J Mol Evol (1991) 33:457–463

Journal of Molecular Evolution



# Presence of Hybridizing DNA Sequences Homologous to Bovine Acidic and Basic β-Crystallins in All Classes of Vertebrates

Geert L.M. van Rens, Frans A. Hol, Wilfried W. de Jong, and Hans Bloemendal

Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands

**Summary.** The eye lens β-crystallins in cow and chicken are encoded by a family of at least six genes. In order to assess the distribution of the corresponding genes among other vertebrates we hybridized β-crystallin sequences (βA2, βA3/A1, βA4, βB1, βB2, βB3), isolated from a bovine lens cDNA library, to Southern blots on which *Eco*R1-digested chromosomal DNA was blotted from different vertebrate species. These included human, chimpanzee, calf, rat, pigeon, duck, monitor lizard, toad, trout, and lamprey. Positive hybridization signals were found in the representatives of virtually all classes of vertebrates. The basic βB-crystallins gave hybridization signals in more species than the acidic βA ones. In monitor lizard and toad the weakest hybridization signals for basic crystallin probes were found. For acidic crystallin probes the distribution pattern was more simple; among cold-blooded vertebrates a signal for βA2 was found in trout and lamprey, for βA4 in trout, and for βA3/A1 only in toad. The results demonstrate that the duplications leading to the β-crystallin gene family occurred before or during the earliest stages of vertebrate evolution.

**Key words:** Eye lens proteins — Molecular evolution — Gene family

## Introduction

The major proteins of the vertebrate eye lens are called crystallins, which constitute 95% of the proteins present (Bloemendal 1981). Crystallin function is supposed to be mainly structural. Lens fiber cells are filled with crystallins to a high concentration in a tight and stable packing in order to impart adequate optical properties to the lens (Tardieu and Delaye 1988). The crystallins can be divided into four major classes, α-, β-, γ-, and δ-crystallins, of which the β- and γ-crystallins belong to the same multigene family. Occurrence of δ-crystallin is restricted to birds and reptiles. Some species possess in addition so-called taxon-specific crystallins, which often have enzymatic activity (Wistow and Piatigorsky 1988; De Jong et al. 1989).

The most heterogeneous group of crystallins is the β-crystallin family. They can be divided into two structurally distinct groups: the basic βB and acidic βA ones (Berbers et al. 1982; Slingsby et al. 1988). This distinction forms the basis for the β-crystallin nomenclature (Bloemendal et al. 1989). The oligomeric β-crystallins are distinguished from the monomeric γ-crystallin family on the basis of structural and immunological criteria (Lubsen et al. 1988). In the bovine lens there exist at least seven primary gene products assigned to the β-crystallin family (βA1, βA2, βA3, βA4, βB1, βB2, and βB3). These paralogous β-crystallins (i.e., that have arisen via gene duplications) are encoded by six different RNAs (Berbers et al. 1982; Van Rens et al. 1991). Also the chicken contains seven primary gene products (Ostrer et al. 1981; Hejtmancik et al. 1985). Two of them, βA3 and A1, are, as in calf and human, translated from the same messenger by the use of two different initiation codons (Gorin and Horwitz 1984; Quax-Jeuken et al. 1984; Hogg et al. 1986; Peterson and Piatigorsky 1986).

By now the primary structures of all bovine β-crystallins have been elucidated, mainly by inference from cDNA sequences (Quax-Jeuken et al. 1984; Van Rens et al. 1991), but also by protein

*Offprint requests to:* H. Bloemendal

sequencing (Driessen et al. 1981; Berbers et al. 1984). Like γ-crystallins they have four internal repeats, corresponding with four structural motifs (Greek keys; Blundell et al. 1981). Also at the gene level this fourfold repeat can be detected; in the β-genes four exons encode the four different structural motifs (Piatigorsky 1984). The motifs are arranged in two compact domains. Recently, the crystal structure of the βB2 homodimer has been elucidated by x-ray analysis (Bax et al. 1990). It has been shown that, as compared with the γ-crystallins, the peptide connecting the domains of βB2 crystallin is extended. The two domains are separated in a way unlike that in the γ-crystallins; the domain interactions in the homodimer of βB2 are antiparallel and intermolecular instead of intramolecular.

