Ethylnitrosourea-Induced Mutation in Mice Leads to the Expression of a Novel Protein in the Eye and to Dominant Cataracts

Jochen Graw,* Norman Klopp,* Jana Löster,* Dian Soewarto,† Helmut Fuchs,† Johannes Becker-Follmann,‡ André Reis,‡,[1] Eckhard Wolf,§ Rudi Balling* and Martin Hrabé de Angelis†

**GSF-National Research Center for Environment and Health, Institute of Mammalian Genetics and †Institute of Experimental Genetics, D-85764 Neuherberg, Germany, ‡Institute of Molecular Genetics, Max-Delbrück-Center for Molecular Medicine, D-13122 Berlin, Germany and §Lehrstuhl für Molekulare Tierzucht und Haustiergenetik, Ludwig-Maximilians-Universität München, D-81377 Munich, Germany*

Manuscript received August 17, 2000
Accepted for publication December 4, 2000

ABSTRACT

A novel ENU-induced mutation in the mouse leading to a nuclear and zonular opacity of the eye lens (*Aey1*) was mapped to chromosome 1 between the markers *D1Mit303* and *D1Mit332*. On the basis of the chromosomal position, the γ-crystallin encoding gene cluster (*Cryg*) and the βA2-crystallin encoding gene *Cryba2* were tested as candidate genes. An A → T mutation destroys the start codon of the *Cryge* gene in the mutants; this mutation was confirmed by the absence of a restriction site for *Nco*I in the corresponding genomic fragment of homozygous mutants. The next in-frame start codon is 129 bp downstream; this predicted truncated γE-crystallin consists of 131 amino acids, resulting in a molecular mass of 14 kD. However, another open reading frame was observed just 19 bp downstream of the regular *Cryge* start codon, resulting in a protein of 119 amino acids and a calculated molecular weight of 13 kD. Western blot analysis using polyclonal antibodies against γ-crystallins or the novel Aey1-specific protein demonstrated the specific expression of the Aey1 protein in the cataractous lenses only; the truncated form of the γE-crystallin could not be detected. Therefore, it is concluded that the novel protein destroys the sensitive cellular structure of the eye lens.

THE β- and γ-crystallins were first characterized by Mörner (1893) more than 100 years ago. Nowadays, they are recognized as members of one β/γ-crystallin superfamily. The corresponding genes are expressed preferentially in the eye and mainly in the ocular lens; low expression can be found also in the retina (Head *et al.* 1995; Jones *et al.* 1999), brain, and testes (Mugabo *et al.* 2000). The common characteristic of all β- and γ-crystallins is the so-called Greek key motif, which allows a dense packing of proteins in the ocular lens. The *Cryg* genes in all mammals consist of 3 exons: the first one codes only for three amino acids, and the subsequent two are responsible for two Greek key motifs each. Biochemically, the γ-crystallins are characterized as monomers with a molecular mass of 21 kD (for reviews see Wistow and Piatigorsky 1988; Graw 1997; Slingsby and Clout 1999).

Six members of the *Cryg* family (*Cryga* → *Crygf*) are located in a cluster on mouse chromosome 1 or human chromosome 2q33–35, whereas the seventh *Cryg* gene (*Crygs*) maps on mouse chromosome 16 and human chromosome 3, respectively. The *Cryba2* gene encoding the βA2-crystallin is located ~8 cM distal to the mouse *Cryg* gene cluster; in human, the relative map positions of the *CRYG* gene cluster and the *CRYBA2* gene are similar, with the *CRYBA2* located at chromosome 2q34–36 (Hulsebos *et al.* 1995).

In mice, several mutations in the *Cryg* genes have been identified and demonstrated to lead to cataracts: the mutation ethylnitrosourea (*ENU)-436* affects the *Cryga* gene, the *Nop* mutation affects the *Crygb* gene (Klopp *et al.* 1998), and *Lop12* affects the *Crygd* gene (Smith *et al.* 2000). Two cataract-causing alleles of *Cryge* have been reported so far in the mouse: *Elo* (Cartier *et al.* 1992) and *Cat2*^{t} (Klopp *et al.* 1998). Several hereditary cataracts in man have also been shown to be caused by mutations in *CRYG* genes (Héon *et al.* 1999; Stephan *et al.* 1999; Kmoch *et al.* 2000; Ren *et al.* 2000), while the human *CRYGE* and *CRYGF* genes are pseudogenes.

While analyzing mice obtained from a large-scale ENU mutagenesis program (Hrabé de Angelis and Balling 1998; Hrabé de Angelis *et al.* 2000; http://www.gsf.de/ieg/groups/enu-mouse.html), we identified several mutants with dominant cataracts. Here we report the map position and identification of the underlying mutation in *Aey1*; this mutation maps to mouse chromosome 1 and is a mutation affecting the start codon of the *Cryge* gene. Interestingly, instead of a (modified) γE-crystallin, a novel protein is formed, which does not exhibit any

Corresponding author: Jochen Graw, GSF-National Research Center for Environment and Health, Institute of Mammalian Genetics, D-85764 Neuherberg, Germany. E-mail: graw@gsf.de

[1]*Present address:* Institute of Human Genetics, University of Erlangen-Nürnberg, D-91054 Erlangen, Germany.

