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Identification of a lens-specific regulatory region (LSR) of the murine αB-crystallin gene

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ABSTRACT

Previous studies have shown that the −661/+44 sequence of the murine αB-crystallin gene contains a muscle-preferred enhancer (−426/−257) and can drive the bacterial chloramphenicol acetyltransferase (CAT) gene in the lens, skeletal muscle and heart of transgenic mice. Here we show that transgenic mice carrying a truncated −164/+44 fragment of the αB-crystallin gene fused to the CAT gene expressed exclusively in the lens; by contrast mice carrying a −426/+44 fragment of the αB gene fused to CAT expressed highly in the lens, skeletal muscle and heart, and slightly in the lung, brain, kidney, spleen and liver. DNase I protection experiments indicated that the −147/−118 sequence is protected by nuclear proteins from αTN4-1 lens cell line, but not by nuclear proteins from myotubes of the C2C12 cell line. Site directed mutagenesis of this sequence decreased promoter activity in transiently-transfected lens cells, consistent with this sequence being a lens-specific regulatory region (LSR). We conclude that the −426/−257 enhancer is required for expression in skeletal muscle, heart and possibly other tissues, and that the −164/+44 sequence of the αB-crystallin gene is sufficient for expression in the lens of transgenic mice.

INTRODUCTION

The crystallins comprise approximately 90% of the water soluble proteins of the transparent eye lens and contribute to its optical properties (1,2). The α, β and γ crystallins are present in all vertebrate lenses, while the taxon-specific crystallins are found only in the lenses of certain species. The latter are closely related or identical to common metabolic enzymes (3–7).

The two α-crystallin genes (αA and αB) are highly conserved members of the small heat shock protein (sHSP) family (3, 9) and are situated on different chromosomes (10–12). Both α-crystallin genes are expressed most highly in the lens. The αA-crystallin gene is also expressed at lower levels in spleen and thymus (13, 14). The αB-crystallin gene is constitutively expressed in many non-lens tissues (15, 16), is associated with numerous diseases, especially neurodegenerative conditions (17, 18, see 19), and is induced under a variety of experimental conditions and physiological stresses (20–24).

Our previous experiments demonstrated that the differential constitutive expression of the murine αB-crystallin gene is under transcriptional control. First, an αB-crystallin minitransgene lacking introns, exon 2 and part of exon 1 and 3 showed a similar pattern of expression as the endogenous αB-crystallin gene in transgenic mice (16). Subsequent transgenic mouse experiments using the bacterial chloramphenicol acetyltransferase (CAT) reporter gene indicated that the −661/+44 sequence of the αB-crystallin gene is capable of directing gene expression in the lens, skeletal muscle and heart (26). Transfection experiments utilizing the *Herpes simplex* thymidine kinase promoter linked to the human growth hormone reporter gene established that the −426/−257 fragment of the αB-crystallin gene comprised an enhancer that functions preferentially in cultured myotubes and less strongly in cultured myoblasts and lens cells (26). Finally, DNase I footprinting, site-specific mutagenesis and transfection experiments have identified three regulatory elements (αBE-1, αBE-2 and αBE-3) that function both in cultured skeletal muscle and lens cells, and one regulatory element MRF (muscle regulatory factor binding site) that functions specifically in cultured muscle cells (25).

In the present investigation we have established the requirement of the −426/−257 enhancer for expression in skeletal muscle and heart by producing transgenic mice containing truncated fragments of the mouse αB-crystallin gene fused to the CAT reporter gene. These transgenic mice experiments also showed that the −164/+44 sequence of the mouse αB-crystallin gene is sufficient to direct lens-specific gene expression. In addition, DNase I footprinting, and site-directed mutagenesis and transient-transfection experiments have implicated the −147/−118 sequence as a critical regulatory element for lens expression within this promoter fragment. Thus, the regulatory elements required for lens-specificity of the diversely expressed mouse αB-crystallin gene can be physically separated from the control elements required for expression in skeletal muscle and heart.

