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Review

Genetics of crystallins: Cataract and beyond

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ABSTRACT

The crystallins were discovered more than 100 years ago by Mörner (1893. Untersuchungen der Proteinsubstanzen in den lichtbrechenden Medien des Auges. Z. Physiol. Chem. 18, 61–106) as the main structural proteins of the vertebrate eye lens. Since that time the major mammalian crystallins referred to as α-, β-, and γ-crystallins were characterized with respect to their genetic organization, regulation of their expression pattern and participation in several diseases. In recent years, more and more crystallins have also been identified outside the lens. Evolutionary analysis has demonstrated the relationship of crystallins to proteins involved in protection against stress. The α-crystallins form large complexes up to 1 Mio Da; they are built up by two subunits referred to as αA- and αB-crystallins. These subunits are encoded by individual genes, *Cryaa* and *Cryab* being localized on different chromosomes and members of the small heat-shock protein family. The αA-crystallin is considered to be a molecular chaperone. It is expressed mainly in the lens – mutations in the *Cryaa* gene lead to recessive or dominant cataracts. In contrast, the αB-crystallin is rather ubiquitously expressed; mutations in the *Cryab* gene are associated with a broad variety of neurological, cardiac and muscular disorders. The β/γ-crystallin super family is encoded by at least 14 genes; the proteins are characterized by four Greek key motifs. In mammals, these genes are not only organized as individual genes (*Cryba1*, *Cryba2*, *Crygf*, *Crygs*, *CrygN*), but also in duplets (*Cryba4–Crybb1* and *Crybb2–Crybb3*) and in one major cluster (*Cryga–Cryge*). The various *Cryb* and *Cryg* genes are considered to have been evolved by various duplications of the Greek key encoding units. The two main families are distinguished by the fact that each Greek key motif in the *Cryb* genes is encoded by one exon, whereas two motifs are encoded by one single exon in the *Cryg* genes. An intermediate between these subfamilies is *CrygN* encoding the first two Greek key motifs by individual exons, but the others by one single exon. Mutations in the *Cryb*/*Cryg* genes lead mainly to an opacification of the eye lens. In some *Cryg* mutants evidence was presented that the formation of large amyloid-like intranuclear inclusions containing the altered γ-crystallins is a key event in cataract formation. Cataract formation, caused by *Cryg* mutations is further characterized by stopping the secondary lens fiber differentiation as indicated by the presence of remnants of cell nuclei, which are usually degraded in secondary fiber cells. Moreover, additional clinical features are being increasingly reported since these crystallins are found outside the eye: the βB2-crystallin (previously referred to the basic principle crystallin) is also involved in neurogenesis and male infertility. For some of the β/γ-crystallins, Ca^{2+}-binding properties have been discussed; however, it is an unsolved question whether these crystallins serve as Ca^{2+} stores *in vivo*. Enzyme crystallins are enzymes, which have been recruited to the lens and are expressed there in high concentrations. The μ- and ζ-crystallins (gene symbols: *Crym* and *Cryz*, respectively) are discussed as examples for mammals. Mutations in the human *CRYM* gene lead to non-syndromic deafness, and mutations in the *Cryz* gene of guinea pigs cause cataracts.

1. Introduction

The eye lens is a unique tissue because of its transparency and flexibility; moreover, it is derived from only one cell type, the ectoderm of the lens placode, and it contains more protein than common cells, ~30–35% of the entire mass of the lens – correspondingly, the water content, which is usually ~95% in a cell, is reduced in the lens to 65–70%. The analysis of these proteins and their characterization has been a challenge since the end of the 19th century. In 1893, Mörner published the fractionation of bovine lens proteins, and he referred to them as crystallins because of their abundance in the crystallin lens. The classification as α-, β- and γ-crystallins followed decreasing molecular weight of the native

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proteins, and in general this nomenclature is still valid. However, genetics have added quite a number of new facts to our knowledge of crystallins, their genomic organization, their evolution, their expression profiles, and their function because of the analysis of mutations.

As mentioned above, lens development starts by forming the lens placodes at both sides of the prospective forebrain, followed by the invagination of the lens pit. This initial process takes place in the mouse embryo at day 9.5 of embryonic development (E9.5). The lens pit develops to the lens vesicle at E11.5, and from its posterior side the primary lens fiber cells grow into its lumen at E13. In man, this process takes place between the fourth and sixth week of gestation. From that time on, a life-long process of formation of secondary fiber cells is initiated. At the germinative zone of the anterior lens epithelium the cells divide (in today's terminology it might be referred to as a stem cell niche) and move to the lens equator, where they elongate to the anterior and posterior pole of the lens surrounding the earlier fiber cells. Since this process takes place throughout life, sections of the lens look like annual rings of a tree with the outermost, superficial fiber cells being the youngest (Graw, 2004).

During this differentiation of the lens epithelial cells to fiber cells, all cell organelles are finally degraded, leading to cells without nuclei and mitochondria in the center of the lens. The ongoing process of nuclear breakdown in the permanently differentiating secondary fiber cells has some similarities with the initial steps in apoptosis in other tissues. It is a prerequisite for lens transparency and functional integrity; its disturbance leads to cataracts.

The crystallins are expressed in the lens from the beginning of its development: *Cryaa* expression is observed in the mouse lens cup at E10–E10.5, and later on, αA-crystallin becomes very abundant in lens fiber cells. αB-crystallin can first be detected in the mouse lens from E9.5 on, and later, it is found preferentially in the epithelial cells (Robinson and Overbeek, 1996). The expression of β-crystallins 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 (Graw, 1997). The *Cryg* genes are expressed in mouse lenses from E13.5 onwards in the primary fibers and later on in the secondary fiber cells, but not in the epithelial cells (van Leen et al., 1987; Santhiya et al., 1995). The expression of *Cryg* genes reaches the maximum in mice at birth, and is decreasing during the first weeks after birth (Goring et al., 1992).

2. α-Crystallins

The α-crystallin protein complexes are in their native form the largest among the crystallins with a molecular weight of ~1 Mio Da. They are mainly composed of two related proteins, αA- and αB-crystallins in a molar ratio of roughly 3:1 (Bloemendal, 1981). They are encoded by two genes, *Cryaa* and *Cryab*, which are located on different chromosomes. The basic characteristics of α-crystallins are summarized in Table 1. The main feature of the native α-crystallin complex in the lens is its chaperone function, which was discovered by Horwitz (1992) and characterized in many details thereafter (Sun and MacRae, 2005; Horwitz, 2003; Derham and Harding, 1999; Groenen et al., 1994). Moreover, further experimental evidence suggests that α-crystallin is involved in remodeling and protection of the cytoskeleton, inhibition of apoptosis and the resistance to stress (for a recent review see Andley, 2007).

2.1. Evolutionary aspects

The α-crystallins belong to the family of the small heat-shock proteins, with which they share a common central domain of about 90 amino acid residues. It is folded into a β-sandwich conformation and has variable N- and C-terminal extensions. Since this domain was first recognized in the α-crystallin, it is referred to as the "α-crystallin domain" (de Jong et al., 1998; Augusteyn, 2004). The small heat-shock proteins are important components of the cellular chaperoning machinery; they are involved in the remodeling of the cytoskeleton and in inhibiting apoptosis. Actually, the vertebrate gene family consists of 15 members formally referred to as *HspB1–15*; in this nomenclature, the *Cryaa* gene is referred to as *HspB4* and the *Cryab* as *HspB5*. Most information is available for 10 *HspB* genes: with exception of *Cryab* and *HspB2*, they are all located on different chromosomes. The chromosomal distribution and their amino acid sequence similarity (45–85%) suggest that the *HspB* gene family evolved before the earliest divergence of the existing vertebrates. The *Cryab* and *HspB2* genes are located in a head-to-head orientation on the same chromosome and separated just by 863 bp (in the mouse); they share some common regulatory elements (for details, see below). Their head-to-head orientation is maintained throughout vertebrate evolution (Franck et al., 2004).

Of particular interest might be the (partial) genome duplication in the zebrafish. In this species, also the *Cryab* gene was duplicated. The two *Cryab* genes in zebrafish, however, show some differences in their primary structure, their expression pattern and chaperone-like activity. The actual analyses suggest that *Cryab1* adopted a more restricted, non-chaperone role in the lens, while *Cryab2* maintained the widespread protective role found also in the mammalian αB-crystallins (Smith et al., 2006).

A new classification scheme for small heat-shock proteins in mammals separates this family of *HspB* genes according to their expression pattern into two classes: class I *HspB* genes are ubiquitously expressed and include *Cryab*; most members of this family are predominantly heat-inducible. In contrast, class II *HspB* genes

Table 1
The α-crystallins.

Gene	Chromosome[a]		Mouse cDNA sources[b]	Protein	Function
	Mouse	Human			
Cryaa	17 (31.8)	21q22 (43.5) OMIM: 123580	Lens, extraembryonic tissue; pituitary gland; placenta; spleen	αA-crystallin; 173 aa; 20 kDa αA^{ins}-crystallin; 196aa; 25 kDa	Structural protein Chaperone Autokinase Gene activator
Cryab	9 (50.6)	11q22 (111.3) OMIM: 123590	Lens, brain; kidney; heart; extraembryonic tissue; mammary gland; liver; muscle; testis; thymus; lung; pancreas; thyroid; skin; uterus; ovary; colon; diaphragma; limb; placenta	αB-crystallin; 175aa; 22 kDa	Structural protein Heat-shock protein

[a] MB according to ENSEMBL databases (http://www.ensembl.org; 2008) in brackets.
[b] According to the Jackson Lab database (http://www.informatics.jax.org).

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display tissue-restricted patterns of expression and include *Cryaa*; available evidence suggests that these genes are not induced by stress (Taylor and Benjamin, 2005).