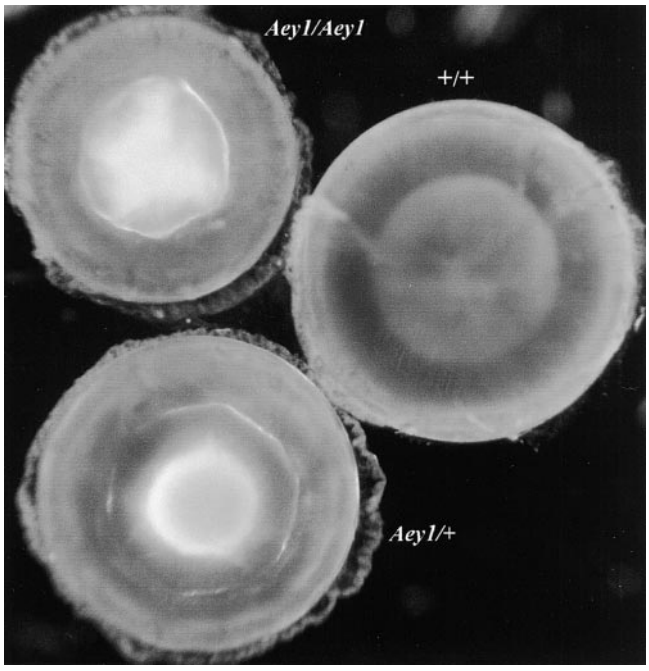

FIGURE 1.—Morphology of cataract formation in the *Aey1* mutant. Gross appearance of unfixed lenses from 3-week-old mice under the dissecting microscope. Right, a clear, wild-type lens; lower left, a slightly smaller lens of a heterozygous mutant with a nuclear cataract and a moderate zonular opacity; top left, a strong nuclear opacity from a homozygous *Aey1* mutant.

remarkable similarity to another previously described protein.

MATERIALS AND METHODS

Animals: C3Heb/FeJ male mice were treated with ENU (160 mg/kg) at the age of 10 wk according to EHLING *et al.* (1985). Treated mice were mated with untreated female C3HeB/FeJ mice and kept under specific pathogen-free conditions at the GSF Research Center according to the German law on the protection of animals. The resulting offspring were monitored for cataract phenotypes within the ENU mouse mutagenesis screen project (HRABÉ DE ANGELIS and BALLING 1998; HRABÉ DE ANGELIS *et al.* 2000).

Cataracts were identified at weaning using a slit lamp (SLM30; Zeiss, Oberkochen, Germany). Homozygous mutant lines were then established and have been maintained by brother × sister matings.

The mutation was mapped by mating heterozygous carriers (first generation) to wild-type C57BL/6J mice; offspring (second generation) with cataracts were backcrossed to wild-type C57BL/6J mice. DNA was prepared from tail tips of 42 cataractous offspring of the third generation (G3) according to standard procedures. For the genome-wide linkage analysis, several markers were used for each chromosome (GRAW *et al.* 1999). All data concerning the linkage of genes or markers are taken from the Mouse Genome Informatics database (http://www.informatics.jax.org).

Morphological analysis: For gross documentation, lenses were enucleated under a dissecting microscope (Leica MZ APO) and photographed. For detailed histological analysis, eye globes were fixed for 3 hr in Carnoy's solution and embedded in JB-4 plastic medium (Polysciences, Eppelheim, Germany) according to the manufacturer's procedure. Sectioning was performed with an Ultratom OMU3 (Reichert, Walldorf, Germany). Serial 2-μm transverse sections were cut with a dry glass knife, collected in water drops on glass slides, and, after drying, the sections were stained with Methylene blue and basic Fuchsin. The sections were evaluated using a light microscope (Axioplan; Zeiss). Images were acquired by means of a scanning camera (Progress 3008; Jenoptik, Jena, Germany) equipped with a screen-capture program (KS100; Carl Zeiss Vision, Hallbergmoos, Germany) and were imported into an image processing program (Photoshop V5.0; Adobe, Unterschleißheim, Germany). All wild-type controls were of the strain C3H/El.

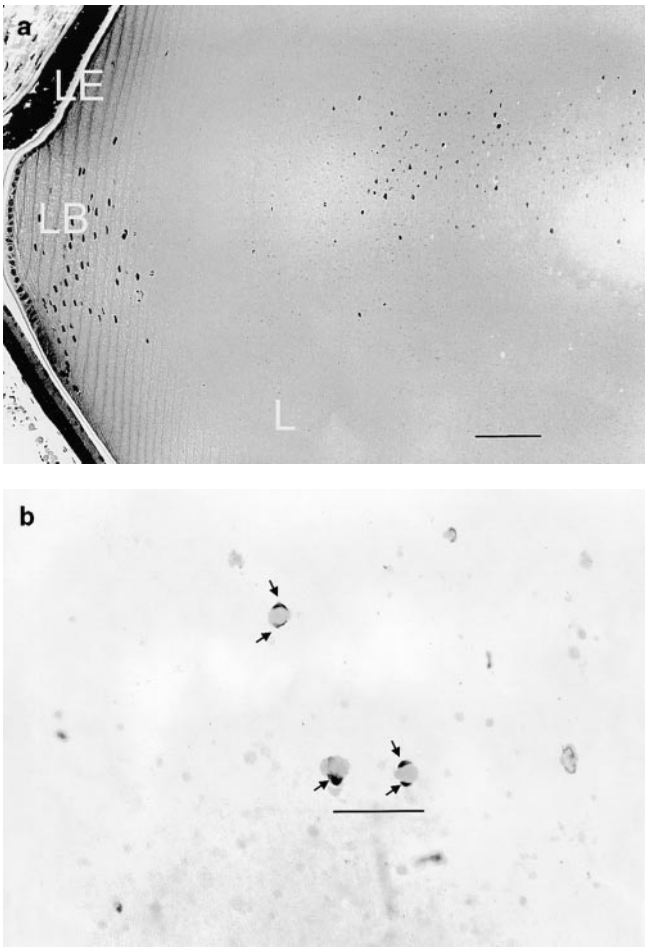

FIGURE 2.—Histology of a juvenile lens. Section through the lens of a 12-week-old homozygous *Aey1* mouse; staining was done with Methylene blue and basic Fuchsin. (a) No irregularities in the arrangement of the lens epithelium or lens fibers become apparent. Remnants of the fiber cell nuclei are visible only in the core of the lens. Bar, 100 μm. L, lens; LB, lens bow; LE, lens epithelium. (b) High-power view of the abnormal fiber cell nuclei in the core of the lens. Arrows point to the condensed chromatin at the nuclear poles. Bar, 20 μm.

