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Cloning and Mapping the Mouse *Crygs* Gene and Non-lens Expression of γS-Crystallin

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Purpose: γ-Crystallins are major structural proteins of the eye lens. While other crystallins have revealed distinct non-lens functions and patterns of expression, γ-crystallins have generally appeared to be the most lens-specific of the crystallins. Here we examine the mouse γS-crystallin (γS) gene and its expression.

Methods: The cDNA and gene for mouse γS were cloned and sequenced. The *Crygs* gene was mapped using genetic crosses. Expression patterns in mouse and cow were examined by northern blot, PCR and western blot using a specific peptide antibody.

Results: The *Crygs* gene was sequenced and mapped to mouse chromosome 16, at or near the locus for the genetic cataract *Opj*. Northern blots of tissues from new born mice, showed lens specific expression of γS. However, in the mature mouse eye there was, in addition, clear non-lens expression of γS. In the adult bovine eye RT-PCR shows that γS is expressed in lens, retina and cornea. A peptide antibody directed against γS detects bands of the expected size in western blots of mouse lens and in 33 day old mouse retina.

Conclusions: These results suggest that γ-crystallins have a non-crystallin role outside the lens, one which may predate the lens in evolutionary terms. Non-lens expression seems to increase with age in young mice, hinting that γS may have a role similar to that of a stress protein in tissues of the eye, perhaps related to accumulating insults resulting from light exposure.

The optical and structural properties of the lens are largely determined by the expression of very high levels of several classes of soluble proteins, the crystallins [1-4]. It is now clear that crystallins arose from proteins with pre-existing functions which underwent direct gene recruitment to acquire additional roles as structural proteins in the lens [3-5]. In many species, the composition of the lens has been modified by relatively recent events involving the gene recruitment of enzymes as taxon-specific crystallins [3,4]. In contrast, the α, β, and γ families are ubiquitously represented in all vertebrates and must have been recruited to the evolving lens in an early common ancestor of modern vertebrate species [3,4]. The α-crystallins clearly arose from the small heat shock protein superfamily and, in mammals, αB-crystallin is expressed as a stress protein [6,7]. The β- and γ-crystallins are evolutionarily and structurally related members of a βγ superfamily [8], which also includes micro-organism stress proteins as well as vertebrate proteins that appear to be associated with processes of cell differentiation and morphological change [4,9]. It has been shown that in addition to their expression in lens, β-crystallins are widely expressed in other tissues at lower levels [10], although their non-lens function is unknown. Currently, γ-crystallins appear to be the most lens-specific and specialized of the crystallins. γ-Crystallin gene transcripts have been detected by sensitive methods outside the lens in amphibian larval stages [11], but this expression could represent "leakage" during early development; no evidence has been presented for functional expression at the level of protein.

In mammals there are six γ-crystallin genes (called γA-F in most species) that are highly similar to each other and closely clustered in the genome. These six genes are expressed mainly during embryonic lens development [4,12,13] and are specific to the fiber cells, the most specialized, terminally differentiated lens cells. As the lens grows throughout life, the cells containing γ-crystallins form the lens nucleus, the most central part of the lens with the highest protein concentration and highest refractive index. Indeed, γ-crystallin expression is generally associated with the most densely packed lenses and regions of lenses. γ-Crystallins are highly abundant in the very hard lenses of fish and many rodents while γA-F are absent from birds, which have very soft, highly accommodating lenses [4,12,14].

However, in addition to the γA-F genes, all vertebrates contain another member of the family that is well-conserved in sequence from fish to mammals and birds [15-18]. Formerly known as βs-crystallin, γS-crystallin (γS) was renamed when the structure of the bovine gene was determined and proved to be characteristic of the γ rather than the β family [18]. In contrast to other γ-crystallins, γS expression increases to high levels only late in lens development and γS appears to replace the embryonic γ-crystallins in the secondary fiber cells of the adult lens [2,19]. In sequence, γS is an outlier of the γ family and possesses a short N-terminal arm and a blocked N-terminus [16,18], characteristics more typical of β-crystallins. In many ways, γS is a good candidate to represent the precursor of the γ-crystallins and possibly a link between the β- and γ-families. As such, γS might be expected to be the member of the family

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most likely to retain a non-lens function distinct from the bulk role as crystallin. Here we examine the gene sequence and expression of mouse γS (*Crygs*) and find evidence for expression outside the lens, particularly in mature retina and cornea.

