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Conservation of Mouse αA-Crystallin Promoter Activity in Chicken Lens Epithelial Cells

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Summary. Previous transfection experiments have shown that 162 base pairs (bp) of the 5′ flanking sequence of the chicken αA-crystallin gene are required for promoter activity in primary chicken lens epithelial cells (PLE), while only 111 bp of the 5′ flanking sequence are needed for activity of the mouse αA-crystallin promoter in transfected chicken PLE cells or in a SV40 T-antigen-transformed transfected mouse lens epithelial cell line (αTN4-1). The effect of site-directed mutations covering positions −111 to −34 of the mouse αA-crystallin promoter fused to the bacterial chloramphenicol acetyltransferase (CAT) gene was compared in transfected chicken PLE cells and mouse αTN4-1 cells; selected mutations were also examined in a nontransformed rabbit lens epithelial cell line (N/N1003A). In general, the same mutations reduced promoter activity in the transfected lens cells from all three species, although differences were noted. The mutations severely affected regions −111/−106 and −69/−40 regions in all the transfected cells examined; by contrast, mutations at positions −105/−99 and −87/−70 had a somewhat greater effect in the chicken PLE than the mouse αTN4-1 cells, while mutations of the −93/−88 sequence reduced expression in the αTN4-1 but not the PLE cells. A partial cDNA with sequence similarity to αA-CRYPB1 of the mouse has been isolated from a chicken lens library; mouse αA-CRYBP1 is a putative transcription factor which binds to the −66/−55 sequence of the mouse αA-crystallin promoter. Thus, despite differences in the molecular mechanisms of expression of the chicken and mouse αA-crystallin gene, the present results indicate numerous similarities in the behavior of the mouse promoter in the transfected lens cells of chicken, mouse, and rabbit.

Introduction

αA-crystallin is one of the major soluble proteins of the lens, and is expressed in all vertebrate species. (See Piatigorsky 1989 for review). The accumulation of αA-crystallin in the lens is tightly regulated both spatially and temporally during development (Zwann and Ikeda 1968; Zwann 1983). Lens expression is conferred by sequences within the 5′-flanking region of both the mouse and chicken αA-crystallin genes in transgenic mice (Klement et al. 1989; Overbeek et al. 1985; Wawrousek et al. 1990) and in transfected lens cells (Chepelinsky et al. 1985, 1987; Klement et al. 1989; Nakamura et al. 1990; Matsuo et al. 1991). A high degree of sequence similarity exists between the mouse and chicken αA-crystallin promoters within the −120 to +23 region (Jaworski et al. 1991; Klement et al. 1989). However, despite the similarities in the expression of the mouse and chicken αA-crystallin genes, they also exhibit differences in their basic mechanisms of transcriptional regulation. While 162

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bp of the chicken 5′-flanking sequence are required to direct optimal transcription of the CAT fusion gene in transfected primary embryonic chicken lens epithelial (PLE) cultures (Klement et al. 1989; Matsuo et al. 1991), only 111 bp of the mouse 5′-flanking sequence are required in the same system (Chepelinsky et al. 1987).

Previous deletion and mutagenesis studies of the mouse αA-crystallin promoter suggested that the −111 to −106 and −69 to −40 regions are critically important for transcriptional regulation when transfected into a cloned SV40 T-antigen-transformed mouse lens cell line (αTN4-1; Nakamura et al. 1990). The mouse −111 to −106 sequence is identical to the −109 to −104 sequence of the chicken αA-crystallin promoter, and site-directed mutation of that sequence within the chicken αA-crystallin promoter eliminates transcriptional activity in transfected chicken PLE cultures (Klement and Piatigorsky, unpublished).

Particular attention has been focused on the −66 to −57 region of the mouse promoter, known as the αA-CRYBP1 site (Nakamura et al. 1990). This is similar to the consensus sequence for the ubiquitously expressed transcription factors PRDII-BF1 (Fan and Maniatis 1990), MBP-1 (Baldwin et al. 1990), HIV-EP1 (Maekawa et al. 1989a,b), NF-kB (for review see Lenardo and Baltimore 1989), H2TF1 (Baldwin and Sharp 1987, 1988), KBF1 (Yano et al. 1987), EBP-1 (Clark et al. 1988), HIVEN86 (Bohnlein et al. 1988), and AGIE-BP1 (Ron et al. 1991). We have previously isolated a cDNA from a mouse lens cell line whose encoded protein specifically binds to the αA-CRYBP1 site and shares 75% sequence identity with the human transcription factors PRDII-BF1, MBP-1, and HIV-EP1 (Nakamura et al. 1990). Mutation of the mouse αA-CRYBP1 site strongly reduces promoter activity in transfection experiments (Nakamura et al. 1990). Moreover, an oligodeoxynucleotide composed of the αA-CRYBP1 binding sequence enhances activity of the HSV tk promoter in transfected αTN4-1 cells (Sax et al. 1990). The chicken αA-crystallin promoter has an αA-CRYBP1-like sequence that differs from the mouse sequence by a single base pair and does not increase tk promoter activity in transfection experiments (Sax et al. 1990).