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MATERIAL AND METHODS

Isolation of DNA for pronuclear injections and transgenic mice production

Approximately 3 kbp *Nde*I−*Pst*I DNA fragments containing the mouse αB-CAT fusion genes were isolated from plasmids p71-3 and p65-7 (26) by polyacrylamide gel electrophoresis followed by electro-elution, phenol-chloroform extraction and ethanol precipitation. Each fragment contained 60 bp of pBR322 sequence at its 5′ end, murine αB-crystallin promoter sequences, the bacterial CAT gene, and SV40-derived sequences including the small-t antigen splice sites and polyadenylation signal. The αB426-CAT fusion gene isolated from p71-3 contained the −426/+44 sequence of the αB-crystallin gene, and the αB164-CAT fusion gene isolated from p65-7 contained the −164/+44 sequence of the αB-crystallin gene.

Linear DNA fragments were injected into one pronucleus of a single celled mouse embryo (27). The embryos were obtained from superovulated FVB/N females. Injected embryos were transferred into FVB/N females made pseudopregnant by mating to vasectomized FVB/N males. Transgenic mice were created by the National Eye Institute Centralized Transgenic Facility.

Analysis of transgenic mice

DNA was isolated (28) from tails of founder Fo mice and analyzed by Southern blot and PCR analyses for the presence of the transgene using a 5′ oligodeoxynucleotide primer (# 7577 5′TAGGACTCCACAAAGAGTTAATGTC3′, for αB426-CAT and oligo # 7746 5′TCTCTTTTCTTAGCTCAGTGAGTAC3′, for αB164-CAT) specific for the murine αB-crystallin promoter and a 3′ oligodeoxynucleotide primer (# 7576 5′CGGTCTGGTTATAGGTACATTGAGC3′) specific for the CAT gene. Fo containing the transgene were mated to nontransgenic FVB/N mice to obtain F1 offspring, and sibling matings were used to establish homozygous mouse lines. The transgene copy number for each mouse was estimated by hybridization intensity in a slot blot analysis of the genomic DNA relative to the standard samples representing 0−50 copies of the transgene, using the ECL 3′-Oligo Labelling Detection System (RPN 2130/2131, Amersham) and the 1.6 kbp *Nde*I/*Bam*HI fragment from pSVO-CAT as the labelled probe. Blots were exposed at room temperature for 1 min with an intensifying screen.

Tissue extraction and CAT assays

Hemizygous mice between 1 and 4 months of age were sacrificed by CO_2 asphyxiation and the lenses, lung, heart, liver, kidney, spleen, brain (cerebrum) and skeletal muscle (from thigh) were homogenized with 0.25 M Tris−HCl (pH 7.8) in Duall glass homogenizers (Knotes, 0020). The lenses were homogenized in polypropylene Eppendorf microfuge tubes, centrifuged for 10 min at 4°C and the supernatant fraction was heated at 65°C for 15 min followed by centrifugation for 10 min at 4°C. Protein concentrations were determined in the final supernatant fraction with the Bio-Rad protein assay kit according to the manufacturer's instructions using bovine serum albumin for the standards. Quantities of tissue extract containing 0.5 to 4 μg of total protein were analyzed for CAT activity by the biphasic assay (29). The level of CAT activity was taken as an indirect measure of promoter strength.

Nucleic acid isolation and primer extension

Plasmid DNA was prepared by the alkaline lysis method (30) followed by ultracentrifugation banding in CsCl−ethidium bromide. Mouse tail DNA was isolated by modification of the procedure of Hogan *et al.* (28). RNAs from 4-month-old transgenic mice and non-transgenic siblings were prepared using RNAzol (TEL-TEST, inc.) as suggested by the manufacturers. Primer extension analysis was performed using a 30-mer oligodeoxynucleotide complementary to the 5′ coding region of the CAT gene (# 8698 5′TCCACTGATTTTTTTCTCCATTTTAGCTTC3′). The primer was 5′ end-labelled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Approximately 2 ng of primer (2×10^5 cpm) was annealed to 5−20 μg of total RNA at 70°C for 5 min, reverse transcribed using thermostable rTth (Gene Amp Thermostable rTth Reverse Transcription PCR Kit, Perkin Elmer Cetus Instruments) at 70°C for 20 min and ethanol precipitated. Extended products were analyzed on a 8% polyacrylamide-8 M urea sequencing gel. ^{32}P-labelled, MspI-digested pBR322 DNA fragments were used as size markers. Oligonucleotide primers were chemically synthesized (Applied Biosystems).