2.2. The structure of the Crya genes

The *Cryaa* and *Cryab* genes have been characterized in a variety of species (e.g. mouse, man, hamster, rat, chicken, and rabbit); both genes contain three exons of similar size. In rodents (mouse, rat, hamster, rabbit), an alternative splice product can be observed in 10–20% of the αA-crystallin encoding gene: additional 69 bp are included from intron 1 into the mature mRNA leading to a protein 23 amino acids longer than the usual αA-crystallin. At the protein level, the insertion is present between the amino acid residues 63 and 64; it is referred to as αA^{ins}-crystallin. There is some evidence that the αA^{ins}-crystallin has decreased chaperone function (Derham et al., 2001), and the recombinant αA^{ins}-crystallin (but not the canonical αA-crystallin) inhibits bacterial growth (Bhat et al., 1996). The advantage of the αA^{ins}-crystallin is not yet clear.

In humans, the αA^{ins}-crystallin was not found, but database predictions (ENSEMBL, release 50, July 2008: http://www.ensembl.org/Homo_sapiens/index.html) suggest four other splice variants; two of them are obviously not translated into proteins. The other two, however, are confirmed by EST sequences – one from the lens (acc. no BM706162), the other (80%) from pooled organs consisting of colon, kidney and stomach (acc. no. BM923671). Both alternative splice products affect only exon 1; they are completely different and shorter in their N terminus and have the 2nd and 3rd exon in common (i.e., they share the major part of the protein beginning at amino acid pos 64; Fig. 1). For the human *CRYAB* the predictions of the databases are conflicting: the VEGA database (version 32; July 2008:http://vega.sanger.ac.uk/Homo_sapiens/index.html) suggests several alternative transcripts, but the ENSEMBL database lists only the well-known one; this discrepancy needs to be clarified experimentally.

2.3. The expression of the Crya genes

The expression pattern of the two *Crya* genes is quite different, although both are expressed at very high levels in the lens. The αA-crystallin was considered for a long time as a lens-specific protein; however, transcripts have been found also in other ocular tissues, particularly in the retina. On the other hand, *Cryab* is expressed rather ubiquitously (Table 1).

To address the regulation of the expression of *Cryaa* and *Cryab*, both promoters have been investigated in some detail. For *Cryaa*, the promoter from −364 to +45 was demonstrated frequently to direct different reporter genes in transgenic mice exclusively to the lens (Overbeek et al., 1985). This region consists of a complex array of positive and negative elements for a αA-crystallin binding protein, Maf and Pax6; moreover a cAMP-responsive element was identified. Moreover, rather recently, the lab of Ales Cvekl (Yang et al., 2006, 2007; Yang and Cvekl, 2005) characterized additional upstream- and downstream regulatory elements (DCR1–3) being rather 2–8 kb upstream and 3 kb downstream of the coding sequence. The 5′-DCR1 enhancer mediates the regulation of *Cryaa* expression in the mouse through Fgf signaling, and together with the 3′-DCR3 enhancer it forms a chromatin domain, which is characterized in the lens by histone H3 K9 acetylation. This acetylation is suggested to be responsible for the lower density of nucleosomes 2 kb upstream from the promoter region keeping the promoter in its active state. Histone acetylation can be performed by a broad variety of histone acetyltransferases (HATs); Yang et al. (2007) clearly showed that two HATs, CBP (CREB binding protein) and p300, are responsible for histone acetylation of the *Cryaa* regulatory domain in the lens.

In contrast to *Cryaa*, the *Cryab* gene is expressed in many more tissues and organs and also at higher levels. To understand its regulation, it is also important to know its genomic organization, which is characterized by the fact that it is separated only by 863 bp (in the mouse) from the *HspB2* gene encoding another small heat-shock protein (also referred to the myotonic dystrophy kinase binding protein; gene symbol *Mkbp*); these two genes are arranged in a head-to-head orientation. Therefore, it is not surprising that both genes share some common regulatory elements – nevertheless, they have different patterns of tissue-specific expression. A very short fragment of the *Cryab* promoter (−164 to +44) was sufficient to activate *CAT* reporter gene expression in transgenic lenses. Further dissection of this element demonstrated immediately upstream of the TATA-box two lens-specific regions (LSR1 and LSR2), both containing Pax6-binding sites, retinoic responsive elements (RAREs) and putative Maf-binding sites (MAREs; Cvekl and Duncan, 2007).

A bit more upstream of this core-promoter, an enhancer activity was determined (−427/−255) driving the *Cryab* expression in

