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

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Received March 6, 1995; accepted May 25, 1995

By using primers synthesized on the basis of the bovine βA2 crystallin gene sequence, we amplified exons 5 and 6 of the human gene (*CRYBA2*). *CRYBA2* was assigned to human chromosome 2 by concordance analysis in human × rodent somatic cell hybrids using the amplified PCR products as probe. Regional localization to 2q34–q36 was established by hybridizing the *CRYBA2* probe to microcell and radiation hybrids containing defined fragments of chromosome 2 as the only human contribution. The *CRYBA2* probe was also used to localize, by interspecific backcross mapping, the mouse gene (*Cryba2*) to the central portion of chromosome 1 in a region of known human chromosome 2 homology. Finally, we demonstrate that in both species the βA2 crystallin gene is linked but separable from the γA crystallin gene. The βA2 crystallin gene is a candidate gene for human and mouse hereditary cataract.

INTRODUCTION

The α, β, and γ crystallins account for approximately 90% of the water-soluble protein of the lens. These specialized proteins are thought to be important for the transparency and light reflection properties of the lens (Wistow and Piatigorsky, 1988). Transparency is impaired in cataract. Many hereditary forms of cataract have been described in human as well as mouse (see Green, 1989; Lund *et al.*, 1992). Because of the important structural role of crystallins in the lens, the crystallin genes are obvious candidate genes for human and mouse cataract. Indeed, in both species, hereditary cataracts that are very closely linked to crystallin genes or result from mutations in these genes have been reported (Chambers and Russell, 1991; Cartier *et al.*, 1992; Brakenhoff *et al.*, 1994; Everett *et al.*, 1994; Löster *et al.*, 1994).

To determine whether β crystallin genes are involved in human and mouse hereditary cataracts, we are identifying all β crystallin genes and establishing their location in human and mouse. The β crystallin family consists of four acidic (A) and three basic (B) forms. βA1 and βA3 crystallin are encoded by one gene. The human βA3/A1 crystallin gene (*CRYBA1*) has been mapped to region q11.2–q12 of chromosome 17 and the mouse homologue (*Cryba1*) to the distal half of chromosome 11 (Van Tuinen *et al.*, 1987; Buchberg *et al.*, 1990). The human βA4 crystallin gene (*CRYBA4*) is located in 22q11.2–q12.1 and the mouse homologue (*Cryba4*) in the central region of chromosome 5 (Van Rens *et al.*, 1992; Bijlsma *et al.*, 1993; Hulsebos *et al.*, 1995).

Here we report the assignment of the βA2 crystallin gene to region q34–q36 of human chromosome 2 and to the central part of mouse chromosome 1 in a region of known chromosome 2 homology.

MATERIALS AND METHODS

Cross-species PCR. Hot-start PCR was performed with 1 μg of genomic DNA, 30 pmol of each primer, 200 μ*M* each dATP, dGTP, dCTP, dTTP, 10 m*M* Tris–HCl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.001% (w/v) gelatin, and 2 U of AmpliTaq polymerase (Perkin–Elmer) in a final volume of 50 μl. Mixtures of 47 μl, without $MgCl_2$, were heated to 80°C, and PCR was started by the addition of 3 μl of 25 m*M* $MgCl_2$. PCR conditions were 3 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C, and one final incubation for 5 min at 72°C. The PCR products were excised from a low-melt-temperature agarose gel and cloned into the *Sma*I site of pUC18 using a commercially available kit (SureClone ligation kit, Pharmacia).

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos. X86395 and X86396.

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0888-7543/95 $12.00

Somatic cell hybrids. For chromosomal assignment, we used human × Chinese hamster and human × mouse hybrid cell lines with known human constitution. The construction of these cell lines has been described previously (Geurts van Kessel *et al.*, 1983 and references therein).

