Targeted Knockout of the Mouse βB2-crystallin Gene (Crybb2) Induces Age-Related Cataract

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PURPOSE: The βB2-crystallin gene (Crybb2) is expressed at an increasing level in the postnatal lens cortex. It has been assumed that the gene functions as structural protein, although this has not been directly tested. Here the in vivo functions of βB2-crystallin are examined via the generation of mice with a targeted disruption of Crybb2.

METHODS. Gene targeting in embryonic stem cells was used to generate mouse lines in which Crybb2 was deleted. Gene structure and protein expression were analyzed by PCR, immunoblot, and two-dimensional gel electrophoresis. Knockout mice were screened for cataract with slit lamp biomicroscopy. Microstructure of lens was analyzed by scanning and transmission electron microscopy. The resistance of crystallins in knockout mice to heat-induced denaturation and oxidative stress was examined.

RESULTS. The lens appeared to develop normally in the first months of life. In older animals, the weight and axial diameter of the lenses of knockout mice were significantly smaller than in wild-type mice. Cataracts were formed in the posterior and anterior cortex several months after birth and cataract severity increased with age. The thermal stability of the supernatant of a lens homogenate was mildly compromised. The knockout lenses also showed decreased resistance to oxidative stress.

CONCLUSIONS. βB2-crystallin is not essential for the normal development of a transparent lens in the mouse. It plays an increasingly important role in maintaining the transparency of the lens after birth, possibly by interacting with other crystallins to increase their resistance to thermal denaturation and oxidative stress. (Invest Ophthalmol Vis Sci. 2008; 49:5476–5483) DOI:10.1167/iovs.08-2179

The ocular lens is characterized by its transparency and high refractive index. Lens clarity derives from crystallins—abundant water-soluble proteins. Crystallins produce a gradient of refractive index from the center to the periphery of the lens.1

Three classes of “classical” crystallins are present in vertebrate lenses: α, β, and γ. α-Crystallins compose up to 50% of the total protein mass of the mammalian lens. When isolated under physiologic conditions, they form a high-molecular mass aggregates of ∼800,000 Da.1 There are two α-crystallins, αA and αB, which share approximately 60% sequence identity.2,3 The α-crystallins function as both structural proteins and chaperones in the lens. The β-crystallins are a family of basic (βB1, -2, and -3) and acidic (βA1, -2, -3, and -4) polypeptides.4–7 The sequences of their corresponding globular domains exhibit between 45% and 60% identity with each other, and approximately 30% with γ-crystallins. The mouse genome contains seven γ-crystallin genes. The γA to γF-crystallin genes are closely linked in a tandemly repeated gene cluster and are highly similar in sequence, particularly in their second exon, which encodes the N-terminal domain of the protein. The seventh gene, encoding γS-crystallin, is located on another chromosome and has a more divergent sequence. β- and γ-crystallins form a superfamily of proteins because of their structural similarity.7 They are presumed to function as structural proteins.

In the past few years, several α-crystallin gene knockout mice have been generated to study the function of these proteins. Cataract formed in the αA-crystallin-null mouse due to inclusion bodies containing αB-crystallin1 and γ-crystallins,8 indicating a role for αA-crystallin in maintaining the solubility of other crystallins. These lenses are also smaller than those of wild-type mice as a result of higher levels of apoptosis in dividing epithelial cells.9 αA/βB-crystallin double knockout mouse lenses are significantly smaller than wild-type, and fiber cell formation is severely disturbed10 due to caspase-dependent fiber cell degeneration.11 Lenses of the αB-crystallin-null mice developed normally and were remarkably similar to wild-type. However, these mice died before six months of age, precluding assessment of whether αB-crystallin protects against cataract formation in older animals.12 αB-crystallin is expressed at low levels in the lens epithelium and numerous other tissues, and its expression is enhanced in stress conditions. Using αB-crystallin knockout mice αB and/or the closely-related gene, Hspb2, is required to maintain cellular integrity in some skeletal muscles.12 Further studies showed hyperproliferation of lens epithelial cells,13 exacerbation of inflammation, and demyelination14 in αB knockout mice. Interestingly, αB-crystallin also appears to play a role in the promotion of tumor angiogenesis.15