Isolation of RNA, DNA, and PCR conditions: Genomic DNA was prepared from spleen or tail tips of 3-week-old mice according to standard procedures. RNA was isolated from lenses (stored at −80°) of newborn mice. cDNA synthesis and PCR for mouse *Cryg* genes using genomic DNA or cDNA as tem-

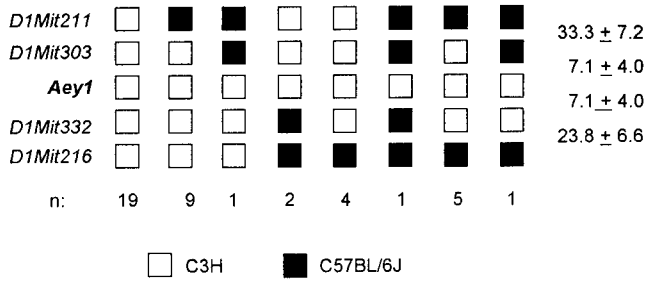

FIGURE 3.—Haplotype analysis of the *Aey1* mutant. Forty-two heterozygous *Aey1* mutants from G3 of an (*Aey1* × C57BL/6J) backcross have been genotyped with respect to the markers *D1Mit211*, *D1Mit216*, *D1Mit303*, and *D1Mit332*. The analysis demonstrated that *Aey1* is located between the markers *D1Mit303* and *D1Mit332*. Seven of the 42 G3 offspring showed double recombinations; 5 of them were observed between the most distant markers *D1Mit211* and *D1Mit216*.

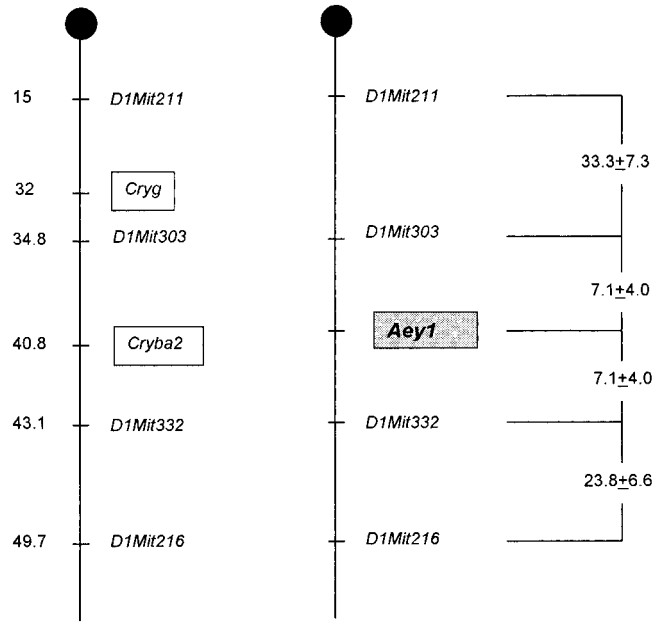

FIGURE 4.—Partial map of mouse chromosome 1. The location of the *Aey1* mutation in relation to the markers tested, and to the candidate genes *Cryba2* and the *Crygc* cluster (right), is shown. Left, the sequence of markers and genes is depicted as outlined by the 2000 Chromosome 1 Committee Report, representing a composite map derived by combining data from various sources. This might explain the different position of the marker *D1Mit303* in the Committee Report and the particular cross reported here. Numbers to the left of the chromosome indicate the genetic distance in centimorgans from the centromere.

plate were performed essentially as described previously (KLOPP *et al.* 1998).

PCR for *Cryba2* used the primer pair 5′-AGCGAACAC CAGGGTCGTGC-3′ (for the left side) and 5′-GAGCTTTTAT TGAGAATCTTSCTGGTGATGAC-3′ (for the right side). Using an annealing temperature of 55°, a 690-bp fragment was amplified.

PCR products were sequenced commercially (SequiServe, Vaterstetten, Germany), either after cloning into the pCR2.1 vector (Invitrogen, Leek, The Netherlands) or directly after elution from the agarose gel using kits from QIAGEN (Hilden, Germany) or Bio-Rad (Munich, Germany), and subsequent precipitation by ethanol and glycogen.

Biochemical analysis of the lens extracts: Computer-assisted prediction of the biochemical properties of the mutated protein were performed using the Proteomics tools of the ExPASy Molecular Biology server (http://www.expasy.ch). Western blot analysis was performed according to standard procedures (KLOPP *et al.* 1998). The γ-crystallin antibody was a gift from Dr. Roy Quinlan (Dundee, Scotland, United Kingdom). To detect the novel protein, a specific antibody was made commercially against the peptide STSERTTEAKWWRSQ corresponding to amino acids 86–100 shown in Figure 5 (Sequence Laboratories, Göttingen, Germany).

General: Chemicals were from Merck (Darmstadt, Germany) or Sigma Chemicals (Deisenhofen, Germany). The enzymes used for cloning and reverse transcription were from Roche (Mannheim, Germany), and restriction enzymes were from MBI Fermentas (St. Leon-Rot, Germany), if not otherwise mentioned.

RESULTS

Phenotype and lens morphology: The *Aey1* mutant was initially identified as a nuclear and zonular cataract by slit lamp analysis of 3-week-old mice. This phenotype is also demonstrated by a gross morphological analysis of enucleated lenses. The lenses of homozygous mutants are smaller than those of heterozygotes and the nuclear opacity is more severe. Associated with the cataract formation is a slight microphthalmia (Figure 1). Histological analysis of the cataractous *Aey1* lenses in juvenile homozygous mutants demonstrates the presence of pycnotic cell nuclei in deep cortical lens areas (Figure 2). The pycnotic nature of these nuclei is characterized by a polar distribution of chromatin. Moreover, it should be noted that the cell nuclei in the more peripheral region have been degraded as usual. Therefore, there may be a developmental difference in fiber cell maturation: fiber cells produced in early stages of development may not lose their nuclei completely, while those produced at later stages may develop normally. This temporal difference in fiber cell nuclei degradation might be related to the temporal expression pattern of *Cryge* expression. Since the cataract is stable from the onset of observation (3 weeks of age), this observation supports the idea that only early fiber cells are formed abnormally. The lens bow and the anterior lens epithelium as well as other ocular tissues besides the lens, in particular the cornea and the retina, are formed regularly.