The β-crystallins have distant structural and sequence homology with some small proteins like the bacterial spore coat protein S (Wistow et al. 1985) and spherulin 3a from the slime mold *Physarum polycephalum* (Wistow 1990). In early vertebrate evolution the β-crystallins have possibly been recruited from preexisting housekeeping genes to function as eye lens proteins, as has been the case with other crystallins (Wistow and Piatigorsky 1988; De Jong et al. 1989). Hitherto, several β-crystallin sequences have been detected in different warm-blooded species and also in frogs.

The availability of the complete set of acidic and basic bovine β-crystallin cDNA sequences as molecular hybridizing probes made it possible for us to study the presence of presumed orthologous β-crystallin sequences (i.e., those that have arisen via speciation) within a wide evolutionary range of vertebrate classes. For this purpose genomic DNA was isolated from different vertebrate species and digested with the enzyme *Eco*R1. The results of hybridizing the β-crystallin probes to Southern blots are evaluated in the present study.

## Materials and Methods

*Chromosomal DNA: Isolation and Blotting.* The isolation of chromosomal DNA from cell cultures or animal tissues was done as described by Maniatis et al. (1982). Cell pellets were used directly for isolation of chromosomal DNA. Tissues isolated from animals were immediately frozen in liquid nitrogen. The species and tissues used to isolate DNA were human (*Homo sapiens*) placenta cells; chimpanzee (*Pan troglodytes*) brain; cow (*Bos taurus*) thymus; rat (*Rattus norvegicus*) spleen; pigeon (*Columba livia*) brain; duck (*Anas platyrhynchos*) brain; monitor lizard (*Varanus exanthematicus*) liver; toad (*Xenopus laevis*) ovary; trout (*Salmo trutta*) spleen; lamprey (*Petromyzon marinus*) ovary. Aliquots of 10 μg of genomic DNA were taken for digestion with *Eco*R1 in 100 μl of the reaction mixture for 3 h as prescribed by the supplier (Boehringer Mannheim). After addition of the enzyme a 10-μl sample was taken from each microtube. To these 10-μl aliquots 0.5 μg of λDNA was added. When the λDNA was digested to completion, it was assumed that the genomic DNA was completely digested too; the appearance of discrete hybridization bands on the incubated blots was an additional indication of complete digestion. The digests were loaded on a 0.7% agarose gel, and size-fractionated for 72 h at 6 mA (Maniatis et al. 1982). Transferral of the DNA to nitrocellulose (Schleicher and Schuell BA 85, 0.45 μm) was accomplished with 10% standard saline citrate SSC: 0.15 M NaCl, 0.015 M sodium citrate) (Maniatis et al. 1982). Finally, after air drying, Southern blots were baked for 2 h under vacuum at 80°C to immobilize the chromosomal DNA.

*Probes: Labeling and Hybridization.* All probes used in the present study were prepared in our laboratory from cDNA clones containing the complete or nearly complete coding sequences of the β-crystallin messengers. All clones were isolated from bovine cDNA libraries. The cDNAs used to synthesize the probes for the acidic β-crystallins were βA2 (520 bp), βA4 (790 bp), and βA3/A1 (200 bp) (Quax-Jeuken et al. 1984; Van Rens et al. 1991). The basic β-crystallin probes used were βB1 (900 bp), βB2 (250 bp), and βB3 (850 bp) (Quax-Jeuken et al. 1984; Van Rens et al. 1989). The probes were labeled by random priming (i.e., Klenow elongation after priming with random hexamer primers; Feinberg and Vogelstein 1983) except for the βA3/A1 and βB2 probes, which were labeled by downstream priming (i.e., Klenow elongation of a primer template) with a cloned fragment from the third and fourth domain of βB2 or βA3/A1 in pTZ18R (Pouwels et al. 1985). Hybridization was done according to Church and Gilbert (1984), in a mixture of 0.25 M anhydrous disodiumhydrogenphosphate, 7% SDS, 1% BSA, and 1 mM EDTA, with the addition of 100 μg of herring sperm DNA/ml of hybridization mixture. The nitrocellulose blots were preincubated in hybridization mixture for at least 1 h at 65°C. The volume of this mixture was the minimum amount required (60 μl/cm²). The activity used was at least $10^6$ cpm/ml. Hybridization (at 65°C) was done for at least 14 h. The blots were washed consecutively in four solutions (each one for half an hour) containing 1% SDS, 1 mM EDTA, and 0.25 M, 0.125 M, 0.05 M, and 0.025 M anhydrous disodiumhydrogenphosphate, respectively. Blots hybridized with the basic β-crystallins were washed to the highest stringency. The blots for the acidic β-crystallins were at first washed to the highest stringency (0.025 M $Na_2HPO_4$), and during a subsequent hybridization experiment to a concentration of 0.05 M $Na_2HPO_4$.