Regional localization was performed with two independently derived sets of somatic cell hybrids containing defined portions of human chromosome 2. The F(2n) series microcell hybrids were prepared by transferring human chromosome 2 from the monochromosomal hybrid, HA(2)A47, into FTO-2B rat hepatoma cells by microcell fusion (Cerosaletti and Fournier, submitted for publication). Human chromosome 2 was derived from a human diploid fibroblast and is marked with the neomycin resistance gene, pSV2neo, in the proximal short arm. The genotypes of the F(2n) hybrids were determined by fluorescence *in situ* hybridization (FISH) and by marker analysis using chromosome 2-specific PCR primers and Southern blot hybridization (Cerosaletti and Fournier, submitted for publication). FTO-2B is a rat hepatoma cell line derived from H411EC3 (Killary and Fournier, 1984). Normal human diploid fibroblasts (HDF) were isolated as described (Reigner *et al.*, 1976). The IB2 series of hybrids are radiation hybrids generated from hybrid GM10826B (NIGMS Human Genetic Mutant Cell Repository, Camden, NJ), containing chromosome 2 as the only human contribution. The cells were irradiated (5 kRad) and fused to the TK^- B14-150 CHO cell line. The resulting HAT-selected clones were characterized for extent and subchromosomal location of the retained chromosome 2 fragments as described previously (Antonacci *et al.*, 1995).

Southern blot analysis. The cloned PCR products of exons 5 and 6 of the human *CRYBA2* gene were liberated by *Eco*RI and *Hin*dIII digestion and used as hybridization probe. Southern blot procedures were all performed as described previously (Hulsebos *et al.*, 1991).

Interspecific mouse backcross mapping. Interspecific backcross progeny were generated by mating (C57BL/6J × *Mus spretus*)F_1 females and C57BL/6J males as described (Copeland and Jenkins, 1991). A total of 205 N_2 mice were used to map the *Cryba2* locus (see text for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described (Jenkins *et al.*, 1982). All blots were prepared with Hybond-N^+ nylon membrane (Amersham). After hybridization of the probe, exons 5 and 6 of the human *CRYBA2* gene (see below), washing was performed to a final stringency of 0.8× SSCP, 0.1% SDS, 65°C. A fragment of 9.4 kb was detected in *Kpn*I-digested C57BL/6J DNA, and a fragment of 7.0 kb was detected in *Kpn*I-digested *M. spretus* DNA. The presence or absence of the 7.0-kb *Kpn*I *M. spretus*-specific fragment was followed in backcross mice.

A description of the probes and RFLPs for the loci linked to *Cryba2* including γ crystallin (*Cryg*), villin (*Vil*), and acetylcholine receptor γ subunit (*Acrg*) has been reported previously (Cerretti *et al.*, 1993). Recombination distances were calculated as described (Green, 1981) using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

RESULTS

Identification of the Human βA2 Crystallin Gene (CRYBA2)

Bovine-derived primers were used to amplify exons 5 and 6 of the human *CRYBA2* gene by hot-start PCR. The positions of the primers in the relevant portion of the cDNA sequence of the bovine βA2 crystallin gene are indicated in Fig. 1. The exon 5 and exon 6 PCR products had the expected lengths of approximately 140 and 125 bp, respectively. To verify their identity, we sequenced the two PCR products. The human *CRYBA2* sequences were found to be highly homologous to the corresponding bovine sequences (Fig. 1). They differed at only 21 of 186 positions. Many of the differences involved the third base of codons, without changing the encoded amino acid residue. The two PCR products were used as probe for *CRYBA2* in all subsequent experiments.

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                351
bovine   AAC CAC AGT GAC AGT CGT GTG ACA CTG TTT GAG GGG GAA AAC
human                              ACA CTG TTT GAG GGG GAC AAC

                     402
bovine   TTC CAG GGC TGC AAG TTT GAA CTC AAT GAT GAC TAC CCA TCC
human    TTC CTT GGC TGC AAG TTT GAC CTC GTT GAT GAC TAC CCA TCC

                          450
bovine   CTG CCT TCC ATG GGC TGG GCC AGC AAG GAT GTG GGT TCC CTC
human    CTG CCC TCC ATG GGC TGG GCC AGT AAG GAT GTG GGT TCC

