b); it could be associated with an inhibition of DNAse activity in the cataractous lenses (Graw and Liebstein 1993). A key feature during this process is the formation of intranuclear inclusions containing the altered γ-crystallins in the primary fibre cells. It precedes not only the first gross morphological changes in the lens, but also the first signs of cataract. The inclusions contained filamentous material that could be stained with the amyloid-detecting dye and congo red. In addition with further *in vitro* data, these findings indicated that this

type of cataract is caused by a mechanism involving nuclear targeting and disrupting of nuclear functions via deposition of amyloid-like inclusions (Sandilands *et al*. 2002).

Intermediate members of the β/γ-crystallin superfamily are the γS-crystallins and γN-crystallins. The cataract mutation *Opj* (opacity due to poor junctions; Everett *et al*. 1994; Kerscher *et al*. 1996) was characterized as a semi-dominant progressive cataract. It was mapped close to *Crygs* and subsequent sequence analysis of *Opj* mice revealed a mutation in the *Crygs* gene affecting a key residue of the core of the N-terminal domain of the protein (Sinha *et al*. 2001). An additional mouse mutant suffering from a premature stop codon in the *Crygs* gene is characterized by a recessive nuclear cataract (Bu *et al*. 2002). For *CrygN*, no mutation has been reported so far. This might be due to the relative low expression level in the mouse; in human, *CRYGN* underwent major changes leading to different splice forms and expression in the retinal pigmented epithelium, hippocampus and testes, but not in the lens (Wistow *et al*. 2005).

In man, also a rapidly increasing number of families suffering from hereditary congenital dominant cataracts were characterized as having mutations in the *CRYG* genes. It might be of evolutionary interest that in man two of the six *CRYG* genes are pseudogenes; they are obviously not necessary for optimal human lens function (Brakenhoff *et al*. 1990). Moreover, it is noteworthy that all mutations characterized so far in human *CRYG* genes were found in *CRYGC*, *CRYGD* and *CRYGS*, but none in *CRYGA* or *CRYGB*. The family of β-crystallins can be divided into more acidic (βA-) and more basic (βB-) crystallins. Each subgroup is encoded by three genes (*Cryba1, Cryba2, Cryba4; Crybb1, Crybb2, Crybb3*); however, *Cryba1* codes for two proteins (βA1- and βA3-crystallin). This feature is conserved among all mammals, birds and frogs. In mouse and man, the six *Cryb* genes are mapped to three different chromosomes. Although β-crystallins are expressed from early developmental stages in the eye lens, their expression continues and rises after birth so that the highest concentrations are usually found in the lens cortex. However, the expression pattern varies among the individual β-crystallins (for review see Graw 2003). There is increasing evidence that *Crybb2* is expressed not only in the lens, but also in the testes, retina and the brain (Magabo *et al*. 2000; DuPrey *et al*. 2007; Liedtke *et al*. 2007; Ganguly *et al*. 2008).

In mouse, three mutant lines have been reported to affect the *Crybb2* gene till now: the Philly mouse was the first cataract mutant to be characterized at the molecular level (Chambers and Russell 1991); further *Crybb2* alles are the *Aey2* mutant line (Graw *et al*. 2001c) and the O377 line (Ganguly *et al*. 2008). All are progressive cataracts with slightly different morphologies. The Philly mouse phenotype is characterized by an anterior and posterior subcapsular opacity. The formation of cataracts begins after birth and continues progressively. This phenotype is caused by an in-frame deletion of 12 bp in the 3′ end of the *Crybb2* gene; it is suggested that the formation of the 4th Greek-key motif is affected (Chambers and Russell 1991). Recently, it was also demonstrated that the Philly mice suffer from reduced fertility since the *Crybb2* mutation affects also the function of the testes (DuPrey *et al*. 2007). The *Aey2* mutants exhibit a cortical opacification at eye opening, which progresses to an anterior suture cataract and reaches its final phenotype as total opacity at eight weeks of age; the typical histology is given in figure 2. The cataract is caused by a T→A mutation at position 553 of the *Crybb2* gene changing the Val at position 187 to a Glu (V187E) and affects, therefore, the same region as the Philly allele (Graw *et al*. 2001c).

The third *Crybb2* allele in the mouse, O377 is characterized by a progressive, semi-dominant cataract and small lens. Histological analysis of the mutant eyes shows the heterozygous lens to be highly vacuolated and patchy at the equatorial and the posterior regions. The underlying mutation is an A→T substitution at the end of intron 5 of the *Crybb2* gene leading to alternative splicing with a 57-bp insertion in the mRNA and to additional 19 amino acids (it is the same region like the two other *Crybb2* alleles described above). Since wild-type and mutant βB2-crystallin is expressed in the cerebellum, olfactory bulb, cerebral cortex and hippocampus, additional effects might be expected (Ganguly *et al*. 2008).

Several human cataract mutations have been currently described to be associated to the *CRYB* genes, most of them are dominant, but two are recessive (one allele each in *CRYBB1* and *CRYBB3*; for a recent review see Graw 2009).

Mutations in α-crystallin encoding genes

The α-crystallin complexes are mainly composed of two related proteins, αA-crystallin and βB-crystallin. They are encoded by two genes, *Cryaa* and *Cryab*, on mouse chromosomes 17 and 9, respectively. The two *Crya* genes are expressed at very high levels in the lens. The αA-crystallin (gene symbol: *Cryaa*) is expressed in the mouse lens cup at E10.0–E10.5, later in the posterior half of the lens vesicle, and after birth, αA-crystallin becomes very abundant in lens fibre cells; αA-crystallins are found also in the retina (for a recent review see Graw 2009). Mutations in the *Cryaa* gene show features, which are interesting because of their phenotypic diversity. The first surprise was the report of the *Cryaa* knockout mice by Brady *et al*. (1997). Only in the homozygous state, the null mutants exhibit a cataractous phenotype, which was shown to be caused by inclusion bodies containing the highly related αB-crystallin. Usually, the αA-crystallin and αB-crystallin form large aggregates with a molecular mass of approximately 800–1000 kDa. Obviously, these mixed complexes cannot be formed if the αA-crystallin subunit is completely missing. In the heterozygous condition, the reduced amount of αA-crystallin might be sufficient to keep the complex soluble. Similarly, also one point mutation in the *Cryaa* gene revealed a similar recessive phenotype in the mouse (*lop18*): a missense mutation in the first exon of the *Cryaa* gene (161G→A) was demonstrated as a cause for a

