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Regulation of the Mouse αA-Crystallin Gene: Isolation of a cDNA Encoding a Protein That Binds to a *cis* Sequence Motif Shared with the Major Histocompatibility Complex Class I Gene and Other Genes

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We have shown by site-directed mutagenesis that the sequence between positions −69 and −40 of the mouse αA-crystallin gene is crucial for tissue-specific gene expression in a transfected mouse lens epithelial cell line transformed with the early region of simian virus 40. Gel retardation experiments with synthetic oligodeoxynucleotides revealed a mouse lens nuclear protein which bound specifically to the palindromic sequence 5′-GGGAAATCCC-3′ at positions −66 to −57 in the αA-crystallin promoter. By screening a bacteriophage λgt11 expression library of the transformed lens cells, we isolated a 2.5-kilobase-pair cDNA encoding a fusion protein which bound to this sequence and to the regulatory element of the major histocompatibility complex (MHC) class I gene. This cDNA hybridized to a 10-kilobase-pair polyadenylated RNA present in many different tissues, including lens. It encoded a protein, tentatively called αA-CRYBP1, containing at least two zinc fingers. αA-CRYBP1 is either homologous or very similar to the human nuclear proteins MBP-1 (Baldwin et al., Mol. Cell. Biol. 10:1406–1414, 1990), PRDII-BFI (Fan and Maniatis, Genes Dev. 4:29–42, 1990), and HIV-EP1 (Maekawa et al., J. Biol. Chem. 264:14591–14593, 1989), which bind to regulatory elements of the MHC class I, beta interferon, and human immunodeficiency virus genes, respectively. Our results suggest that the lens-specific αA-crystallin, MHC class I, beta interferon and other genes have a similar *cis*-acting DNA regulatory motif that shares αA-CRYBPI, MBP-1, PRDII-BF1, HIV-EP1, or other closely related proteins as *trans*-acting factors.

Crystallins are a diverse group of soluble proteins that accumulate in the eye lens and are responsible for its transparency (15, 37). The two α-crystallins (αA and αB), related to the small heat shock proteins (16), are present in all vertebrates. The αA gene is lens specific, while the αB gene is expressed in many tissues (28). Previously we have identified a promoter sequence (positions −366 to +46 [−366/+46]) of the mouse αA-crystallin gene which functions with tissue specificity in transfected embryonic chicken lens epithelia (7) and transgenic mice (27). Further studies showed that efficient promoter activity can be obtained by the −111/+46 fragment in transfected chicken lens cells (8). Furthermore, lens- and development stage-specific expression was directed by the −88/+46 fragment in transgenic mice (36). Recently, we have established several crystallin-producing lens epithelial cell lines (25a, 38), which should be useful for additional studies on the expression of the αA-crystallin gene.

In the present investigation, by using the −84/−32 sequence of the αA-crystallin promoter as a probe to screen a bacteriophage λgt11 expression library constructed from the mouse lens cell line, we have isolated a cDNA encoding a zinc finger protein that binds to a 19-base-pair (bp) sequence resembling the regulatory region of the major histocompatibility complex (MHC) class I gene (32) and the NF-kB motif (20). This protein, tentatively called αA-CRYBP1, is either homologous or extremely similar to human MBP-1 (1), PRDII-BF1 (11), and HIV-EP1 (23), ubiquitous zinc finger proteins capable of binding to NF-kB DNA sequence motifs found in a number of gene-regulatory elements. Finally, by site-directed mutagenesis, we show that the αA-CRYBP1 binding region is critical for the function of the αA-crystallin promoter in the mouse lens cell line.

MATERIALS AND METHODS

Lens cell lines. Two mouse lens epithelial cell lines producing αA-crystallin were used, αTN6 and αTN4-1. Both were derived from lenses of transgenic mice carrying the αA-crystallin promoter–simian virus 40 (SV40) T antigen transgene (24). αTN6 was established from line 6 of αT2 mice at 11 months of age (25a); αTN4-1 was cloned from the αTN4 cells (38), which were established from line 4 lens epithelial cells of αT2 mice at 2 months of age (25a).

Construction of a λgt11 cDNA library and screening with the αA-crystallin gene regulatory region. All oligodeoxynucleotides were synthesized in an automated synthesizer and purified by high-pressure liquid chromatography (32). Polyadenylated [poly(A)$^+$] RNA from the αTN6 cell line was prepared by using Hybond mAP (Amersham). cDNA and a λgt11 expression library were constructed by using the cDNA Synthesis System Plus and the cDNA Cloning System λgt11 from Amersham according to the manufacturer's instructions. The ^{32}P-labeled, concatenated duplex oligodeoxynucleotide corresponding to −84/−32 of the αA-crystallin gene (see Fig. 2) was used to screen the λ phage library as described before (14, 35). A λgt11 fusion protein from the

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positive clone (YTN) was tested with other regions of the mouse αA-crystallin promoter and *cis* sequences (CRE and ICS; see below) of the MHC class I gene by a filter-binding assay as described before (14).

