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Identification of the Human \(\beta A2\) Crystallin Gene (\(CRYBA2\)):
Localization of the Gene on Human Chromosome 2 and
of the Homologous Gene on Mouse Chromosome 1

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By using primers synthesized on the basis of the bovine \(\beta A2\) 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 \(\times\) 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 \(\beta A2\) crystallin gene is linked but separable from the \(\gamma A\) crystallin gene. The \(\beta A2\) crystallin gene is a candidate gene for human and mouse hereditary cataract. © 1995 Academic Press, Inc.

INTRODUCTION

The \(\alpha\), \(\beta\), and \(\gamma\) 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 \(\beta\) crystallin genes are involved in human and mouse hereditary cataracts, we are identifying all \(\beta\) crystallin genes and establishing their location in human and mouse. The \(\beta\) crystallin family consists of four acidic (A) and three basic (B) forms. \(\beta A1\) and \(\beta A3\) crystallin are encoded by one gene. The human \(\beta A3/A1\) 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 \(\beta A4\) 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 \(\beta A2\) 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 \(\mu\)g of genomic DNA, 30 pmol of each primer, 200 \(\mu\)M each dATP, dGTP, dCTP, dTTP, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl\(_2\), 0.001% (w/v) gelatin, and 2 U of AmpliTaq polymerase (Perkin-Elmer) in a final volume of 50 \(\mu\)l. Mixtures of 47 \(\mu\)l, without MgCl\(_2\), were heated to 80°C, and PCR was started by the addition of 3 \(\mu\)l of 25 mM 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 Smal site of pUC18 using a commercially available kit (SureClone ligation kit, Pharmacia).

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

Regional localisation 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 (Cerosi 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 (Cerosi 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 \textit{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\textsuperscript{–} 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 \textit{et al.}, 1995).

**Southern blot analysis.** The cloned PCR products of exons 5 and 6 of the human CRYBA2 gene were liberated by EcoRI and HindIII digestion and used as hybridization probe. Southern blot procedures were all performed as described previously (Hulsebos \textit{et al.}, 1991).

**Interspecific mouse backcross mapping.** Interspecific backcross progeny were generated by mating (C57BL/6J \* Mus spretus\textsuperscript{P1}, females and C57BL/6J males as described (Copeland and Jenkins, 1991). A total of 205 N\textsubscript{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 \textit{et al.}, 1982). All blots were prepared with Hybond-N- nylon membrane (Amer sham). After hybridization of the probe, exons 5 and 6 of the human CRYBA2 gene (see below), washing was performed to a final stringency of 0.8X SSC, 0.1% SDS, 65°C. A fragment of 9.4 kb was detected in KpnI-digested C57BL/6J DNA, and a fragment of 7.0 kb was detected in KpnI-digested \textit{M. spretus} DNA. The presence or absence of the 7.0-kb KpnI \textit{M. spretus}-specific fragment was followed in backcross mice.

A description of the probes and RFLPs for the loci linked to Cryba2 including \(\gamma\) crystallin (Cry\(\gamma\)), villin (Vil), and acetylcholine receptor \(\gamma\) subunit (Acgr) has been reported previously (Cerretti \textit{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 \(\beta\)A2 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 \(\beta\)A2 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.

**Chromosomal Localization of CRYBA2**

To determine the chromosomal location of CRYBA2, we hybridized the CRYBA2 probe to a panel of human \*X\* rodent somatic cell hybrids. On Southern blots of HindIII 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...
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 PstI-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 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.

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 ideogram 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.
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 HindIII fragment (arrow) in IB2L22 and its absence in IB2L25 strongly suggest that CRYBA2 is located within 2q34–q36 (Fig. 3B).

**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)F₁ × 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 Kpn1 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 HindIII fragments ranging in size from about 12.3 to 2 kb (Den Dunnen et al., 1985b). The CRYBA2 probe specifically recognizes one large HindIII 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,
chromosome 1 linkage map showing the location of a presence of a C57BL/6J allele, and white boxes represent the presence of chromosome is listed at the bottom of each column. A partial analysis of animals were typed (see text). Each column represents the 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 crossovers were analyzed. The segregation patterns of position of the human homologue of Cryg and distances between loci in centimorgans are shown to the left of the map.

10.6

9.1

Cryg 2q33-q35

Vil 2q35

Cryba2 2q34-q36

Acrg 2q37.1

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. spreitus)F1 parent. The shaded boxes represent the presence of a C57BL/6J allele, and white boxes represent the presence of an M. spreitus 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 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 three 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 a candidate gene for the Cat-2 series of mouse cataracts.

In human, hereditary Copock-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 Copock-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.

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