METHODS

cDNA and Gene Cloning and Sequencing— cDNA cloning was performed using polymerase chain reaction (PCR) techniques. Primers were designed from the published bovine γS sequence (GenBank accession number X03006) [18] and were used for 5' and 3' RACE [20]. For RACE, 1 µg of adult mouse lens total RNA was transcribed with the appropriate primer using Superscript RT (Life Technologies, Gaithersburg, MD) following the manufacturer's protocols. Ten percent of the resulting cDNA template then was used for PCR amplification with Taq polymerase (Boehringer Mannheim, Indianapolis, IN) with 30 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, finishing with a final 10 min extension at 72 °C. Magnesium concentrations were optimized for each reaction. For 3' RACE, oligo(dT) and γS-specific primers were used. For 5' RACE, γS-specific primers were used for first strand synthesis and cDNA was G-tailed using terminal transferase. Reagents for RACE were taken from the Life Technologies 5' RACE system kit, following the manufacturer's instructions. Primer sequences are available on request. PCR fragments were sequenced directly using the PRISM dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and AmpliTaq polymerase FS (Perkin Elmer, Norwalk, CT), following manufacturers' protocols. To identify the mouse γS gene, two primers GSG1: GGTCAGATGTACGAAACCACGGAAGACTGT and GSG2:CAATGCTTTTATTTACGTTGTCTATTTGGAC, designed from the cDNA sequence, were used to amplify a γS genomic fragment predicted to contain intron 2. These were tested on mouse genomic DNA and then supplied to Genome Systems, Inc. (St. Louis, MO) to identify P1 clones from 129/svJ strain mouse genomic DNA. A clone was obtained and PCR, using primer pairs specific to 5' and 3' ends of the gene transcript, was used to ensure that the complete gene was present.

Fragments of the gene were amplified from the P1 clone by PCR using primers from the cDNA sequence. These were gel purified and used as direct sequencing templates as described above. Flanking sequences were obtained by cycle sequencing of the P1 clone itself. New primers were designed as needed to walk through the sequence. Primer sequences are available on request. Introns were amplified using primers in the flanking exons. For intron 1, long range PCR was necessary, and was performed using the Expand system (Boehringer Mannheim).

Southern Blotting— Southern blotting followed standard methods [21]. Mouse genomic DNA was extracted from cultured NIH 3T3 cells and human genomic DNA from Hs27 cultured human foreskin fibroblasts by treatment with proteinase K, SDS, and RNase A followed by ethanol precipitation. Genomic DNA (10 µg) was digested by EcoRI, BamHI, and PstI restriction enzymes (Life Technologies). A 320 bp mouse γS cDNA probe was labeled by random priming and hybridization was carried out at 65 °C.

Chromosomal localization— *Crygs* was mapped by analysis of two genetic crosses: (NFS/N or C58/J X *M. m. musculus*) X *M. m. musculus* [22] and (NFS/N X *M. spretus*) X *M. spretus* or C58/J [23]. Progeny of these crosses have been typed for over 1200 markers distributed on all 19 autosomes and the X chromosome. Recombination was calculated according to Green [24], and genes were ordered by minimizing the number of recombinants.

Northern Analysis of Mouse Tissues— Total RNA was extracted from dissected 2 day, 8.5 week, and 10 month old mouse tissues using RNA STAT-60 (Tel-Test Inc., Friendswood, TX). Northern blotting followed standard methods [21], with 20 µg of total RNA loaded per lane on a 1.5% agarose gel, using formaldehyde buffer. A 320 bp mouse