In order to analyze further the similarities and differences between murine and chicken αA-crystallin gene regulation, we have transfected a series of recombinant DNA plasmids harboring mutations within the mouse αA-crystallin promoter (Nakamura et al. 1990) into chicken PLE cells; selected mutations were also examined in a nontransformed rabbit lens cell line (N/N1003A). Our results implicate the −111 to −100 and −87 to −40 regions for transcriptional regulation of the mouse αA-crystallin promoter in the transfected chicken and rabbit lens cells, as was similarly observed in the homologous αTN4-1 mouse lens cell line (Nakamura et al. 1990). In addition, we isolated a partial cDNA from a chicken embryonic lens cDNA library which is 69% similar to the comparable region of the mouse αA-CRYBP1 cDNA (Nakamura et al. 1990). Thus, although the chicken αA-crystallin promoter requires more upstream sequences for activity in the chicken PLE cells than does its mouse homologue (Klement et al. 1989; Matsuo et al. 1991), the promoter activity of the mouse αA-crystallin gene shows many similarities in transfected lens cells derived from chicken, mice, and rabbits.

Materials and Methods

Plasmids. Site-directed mutations of the murine αA-crystallin promoter fused to the CAT gene were previously described (Nakamura et al. 1990). Each of the 13 mutant promoters contains a 6-bp block whose sequence was altered to an XbaI restriction enzyme site (TCTAGA), and the entire mutagenesis series spanned the −111 to −34 region of the mouse αA-crystallin promoter. Point mutations in the −111/−84 distal domain (Chepelinsky et al. 1987; M1, M2, and M3) were constructed as shown in Fig. 1. Single-stranded oligodeoxynucleotides containing wild-type and mutant sequences were synthesized on an Applied Biosystems Oligonucleotide Synthesizer, annealed to their respective complementary oligodeoxynucleotides (Sommer et al. 1988), and ligated to NdeI-digested pαA88a-CAT (Chepelinsky et al. 1985). Resultant constructs were analyzed by standard DNA sequence analysis. α111, α88, and α60 contain −111/+46, −88/+46, and −60/+46 of the mouse αA-crystallin promoter fused to the CAT gene, and correspond to pαA111$_a$-CAT, pαA$_a$88-CAT, and pαA60$_a$-CAT, respectively, described by Chepelinsky et al. (1985, 1987). All plasmids used in transfections were purified twice by cesium chloride centrifugation.

Primary Cultures, Transfections, CAT and β-Galactosidase Assays. Primary embryonic chicken lens epithelial cultures (PLE) were prepared from 14-day embryonic chicken lenses according to Borras et al. (1988). The rabbit lens epithelial cell line N/N1003A was originally established from the lenses of newborn rabbits by Reddan et al. (1986). N/N1003A cells were plated at a density of 4×10^5 cells/60-mm dish 1 day prior to transfection. For each transfection 10 μg of CAT plasmid DNA was cotransfected via calcium phosphate precipitation (Dubin et al. 1989) with 1 μg of pTB1. pTB1 contains the Rous sarcoma virus LTR fused to the bacterial β-galactosidase gene (Borras et al. 1988) and serves as an internal control for differing transfection efficiencies; 48 h posttransfection primary cultures were harvested, washed twice in phosphate-buffered saline, submitted to three freeze/thaw cycles, dounce homogenized, and assayed for CAT (Neumann et al. 1987) and β-galactosidase activities (Nielsen et al. 1983).

Library Screening, Isolation, and DNA Sequencing of Chicken αA-CRYBP1. A total of 200,000 plaques of a 20–21-day embryonic chicken lens cDNA library in lambda gt11 were screened by hybridization using as a probe a fragment of the

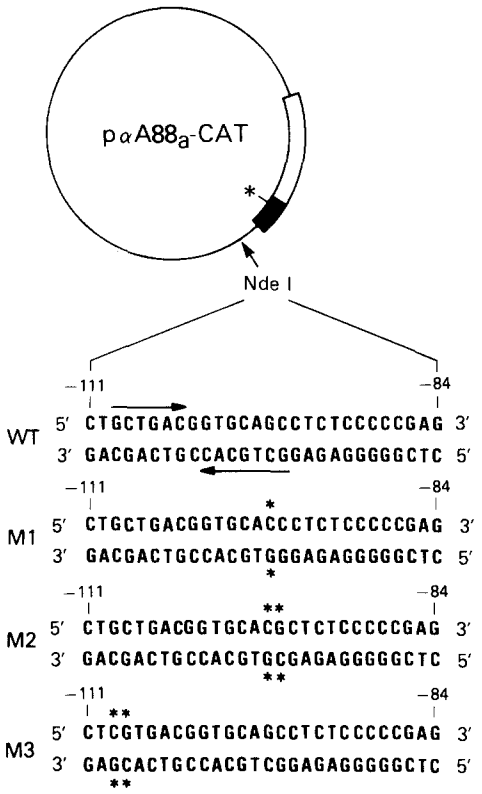


Fig. 1. Point mutations in the murine αA-crystallin promoter. Synthetic complementary oligodeoxynucleotides containing either wild-type (WT) or mutant (M1, M2, M3) −111 to −84 mouse αA-crystallin promoter sequences were cloned 57 base pairs upstream of the wild-type −88 to +46 region fused to CAT in pαA88$_a$-CAT (Chepelinsky et al. 1987). * denotes the changed nucleotides. Arrows denote a dyad of symmetry.