## Results

For our experiments we constructed three Southern blots with *Eco*R1-digested genomic DNA of several vertebrate species (see legends to Fig. 1). The digests were monitored (see Materials and Methods), and, if fully digested, loaded onto an agarose gel and electrophoresed. The size-fractionated DNA was blotted onto nitrocellulose and hybridized consecutively with the different β-crystallin probes. At first each of the blots was screened with one of the probes coding for the three basic β-crystallins (Fig. 1). After dehybridization of the blots, screening was done with the probes for the three acidic β-crystallins (Fig. 2).

In Fig. 1A the results of screening with the βB1 probe are presented. All the lanes show discrete hybridization bands, although the intensities of these bands differ greatly in the various species. The strongest hybridization signal was found in calf, owing to the complete homology between the gene and

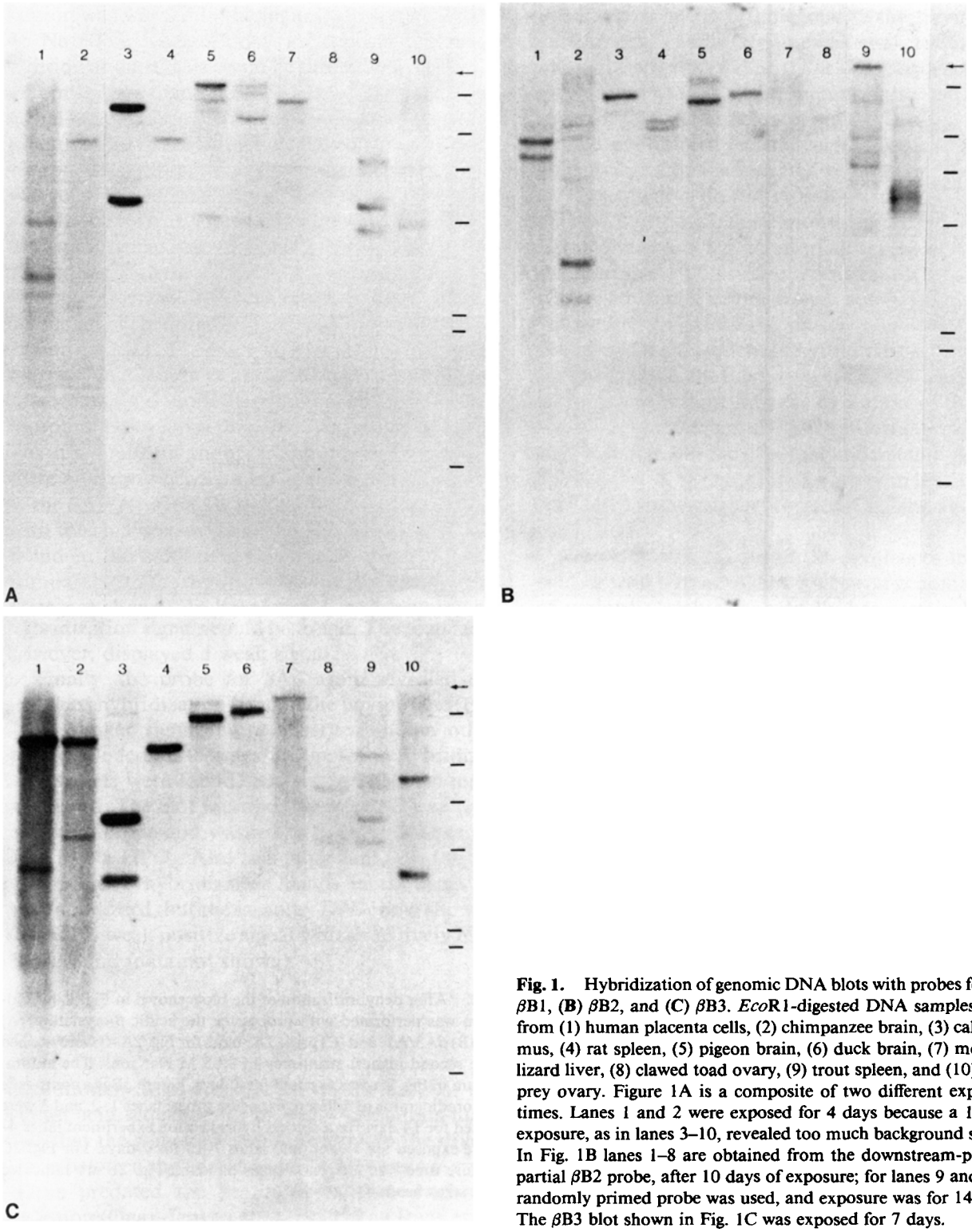


**Fig. 1.** Hybridization of genomic DNA blots with probes for (**A**) $\beta$B1, (**B**) $\beta$B2, and (**C**) $\beta$B3. *Eco*R1-digested DNA samples were from (1) human placenta cells, (2) chimpanzee brain, (3) calf thymus, (4) rat spleen, (5) pigeon brain, (6) duck brain, (7) monitor lizard liver, (8) clawed toad ovary, (9) trout spleen, and (10) lamprey ovary. Figure 1A is a composite of two different exposure times. Lanes 1 and 2 were exposed for 4 days because a 10-day exposure, as in lanes 3–10, revealed too much background signal. In Fig. 1B lanes 1–8 are obtained from the downstream-primed partial $\beta$B2 probe, after 10 days of exposure; for lanes 9 and 10 a randomly primed probe was used, and exposure was for 14 days. The $\beta$B3 blot shown in Fig. 1C was exposed for 7 days.

the cDNA sequences. In toad the hybridization bands are very weak, reflecting the lower homology of the hybridizing sequences.

Figure 1B combines the results of two consecutive hybridizations. The blot was first hybridized with a randomly primed probe made from a cDNA insert coding for $\beta$B2, which gave a smear of aspecific signals in lanes of some higher vertebrates. The second hybridization was done with a downstream-primed probe, containing only coding sequences for the second domain of $\beta$B2. The hybridization signals displayed in the warm-blooded animals are on average just as strong as in calf. In lane 7 the monitor lizard chromosomal DNA dis-