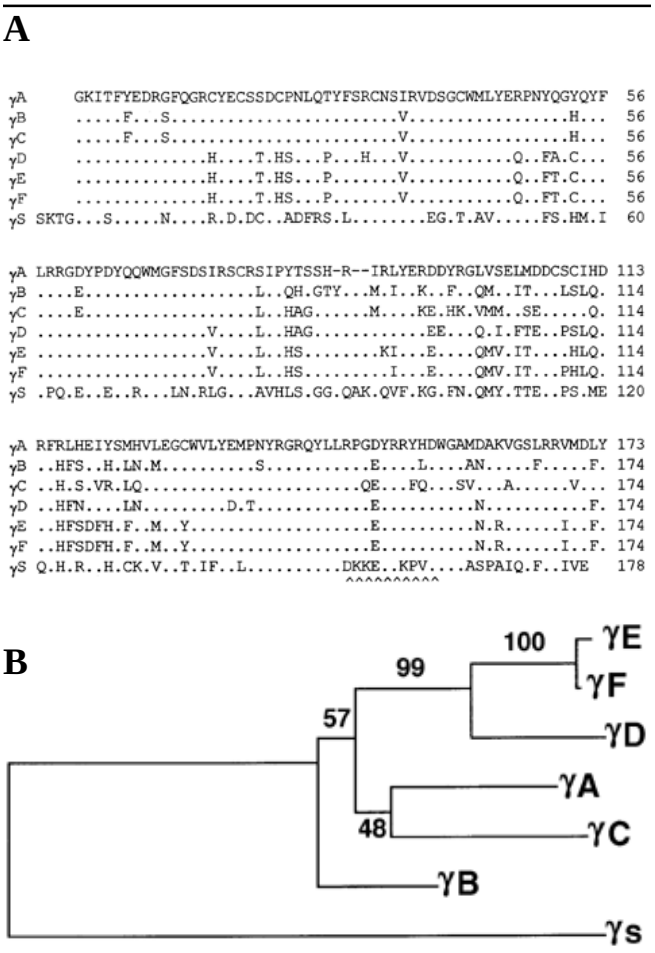

Figure 1. The Mouse γ-crystallin family. A. Alignment of protein sequences for the complete mouse γ-crystallin family, including γS. Identity with γA is shown by a dot, gaps are shown by dashes. The position of the peptide chosen for antiserum production is indicated under the γS sequence. B. Cladogram of the mouse γ-crystallin family. Numbers show the bootstrap values, a measure of the confidence level, for each branchpoint. Multiple replications of the analysis are performed using different samples of the alignment. The bootstrap values are the percentage of these analyses which generate each branchpoint. This is to test against bias resulting from a small number of matches or mismatches [29].The distance calculations upon which this cladogram is based place γS as the outgroup and as such it has no bootstrap value.

γS cDNA fragment derived by RT-PCR was labeled with ^{32}P by random priming (Life Technologies) for use as a probe. Hybridization in aqueous buffer, without formamide, was carried out at 65 ^{O}C. For localization within mouse eye, RNA was also extracted from lenses and retinas dissected from 12 and 33 day mice. In this case, 5 µg of RNA was loaded for lens and 20 µg for retina. Blots were hybridized by a similar protocol and visualized using a phosphoimager. Lens signals were imaged using a range of 5-10,000 (10,000 is the maximum signal) while retina imaging used a range of 1-2894.

Bovine RT-PCR— One year old bovine eyes were obtained from a local slaughterhouse. Tissues were dissected and RNA was extracted from lens, retina, iris (including ciliary body) and cornea, as above. Primers for bovine γS were designed from the published sequence. For reverse transcription-polymerase chain reaction (RT-PCR) amplification [25], 200 ng of each total RNA sample was reverse transcribed using Superscript 1 (Life Sciences, Gaithersburg, MD) as described by others [26]. Specific primers were designed for each sequence and synthesized on an Applied Biosystems DNA synthesizer. PCR amplification used 30 cycles of 1 min 94 ^{O}C, 1 min 55 ^{O}C, 1 min 72 ^{O}C. Products were visualized on agarose gels (1.5-4%). A no-RT control was performed for retina to test for cDNA contamination.

Computer Methods— Sequence analysis was performed using programs of the GCG package [27] implemented at the Advanced Scientific Computing Laboratory, FCRDC, Frederick MD and through the Internet at the National Center for Biotechnology Information (NCBI). Sequence databases were searched using BLAST programs [28].

A cladogram of the mouse γ-crystallin family was drawn using the Neighbor-joining option of the MEGA (Molecular Evolutionary Genetics Analysis) program version 1.01 [29]. Distance calculations used the Poisson correction, and one thousand (1000) bootstrap replications were performed. Protein sequences were extracted from GenBank databases maintained at the Frederick Cancer Research and Development Facility, Frederick, MD. Matching sequences were identified using BLAST [28] and were aligned using the PILEUP program of the GCG package [27]. BLAST output was formatted for input to PILEUP using the program BTF (Mark Gunnell, FCRDF).

Antiserum to γS and Western Analysis— Amino acid sequences for γS and other γ-crystallins from different species were aligned. A peptide was chosen for its conservation in γS, differences with other proteins and probable antigenicity. The peptide, DKKEYRKPVD was synthesized with an N-terminal cysteine for linkage to carrier and was used to produce antisera in rabbits by Lofstrand Labs Limited (Rockville, MD). The eventual antiserum was designated GSP1 (γS peptide antibody 1).