                                    501
bovine   AAA GTC AGC TCT GGA GCG TGG GTG GCC TAC CAG TAT CCG GGC
human

                                          552
bovine   TAC CGG GGC TAC CAG TAT GTG TTG GAG CGG GAC CAC CAC AGT
human    TAC CGA GGC TAC CAG TAT GTG TTG GAG CGG GAC CGG CAC AGC

                                                600
bovine   GGG GAG TTC CGT AAC TAC AGC GAA TTC GGC ACG CAG GCC CAC
human    GGA GAG TTC TGT ACT TAC GGT GAG CTC GGC ACA CAG GCC CAC

                               621
bovine   ACC GGG CAG CTG CAG TCC ATC
human
```

FIG. 1. DNA sequence of part of exons 5 and 6 of the human βA2 crystallin gene and comparison with the corresponding sequence in the bovine βA2 crystallin gene. The nucleotide sequence of the bovine βA2 crystallin cDNA was taken from Van Rens *et al.* (1991). Numbering of nucleotide residues is according to this sequence. The most probable boundaries of exons 5 and 6 in the bovine cDNA sequence were deduced by comparison with the evolutionarily related rat βB1 crystallin gene, of which the intron–exon boundaries have been determined (Den Dunnen *et al.*, 1985c, 1986) Exon 5 starts at residue 346 and ends at residue 491. The 3′-terminal exon 6 starts at residue 492 and continues over residue 621. Bovine-derived primers were used to amplify the homologous segments of exons 5 and 6 in the human βA2 crystallin gene. The positions of the forward and reverse primers in the respective exons of the bovine βA2 crystallin gene sequence are indicated by underlining. Nucleotide differences between the human and the bovine βA2 crystallin gene sequences are indicated with dots.

Chromosomal Localization of CRYBA2

To determine the chromosomal location of *CRYBA2,* we hybridized the *CRYBA2* probe to a panel of human × rodent somatic cell hybrids. On Southern blots of *Hin*dIII digests, the probe hybridized to a large restriction fragment of more than 24 kb in human DNA (see below) and cross-hybridized with a 3.7-kb fragment in hamster DNA (see below) and a 7.2-kb fragment in

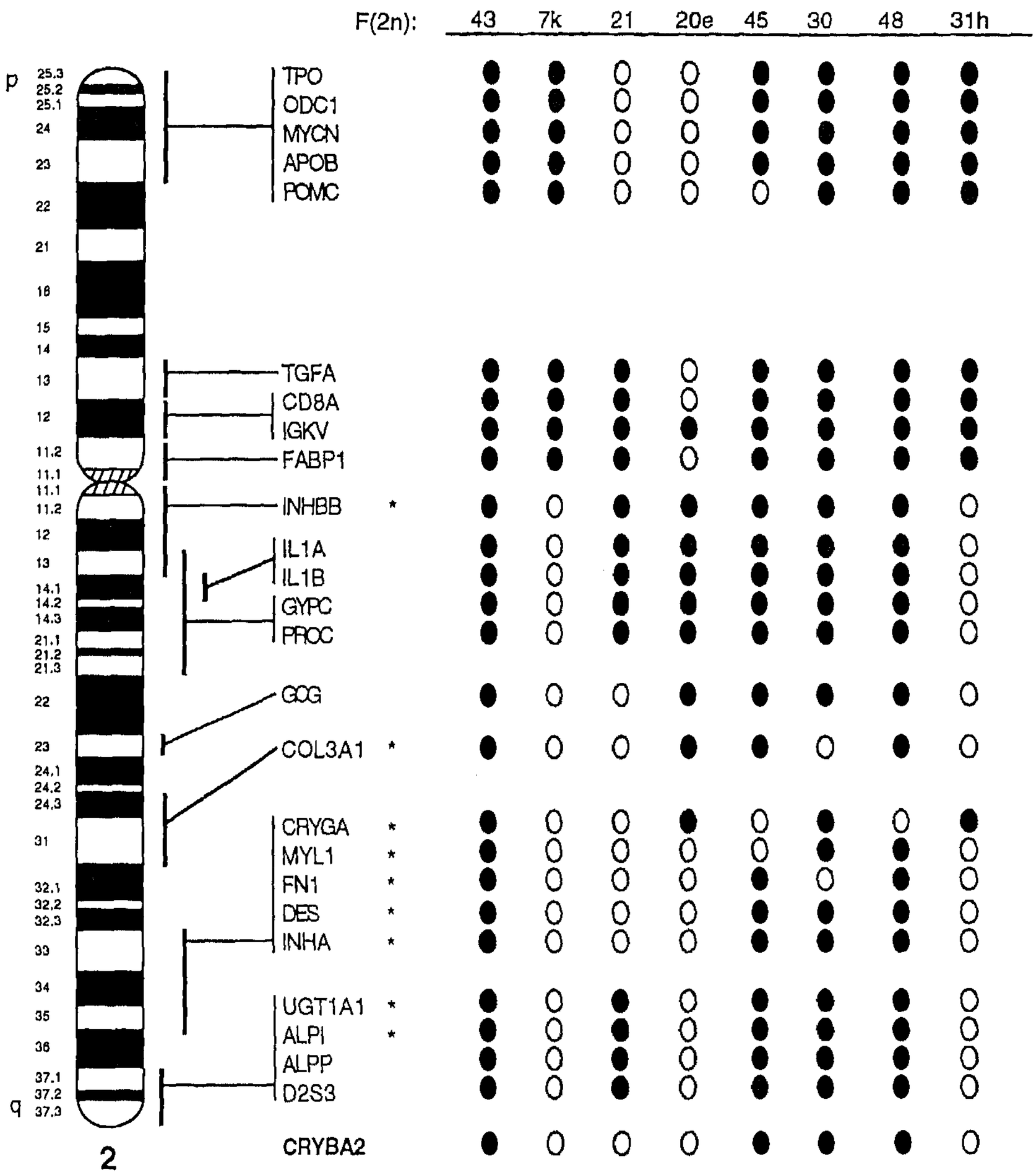