mouse αA-CRYBP1 cDNA. A 1300-bp Hind III fragment of the mouse αA-CRYBP1 cDNA clone pYTN (Nakamura et al. 1990) was labeled with [α^{32}P]dCTP using a nick-translation kit (Boehringer Mannheim Biochemicals) and spin-column elution to remove unincorporated [α^{32}P]dCTP. Filters were prehybridized in 6 × SSC (1× = 150 mM NaCl, 15 mM sodium citrate pH 7.0), 5 × Denhardt's solution, 0.5% SDS, 100 μg/ml denatured herring sperm DNA at 55°C for 2 h, and then hybridized with 1 × 10^6 cpm/ml of radiolabeled probe for 16 h. Filters were washed three times in 2 × SSC, 0.2% SDS for 20 min at room temperature and then twice at 65°C for 20 min each. Secondary and tertiary screenings were performed using the same method. Three phages with unique inserts which hybridized to the mouse αA-CRYBP1 cDNA probe were isolated. A 1.9-kb DNA fragment, which hybridized most strongly with the mouse αA-CRYBP1 cDNA probe, was isolated from one of the positive phages and subcloned as an EcoRI restriction fragment into pUC19. The resulting plasmid was designated pJFK60. DNA sequencing of both strands of pJFK60 was performed by the dideoxy chain termination method with synthetic oligodeoxynucleotides by Lark Sequencing Technologies Inc. (Houston, TX).

PCR Analysis of Chicken RNA Samples. Total RNA was prepared from the lenses of 6-, 14-, and 19-day embryonic chickens using RNAzol (Cinna/Biotecx Laboratories). Poly A$^+$ RNA was prepared from 8 μg of 6-day total RNA and 5 μg each of 14- and 19-day total RNA using the Dynabeads mRNA Purification Kit (Dynal #610.01). Reverse transcription was carried out in 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 500 μM each of dNTPs and 2 μM of the 3′ PCR primer #6008 (5′-ACCGGGCTGATGGAGGGGATGAGGG-3′, see Fig. 4) in a total volume of 25 μl. Following annealing at 70°C for 5 min, 500 units of M-MLV reverse transcriptase (BRL) were added, and the sample was incubated at 37°C for 20 min and at 70°C for 10 min and then immediately cooled on ice; PCR reactions contained 10 μl of cDNA template from the reverse transcription reaction, 50 mM KCl, 10 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 250 μM each dNTP, 2.25 units AmpliTaq (Perkin-Elmer Cetus), and 1 μM each of 5′ #6007 (5′-CAGACACACCTCTTCAGCCACCTGC-3′, see Fig. 4) and 3′ primer #6008 in a final volume of 50 μl. Following an initial denaturation at 94°C for 5 min, amplification was carried out at 94°C for 1 min, 65°C for 1 min, and 72°C for 1 min for a total of 30 cycles, followed by extension at 72°C for 7 min. A positive control PCR was performed using pJFK60 as the template, while a negative control was performed using all reagents except template mRNA.

The identity of PCR products was verified via Southern blot analysis. Following electrophoresis of PCR products on a 1.2% agarose gel, the gel was soaked in 0.5 M NaCl, 0.05 N NaOH for 30 min, twice in 5 × TBE (1× = 89 mM Tris, 89 mM boric acid, 0.2 mM EDTA pH 8.3) for 10 min each, and in 1 × TBE for 10 min. The DNA was electrophoretically transferred to a nylon Gene-Screen membrane (Dupont/NEN) in 1 × TBE using a Bio-Rad TransBlot apparatus. The membrane was treated with UV irradiation in a Stratalinker 2400 (Stratagene) and prehybridized in 10 ml of 6 × SSC (1 × SSC = 150 mM NaCl, 0.015 NaCitrate pH 7.4), 5 × Denhardt's solution, 0.5% SDS, 100 μg/ml sheared, and denatured salmon sperm DNA (BRL #556OUA) for 5 h at 42°C. The oligodeoxynucleotide 5′-ACGAGCGGCCTCGGC-GAGGTGGGTTGCGCC-3′ (#INT1; see Fig. 4), which is located between PCR primers #6007 and #6008, was ^{32}P end-labeled using T4 polynucleotide kinase and used as a probe to hybridize in Southern blots. Hybridization was performed at 40°C for 5 h in prehybridization solution containing 5 × 10^8 cpm/ml probe. The membrane was washed in 2 × SSC, 0.1% SDS for 30 min at room temperature followed by four times at 55°C for 20 min each, and finally subjected to autoradiography.