Mouse lens and retina extracts were prepared essentially as described before [30,31]. Proteins were separated by SDS PAGE [30,32] using 4-20% gradient gels in Tris-glycine SDS for 3 h at 120 V and were transferred to nitrocellulose membranes (S & S, Keene, NH) using the Novex system (Novex, San Diego CA). Western blots were performed as described before [30], except that membranes were blocked in 5% milk powder, 2% goat serum, 0.05% Tween-20, 0.14 M NaCl in Tris buffer (10 mM, pH 7.4) overnight. Membranes were incubated overnight with anti-γS-crystallin serum, diluted 1/4000, processed and visualized using the Vectastain Elite ABC (HRP) kit with DAB substrate for peroxidase (Vector Labs, Burlingame, CA), following the manufacturer's instructions.

RESULTS

Cloning Mouse γS— The full-length cDNA sequence for mouse lens γS was obtained using RACE [20] and RT-PCR [25] methods, thereby completing the family of mouse γ-crystallins. The complete cDNA sequence (GenBank accession number AF032995) is 702 bp in length and predicts a protein of 178 residues (Figure 1a), with a size of 20.8 kDa and a predicted

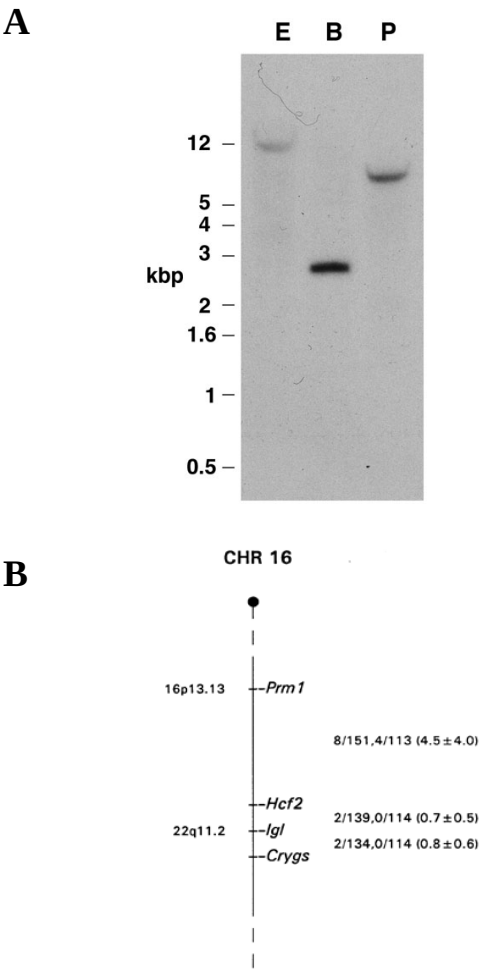

Figure 2. Southern Blotting and Mapping Crygs in the Mouse Genome. A. Southern blot of mouse γS. Hybridization patterns for mouse genomic DNA digested with EcoRI (E), BamHI (B) and PstI (P) and probed with cDNA for mouse γS. B. Localization of *Crygs*. Genetic map location of *Crygs* on mouse chromosome 16. To the right of the map are recombination fractions between adjacent loci with the first fraction from the *M. m. musculus* crosses and the second from the *M. spretus* crosses. Numbers in parentheses are recombinational distances. The chromosome 16 markers used here were typed as described previously [38]. To the left of the map are locations of human homologues. The cataract *Opj*, mapped by different crosses, is in the same region of chromosome 16.

isoelectric point of 7.25, as calculated using the program Peptidesort of the GCG package. Comparison with other γS sequences shows the expected high conservation, 89% identity with human [33], 87% identity with bovine [16], and 67% identity with carp [15]. When the mouse γS sequence was compared with the complete family of mouse γ-crystallins (Figure 1a) [34-37] and the results displayed as a cladogram (Figure 1b), it was clear that while γA-F sequences are clustered, γS is an outlying member of the family.