Nuclear extracts and DNase I footprinting assays

Nuclear extracts were prepared from C2C12 myotubes (31) and αTN4-1 lens cells (32) as described by Dignam (33), except that the ammonium sulfate precipitation step was omitted. αTN4-1 is an SV40 T-antigen transformed cell line (32).

DNase I footprinting was performed by using the *Eco*RI−*Bam*HI restriction fragment of pRD28 (26) spanning positions −666 to +76 of the αB-crystallin gene. Initially, pRD28 was digested with *Bam*HI and the anti-sense strand end-labelled with [γ-^{32}P] dATP (Amersham Corp., Arlington Heights, IL) using T4 polynucleotide kinase. The radiolabelled DNA was digested with *Eco*RI and purified on a polyacrylamide gel; DNase I footprinting was performed as described previously (25).

Site-directed mutagenesis

Mutations were generated within the −164/+44 *Eco*RI/*Pst*I fragment of the mouse αB-crystallin gene obtained from pRD30A (26) and cloned into the *Eco*RI/*Pst*I site of bacteriophage M13mp18; site specific mutations (Mu-9759 to Mu-9764) were introduced by using a deoxyoligonucleotide-directed mutagenesis kit (Sculptur *in vitro* mutagenesis kit, Amersham). Mutagenic deoxyoligonucleotides contained the substitution sequence TCTAGA (*Xba*I site) and 20 bases on each side complementary to the αB-crystallin promoter sequence. The resulting mutated restriction fragments were subcloned into pRD30A at the unique *Bam*HI site. All constructs were confirmed by sequencing the ligation junctions and mutated regions, using Sequenase (U.S Biochemical Corp.) and [α-^{35}S] dATP (1,000 Ci/mmol; Amersham).

Cell culture, transfection and enzyme assay

Mouse αTN4-1 (32) and rabbit N/N1003A (34) lens cell lines were grown in Dulbecco's modified Eagle's medium (GIBCO-BRL) containing 10% fetal calf serum and 50 μg/ml of gentamicin. Transient transfections using calcium phosphate method were performed with 10 μg of promoter-CAT plasmid and 4 μg of pCMVβgal as an internal control for transfection efficiency, as described previously (25). Lens cells were harvested 31 hours after transfection. CAT activities were measured by the biphasic (29) assay and β-galactosidase activities were determined as described previously (25). The transfection data

represent the mean of three separate experiments, with each experiment being conducted with duplicate plates.

RESULTS

DNase I footprinting and site-specific mutations of the αB-promoter: evidence for a lens-specific regulatory region

Although we have previously demonstrated (25) that the −427/−259 enhancer of the αB-crystallin gene contains regulatory sequences that contribute to expression in cultured lens cells, the enhancer also contains a muscle-specific regulatory element (MRF) and functions much more effectively in transfected muscle cells, especially myotubes. Consequently we performed DNase I footprinting in order to investigate the possibility that sequences further downstream may contain lens-specific regulatory elements. A DNase I footprint obtained with a nuclear extract from the αTN4-1 lens cells was compared with that obtained using a nuclear extract derived from C2C12 muscle cells (myotubes). The results showed clearly that the sequences between positions −147 and −118 of the αB-crystallin 5′ flanking region were protected from DNase I digestion in the extracts from αTN4-1 cells but not in the extracts from the C2C12 cells (Fig.1). These data raised the possibility that the −147/−118 sequence is used specifically for expression of the αB-crystallin gene in lens cells. The footprinted sequence and the surrounding nucleotides are shown in Fig. 1. Sequences that resemble the αCE1 and αCE2 sequences, proposed as lens consensus regulatory sequences (34), are overlined (see Discussion).

Site-specific mutations were made throughout the −147/−118 sequence in order to test whether this DNase I footprinted region contains regulatory elements that function in lens cells. p65-7, a plasmid containing the −164/+44 fragment of the αB-crystallin gene fused to the bacterial CAT reporter gene, was used for these experiments; p65-7 produces CAT in transfected lens cells but not in transfected C2C12 muscle cells (26). Each mutation replaced an *Xba*I restriction enzyme recognition sequence for the indicated hexanucleotides in the αB-crystallin sequence between positions −150 and −116 (Fig. 2A). The mutated promoter constructs were compared with the wild type construct for the