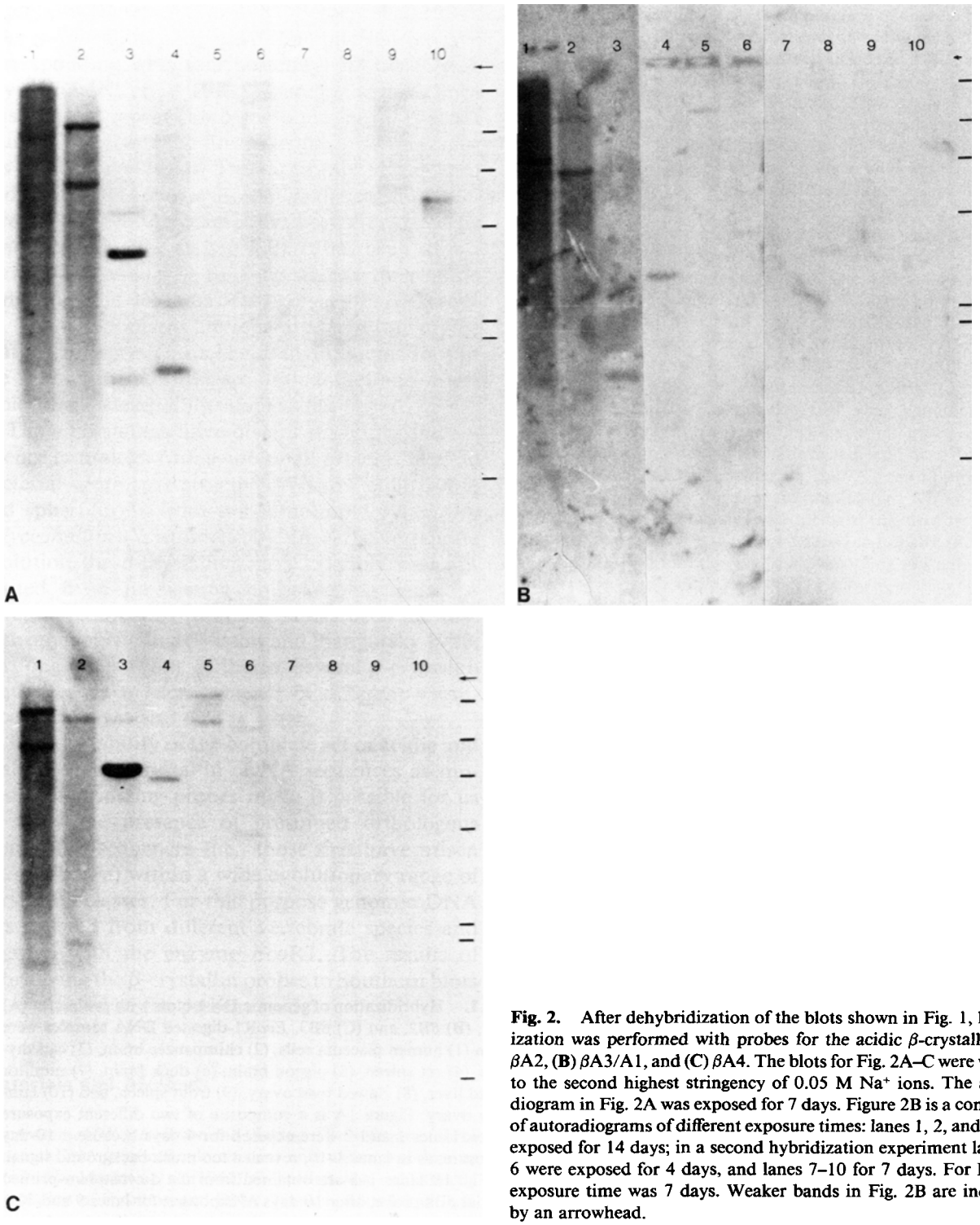


**Fig. 2.** After dehybridization of the blots shown in Fig. 1, hybridization was performed with probes for the acidic β-crystallins (**A**) βA2, (**B**) βA3/A1, and (**C**) βA4. The blots for Fig. 2A–C were washed to the second highest stringency of 0.05 M $Na^+$ ions. The autoradiogram in Fig. 2A was exposed for 7 days. Figure 2B is a composite of autoradiograms of different exposure times: lanes 1, 2, and 3 were exposed for 14 days; in a second hybridization experiment lanes 4–6 were exposed for 4 days, and lanes 7–10 for 7 days. For Fig. 2C exposure time was 7 days. Weaker bands in Fig. 2B are indicated by an arrowhead.

plays two possible hybridization bands; however, the signals are weak and the background is relatively strong. Also in the toad (lane 8) two weak bands can be distinguished. Surprisingly, rather strong signals were found in the lanes loaded with DNA from trout and lamprey.

The βB3 probe gave the best results (Fig. 1C). The strongest hybridization signal was found in the bovine lane. The rest of the mammals also showed discrete and strong hybridization signals. Again, there is some aspecific hybridization found in human and chimpanzee. Slightly less intense hybridization bands were found in birds. The weakest signals were found in the lanes of lizard, toad, and trout. Lamprey showed a relatively strong hybridization signal.