Cloning and mapping the Crygs gene— Southern blot analysis (Figure 2a) of mouse DNA from NIH 3T3 cells was performed and yielded single bands with EcoRI, BamHI, and PstI, consistent with the presence of a single γS gene (*Crygs*) in the mouse genome. *Crygs* was then mapped by RFLP analysis in sets of mouse crosses. HindIII digestion produced *Crygs* fragments of 7.9 and 6.0 in NFS/N and 12.0 kb in *M. m. musculus*. *M. spretus* produced a PstI fragment of 8.0 kb and NFS/N produced a fragment of 9.4 kb. Inheritance of the variant fragments was followed in both crosses and linkage to chromosome 16 markers was detected as shown in Figure 2b. This shows that *Crygs* is on a different chromosome from the other clustered γ-crystallins on chromosome 1 [39]. The human homologue of this gene has been mapped to chromosome 3 [40] and is also on a different chromosome from the human γ-crystallin cluster on chromosome 2 [41]. Most interestingly, the localization of *Crygs* in mouse overlaps with that described for the mouse genetic cataract *Opj* [42]. Work in progress suggests that *Crygs* is indeed a strong candidate for the locus of *Opj*. Another mouse cataract, *Coc* is also on chromosome 16 [43], but from its described location, it does not seem likely that *Crygs* is a candidate for this cataract.

To obtain the γS gene sequence, specific PCR primers were used to select a P1 clone containing the complete γS transcription unit. This was then used as a template for sequence characterization of the gene, promoter region, and introns by PCR methods (Figure 3a). The gene sequence is deposited in GenBank (GenBank accession numbers are AF055702 and AF055703). As expected, the gene has the same general structure as bovine γS [18] and other γ-crystallins [4], with two phase 0 introns (introns which do not interrupt a

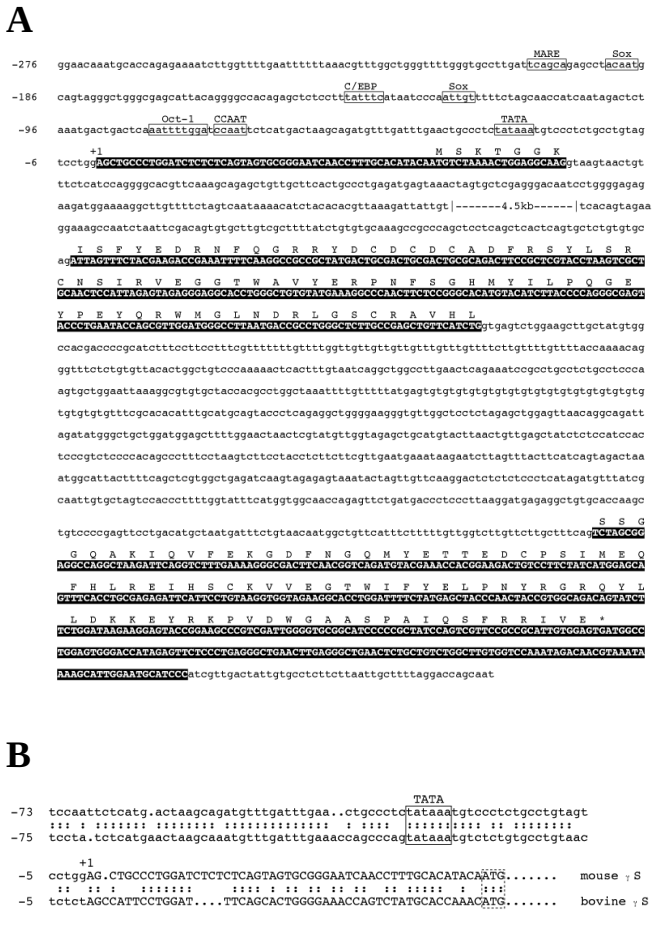

Figure 3. The Crygs Gene. A. Gene sequence. Exons are shown in reverse phase print. Consensus transcription factor sites are boxed. B. Sequence comparison of the mouse γS promoter and 5' UTR with available sequence from the bovine gene. TATA and initiator ATG sequences are boxed. The transcribed 5' UTR is shown in upper case while promoter sequences are in lower case.