Crybb2 (GeneID: 12961) is located in chromosome 5, encoding 5 exons. Its protein product consists of 205 amino acids, and it has a molecular weight of 23,379.20 Da and isoelectric point of 6.54. In mammals, the β-crystallin genes are reported to be specifically expressed in fiber cells. Expression of the β-crystallins is under developmental control. The βB2-crystallin gene is a late-expressed gene, being expressed in
rodents only in the postnatal lens, while the βB1- and βB3-crystallin genes are early genes and their products are found primarily in the lens nucleus. In the human lens, a similar expression pattern is found, but there is a much higher level of βB2-crystallin than in the rodent lens. At high concentrations or in the lens, βB2-crystallin forms hetero-oligomers with other beta crystallins. The interaction domains have been speculated to be the β-sheets, each of which is formed by two or more β-strands. βB2-crystallin consists of 16 β-strands, eight in the N-terminal domain and eight in the C-terminal domain.

βB2-crystallin is also expressed within the retina, including in filopodial protrusions and axons of adult retinal ganglion cells (RGCs). Axonal regeneration appears to be related to βB2-crystallin movement. From lens enhances the growth of axons in retinal explants and primary hippocampal neurons. Moreover, βB2-crystallin mRNA and protein are expressed in tissues outside of the eye including brain, and testis. Extralenticular and extraocular expression of βB2-crystallin suggests that it has nonrefractive functions in these tissues.

Since gene knockout is an important approach to study gene function, we generated the βB2-crystallin knockout mice. This is the first knockout reported for a β-crystallin gene. The function of βB2-crystallin in lens development and in maintaining the transparency of the lens was directly examined in vivo.

METHODS

βB2-crystallin gene knockout mice were produced by the Ingenious Targeting Laboratory, Inc. (Stony Brook, NY) at our request. Briefly, a 10.5 KB mouse genomic DNA fragment was cloned from a mouse C57BL/C BAC library. This genomic fragment contains exons 1 to 4 of Crybb2. A 2942bp sequence, encompassing the first and the second exons of Crybb2, was replaced with the Neo cassette. One side of the Neo cassette was inserted 30 bp upstream of exon 1. The other side of the Neo cassette was inserted 35 bp downstream of exon 2. Ten micrograms of targeting vector was linearized by NotI and transfected by electroporation of IC1 ES cells (C57BL/C) embryonic stem cells. After selection in G418, surviving colonies were expanded and PCR analysis was performed to identify clones that had undergone homologous recombination. PCR was done using primer pairs BBCA1 and Neo1. Primer BBCA1 is located 12 bp upstream of the short arm side, with a sequence of 5’-GGAGAAACTTGTGGGCTAA-3’. Primer Neo1 is located in the 5’ promoter region of the neo gene cassette and has the sequence 5’TGGCGAGCCAGAGCCTTGTTGATCG-3’. Positive clones produced a 2.5 KB PCR fragment. This 2.5 band will also represent the KO band. Chimeric males were mated to C57BL/C females, and heterozygous offspring identified by PCR were interbred to produce mice homozygous for the knockout allele. Mice were maintained at the Laboratory Animal Center of the Second Military Medical University. All procedures were carried out in accordance with the Chinese legislation on the use and care of laboratory animals and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the respective university committees for animal experiments.

PCR Analysis

Genomic DNA was extracted from mouse tail using a kit (Universal Genomic DNA Extraction Kit; Takara Biotechnology (Dalian) Co., Ltd., Dalian, PRC). Primer Neo1 is located in the 5’ promoter region of the neo gene cassette and the first pair of primers was used to detect the inserted Neo cassette. The second pair of primers was used to detect the wild-type. The primers used to identify the genotype of the mice were Neo 1: 5’TGGCGAGCCAGAGCCTTGTTGATCG-3’ and Reverse: 5’CTGAATGCTGGATTTGACATCG-3’. The second pair of primers was Forward: 5’-GGTCCAGAAGGAGGAA-3’ and Reverse: 5’GGAGAAGCTTGGCGCTAA-3’.