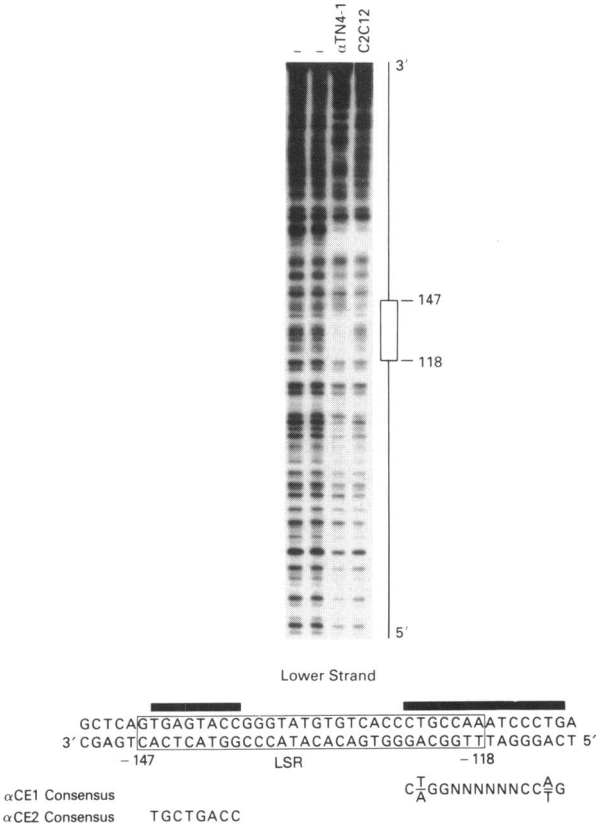

Figure 1. *In vitro* DNase I footprinting of the 5′ flanking region of the murine αB-crystallin gene. Footprinting is shown for the lower (anti-sense) strand of the *Eco*RI−*Bam*HI fragment (−666 to +76) of pRD28 (26). pRD28 was digested with *Bam*HI and the lower strand was 5′-end labelled with [γ-^{32}P]dATP, using T4 polynucleotide kinase. The two lanes on the left (−) contain free DNA; the lane labeled αTN4-1 contains DNA incubated with a nuclear extract from αTN4-1 lens cells; the lane labeled C2C12 contains DNA incubated with a nuclear extract from C2C12 myotubes. The open box indicates the region protected from DNase I digestion after incubation with the αTN4-1 nuclear extract. The protected sequence (LSR, see text) is boxed below the footprint. Similarities to the αCE1 and αCE2 lens consensus sequences (35), which are given below, are overlined in the αB-crystallin promoter.

