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LETTER TO THE EDITORS

Isolation and Partial Characterization of the Human αA-Crystallin Gene

There are several reasons for stressing the importance of isolating α-crystallin genes from different species. From an evolutionary viewpoint, α-crystallin is highly conserved (see de Jong, 1981, 1982), making small changes in primary structure very interesting. Moreover, the unexpected relationship between α-crystallin and *Drosophila* heat-shock proteins (Ingolia and Craig, 1982) is intriguing and requires detailed examination (see Wistow, 1985). From a developmental viewpoint, α-crystallin is the first crystallin to appear in some, but not all, species (see Piatigorsky, 1981). It is also interesting that the two α-crystallin genes (αA and αB) are differentially expressed during lens cell differentiation (Delcour and Papaconstantinou, 1974; Vermorken and Bloemendal, 1978). Another possible benefit from comparative studies of α-crystallin genes is to gain a better understanding of the molecular and biological basis for the selective appearance of the αA^{ins} polypeptide in certain rodents (Cohen, Westerhuis, de Jong and Bloemendal, 1978); the αA^{ins} mRNA is a product of alternative RNA splicing of the αA-crystallin gene (King and Piatigorsky, 1983). Finally, the numerous post-translational modifications associated with α-crystallin (e.g. aggregation, cleavage and racemization) during lens aging and cataract give special importance to the isolation of human α-crystallin genes (see Spector, 1973; Zigler and Goosey, 1981; Hoenders and Bloemendal, 1983; Harding and Crabbe, 1984).

At present, genomic clones of the αA-crystallin gene have been isolated from the mouse (King and Piatigorsky, 1983), hamster (van den Heuval, Hendricks, Quax and Bloemendal, 1985) and chicken (Yasuda, Okazaki, Kondoh, Shimura and Okada, 1983; Thompson, Hawkins and Piatigorsky, in preparation), while a genomic clone of the αB-crystallin gene has been obtained only from the hamster (Quax-Jeuken, Quax, van Rens, Khan and Bloemendal, 1985). Here we have used a murine αA-crystallin cDNA probe to isolate the human αA-crystallin gene.

Two human genomic libraries (partial Mbo I digests of placenta and spleen DNA, respectively, cloned without linkers into bacteriophage λ Charon 28; gift of Dr Philip Leder) were screened with pMαACr2, a murine αA_2-crystallin cDNA (King, Shinohara and Piatigorsky, 1982). The cDNA insert was isolated electrophoretically from pMαACr2 after digestion with Pst I and nick-translated (Maniatis, Jeffrey and Kleid, 1975) before use for hybridization. Each library was screened (total 10^6 bacteriophage plaques per library) by the colony hybridization method of Benton and Davis (1977) using $1{\cdot}5 \times 10^6$ counts per filter. Hybridization (12 hr) was performed at 68 °C in 2 × SSC (standard saline citrate) and Denhardt's solution (1966). The washes for the first screening were at 63 °C with 2 × SSC, while those for the second and third screenings were at 68 °C with 2 × SSC; all washes contained 0·5 % SDS. Ten positive clones were obtained after the three screenings (six from the spleen DNA and four from the placenta DNA). Considering that the DNA library contains overlapping inserts, this number is consistent with the presence of very few, if not a single, αA-crystallin gene.

Restriction analysis of the 10 genomic clones indicated that there were five recombinant bacteriophage containing different inserts (data not shown). These

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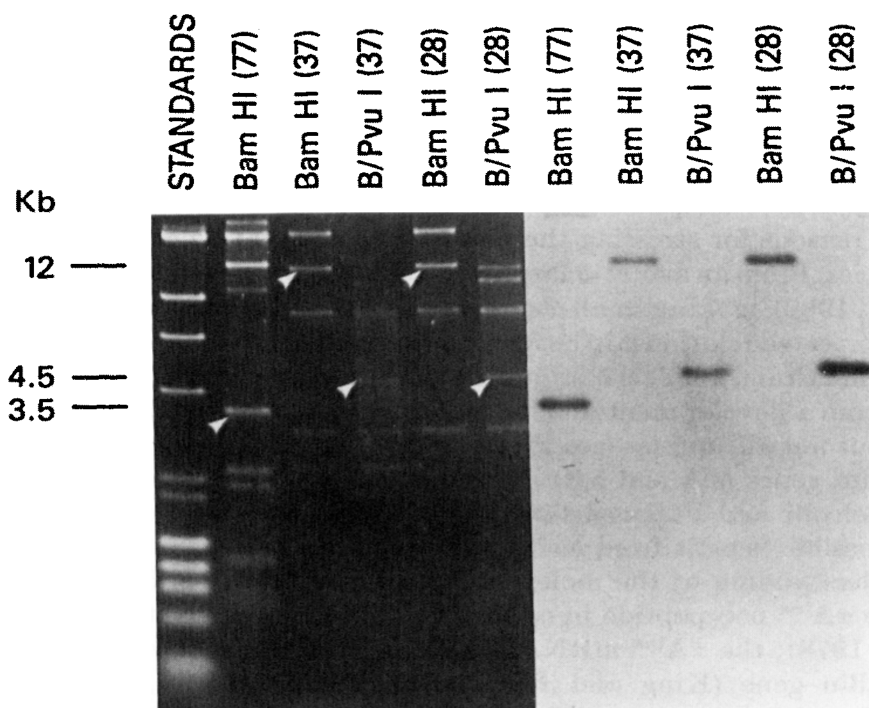