Western Blot Analysis

Isolated lenses were weighed and then homogenized in Tris-HCl buffer (pH 7.4) containing 0.2 M Tris, 0.1 M HCl, and protease inhibitor (Calbiochem, San Diego, CA). Soluble and insoluble fractions were separated by centrifugation at 10,000g at 4°C for 20 minutes. Protein concentration of soluble fraction was determined using a protein assay kit (BCA Protein Assay Kit; Pierce Biotechnology, Rockford, IL). Proteins from soluble fraction were electrophoretically separated in a 12% SDS/polyacrylamide gel and transferred onto nitrocellulose (Mini-PRO-TEIN 3 Electrophoresis Cell; Bio-Rad Laboratories, Hercules, CA). The blots were blocked for 2 hour at room temperature with PBS containing 5% dried nonfat milk. They were then incubated overnight at 4°C with the primary antibody in PBS containing 2.5% dried nonfat milk. Polyclonal antibodies to βB2-crystallin (N-20) were purchased from Santa Cruz Biotechnology, Inc. and were used at 1:2000 dilution. HRP-labeled rabbit Anti-goat IgG (H+L) was purchased from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD). Tissue stain (HRP-DAB) was purchased from TIANGEN Biotech Co. Ltd. (Beijing, PRC). The detailed protocol for PCR and Western blot were as described previously.

Immunohistochemical Studies

Eyeballs were fixed in 4% paraformaldehyde for 24 hours, rinsed in 1X PBS, and paraffin embedded. Four-μm-thick sections were prepared, deparaffinized, and blocked with 1% BSA in 1X PBS for one hour at room temperature. The slides were then incubated with a 1:2000 dilution of monoclonal antibodies to βB2-crystallin (N-20, Santa Cruz Bio-technology, Inc., Santa Cruz, CA) at room temperature for one hour, and the signal was detected using an anti-goat horseradish peroxidase kit (Zymed Laboratories, Burlingame, CA) using diaminobenzidine as a substrate.

Slit-Lamp Biomicroscopy

Pupils were dilated with eye drops containing 1% tropicamide and eye drops containing 5% phenylephrine hydrochloride. Approximately 25 minutes later, the mice were examined with a slit lamp.

Transmission and Scanning Electron Microscopy

Eyes were fixed by immersion for at least 24 hours at room temperature in a freshly prepared solution of 2.5% glutaraldehyde and 0.6% sucrose, buffered to pH 7.2 with 50 mM sodium cacodylate. Lenses were postfixed in OsO4 buffered with 150 mM sodium-potassium phosphate (pH 7.4), embedded, sectioned, and stained for electron microscopy. They were examined by electron microscopy (Hitachi H-800 Transmission Electron Microscope and Hitachi H-520 Scanning Electron Microscope; Hitachi, Tokyo, Japan).

Two-Dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis was carried out as described previously. Briefly, lenses of were homogenized in 250 μL lysis buffer and the soluble supernatants were concentrated as described in Western blot. One hundred-twenty micrograms supernatant was run in the first-dimension isoelectric focusing gel, using ampholine, pH 5–8 (Multiphor II Electrophoresis System; GE Healthcare UK Ltd, Little Chalfont, Buckinghamshire, England). In the second dimension, 12% SDS polyacrylamide gel was used. Gels were stained with Coomassie

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Heat-Induced Denaturation Assay

Fresh lenses from 8-week-old wild-type and knockout mice were disrupted in 2500 μL Tris-HCl buffer (0.2 M Tris, 0.1 M HCl, pH 7.4). The homogenate was centrifuged for 20 minutes at 4°C at 10,000 g in a microfuge. Protein concentrations of supernatant were determined by assay (BCA; Pierce Biotechnology) and samples were diluted to 6 mg/mL protein. Samples were placed in a temperature-controlled (50°C) water bath. Absorbance at 360 nm was monitored by spectrophotometer (Unicam-UV300; Thermo-Forma, San Jose, CA).

Oxidation Exposure

Fresh lenses from 8-week-old mice were removed from the eyeballs and preincubated in TC-199 medium for 2 hours in a CO2 incubator. Lenses were then transferred to 96-well plates containing 200 μL fresh TC-199 medium containing 0.1 mM H2O2 and 2.31 units glucose oxidase (GO) to achieve a final and constant concentration of approximately 0.2 mM H2O2 and incubated for 24 hours.32 Lens images were captured on culture plates at 0, 12, and 24 hours after H2O2 treatments using a dark field microscopy (Olympus BX40; Olympus, Tokyo, Japan).

Soluble fractions of lens protein of wild-type and knockout mice were prepared as for the heat-induced denaturation assay. Protein concentrations of supernatant were determined by assay and samples were diluted to 6 mg/mL protein using the Tris-HCl buffer containing 0.1% glucose, 0.1 mM H2O2, and 2.31 units GO. Samples were placed in a 37°C water bath. Absorbance at 560 nm was monitored by spectrophotometer (Unicam-UV300; Thermo-Forma).