Results

Transfection of Primary Embryonic Chicken Lens Epithelial Cells

A series of site-directed mutations (#1–#13; wild-type sequence changed to an XbaI restriction site) were created through the −111 to −34 region of the murine αA-crystallin promoter and fused to the bacterial CAT gene (Nakamura et al. 1990). Each construct was transfected into chicken PLE cells established from the lenses of 14-day embryonic chickens. CAT activity was assayed to assess the effect of specific mutations upon transcriptional activation driven by the mouse αA-crystallin promoter (Fig. 2). As controls, we also transfected the promoterless plasmid pSVOCAT and a plasmid in which the RSV LTR directs transcription of the CAT gene (pRSVCAT). The positive control pRSVCAT is expressed to a high degree as expected and previously shown for chicken PLE cultures (Sax et al. 1990). The negative control pro-

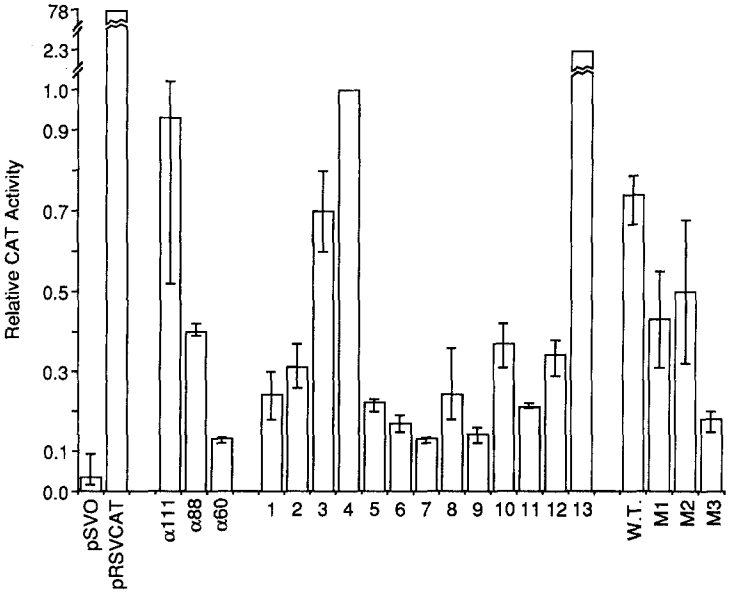


Fig. 2. Relative CAT activity of αA-crystallin promoter CAT fusion plasmids transfected into chicken PLE cultures. Values shown represent averages of CAT activities normalized to β-galactosidase expression and then expressed relative to the activity arising from the wild-type promoter in plasmid α111. Bars denote the range of the normalized CAT activities, for two to four transfections of each plasmid. No range bars are given for mutant #4 because this plasmid consistently yielded the same level of expression. pSVO denotes pSVO-CAT, the promoterless plasmid (Gorman et al. 1982b). pRSV-CAT is a positive control plasmid in which the Rous sarcoma viral LTR is fused to CAT (Gorman et al. 1982a). α111, α88, and α60 contain −111 to +46, −88 to +46, and −60 to +46, respectively, of the mouse αA-crystallin promoter fused to the CAT gene (Chepelinsky et al. 1987). WT, M1, M2, and M3 are described in Materials and Methods and Fig. 1. The site-directed mutations introduced correspond to the following regions: #1, −111/−106; #2, −105/−100; #3, −99/−94; #4, −93/−88; #5, −87/−82; #6, −81/−76; #7, −75/−70; #8, −69/−64; #9, −63/−58; #10, −57/−52; #11, −51/−46; #12, −45/−40; #13, −39/−34.

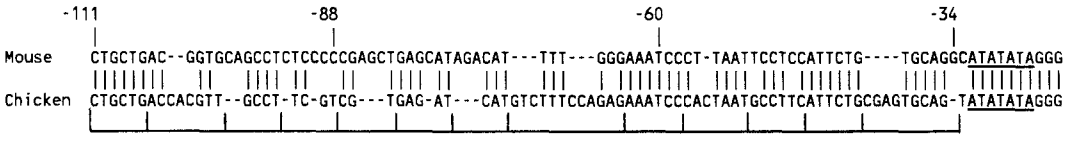
moterless plasmid pSVOCAT represents background levels of CAT activity; in fact the wild-type mouse αA-crystallin −111/+46 promoter (α111) yields a 30-fold activation of CAT over that observed for pSVOCAT (Fig. 2 and Chepelinsky et al. 1987).

Mutations #1 and #2 (spanning −111 to −100) and #5–#12 (spanning −87 to −40) reduced CAT activity in transfected PLE cultures, as compared to the wild-type sequence in α111. Moreover, deletion of the −111 to −89 and −111 to −61 regions in α88 and α60, respectively, reduced promoter activity in transfected PLE cultures (Fig. 2 and Chepelinsky et al. 1987).