FIG. 2. Localization of *CRYBA2* on human chromosome 2 using the F(2n) series of hybrids. Southern blot hybridization and PCR marker analysis were used to determine the presence (filled oval) or absence (open oval) of 25 chromosome 2 markers in genomic DNA from each of eight F(2n) hybrid clones as described (Cerosaletti and Fournier, submitted for publication). The markers are listed in a likely order, p to q, and the general cytogenetic locations are indicated on the idiogram of chromosome 2. Human chromosome 2 markers with known homologues on mouse chromosome 1 are marked with asterisks. The retention of *CRYBA2* in the hybrid cell lines is shown at the bottom of the figure.

mouse DNA (not shown). We found concordant segregation of *CRYBA2* with chromosome 2 in 19 of 20 hybrids (5% discordancy). At least 30% discordant hybrids were detected for all other chromosomes, except for chromosome 5, with 15% discordant hybrids. These data suggest that *CRYBA2* is located on chromosome 2.

Localization of CRYBA2 on Human Chromosome 2

To test further whether *CRYBA2* is encoded on human chromosome 2, we performed Southern blot hybridization using a panel of F(2n) microcell hybrid clones, which retain various portions of human chromosome 2 in a rat hepatoma background (Cerosaletti and Fournier, submitted for publication). The genotypes of the F(2n) hybrids used in these experiments are shown in Fig. 2. Southern blots were prepared from *Pst*I-digested DNA isolated from parental FTO-2B rat hepatoma cells, HDF, a microcell hybrid clone that retains an intact chromosome 2 [F(2n)-43], and seven hybrid clones, F(2n)-7k, F(2n)-21, F(2n)-20e, F(2n)-45, F(2n)-30, F(2n)-48, and F(2n)-31h, which retain fragments of chromosome 2. Filters were hybridized with the *CRYBA2* probe. The probe detected one restriction fragment of approximately 1.2 kb in human DNA, which was clearly distinguishable from the 2.0-kb fragment detected in the rat parent. The 1.2-kb human-specific fragment was retained in F(2n)-43, indicating that *CRYBA2* is encoded on human chromosome 2.

The retention of *CRYBA2* sequences in F(2n) hybrids containing various chromosome 2 markers was compared to localize *CRYBA2* on chromosome 2. *CRYBA2* sequences were not detected in DNA from F(2n)-7k, -21, or -20e, indicating that the gene is not encoded on