Mapping: Heterozygous carriers from the second generation were mated to wild-type C57BL/J6 mice; among 419 offspring of the third generation were 219 cataractous mice and 200 wild types, indicating a dominant mode of inheritance with a complete penetrance of the *Aey1* mutation. The first 42 cataractous mice were used for the genome-wide mapping. The result indicates linkage to chromosome 1 of the mouse; the detailed haplo-

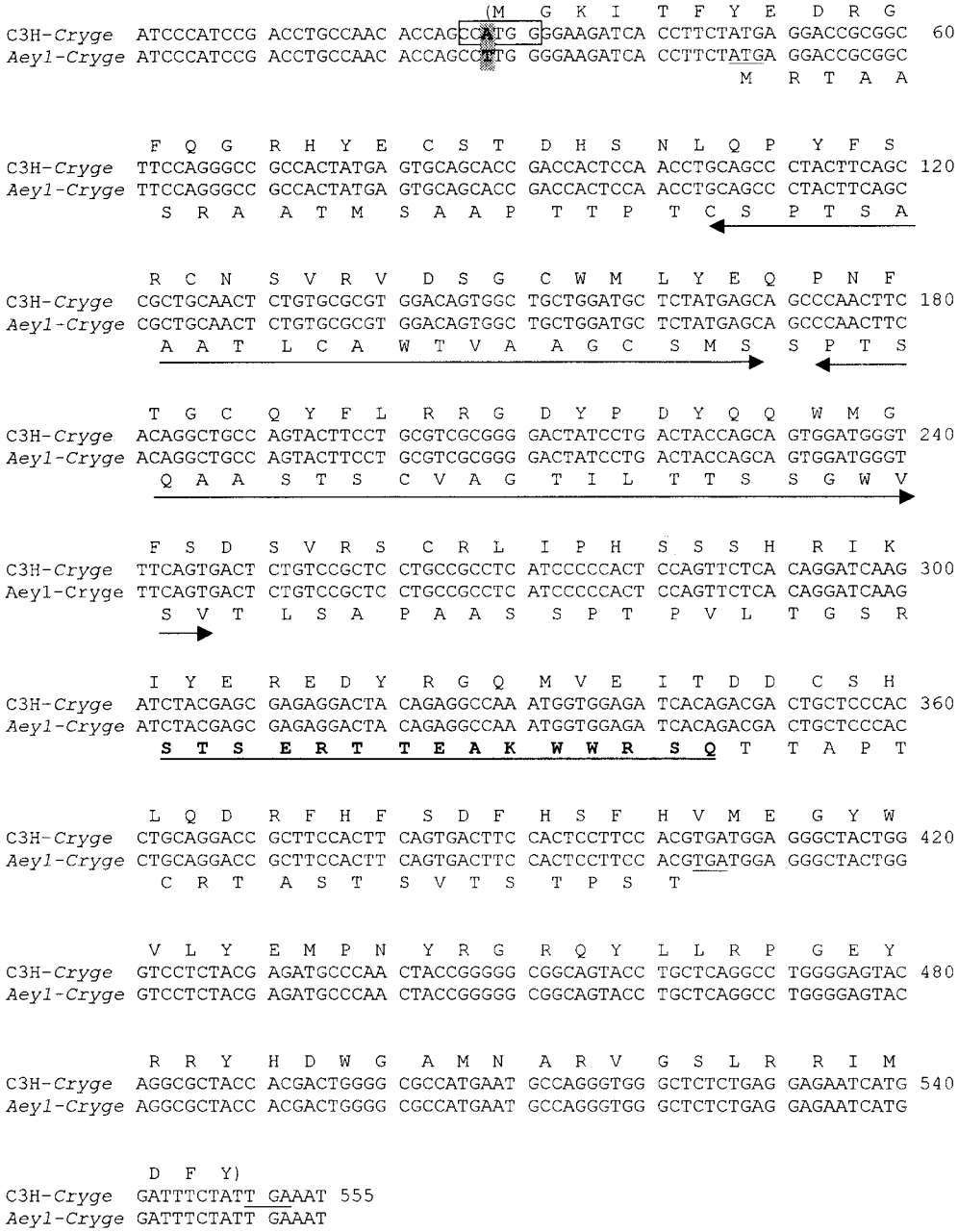

FIGURE 5.—Sequence analysis of the *Aey1* mutant. The *Cryge* DNA sequence from wild-type mice is compared to that from the *Aey1* mutants. The γE-crystallin amino acid composition is demonstrated above the DNA sequence in parentheses. The transversion of A to T in the *Cryge* start codon is shown in boldface and shaded in gray; the *Nco*I restriction site is boxed. The γE-crystallin amino acids are above the cDNA sequence. Below the cDNA sequence, a further putative ORF is predicted starting 19 bp downstream of the regular *Cryge* translation start codon. The small peptide used for specific antibody production is underlined and shown in boldface. The two predicted transmembrane domains are indicated by arrows. The stop codons are underlined.

type analysis for this chromosome and the four markers used is given in Figure 3. It turned out that *Aey1* maps between the markers *D1Mit303* and *D1Mit332*.

The mapping of *Aey1* between the markers *D1Mit303* and *D1Mit332* makes the *Cryba2* gene an interesting candidate gene; however, the *Cryg* gene cluster was also possible, since this cluster is only 8 cM apart from *Cryba2* (Figure 4). Because of the small number of G3 animals genotyped and the resulting large standard deviation, a mutation in the *Cryg* cluster could not be ruled out.