Predicted amino-acid sequences of human *CRYAA* splice variations.

```
CRYAA004        MPVCPGDSHRPPKALPHLVCGRRGRQ---------------------------------- 24
CRYAA003        MSSACPRLAKLLASLLRCPAKAKRTGNGRPPPHPTTGLLSEPR----------------- 40
CRYAA001        MDVTIQHPWFKRTLGPFYPSRLFDQFFGEGLFEYDLLPFLSSTISPYYRQSLFRTVLDSG 60

CRYAA004        ---VRSDRDKFVIFLDVKHFSPEDLTVKVQDDFVEIHGKHNERQDDHGYISREFHRRYRL 83
CRYAA003        ---VRSDRDKFVIFLDVKHFSPEDLTVKVQDDFVEIHGKHNERQDDHGYISREFHRRYRL 100
CRYAA001        ISEVRSDRDKFVIFLDVKHFSPEDLTVKVQDDFVEIHGKHNERQDDHGYISREFHRRYRL 120

CRYAA004        PSNVDQSALSCSLSADGMLTFCGPKIQTGLDATHAERAIPVSREEKPTSAPSS 136
CRYAA003        PSNVDQSALSCSLSADGMLTFCGPKIQTGLDATHAERAIPVSREEKPTSAPSS 153
CRYAA001        PSNVDQSALSCSLSADGMLTFCGPKIQTGLDATHAERAIPVSREEKPTSAPSS 173
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Fig. 1. Predicted amino acid sequences of human *CRYAA* splice variations. The αA-crystallin (CRYAA001) and two other transcripts of the human *CRYAA* gene are shown. They differ only in the first exon and in the first and second of the seven fingerprint motifs (highlighted in yellow); according to PRINTS Protein Fingerprint Database (http://www.bioinf.manchester.ac.uk/fingerPRINTScan/). The sequences are received from the ENSEMBL database (http://www.ensembl.org) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

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lenses and non-lens tissues. Detailed analysis of this enhancer demonstrated that it acts only with the *Cryab* gene leading to the hypothesis of an insulator or boundary activity between this enhancer and the *HspB2*/*Mkbp* gene. This enhancer element contains several elements, which are used in several tissues: αBE1–4 are the αB-crystallin elements – αB1 has been shown to interact with the glucocorticoid receptor, whereas αB3 is a classical SP1 binding site. The glucocorticoid binding to αBE1 activates also the *HspB2* gene (Swamynathan and Piatigorsky, 2007), which is in contrast to the insulator hypothesis of the enhancer element. In the rat, it was shown recently that the deletion of the middle of the intergenic region including the enhancer element affected the activity of both genes (Doerwald et al., 2004). αBE4 contains a heat-shock element (HSE), which may be responsible for the activation of *Cryab* expression by heat-shock (Somasundaram and Bhat, 2004). The MRF site is important for interaction with MyoD (a basic helix–loop–helix transcription factor during myogenesis) and, therefore, responsible for the muscle-specific expression of *Cryab*. Partially overlapping with the MRF site, two E-box sequences were identified (−726/−721; −702/−697; Swamynathan and Piatigorsky, 2007). Recently, Duncan and Zhao (2007) identified a 30-bp element required for mediating the activation of *Cryab* by the product of the *brahma-related gene 1* (*Brg1*). This Brg1-response element is located immediately upstream of the *Cryab* transcription start site and contains an AT-rich sequence, which is bounded by the high-mobility group AT-hook 1 (HMGA1). Moreover, the Brg1-response element is obviously located within a positioned nucleosome and may thereby stabilize nucleosomal structures responsible for an active state of the *Cryab* gene. From a clinical point of view, three polymorphic sites in the human *CRYAB* promoter (−C249G, −C650G and −A652G) are interesting. They have been studied in sporadic cases of multiple sclerosis (MS), and it turned out that the rare allele −C650 has an increased likelihood of a non-inflammatory neurodegenerative phenotype of MS, characterized by a relatively rapid, primary progressive clinical disease course (van Veen et al., 2003).

In this context, the blind mole rat (*Spalax ehrenbergi*) is an interesting model system. This animal has a highly degenerated visual system because of its adaptation to a subterranean environment for the past 25 mio years. It is characterized by degenerated eyes; the lenses contain disorganized, vacuolated cells expressing α-crystallins at a very low level – however, the promoter is strongly active in muscle cells (Hough et al., 2002). The intergenic region between *Cryab* and *HspB2* is roughly 80% identical to the mouse (Li et al., 2007). Among the 20% differences, it turned out that the exchange of 2 bp (−273 CA→G) in the mouse promoter converting it to the mole rat sequence is able to abolish its lens-specific promoter activity. However, the reciprocal experiment, the insertion of a CA at the mole rats promoter position G272 did not enhance the promoter activity to the mouse level.

All these investigations using the very small fragment of less than 1 kb indicate strongly that the particular regulatory elements in a promoter are context-dependent in their activity. This interpretation has important implications for the understanding of evolutionary processes, because it demonstrates that small alterations being without consequences in one organism night be important in another one because of the different context, which this alteration is embedded in.

2.4. The α-crystallins: function and diseases

As mentioned above, the α-crystallins represent the major class of water soluble proteins in the lens (about 30%). Recent work in the past concentrated mainly on three functions of the α-crystallins (mainly in the lens):

- their ability to protect the lens against stress, particularly against oxidative stress, acting as a small heat-shock protein;
- their ability to act as molecular chaperones; and
- their interaction with the cellular cytoskeleton.

Since these aspects have been widely reviewed recently from a rather biochemical point of view (Bloemendal et al., 2004; Andley, 2007), I would like to concentrate here on the genetic aspects which become obvious from genetic modifications in the *Cryaa* and *Cryab* genes, and their clinical consequences.

2.4.1. The αA-crystallin

Because of the expression pattern of the α-crystallins (Table 1), it is expected that mutations in the *Cryaa* gene should have pleiotropic effects. Surprisingly, this does not seem to be true. The only disorder, which seems to be caused by mutations in the *Cryaa* gene, is cataract (of different forms) sometimes associated with microcornea or microphthalmia. Besides the already mentioned chaperone activity, it might be due to the altered interaction with the lens cytoskeleton (actin, tubulin or intermediate filaments; for recent reviews see Andley, 2007; Quinlan, 2002), or the disturbed leading function in migrating lens epithelial cells (Maddala and Rao, 2005).

The first dominant cataract mutation affecting the human *CRYAA* gene was characterized by Litt et al. (1998) as the Arg116Cys mutation. In the meantime, the same mutation was reported independently in several other unrelated families (Table 2) suggesting this position as a mutational hotspot in the *CRYAA* gene. Among them, a French family shows a new phenotype, adding an iris coloboma to the nuclear cataract (Beby et al., 2007). This might be the only case showing an additional phenotype outside the lens. However, iris colobomata reflect disturbances during eye development and are caused by mutations in several genes, e.g. in *GDF6* (Asai-Coakwell et al., 2007), *Pax2* (Favor et al., 1996), *PAX6* (Azuma et al., 2003), or *SOX2* (Wang et al., 2008). Therefore, in such cases, a second mutation in another gene might be considered, too. A similar case was reported by Graw et al. (2006): the cataract could be attributed to an Arg21Lys mutation in *CRYAA*, however, the additional macular hypoplasia is most likely caused by compound heterozygosity in the *OCA2* gene, the human homolog to the mouse gene *pink-eyed dilution* (alternative gene symbol *P*).

In addition to the nine *CRYAA* mutations in human, there are also four mouse *Cryaa* mutants characterized. One mutation, 160C→T, was observed both in mouse and human (leading to an Arg→Cys exchange at codon 54), however, the human cataract is inherited in a *recessive* way, but the mouse cataract as a *dominant* trait. In contrast, the mutation in the mouse changing the next base (161G→A, but affecting the same codon) replaces the Arg by His, but this cataract is transmitted in a recessive mode of inheritance. In human, another recessive cataract is caused by 27G→A (Trp9Ter), which might be understood as a classical loss-of-function mutation like the corresponding recessive knock-out model in the mouse (Brady et al., 1997). The only pathological consequence found in the homozygous mutant mice is an opacification of the lens resulting from inclusion bodies containing αB-crystallin. This occurrence of recessive and dominant alleles in a single gene might indicate different functional aspects, because a dominant mode of inheritance frequently is associated with structural functions, whereas recessive alleles indicate rather enzymatic activities (Wilkie, 1994).

In 10 out of these 13 mutations, an Arg residue is involved, which is most frequently changed into a Cys residue. In summary, although *Cryaa* is expressed in remarkable amounts also outside the lens, only cataracts are reported to be caused by mutations in the αA-crystallin encoding gene.

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Table 2
Mutations in mouse and human *CRYAA* genes.

nt Position[a]	bp Exchange	aa Position[a]	aa Exchange	Biological consequence (allele symbol; reference)
Mouse mutations				
160	C→T	54	Arg→Cys	Dominant cataract, small lens, microphthalmia (Xia et al., 2006)
161	G→A	54	Arg→His	Recessive cataract (Chang et al., 1996)
352	T→G	118	Tyr→Asp	Dominant cataracts, small lens (Xia et al., 2006)
371	T→A	124	Val→Gln	Dominant cataracts, microphthalmia (Graw et al., 2001a)
Human mutations				
27	G→A	9	Trp→stop	Recessive cataract (no detailed morphology given; Pras et al., 2000)
34	C→T	12	Arg→Cys	Two cases in distinct families: dominant cataract & microcornea (Hansen et al., 2007; Devi et al., 2008)
61	C→T	21	Arg→Trp	Dominant cataract & microcornea (Hansen et al., 2007); dominant cataract, microcornea and microphthalmia (Devi et al., 2008)
62	G→T	21	Arg→Lys	Dominant dense central cataract (Graw et al., 2006)