the short arm or the proximal long arm of chromosome 2, nor distally at 2q37. These results localize *CRYBA2* to 2q33–q36, between *CRYGA* and *ALPI*. *CRYBA2* sequences were detected in hybrids F(2n)-45 and F(2n)-30, which lack *MYL1* and *FN1*, respectively, within the *CRYGA–ALPI* interval. These results are consistent with a localization of *CRYBA2* between *FN1* and *ALPI* at 2q33–q36; however, a more proximal location between *MYL1* and *FN1* at 2q33–q35 cannot be ruled out. Importantly, detection of the *CRYBA2*-specific restriction fragment in hybrids F(2n)-45 and F(2n)-48, which lack the γ crystallin gene *CRYGA* at 2q33–q35, and the absence of hybridization to F(2n)-20e and F(2n)-31h, which retain *CRYGA*, clearly demonstrate that *CRYBA2* maps to a location unique from that of *CRYGA*.

The location of *CRYBA2* on human chromosome 2 was confirmed and further narrowed down by hybridizing the *CRYBA2* probe to human × hamster hybrids selected from an independently derived series of hybrids, containing various portions of chromosome 2 (Antonacci *et al.*, 1995; Rochhi *et al.*, unpublished results). The chromosome 2 content of hybrids IB2L22 and IB2L25 is indicated in Fig. 3A. The presence of the *CRYBA2*-specific *Hin*dIII fragment (arrow) in IB2L22 and its absence in IB2L25 strongly suggest that *CRYBA2* is located within 2q34–q36 (Fig. 3B).

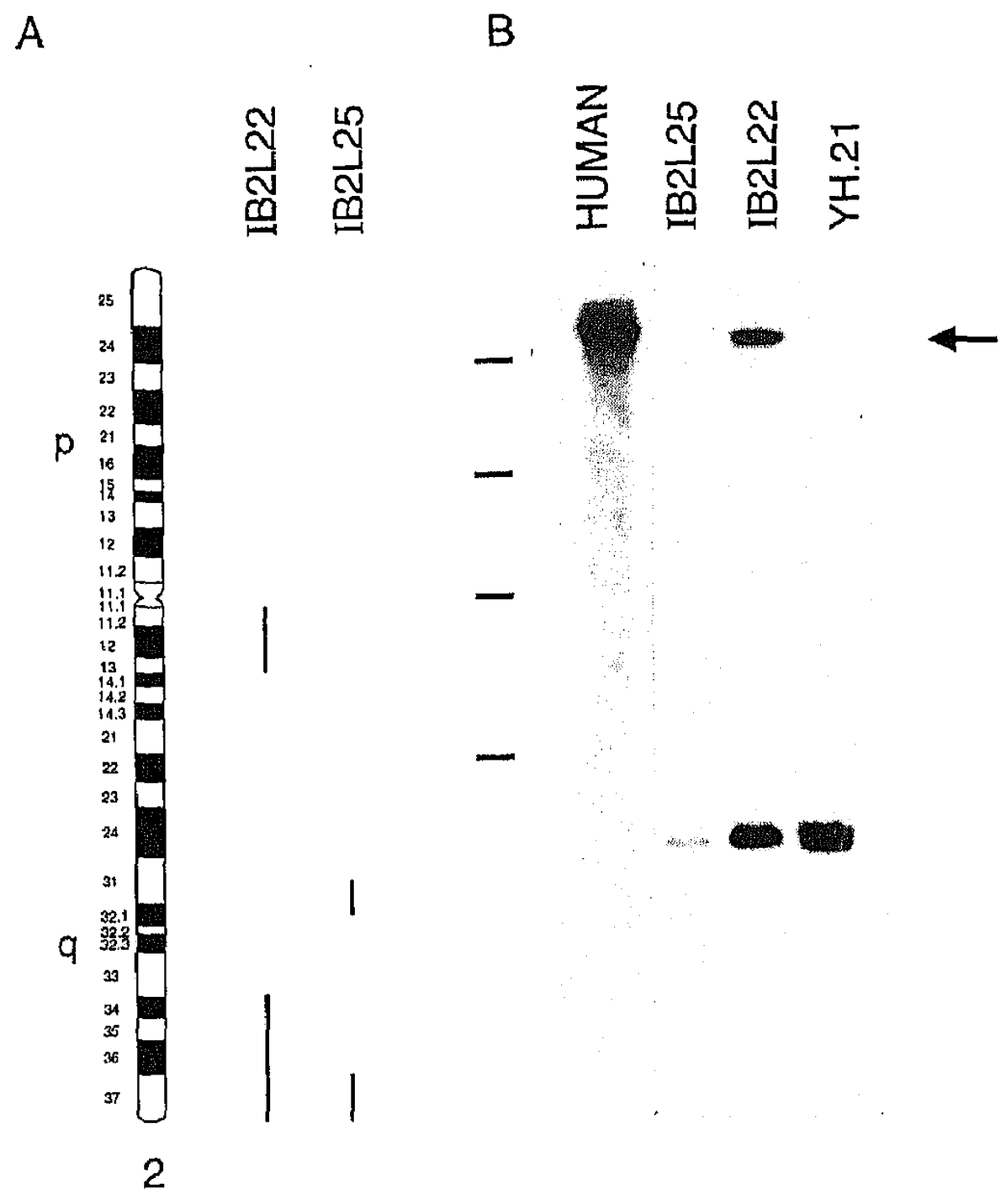

FIG. 3. Localization of *CRYBA2* on human chromosome 2 using selected IB2 hybrids. (**A**) Extent and subchromosomal location of the chromosome 2 fragments retained in human × hamster hybrids IB2L22 and IB2L25 determined by *in situ* hybridization procedures as described in Antonacci *et al.* (1995). (**B**) Southern blot hybridization was used to narrow down the location of *CRYBA2* to 2q34–q36. Genomic DNA was isolated from normal peripheral blood leukocytes (HUMAN), hybrids IB2L22 and IB2L25, and a hamster-only cell line (YH.21). DNA was digested with *Hin*dIII, electrophoresed through an 0.8% agarose gel, and transferred to a nylon membrane. The *CRYBA2* probe, labeled by random priming, was hybridized to the blot. Horizontal lines indicate positions of the 23-, 9.4-, 6.6-, and 4.4-kb *Hin*dIII fragments of λ DNA. The arrow points to the location of the human-specific *CRYBA2* fragment.