Genomic analysis: At first, the *Cryba2* gene was tested as a candidate for the *Aey1* phenotype. However, sequencing of PCR products derived from cDNA from the lenses of homozygous cataractous mice revealed no sequence alterations as compared to the database (accession no. AJ272228). Moreover, there was no apparent alteration in the amount of amplified *Cryba2* cDNA, making a promoter mutation unlikely. Thus, the *Cryba2* gene was eliminated as a candidate for *Aey1*.

Therefore, all six *Cryg* genes were amplified specifically by PCR using genomic DNA or lens cDNA as a template and sequenced. Several polymorphic sites were observed in the *Cryga* and *Crygd* genes, which were not associated with the cataractous phenotype. The only difference between wild-type C3H and mutant *Aey1* cDNA, which could be correlated to the cataractous phenotype, was identified in the *Cryge* gene (acc. no. NM_007777) as a transversion of an A at position 1 of the cDNA to a T (Figure 5). The mutation in *Cryge* was confirmed by sequencing exon 1 from genomic DNA

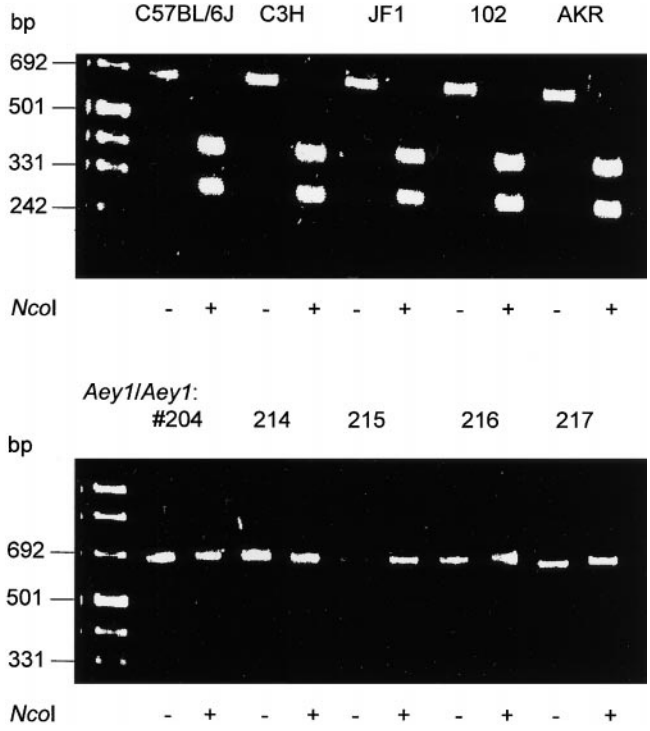

FIGURE 6.—*Cryge* digest by *Nco*I. The exons 1 and 2 as well as the connecting intron A were amplified from genomic DNA. The PCR fragment was analyzed by agarose electrophoresis with (+) and without (−) subsequent digestion by *Nco*I. The genomic DNA from all wild types (derived from the strains C57BL/6J, C3H, JF1, 102, and AKR) can be digested, but not the DNA from five homozygous *Cryge*^{Aey1} mutants.

of homozygous mutants. It destroys a restriction site for *Nco*I, which was demonstrated in five homozygous mutants. This site is still present in five wild-type mice from different strains (Figure 6). Therefore, we conclude that this point mutation in the *Cryge* gene is responsible for the cataractous phenotype; the new allele symbol is suggested as *Cryge*^{Aey1}.

Biochemical analysis: The deduced amino acid sequence of Cryge suggests that the *Aey1* phenotype is caused by the destruction of the start codon of the *Cryge* gene. Since a second in-frame ATG is present 129 bp downstream, it is possible that a truncated γE-crystallin of 14 kD is expressed. To investigate whether this N-terminally truncated γE-crystallin is present in the eye lens, a polyclonal antibody against γ-crystallins was used for Western blot analysis of the water-soluble lens proteins. However, no immunoreactivity for γ-crystallin was apparent at 14 kD in either wild-type or *Aey1* homozygous lenses (data not shown). Thus, it appears unlikely that a truncated γE-crystallin is present and responsible for cataract formation.

However, sequence analysis suggested that another long open reading frame (ORF) starts only 19 bp downstream of the destroyed ATG. The phase of this ORF is shifted by +1 as compared to the *Cryge* ORF and a protein consisting of 119 amino acids with a calculated molecular weight of 13 kD was predicted. Analysis for hydrophobicity using the Kyte-Doolittle algorithms (KYTE and DOOLITTLE 1982) revealed two hydrophobic regions (Figure 7), which might be interpreted as membrane spanning segments. The TMpred program—and similarly also the TopPred2 program—strongly prefer the model of N terminus inside and the first transmembrane domain (amino acids 20–41) from inside to outside; correspondingly, the second transmembrane region (amino acids 43–67) runs from outside to inside. In the first putative transmembrane domain and in the regions between amino acids 8–12 and 91–98, α-helical regions are suggested. All other regions are suggested to be randomly coiled (63%) or extended β-strands (21%; GOR4, GARNIER *et al.* 1996). As outlined by the PROSCAN program, additional putative biochemical features of the novel protein are three *N*-myristoylation sites and three phosphorylation sites (one for Casein kinase II and two for protein kinase C).

Using a specific antibody against the most hydrophilic region (−1.5 to −2.0 according to the hydrophobicity plot; Figure 7), we could demonstrate that this particular protein and a somewhat smaller degradation product are present only in the cataractous lenses but not in the lenses of wild-type mice (Figure 8). It might be proposed that the novel protein destroys the highly organized cellular structure of the lens fiber cells.

DISCUSSION

In this article, we describe the molecular characterization of an ENU-induced mouse cataract mutation, *Aey1*. The ENU-induced point mutation changes the start codon of the *Cryge* gene. Since this mutation segregates with the phenotype, this strongly suggests that the mutation in the *Cryge* gene is responsible for the cataractous phenotype. Since the 2000 Chromosome Committee Report places the *Cryg* gene cluster proximal to the marker *D1Mit303*, this position of the *Cryg* gene cluster should be changed on the basis of our mapping data to between the markers *D1Mit303* and *D1Mit332*.