Gel retardation assay. αTN6 nuclear extracts were prepared by the method of Dignam et al. (10) with modifications (32). The cDNA insert of positive phage YTN was cut out by digestion with *Eco*RI and *Kpn*I and subcloned into the pBS plasmid (Stratagene), digested with *Bam*HI and filled in with Klenow fragment and then digested with *Kpn*I. The resulting plasmid was called pYTN. Note that in this subcloning, the *Kpn*I site of λgt11 was used in liberation of the cDNA due to destruction of one of the *Eco*RI sites in construction of the phage YTN. Crude extracts from pYTN-transformed *Escherichia coli* strain XL1 were prepared as described before (14). Gel retardation assays were performed with 2 fmol of ^{32}P-labeled oligodeoxynucleotides corresponding to −84/−32 of the αA-crystallin gene as described before (14), in the presence of 10 μg of αTN6 nuclear extract, 1.3 μg of crude pYTN-transformed XL1 cell extract, or 5.6 μg of crude pBS-transformed XL1 cell extract. One picomole of unlabeled oligodeoxynucleotide was used in the competition experiments.

Site-directed mutagenesis. Mutants were constructed by using an oligonucleotide-directed in vitro mutagenesis system. Recombinant single-stranded M13 phage DNAs harboring the αA111$_a$-CAT fusion gene (8) on a 561-bp *Hae*III restriction fragment were annealed separately to 13 oligodeoxynucleotides containing an *Xba*I site and 20 bases of homologous flanking sequences on each side of the site. Reagents for the second-strand synthesis (with the mutant oligodeoxynucleotide as a primer) and subsequent first-strand removal and resynthesis were performed with Amersham kit RPN.1523. After transformation into *E. coli* JM109, the M13 phage were screened for the introduction of the *Xba*I site. A 360-bp *Nde*I-*Pvu*II restriction fragment containing the mutation from each of the 13 constructs was isolated from replicative-form DNA and cloned into pSVO-CAT (12). The resultant plasmids are identical to pαA111$_a$-CAT except for the new *Xba*I site in the promoter region of the αA-*cat* fusion gene (Fig. 1). All constructs were screened for this unique *Xba*I restriction site within the *Nde*I-*Pvu*II fragment and sequenced by standard double-stranded DNA-sequencing methods.

Transfection assays. αTN4-1 lens cells were maintained in Dulbecco modified Eagle medium, high glucose, with 10% fetal calf serum and 100 U of penicillin G and 100 μg streptomycin sulfate per ml in a 5% CO_2–air environment (all materials from GIBCO). Plasmids (10 μg) were transfected by the calcium phosphate precipitation method as described by Chepelinsky et al. (7), except that the glycerol shock was omitted. pTB1 (5) (3 μg) was cotransfected as an internal control for transfection efficiency. pTB1 has the β-galactosidase gene expressed from the Rous sarcoma virus long terminal repeat (LTR). Chloramphenicol acetyltransferase (CAT) assays were performed as described by Neumann et al. (26) with 5.0 μCi of ^{3}H-labeled acetyl coenzyme A. β-Galactosidase activity was determined as described by Borras et al. (5). Each plasmid was tested in three experiments, and the data were normalized with respect to β-galactosidase activity.

Northern (RNA) blot hybridization. From 2 to 6 μg of poly(A)$^+$ RNA, obtained by oligo(dT)-cellulose chromotography, was electrophoresed in a 1.5% agarose–formaldehyde gel, blotted onto nitrocellulose paper, and hybridized overnight at 42°C in 50% formamide–1 M NaCl to either a 614-bp *Hin*dIII fragment spanning nucleotides 1231 to 1845 (see Fig. 6) of the pYTN cDNA insert or a human β-actin cDNA (13). The fragments were labeled by random priming with [α-^{32}P] dCTP. The blot was washed twice with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.4) at room temperature and twice with 0.5× SSC at 65°C, each time for 30 min, and exposed for autoradiography at −70°C for the times listed in the figure legends.

DNA sequencing. DNA sequencing of both strands was performed by the dideoxy chain termination method with synthetic oligodeoxynucleotides, using the Sequenase DNA sequencing kit (United States Biochemical Corp.).

RESULTS

Oligonucleotide-directed mutations in the αA-crystallin promoter. Earlier studies with transgenic mice established that the sequence between positions −88 and −34 of the αA-crystallin promoter is sufficient to direct lens- and development stage-specific expression of an αA-crystallin-*cat* fusion transgene (36). Oligonucleotide-directed mutations were made in the αA-crystallin promoter of the pαA111$_a$-CAT plasmid (8). pαA111$_a$-CAT contains the sequence −111/+46 of the mouse αA-crystallin gene fused to the *cat* gene and is functional in transfected cultured embryonic chicken lens epithelial cells (8) and transgenic mice (36). Each mutation substituted directly an *Xba*I restriction enzyme recognition site for 6 bp in the sequence between positions −111 and −34 of the αA promoter (Fig. 1B); the TATA box at position −32 was not interrupted by these mutations. The results showed that the lens cells transfected with plasmids bearing mutations in and around a sequence resembling the MHC class I cyclic AMP-responsive element (CRE) (32) (mutations 8 to 12, spanning −69 to −40 of the αA-crystallin promoter) had CAT activities barely above those of cells transfected with pSVO-CAT. The cells transfected with mutations upstream (mutations 2 to 7) or downstream (mutation 13) of this region had CAT activities generally similar to those of the unmodified pαA366$_a$-CAT and pαA111$_a$-CAT plasmids (Fig. 1). Mutation 1, the most-upstream modification, was exceptional in that it reduced CAT activity in the transfected lens cells.