We have also inserted wild-type and mutant versions of the distal-region oligodeoxynucleotide (spanning −111 to −84) upstream of the wild-type −88 to +46 (α88) region fused to CAT (Fig. 1) and transfected these constructs into PLE cultures. These point mutations were chosen because they disrupted a dyad of symmetry within the sequence of the mouse αA-crystallin promoter (denoted by arrows in Fig. 1). The point mutant M3 (Fig. 1) falls within the region mutated to an XbaI site in mutant construct #1 (Fig. 2) and also severely reduced promoter activity from wild-type levels (W.T.; Fig. 2). Mutations M1 and M2 (Fig. 1) fall within the region mutated in XbaI mutant construct #3 (Fig. 2) and, as mutant #3, did not significantly reduce transcription from wild-type levels (W.T.; Fig. 2).

A comparison of the mouse and chicken αA-crystallin 5′-flanking sequences indicates a high degree of sequence conservation (Fig. 3) in those regions identified above as functionally important. Comparison of the CAT activity levels generated from the site-directed mutants in the present transfection experiments in the chicken PLE cells with that generated previously (Nakamura et al. 1990) in the mouse αTN4-1 lens cell line indicates that these conserved regions are important for promoter function in both systems (Fig. 3). Mutations #1 and #8–#12 behaved similarly in the αTN4-1 and PLE cultures by greatly reducing promoter activity. In addition, mutations #1, #3, #9, and #11 transfected into an untransformed rabbit lens cell line (N/N1003A) behaved the same as in the transfected αTN4-1 and PLE cells (Fig. 3). Thus, the importance of regions −111/−106 (mutation #1), −63/−58 (mutation #9), and −51/−46 (mutation #11) has been demonstrated in primary lens cultures, and transformed and untransformed lens cell lines derived from three different species. These findings suggest a conservation of factors acting at these sites across species.

Despite the similarity in the behavior of the promoter mutants in the transfected chicken and mouse lens cells, some differences were noted. Mutations #2 and #5–#7 reduced promoter activity more strongly in the chicken PLE cells than in the mouse αTN4-1 lens cell line (Fig. 3). By contrast, mutation #4 reduced promoter activity by 50% in the αTN4-1 cells but not in the PLE cells (Fig. 3). In addition, mutant #13 gives a 2.3-fold greater CAT activity than the wild-type mouse αA-crystallin promoter-CAT fusion gene (αA111) in transfected PLE cells (Fig. 3).



	1	2	3	4	5	6	7	8	9	10	11	12	13
TN4-1	0.26	0.80	1.0	0.52	0.61	0.56	0.61	0.23	0.23	0.16	0.16	0.26	0.9
PLE	0.24	0.31	0.70	1.0	0.22	0.17	0.13	0.24	0.14	0.37	0.21	0.34	2.3
N1003	0.20	-	0.70	-	-	-	-	-	0.20	-	0.30	-	-

Fig. 3. Comparison of mouse and chicken αA-crystallin promoter sequences and relative CAT activities of promoter mutants. The nucleotide sequences of the mouse and chicken αA-crystallin promoters are aligned to demonstrate regions of identity. −111, −88, −60, and −34 refer to nucleotide positions upstream of the transcription start site of the mouse αA-crystallin gene. Underlined sequences represent TATA boxes. The site-directed mutations created through the mouse αA-crystallin promoter (Materials and Methods, Nakamura et al. 1990) are numbered 1–13. The relative CAT activity observed for each mutation when transfected into either a mouse lens cell line (αTN4-1; data from Nakamura et al. 1990), into primary chicken lens epithelial cultures (PLE, Fig. 2), or into a rabbit lens epithelial cell line (N/N1003A) is shown below each mutation. Each plasmid was transfected three times for αTN4-1 cells (Nakamura et al. 1990), two to four times for PLE cultures (Fig. 2), and three times for N/N1003A cultures. The standard deviations do not overlap across αTN4-1 and PLE cells for mutations #2, #4–#7, and #13, indicating a statistical difference in relative CAT activity across the two cell types. The mean and standard deviation values are as follows: mutant #2: 0.80 ± 0.14 in αTN4-1, 0.31 ± 0.07 in PLE; mutant #5: 0.61 ± 0.20 in αTN4-1, 0.22 ± 0.01 in PLE; mutant #6: 0.56 ± 0.17 in αTN4-1, 0.17 ± 0.02 in PLE; mutant #7: 0.61 ± 0.28 in αTN4-1, 0.13 ± 0.01 in PLE; mutant #13: 0.90 ± 0.37 in αTN4-1, 2.3 ± 0.43 in PLE. Standard deviations for each of the other mutants overlapped, indicating no difference in expression in these cell types.