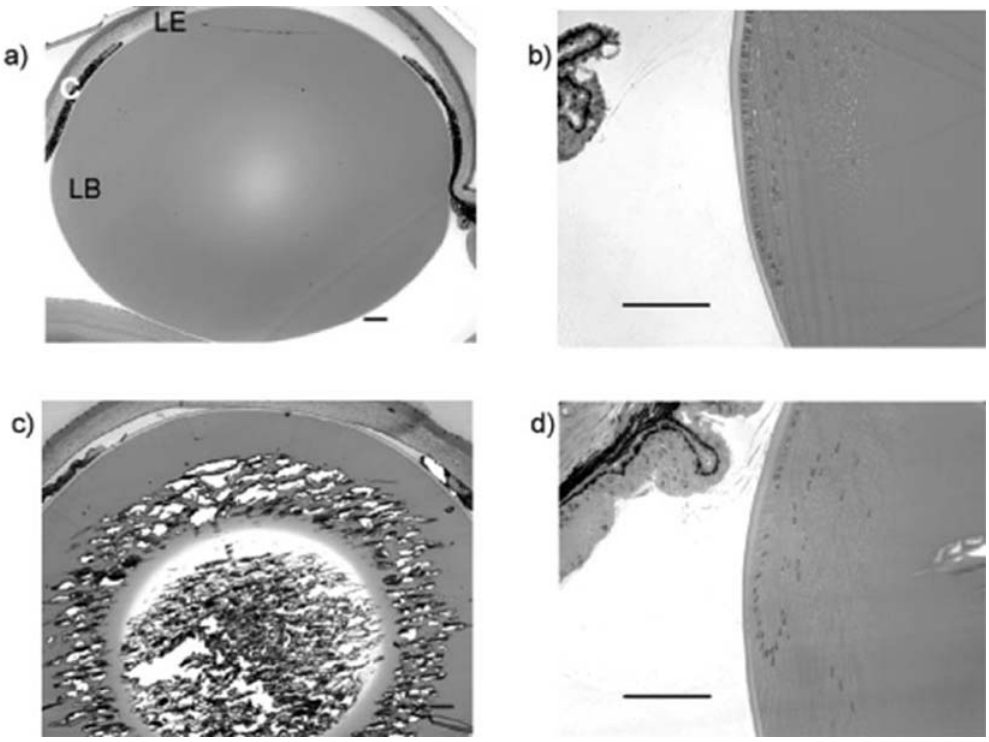

Figure 2. A cataractous *Cryaa*^{Aey7} lens. Histologic analysis of lenses of the *Cryaa*^{Aey7} mutant. Sections through lenses of seven-week-old wild type (a, b) or homozygous mutant mice (c, d) are shown; the tissue is stained by methylene blue and basic fuchsin. The mutants showed numerous clefts in the lens core and in the subcortical region (c). The age-matched control did not show abnormalities. Magnification of the lens bow region demonstrated the regular arrangement of the epithelial cells and the novel secondary fibre cells as well as the presence of cell nuclei in a well-shaped order, both in the wild type (b) and in the homozygous mutant (d). C, cornea; LB, lens bow; LE, lens epithelium. Scale bars, 100 mm (with permission from *Invest. Ophthalmol. Vis. Sci*. (Graw *et al*. 2001a)).

cataract converting codon 54 Arg to His (Chang *et al*. 1999). Surprisingly, a point mutation at position 160 (C→T) leading to the same Arg54His exchange was characterized as a dominant cataract mutation in the mouse (Xia *et al*. 2006). In man, the same mutation (160C→T) but leading to an Arg→Cys exchange at position 54 (R54C) was described to cause dominant (Devi *et al*. 2008) and semi-dominant cataracts (Khan *et al*. 2007). A 'true' recessive cataract is caused by a nonsense mutation affecting the N-terminal region of the αA-crystallin (WX9; Pras *et al*. 2000). It is of great interest from a genetic point of view that mutations in the *Cryaa/CRYAA* gene lead to both recessive and dominant phenotypes although the mechanisms influencing this remain unknown.

There are two other mouse models leading to dominant cataracts; in both cases, the C-terminal part is affected. The *Cryaa*^{Aey7} allele was characterized as a nuclear opacity associated with a posterior suture anomaly, which is visible at eye opening. It progresses to a nuclear and zonular cataract at two months of age and is associated with microphthalmia; homozygotes are more severely affected than heterozygotes. The lenses of these mutants show numerous clefts in the lens nucleus and in the subcortical region, whereas the outer cortex and the epithelial cells seem to be unaffected (figure 3). Sequence analysis identified a T→A exchange at *Cryaa* cDNA position 371 resulting in a replacement of Val by Glu at codon 124 (V124E; Graw *et al*. 2001a).

In contrast to *Cryaa*, *Cryab* is expressed rather ubiquitously. In mouse lenses, αB-crystallin is present at E9.5 and later on found preferentially in the epithelial cells (Robinson and Overbeek 1996). Unlike *Cryaa*, the mouse knockout of the *Cryab* gene revealed a phenotype, which could not be distinguished from the wild type (Brady *et al*. 2001). The lenses developed normally, and all other crystallins were present. The authors concluded from these results that *Cryab* is not essential for normal development of a transparent lens in the mouse. In contrast to the mouse, several recessive and dominant cataract mutations in *CRYAB* are described in man. Furthermore, because of the expression pattern of the human *CRYAB*, at least some of these patients suffer from other diseases such as myopathies/cardiomyopathies. It is also well known that αB-crystallin is present in some neurodegenerative disorders (e.g. Creutzfeld–Jacob disease, Alexander's disease, Alzheimer's disease, Parkinson's disease; for a recent review see Graw 2009).

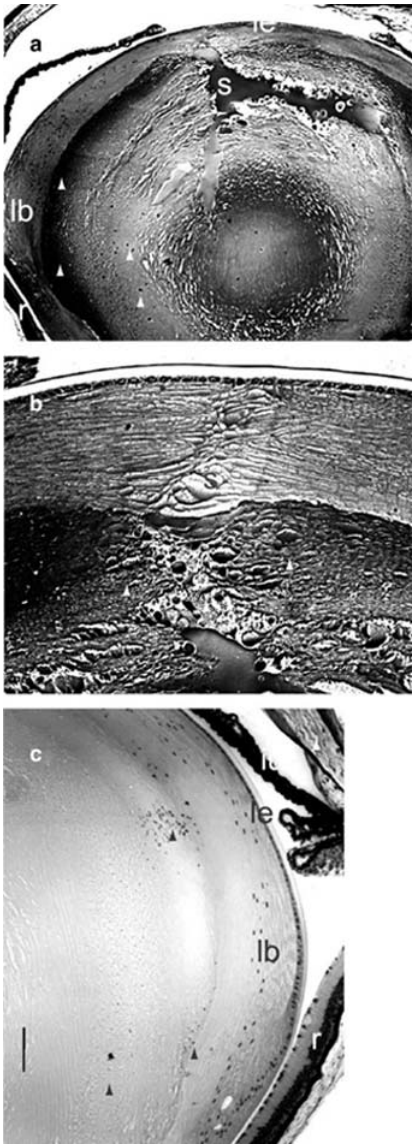

Figure 3. Cataract in *Crybb2*^{Aey2} lenses. Histologic analysis of lenses of the *Crybb2*^{Aey2} mutant at the age of 11 weeks. The section through a lens of an 11-week-old mouse is shown. Irregular remnants of fibre cell nuclei. (a) Striking abnormality of the anterior suture, a layer of dust-like particles in the anterior cortex and intercellular clefts in the lens nucleus appeared in *Crybb2*^{Aey2} mutants. They were caused by a disturbed denucleation process in those fibres, which were completely degraded in the normal inner lens fibre, suggesting impaired chromatin degradation. (b) Higher magnification of the central anterior region of the lens demonstrates the irregular fibre cells in the outer cortical fibre cells and shows a distinct zone of discontinuity between the outer and inner fibre cells. The cellular structure of the inner fibre cells was markedly destroyed. The epithelial cells remained unaffected. (c) The remnants of the fibre cell nuclei were still present in the lens bow of the *Crybb2*^{Aey2} lens. Partially degraded nuclei are obvious behind the zone of discontinuity from the outer cortex to the cataractous inner cortex. c, cornea; i, iris; lb, lens bow; le, lens epithelium; r, retina; s, anterior suture. Staining, methylene blue and basic fuchsin. Scale bars, 100 mm (with permission from *Invest. Ophthalmol. Vis. Sci.* (Graw *et al*. 2001c)).