Isolation of a cDNA encoding an αA-crystallin promoter-binding protein. We next attempted to isolate a putative lens nuclear regulatory protein which binds to this DNA sequence. As a source of material we used the αA-crystallin-producing αTN6 lens cells (see Materials and Methods). Initial gel mobility shift assays showed that a duplex oligodeoxynucleotide identical to the −84/−32 sequence of the αA-crystallin promoter formed a complex with nuclear extracts of αTN6 cells (data not shown).

A λgt11 cDNA expression library was made from the αTN6 cells and screened for the ability to bind the −84/−32 oligodeoxynucleotide. One positive recombinant phage (YTN) was obtained from 1.2 × 10^6 plaques of the library. The fusion protein of this recombinant phage was tested for binding to duplex oligodeoxynucleotides identical to different regions of the αA-crystallin promoter between positions −111 and −32, two regulatory elements of the MHC class I gene (32), and a highly conserved 43-bp sequence from the mouse γ2-crystallin gene (22) (Fig. 2). Strongest binding was obtained with oligodeoxynucleotide αA4 (−73/−55); weaker binding, as judged by the intensity of the signal, was obtained with oligodeoxynucleotide αA2 (−84/−32). By contrast, no binding was observed with oligodeoxynucleotides αA1 (−111/−85), αA3 (−84/−74), or αA5