Isolation and DNA Sequence of a Chicken αA-CRYBP1-Like cDNA Clone

Since the mouse αA-CRYBP1 site (5′-GGGAAATCCC-3′) at positions −66 to −57 was functionally important in both the chicken PLE and mouse αTN4-1 cells (mutations #8–#10), and a factor binding to this site has been cloned from the mouse lens cells (Nakamura et al. 1990), we attempted to clone a similar factor from the chicken lens. A fragment of the mouse αA-CRYBP1 cDNA clone pYTN (Nakamura et al. 1990) was used to probe a 20–21-day embryonic chicken lens cDNA library constructed in λgt11 (constructed by Dr. Mark Thompson). The complete sequence for one positive clone, pJFK60, was obtained. Analysis of this sequence revealed that the cDNA clone was a fusion between a partial δ1-crystallin cDNA (exons 4–13; Nickerson et al. 1985) and a sequence (560 bp in length) similar to that of the mouse αA-CRYBP1 cDNA. Here we compare both the nucleotide (Fig. 4) and deduced amino acid (Fig. 5) sequences of this chicken αA-CRYBP1-like cDNA clone with the analogous region of the mouse sequence (Nakamura et al. 1990). The chicken clone shares 69% and 71% nucleotide identity with the mouse (Nakamura et al. 1990) and human (Fan and Maniatis 1990) cDNA clones, respectively. The deduced amino acid sequence of the chicken clone is 70% and 85% identical to that of the mouse and human clones, respectively. This chicken cDNA does not contain the zinc-finger region found in the mouse αA-CRYBP1 cDNA (Nakamura et al. 1990). Should this 560-bp chicken cDNA clone in fact represent the chicken homologue of mouse αA-CRYBP1 and human PRDII-BF1 it would contain only 5–7% of the entire coding sequence, given the 10-kb mRNA observed for mouse αA-CRYBP1 (Nakamura et al. 1990) and the known complete coding sequence for PRDII-BF1 (Fan and Maniatis 1990).

PCR Analysis of Chicken αA-CRYBP1-Like Expression

Due to the fusion of αA-CRYBP1-like and δ1-crystallin sequences in cDNA clone pJFK60 we attempted to rule out cloning artifacts and verify the expression of the chicken αA-CRYBP1-like gene in lenses by the polymerase chain reaction (PCR). Poly A^+ RNA was isolated from 6-, 14-, and 19-day embryonic chicken lenses and subjected to PCR amplification using oligodeoxynucleotide primers which flank the αA-CRYBP1-like region of pJFK60. (See Fig. 4.) The resulting PCR products were analyzed for sequence specificity by Southern blot analysis using as a probe an oligodeoxynucleotide whose sequence lies between that of the PCR primers in pJFK60. Our results indicate that the chicken αA-CRYBP1-like gene is expressed at all three stages of embryonic lens development (Fig. 6). Both αA-crystallin and the αA-CRYBP1-like genes are expressed in the 14-day embryonic chicken lens, and this timepoint also corresponds to those lenses used to set up PLE cultures (Fig. 2 and Nakamura et al. 1990). The lens cDNA library from