Genes coding for other structural proteins

Genes encoding cytoskeletal proteins: There are three major lens cytoskeletal proteins, filensin (CP94 or beaded filament structural protein 1; gene symbol *Bfsp1*), phakinin (also referred to as CP49 or beaded filament structural protein 2; gene symbol *Bfsp2*) and vimentin. Since vimentin knockout mutants do not show an ocular phenotype, the two *Bfsp* genes are important for lens transparency. CP49 and filensin, together with α-crystallins, have been localized at unique cytoskeletal structures within the lens fibre cells known as beaded filaments. They are considered to be important in facilitating the chaperone activity of α-crystallin assemblies. Mutations in these two genes lead to cataract formation.

Disruption of the *Bfsp1* gene reduced levels of filensin's assembly partner CP49 and prevented the assembly of beaded filaments. These knockouts began to show evidence of light scattering by two months and worsened with age. Heterozygous animals exhibited an intermediate phenotype, showing a moderate light scattering at five months (Alizadeh *et al*. 2003).

However, the knockout of the *Bfsp2* gene does not lead to cataracts, even in the absence of CP49 causes a subtle loss of optical clarity in the lens (Alizadeh *et al*. 2002; Sandilands *et al*. 2003). Moreover, a deletion of the splice-acceptor site in exon 2 of the mouse *Bfsp2* results in a splicing of exons 1 to 3 and causes a frameshift in the reading frame as well as the introduction of a stop codon at position 2 of exon 3 in the *Bfsp2* transcript. The phenotype of this mutation is also subtle as described for the knockout of the entire gene. Since this mutation is present in several mouse strains (129, 101 and CBA), it might interfere with other mutations or targeted deletions and, therefore, it might have important implications for lens studies using these strains (Sandilands *et al*. 2004). In humans, a mutation in the filensin-encoding gene BFSP1 was shown to cause a recessive cataract (Ramachandran *et al*. 2007). In contrast, mutations in the CP49-encoding gene *BFSP2* were shown to be responsible for dominant cataracts (Conley *et al*. 2000; Jakobs *et al*. 2000); their phenotypes, however, seem to be variable ranging from congenital nuclear, sutural or stellate cataracts to juvenile-onset cataracts.

Genes encoding collagens: Collagen is the most abundant protein in animals forming large triple helices based on three subunits. In the eye, collagen fibrils are responsible for transparency of cornea, lens and vitreous body; collagens are present in the sclera and important to keep the eye in its shape. In lens, mainly *Col4a1* and *Col4a5* are expressed and only two cataract-causing mutations in *Col4a1* are reported in mice. They were identified within an ENU-mutagenis screen for dominant mutations; vacuolar cataracts were visible from six to eight weeks of age, when the mice were examined. Besides cataracts, heterozygous *Bru* (*bruised*) and *Svc* (*small with vacuolar cataracts*) mice display as heterozygotes several other ocular defects including corneal opaci-

ties, iris defects and significant iris-corneal adhesions indicating an Axenfeld–Rieger-like anomaly. Homozygous mutants die during embryogenesis. Both mutations affect the central collagen domain consisting of multiple Gly-Xaa-Yaa repeats (G627W; G1064D). They lead to a replacement of the critical glycin residue either by tryptophane or by aspartic acid, respectively; however, these particular Gly residues are important for the formation of the triple helix. There is a third *Col4a1* allele (*Raw*, retinal anteriolar wiring), however this mutation causes an exchange of Lys by Glu (K950E) in the third position of this Gly-Xaa-Yaa triplet; as the gene symbol indicates, the phenotype is less severe and was originally characterized by a shiny reflex from the retinal arterioles. The more severe mutants have in addition to the ocular symptoms some more clinical features including basement-membrane defects affecting mainly the kidney (glomerulopathy), but also other organs (Thaung *et al*. 2002; van Agtmael *et al*. 2005).

Sparc

Sparc encodes a secreted protein, acidic and rich in cysteine (mouse chromosome 11; 29.9 cM; at position 55.2 Mb), which is expressed by many different types of cells. SPARC is a 32-kDa glycoprotein consisting of two domains, an extracellular calcium binding module and a follistatin-like domain. It interacts with some extracellular matrix molecules including collagen IV. SPARC is localized in the nuclear matrix of certain proliferating cells, but in postmitotic neurons it was found in the cytosol. High levels of SPARC have been observed in the adult eye, and homozygous *Sparc*-null mice develop posterior and anterior cortical cataracts at one to two months of age; mature cataracts can be observed at approximately four months of age. The first sign of lens pathology occurs in the equatorial bow region, where vacuoles gradually form within differentiating epithelial cells and cells. Additional complications like dislocation into the anterior chamber, iris pigmentation on the lens or posterior synechiae and rupture of the lens capsule occur at fifth to eighth months (Norose *et al*. 1998). An expression profiling study revealed that five mouse globin genes show persistent downregulation in the cataractous lenses of *Sparc*-null mice (Mansergh *et al*. 2004). Additionally, *Sparc*-mutants have a reduced skin collagen content and show an accelerated wound closure, but they also suffer from osteopenia associated with bone remodelling, and increased growth of implanted tumours (http://www.informatics.jax.org).

Mutations in genes coding for connexins and connexin-related proteins

Connexins are well known as a family of proteins, which have been shown to be structural and functional building blocks of gap junctional intercellular channels. They span two plasma membranes and provide a direct pathway for the movement of signalling molecules and ionic currents between adjacent cells (Stout *et al*. 2004). In the lens, three connexins play major roles during development and adulthood, i.e. connexin43 (Cx43), connexin46 (Cx46) and connexin50 (Cx50). The respective gene symbols are *Gja1, Gja3* and *Gja8* (gap junction subunit α1, α3 or α8, respectively); they are mapped to mouse chromosomes 3 (*Gja3* and *Gja8*) and 10 (*Gja1*). The designation as connexin43, connexin46 or connexin50 is based upon their apparent molecular weight.

Connexin43 (encoded by *Gja1*) can be found in the mouse lens placode (E10) and later on in the optic vesicle, predominantly in the posterior portion that will become the pigmented layer of the retina. When the lens vesicle has been formed, Cx43 can be found primarily in the cells that will give rise to the anterior epithelium, where it remains present in later stages. Cx43 disappears gradually at the margin of the epithelial layer, and only low concentrations can be identified between the fibre cells (Yancey *et al*. 1992).

In *Gja1* knockout mice, the epithelial cells were connected more loosely as compared to the wild type. However, organization of appositional membranes among lens fibre cells and between fibre and epithelial cells differ dramatically in the $Gja1^{-/-}$ lens. In contrast to the close apposition of cells in lenses of wild-type mice, fibre cells in $Gja1^{-/-}$ lenses were largely separated from apical surfaces of epithelial cells, and large vacuolar spaces were apparent between fibre cells, most prominently in deeper cortical regions. These changes suggest that the osmotic balance within these cells is markedly altered (Gao and Spray 1998). To date, no further mutations in mouse or man have been reported in the *Gja1* gene up to now, most likely because it is not compatible with life due to a failure in pulmonary gas exchange caused by a swelling and blockage of the right ventricular outflow tract from the heart (Reaume *et al*. 1995).

Expression of *Gja3* coding for connexin46 begins in the lens vesicle, when it is detaching from the ectoderm. It is present primarily at the apical-most lateral surfaces of the columnar cells, with a higher concentration posteriorly. It is highly accumulated in intercellular maculae in the lens vesicle and at the posterior part of the lens vesicle. When primary fibres elongate, Cx46 is present at both ends, anterior and posterior. In adults, connexin46 has been shown to be part of the fibre-to-fibre junctions and in interepithelial cell gap junctions (Jiang *et al*. 1995).