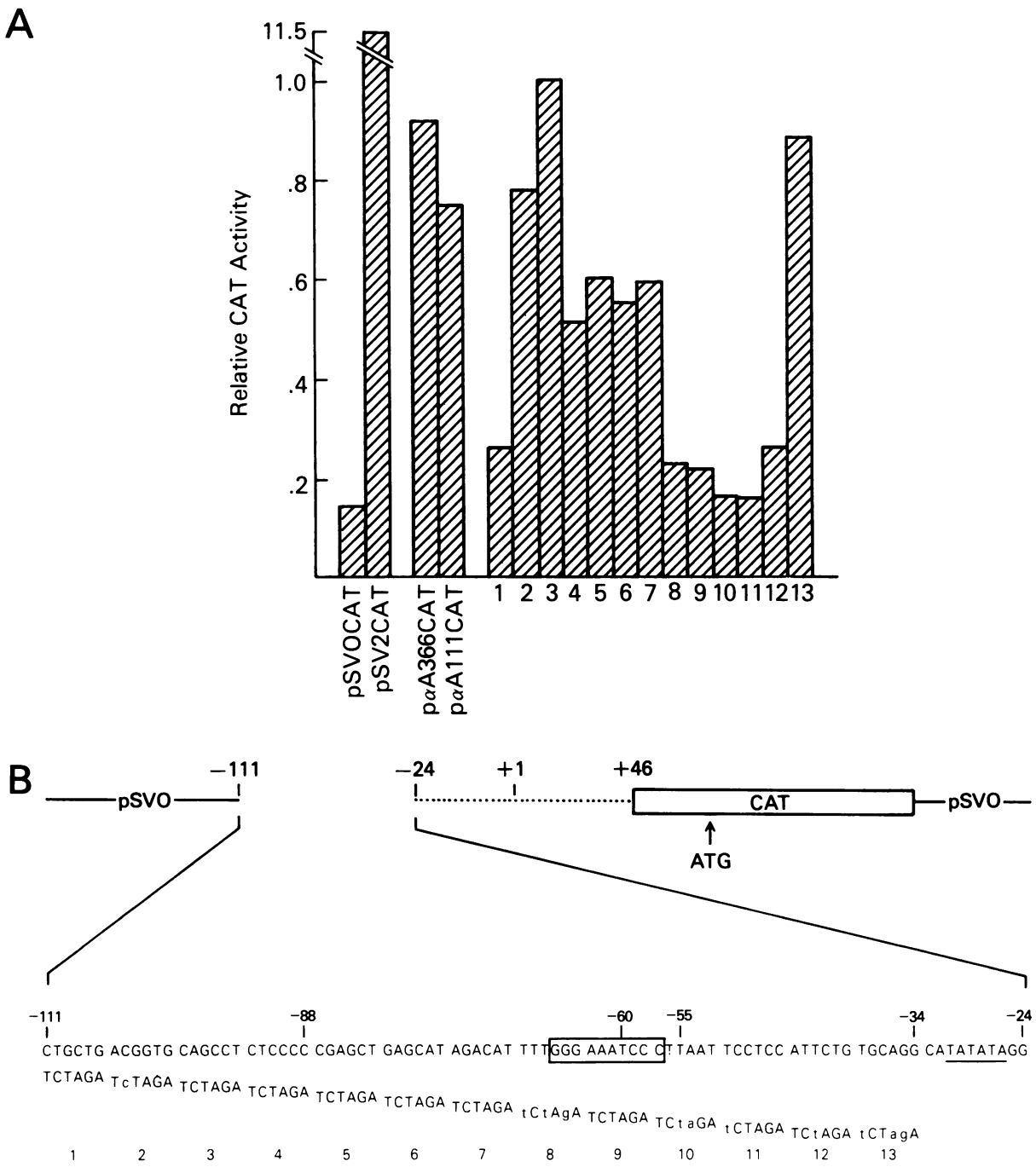

FIG. 1. Site-directed mutagenesis of the mouse αA-crystallin promoter. (A) The effects of mutations in the αA promoter on CAT activity. Each mutation-harboring vector (10 μg) and 3 μg of pTB1 were cotransfected into αTN4-1 mouse lens cells. CAT activities were normalized to β-galactosidase expression and then normalized to the expression of mutation 3, which yielded the highest relative CAT activity for the mutation plasmids in each of the three experiments which were averaged for this figure. All plasmids were grown in *E. coli* HB101 and purified twice from $CsCl_2$ gradients. (B) DNA sequences of site-directed mutations. The mutations (1 to 13) occur in blocks of 6 bp. Lowercase letters represent bases which were not altered when the *Xba*I restriction enzyme recognition site was introduced in place of the wild-type sequence. The parent plasmid is $\alpha A111_a$-CAT. αA-crystallin sequences extend from −111 to +46. The TATA box is underlined. The transcription start site is noted as +1. The boxed sequence is the CRE-like sequence similar to the regulatory region of the MHC class I gene (32).

(−54/−32) of the αA-crystallin promoter or with oligodeoxynucleotide γ2 (−67/−25) of the γ2-crystallin promoter. The fusion protein also bound the regulatory element (CRE) conserved in MHC class I genes (Fig. 2). Region I (GGGG ATTCCCC) of the CRE (32) resembles the αA4 sequence 5′-GGGAAATCCC-3′. The CRE contains an additional upstream sequence called region II (32). Both regions I and II act as enhancers and bind distinct nuclear factors. The region I binding activity expressed in various tissues correlates with the expression of the MHC class I gene (6). Also