FIG. 1. *Left panel.* Agarose gel electrophoresis of DNA from three recombinant bacteriophage clones: gλHαCr77 (77), gλHαCr37 (37) and gλHαCr28 (28). Bam HI and Bam HI (B)/Pvu I double digestions, as noted. DNA size standards include λ DNA digested with Hind III and ΦX-174 DNA digested with Hae III. White arrowheads indicate fragments hybridizing with the αA-crystallin cDNA probe. Electrophoresis was in 0·9% agarose in 40 mM Tris–HCl, pH 8·2, 2 mM EDTA, 20 mM Na acetate and 18 mM NaCl; the gel was stained with ethidium bromide. *Right panel.* Southern blot hybridization of the same gel with the ^{32}P-nick-translated murine αA-crystallin cDNA probe.

clones were examined further (gλHαCr37 from the placenta DNA library, and gλHαCr28 and gλHαCr77 from the spleen DNA library). Southern blot analysis (Southern, 1975) showed that the sequences which hybridized to the αA-crystallin cDNA are situated on a 3·5 kb Bam HI fragment of gλHαCr77 and on a 4·5 Kb Bam HI/Pvu I fragment of gλHαCr37 and gλHαCr28 (Fig. 1). Digestion of gλHαCr37 and gλHαCr28 with Bam HI alone gave a 12 Kb band which hybridized to the nick-translated cDNA (Fig. 1). The relatively large size of this fragment was due to the fact that only one of the end Mbo I sites of the genomic insert was converted to a Bam HI site during the cloning procedure. Thus, only approximately 3·3 Kb of each 12 Kb fragment are genomic in origin, the remainder being derived from the bacteriophage arm. Further restriction analysis revealed that gλHαCr28 and gλHαCr37 have at least 11 Kb 5′ to the 3·3 Kb Bam HI/Pvu I fragment containing the αA-crystallin sequences, while gλHαCr77 has about 12 Kb 3′ to the 3·5 Kb Bam HI fragment containing the αA-crystallin sequences (data not shown).

We next identified the αA-crystallin gene within gλHαCr77 phage by sequencing. This phage was digested with Alu I and the resulting fragments were cloned into a Sma I-cut M13 mp8 vector (Messing and Vieira, 1982). Positive subclones were identified by hybridization with pMαACr2 and sequenced by the dideoxy-chain termination method (Sanger, Nicklen and Coulson, 1977), as described by Biggen, Gibson and Hong (1983). The results established that the 3·5 Kb Bam HI fragment in gλHαCr77 contains the human αA-crystallin gene. The 189 nucleotides given on

Human αA-Crystallin Gene

kb 0 1 2 3

EXON 1 EXON 3

AAG CCC ACC TCG GCT CCC TCG TCC TAA GCA GCA TTC CGT CGG CTG CCT CCC
Lys Pro Thr Ser Ala Pro Ser Ser TER

25 mer

10 20
ATG GAC GTG ACC ATC CAG CAC CCC TGG TTC AAG CGC ACC CTG GGG CCC TTC TAC CCC AGC CGG
Met Asp Val Thr Ile Gln His Pro Trp Phe Lys Arg Thr Leu Gly Pro Phe Tyr Pro Ser Arg — Translated human amino acid sequence
Met Asp Val Thr Ile Gln His Pro Trp Phe Lys Arg Ala Leu Gly Pro Phe Tyr Pro Ser Arg — Mouse amino acid sequence

30 40
CTG TTC GAC CAG TTT TTC GGC GAG GGC CTT TTT GAG TAT GAC CTG CTG CCC TTC CTG TCG TCC
Leu Phe Asp Gln Phe Phe Gly Glu Gly Leu Phe Glu Tyr Asp Leu Leu Pro Phe Leu Ser Ser
Leu Phe Asp Gln Phe Phe Gly Glu Gly Leu Phe Glu Tyr Asp Leu Leu Pro Phe Leu Ser Ser