For the *Gja3* gene knock-out mutants have been described. Although the absence of connexin46 had no obvious influence on the early stages of lens formation and the differentiation of lens fibres, mice homozygous for the disrupted *Gja3* gene developed nuclear cataract, which was associated with the proteolysis of crystallins (Gong *et al*. 1997). Interestingly, the genetic background of the loss of *Gja3* gene activity influences the severity of cataract formation tremendously. While *Gja3* null mutations on two 129 mouse

strains suffered from severe cataracts with γ-crystallin cleavage, *Gja3*$^{-/-}$ mice on the C57Bl6 background had far milder cataracts with no detectable γ-crystallin cleavage (Gong *et al*. 1999). Moreover, cataract formation can be prevented in *Gja3*$^{-/-}$ mice by the general cysteine protease inhibitor E-64. Its primary targets are the calcium-dependent proteases m-calpain and Lp82 (Baruch *et al*. 2001). In human, also several cataract-causing mutations in *GJA3* have been reported (OMIM 121015).

The expression pattern of the Cx50 encoding gene *Gja8* is similar to *Gja3*; it is present mainly in the lens fibre cells. However, Dahm *et al*. (1999) showed that Cx50 (which is identical to the lens membrane protein MP70) can be found also in the epithelial cells forming complexes with Cx43. The importance of Cx50 for lens development and function is demonstrated by four types of dominant congenital cataracts—two in man (Shiels *et al*. 1998; Berry *et al*. 1999) and two in mice (Steele *et al*. 1998; Graw *et al*. 2001b). Additionally, a knock-out mutation of the *Gja8* gene was created. Cx50-null mice exhibited microphthalmia and nuclear cataracts, but also zonular pulverulent cataracts have been observed within the first week of life. Remarkably, the intercellular passage of tracers revealed the persistence of communication between all lens cell types in the *Gja8*$^{-/-}$ mice (White *et al*. 1998).

However, most interesting results came from replacement of *Gja8* by *Gja3* or double-knockouts of *Gja8* with *Gja1*. Even though *Gja1*$^{-/-}$ mice die perinatally, it was possible to breed *Gja1*$^{-/-}$/*Gja8*$^{-/-}$ double knockouts starting from *Gja1*$^{-/+}$ heterozygotes. Lenses from these double knockouts were also histologically normal through E18.5 and synthesized the four lens differentiation markers MIP26, αA-, αB-, and γ-crystallin. Moreover, these lenses retained gap-junction mediated dye transfer between fibre cells, but not between epithelial cells and fibre cells; it was reduced between epithelial cells (White *et al*. 2001). In another type of experiment the *Gja8* gene was replaced by *Gja3*. In these mice, the cellular differentiation defects were corrected and cataract formation was prevented, but normal growth of the lens was not restored as compared to the knockout of *Gja8*. The data indicate that intrinsic properties of Cx50 are required for cellular growth, but non-specific restoration of cell–cell communication by Cx46 maintains differentiation (White 2002).

In the mouse, four point mutations have been reported up to now affecting the *Gja8* gene, *No2* (Steele *et al*. 1998), *Aey5* (Graw *et al*. 2001b), *Lop10* (Chang *et al*. 2002) and *L1* (DeRosa *et al*. 2007). The *No2* mutant was phenotypically characterized as bilateral, congenital nuclear opacity with full penetrance and fertility (Favor 1984). The *No2* cataract is present both in heterozygotes and in homozygous mutants; however, the severity is greater in the latter. The *No2* cataract is caused by an A→C transversion within codon 47. The corresponding exchange of the wild-type Asp to Ala in the mutants affects the first extracellular loop of the Cx50 protein (Steele *et al*. 1998).

The *Aey5* mutant displays nuclear and posterior suture opacity at eye opening and progresses until about two months of age remaining stable as a nuclear and zonular opacity. Histological analysis revealed that fibre cell differentiation continues at the lens bow region, but the cell nuclei do not degrade normally and remain in the deeper cortex. Further, the lens nucleus has clefts of various sizes while the remainder of the eye was morphologically normal. The mutation was mapped to mouse chromosome 3, and sequence analysis of both candidate genes identified only a mutation in the *Gja8* gene (192T→C). This mutation results in a Val→Ala substitution at amino acid 64 of the Cx50 protein. Computer assisted prediction of the biochemical properties suggested that in the *Aey5* mutants also the first extracellular loop will be affected (Graw *et al*. 2001b).

The other two *Gja8* mutants (L1: Ser50Pro; *Lop10*: Gly22Arg) affect also the N-terminal part of the protein. Taking all the available data together, three out of the four dominant mutants reported so far lead to amino-acid exchanges in the first extracellular domain. In contrast to the knockout mutants, the point-mutations affecting the protein structure might interfere with the interactions of the changed Cx50 protein with other proteins in the channel complex suggesting a dominant-negative role of the mutation. However, the differences between the resulting effects from the point mutations and the gene knockout might be due to the loss of the gene function in the knockout mice, which might be assisted in part by other channel proteins. Further and more detailed studies are necessary to answer this question for the distinct modes of inheritance. Finally, it should be noticed that there are also several dominant cataracts in man caused by mutations in the *GJA8* gene (OMIM 600897).

Gjf1 (also referred to as *Gje1*) encodes a connexin-like protein of 23.8 kDa in the mouse. Mutants were identified primarily based upon their small eyes. Detailed phenotypic analysis showed also a variable feature of lens and cornea opacities. Histological analysis during embryonic development indicated the presence of a lens vesicle without proper elongation of the primary lens fibre cells (figure 4). The mutation was mapped to the proximal region of chromosome 10 and finally, the mutation was identified in an EST clone (D230044M03Rik) encoding a connexin-like protein. A G→T point mutation was identified at cDNA position 96 resulting in an R32Q amino acid exchange in a transmembrane domain. The gene is expressed in the posterior part of the lens vesicle, where the primary fibre elongation starts. In the mutants, the expression pattern of *Pax6, Prox1, Six3* and *Crygd* are modified, but not the pattern of *Pax2*. The new gene *Gjf1* is thought to be essential for the formation of the primary lens fibres (Puk *et al*. 2008) and might be considered a downstream target of the transcription factor c-Maf (given here); mutations in the corresponding *Maf* gene lead to a similar phenotype in the mouse (Lyon *et al*. 2003; Perveen *et al*. 2007). At present, it is not clear whether there is a functional human counterpart of the mouse *Gjf1*-gene.