149	C→T	49	Arg→Cys	Dominant nuclear cataract (Mackay et al., 2003)
160	C→T	54	Arg→Cys	*Recessive* total cataract, microcornea (Khan et al., 2007); *dominant* cataract and microcornea (Devi et al., 2008)
292	G→A	98	Gly→Arg	**Dominant** progressive total cataract (Santhiya et al., 2006)
347	C→T	116	Arg→Cys	Six independent cases of dominant cataract with variable phenotype (Litt et al., 1998; Vanita et al., 2006; Beby et al., 2007; Hansen et al., 2007; Gu et al., 2008; Richter et al., 2008)

[a] Beginning at coding sequence (start codon ATG).

In the context of the functional roles of different mutations, other aspects of genomic variability might be considered. This is particularly true for genetic modifications like single nucleotide polymorphisms (SNPs) occurring in a given frequency in a population. Some of them change the amino acid composition, while others do not. The SNPs, which have been identified in the coding region of the *Cryaa* gene, are "silent" and do not alter the amino acid sequence. However, it is not known, whether they may lead to a different amount of protein because of differences in the codon usage by the corresponding tRNAs. Another putative function of such "silent" SNPs might be in an allele-specific methylation of the entire gene (Kerkel et al., 2008). It remains an open question, which might be addressed in the near future by groups interested into this topic.

Since the Arg116Cys mutation was the first human *CRYAA* mutation reported, a broad variety of experimental approaches have been undertaken to find out the functional consequences of this mutation. The mutant protein shows reduced chaperone activity (Kumar et al., 1999), interaction with βB2- and γC-crystallins (Fu and Liang, 2003) as well as with actin (Brown et al., 2007), but the membrane binding capacity was shown to be increased (Cobb and Petrash, 2000). However, a decreased chaperone activity is obviously no common feature of the cataract-causing *CRYAA* mutations. In case of the G98R mutation, the resulting protein forms larger oligomers than the wild-type forms, and the chaperone activity is dependent on the substrate used in the *in-vitro* assays: it shows enhanced chaperone activity, when citrate synthase or alcohol dehydrogenase is used, but a reduced activity toward aggregating α-lactalbumin (Murugesan et al., 2007). Moreover, the αA-crystallin is also considered to have anti-apoptotic properties by binding to Bax and Bcl-S. The Arg120Cys mutation shows also a weaker binding to these proteins which, in turn, might result in a weaker anti-apoptotic effect (Mao et al., 2004). A similar functional consequence was suggested by Xi et al. (2008) expressing the human Arg49Cys mutation as a knock-in gene in the mouse *Cryaa* locus. In heterozygotes, it leads to early nuclear cataracts, but in homozygous mutants to small eyes (with cataract) caused by an increase in cell death by apoptosis.

Similar like for the *Cryaa* promoter, the comparison of the mouse (or rat) αA-crystallin sequence to that of the blind mole rat, *S. ehrenbergi*, gave some interesting functional insights. As mentioned above, αA-crystallin is still present in the mole rat as a viable protein; in terms of secondary and quaternary structure as well as thermostability it is comparable to the rat protein. There are in total nine amino acids changed – but only one in the C-terminal α-crystallin domain. The chaperone activity is reduced, which is believed to be caused by the S172L and S173F replacements in the middle of the αA-crystallin extension of the mole rat (as compared to the rat sequence; Smulders et al., 2002). The authors suggest another, yet unknown function of αA-crystallin.

2.4.2. αB-crystallin: cataracts and myopathies

In contrast to the clear relationship of *Cryaa* mutations and the formation of cataracts, the role of αB-crystallin is more heterogeneous. In 1997, Brady and Wawrousek reported on the knock-out of *Cryab*, the αB-crystallin encoding gene, in the mouse. Surprisingly, even the homozygous mutants do not display an overt disease phenotype. Histological analysis of eyes, cardiac muscle and skeletal muscles from *Cryab*$^{-/-}$ revealed no apparent structural abnormalities detectable by light microscopy. Immunohistochemistry showed a regular distribution of αA-, β-, and γ-crystallins. In contrast to wild-type mice, the *Cryab*-knock-out mice show a general loss of body weight, muscle degeneration and abnormal skeleton development (Brady et al., 2001). However, there is no further mouse *Cryab* mutant reported so far; the two known SNPs do not cause an amino acid exchange.

On the human side, the situation is somewhat different. There are also a few silent SNPs reported in the coding region of the *CRYAB* gene, however, also a few SNPs are listed in the databases leading to amino acid exchanges (Table 3). In one case, the allele frequency for the major allele is 100%, and for the other three SNPs no population data are available up to now. Therefore, it is an open question whether they contribute to any disease or not. Moreover, in addition to the SNPs in coding regions, SNPs occur also in the noncoding regions like the promoter and in introns. Recently, Stoevring et al. (2007) could demonstrate in a Danish case-control study of 233 patients suffering from multiple sclerosis (but only 96 controls) that some of these SNPs (in particular from the promoter: −249:C→G) are obviously associated with multiple sclerosis. In this context it is important to know that *CRYAB* expression is enhanced in the earlier stages of multiple sclerosis in oligodendrocytes and in astrocytes; the C→G exchange at pos. −249 is believed to create a new binding site for the transcription factor Nrf2; however, it remains to be proven experimentally (August 2008).

Moreover, in human nine mutations are reported affecting the *CRYAB* gene (Table 3). Only a few of them are associated with

Table 3
Mutations and polymorphic sites in mouse and human *CRYAB* genes.

nt Position[a]	bp Exchange	aa Position[a]	aa Exchange[b]	Biological consequence (allele symbol; reference)
Human SNP[b]				
88	G→T	30	Glu→stop	Allele frequency for G: 100%
122	C→A	41	Ser→Tyr	Unknown, no population data
152	C→T	51	Pro→Leu	Unknown, no population data
270	ΔA	91	Frameshift	Unknown, no population data
Human mutations				
58	C→T	20	Pro→Ser	Dominant posterior polar cataract (Liu et al., 2006a)
358	A→G	120	Arg→Gly	Desmin-related myopathy and cataract (Vicart et al., 1998)
418	G→A	140	Asp→Asn	Dominant lamellar cataract (Liu et al., 2006b)
450	ΔA	150	Frameshift	Dominant posterior polar cataract (Berry et al., 2001)
451	C→T	151	Gln→stop	Desmin-related myopathy (Selcen and Engel, 2003)
460	G→A	154	Gly→Ser	Dilated cardiomyopathy (Pilotto et al., 2006)
464	ΔCT	155	Frameshift	Desmin-related myopathy (Selcen and Engel, 2003)
470	G→A	157	Arg-His	Dilated cardiomyopathy (Inagaki et al., 2006)
514	G→A	171	Ala→Thr	Dominant lamellar cataract (Devi et al., 2008)

Δ, deletion.
[a] Beginning at coding sequence (start codon, ATG).
[b] According to dB SNP: http://www.ncbi.nlm.nih.gov/SNP/; only such SNPs are listed leading to amino acid exchanges.

dominant cataracts only, but some are suggested to be causative also for desmin-related myopathy or dilated cardiomyopathy. This feature is distinct from the mutation spectrum of the *CRYAA* gene and reflects to some extent the different expression pattern of the *CRYAB* gene. The regions being affected by mutations in the human *CRYAB* gene are given in Fig. 2: eight out of nine mutations affect the third exon, and there is no domain obvious being particularly responsible for cataract or myopathies.

Nevertheless, particular binding areas could be attributed to the two major domains of the αB-crystallin: the N-terminal domain is responsible for the interaction with the kidney-specific cadherin 16 (Thedieck et al., 2008), and the C-terminal α-crystallin domain is responsible for the interaction with free cytoskeletal proteins (Ohto-Fujita et al., 2007) and the actin polymerization (Ghosh et al., 2007); the R120G mutation was shown to be important for the desmin aggregation (Perng et al., 2004).

2.7. αB-crystallin and neurological disorders

Moreover, none of the reported mutations in the *CRYAB* gene seems to be associated with neurological disorders, even if *CRYAB* is also expressed in the brain mainly in oligodendroglia cells (Renkawek et al., 1992). However, very early reports demonstrated that the αB-crystallin protein is associated with a broad variety of neurodegenerative diseases. First, αB-crystallin was found to be accumulated in scrapie-infected hamster brain cells (Duguid et al., 1988) and later on in brains from humans suffering from Creutzfeld–Jacob-disease (CJD; Renkawek et al., 1992); it was also shown in bovine to directly interact with PrP^C, the cellular normal prion protein (Sun et al., 2005a). Furthermore, αB-crystallin accumulates in the brain of patients with Alexander's disease (Iwaki et al., 1989). Alexander's disease is a rare sporadic encephalopathy caused by mutations in the gene coding for the glial fibrillary acidic protein (gene symbol: *GFAP*); the disease is characterized by macrocephaly, extensive proliferation of abnormal astrocytes, and formation of inclusions in astrocytes and their processes (Rosenthal fibers). The sequestration of αB-crystallin into the Rosenthal fibers is one of the key processes during the pathogenesis in Alexander's disease (for a recent review see Quinlan et al., 2007).

Additionally, αB-crystallin was demonstrated in about 10% of Lewy bodies (Lowe et al., 1990). Lewy bodies are aggregated protein deposits found in neuronal and glial cytoplasm; they are associated with particular forms of neurodegenerative disorders like Parkinson's or Alzheimer's disease. The main component of the Lewy bodies is α-synuclein. Recently, Rekas et al. (2004) could show that αB-crystallin directly interacts with α-synuclein thereby

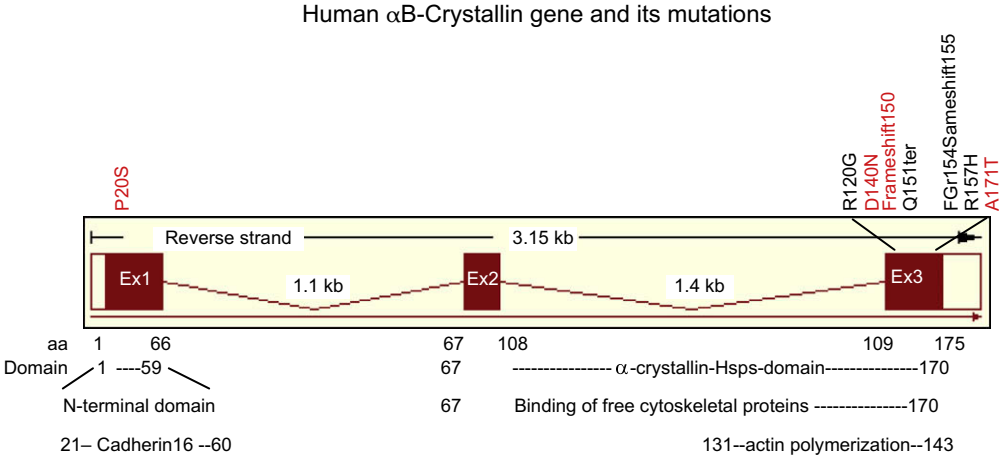