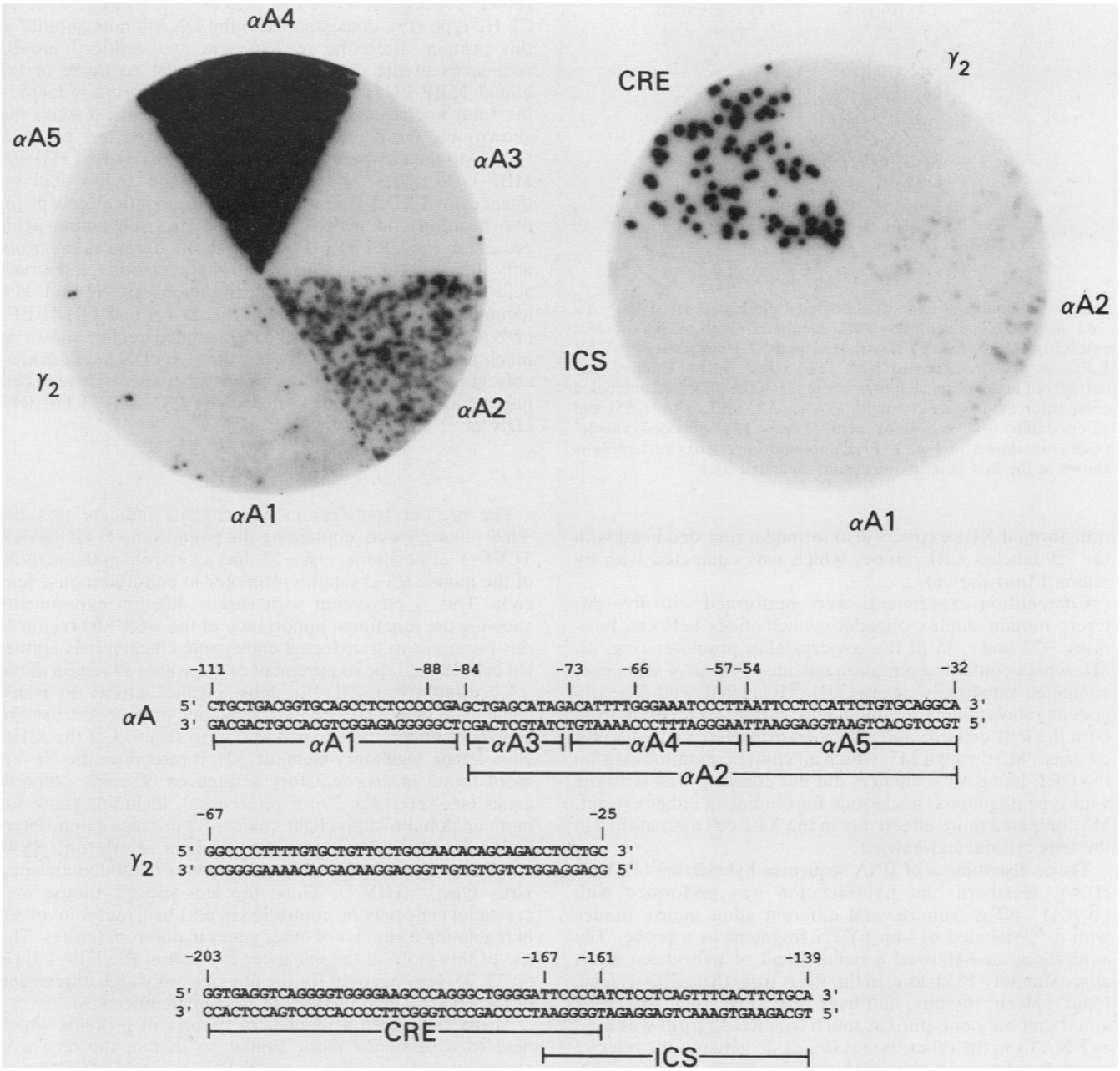

FIG. 2. Purified YTN phage was plaqued onto a Y1090 lawn, and the cDNA-encoded fusion protein was screened for its ability to bind the indicated double-stranded radiolabeled oligodeoxynucleotides representing promoter sequences of the mouse αA-crystallin (αA1, αA2, αA3, αA4, αA5), mouse γ2-crystallin (γ2), and mouse MHC class I (CRE, ICS) genes.

shown in Fig. 2, the fusion protein bound poorly the interferon consensus sequence (ICS) involved in transcriptional induction of MHC class I genes by interferons (31).

To further study binding of the fusion protein, the cDNA was subcloned into the pBS plasmid; this construct, called pYTN, contains, 3′ to the 2.5-kbp cDNA insert, approximately 1 kbp of λgt11, including 53 bp of the *lacZ* gene, encoding the C-terminus of β-galactosidase. As a result of this subcloning, the cDNA was cloned within the *lacZ* gene of pBS so that the pBS *lacZ* initiation codon is utilized in expression of the resulting fusion protein. Extracts from *E. coli* XL1 cells transformed with pYTN formed a gel-retarded complex with the −84/−32 oligodeoxynucleotide of the αA-crystallin promoter, albeit smaller than that observed with nuclear extract from the αTN6 lens cells (Fig. 3). This faster migration suggests that the YTN cDNA does not encode the complete −84/−32 binding protein sequence. An extract from pBS-transformed cells did not form a complex with the oligodeoxynucleotide. The complexes formed with the extracts from both the mouse lens cell line and the pYTN-transformed bacterial cells were specifically eliminated by competition with a 500-fold molar excess of the αA4 oligodeoxynucleotide but not by competition with the αA1, αA3, αA5, and γ2 oligodeoxynucleotides. pYTN-

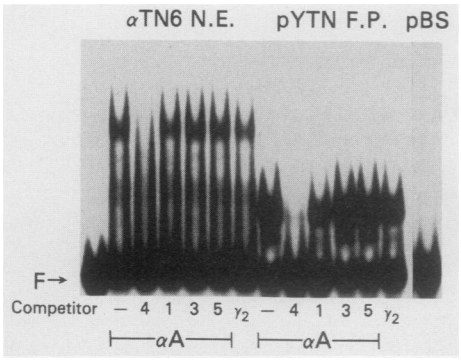

FIG. 3. Double-stranded radiolabeled mouse αA-crystallin −84/−32 oligodeoxynucleotides were incubated with αTN6 nuclear extract (αTN6 N.E.), pYTN-transformed XL1 cell extract (pYTN F.P.), or pBS-transformed XL1 cell extract (pBS). Binding was carried out in the absence (—) or presence of the indicated unlabeled competitor mouse αA-crystallin (αA1, αA2, αA3, αA4, αA5) and γ2-crystallin (γ2) oligodeoxynucleotides. The oligodeoxynucleotides are shown in Fig. 2. Free/unbound oligodeoxynucleotide is shown in the first lane, its migration indicated by F.

transformed XL1 extracts also formed a retarded band with the ^{32}P-labeled CRE probe, which was competed with by region I (not shown).

Competition experiments were performed with five different mutant duplex oligodeoxynucleotides between positions −73 and −55 of the αA-crystallin promoter (Fig. 4). M1, which contains a mutation outside of the αA4 sequence, competed effectively against the ^{32}P-labeled wild-type oligodeoxynucleotide for binding to extracts prepared from both the lens cell line and pYTN-transformed XL1 cells. By contrast, M2 through M5, which all contain mutations within the CRE-like αA4 sequence, did not compete well with the wild-type oligodeoxynucleotide for binding in either extract. M3 competed more effectively in the XL1 cell extract than in the lens cell nuclear extract.

Tissue distribution of RNA sequences hybridizing to pYTN cDNA. Northern blot hybridization was performed with poly(A)$^+$ RNA from several different adult mouse tissues with a ^{32}P-labeled 614-bp pYTN fragment as a probe. The autoradiograms showed a major band of hybridized RNA approximately 10 kb long in the RNA from the αTN4-1, lens, brain, spleen, thymus, and liver cells (Fig. 5). It should be noted that only one-third as much lens RNA (2 μg) was used as RNA from the other tissues (6 μg). In general, the relative amounts of the 10-kbp mRNA hybridizing to the pYTN probe paralleled the intensity of the band hybridizing to the actin probe. The specificity of this signal for a YTN-encoded mRNA and not another related mRNA is strengthened by the pYTN fragment choosen as a probe. This 614-bp pYTN fragment spans a region in which the YTN, PRDII-BF1 (11), and MBP-1 (1) nucleotide sequences share only 58 to 64% similarity (see below and Fig. 6 and 7). Hybridization of these RNA samples to the 5′-most 1,231-bp pYTN fragment, which spans the most homologous region of YTN, PRDII-BF1 (11), and MBP-1 (1), yielded the same 10-kbp band (data not shown).