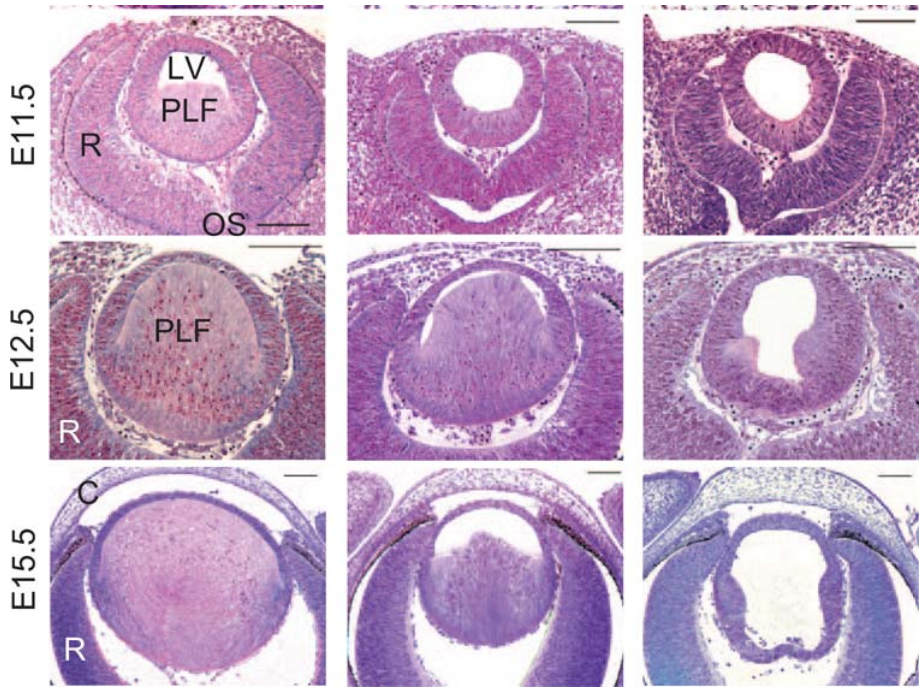

Figure 4. Histology of the *Gjf1*^{Aey12} mutant eyes. Histologic data are given from E11.5 to E15.5. At E11.5, the lens vesicle was formed, which was usually filled by primary lens fibres at E12.5 in the wild type (+/+), but not in the homozygous *Gjf1*^{Aey12} mutants (-/-): they revealed retarded lens fibre elongation and the lens vesicle remained empty. The heterozygous mutants (+/-) exhibited an intermediate phenotype with shortened primary lens fibres. C, cornea; L, lens; LP, lens placode; LV, lens vesicle; OS, optic stalk; PLF, primary lens fibres; R, retina. Scale bars 100 μm (with permission from *Invest. Ophthalmol. Vis. Sci*. (Puk *et al*. 2008)).

Another protein, which is associated with the lens cytoskeleton and epithelial cell junctions, is the Nhs1 protein, which, when mutated, causes the Nance–Horan Syndrome (NHS) in children. Recently, a large insertion between exons 1 and 2 of the mouse *Nhs*1 gene was shown to underlie the X-linked dominant cataract *Xcat*, which was recovered after parental radiation. Histological analysis during embryonic development revealed that in the affected embryos the primary fibre cells are irregularly arranged and show small foci of cellular disintegration; the fibres progressively degenerate. At the molecular level, the insertion inhibits the expression of the *Nhs*1 isoform containing exon 1 and results in exclusive expression of the alternative isoform containing exon 1A. The presence of *Nhs*1 exon 1 is critical for localization of the protein to the cytoplasm. Proteins lacking *Nhs*1 exon 1 are predominantly nuclear. These results indicate that the first exon of Nhs1 contains crucial information required for the proper expression and localization of Nhs1 protein (Huang *et al*. 2006).

Genes coding for other membrane and membrane-associated proteins

Aquaporin/MIP: One of the first detected cataract mutations is the cataract Fraser (*Cat*^{Fr}; Fraser and Schabtach 1962). In this mutant, the cell nuclei in the deep cortex become abnormally pycnotic (beginning at E14) degeneration of cytoplasm and destruction of the lenticular nucleus then follows (Zwaan and Williams 1969). *Cat*^{Fr} was shown to be allelic with another mouse mutant, referred to as lens opacity (*Lop*). The two alleles, *Cat*^{Lop} and *Cat*^{Fr}, were mapped to chromosome 10 (Lyon *et al*. 1981; Muggleton-Harris *et al*. 1987); sequence analysis revealed that the *Cat*^{Fr} mutation is due to a transposon-induced splicing error leading to a truncated form of *Mip* transcripts (*Mip* encodes the major intrinsic protein of lens fibre). Later, the mutation in the *Cat*^{Lop} allele was shown to cause a single amino-acid substitution, which inhibits targeting of Mip to the cell membrane (Shiels and Griffin 1993; Shiels and Bassnett 1996). A third mutant allele (*Cat*^{Tohm}) was reported, which is characterized by a 12-bp deletion and a corresponding loss of four amino acids within the second transmembrane region (Okamura *et al*. 2003). Mip forms specialized junctions between the fibre cells and can first be detected in the primary fibre cells of the early lens vesicle. *In situ* hybridization demonstrated that *Mip* expression is highest in the elongating fiber cells in the bow region of the lens; Mip antiserum specifically decorates fibre cell membranes, highlighting their regular anterior to posterior organization (Shiels and Griffin 1993; Zhou *et al*. 2002). Mip is also referred to as aquaporin 0; a recent review was published by Chepelinsky (2009).

LIM: The mouse mutant total opacity (*To3*) is located on chromosome 7 (Kerscher *et al*. 1996). Mice heterozygous or homozygous for the *To3* mutation exhibit a total opacity of the lens with a dense cataract. Additionally, homozygotes have microphthalmia. Histological analysis revealed vacuolization of the lens and gross disorganization of the fibres; posterior lens rupture is observed only in homozygotes. The *To3* mutation was characterized as a single G→T transversion within the first exon of the *Lim2* gene coding for a lens-specific integral membrane protein, MP19. It was predicted that this DNA change results in a nonconservative substitution of a valine for the normally encoded glycine at amino acid #15 of the MP19 protein (Steele *et al*. 1997). *Lim2* mRNA can be found in the head region of mouse embryos from embryonic day 12 on (Zhou *et al*. 2002). In humans, two mutations in the *LIM2* gene are associated with recessive cataracts (Pras *et al*. 2002; Ponnam *et al*. 2008).

Ephrin-A5: Ephrin-A5 (gene symbol: *Efna5*) is well known as a ligand of the ephrin receptor tyrosine kinase and to be involved in axonal guidance and cell differentiation. It is also vital for regular lens development and differentiation, since knockout mutants of *Efna5* develop cataract in ~87% of the mutant carriers. The lens fibre cells appear rounded and irregular in cross-section (Cooper *et al*. 2008). Furthermore, ephrin-A5 was shown to interact with the Ephrin-A2 receptor (gene symbol *Epha2*) to regulate the adherens junction complex by recruitment of β-catenin to N-cadherin. It should be noted that mutations in the human *EPHA2* gene lead to childhood cataracts (Shiels *et al*. 2008; Zhang *et al*. 2009), but also to age-related cataracts (Jun *et al*. 2009). Moreover, similar like the transcription factor Pitx3 (which is crucial for very early lens development), ephrin-A5 is also involved in the formation of dopaminergic neurons in the *substantia nigra* (Cooper *et al*. 2009). It might be speculated that *Pitx3* and *Efna5* are acting in the same signalling cascade both in the eye and brain.

NrCam and ankyrin-B: NrCAM is a transmembrane glycoprotein and related to NgCAM (neuron-glia cell adhesion molecule); it belongs to the immunoglobulin superfamily. The *Nrcam* gene knockout leads to cataracts in mice. Histologically, the first observation identified disorganized lens fibre cells during the late phase of embryonic development (E18). At the age of one month, isolated lenses are almost clear but become progressively opaque; the cataracts are visible by naked eye at four months of age. Since NrCam interacts with ankyrin-B, it is not surprising that the corresponding *Ank2*-knockout mice show a similar disorganization of lens fibre cells at birth as the *Nrcam* mutant mice. However, since the loss of *Ank2* is not compatible with life after birth, the development of cataract cannot be observed in the *Ank2*-knockouts (Moré *et al*. 2001).