The novel *Cryge* allele *Cryge*^{Aey1} leads to a nuclear and zonular cataract. At the histological level it is obvious that only the lens nucleus is affected by the presence of pycnotic fiber cell nuclei and the cortical regions of the lens remain intact. Comparing the histological observations at this stage, *Cryge*^{Aey1} is very similar phenotypically to the *Crygb*^{nop} mutants described previously. Particularly, the nuclear fibers do not denucleate in either *Cryge*^{Aey1} or *Crygb*^{nop} (GRAW *et al.* 1984, 1990b).

Cryge^{Aey1} is the seventh mouse mutation reported to affect a gene of the *Cryg* gene cluster and to lead to cataracts; these include the *Cryge*^{elo} (CARTIER *et al.* 1992), the *Crygd*^{Lop12} (SMITH *et al.* 2000), as well as the *Cryga*^{1Neu}, *Crygb*^{nop}, and the *Cryge*^{t} genes (KLOPP *et al.* 1998). The deletion in the *Cryge*^{ns} mutant is >2 kb and is not yet

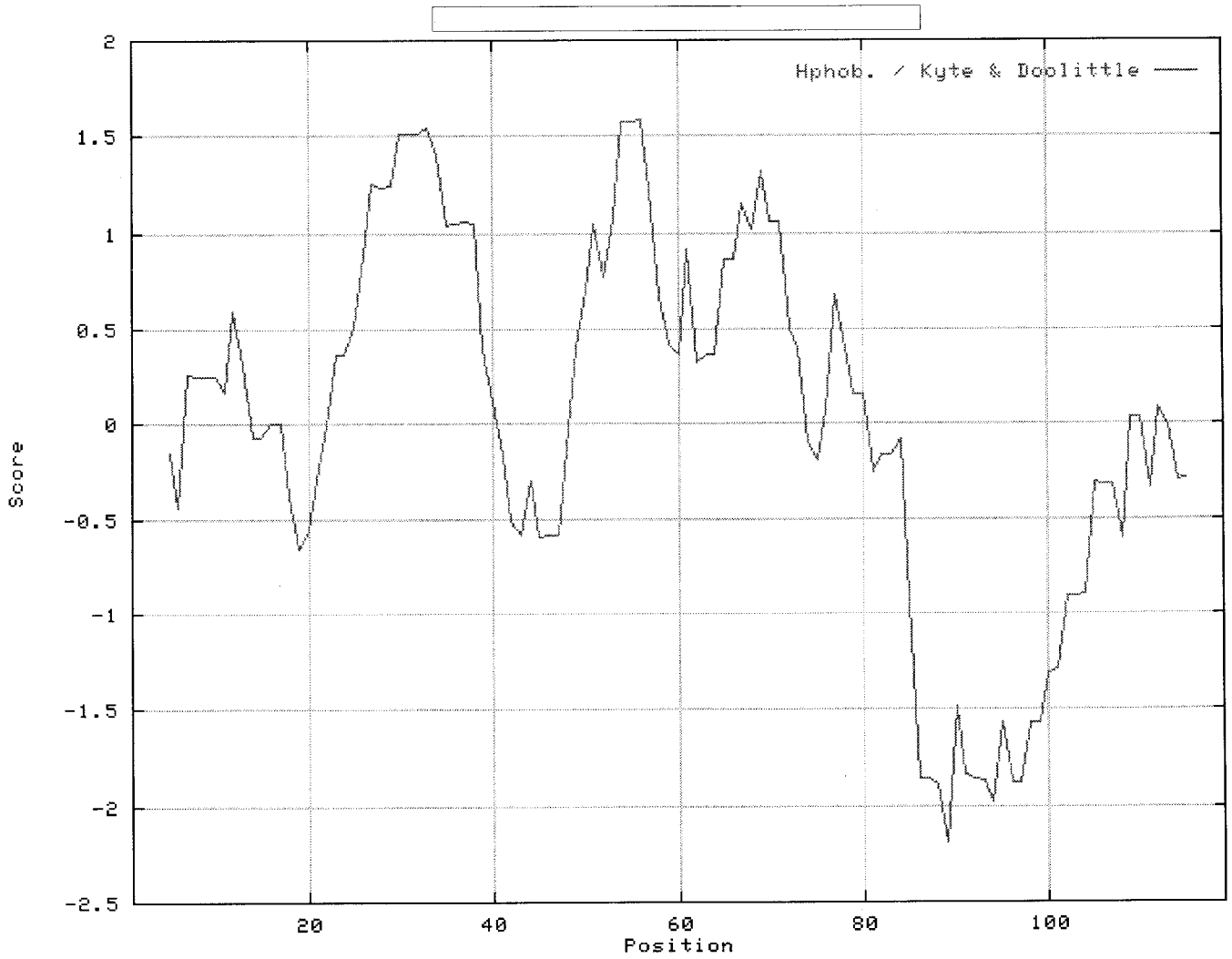

FIGURE 7.—Hydrophobicity of the predicted Aey1 protein was calculated using the ProtScan program (http://expasy.cbr.nrc.ca). Positive scores indicate increasing hydrophobicity; negative scores indicate increasing hydrophilic character. The most hydrophilic region lies between the amino acid residues 86–100, which were used for the antibody production.

characterized in detail (GRAW 1999). The novel *Cryge*^{Aey1} mutation, however, causes a less severe phenotype than observed in other *Cryge* alleles like *Cryge*^{t} or the *Cryge*^{elo}. Also, the recently described cataract mutation *Lop12*, which was characterized as a mutation in *Crygd* (SMITH *et al.* 2000), had a more severe phenotype and was similar to that of the *Cryge*^{t} (GRAW *et al.* 1990a). All these *Cryg* mutants are characterized by an amino acid exchange at an important region of the corresponding γ-crystallin or contain a truncated form of the γ-crystallin with or without a few new amino acids. The *Cryge*^{Aey1} mutation is the only one that leads to the formation of an entirely novel protein in the eye lens.