Sequence of the pYTN cDNA. Starting from the 5′ end of the cDNA insert in pYTN, the sequence contains an open reading frame encoding 665 amino acids followed by a termination codon, 513 nucleotides of 3′ nontranslated sequence, and a poly(A) tail (Fig. 6). The atypical, putative polyadenylation signal is underlined in Fig. 6. The deduced protein sequence has two zinc fingers (boxed in Fig. 6) of the C2-H2 type (19), consistent with the DNA-binding ability of this protein. Both the nucleic acid and deduced protein sequences of the cDNA are very similar to those of the human MBP-1 (1) and PRDII-BF1 (11) transcription factors; the total nucleic acid sequence is 77% identical (data not shown) and the deduced protein sequence is 75% identical (Fig. 7) to the comparable regions of the PRDII-BF1 (11) and MBP-1 (1) genes. Within this region the amino acid sequences of PRDII-BF1 and MBP-1 are identical except for two positions, at which MBP-1 matches the amino acids present in αA-CRYBP1 (Fig. 7). These differences are probably the result of polymorphism. The nucleotide and amino acid sequences of the zinc finger regions are 92 and 98% identical, respectively, between the pYTN and PRDII-BF1 cDNAs. Surprisingly, the 3′ untranslated region shows as much as 91% similarity between the two cDNAs, considerably greater than the 73% similarity between the non-zinc finger-coding nucleotides of the pYTN and PRDII-BF1 cDNAs.

DISCUSSION

The present transfection experiments indicate that the −69/−40 sequence, containing the palindrome 5′-GGGAAA TCCC-3′ at positions −66/−57, has a key role in the activity of the mouse αA-crystallin promoter in cultured mouse lens cells. This is consistent with earlier deletion experiments showing the functional importance of the −88/−60 region of this promoter in transfected embryonic chicken lens epithelial cells (8) and the requirement of the −88/−34 region of the αA-crystallin promoter for lens-specific activity in transgenic mice (36). The −66/−57 sequence of the αA-crystallin gene is of interest, since in addition to region I of the MHC class I gene regulatory element (32), it resembles the NF-kB motif found in *cis*-regulatory sequences of many different genes (see reference 20 for references), including those for immunoglobulin kappa light chains, β2-microglobulin, interleukin-2, interleukin-2 receptor α, beta interferon, SV40 enhancer, cytomegalovirus, and human immunodeficiency virus type 1 (HIV-1). Thus, the lens-specific mouse αA-crystallin gene may be controlled in part by a factor involved in regulating a number of other genes in different tissues. The use of this motif in the enhancer element of the HIV-1 LTR (4, 17, 25) may account for the unexpectedly high expression of the HIV LTR in the lens of transgenic mice (18).

There have been a number of reports of proteins which bind to a sequence motif similar to that in the lens αA-crystallin gene studied here. PRDII-BF1 (11), MBP-1 (1), and HIV-EP1 (23) are zinc finger proteins (19) whose cDNAs have been cloned from human cells. They are either homologous or very similar to our mouse lens cDNA. PRDII-BF1, MBP-1, and our mouse lens cDNA hybridize to an approximately 10-kb RNA in a variety of tissues. Southern blot hybridization with MBP-1 (33) and our pYTN cDNA (our unpublished data) suggests at most a few genes for this protein in humans, mice, and rats. Since our lens cDNA is incomplete and shows appreciable differences in the non-zinc finger-coding regions with PRDII-BF1 and MBP-1, we cannot be certain whether it is homologous to or a closely related family member of the human factor(s). We thus tentatively name the lens protein αA-CRYBP1 (for αA-crystallin-binding protein 1).

NF-kB (17, 20, 21, 30), H2TF1 (2, 3), KBF1 (39), EBP-1 (9), and HIVEN86 (4) also bind to the same motif but appear to differ from PRDII-BF1, MBP-1, and αA-CRYBP1 in being