50 60
ACC ATC AGC CCC TAC TAC CGC CAG TCC CTC TTC CGC ACC GTG CTG GAC TCC GGC ATC TCT GAG
Thr Ile Ser Pro Tyr Tyr Arg Gln Ser Leu Phe Arg Thr Val Leu Asp Ser Gly Ile Ser Glu
Thr Ile Ser Pro Tyr Tyr Arg Gln Ser Leu Phe Arg Thr Val Leu Asp Ser Gly Ile Ser Glu

FIG. 2. Deduced partial structure of the human αA-crystallin gene in gλHαCr77. The protein coding sequence in exon 1 and for the C-terminal end of the αA-crystallin polypeptide are shown. These sequences were derived from a single strand of DNA. The nucleotide sequence for the C-terminus of the protein is assumed to be in exon 3 by analogy with the αA-crystallin gene from other species (see text). The thr-ala difference between the mouse and human α-crystallin polypeptide at position 13 is boxed. The 25-mer oligonucleotide used in Fig. 4 is indicated. The numbers above the sequence refer to amino-acid residue number in the protein. See text for further discussion.

the left side of Fig. 2 encode the first 63 amino acids of the human αA-crystallin polypeptide (de Jong, Terwindt and Bloemendal, 1975). By analogy with the known structure of the αA-crystallin gene of the mouse (King and Piatigorsky, 1983) and hamster (van den Heuval et al., 1985), we assume that this human coding sequence is located on exon 1. Experiments to be presented elsewhere support this assumption.

Interestingly, the 3′ splice site for exon 1 in the murine and human αA-crystallin gene occurs after the codon for amino acid 63 (glu) (Fig. 2). There is evidence that this exon has resulted from duplication and fusion of an ancestral sequence (Barker, Ketchan and Dayhoff, 1978; Wistow, 1985; van den Heuval et al., 1985). As noted earlier by protein sequencing (de Jong, 1982; de Jong and Goodman, 1982), there is only a single amino acid difference (thr in the human and ala in the mouse at position 13) of the encoded αA-crystallin protein in exon 1 (boxed in Fig. 2). Position 13 is occupied by thr also in the tapir, rhinoceros, ox and rabbit, by pro in the lemur, galago and potto, and by ser in the dogfish, indicating that this position is not invariant (de Jong, 1982).

Figure 3 compares the nucleotide sequence of the coding region of exon 1 of the human and murine αA-crystallin gene. There are only 12 nucleotide differences between these sequences (6·3 % change) despite the 70–80 million years of divergence between mouse and man (de Jong, 1982). All but one of these occur at the third position of the codons and do not result in an amino acid change. The ala → thr change noted above is due to a G → A change at position one of codon 13 (boxed in Fig. 3).