Localization of the Mouse Homologue of CRYBA2

The chromosomal location of the mouse homologue of *CRYBA2, Cryba2,* was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × *M. spretus*)F_1 × C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 1800 loci that are well distributed among all of the autosomes as well as the X chromosome (Copeland and Jenkins, 1991). C57BL/6J and *M. spretus* DNAs were digested with several restriction enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms using the human *CRYBA2* probe. The 7.0-kb *Kpn*I *M. spretus* RFLP (see Materials and Methods) was used to follow the segregation of *Cryba2* in backcross mice. The mapping results indicated that *Cryba2* is located in the central region of mouse chromosome 1 linked to *Cryg, Vil,* and *Acrg*. Although 144 mice were analyzed for every marker and are shown in the segregation analysis (Fig. 4), up to 186 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are centromere–*Cryg*–16/151–*Vil*–0/184–*Cryba2*–17/186–*Acrg*. The recombination frequencies (expressed as genetic distances in centimorgans ± the standard error) are *Cryg*–10.6 ± 2.5–(*Vil, Cryba2*)–9.1 ± 2.1–*Acrg*. No recombinants were detected between *Vil* and *Cryba2* in 184 animals typed in common, suggesting that the two loci are within 1.6 cM of each other (upper 95% confidence limit).

DISCUSSION

In human and mouse, we have localized the βA2 crystallin gene at a separate position from the γA crystallin gene. The human γA crystallin gene (*CRYGA*) is part of the γ crystallin gene cluster, which has been assigned to 2q33–q35 (Willard *et al.*, 1985; Den Dunnen *et al.*, 1985a; Shiloh *et al.*, 1986). The human γ crystallin genes reside on *Hin*dIII fragments ranging in size from about 12.3 to 2 kb (Den Dunnen *et al.*, 1985b). The *CRYBA2* probe specifically recognizes one large *Hin*dIII fragment of more than 24 kb and does not cross-hybridize to any of the γ crystallin genes containing fragments (see Fig. 3). Although the γ crystallin gene cluster is genetically considered one locus,