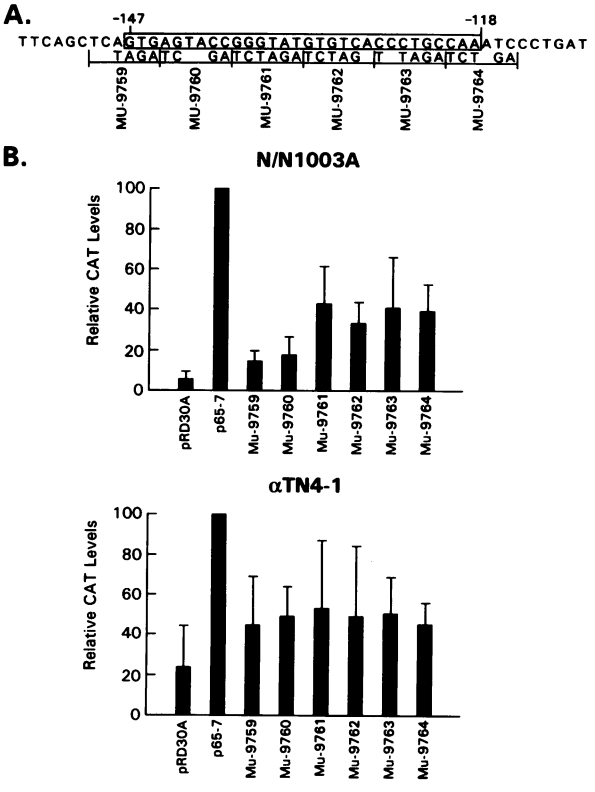

Figure 2. Site-directed mutagenesis and transfection of αB-crystallin promoter−CAT constructs in lens cell lines. (**A**) DNA sequence of the αB-crystallin promoter sequence used for site-directed mutations. The region protected from DNase I digestion is boxed. The location of the mutations (TCTAGA, an *Xba*I site) are shown below the sequence. Gaps represent bases which were not altered when *Xba*I restriction enzyme recognition site was introduced. (**B**) Plasmids containing the mutated αB-crystallin constructs were transfected into rabbit N/N1003A and mouse αTN4-1 lens cell lines. Lens cells were harvested 31 hours after DNA removal; CAT activity was determined by the biphasic assay (29) and normalized with respect to the activity of β-galactosidase, which resulted from co-transfection of pCMVβgal (see Material and Methods). Relative CAT levels (± standard deviations) are expressed as a percentage of the levels expressed by the plasmid p65-7 containing the wild type promoter −164/+44 fragment of the mouse αB-crystallin gene.

ability to direct expression of the reporter CAT gene in transfected mouse αTN4-1 (32, SV40 T-antigen transformed) and rabbit N/N1003A (34, untransformed) lens cell lines. All of the mutant constructs showed a decreased promoter activity relative to the wild type plasmid in both cell lines tested (Fig. 2B). The largest decreases obtained were 5 (Mu-9760) to 10 (Mu-9759) fold in the N/N1003A cells; all the other transfections resulted in 2 to 2.5 fold losses in promoter strength in both cell lines. Thus the sequences between positions −147 and −118 contribute to αB-crystallin expression in transfected cells derived from the lens. These data are consistent with the DNase I footprinting results and indicate that the −147/−118 sequence is a cis-regulatory region for lens-expression. Consequently, we have called this sequence LSR for lens-specific region.

Production of transgenic mice

Two constructs of αB-crystallin-CAT fusion genes (Fig. 3) were used as transgenes in transgenic mice (FVB/N strain). The αB426-CAT construct contains the αB-crystallin muscle-preferred enhancer and the LSR sequence. By contrast, the αB164-CAT construct contains only the LSR sequence and not any of the previously identified αB-crystallin muscle/lens cis-regulatory elements (25) in the enhancer.

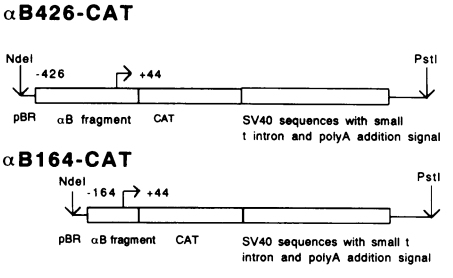

Figure 3. Structure of the murine αB-crystallin−CAT chimeric transgene. Approximately 3 kbp *NdeI*/*PstI* fragments isolated from p71-3 and p65-7 (26) containing a −164/+44 and a −426/+44 fragment of αB-crystallin gene, respectively, linked to the bacterial CAT gene were used as transgenes in transgenic mice. Approximately 60 bp of pBR sequences are present 5′ to the αB fragments in the transgene and approximately 1,400 bp of simian virus 40 (SV40) sequences, including the small intron and poly(A) addition signal, are present 3′ to the CAT gene.

PCR analyses of tail DNA were performed from the mice derived from the embryos injected with αB426-CAT and αB164-CAT constructs, to establish the presence of the transgene using primers # 7577, # 7746 and # 7576. 6 of the 33 Fo mice carried the αB164-CAT transgene in their genome, while 7 out of 43 Fo mice contained the αB426-CAT transgene. Three lines (for αB426-CAT) and five lines (for αB164-CAT) which originated from Fo mice containing 1 integration site were chosen for further analysis. Slot blot analyses of the genomic DNA were performed to estimate the transgene copy number, using standard samples representing 0−50 copies of the transgene. The transgene data are summarized in Table I.

Expression of the αB-crystallin promoter-CAT fusion genes in transgenic mice

To analyze the tissue specifity of αB-crystallin promoter-CAT sequences, transgenic mice were sacrificed and their tissues (heart, lung, liver, spleen, kidney, skeletal muscle, brain and lens) were assayed for CAT activity. Mice from multiple independent lines (different founders), containing the αB164-CAT transgene expressed CAT activity significantly above background exclusively in the lens. This is shown for one experiment in the right panel of Fig. 4. Table 1 shows that the average CAT activity in the lenses of the transgenic mice were significantly higher than the average background CAT activity observed in the lenses of non-transgenic mice. The average non-lens CAT activities of the transgenic mice were similar to the background levels observed in non-transgenic mice (see Table 1).