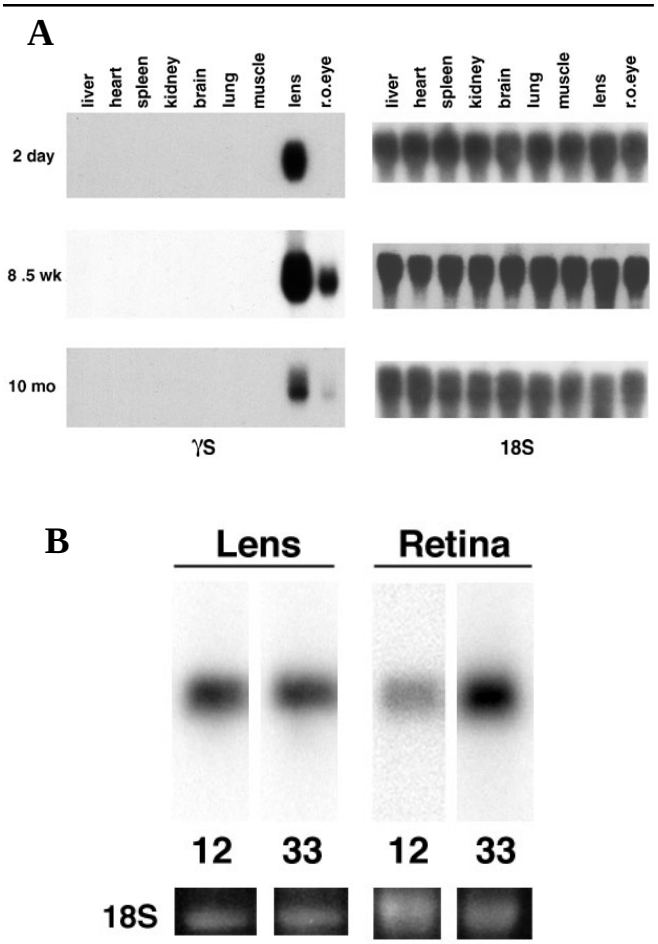

Figure 4. Lens and Non-lens Expression of Mouse γS in mouse. A. Northern analysis of tissues from 2 day, 8 week and 10 month old mice. Equal amounts of RNA were loaded as shown by 18S hybridization. The lane "r.o.eye" contains RNA from the rest of the eye after removal of the lens. B. Northern analysis of lens and retina from 12 and 33 day old mice. Results are for 5 μg of lens RNA and 20 μg of retina RNA. Relative loading is indicated by the staining pattern for 18S RNA shown below. Retina and lens blots were separately scanned using a phosphoimager. Absolute levels of γS hybridization are estimated to be over 20 fold higher in lens than in retina.

codon). Long range PCR showed that intron 1 is approximately 4.8 kb in length (not shown) and this was not completely sequenced. The second intron is 827 bp in length.

Little 5' flanking sequence for bovine γS is present in the databases. However, when mouse and bovine sequences are compared over this short region (Figure 3b), the promoter and 5' UTR sequences are 79% identical, with the insertion of 6 small gaps. The putative TATA box sequences align and are well conserved. Overall, proximal promoter sequences are better conserved than the 5' UTR, presumably reflecting conservation of functional elements. When compared with the other γ-crystallin promoters that have been extensively characterized, mouse γF [44-46] and rat γD [47-49], the γS promoter shows no obvious sequence similarity. Recent results have suggested that Maf-response elements (MARE) [50,51] and Sox consensus binding sites [52] are important for lens-expression of the mouse γF gene. The γS promoter contains consensus MARE and Sox sites (Figure 3) but whether these have functional significance remains to be seen. The γS gene has a CCAAT box, immediately preceded by a consensus Oct-1 site, 38 bp upstream of the TATA box. Other sites of interest are also shown in Figure 3.

Northern Blot— Using a mouse γS cDNA probe, tissues from 2-day, 8.5 week, and 10 month old mice were examined for γS expression (Figure 4a). As expected, 2-day mice showed a strong signal for γS in lens and no detectable signal in other tissues. Surprisingly however, by 8.5 weeks a strong signal also appeared in the rest of the eye. By 10 months γS signal declined, but was still apparent in both lens and the rest of eye. This clearly suggested that between 2-days and 8.5 weeks, the gene for γS begins to be expressed specifically in non-lens tissues of the eye. Northern blots were then performed on RNA extracted from lens and retina of 12 and 33 day old mice (Figure 4b). γS hybridization was detected at similar levels in lenses of both ages. In retina, γS was weakly detected at 12 days, suggesting that the gene is activated in retina between day 2 and day 12 after birth. Day 33 retina gave a stronger signal than day 12 for equal loading of RNA, suggesting that expression increases with age in young mice.

RT-PCR of Dissected Eye Tissues— Mature bovine eyes were used to extend these observations to another species. RT-PCR detected γS mRNA in lens, retina, and cornea but not in iris, even though iris and ciliary body are physically the closest tissues to the lens (Figure 5). An RT(-) control for retina showed that amplified signals originated from RNA and not from contaminating cDNA. Attempts to extract RNA for RT-PCR from vitreous, to test for possible leakage from lens, yielded extremely low levels of RNA and no detectable PCR product (not shown).