Transcription factors

Pax6: One of the central genes in eye development is the paired-box gene *Pax6*, which was recognized as being affected in the mouse and rat *Small eye* (*Sey*) mutants (Hill *et al*. 1991; Matsuo *et al*. 1993). *Pax6* maps to mouse chromosome 2; the actual list of the Jackson Laboratory (http://www.informatics.jax.org; September 2009) contains 39 alleles in the mouse, 10 of them are targeted mutations. Usually, the expressivity of heterozygous *Pax6*-mutations is variable with mutant carriers exhibiting a range of phenotypes from small anterior polar cataracts to the more extreme phenotype of anterior polar opacity, corneal adhesions, iris abnormalities and microphthalmia. Furthermore, the degree of phenotype expressed between the two eyes of an affected individual is variable (Favor *et al*. 2008, , and references therein)). In humans, *PAX6* mutations lead frequently to aniridia and sometimes to cataracts. However, there is a growing body of evidence that *PAX6* mutations cause in addition to the ocular diseases behavioural and neurodevelopmental phenotypes (Davis *et al*. 2008; Tsonis and Fuentes 2006).

Pitx3: Another interesting gene in the context of early lens development is *Pitx3*. In the mouse mutant aphakia (*ak*; Varnum and Stevens 1968), the promotor of the *Pitx3* gene is affected by two deletions (Semina *et al*. 2000; Rieger *et al*. 2001). The phenotype is characterized at early stages of development by a small lens vesicle with a stable contact to the cornea (lens stalk). In later stages, the lens vesicle is degraded leading to the formation of a lens-less eye giving this mutant its name. Another mutant line, *Cat4*^{a}, shares one aspect with the aphakia mutant, the inhibition of the separation of the lens vesicle from the surface ectoderm (Grimes *et al*. 1998). However, *Cat4*^{a} is mapped on mouse chromosome 8 (Favor *et al*. 1997) and is therefore different from *Pitx3*.

In contrast to the mouse situation, mutations in the human *PITX3* gene are causative for congenital cataracts; in some cases the phenotype includes also anterior segment mesenchymal dysgenesis (Semina *et al*. 1998). Moreover, there are recent reports that *Pitx3* is also expressed in the dopaminergic neurons of the substantia nigra in the brain (Nunes *et al*. 2003); therefore, it is not surprising that aphakia mice suffer also from a selective loss of these particular neurons (Hwang *et al*. 2003) and a malformation of the mesencephalic dopamine system (Smidt *et al*. 2004). Recently, the aphakia mouse has been discussed as a model of Parkinsonism (Ardayfio *et al*. 2008).

Fox, Maf and Sox: There are some other genes coding for transcription factors that are important in eye and lens development, *Maf, Sox1, Sox2, FoxC1* and *FoxE3*. Particularly, Maf and Sox1 act as transcription factors on the promoters of the γ-crystallin encoding genes (*Cryg*).

The Fox-transcription factors are characterized by a 110-amino-acid motif originally defined as a DNA-binding do-

main in the *Drosophila* transcription factor forkhead (Fox: forkhead box). Blixt *et al*. (2000) described a mutation in *FoxE3* as causative for the phenotype in an ancient mouse mutant, *dysgenic lens* (*dyl*). In this mutant the lens vesicle fails to separate from the ectoderm causing the lens and the cornea to fuse. In human, mutations in *FOXE3* lead to anterior segment ocular dysgenesis (Semina *et al*. 2001). Similarly, mutations in the human *FOXC1* and corresponding mouse mutant lead to a similar phenotype with additional glaucoma (Nishimura *et al*. 2001; Hong *et al*. 1999).

The Maf family of basic region leucine zipper (bZIP) transcription factors was first identified through the *v-maf* oncogene, an avian retrovirus transforming gene (Nishizawa *et al*. 1989). In chicken and *Xenopus*, it was demonstrated by several authors that L-*MAF* is involved in the regulation of crystallin expression. L-*Maf* is first expressed at the lens placode and is maintained specifically in lens cells (Shimada *et al*. 2003). The mouse homologue to the *Xenopus L-Maf* is obviously *Nrl* coding for a neural retina-specific leucine zipper protein; whereas the mouse homologue to the chicken L-*Maf* is *Maf*-B (NCBI UniGene). The third mammalian member of the Maf family demonstrating important properties influencing lens development is c-*Maf*. The targeted deletion of c-Maf in the mouse leads to a stop of lens primary fibre cell elongation at the lens vesicle stage (Ring *et al*. 2000); the same mutation was published recently as causing a mild pulverulent cataract mutant in mouse (opaque flecks in the lens, *Opj*; Lyon *et al*. 2003). The point mutation affects the basic region of the DNA-binding domain.

In general, Maf binds as homodimer or heterodimer to two known Maf responsive elements (MAREs), with varying affinities and transactivation potentials. MAREs are found in the promoters of the crystallin-encoding genes and *Pitx3* (Ogino and Yasuda 1998; Semina *et al*. 2000). In human, two families were identified suffering from ocular developmental abnormalities (in one case, cataract is associated with anterior segment dysgenesis and microphthalmia; in the other case, cataract is associated with microcornea and iris coloboma) (Jamieson *et al*. 2002).

The Sox-family of transcription factors has a HMG domain (high mobility group) in common; the founder of this family is the *Sry* gene (sex-determining region of Y chromosome; Koopman 1999). The genes *Sox1, Sox2* and *Sox3* belong to subgroup B (Kamachi *et al*. 2000); they are expressed in the mouse in the central nervous system and in the sensory placodes. Particularly, *Sox2* is expressed during early eye development in the lens placode in the portion of the ectoderm that is in contact with the optic cup and invaginates to form the lens vesicle. This invagination coincides with the onset of *Sox1* expression in the mouse lens placode. At later stages, *Sox2* is downregulated and *Sox1* expression increases (Kamachi *et al*. 1998).

A targeted deletion of *Sox1* in mice caused microphthalmia and cataract. Mutant lens fibre cells fail to elongate, probably as a result of an almost complete absence of *Cryg* transcripts (Nishiguchi *et al*. 1998). The phenotype of the homozygous *Sox1* deletion mutant is very similar to a very severe *Cryg* mutation, *Cryge*^{t}.

Six5: To determine whether *Six5* deficiency contributes to cataract formation, the *Six5* gene was knocked out by replacing the first exon with a beta-galactosidase reporter. *Six5*-mutant mice showed reporter expression in multiple tissues, including the developing lens. Homozygous mutant mice had no apparent abnormalities of skeletal muscle function, but developed lenticular opacities at a higher rate than controls. These results suggest that *Six5* deficiency contributes to the cataract morphology. Moreover, it has been suggested that the resulting phenotype is a result of *Six5* acting closely with a partially overlapping gene, *Dmpk*, responsible for myotonic dystrophy (Sarkar *et al*. 2000).

HSF4: Heat-shock transcription factor 4 (Hsf4) is considered to be involved in lens development and differentiation. The *Hsf4* knockout mouse (*Hsf4*$^{-/-}$) develop cataract. Further analysis of the cataractous lenses of the *Hsf4*$^{-/-}$ mutant mice demonstrated the down-regulation of several genes considered to be relevant for cataract formation including the genes encoding γS-crystallin (*Crygs*) and beaded filament proteins 1 and 2 (*Bfsp1* and *Bfsp2*). Further detailed analysis suggested that these cataract-relevant genes are direct downstream targets of Hsf4 (Shi *et al*. 2009). Recently, Mou *et al*. (L. Mou, J. Y. Xu, W. Li, X. Lei, Y. Wu, G. Xu, X. Kong and G. T. Xu, unpublished data) reported the identification of the gene coding for the cytoskeletal protein vimentin as a novel target gene of the Hsf4 protein. In humans, mutations in *HSF4* are associated with various recessive and dominant forms of congenital and age-related cataracts (Bu *et al*. 2002; Smaoui *et al*. 2004; Forshew *et al*. 2005; Ke *et al*. 2006; Sajjad *et al*. 2008; Shi *et al*. 2008; Hansen *et al*. 2009).