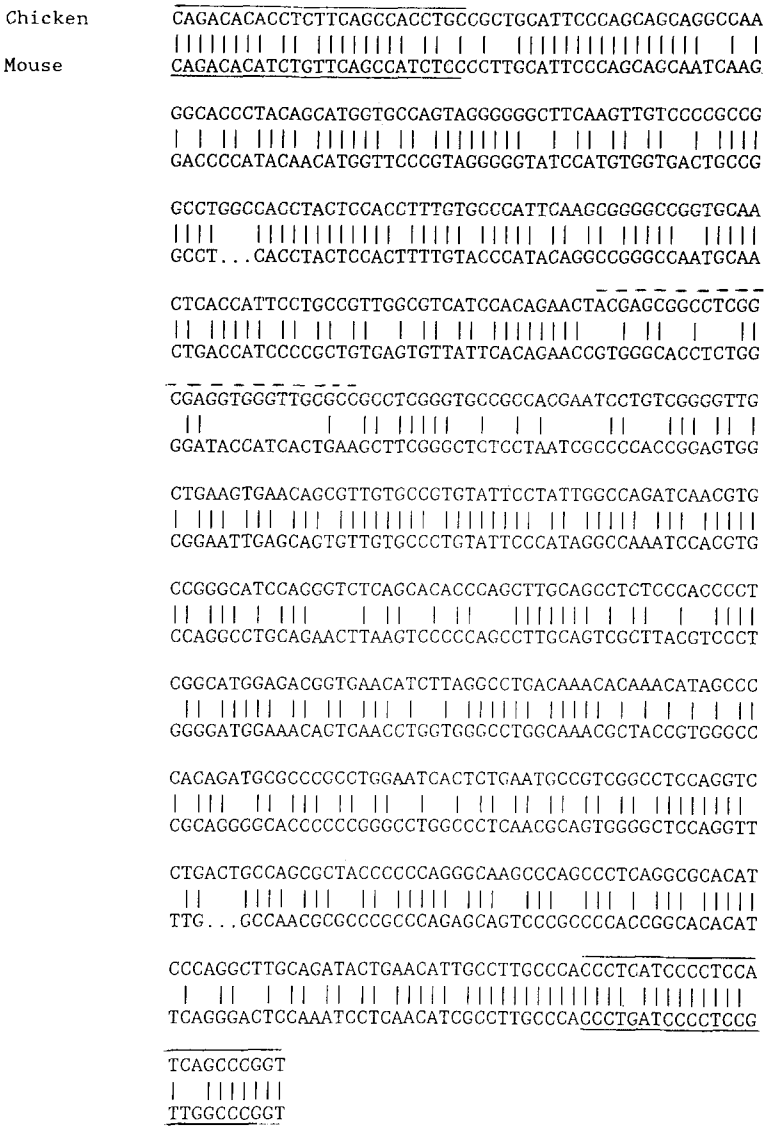


Fig. 4. Comparison of chicken and mouse αA-CRYBP1 cDNA sequences. The αA-CRYBP1-like sequence of the clone pJFK60 is aligned with the analogous portion of the mouse αA-CRYBP1 cDNA sequence (positions 1026–1579; Nakamura et al. 1990) to show regions of identity (|). The position of primers #6007 (5′) and #6008 (3′) used for PCR amplification of lens RNA samples (Fig. 6) is denoted by solid over- and underlines, while the oligodeoxynucleotide (#INT1) used as a probe in Southern blot analysis of PCR amplified lens RNA samples (Fig. 6) is denoted by a dashed overline.

which pJFK60 was isolated was derived from chicken embryos approximately 19 days old.

Discussion

The chicken and mouse αA-crystallin promoters exhibit both similarities and differences in their regulatory mechanisms. The mouse αA-crystallin promoter contains an αA-CRYBP1 site (5′-GGGAAATCCC-3′) at positions −66/−57, while the chicken gene contains an αA-CRYBP1-like sequence (5′-GAGAAATCCC-3′) at positions −67/−58. Mutagenesis of the αA-CRYBP1 site within the mouse promoter eliminated promoter activity in transfected primary chicken lens cultures (present study), as it did in a transfected mouse SV40 T-antigen–transformed lens epithelial cell line, αTN4-1 (Nakamura et al. 1990), and in an untransformed rabbit lens epithelial cell line, N/N1003A (present study). Moreover, we have isolated here a partial cDNA derived from chicken lens mRNA which is 69% identical at the nucleotide sequence level and 70% identical at the deduced amino acid sequence level to a mouse αA-CRYBP1 cDNA (Nakamura et al. 1990) throughout the cloned regions. Although the chicken αA-CRYBP1 cDNA was fused to a δ1-crystallin cDNA, PCR experiments using polyadenylated confirmed that this gene is expressed in the chicken lens. Thus, the chicken lens does express an αA-CRYBP1-like gene whose encoded protein may function as a transcription factor. While we have not yet analyzed the tissue distribution of the chicken αA-CRYBP1-like mRNA, we expect it to be ubiquitous as is the mouse αA-CRYBP1 mRNA (Nakamura et al. 1990).

The question as to whether the αA-CRYBP1-like sequence in the chicken αA-crystallin gene is func-

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Chicken     QTHLFSHLPLHSQQQAKAPYSMVPVGGLQVVPAGLATYSTFVPIQAGPVQ
                ||||||||||||||.:.||.||||||::||.||| ||||||||||:|
Mouse       QTHLFSHLPLHSQQQSRTPYNMVPVGGIHVVTAGL.TYSTFVPIQAGPMQ

            LTIPAVGVIHRTTSGLGEVGCAASGAATNPVGVAEVNSVVPCIPIGQINV
            ||||||:|||||.:. |:. ..|||.:..|.||||:.||||||||||:|
            LTIPAVSVIHRTVGTSGDTITEASGSPNRPTGVAELSSVVPCIPIGQIHV

            PGIQGLSTPSLQPLPPLGMETVNILGLTNTNIAPQMRPPGITLNAVGLQV
            ||:|.||.|.||.|..|||||||::||.|..::|| :|||:.|||||||
            PGLQNLSPPALQSLTSLGMETVNLVGLANATVGPQGHPPGLALNAVGLQV

            LTASATPQGKPSPQAHIPGLQILNIALPTLIPSISP
            | |.|.::|:.|.|.|||.|||||||||||||::|
            L.ANAPAQSSPAPPAHIQGLQILNIALPTLIPSVGP
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Fig. 5. Comparison of chicken and mouse αA-CRYBP1 deduced amino acid sequences. The deduced amino acid sequence of the chicken and mouse αA-CRYBP1 nucleotide sequences shown in Fig. 4 are aligned here to show regions of identity (|) A double dot (:) between amino acids denotes greater similarity between those amino acids than does a single dot (.) (Gribskov and Burgess 1986).

tionally important remains open despite the requirement of this sequence in mouse αA-crystallin promoters transfected into the chicken PLE cells. That mutations of the chicken sequence do not reduce activity of the promoter in transfections (Klement and Piatigorsky, unpublished) and the chicken αA-CRYBP1-like sequence does not activate the tk promoter in transfected PLE cells (Sax et al. 1990) argue against its utilization as a *cis*-regulatory element in the chicken gene although it does not eliminate that possibility. It is also possible that the chicken αA-CRYBP1-like protein regulates other genes expressed in the lens. As mentioned above, αA-CRYBP1 is a ubiquitously expressed protein (Nakamura et al. 1990) and may regulate the expression of many genes.