The initial hybridization for βA2 was done with a randomly primed probe (Fig. 2A). This hybrid-

ization was washed to the highest stringency (0.025 M $Na_2HPO_4$). After 7 days of exposure, positive hybridization signals could be observed in all lanes, except for the toad and lizard lanes. The strongest signal was observed in the bovine lane. Also the other mammalian samples displayed strong hybridization. In lamprey, one of the hybridizing bands was relatively strong as compared to the other species. In the trout, many bands were visible, but they were poorly defined. A second hybridization was carried out with the same probe, but by adding twice as many cpm/ml of hybridization mixture. Again, no signals were found in toad and in lizard.

For βA3/A1, the most widely sequenced acidic β-crystallin (Lubsen et al. 1988), hybridization signals were found in the mammalian and avian chromosomal DNA lanes (Fig. 2B). The strongest signal was not found in the calf lane, probably because there are many *Eco*R1 sites in the noncoding parts of the βA3/A1 gene. In another blotting experiment with *Xba*1-digested DNA the strongest signal was found in the calf lane. The rest of the data were comparable to the results found with the *Eco*R1 blot (data not shown). In lizard, trout, and lamprey no hybridization signals could be found. The toad lane, however, displayed a weak signal.

Finally, the probe for βA4 again revealed the strongest hybridization band in the bovine lane (Fig. 2C). Weaker signals were observed in the other warm-blooded vertebrates. In the trout, hybridization signals were found, but not in lamprey, toad, and lizard. The blot was once more hybridized with the same probe and washed to a lower stringency, 0.05 M $Na_2HPO_4$. Also this procedure failed to reveal positive hybridization bands in the lanes of toad and lizard, but the lamprey DNA now showed a possibly weak positive signal with a relatively high background (data not shown).

## Discussion

Evolutionary trees constructed on the basis of the amino acid sequences of β- and γ-crystallins indicate that the gene duplication leading to the divergence between the ancestral acidic and basic β-crystallins predated the beginning of the vertebrate radiation (Quax-Jeuken et al. 1985; Van Rens et al. 1991). The sequence differences among the three acidic and the three basic chains from cow also suggest that the subsequent gene duplications resulting in the present-day β-crystallin family may well have occurred during the earliest stages of vertebrate evolution (Berbers et al. 1984; Aarts et al. 1989). It is therefore likely that orthologues of the mammalian β-crystallins will be present in lower vertebrates. The two sequenced chicken β-crystallin cDNAs indeed appear to be orthologous to the bovine βB1 and βA3/A1 chains (Hejtmancik et al. 1985; Peterson and Piatigorsky 1986). The only sequenced frog β-crystallin cDNA is presumably also the orthologue of βA3/A1 (Luchin et al. 1985).

The evolutionary distance between the paralogous β-crystallins is reflected by the fact that their DNA sequences do not cross-hybridize (Lubsen et al. 1988). Although this seriously hampered the isolation and characterization of all representatives of the mammalian β-crystallin gene family, it actually was an advantage in the present study. In this study we wanted to assess the presence in lower vertebrates of DNA sequences hybridizing with probes for the six different bovine β-crystallins. This would increase our insight into the evolution of the multigene β-crystallin family. Such information, again, may be helpful in the eventual understanding of the significance of the enormous variety in lens protein composition among vertebrates (De Jong and Hendriks 1986).

Our approach, to search for sequences that hybridize with bovine cDNA probes on genomic blots of various vertebrates, actually has certain limitations. The inability to detect hybridization sequences in lower vertebrates does not prove the absence of homologous genes, but may as well reflect that sequences have diverged beyond the limits of detection. On the other hand, positive hybridization signals tell nothing about the actual expression and significance of the corresponding β-crystallin protein in that particular species. Redundant and dispensable genes may still be recognizable and capable of hybridizing over tens of million of years because of the relatively low rate of unhindered spontaneous base substitutions (Ohno 1985). The intensity of hybridization bands is in part an indication of the extent of sequence homology, and thus of evolutionary distance, but additional factors seriously interfere with such an interpretation. Similarly, the complexity of the pattern of hybridization bands suggests the occurrence of more recent duplication events. A relatively recent duplication of the βB2 gene has indeed been found in the human genome (Aarts et al. 1987). It is assumed to have arisen in the primate lineage and it may relate to the complexity in the chimpanzee lane 2 of Fig. 1B.