Cataract mutants with unknown chromosomal position

There is a (decreasing) number of mouse mutants listed in the Mouse Genomics database (http://www.informatics.jax.org) without a known chromosomal region. Besides several transgenic mice with an unknown region of the corresponding insertion, these loci mainly represent ancient mutants, which might be extinct or deposited in only one of the distribution centres, like at the Jackson Laboratory in the US or in the European Mouse Mutant Archive (EMMA) in Neuherberg/Germany or Rome/Italy. Some of them are listed here; further details of these mutants can be extracted from the Mouse Genomics database (search term was 'cataract' in the 'Genes and Markers' section). *Acc* (anterior capsular cataract), *act* (adult cataract), *Alm* (anterior lenticonus with microphthalmia), *Apo* (anterior polar opacity), *Apoca* (anterior polar cataract), *Asc1* (anterior suture cataract 1), *Asc2* (anterior suture cataract 2), *cac* (recessive cataract), *Cat6* (dominant cataract 6), *Cts* (cataract and small eye), *dcm* (dense cataract and microphthalmia), *Enc* (embryonic nu-

cleus cataract), *eob* (eyelids open at birth), *Iac* (iris anomaly with cataract), *Idc* (iris dysplasia with cataract), *Lcl* (lens cloudy), *lop2* (lens opacity 2), *lop13* (lens opacity 13), *lr* (lens rupture), *nmf131* (neuroscience mutagenesis facility, 131), *Nuca* (dominant nuclear cataract), *pcat* (perinatal cataract 6), *Pcs1* (polar cataract and small eyes 1).

If linkage analysis of these mutants were possible, it might be expected that some would be novel and others would map to regions already known to be responsible for cataract formation.

Mapped cataract mutants without identification of the causative mutation

Based upon database searches it is also obvious that there are additional mouse mutants suffering from diverse forms of cataract, which have been attributed to a particular region of a chromosome, but not yet been characterized in detail with respect to the individual gene responsible. Such mutants include the following phenotypes (for further details and references refer to the web side of the Jackson Laboratory at http://www.informatics.jax.org/).

(i) The mutation vacuolated lens (*vl*) is mapped to mouse chromosome 1 and leads to opaque white lenses. Additionally, the mutants are characterized by a white belly spot and spina bifida. Small lens vacuoles are present at birth. (ii) The mutant blind-steril (*bs*) is characterized by bilateral nuclear cataracts, microphthalmia and glossy coats. The cataracts are detectable at E16. Females are fertile, but males are sterile. The mutation was mapped to mouse chromosome 2. (iii) The *Tcm* mutation (total cataract with microphthalmia), a cataract with iris dysplasia and coloboma and the *Ccw* mutation, cataract and curly whiskers, are mapped to mouse chromosome 4. (iv) The *Tim* (translocation-induced circling mutation) is associated with a reciprocal chromosomal translocation between chromosomes 4 and 17. Affected mice develop an anterior subcapsular cataract that appears after birth and is progressive, accompanied by abnormal head tossing and circling behavior. The translocation breakpoint must have disrupted a gene or its regulation but the details have yet to be determined (Smith *et al*. 1999). (v) The nuclear-posterior polar opacity (*Npp*) maps to chromosome 5. (vi) *Cat5* (previously *To2*), a total opacity, is located proximal to the centromer on chromosome 10; this position is close to the *Gjf1* gene described above. (vii) Two alleles of *Cat3* ($Cat3^{vl}$, vacuolated lens; $Cat3^{vao}$, cataract with anterior opacity) arose independently in the F_1 generation after paternal γ-irradiation and map to the central region of chromosome 10. (viii) The rupture of lens cataract (*rlc*) was mapped to chromosome 14; a similar form, *lr2* (lens rupture 2) was mapped nearby. The opacity in the *rlc/rlc* mice becomes apparent at 35–60 days of age; there are no developmental changes reported. (ix) Finally, a form of cataract, which is formed postnatally without any observed developmental alterations, is the Nakano cataract (*nct*). The mutation was mapped to chromosome 16.

In all of the above cases there are no obvious candidate genes making identification of the genes responsible a laborious task of exclusion. We shall have to wait for the 'next generation sequencing technology' to make gene sequencing faster and significantly cheaper to aid this process.

Mouse models for senile cataracts

SAM

Another genetic mouse model for senile cataracts is the senescence-accelerated mouse (SAM), which was identified at Kyoto University in 1970 on a AKR/J background strain. There are eight senescence-prone (SAM-P) strains, which are characterized by an earlier onset and more rapid advancement of senescence resulting from a significantly shorter lifespan. Cataracts have been found in the SAM-P/1 and SAM-P/9 strains. The earliest change was the appearance of a ripple-mark body at about three months of age. The number of rippled rings increased with age. These changes later induced refractive distortion of retinal vessels. Whole-mount flat preparations of the epithelium showed that the number of cells was markedly decreased at the advanced stages of cataract. At the late stages of life the lens cortex became liquefied and developed into a mature cataract (Nishimoto *et al*. 1993). The mode of inheritance and the linkage to a particular chromosome still remains to be investigated.

Emory

The Emory mouse is a well-characterized genetic model for age-onset cataract. It has been first described in 1981/1982 by Kuck *et al*. (1981) as a spontaneous dominant cataract, which appears between five and eight months. The Emory cataracts increase in severity with age and first develop in the anterior superficial cortex region of the lens. They eventually progress into the anterior deep cortex region and ultimately result in complete lens opacification. Emory mouse cataracts are also associated with changes in numerous biochemical parameters and gene expression levels in the lens (Kuck 1990; Sheets *et al*. 2002, references therein), however, the primary genetic defect remains to be elaborated.

Mouse models for metabolic cataracts

Sugar-induced cataracts in the mouse: Inborn errors in the galactose pathway and diabetes are known risk factors for cataractogenesis in humans. Sugar is converted to the corresponding sugar alcohol, which accumulates in the lens and creates osmotic problems leading eventually to a cataract. Mutations affecting enzymes of the sugar metabolism are not only involved in childhood cataracts, but first data indicated that particular alleles might also be risk factors for age-related cataracts. One of the most prominent alleles is the A198V polymorphism in *GALK* (encoding galactokinase), which has a prevalence of 3%–4% among Asians. It has been shown to be significantly over-represented in Japanese individuals with bilateral cataracts (Okano *et al*. 2001).

For a long time, appropriate mouse models were missing. One hypothesis was that the enzyme aldose reductase (responsible for the conversion of sugar to its alcohol) has a very low activity in the mouse compared to humans. Therefore, it was not surprising that the galactokinase (*Glk1*) knockout in the mouse did not suffer from cataracts at all. However, the introduction of a human aldose reductase transgene into a *Glk1*-deficient background resulted in cataract formation within the first postnatal day (Ai *et al*. 2000). This result highlights the importance of aldose reductase in sugar-dependent cataract formation.