Mutations #2 and #5–#7 of the mouse αA-crystallin promoter reduced promoter activity more strongly in the primary chicken PLE cultures than in the mouse αTN4-1 cell line, while mutation #13 increased promoter activity in the transfected PLE but not the transfected αTN4-1 cells. These differences may result from the existence of different factors in mouse and chicken lens cells, or similar factors which interact with the murine promoter in a slightly different manner. Alternatively, the new nucleotide sequence of certain mutants may create a site with a different affinity for a transcription factor or for an unidentified factor(s) present in the cultured chicken lens cells but not in the αTN4-1 mouse lens cells used previously (Nakamura et al. 1990). For instance, in the case of mutation #13, the mutated sequence may result in an increased affinity with which TFIID binds to this promoter. It is also possible that the transformed state of the αTN4-1 cells affects the utilization of the αA-crystallin promoter.

Previous analysis indicated that regulation of the murine αA-crystallin promoter in chicken lens cultures requires an interaction of a distal (−111 to −88) and a proximal (−87 to −55) sequence (Chepelinsky et al. 1987; Sax et al. 1990). The present results indicate that the −111/−100 sequence of the mouse αA-crystallin promoter is critical for function of the distal region. The −111 to −106 sequence (5′-CTGCTG-3′) of the mouse αA-crystallin promoter is identical to the −109 to −104 sequence of the chicken promoter. Mutations within this sequence in both the chicken (Klement and Piatigorsky, unpublished) and mouse (present study) αA-crystallin 5′-flanking region eliminated promoter activity in transfected chicken PLE cultures and mouse αTN4-1 cells (Nakamura et al. 1990). The demonstration of both sequence and functional conservation of promoter elements sug-

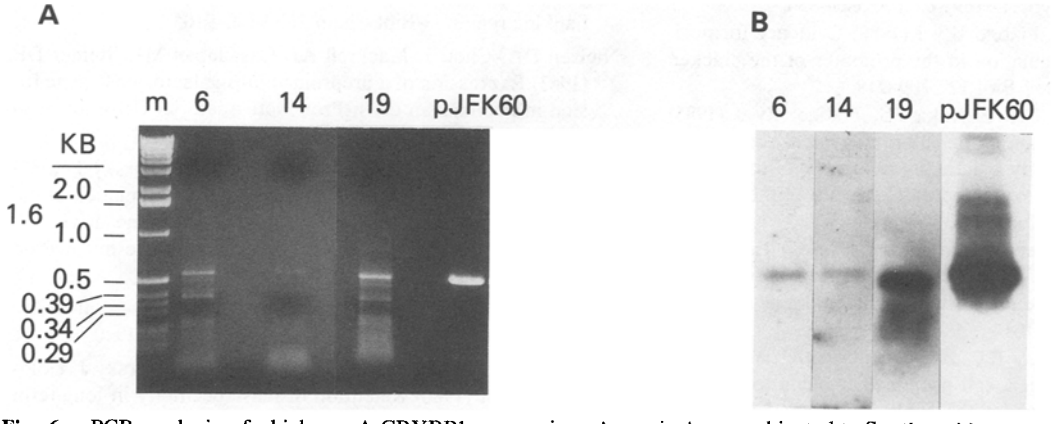

Fig. 6. PCR analysis of chicken αA-CRYBP1 expression. **A** Poly A$^+$ RNA from 6-, 14-, and 19-day embryonic chicken lenses was subjected to PCR amplification using primers #6007 and #6008 (see Fig. 4) and the resulting PCR products were electrophoresed on a 1.2% agarose gel and then stained with ethidium bromide. As a control the cDNA clone pJFK60 was also subjected to PCR amplification using the same primers. **B** The DNA in **A** was subjected to Southern blot analysis using as a probe the radiolabeled oligodeoxynucleotide #INT1 (see Fig. 4), whose sequence in pJFK60 is located between primers #6007 and #6008 used in PCR amplification. The autoradiography panels shown are as follows: 20 min for pJFK60 lane, 4.5 h for 6-day and 19-day, and 15.5 h for 14-day.

gests conservation of transcription factors specific for this distal site in both the mouse and chicken lens. This suggestion is supported by the formation of specific DNA–protein complexes between the mouse αA-crystallin promoter distal region and chicken lens nuclear extracts (Sommer et al. 1988), and by similar gel shift patterns using crude nuclear extracts from both mouse and chicken lenses (Sax and Piatigorsky, unpublished). A detailed comparison of mouse and chicken αA-crystallin gene expression is under further study and may provide insight to the evolutionary processes which shape mechanisms of gene expression in different species.

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