Fig. 2. Human αB-crystallin gene and its mutations. The genomic structure of the human *CRYAB* gene is given. It consists of three exons; the N-terminal domain is encoded by the first exon, whereas the α-crystallin/heat-shock protein domain is encoded by exons 2 and 3. Interaction partners of the domains are indicated. Most of the mutations identified in the human *CRYAB* gene affect exon 3. There is no particular region responsible for cataract-causing mutations (red) or mutations affecting the heart and/or the muscle (black). Drawings are according to the ENSEMBL release 50 (July 2008; http://www.ensembl.org) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

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inhibiting – at least *in vitro* – the fibrillization of α-synuclein. These authors suggest that αB-crystallin redirects α-synuclein from a fibril-formation pathway towards an amorphous aggregation pathway to keep it in a form which might be easily degraded.

In case of Alzheimer' disease, it was shown in several studies that αB-crystallin might be directly involved into its pathogenesis. In Alzheimer patients, αB-crystallin was present in limbic and paralimbic region, which are commonly affected in Alzheimer's disease. The number of B-crystallin positive neurons increased in parallel with the neuronal loss (Mao et al., 2001). In biochemical *in-vitro* experiments, it was shown that αB-crystallin (and the small heat-shock protein HSP27) prevents fibril-formation of β-amyloid$_{1-40}$. However, in cultured neurons this effect leads to an increased toxicity rather than a protective effect (Stege et al., 1999). In a more sophisticated study using NMR spectroscopy it was shown recently, that this effect is caused by an efficient competition of αB-crystallin for monomer–monomer interactions with β-amyloid$_{1-40}$. Interactions between β-amyloid$_{1-40}$ involve the core residues 17–21 as well as the residues 31–32 of β-amyloid$_{1-40}$. In the presence of αB-crystallin, Met35 in β-amyloid$_{1-40}$ becomes efficiently oxidized, which is discussed as the cause for the observed increased neurotoxicity and the inability of β-amyloid$_{1-40}$ to form fibrillary structures (Narayanan et al., 2006). Recently, Ecroyd and Carver (2008) explored the potential for small molecules such as arginine and guanidine to affect the chaperone activity of αB-crystallin against disordered (amorphous) and ordered (amyloid fibril) forms of protein aggregation. They could show that the effect of these additives is highly dependent upon the target protein undergoing aggregation. In particular, the chaperone action of αB-crystallin against aggregation of the disease-related amyloid fibril forming protein α-synuclein A53T is enhanced in the presence of arginine and similar positively charged compounds such as lysine and guanidine.

3. β/γ-Crystallins

The β- and γ-crystallins share a common feature of antiparallel β sheets in the protein, which is referred to as "Greek key motif" because of its similarity (in schematic drawings) to paintings on ancient Greek pottery. In all members of the β- and γ-crystallin super family, this motif occurs four times. According to the original finding of three main protein fractions in the eye lens (Mörner, 1893), the β-crystallins were characterized as oligomers (the molecular mass of the monomers is between 22 and 28 kDa) with native molecular masses ranging up to 200 kDa for octameric forms.

3.1. Evolutionary aspects

Based on the repetition of the four Greek key motifs it is widely accepted that β/γ-crystallins evolved in several duplication steps from an ancestral gene coding for just one Greek key motif. However, the one-motif ancestor was not (yet) observed, but some examples exist for proteins consisting of two Greek key motifs, like spherulin 3a from *Physarum polycephalum* (involved in the formation of dehydrated spores), the yeast killer toxins WmKT (Antuch et al., 1996) and SKLP (Ohki et al., 2001) and the *Ci-βγ-crystallin* in the sea squirt *Ciona intestinalis* (Shimeld et al., 2005). There exist also proteins of four Greek key motifs like the protein S from *Myxococcus xanthus*, the epidermis differentiation-specific protein (EDSP or ep37) of the amphibian *Cynops pyrrhogaster* (Wistow et al., 1995) or the βγ-crystallin-and-trefoil factor from the skin of the frog *Bombina maxima* (Liu et al., 2008); AIM1 (absent in melanoma) consists of even 12 Greek key motifs (Ray et al., 1997).

There are also some differences among the genes coding for the lens βγ-crystallins, which are interesting from an evolutionary point of view. In the β-crystallins, individual Greek key motifs are encoded by separate exons, whereas in the γ-crystallin genes two motifs are encoded by one exon reflecting the modular nature of the proteins during evolution. However, there are two genes that are obviously intermediates: the previously designated βs-crystallin showed a slow chromatographic migration among the β-crystallin fraction. However, since it has the typical structure of a γ-crystallin encoding gene, it is referred today as γS-crystallin (gene symbol *Crygs*). It should be mentioned that the ENSEMBL database suggests an alternative splice product affecting the N-terminal part of the protein.

The second intermediate gene has been designated *CrygN*: it has a hybrid gene structure, half *Cryg* (one exon encoding two Greek key motifs) and half *Cryb* (two exons encoding a single Greek key motif each; Wistow et al., 2005). Besides the eye, *CrygN* transcripts can be found in the mouse thyroid gland (MGI database). In this context, it might be of particular interest that the sponge *Geodia cydonium* forms an intronless βγ-crystallin-type gene; however, there are proto-splice sites identified in the nucleotide sequence at those positions, where introns are located in the homologous vertebrate genes (di Maro et al., 2002).

3.2. Common functional aspects

The function of the Greek key motif is still a matter of debate. Computer-based analysis suggests that they form an interdomain association – intramolecular in the γ-crystallins, and intermolecular in the β-crystallins. In this way, they allow a dense packaging of the proteins minimizing light scattering, but providing an optimum in transparency of the eye lens. The detailed analysis of the mutations in the βγ-crystallin encoding genes might help to identify those amino acids which are the important corner stones for this function.

A second functional aspect of the Greek key motif is its Ca^{2+}-binding properties (Sharma et al., 1989). Later on, Ca^{2+}-binding activity was found also in other proteins with Greek key motifs as mentioned above, like protein S (Wenk et al., 1999) or spherulin 3a (Clout et al., 2001), and also in γ-crystallins (Rajini et al., 2001; Jobbi and Sharma, 2007). This finding might have important implications not only for the understanding of cataractogenesis, but also for other pathological processes, in which βγ-crystallins are involved. The role of Ca^{2+} in cataractogenesis has been extensively elaborated by Duncan and his coworkers (Duncan and Wormstone, 1999; Sanderson et al., 2000).

Recently, Fischer et al. (2008) reported on the effects of β/γ-crystallins on axon regeneration of retinal ganglion cells. In particular, intravitreal injections of β- or γ-crystallins (or their addition to the tissue culture medium) strongly enhanced axon regeneration in corresponding retinal explants in culture. Additionally, they induce the release of the ciliary neurotrophic factor (CTNF) in retinal astrocytes and lead to the activation of the major downstream JAK/STAT3 signaling pathway.

3.3. β-Crystallins

3.3.1. Genes and gene expression

The β-crystallins can be divided into more acidic (βA-) and more basic (βB-) crystallins. Each subgroup is encoded by three genes (*Cryba1*, *–2*, *–4*; *Crybb1*, *–2*, *–3*); however, *Cryba1* is encoding two proteins (βA1- and βA3-crystallins). This feature is conserved among all mammals, birds and frogs. In man, a partly duplicated *Crybb2* gene was found; however, no transcripts of this particular gene could be detected, therefore, it is referred to as a pseudogene (Brakenhoff et al., 1992).

In mouse and man, the *Cryb* genes are distributed among three chromosomes; *Cryba4*/*Crybb1* and *Crybb2*/*Crybb3* form a pair-wise

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Table 4
The β/γ-crystallins.

Gene	Chromosome[a]		Protein
	Mouse	Human	
Cryba1	11 (77.5)	17q11 (24.6)	βA1/A3-crystallin; 215aa, 23/25 kDa
Cryba2	1 (74.9)	2q34 (219.6)	βA2-crystallin; 196aa, 22 kDa
Cryba4[b]	5 (112.7)	22q11 (25.4)	βA4-crystallin; 186aa, 22 kDa
Crybb1[b]	5 (112.7)	22q11 (25.3)	βB1-crystallin; 252aa, 28 kDa
Crybb2	5 (113.5)	22q11 (23.9)	βB2-crystallin; 205aa, 23 kDa
Crybb3	5 (113.5)	22q11 (23.9)	βB3-crystallin; 211aa, 24 kDa
ψCRYBB2	–	22q11	Additional Pseudogene
CrygN	5 (24.3)	7q36 (150.8)	γN-crystallin; 182aa, 21 kDa
Crygs	16 (22.8)	3q27 (187.7)	γS-crystallin; 178aa, 21 kDa
Cryga→Crygd	1 (65.1)	2q33 (208.7)	γA→γD-crystallin; 175aa, 21 kDa
Cryge	1 (65.0)		γE-crystallin; 174aa, 21 kDa
ψCRYGE	–	2q33	γE-crystallin; pseudogene
Crygf	1 (65.9)		γF-crystallin; 174aa, 21 kDa
ψCRYGF	–	2q33	γF-crystallin; pseudogene

[a] MB according to ENSEMBL databases (http://www.ensembl.org; 2008) in brackets.
[b] Head-to-head orientation.

cluster on the same chromosome separated by ~1 MB and a few other genes (Table 4). It is noteworthy that the *Cryba4*/*Crybb1* genes are organized head-to-head, whereas the other pair of *Cryb* genes has a head-to-tail orientation. The ENSEMBL database suggests that the human *CRYBA2* and *CRYBB3* genes might be alternatively spliced affecting either the N-terminal region (*CRYBA2*) or the C terminus (*CRYBB3*).

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. Because of the high level of expression in the lens, the smaller level of expression in other organs was not recognized for a long time. One of the first reports on the presence of β-crystallin in non-lens tissue was from de Pomerai and Clayton (1978) demonstrating trace amounts of β-crystallin (and α-crystallin) in 60-days culture of 17-day embryonic neural retina. In a more detailed study, Xi et al. (2003) detected transcripts of all β-crystallin encoding genes in the mouse retina and the corresponding proteins by immunofluorescence in its outer and inner nuclear layer as well as in the inner segments of the photoreceptors.