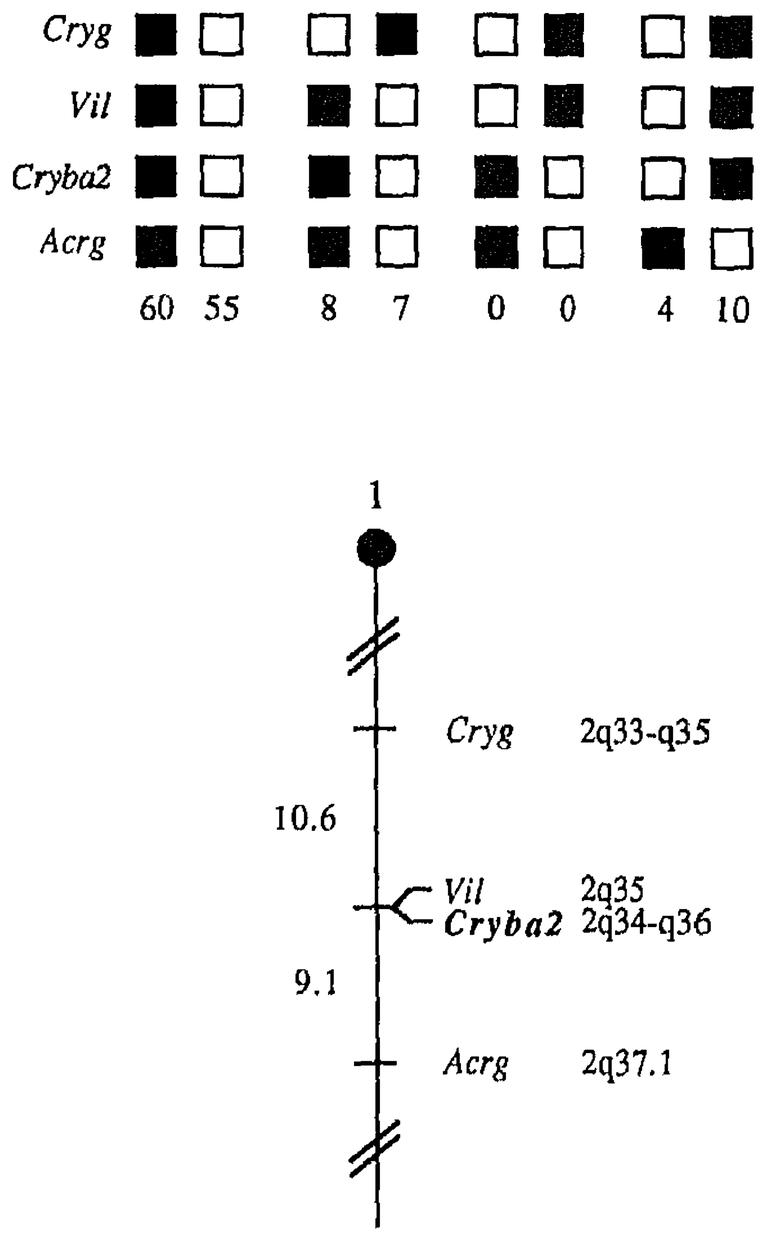

FIG. 4. *Cryba2* maps to the central region of mouse chromosome 1. *Cryba2* was placed on mouse chromosome 1 by interspecific backcross analysis. The segregation patterns of *Cryba2* and flanking genes in 144 backcross animals that were typed for all loci are shown at the top of the figure. For individual pairs of loci, more than 144 animals were typed (see text). Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*)F_1 parent. The shaded boxes represent the presence of a C57BL/6J allele, and white boxes represent the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome 1 linkage map showing the location of *Cryba2* in relation to linked genes is shown at the bottom of the figure. Recombination distances between loci in centimorgans are shown to the left of the chromosome, and the positions of loci in human chromosomes are shown to the right. The human map positions of the homologues of *Cryg* and *Vil* were taken from GDB (Genome Data Base). The map position of the human homologue of *Acrg* was taken from Lu-Kuo *et al.* (1993).

the physical linkage of its gene members has only partly been established (Den Dunnen *et al.*, 1985b; Meakin *et al.*, 1985). It is clear from our hybrid mapping data that *CRYBA2* maps separate from and distal to *CRYGA*. The mouse γ crystallin gene *Cryg* is the homologue of human *CRYGA*. It is part of the γ crystallin gene cluster in the central region of chromosome 1 (Buchberg *et al.*, 1990). This cluster contains at least five genes, between which recombination events have been detected (Quinlan *et al.*, 1987). Our backcross mapping data indicate that *Cryg* and the mouse βA2 crystallin gene (*Cryba2*) are located at a considerable distance (10.6 cM) from each other. Thus, the separate position, in human and mouse, of the βA2 crystallin gene from the γ crystallin gene cluster has been firmly established only for the γA crystallin gene member of that cluster.