Corresponding to the increasing number of characterized cataract mutants in mice, mutations in human *CRYG* genes have been shown to be associated with cataract formation: the Coppock-like cataract (HÉON *et al.* 1999) and the variable zonular pulverulent cataract (REN *et al.* 2000) are associated with the *CRYGC* gene, whereas the aculeiform cataract (HÉON *et al.* 1999), a punctate cataract (STEPHAN *et al.* 1999), and a crystal-deposition cataract (KMOCH *et al.* 2000) are associated with mutations in the *CRYGD* gene. Finally, a polymorphic congenital cataract was mapped very close to the *CRYGB* gene (ROGAEV *et al.* 1996).

A common feature of all these mutations are changes in the amino acid sequence, which are considered to lead to altered folding properties. None of the altered proteins can behave like the intact γ-crystallin with the four Greek key motifs. The cataract mutation, which is reported here, is the only one that leads to the formation of a completely novel protein in the eye lens, because of the destruction of the regular start codon and the use of another ORF immediately downstream. The comparison of the deduced Aey1 amino acid sequence to the SwissProt database revealed as the best match just

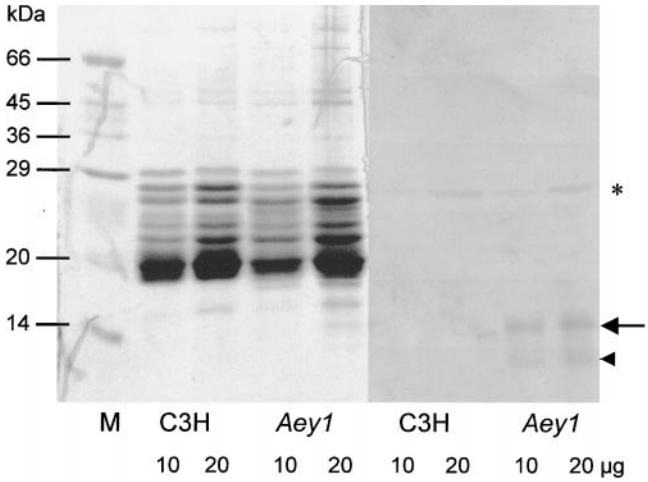

FIGURE 8.—Western blot of lens extracts. Water-soluble lens proteins from wild-type or homozygous *Aey1* lenses were separated by polyacrylamide gel electrophoresis and analyzed for the presence or absence of the novel Aey1-specific protein using a corresponding antibody. The arrow points to the specific band at the appropriate size; it is visible only in extracts from mutant lenses. The lower band (arrowhead) most likely represents a degradation product. The asterisk (*) indicates an unspecific interaction of the antibody, which is present in all extracts. The amounts of lens proteins are indicated at the bottom. Left, Coomassie staining of the lens extracts; right, Western blot of lens proteins; the membrane was incubated with the Aey1-specific antibody.

45% identity to the chick octamer-binding transcription factor 1 (PETRYNIAK *et al.* 1990) within a short stretch between amino acids 44 and 76. The same region of the Aey1 protein (between amino acids 37 and 77) shows just 39% identity to the Drosophila POU-domain protein dPOU-19 (termed POU for its presence in the Pit-1, Oct-1/Oct-2, and Unc-86 genes) (DICK *et al.* 1991); these weak homologies are not considered to allow speculations on functional similarities. Moreover, this short region of similarity is outside the conserved sequences of the POU-specific domain and the more C-terminal POU homeodomain, but is part of one of the predicted transmembrane domains. Further biochemical characterization of the new protein will be necessary to prove such similarities in detail.

The numerous mutations affecting *Cryg* genes that cause cataracts makes this cluster very interesting. On one hand, this high number of pathological phenotypes supports the importance of these genes for lens transparency. On the other hand, the evolutionary "knockout" of two of these six genes in man (ψ*CRYGE* and ψ*CRYGF*) demonstrated that the loss of at least a few of them seems to be without any biological relevance. Additionally, there are several reports for polymorphic sites within these genes in both mouse and human. At these sites, sequences are allowed to be changed without any effect on the function of the proteins to keep the lens transparent (KLOPP *et al.* 1998; HÉON *et al.* 1999; SMITH *et al.* 2000). On the basis of these studies and additional pathological and nonpathological alleles of the *Cryg* genes, we will be able to characterize the domains of the corresponding γ-crystallins that are important for their biochemical function.

In summary, we demonstrated here that an ENU-induced mutation in the start codon of the *Cryge* gene leads to the use of an alternative start codon in another ORF and to the subsequent translation of a novel, but pathological, protein in the eye lens. To our knowledge, it is the first report of such consequences of a mutation in cataracts.

The *Aey1* mutant was kindly provided by Ingenium Pharmaceuticals AG (Martinsried, Germany). The expert technical assistance of Erika Bürkle, Gerlinde Bergter, Andreas Mayer, Nicole Hirsch, Sabine Manz, Sylvia Prettin, Dagmar Reinl, and Monika Stadler is gratefully acknowledged. The authors also thank Dr. Melinda K. Duncan (University of Delaware, Newark, Delaware) for critical comments on the manuscript. Oligonucleotides were obtained from Utz Linzner (GSF-AG BIODV). Part of this work was supported by a grant from the German Human Genome Project (DHGP) to R.B., E.W., and M.H.d.A. (01KW9610/1).

LITERATURE CITED

CARTIER, M., M. L. BREITMAN and L.-C. TSUI, 1992 A frameshift mutation in the γE-crystallin gene of the *Elo* mouse. Nat. Genet. **2:** 42–45.

DICK, T., X. YANG, S. YEO and W. CHIA, 1991 Two closely linked *Drosophila* POU domain genes are expressed in neuroblasts and sensory elements. Proc. Natl. Acad. Sci. USA **88:** 7645–7649.