Statistical Analysis

Each assay was performed in triplicate, and all data are expressed as mean or mean ± SD. Statistical analyses were performed using independent samples and Student’s t-test. Two-tailed values of P < 0.05 were considered statistically significant.

RESULTS

Phenotypic Characterization of Knockout Mice and Evaluation of βB2-crystallin Expression

The mouse βB2-crystallin gene was disrupted by inserting a neo expression cassette to replace the first and the second exons and preventing the production of a functional transcript from this locus. Lines of knockout mice were established from targeted embryonic stem cells. PCR analysis of the resulting mice confirmed that the expected recombination event had occurred (Fig. 1). To verify that no βB2 protein was produced by the knockout mice, the soluble fraction of lens homogenates from 6-week-old mice were analyzed by SDS-PAGE and immunoblotting with polyclonal antibodies to βB2-crystallin. The major band corresponding to βB2-crystallin was not evident in the knockout lane of the Coomassie blue-stained gel (Fig. 2A). Similarly, Western blotting also failed to detect any βB2-crystallin in the lenses of knockout mice (Fig. 2B). No cross-reactivity of this antibody with other proteins was observed, even using a more sensitive enhanced chemiluminescence (ECL) method (data not shown). By immunohistochemistry using a polyclonal antibody raised against βB2-crystallin, we detected that βB2-crystallin was expressed mainly in the cortex of wild-type lenses (Fig. 2C). βB2-crystallin expression was not detected in the knockout mice (Fig. 2D). These data suggest that the intended alteration of Crybb2 was successful in eliminating βB2-crystallin from the lens.

Characterization of Lens Structure and Development

The external morphologies of whole eyes and dissected lenses from the Crybb2 knockout mice did not appear different from wild-type at birth. With aging, however, significant difference in both lens weight and diameter was observed between the knockout mice and wild-type; the difference grew bigger with increasing age (Fig. 3). At 18 months of age, Crybb2 knockout mice were significantly smaller than wild-type. At this age, lenses from knockout eyes weighed 34% less than those of wild-type littermates (Fig. 3A), and their axial diameters were 5% smaller than wild-type lenses (Fig. 3B).

Slit-lamp micrographs showed development of lens opacities at 6 to 8 weeks of age, which progressed in severity with age. Punctate cataracts located in the posterior portion of the cortex could be observed in Crybb2 knockout mice at 6 to 8 weeks of age and became obvious by 4 months of age (Fig. 4A). Punctate anterior cortical cataracts began to appear at 6 months of age (Fig. 4B). At the age of about 1 year, the cataract became more severe and the opaque area included the Y-sutures, forming a fishbone pattern (Fig. 4C). The whole lens became opaque by 18 months of age (Fig. 4D). No obvious nuclear cataract was observed in younger Crybb2 knockout lenses. No cataract was observed in the wild-type mice before 12 months of age (Figs. 4E, 4F).

Transmission and Scanning Electron Microscopy

Scanning electron microscopy showed that irregularly shaped fiber cells with numerous cell-surface projections can be observed in the posterior subcapsular regions and the anterior cortical region of 6-month-old mice (Fig. 5A). In contrast, radial columns of elongated fiber cells of uniform size and shape, with ball and socket interdigitations, were present in the posterior subcapsular regions and the anterior cortical regions of 6-month-old wild-type lenses (Fig. 5B).

Irregularly shaped fiber cells could be observed in the posterior subcapsular regions and in the anterior cortical region of 6-month-old mice (Fig. 5C) by transmission electron microscopy. In contrast, uniform fiber cells were present in the posterior subcapsular regions and the anterior cortical regions of 6-month-old wild-type lenses (Fig. 5D).
Two-Dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis of lens proteins (Fig. 6) revealed that all the major and minor crystallin spots that were present in wild-type mice were also present in the knockout mice, except, of course, βB2. The typical age-related changes were seen in the lenses of both genotypes.

Heat-Induced Denaturation Assay

The ability of lens proteins to resist heat-induced denaturation was studied (Fig. 7). When the soluble fraction of lens homogenate from wild-type mice was heated to 50°C, there was moderate increase in turbidity of the solution over the 60-minute experiment. The lens homogenate supernatant without βB2 showed a dramatic increase in turbidity (and therefore, protein denaturation) and reached twice the level of wild-type lenses at 60 minutes, suggesting that the overall protective activity in the βB2 knockout lens was mildly impaired.