Detection of γS Protein— While expression of γS mRNA outside of the lens is interesting, to have functional significance it is important that protein is also present. To detect γS protein, specific polyclonal antisera were produced against a peptide sequence. Protein sequences of γS and other γ-crystallins were aligned and a peptide that was specific to γS sequences and that had good antigenic potential, was selected and used to raise polyclonal antisera in rabbits. Anti-γS serum (GSP1) was used on mouse lens and retina extracts. A band of the expected size for γS was detectable in newborn mouse lens extract (Figure 6). In retina, γS was undetectable at P7 and P17 but at P33 a clearly detectable band, identical in size to that seen in lens appeared in retina extract (Figure 6). In both retina and lens, there is evidence to suggest that post-translational modification of γS may occur (Figure 6 and unpublished). This is commonly observed for other crystallins [2,53] and future experiments will attempt to identify any such modifications for possible functional significance.

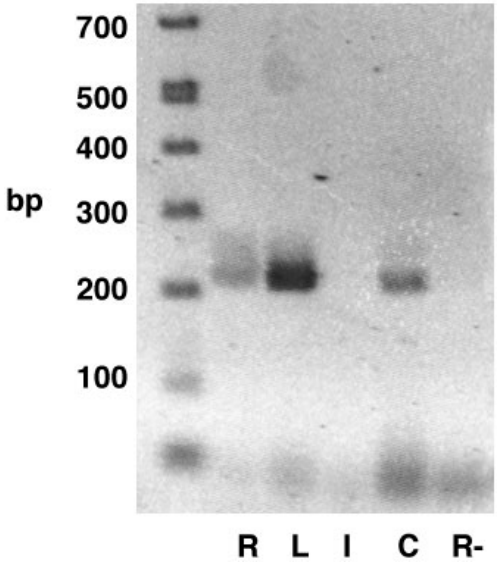

Figure 5. Lens and Non-lens Expression of γS in Bovine Eye. γS specific RT-PCR of total RNA from dissected bovine eye tissues. Results are shown for lens, retina, cornea and iris with an RT(-) control shown for retina.

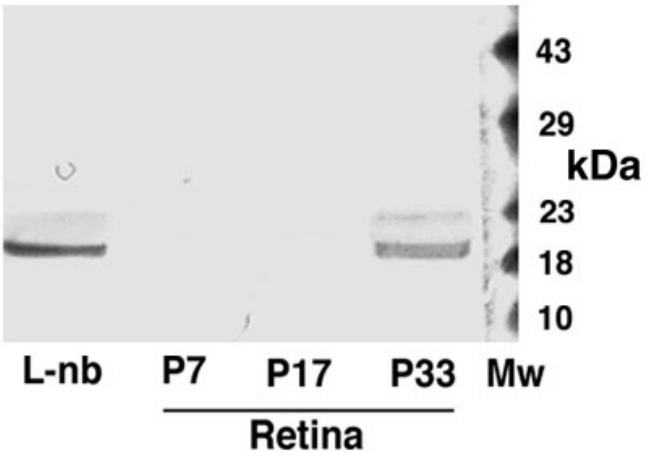

Figure 6. Detection of γS Protein in Lens and Maturing Mouse Retina. Western blot of soluble extracts from newborn mouse lens (L-nb) and mouse retina of ages P7, 17 and 33 (days after birth) using anti-serum GSP1. Equal loading of retina extracts (approximately 20 μg) was adjusted according to Coomassie staining patterns (not shown). No immunoreactivity could be detected earlier than P33 even at loadings of 100 μg of retina extracts (not shown).

DISCUSSION

Crystallins constitute the bulk soluble component of the eye lens, but they are not mere "filler". Non-lens roles have been identified for many crystallins, particularly those that are taxon-specific and that, for the most part, serve as metabolic enzymes in other tissues [3,4]. It has been suggested that all crystallins arose from proteins with functions that predate the existence of the vertebrate eye lens [3,4]. They may have had various roles in stress responses or in control of cell morphology, elongation, and differentiation. When it became advantageous to recruit proteins to high level expression in the lens to improve its optical properties, these proteins were available and furthermore were either functionally neutral or even slightly beneficial to the lens when present at high concentrations.