The molecular basis for expression of the *Cryb* genes has not yet been fully established; in mammals, most work was done on the promoters of the *Crybb1* and *Crybb2* genes (for an excellent review see Cvekl and Duncan, 2007). Regulatory elements determining the activity of these genes in the rat are located in upstream regions and also in the first intron. It is obvious that Maf, Pax6, Sox2 and the canonical Wnt pathway are involved in the regulation of the β-crystallins; however, further detailed studies are necessary to identify further relevant transcription factors and their binding sites.

3.3.2. *β-Crystallins: functions and diseases*

The native β-crystallin proteins can be found as multiple forms of oligomers with a general isoelectric point ranging between pH 5.7 and pH 7.0. Moreover, β-crystallins are acetylated at their N-termini; further post-translational modifications are glycosylation and phosphorylation (Graw, 1997). Another reason is the heterogeneity of the proteins in the different cell types in the lens (epithelia cells vs. fiber cells). Therefore, the proteomic analysis of lenses and the comparative analysis of their results with respect to ageing processes remain somewhat conflicting. Additionally, the β-crystallins are also genetically heterogeneous as indicated by the list of their polymorphic sites in mice and more impressively in humans (Table 5). There are several SNPs affecting the coding sequence of the *Cryb* genes. Since some of them have an allele frequency of 100%, the corresponding ones might be considered as sequencing artifacts. However, for the remaining SNPs, it might be interesting to investigate in detail how they influence the folding and the possibility of post-translational modifications of the corresponding β-crystallins.

The first mutation in a *Cryb* gene was described in 1991 by Chambers and Russell for the Philly-cataract in the mouse; a few years later, a mutation in the human *CRYBA1* gene was identified to be causative for a congenital zonular cataract. In the meantime some additional mutations have been identified both in mouse and human (for an overview see Table 5) affecting all *Cryb* genes except *Cryba2*. There seem to be some mutational hot spots: in human *CRYBA1*, the ΔG91 mutation occurs in four different families, and position 1 of its third intron is also affected in four other distinct families. Even if the mutations all lead to cataracts, the individual phenotypes are different and no conclusive genotype–phenotype correlation might be drawn.

Among the *Cryb* genes, the *Crybb2* gene is the most important one and might be understood as a paradigm for the β-crystallin encoding genes (like for lens biochemists, who coined in the past the term "basic principle β-crystallin", β_{Bp}-crystallin). In human, the most frequent mutation in the *CRYBB2* gene is a gene conversion to its pseudogene. Gene conversion is a process occurring frequently after gene duplication: the newly generated gene copies are prone to unequal reciprocal crossover because of the high degree of homology between these two genes. As a consequence, the 'acceptor' sequence is replaced by a sequence that is copied from the 'donor'. Today, the gene conversion mechanism in *CRYBB2* is a text-book example of this mechanism (Chen et al., 2007); by replacing 9–104 of the original bases in the *CRYBB2* gene it leads to two bp exchanges (C475T, C483T). The first one leads to a stop codon (Q155X) and is, therefore, considered to be causative for the cataract (Vanita et al., 2001).

Besides the conversion to its pseudogene, the human *CRYBB2* is also involved in another interesting mechanism, since it belongs to those genes that are characterized by copy number variations (CNV: a DNA segment, longer than 1 kb, with a variable copy number compared with a reference genome). Actually, it is not really clear how CNVs might contribute to clinical phenotypes; however, they should be considered as possible disease mechanisms (Lee et al., 2007). Actually, there is a discussion going on whether the gene dosage of the *CRYBB2* pseudogene might be related to neurofibromatosis 2 (NF2). These patients frequently display cataracts as part of the disease, but this feature apparently does not correlate to the mutational status of the *NF2* gene, which is just ~3.8 MB away from the *CRYBB2* and its pseudogene (de Bustos et al., 2006).

In the mouse, the three alleles of the *Crybb2* have been reported to lead to progressive cataracts (Table 5); all of them affect the beginning of exon 6 (and therefore the fourth Greek key motif). The most prominent one is the Philly mouse; it was the first cataract mutant, which was characterized at a molecular level (Chambers and Russell, 1991). After the observation that *Crybb2* is expressed also in brain and testes (Magabo et al., 2000), a more detailed study described also subfertility in homozygous Philly mice on a Swiss Webster background (but not on the C57BL/6 genetic background; DuPrey et al., 2007). Moreover, in a new *Crybb2* mouse mutant, Ganguly et al. (2008) reported recently the detailed expression pattern of *Crybb2* in the brain (olfactory bulb, cortex, hippocampus and cerebellum) together with its influence on the number and size of the Purkinje cells in the cerebellum in the homozygous mutants. We may expect further reports of additional phenotypes in these mutants.

However, *Crybb2* is obviously also involved in the elongation of axons in retinal ganglion cells in retinal regeneration (Liedtke et al., 2007). The authors discuss βB2-crystallin as a neurite-promoting

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Table 5
Mutations and polymorphic sites in mouse and human *CRYB* genes.

nt Position[a]	bp Exchange	aa Position[a]	aa Exchange	Allele symbol (comment)
Mouse SNPs[b]				
Cryba2				
313	G→C	105	Asp→His	rs50671503
Crybb2				
500	G→A	167	Arg→His	rs32270282 (Validated[c])
Human SNPs[b]				
CRYBA1				
346	A→T	116	Ile→Phe	rs1129656
521	C→T	174	Pro→Leu	rs1129658
CRYBA2				
326	C→T	109	Thr→Ile	rs41272691
CRYBA4				
77	G→A	26	Arg→Gln	rs12053788
106	G→A	36	Val→Met	rs35520672 (Allele frequency "A" in African Americans ~3%)
251	C→T	84	Thr→Met	rs4277 (Allele frequeny "T" ~2%)
437	C→G	146	Ser→Cys	rs28412604
CRYBB1				
52	G→A	18	Gly→Arg	rs5761634 (Allele frequency "G" 100%)
CRYBB2				
13	A→T	5	His→Leu	rs7291633 (Allele frequency "A" 100%)
193	G→T	65	Ala→Ser	rs16986560 (Allele frequency "T" ~2%)
433	C→T	145	Arg→Trp	rs2330991
440	A→G	147	Gln→Arg	rs2330992 (Allele frequency "A" 100%)
449	C→T	150	Thr→Met	rs4049504
CRYBB3				
314	G→A	105	Arg→Gln	rs17670505 (Allele frequency "A" 100%)
337	C→G	113	His→Asp	Rs9608378 (Allele frequency "C" 10–65%)
475	G→A	159	Val→Ile	Rs4455261 (Allele frequency "A" ~2%)
Mouse mutations				
Cryba1				
502	T→A	168	Trp→ Arg; splicing Δ168	Zonular, progressive cataract (Graw et al., 1999)
Crybb2				
Intron 5:−57	A→T	Splicing: 19 new amino acids in front of exon 6		Progressive cataract (Ganguly et al., 2008)
560	T→A	187	Val→Glu	Progressive cataract (Graw et al., 2001b,c)
585–587	Deletion	195–198	ΔQSVR	Progressive cataract (Chambers & Russell, 1991); subfertility (DuPrey et al., 2007)
nt Position[a]	bp Exchange	aa Position[a]	aa Exchange	Biological consequence (reference)
Human mutations				
CRYBA1				
279–281	ΔGAG	91	ΔG	Four different families with distinct cataracts (Ferrini et al., 2004; Lu et al., 2007; Qi et al., 2004)
Intron 3, +1	G→A	Splicing	Premature stop?	Five different families with distinct cataracts (Devi et al., 2008; Burdon et al., 2004; Kannabiran et al., 1998)
Intron 3, +1	G→C	Splicing	Premature stop?	Pulverulent cataract (Bateman et al., 2000)
CRYBA4				
242	T→C	69	Leu→Pro	Microphthalmia (Billingsley et al., 2006)
317	T→C	94	Phe→Ser	Dominant lamellar cataract and microphthalmia (Billingsley et al., 2006)
CRYBB1				
667	C→T	223	Gln→Val	Nuclear cataract (Yang et al., 2008)
682	T→C	228	Ser→Pro	Nuclear cataract (Wang et al., 2007a)
168	ΔG	57	Frameshift	Recessive nuclear cataract (Cohen et al., 2007)
CRYBB2				
383	A→T Gene conversion to pseudogene (Q155X)	128	Asp→Val	Dominant ring-shaped cortical cataract (Pauli et al., 2007) Eight independent families with dominant cataracts with phenotypic variations (Li et al., 2008c; Devi et al., 2008; Bateman et al., 2007; Yao et al., 2005; Vanita et al., 2001; Gill et al., 2000; Litt et al., 1997)
CRYBB3				
493	G→C	165	Gly→Arg	Recessive nuclear cataracts in two families (Riazuddin et al., 2005)

[a] Beginning at coding sequence (start codon ATG).
[b] According to dB SNP: http://www.ncbi.nlm.nih.gov/SNP/; only such SNPs are listed leading to amino acid exchanges.
[c] Validation included the mouse strains A/J; C57BL/6J; DBA/2J; 129X1/SvJ; 129S1/SvImJ.

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factor operating through an autocrine mechanism, which could be used in neurodegenerative diseases.

A quite unexpected result was reported recently from the rat mutant *Nuc1*: the homozygous mutants suffer not only from cataracts, but also from severe retinal and other ocular abnormalities including the persistence of the fetal intraocular vessels. The underlying mutation affects the *Cryba1* gene, and more detailed studies showed that *Cryba1* is expressed in retinal astrocytes; the mutation destroys their normal structure and function leading to abnormalities in the development and maturation of the retinal vasculature. It should be noted that these retinal abnormalities are apparent only in the homozygous mutants indicating a recessive mode of inheritance; the cataracts are present in the heterozygotes and are considered as a dominant feature (Sinha et al., 2008).

3.4. γ-Crystallins

3.4.1. Genes and gene expression