The 3′ end of the coding region of the αA-crystallin gene was found in another Alu I fragment derived from gλHαCr77 (Fig. 2, right side). Again, by analogy with the

```
                                     30                            60
Human  ATGGACGTGACCATCCAGCACCCCTGGTTCAAGCGCACCCTGGGGCCCTTCTACCCCAGCCGG
       :::::::: ::::: ::::: :: :::::::::::  ::::::::::::::::::::::::: 
Mouse  ATGGACGTCACCATTCAGCATCCTTGGTTCAAGCGTGCCCTGGGGCCCTTCTACCCCAGCCGA

                                     90                            120
Human  CTGTTCGACCAGTTTTTCGGCGAGGGCCTTTTTGAGTATGACCTGCTGCCCTTCCTGTCGTCC
       :::::::::::::: :::::::::::::::::::::: ::::::::::::::::::::: :::
Mouse  CTGTTCGACCAGTTCTTCGGCGAGGGCCTTTTTGAGTACGACCTGCTGCCCTTCCTGTCTTCC

                                     150                           180
Human  ACCATCAGCCCCTACTACCGCCAGTCCCTCTTCCGCACCGTGCTGGACTCCGGCATCTCTGAG
       :::::::::::::::::::::::::::::::::::::: ::::::::::: ::::::::::::
Mouse  ACCATCAGCCCCTACTACCGCCAGTCCCTCTTCCGCACTGTGCTGGACTCGGGCATCTCTGAG
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FIG. 3. Comparison of the coding regions in exon 1 of the human and mouse. The only non-third position difference is boxed and occurs in codon 13. See text. Numbers above sequence refer to nucleotide position.

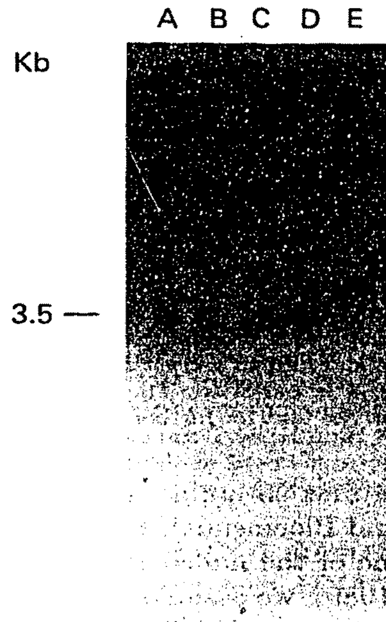

FIG. 4. Southern blot hybridization of Bam HI-digested genomic DNA from five different individuals. The 25-mer oligonucleotide probe indicated in Fig. 2 was synthesized by OCS Labs, Denton, Texas. It was labeled to a specific activity of about 10^{9} cpm μg^{-1} DNA by hybridization with a 15-mer complement followed by extension with Klenow fragment of DNA polymerase using four α-^{32}P-dNTPs. Hybridization procedures are given by Miyada, Klofelt, Reyes, McLaughlin-Taylor and Wallace (1985). This probe was hybridized with a dried denatured, neutralized and rehydrated 0·9% agarose gel using $2{\cdot}0 \times 10^{6}$ cpm ml^{-1}. Hybridization was carried out at 68 °C for 3 hr followed by two 15-min washes at 25 °C, one 2-hr wash at 25 °C and one 90-sec wash at 68 °C. All washes were in 6 × SSC (0·9 M NaCl, 90 mM sodium citrate, pH 7·2). Autoradiography was with Kodak XAR-5 film for 15 hr at −70 °C with an intensifying screen.

known structure of the hamster αA-crystallin gene (van den Heuval et al., 1985) we assume that the sequences encoding the C-terminus of the protein are located on exon 3. The numbering of these exons depends on the assumption that the insert exon present in the mouse (King and Piatigorsky, 1983) and hamster (called exon 2 in this species, van den Heuval et al., 1985) is absent in the human.

Finally, we performed a Southern blot on Bam HI-digested DNA from tissue samples of five individuals in order to provide further evidence that there is only a single αA-crystallin gene in humans. Hybridization was performed with a radioactively labeled 25-mer oligonucleotide probe encoding amino acids 10 to 17 of the αA-crystallin

polypeptide (indicated in Fig. 2) on the dried agarose gel. The 25-mer probe has minimum and maximum mismatches with the human αB-crystallin gene of 32 % and 64 %, respectively, as calculated from the known sequence of the human αB protein (Kramps, de Man and de Jong, 1977) and the ambiguity of the genetic code. The oligomeric probe hybridized exclusively to a 3·5 Kb Bam HI DNA fragment from each individual (Fig. 4). In another test, this 3·5 Kb Bam HI fragment co-migrated with the 3·5 Kb insert in gλHαCr77 which contains the αA-crystallin gene. This result is consistent with the interpretation that there is a single αA-crystallin gene in the human and that this gene is present in gλHαCr77. Additional experiments using pMαACr2 (the cDNA probe) revealed the presence of this hybridizing 3·5 Kb Bam HI DNA fragment in 16 individuals. Thus, it appears as if the human αA-crystallin gene is not highly polymorphic.

In summary, we have isolated the αA-crystallin gene in humans by using a nearly full-length cDNA probe from mice. There appears to be only one αA-crystallin gene in man as in mouse (King and Piatigorsky, 1983), hamster (van den Heuval et al., 1985) and chicken (Thompson et al., in preparation). A recent investigation using somatic cell hybrids indicates that this gene is located on human chromosome 21 (Quax-Jeuken et al., 1985). Our data indicate that the size and structure of the human αA-crystallin gene are generally similar to that in the other organisms examined. The first intron of the human gene is situated after codon 63 as it is in the mouse (King and Piatigorsky, 1983), hamster (van den Heuval et al., 1985) and chicken (Yasuda et al., 1983; Thompson et al., in preparation). We presume that the human αA-crystallin gene lacks an insert exon (King and Piatigorsky, 1983) and that codons 64–104 are contained on a separate exon as in the other organisms, but this remains to be established. The present isolation of the human αA-crystallin gene extends our ability to conduct comparative studies on this highly conserved lens protein, which should deepen our understanding of its selective expression (Chepelinsky, King, Zelenka and Piatigorsky, 1985; Okazaki, Yasuda, Kondoh and Okada, 1985; Overbeek, Chepelinsky, Khillan, Piatigorsky and Westphal, 1985) and alternative RNA splicing (King and Piatigorsky, 1983). The availability of the human αA-crystallin gene should also facilitate studies concerning the possible involvement of αA-crystallin in cataract.

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