Similarly, sorbitol dehydrogenase (SDH; gene symbol: *Sord*) is involved in diabetic-induced cataracts. SDH deficient mice develop cataracts, which might be due to significantly increased lenticular sorbitol level in *Sord*-deficient mice. Moreover, blocking sorbitol accumulation by the AR null mutation prevents cataract development demonstrating the contribution of osmotic stress in cataract development. Furthermore, treatment with vitamin E significantly reduced the incidence of cataract, and *Gpx1* deficiency exacerbated cataract development in these mice. This osmoregulatory dysfunction model is supported by the fact that the activity of Na^+/K^+-ATPase, the key regulator of cellular ions and water balance, was dramatically reduced in the precataractous lenses of the *Sord*-deficient mice. This osmoregulatory dysfunction model might explain why diabetic patients who control their blood glucose moderately well are still susceptible to develop cataract (Chan *et al*. 2008).

Another candidate for the diabetes-specific cataract formation came from the investigation of the bi-functional protein DCoH (Dimerizing Cofactor for HNF1). It acts as an enzyme in intermediary metabolism (gene symbol *Pcbd1*: pterin 4α-carbinolamine dehydratase) and as a binding partner of the HNF1 family of transcriptional activators. Knockout mutants of *Pcbd1* are viable and fertile, but display hyperphenylalaninemia and a predisposition to cataracts. Lens opacities were visually detectable in ~20% of the *Pcbd1* null mice, if maintained on the outbred CD1 genetic background. The age of onset varied widely with the earliest detection at 12 days; most of the affected animals presented with cataracts by the age of 24 weeks. The incidence of cataract formation was reduced in the C57BL/6J inbred genetic background (Bayle *et al*. 2002).

Protein-bound carbohydrates and cataract: Besides the free sugars of the intermediate metabolic pathways, sugar residues are present in a variety of glycoproteins. One of the corresponding enzymes is the α(1,3)-galactosyltransferase, which catalyses the addition of galactose in an α(1,3) configuration to particular glycoproteins. It is also referred to as glycoprotein galactosyltransferase-α1,3 (gene symbol *Ggta1*); the corresponding gene is mapped on chromosome 2. The transfer of galactose to particular glycoproteins creates a highly immunogenic epitope, which is present in all mammals except humans, apes and Old World monkeys. *Ggta1*-knockout mice have impaired glucose tolerance, and decreased insulin sensitivity, and additionally, develop cataracts. A white pinhead opacity was observed at an average age of 36 to 40 days. Rapid progression to full opacities occurred, on average, within seven to eight days. Early nuclear and posterior cortical changes as well as fibre folds and swollen sutures have been observed (Dahl *et al*. 2006, and references therein).

Another mouse model for congenital cataracts was characterized by targeting the gene coding for perlecan (*Hspg2*, mapped to chromosome 4). Perlecan is a large multidomain, heparan-sulfate proteoglycan found in all basement membranes; besides type IV collagen and laminin, it is a core protein of the lens capsule. Therefore, it is not surprising that the homozygous deletion of the exon 3 by gene targeting leads to leakage of cellular material through the lens capsule and degeneration of the lens within three weeks of birth. In detail, loss of exon 3 removes the attachment sites for three heparan-sulfate side chains, which are replaced by linear polysaccharides. It is possible that this deletion changes the affinity of perlecan to the basic fibroblast growth factor. The cataractogenic potential of deletion within the *Hspg2* gene is dramatically enhanced in double-knockouts including both, *Hspg2* and *Col18a1*; the *Col18a1* knockouts do not have any eye phenotype (Rossi *et al*. 2003).

Cholesterol metabolism and cataract: One of the 'old' syndromic, dominant mouse cataract mutants is the X-linked bare patches (*Bpa*). Whereas hemizygous males die before birth, heterozygous females have patches of bare skin. Lens cortical 'frost figure' opacities are present. Molecular analysis revealed that mutations in the gene *Nsdhl* (encoding a NAD(P)H steroid dehydrogenase-like protein) are responsible for the phenotype in two independent *Bpa* and three independent striated (*Str*) alleles (Liu *et al*. 1999). At that time, it was the first mammalian locus associated with an X-linked dominant, male-lethal phenotype. To date, 10 alleles have been reported (three spontaneous, two chemically induced and five radiation induced). It is also the first cataract phenotype, which has been shown to be related to the cholesterol pathway.

Genes involved in DNA repair and cell-cycle control

Tumour suppressor genes: The lens is the only organ without developing tumours. This might be due to the final differentiation of lens fibre cells losing their nuclei. However, absence of tumour suppressor genes have been shown to lead to cataract formation in a few cases. One example is the *Cdkn2a* locus (cyclin-dependent kinase inhibitor 2A) encoding the two tumour suppressor proteins p16 and p19 (in human, the corresponding locus is referred to as *INK4a*). The corresponding mouse knockout mutants in the mouse show cataracts with numerous vacuoles and have been associated

with retinal dysplasia and defects in the regression of the embryonic hyaloid vascular system. The first alterations in the lens occur at embryonic day 15.5 as altered migration of lens fibre cells resulting in disturbed differentiation of the lens (Cheong *et al*. 2006).

In rat, a recessive cataract mutant of spontaneous origin was identified (white eye, gene symbol *we*; Fritz *et al*. 2002), which was finally characterized by a mutation in the $p27^{kip1}$ gene. In addition to the cataracts, these mutant rats suffer later from a broad variety of endocrine tumours (Pellegata *et al*. 2006).

Atm*$^{-/-}$*-mice: increased sensitivity for radiation-induced cataracts: The lens is considered as being very sensitive to ionizing radiation (for a recent review see Ainsbury *et al*. 2009). Since ionizing radiation is believed to produce double strand DNA breaks which should be repaired within a given time (Wolf *et al*. 2008), mouse models with genetic defects in these repair system might be more sensitive to radiation than others. This is illustrated in a study by Worgul *et al*. (2002) using mice defective in the *Atm* gene. Mutations in the human *ATM* gene are responsible for ataxia telangiectasia. Therefore, they exposed one eye of wild type, *Atm* heterozygous and homozygous knockout mice to 0.5-, 1.0-, 2.0-, or 4.0-Gy X rays and showed that cataract development in all three groups was strongly dependent on dose. However, the lenses of homozygous mutant mice were the first to opacify at any given dose. Most important in the present context is that cataracts appeared earlier in the heterozygous versus wild-type animals. The data suggest that *ATM* heterozygotes in the human population may also be more sensitive to radiation-induced cataracts at low doses.

Nbn: The phenotypic characterization of a conditional knockout of the murine *Nbn* gene (encoding nibrin) has been reported. The *Nbn* gene is the mouse homologue for the human Nijmegen breakage syndrome gene. All *Nbn*-deficient lenses develop cataracts at an early age due to altered lens fibre cell differentiation, including disruption of normal lens epithelial and fibre cell architecture and incomplete denucleation of fibre cells. In addition, *Nbn*-deficient lenses show dysregulated transcription of various crystallin genes. These features implicate a function of *Nbn* in terminal differentiation of the lens fibre cells and cataractogenesis (Yang *et al*. 2006). The encoded protein nibrin has a role in identifying breaks in double stranded DNA in response to DNA damage. Since defects in DNA repair are frequently associated with premature ageing processes, the mutation in the *Nbn* gene might be further evidence for the participation of the corresponding repair and cell cycle control proteins in the formation of age-related cataracts.

Concluding remarks

Over the past decade we have learned a great deal from mouse models about the underlying molecular mechanisms involved in lens formation. Specific gene mutations in the mouse have led scientists to identify the genes involved in the formation of cataracts in man. The list of genetically characterized murine mutants (and locus-only determined mutants) is increasing with on-going mutagenesis screens and improved phenotyping strategies. But there is still an enormous amount to discover and in particular further work needs to be directed at revealing the underlying processes involved in age-related cataracts. Animal models provide a very powerful tool to further our understanding of the pathological processes involved.

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