The central region of mouse chromosome 1 has syntenic homology with the distal half of the long arm of human chromosome 2. In mouse, we established the gene order cen–*Cryg*–*Cryba2*–*Acrg*–tel. In human, we have localized *CRYBA2* distal to *CRYGA* in region q34–q36 of chromosome 2. By using *in situ* hybridization, Lu-Kuo *et al.* (1993) have recently mapped *CHRNG*, the human homologue of *Acrg*, to 2q37.1. Thus, with regard to these three genes, gene order seems to be conserved between human and mouse.

A number of dominant cataract mutations (*Cat-2*) have recently been assigned to the mouse γ crystallin gene cluster region (Everett *et al.*, 1994; Löster *et al.*, 1994). The *Cat-2* alleles display diverse phenotypes, and it was suggested that these represent various mutations within the γ crystallin gene cluster (Löster *et al.*, 1994). However, considering the uncertainty about the positions of the γ crystallin genes (other than the γA crystallin gene) in relation to the position of the βA2 crystallin gene, the latter should be included as candidate gene for the *Cat-2* series of mouse cataracts.

In human, hereditary Coppock-like cataract has recently been associated with reactivation of the γE crystallin pseudogene (Brakenhoff *et al.*, 1994). The location of the human βA2 crystallin gene in relation to this gene is unknown. We are currently searching for polymorphisms associated with the βA2 crystallin gene. These will be used for the study of linkage between the βA2 crystallin gene and the Coppock-like cataract. Moreover, these polymorphisms will enable us to test the βA2 crystallin gene as a candidate gene for currently unmapped forms of human hereditary cataract.

ACKNOWLEDGMENTS

We thank Debbie Barnhart and Engelien Bijleveld for excellent technical assistance. This work was supported, in part, by the National Cancer Institute, DHHS, under Contract NO1-CO-46000 with ABL, by the Dutch Cancer Society (Grant AMC 94-700), and by AIRC and Telethon. K.M.C. was supported by Grant PF3707 from the American Cancer Society. R.E.K.F. was supported by Grant GM26449 from the National Institute of General Medical Sciences.

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