Oxidation Exposure

Lenses exposed to 0.2 mM H$_2$O$_2$ maintained clarity in the first 3 hours of culture and then developed opacity at the anterior and posterior cortical regions, with the affected area expanding with increasing time. Haziness in these lenses started from the polar regions and extended to the entire cortex as the exposure time was prolonged. The degree of opacity in the Crybb2 knockout mice was significantly more severe than that in the wild-type mice (Fig. 8).

The ability of soluble lens proteins to resist oxidation-induced denaturation was studied (Fig. 9). There was mild increase in turbidity of the solution over the 5-hour experiment. The lens homogenate supernatant without βB2 showed a moderate increase in turbidity (and therefore, protein denaturation), suggesting that the overall resistance to protein oxidation was mildly impaired in the Crybb2 knockout lens.

DISCUSSION

We generated knockout mice that produced no detectable βB2-crystallin protein in their lenses. Our principal finding

Figure 2. Evaluation of βB2-crystallin expression by gel electrophoresis, immunoblot, and immunohistochemistry. Soluble fractions of lens homogenates from Crybb2 knockout and wild-type mice (1-, 2-, 3-, and 4-months-old) were subjected to SDS-PAGE. Each lane contains 60 µg of total protein. Coomassie blue staining (A) showed that the prominent βB2 band seen in wild-type lenses was not detected in Crybb2 knockout lenses (arrow). Immunoblot analysis with polyclonal antibodies to βB2-crystallin confirmed the absence of βB2 in the knockout lenses (B). No cross-reactivity of this antibody with other proteins was observed, even using a more sensitive ECL method (data not shown). Immunohistochemical studies found that βB2-crystallin was mainly expressed in the cortex of wild-type lenses (C), but staining was not detected in the knockout (D). Bar: (C, D) 100 µm.

Figure 3. The weight and axial diameter of knockout and wild-type lenses. Data are mean ± SD. Lenses from the eyes of Crybb2 knockout mice weighed 10% to 34% less than those of wild-type littermates (P < 0.05 or P < 0.01) (A), and their axial diameters were 3% to 5% smaller than wild-type lenses (P < 0.05 or P < 0.01) (B).
Crybb2 from these animals is that, in the absence of βB2-crystallin, cataract forms slowly after birth and progresses in severity with aging. βB2-crystallin begins to be expressed after birth in rodents, which means that it does not contribute to the development of the fetal lens. Our finding that Crybb2 KO lens develops normally and maintains transparency for months after birth is consistent with this later function of βB2-crystallin.

With aging, the crystallins undergo extensive modification under the effects of oxidation, heat, proteolysis, deamidation, etc. These effects can convert the water-soluble crystallins into water-insoluble aggregates. It was reported that βB2-crystallin is the most thermally stable and resistant to modification of the beta crystallins. Based on this observation, βB2-crystallin was hypothesized to be crucial to maintain the solubility of other crystallins in the adult. In that way, βB2-crystallin seems to be not only a structural protein but also functions to protect other crystallins. In the knockout mouse lens, this function of βB2-crystallin is abolished, so cataract tends to form.

Mutations have been reported in the human and mouse βB2-crystallin genes. For example, a dominant mutation caused by an in-frame deletion of 12 bp in Crybb2 is responsible for the Philly cataract in the mouse. Aey2, which is associated with an A→T exchange within exon 6 of Crybb2, also leads to progressive dominant cataract. Q155X mutation of Crybb2 leads to a progressive polymorphic congenital cataract in a Chinese family and D128V mutation outside exon 6 of the human Crybb2 is the reason for cataract in a family of German descent.

In each of these cases, the mutations are dominant. This raises the possibility that the altered structure of the resulting protein is the cause of increased aggregation and cataract formation. Cataract-causing mutations of Crybb2 usually affect functional motifs that are essential for normal protein folding. Graw et al. speculate that interaction of mutated βB2-crystallin with other lens proteins causes rapid aggregation, leading to the formation of insoluble, high-molecular-weight molecules, finally resulting in cataract. In other words, these dominant cataracts could result from a ‘gain of function’ rather than loss of the normal function of βB2-crystallin. In the Crybb2 knockout mice, no protein product was observed. In this case, the cataracts that form must result from loss of function. This result shows that βB2-crystallin is not only just a structural protein, but also a functional protein in maintaining the transparency of the lens possibly by its interaction with other crystallins.