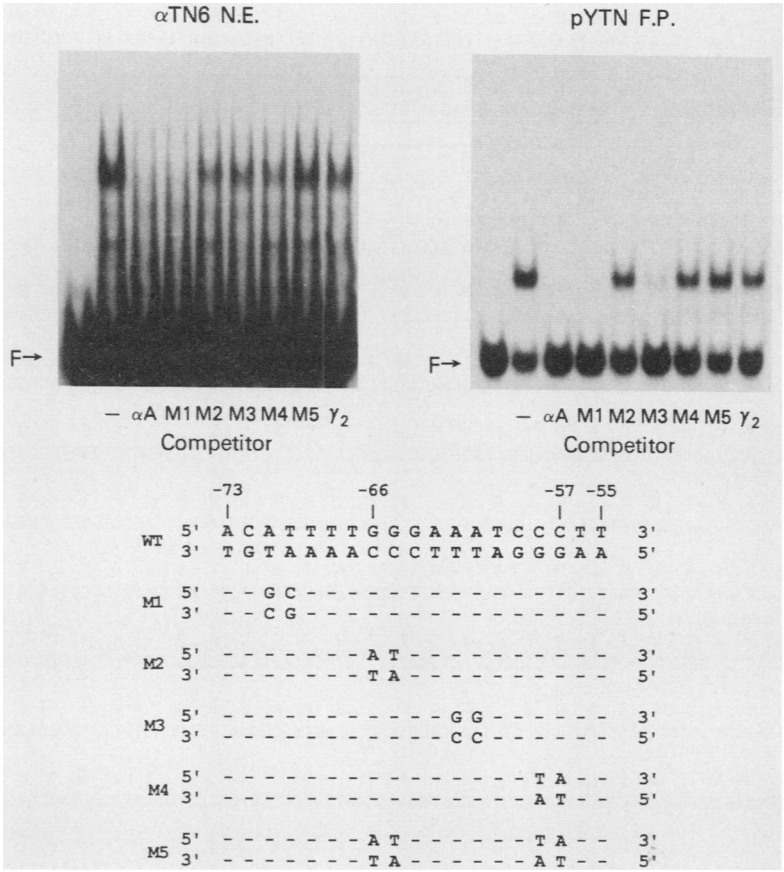

FIG. 4. Double-stranded radiolabeled mouse αA-crystallin −84/−32 oligodeoxynucleotides were incubated with αTN6 nuclear extract (αTN6 N.E.) or pYTN-transformed XL1 cell extract (pYTN F.P.). Binding was carried out in the absence (—) or presence of the indicated unlabeled competitor γ2-crystallin (γ2) or mouse αA-crystallin oligodeoxynucleotides: wild type −73/−55 (WT; denoted as αA on the gels) and mutants M1, M2, M3, M4, and M5. Free/unbound oligodeoxynucleotide is shown in the first lane of each panel, its migration position indicated by F.

significantly smaller, as judged by the large mRNAs of PRDII-BF1, MBP-1, and αA-CRYBP1. NF-kB, H2TF1, and KBF1 appear to be distinct proteins with characteristic differences in their tissue distribution, binding specificities, and patterns of methylation interference for binding (3; see references 1 and 11 for further discussion). Clarification of the relationships of these various factors awaits further characterization of the cDNAs and natural proteins.

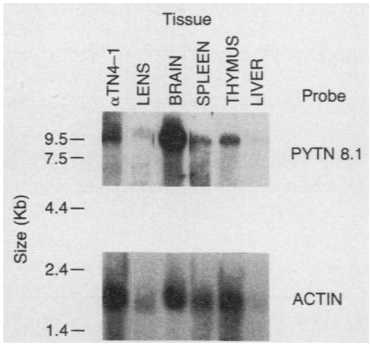

FIG. 5. Northern blot hybridizations. Either 2 (lens) or 6 (all other tissues) μg of poly(A)$^+$ RNA was electrophoresed in each lane and probed with either the 614-bp *Hin*dIII fragment derived from the pYTN cDNA or a human β-actin cDNA. The autoradiogram with the pTYN probe was exposed for 4 days, while that with the actin probe was exposed for 1 day. Lens was from newborn mice; the other tissues were from adults.

The presence of αA-CRYBP1 in the lens and its ability to bind to the 5′-flanking region of the αA-crystallin gene in a sequence critical for lens function in transgenic mice (36) and transfected lens cells from embryonic chickens (8) and mice (present study) suggest that it is required for the lens-specific expression of the αA-crystallin gene. Additional studies in our laboratory suggest that the αA-crystallin αA-CRYBP1 site is sufficient to activate transcription of the thymidine kinase promoter in αTN4-1 cells but not in a mouse fibroblast cell line (Sax, unpublished). We cannot, however, rule out the possibility that a similar but different factor is involved in vivo. Indeed, it is not clear whether transcription of the other genes with the same sequence motif in their regulatory regions is controlled by PRDII-BF1, MBP-1, HIV-EP1, and αA-CRYBP1. For example, even though PRDII-BF1, MBP-1, and αA-CRYBP1 mRNAs are expressed in brain tissue at high levels, binding activity to region I of the MHC class I gene is not observed in brain tissue (6). αA-CRYBP1 appears to represent a lower percentage of the poly(A)$^+$ mRNA fraction in the mouse lens than in αTN4-1 cells. This lower apparent percentage may be