The γ-crystallin encoding genes (*Cryg*) are organized as a cluster of five genes (γA→γE-crystallin; gene symbols *Cryga*→*Cryge*) within approximately 50 kb; these genes are all arranged in a head-to-tail orientation. Close to this cluster, but clearly separated, a sixth gene (*Crygf*) is present; in the mouse, it is ~0.9 MB apart from the cluster and inversely oriented. Between *Cryga* (the most 3′-oriented gene) and *Crygf* a few other genes are located (e.g. *Idh1*, *Pip5k3*). This gene cluster is found on mouse chromosome 1 and on human chromosome 2. The six genes are very similar; the protein sequences deduced from the mouse *Cryge* and *Crygf* genes are even identical (Graw et al., 1991; Graw et al., 1993).

A typical *Cryg* gene is composed of three exons; the first exon contains only 9 bp and is followed by a short intron of about 100 bp. The second exon (243 bp) and the third exon (273–276 bp) are separated by a large intron (1–2 kb). The non-translated 3′-end is short (about 40 bp). The *Cryge* and *Crygf* 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 the mouse, alternative splicing has to be considered only for the *Cryge* gene (ENSEMBL database).

The *Cryg* genes are expressed in mouse lenses from E13.5 onwards in the primary fibers and later on in the secondary fiber cells, but not in the epithelial cells. The expression of *Cryg* genes reaches the maximum in mice at birth, and is decreasing during the first weeks after birth. In human, the *Cryg* expression is restricted to prenatal development, because of the different time scale of mouse and human intrauterine life (Graw, 1997).

In contrast to mammals, γ-crystallins appear in amphibians first even before the α-crystallins. Moreover, Brunnekreef et al. (1997) reported that in *Xenopus* some *Cryg* genes (and *Cryba4*) are expressed in the animal cap of the *Xenopus laevis* gastrula. The non-lens expression of *Cryg* mRNA is not restricted to early embryogenesis, but continues in other differentiated tissues by tadpole stages in middle and posterior regions (Smolich et al., 1994). Therefore, it might be suggested that the γ-crystallins play a role in general cellular processes in *Xenopus*.

The regulation of the expression of the very similar *Crygd*, *Cryge* and *Crygf* genes has been intensively studied in mouse and rat. In the mouse, previous studies have established that the 5′-flanking region −226/+45 of the *Crygf* crystallin gene is sufficient for optimal promoter activity in the lens. Of major importance are the binding sites for retinoic acid receptors (retinoic acid response elements, RARE), for heat-shock factors (heat-shock element, HSE), and for the transcription factors Pax6, Six3, Sox1, Sox2, and Maf (for a recent review see Cvekl and Duncan, 2007); Six3 has been shown to antagonize the activating potential of the transcription factor Prox1 at the *Cryge* promoter (Lengler et al., 2001).

3.4.2. γ-Crystallins: functions and disease

γ-Crystallins appear in the gel-chromatography of lens proteins as the last peak because of their low molecular mass of 20 kDa and of their monomeric structure. They are the most basic crystallins in mammals with isoelectric points ranging from pH 7.1 to pH 8.6. They are characterized by a high content of free Cys residues (4–7 per molecule) making them highly sensitive to the formation of mixed disulfides during oxidative stress. Because of sequence alignment studies the six γ-crystallins can be clustered into two groups of γA/B/C-crystallin and γD/E/F-crystallin (Graw, 1997). It might be noteworthy that this clustering reflects differences in their behavior in phase separation. The critical temperature, at which phase separation occurs, is low for the γA/B/C-crystallins and high for the γD/E/F-crystallins. This finding might be explained by distinct states of hydration observed between members of the two groups. The difference in hydration state could be attributed to three amino acid residues in the γD-crystallin (Leu^{51}, Ile^{103} and His^{115}), leading to a change of hydrophobicity/hydrophilicity compared to γB-crystallin (Slingsby et al., 1997).

Genetic research identified various mutations at the *Cryg* genes leading to various forms of cataract. In mice, the *Elo* mutant (*Eye lens obsolesence*) was the first being characterized as a mutation in a *Cryg* gene: a single nucleotide deletion in *Crgye*. The mutation destroys the reading frame affecting the fourth Greek key motif of the protein (Cartier et al., 1992). Today, more than 20 mutations in all of the six genes of the *Cryg*-cluster at mouse chromosome 1 have been reported (see Table 6); all of them are dominant and lead to cataracts without any other obvious anomaly. In Fig. 3, the regional distribution of the mutations is shown for the mouse *Crygd* gene and compared to the mutations in the corresponding human gene. It should be noted that the phenotype of the mutants varies significantly from very mild opacities to severe cataracts with microphthalmia. There is no genotype–phenotype correlation possible. In contrast to the dominant cataracts caused by mutations in the "classical" *Cryga–Crygf* genes, mutations in the intermediate gene *Crygs* reveal a recessive or semi-dominant mode of inheritance.

Usually, the first alterations in these mutants can be observed after development of the lens around embryonic days 13–14 (Oda et al., 1980; Santhiya et al., 1995). The mechanism of how the cataracts develop has been studied in detail by Sandilands et al. (2002) presenting evidence that the formation of intranuclear inclusions is a key event in this process. In three different inherited, congenital cataracts in the mouse involving *Cryg* gene mutations, large inclusions containing the altered γ-crystallins were found in the nuclei of the primary lens fiber cells. Their formation preceded 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, Congo red. *In vitro*, recombinant mutant γB-crystallin readily formed amyloid fibrils under physiological buffer conditions, unlike wild-type protein. These data suggest 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). In a recent study, Papanikolopoulou et al. (2008) confirmed the formation of amyloid fibril *in vitro* using isolated N- and C-terminal fragments of the human γD-crystallin upon incubation at acid pH. Similar results have been published recently by Talla et al. (2008) for the human W165X mutation.

In contrast to the mouse, cataract-causing mutations in humans have been detected in the *CRYGC* and *CRYGD* genes only (the *CRYGE* and *CRYGF* genes are pseudogenes; Table 6). However, similar to the mouse, the cataract phenotypes are quite different and do not allow any genotype–phenotype correlations. In human, however, the situation is much more complicated than in the mouse (using inbreed strains) because of the general genetic diversity; modifier

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Table 6
Polymorphic sites and mutations in mouse and human *CRYG* genes

nt Position[a]	bp Exchange	aa Position[a]	aa Exchange	Allele symbol (comment)
Mouse SNPs[b]				
Crygb				
160	C→T	54	His→Tyr	rs48637499
238	C→T	80	Arg→Cys	rs32224474 (Validated[c])
368	A→G	123	His→Arg	rs48761726
Crygc				
464	T→A	155	Phe→Tyr	rs50495755
Cryge				
154	A→G	80	Arg→His	rs33471083 (Validated[c])
Crygf				
238	G→A	80	Arg→His	rs3666875 (Validated[d])
493	A→G	165	Ser→Gly	rs31714794 (Validated[c])
CrygN				
99	A→C	33	Gln→His	rs32000514
Crygs				
335	C→T	112	Thr→Met	rs47659975
Human SNP[b]				
CRYGB				
268	G→C	90	Arg→Thr	rs2241980 (C 100% in all populations)
331	C→A	111	Leu→Ile	rs796287 (overall allele frequency of C: ~64%
CRYGC				
18	C→A	6	Phe→Leu	rs2242072 (allele frequency of A in Asia: ~1%)
nt Position[a]	bp Exchange	aa Position[a]	aa Exchange	Biological consequence (allele symbol; reference)
Mouse mutations				
Cryga				
127	T→C	43	Trp→Arg	Severe dominant total cataract with vacuoles (Graw et al., 2004)
222	A→G	74	Asp→Gly	Diffuse dominant nuclear opacity (Klopp et al., 1998)
Crygb				
10	A→T	4	Ile→Phe	Dominant lamellar cataract (Liu et al., 2005)
31	A→C	11	Ser→Arg	Dominant dense nuclear cataract (Li et al., 2008a)
417–424	Δ417–427/Ins4	139	Ser, 6 new aa	Dominant nuclear opacity of medium severity (Klopp et al., 1998)
Crygc				
420–425	Deletion	141–142	ΔG, Arg	Dominant total and lamellar cataract (Graw et al., 2002a)
471	G→A	157	Trp→stop	Dominant total cataract with vacuoles (Graw et al., 2004)
Crygd				
134	T→C	45	Leu→Pro	Dominant cloudy nuclear cataract (Graw et al., 2004)
227	T→A	76	Val→Asp	2 independent dominant cataracts: nuclear and cortical opacity (Graw et al., 2002b); total cataract with small lenses (Wang et al., 2007b)
275	A→T	90	Ile→Phe	Dominant diffuse total cataract – mild (Graw et al., 2004)
432	C→G	144	Tyr→stop	Total dominant cataract (Graw et al., 2004)
470	G→A	157	Trp→stop	Dominant irregular nuclear cataract (Smith et al., 2000)
Cryge				
1	A→T	1	New protein	Dominant nuclear cataract (Graw et al., 2001a–c)
Intron 1, 66	A→G	4	Splicing, new protein	Dominant nuclear and lamellar cataract (Graw et al., 2002c)
12–21	Deletion	4	New protein	Dominant total lamellar cataract (Graw et al., 2004)
89	Deletion	30	hybrid protein	Dominant nuclear and zonular cataract (Klopp et al., 2001)
134	T→C	45	Leu→Pro	Dominant zonular cataract (Graw et al., 2004)
376	G→A	126	Val→Met	Dominant capsular opacity – mild (Graw et al., 2004)
403	Deletion	135	hybrid protein	Dominant eye lens obsolescence (Cartier et al., 1992)
432	C→G	144	Tyr→stop	Dominant total cataract – severe (Klopp et al., 1998)
427	Retroviral insertion	142	Hybrid protein	Dominant nuclear cataract (Nag et al., 2007)
>2 kb	Insertion/deletion	n.d.	n.d.	Dominant suture cataract (Graw, 1999)
Crygf				
113	T→A	38	Val→Glu	Dominant radial opacity (Graw et al., 2002d)
Crygs				
17	T→C	9	Phe→Ser	Semi-dominant progressive cataract (Sinha et al., 2001)
489	G→A	163	Trp→stop	Recessive nuclear cataract (Bu et al., 2002)
Human mutations				
CRYGC				
13	A→C	5	Thr→Pro	Coppock-like cataract, dominant (Héon et al., 1999)
212–222	Duplication GCGGC	59	hybrid protein	Dominant variable zonular pulverulent cataract (Ren et al., 2000)
327	C→A	109	Cys→stop	Dominant nuclear cataract (Yao et al., 2008)
502	C→T	168	Arg→Trp	Three independent families with lamellar dominant cataract (Devi et al., 2008; Santhiya et al., 2002)