EHLING, U. H., D. J. CHARLES, J. FAVOR, J. GRAW, J. KRATOCHVILOVA *et al.*, 1985 Induction of gene mutations in mice: the multiple endpoint approach. Mutat. Res. **150:** 393–401.

GARNIER, J., J.-F. GIBRAT and B. ROBSON, 1996 GOR secondary structure prediction method version IV. Methods Enzymol. **266:** 540–553.

GRAW, J., 1997 The crystallins: genes, proteins, and diseases. Biol. Chem. **378:** 1331–1348.

GRAW, J., 1999 Cataract mutations and lens development. Prog. Retin. Eye Res. **18:** 235–267.

GRAW, J., J. KRATOCHVILOVA and K.-H. SUMMER, 1984 Genetical and biochemical studies of a dominant cataract mutant in mice. Exp. Eye Res. **39:** 37–45.

GRAW, J., W. BORS, P. M. GOPINATH, C. MICHEL, P. REITMEIR *et al.*, 1990a Characterization of *Cat-2*^{t}, a radiation-induced dominant cataract in mice. Invest. Ophthalmol. Vis. Sci. **31:** 1353–1361.

GRAW, J., T. WERNER, S. MERKLE, P. REITMAIR, E. SCHÄFFER *et al.*, 1990b Histological and biochemical characterization of the murine cataract mutant *Nop*. Exp. Eye Res. **50:** 449–456.

GRAW, J., M. JUNG, J. LÖSTER, N. KLOPP, D. SOEWARTO, *et al.*, 1999 Mutation in the βA3/A1-crystallin encoding gene *Cryba1* causes a dominant cataract in the mouse. Genomics **62:** 67–73.

HEAD, M. W., K. SEDOWOFIA and R. M. CLAYTON, 1995 βB2-crystallin in the mammalian retina. Exp. Eye Res. **61:** 423–428.

HÉON, E., M. PRISTON, D. F. SCHORDERET, G. D. BILLINGSLEY, P. O. GIRARD *et al.*, 1999 The γ-crystallins and human cataracts: a puzzle made clearer. Am. J. Hum. Genet. **65:** 1261–1267.

HRABÉ DE ANGELIS, M., and R. BALLING, 1998 Large scale ENU screens in the mouse: genetics meets genomics. Mutat. Res. **400:** 25–32.

HRABÉ DE ANGELIS, M., H. FLASWINKEL, H. FUCHS, B. RATHKOLB, D. SOEWARTO *et al.*, 2000 Genome-wide, large-scale production of mutant mice by ENU mutagenesis. Nat. Genet. **25:** 1–4.

HULSEBOS, T. J., K. M. CEROSALETTI, R. E. FOURNIER, R. J. SINKE, M. ROCCHI *et al.*, 1995 Identification of the human βA2-crystallin

gene (*CRYBA2*): localization of the gene on human chromosome 2 and of the homologous gene on mouse chromosome 1. Genomics **10:** 543–548.

Jones, S. E., C. Jomary, J. Grist, J. Makwana and M. J. Neal, 1999 Retinal expression of γ-crystallins in the mouse. Invest. Ophthalmol. Vis. Sci. **40:** 3017–3020.

Klopp, N., J. Favor, J. Löster, R. B. Lutz, A. Neuhäuser-Klaus *et al.*, 1998 Three murine cataract mutants (*Cat2*) are defective in different γ-crystallin genes. Genomics **52:** 152–158.

Kmoch, S., J. Brynda, B. Asfaw, K. Bezouška, P. Novák *et al.*, 2000 Link between a novel human γD-crystallin allele and a unique cataract phenotype explained by protein crystallography. Hum. Mol. Genet. **9:** 1779–1786.

Kyte, J., and R. F. Doolittle, 1982 A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157:** 105–132.

Mörner, C. T., 1893 Untersuchungen der Proteinsubstanzen in den lichtbrechenden Medien des Auges. Z. Physiol. Chem. **18:** 61–106.

Mugabo, K. S., J. Horwitz, J. Piatigorsky and M. Kantorow, 2000 Expression of βB2-crystallin mRNA and protein in retina, brain and testis. Invest. Ophthalmol. Vis. Sci. **41:** 3056–3060.

Petryniak, B., L. M. Staudt, C. E. Postema, W. T. McCormack and C. B. Thompson, 1990 Characterization of chicken octamer-binding proteins demonstrates that POU domain-containing homeobox transcription factors have been highly conserved during vertebrate evolution. Proc. Natl. Acad. Sci. USA **87:** 1099–1103.

Ren, Z., A. Li, B. S. Shastry, T. Padma, R. Ayyagari *et al.*, 2000 A 5-base insertion in the γC-crystallin gene is associated with autosomal dominant variable zonular pulverulent cataract. Hum. Genet. **106:** 531–537.

Rogaev, E. I., E. A. Rogaeva, G. I. Korovaitseva, L. A. Farrer, A. N. Petrin *et al.*, 1996 Linkage of polymorphic congenital cataract to the γ-crystallin gene locus on human chromosome 2q33-35. Hum. Mol. Genet. **5:** 699–703.

Slingsby, C., and N. J. Clout, 1999 Structure of the crystallins. Eye **13:** 395–402.

Smith, R. S., N. L. Hawes, B. Chang, T. H. Roderick, E. C. Akeson *et al.*, 2000 *Lop12*, a mutation in mouse *Crygd* causing lens opacity similar to human Coppock cataract. Genomics **63:** 314–320.

Stephan, D. A., E. Gillanders, D. van der Veen, D. Freas-Lutz, G. Wistow *et al.*, 1999 Progressive juvenile-onset punctate cataracts caused by mutation of the γD-crystallin gene. Proc. Natl. Acad. Sci. USA **96:** 1008–1012.

Wistow, G. J., and J. Piatigorsky, 1988 Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. Annu. Rev. Biochem. **57:** 479–504.

Communicating editor: C. Kozak