It therefore seems likely that the β- and γ-crystallins similarly arose from proteins with specific non-lens functions. Several members of the βγ superfamily have been identified [8]. Protein S of the bacterium *Myxococcus xanthus* [54,55] and spherulin 3a of the slime mold *Physarum polycephalum* [56,57] are both induced by stresses leading to spore formation. EDSP (Ep37) of the amphibian *Cynops pyrroghaster* [58,59] and the remarkable AIM1 [9], which is associated with suppression of malignancy in human melanoma and which contains 12 βγ motifs, are both expressed in ectodermal tissues and have plausible connections with cytoarchitecture. This has led to the idea that β- and γ-crystallins may have a role associated with control of cell morphology, perhaps involving assembly or protection of the cytoskeleton. Another plausible function for several crystallins is in protection against the oxidizing effects of light exposure or similar stresses [4]. Indeed, such functions could certainly be beneficial for the retina and cornea as well as lens.

β- and γ-Crystallins are closely related in structure and clearly share an evolutionary origin [4,12,13]. In β-crystallins, each of four repeated structural motifs is encoded in a separate exon, while in γ-crystallins, the four motifs are coded as fused pairs in only two exons. γS-crystallin is an outlying member of the γ family. It resembles γ-crystallins in gene structure and sequence [16,18], but is the most divergent member of the family. As in human, the mouse gene for γS is on a different chromosome from the other clustered γ-crystallins. γS also is widely distributed in vertebrates [15,17,18] and more highly conserved in sequence from fish to mammals than are the other γ-crystallins. Such characteristics make γS a candidate to represent the ancestral, non-lens forerunner of the γ-crystallins.

It has been shown that several β-crystallins have non-lens expression and therefore probably have non-crystallin roles. Transient, low level expression of γ-crystallin transcripts in amphibian embryos has been observed [11], but it is not clear whether this has functional significance; no expression of γ-crystallin proteins outside the lens has been reported in any species. In the lens, both β- and γ-crystallins are expressed only in the differentiated lens fiber cells, but β-crystallins seem to be activated at a slightly earlier stage during differentiation. In this and in other ways, γ-crystallins seem to be more specialized for the lens. A plausible scenario for the evolutionary history of these proteins in the lens is that an ancestral β-crystallin was recruited to the evolving lens at a very early stage, underwent gene duplications to generate first the β family, and later the γ family for the most highly specialized roles in lens.

However, the results presented here suggest that at least one γ-crystallin also may have a non-lens role in the eye, one which could have pre-dated recruitment to the lens. γS crystallin is expressed outside the lens, specifically in the maturing eye. In the adult bovine eye, γS mRNA is detectable by RT-PCR in lens, retina, and cornea, while none is detected in iris or vitreous. It was shown previously that within the bovine lens, γS mRNA is expressed preferentially in secondary cortical fiber cells [19]. In the mouse eye, γS appears first in the lens and is always at higher relative levels in the lens than in the rest of the eye. Furthermore, according to northern blots, γS expression is restricted to the eye but increases with age in the maturing retina. Western blots also show γS immunoreactivity in retina, although protein is not detectable until 33 days after birth, again suggesting an increase in expression in the maturing retina. Eye-specific expression, increasing with age, raises the possibility of a protective role, perhaps a response to light exposure or some other stress peculiar to the eye. Indeed, experiments in progress suggest that γS is stress-inducible in the retina (data not shown).

At present we do not know if other γ-crystallins also are present as protein outside the lens, although preliminary results suggest that γB-crystallin mRNA can be detected by RT-PCR in adult bovine non-lens eye tissues (Jaworski, unpublished). Furthermore, examination of the dbEST databases (maintained at NCBI) show that sequence tags for γC-crystallin (GenBank accession number AA457298 and AA457297) were found in a human fetal retina cDNA library. Indeed, since our first observations of γS mRNA outside of the lens, an EST for γS (GenBank accession number AA457402) has appeared in a human fetal retina library. Interestingly, another probable γS EST (GenBank accession number AA657934) was reported in a CGAP (Cancer Genome Anatomy Project) EST library of prostate cancer PIN2 cells. While cancer cells clearly have atypical gene expression, this may reflect induction of the γS gene in cells under stress. The possible association with cancer cells is intriguing in view of the superfamily relationship between βγ-crystallins and AIM1, a protein whose expression is associated with suppression of malignancy in melanoma [9].

With these observations it now becomes clear that all the families of crystallins have functions distinct from their bulk role in lens. This has significance both for their contribution to other tissues, but also for the lens itself where their functional side should not be ignored. In the case of γS, the mapping of *Crygs* to a position close to the cataract *Opj* is particularly suggestive, since this cataract was originally described as a defect in junctions between secondary fiber cells [42], perhaps indicating that the protein involved may have a role in maintenance of proper cell morphology or cell-cell contacts.

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