(*continued on next page*)

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Table 6 (*continued*)

nt Position[a]	bp Exchange	aa Position[a]	aa Exchange	Allele symbol (comment)
CRYGD				
43	C→T	14	Arg→Cys	Dominant progressive juvenile-onset punctuate cataract (Stephan et al., 1999)
70	C→A	24	Pro→Thr	Independent dominant cataracts in 3 families (Santhiya et al., 2002; Nandrot et al., 2003; Burdon et al., 2004)
70	C→T	24	Pro→Ser	Dominant non-nuclear polymorphic congenital cataract (Plotnikova et al., 2007)
109	C→A	36	Arg→Ser	Dominant symmetric crystal deposition and greyish opacity (Kmoch et al., 2000)
176	G→A	58	Arg→His	Aculeiform dominant cataract (Héon et al., 1999)
181	G→T	61	Gly→Cys	Dominant corraliform cataract (Li et al., 2008b)
403	C→A	134	Tyr→stop	Dominant cataract (no detailed data; Hansen et al., 2007)
418	C→T	140	Arg→stop	Dominant nuclear cataract (Devi et al., 2008)
470	G→A	157	Trp→stop	Dominant central nuclear cataract (Santhiya et al., 2002)
494	ΔG	165	Frameshift (2 amino acids	Dominant nuclear cataract (Zhang et al., 2007)
CRYGS				
105	G→T	18	Gly→Val	Polymorphic dominant cortical cataract (Sun et al., 2005b)
116	C→G	39	Ser→Cys	Dominant progressive juvenile-onset cataract (Devi et al., 2008)

Δ, deletion.
[a] Beginning at coding sequence (start codon ATG).
[b] According to dB SNP: http://www.ncbi.nlm.nih.gov/SNP/; only such SNPs are listed leading to amino acid exchanges.
[c] Validation included the mouse strains A/J; C57BL/6J; DBA/2J; 129X1/SvJ; 129S1/SvImJ.
[d] Validation included the mouse strains C3H/HeJ; C57BL/6J; BALB/cByJ; 129S1/SvImJ; CZECHII/Ei.

genes might influence the severity of cataract formation, too. In human, the *CRYGD* gene is mainly affected among the γ-crystallin encoding genes; Fig 2 shows the regional distribution of the mutations within the *CRYGD* gene; it is compared also to the corresponding *Crygd* gene of the mouse. In contrast to the *CRYAB* gene, the mutations affect the two major exons rather randomly. Nevertheless, it is obvious that termination mutations occur only in exon 3, both in man and mouse.

There is one mutation among the cataract-causing mutations in the *CRYGC* gene (R168 W), which was stated by Santhiya et al. (2002) to be causative for cataracts because it co-segregated within the family and it did not occur in an appropriate population control. However, in the OMIM database (online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim) it is considered as an SNP (ss38341593). Similarly, the *CRYGD* mutation P24T was recognized in three independent families as very likely to be causative for cataracts based on the same criteria; nevertheless, it is considered as SNP (ss38341594), too. In both cases, no data are available on allele frequencies in any population. Since the annotation process is still under construction, it might be expected that such inconsistencies might be cleaned up soon. As long as no further population data of these alleles are given, they should be

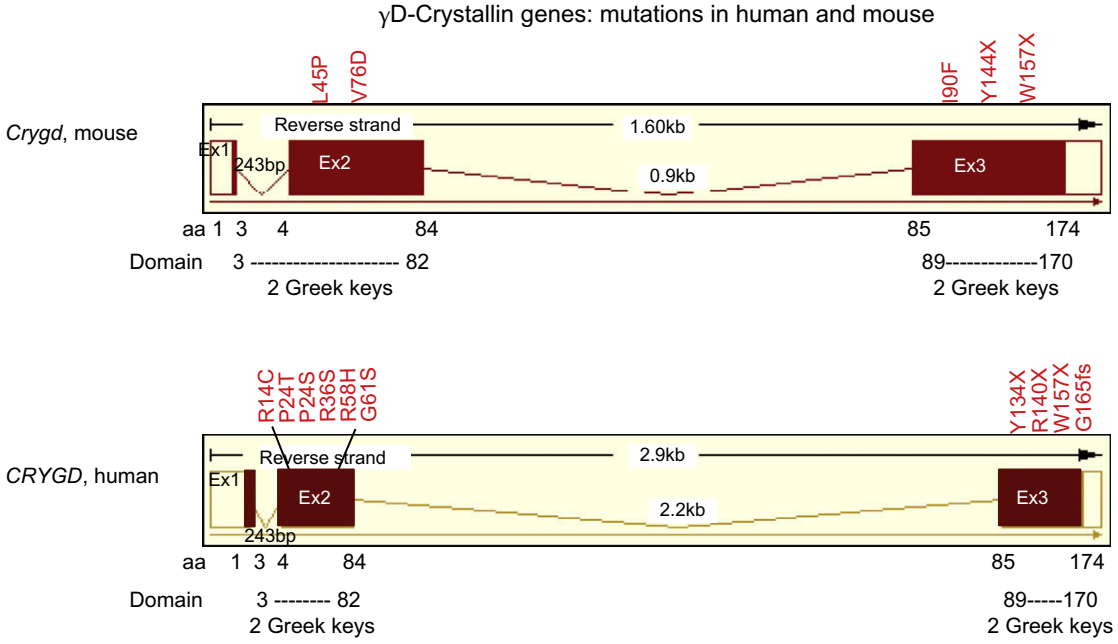

Fig. 3. γD-crystallin genes: mutations in human and mouse. The genomic structure of the mouse (top) and the human (bottom) human *CRYGD* genes are given. Both consist of three exons: in the mouse, intron two is remarkably smaller. Each of the large exons 2 or 3 encodes one γ-crystallin domain (consisting of two Greek key motifs each). There are only cataract-causing mutations observed (red). One of them is identical between mouse and human (W157X). The mutations in exon 3 mainly lead to premature stop codons. Drawings are according to the ENSEMBL release 50 (July 2008; http://www.ensembl.org) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

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discussed as causative Mendelian mutations instead of putative polymorphic sites.

Besides these conflicting data, there are three further SNPs reported in human affecting the amino acid sequence of γ-crystallins. One of them occurs in the *CRYGC* gene with an allele frequency of ~1% in Asia leading to a replacement of Phe by Leu at amino acid position 6. One other SNP might be a sequencing artifact, since only one allele is found in all populations analyzed so far. The third SNP (rs796287) affects the *CRYGD* gene; the G allele has a frequency in African and European populations of ~80% (homozygotes: ~67%), but in Asian population only of ~50% (homozygotes only ~17%). Moreover, there are some additional SNPs in the databases without direct effects on the amino acid composition and in the v5′-upstream regulatory regions. These population-based differences in various SNPs should be tested in large epidemiological studies for a contribution to the formation of age-related cataracts.

4. Enzyme crystallins

The intensive investigation of the evolution of the classical α-, β- and γ-crystallins and their relationship to genes expressed outside the lens leads to the concept of recruitment of lens crystallins by gene sharing. This concept was first described by Wistow and Piatigorsky (1987) and further elaborated by Wistow (1993) and Piatigorsky (1998). A paradigm is the δ-crystallin, which has close similarity to argininosuccinate lyase (Piatigorsky, 1989); however, its presence in the lens is restricted to birds only. Due to focus of this review, only two other enzyme crystallins will be discussed here, the μ-crystallin (gene symbol *Crym*) and the ζ-crystallin (gene symbol *Cryz*), which are present in mouse and human.

4.1. μ-Crystallin (Crym)

The μ-crystallin was first identified as a major lens structural protein in kangaroos (Kim et al., 1992). Later, it was characterized as an NADPH-dependent cytosolic 3,5,3′-triiodo-L-thyronine (T3) binding protein with a molecular weight of 34 kDa (Vié et al., 1997). The corresponding gene (*Crym*) was also found in other mammals like mouse and human, where it is expressed in eye, ear, heart, brain, muscle, skin, thymus and kidney; the expression level in the lenses of mice and humans, however, is much lower than in the kangaroo's lens. The human *CRYM* gene is localized at chromosome 16p13 and the mouse *Crym* at chromosome 7.

Actually, there are two human mutations known in *CRYM*, both are discussed to be causative for non-syndromic, dominant deafness; there were no other pathological effects (including the eye) reported. Both mutations affect the very C-terminal part of the protein (K314T, X315Y) and lead to a loss of the binding capacity for T3. Moreover, in Cos1 cells both mutant proteins show a subcellular localization different from the wild-type protein (Oshima et al., 2006). *In-situ* hybridization experiments in the mouse demonstrated the expression of *Crym* in the lateral region of the spiral ligament and the fibrocytes of the spiral limbus of the inner ear (Abe et al., 2003). Recently, Suzuki et al. (2007) analyzed the mechanism of the disease in a mouse knock-out mutant of *Crym*. Because of the loss of T3 binding capacity in these mutants and the rapid T3 turnover, the decreased concentration of T3 in tissues and serum does not alter the peripheral T3 action *in vivo*.

4.2. ζ-Crystallin (Cryz)

The z-crystallin was found at first in the lenses of guinea pig (Huang et al., 1987); a mutation in the *Crygz* gene leads to an autosomal dominant cataract in the 13/N mutant line of guinea pigs. The mutation is characterized by a deletion of two nucleotides at the acceptor splice site of intron 6 and results in the elimination of exon 7; the corresponding mutant ζ-crystallin is shortened by 34 amino acids (Rodriguez et al., 1992). The ζ-crystallin is structurally related to alcohol dehydrogenase (Borras et al., 1989) and has a quinone-oxidoreductase activity (Rao et al., 1992). The human *CRYZ* gene is assigned to chromosome 1p22–31 and the mouse *Cryz* gene to chromosome 3 (another related gene, ζ-crystallin-like 1 [gene symbol *Cryzl1*] is mapped to mouse chromosome 16); there are no mutations known neither in mouse nor in humans. In the mouse, in addition to being expressed in the eye, *Cryz* is also expressed in the brain, limbs, germ cells, kidney, liver, muscle and mammary glands.

In the guinea pig as well as in the llama, *Cryz* is highly expressed in the lens (Gonzalez et al., 1995) – in contrast, in the mouse its expression in the lens occurs only at enzymatic levels. Gonzalez et al. (1994a) reported the presence of two promoters in the guinea pig *Cryz* gene, one responsible for expression at enzymatic levels and the other responsible for the high expression in the lens. Detailed sequence analysis suggests that the new lens promoter evolved from non-functional intron sequences by accumulation of several mutations (Gonzalez et al., 1995). The guinea pig lens promoter is present neither in the mouse gene nor in the human gene (Gonzalez et al., 1994b). The authors pointed out that this was the first example in which the recruitment of an enzyme as a lens crystallin could be explained by the acquisition of an alternative lens-specific promoter. Additionally, Rao et al. (1997) isolated the ζ-crystallin from bovine lenses. Although the bovine ζ-crystallin has similar biophysical properties, it binds single-stranded DNA instead of quinones.

5. Concluding remarks

This overview on our current knowledge of crystalline genes, their polymorphic sites and disease-causing mutations in mice and humans demonstrates that the crystallins are not only involved in pathogenic processes affecting the ocular lens – as considered for almost 100 years – but they also play important roles in many other organs; therefore, they are involved in a broad variety of other diseases besides cataract. Accepting this point of view, cataract might be understood as a "bio-indicator" for less obvious, but more severe disorders.

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Several databases have been used for the chromosomal localization and expression data of genes, like OMIM (http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM) or MGI (http://www.informatics.jax.org).

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