Despite the limitations of the method, a number of conclusions can be drawn concerning the distribution of acidic and basic β-crystallin sequences in the genomes of vertebrates. First of all the basic β-crystallins appear to be more conserved than the acidic ones. They display hybridizing bands in more species than the acidic probes do. Moreover, all the patterns of the autoradiograms of the blots screened with the basic crystallins differ from each other. The different probes hybridize with different homolo-

gous DNA sequences in each of the lower vertebrate species. Probably all lower vertebrates possess conserved basic β-crystallin sequences for βB1, βB2, and βB3. However, lower vertebrates do show weaker hybridization signals than the warm-blooded vertebrates. Autoradiograms from hybridization experiments with acidic β-crystallin probe showed less clear results. In all lanes loaded with DNA isolated from warm-blooded animals positive hybridization signals were found. In cold-blooded animals, however, only weak hybridization signals were found in some species. The hybridization patterns differ from each other and also from the patterns found for the basic β-crystallin probes. The only species that does not have any positive hybridization signal with probes for the acidic crystallins is the monitor lizard. In the clawed toad only βA3/A1 was found. Indeed, in another amphibian species, the frog *Rana temporaria*, a βA3/A1 cDNA supposedly orthologous to the sequence found in higher vertebrates, was sequenced by Luchin et al. (1985).

The weak hybridization signals found in the lower vertebrates may be due to the evolutionary distance of the cold-blooded species from the cow. A faster rate of evolution of the crystallin sequences may even totally obliterate hybridization in certain species. It has indeed been shown that conspicuous differences do exist in the rates of evolutionary change of the β-crystallins (Aarts et al. 1989). At variance with our results these authors found, in a restricted number of species, a higher conservation for βA3/A1 compared to the basic β-crystallin sequences. If the absence of hybridization signals would indeed signify the lack of particular β-crystallins in certain taxa, it would be interesting to see whether this correlates with an increase of taxon-specific crystallins, as has been observed for δ-crystallin in the chimney swift (Wistow et al. 1990). To ensure that the sequences found to be homologous to bovine β-crystallin are not just remnants of ancient relatives of the present-day crystallins, but still functioning and expressed in modern lower vertebrates, the corresponding crystallin cDNAs should be isolated and sequenced from the eye lenses of these lower vertebrates.

## References


Aarts HJM, Den Dunnen JT, Lubsen NH, Schoenmakers JGG (1987) Linkage between the βB2- and βB3-crystallin genes in man and rat: a remnant of an ancient gene cluster. Gene 59:127–135

Aarts HJM, Jacobs EHM, Van Willigen G, Lubsen NH, Schoenmakers JGG (1989) Different evolution rates within the lens-specific β-crystallin gene family. J Mol Evol 28:313–321

Bax B, Lapatto R, Nalini V, Driessen H, Lindly PF, Mahadevan D, Blundell TL, Slingsby C (1990) X-ray analysis of βB2-crystallin and evolution of oligomeric proteins. Nature 347: 776–780

Berbers GAM, Boerman OC, Bloemendal H, De Jong WW (1982) Primary gene products of bovine β-crystallins and reassociation behavior of its aggregates. Eur J Biochem 125:495–502

Berbers GAM, Hoekman WA, Bloemendal H, De Jong WW, Kleinschmidt T, Braunitzer G (1984) Homology between the primary structures of the major bovine β-crystallin genes. Eur J Biochem 139:467–479

Bloemendal H (ed) (1981) Molecular and cellular biology of the eye lens. John Wiley and Sons, New York

Bloemendal H, Piatigorsky J, Spector A (1989) Recommendations for crystallin nomenclature. Exp Eye Res 48:465–466

Blundell T, Lindley P, Miller L, Moss D, Slingsby C, Tickle I, Turnell B, Wistow G (1981) The molecular structure and stability of the eye lens: x-ray analysis of γ-crystallin II. Nature 289:771–777

Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81:1991–1995

De Jong WW, Hendriks W (1986) The eye lens crystallins: ambiguity as evolutionary strategy. J Mol Evol 24:121–129

De Jong WW, Hendriks W, Mulders JWM, Bloemendal H (1989) Evolution of eye lens crystallins: the stress connection. Trends Biochem Sci 14:365–368