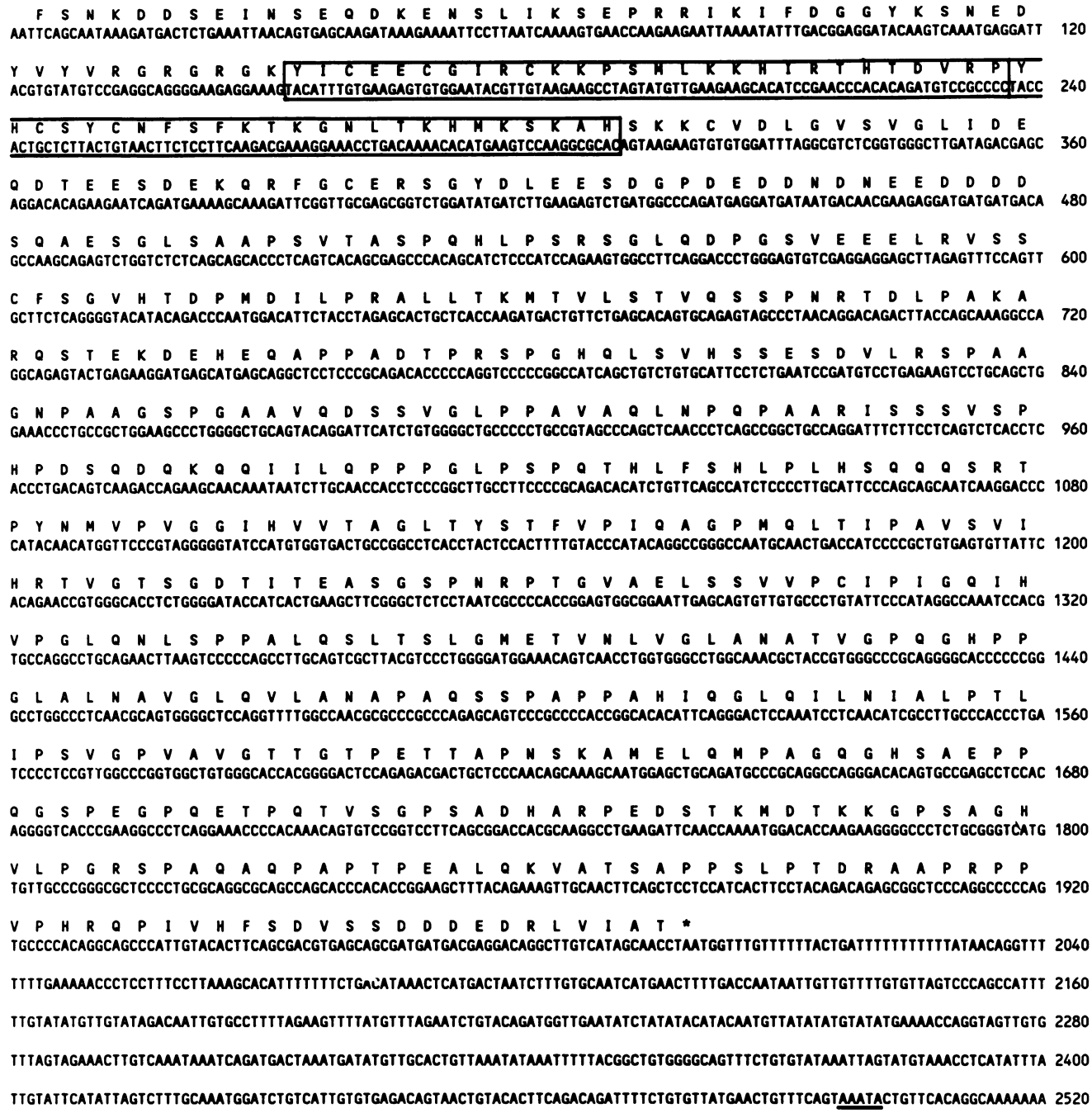

FIG. 6. Nucleotide and deduced protein sequences of the pYTN cDNA. The single-letter symbol for each amino acid is placed above the central nucleotide of its codon; an asterisk (*) designates the termination codon. The two zinc fingers are boxed, and the putative polyadenylation signal is underlined. The *Hin*dIII fragment used in Northern blot hybridizations spans nucleotide sequence positions 1231 to 1845.

the result of an extreme abundance of the crystallin mRNAs in the intact lens or the loss of nuclei in lens fiber cells, resulting in lower levels of transcription.

In general, tissue-specific gene expression involving a ubiquitous transcription factor may require posttranslational modifications and/or contributions by other factors. With respect to the interaction of multiple factors, gel retardation experiments have shown that at least two complexes form with oligodeoxynucleotides identical to the sequence between positions −111 and −55 in the 5′-flanking region of the αA-crystallin gene when incubated with nuclear proteins from chicken lenses, chicken erythrocytes, and HeLa cells (34). It may be significant in this connection that the mutation between positions −111 and −106 in the present study (Fig. 5) decreases expression of the αA-crystallin promoter-*cat* fusion gene in the transfected mouse lens cells, as do those involving the downstream αA4 motif and its associated sequences. In any event, the use of general transcription

FIG. 7. Amino acid comparison of αA-CRYBP1 and PRDII-BF1. The deduced amino acid sequence of αA-CRYBP1 is aligned with the corresponding region of PRDII-BF1 (14). Amino acid similarities are denoted by :, while gaps in the sequence are denoted by —. An asterisk (*) denotes the positions at which the amino acid sequences of PRDII-BF1 (14) and MBP-1 (13) differ. The zinc finger regions are boxed. The region encoded by the 614-bp *Hin*dIII fragment used in Northern blotting (Fig. 5) lies between the two triangles.

factors for high gene expression in the lens fits well with the fact that crystallins have been recruited from ubiquitously expressed genes and continue to share genes encoding metabolic enzymes and other proteins with unknown functions (i.e., αB-crystallin) in nonlens cells (29).

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