Biochemical studies indicated that the cataractous process in the Philly mouse is associated with a variety of osmotic changes. Lenticular sodium rapidly increased and potassium levels decreased. Concomitant with cataract formation, there was an increase in total lenticular calcium and a decrease in reduced glutathione and adenosine triphosphate. We noted no changes in lens sodium, potassium, calcium, and glutathione levels in the Crybb2 knockout mouse (data not shown). This provides further evidence that the mechanism of cataract formation is different in these cataract models.

Two-dimensional electrophoresis revealed that βB2-crystallin accounted for approximately 0.2% of the total lens protein.

**FIGURE 4.** Slit-lamp biomicroscopy examination of wild-type and Crybb2 knockout lenses. Mice were examined with a slit lamp after dilatation of the pupils. A punctate cataract was first observed in the posterior portion of the cortex at 6 to 8 weeks of age and became more obvious at 4 months (A). Punctate anterior cortical cataracts began to appear at 6 months of age (B). At the age of 1 year, the cataract region includes the Y-sutures and looks like a ‘fishbone’ pattern (C). The whole lens becomes opaque by 18 months of age (D). No cataract was observed in 12-month-old wild-type mice (E, F).

**FIGURE 5.** Representative scanning and transmission electron micrographs of 6-month-old Crybb2 knockout mice. Scanning electron microscopy showed irregularly-shaped fiber cells with numerous cell surface projections in the posterior subcapsular regions and the anterior cortical regions of 6-month-old lenses (A). In contrast, radial columns of elongated fiber cells of uniform size and shape, with ball and socket interdigitations, were present in the posterior subcapsular regions and the anterior cortical regions of 6-month-old wild-type lenses (B). Irregularly shaped fiber cells were observed in the posterior subcapsular and the anterior cortical regions of 6-month-old lenses (C) by transmission electron microscopy. In contrast, uniformed fiber cells were present in the posterior subcapsular regions and the anterior cortical regions of 6-month-old wild-type lenses (D). Bars: (A, B) 20 μm; (C, D) 1 μm.
at birth. However, βB2-crystallin accumulated with age, until it became the major crystallin by 51 weeks of age. We found that Crybb2 knockout lenses were 10% smaller than wild-type at 4 weeks of age, a value that compared well with the relative abundance of βB2-crystallin reported by Ueda et al. at this age (9.8%).

Our immunohistochemistry results indicated that βB2-crystallin is mostly expressed in the lens cortex. This observation is in agreement with the location of the cataracts in the Crybb2 knockout lenses, which first formed in the posterior and anterior cortex, while the lens nucleus remained clear.

Irregularly shaped fiber cells with numerous cell surface projections were observed in the Crybb2 knockout lenses using scanning electron microscopy. Transmission electron microscopy showed distorted fiber cells, especially in junctional areas. Lens fiber cells are filled with properly folded crystallins. In the knockout mice, expression of βB2-crystallin is abolished, reducing the intracellular crystallin concentration. Reduced protein content may be one reason for the distorted appearance of the fiber cells. Whatever the cause, our results show that βB2-crystallin plays an important role in the later development of lens fiber cells.

Oxidative stress and protein denaturation are thought to contribute to age-related cataract. We tested the stability of the crystallins remaining in the Crybb2 knockout mice. The results showed that their resistance to heat or oxidative stress was moderately diminished. Proteins must resist thermal denatur-
than wild-type. Data are mean ± SD.

FIGURE 9. Oxidation-induced denaturation of proteins in knockout lenses. Lens supernatants containing similar amounts of protein were diluted to 6 mg/mL protein using the TC-199 medium to achieve a final concentration of 0.1 mM H$_2$O$_2$ and 2.51 units of glucose oxidase (GO). Samples were placed in a 37°C water bath and absorbance at 360 nm was monitored. Supernatants from Crybb2 knockout lenses showed a more rapid increase in turbidity (and therefore, protein denaturation), suggesting that the KO lens had less overall resistance to oxidation than wild-type. Data are mean ± SD.

In conclusion, Crybb2 knockout mice develop cataracts 6 weeks after birth. The cataract lesions begin in the anterior cortex, progressing in severity and extent with increasing age. The remaining lens proteins showed reduced stability to heat-induced denaturation and oxidative stress, suggesting that the KO lens had less overall resistance to oxidation than wild-type. Data are mean ± SD.

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