Driessen HPC, Herbrink P, Bloemendal H, De Jong WW (1981) Primary structure of the bovine β-crystallin Bp chain. Internal duplication and homology with γ-crystallin. Eur J Biochem 121:83–91

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Gorin MB, Horwitz J (1984) Cloning and characterization of a cow β-crystallin cDNA. Curr Eye Res 3:939–948

Hejtmancik JF, Beebe DC, Ostrer H, Piatigorsky J (1985) δ- and β-crystallin mRNA levels in embryonic and posthatched chicken lens: temporal and spatial changes during development. Dev Biol 109:72–81

Hogg D, Tsui LC, Gorin M, Breitman ML (1986) Characterization of the human β-crystallin gene HuβA3/A1 reveals ancestral relationships among the βγ-crystallin superfamily. J Biol Chem 261:12420–12427

Lubsen NH, Aarts HJM, Schoenmakers JGG (1988) The evolution of lenticular proteins: the β- and γ-crystallin gene superfamily. Progr Biophys Mol Biol 51:47–76

Luchin SV, Tomarev SI, Dolgilevich SM, Krayev AS, Gause GG (1985) Isolation and structure of a clone of recombinant cDNA encoding β-crystallin of the eye lens of the frog *Rana temporaria*. Dokl Biochem 279:1408–1414

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor NY

Ohno S (1985) Dispensable genes. Trends Genet 1:160–164

Ostrer H, Beebe DC, Piatigorsky J (1981) β-crystallin mRNAs: differential distribution in the developing chicken lens. Dev Biol 86:403–408

Peterson CA, Piatigorsky J (1986) Preferential conservation of the globular domains of the βA3/A1-crystallin polypeptide of the chicken eye lens. Gene 45:139–147

Piatigorsky J (1984) Lens crystallins and their gene families. Cell 38:620–621

Pouwels PH, Enger-Valk BE, Brammar WJ (1985) Cloning vectors: a laboratory manual. Elsevier, Amsterdam

Quax-Jeuken Y, Jansen C, Quax W, Van den Heuvel R, Bloemendal H (1984) Bovine β-crystallin complementary DNA clones. Alternating proline/alanine sequence of βB1 subunit originates from a repetitive DNA sequence. J Mol Biol 180: 457–472

Quax-Jeuken Y, Driessen H, Leunissen J, Quax W, De Jong W,

Bloemendal H (1985) βs-crystallin: structure and evolution of a distinct member of the βγ-superfamily. EMBO J 10: 2597–2602

Slingsby C, Driessen HPC, Mahadevan D, Bax B, Blundell TL (1988) Evolutionary and functional relationships between the basic and acidic β-crystallins. Exp Eye Res 46:375–403

Tardieu A, Delaye M (1988) Eye lens proteins and transparency: from light scattering transmission theory to solution x-ray structural analysis. Annu Rev Biophys Chem 17:47–70

Van Rens GLM, Raats JHM, Driessen HPC, Oldenburg O, Wijnen J, Meera Khan P, De Jong WW, Bloemendal H (1989) Structure of the bovine eye lens γs-crystallin gene (formerly βs). Gene 78:225–233

Van Rens GLM, Driessen HPC, Nalini V, Slingsby C, De Jong WW, Bloemendal H (1991) Isolation and characterization of cDNAs encoding βA2- and βA4-crystallin: heterologous interactions in the predicted βA4–βB2 heterodimer. Gene 102: 179–188

Wistow G (1990) Evolution of a protein superfamily: relationships between vertebrate lens crystallins and microorganism dormancy proteins. J Mol Evol 30:140–145

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

Wistow G, Summers L, Blundell T (1985) *Myxococcus xanthus* spore coat protein S may have a similar structure to vertebrate lens βγ-crystallins. Nature 316:771–773

Wistow G, Anderson A, Piatigorsky J (1990) Evidence for neutral and selective processes in the recruitment of enzyme-crystallins in avian lenses. Proc Natl Acad Sci USA 87:6277–6280

Received March 11, 1991/Revised May 27, 1991/Accepted June 11, 1991