A typical experiment showing the tissue distribution of CAT activity in transgenic mice containing the αB426-CAT transgene is shown in the left panel of Fig. 4. In contrast to the results obtained with the αB164-CAT transgene, the mice containing the αB426-CAT transgene had significant amounts of CAT activity in the heart, skeletal muscle and lens. In addition, there was a low level of CAT activity in the lung, brain, kidney, spleen and liver in the αB426-CAT transgenic mice (Table 1). All the lines tested exhibited the same pattern of expression. The data are summarized in Table 1. The overall levels of CAT activity were 6 to 7 fold higher in the lenses of the αB426-CAT lines than in the lenses of the transgenic mice harboring the αB164-CAT transgene.

To test whether the transcription initiation site of the transgene occurred at the same location in the αB-crystallin promoter

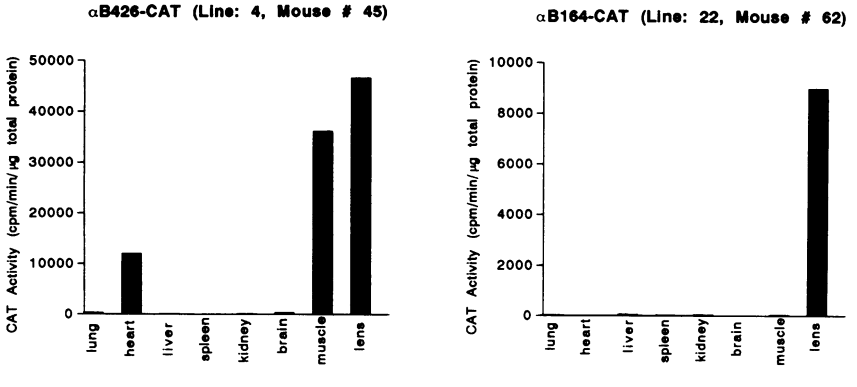

Figure 4. Analysis of CAT activity in transgenic mice. Tissue extracts were prepared from F1 hemizygous mice and were assayed for CAT activity. Representative samples from line 4 (αB426-CAT) and line 22 (αB164-CAT) are shown here.

fragment as that in the endogenous αB-crystallin gene (16), primer extension experiments were conducted using oligodeoxynucleotides complementary to the CAT gene (# 8698) and total RNA from lenses, heart and skeletal muscles from several lines of αB426 and αB164 transgenic mice. A major primer extended product of 115 nucleotides was obtained in every case, which corresponds to the transcription initiation site at position +1 in the endogenous gene (results for mouse # 55, line: 26 is shown in Fig. 5).

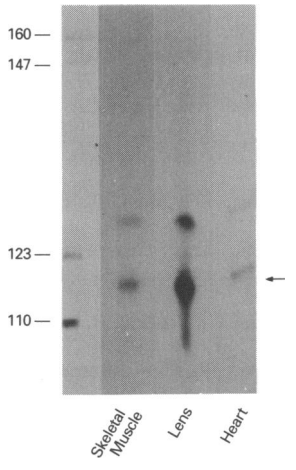

Figure 5. Primer extension analysis of RNA from a transgenic mouse (line: 26, mouse # 55). Primer extension analysis was performed with RNA isolated from the lens, skeletal muscle and heart of F1 transgenic mouse using a CAT primer as described in Materials and Methods. The extended products were analyzed on a 8% acrylamide sequencing gel. *Msp*I-digested ^{32}P labelled pBR322 DNA was used as a size marker; sizes are indicated in base pairs.

DISCUSSION

We have previously identified four cis-acting regulatory elements (αBE-1, αBE-2, αBE-3 and MRF) located within the αB-crystallin enhancer (−427/−259) (25). αBE-1, αBE-2 and αBE-3 are shared by both transfected lens and muscle cells, but the MRF element is used only by the transfected muscle cells.

In the present DNase I footprinting, site-directed mutagenesis, transient transfection and transgenic mouse experiments we have identified a lens-specific regulatory sequence between positions −147 and −118 of the mouse αB-crystallin gene that is proximal to the muscle-preferred enhancer. Previous deletion and transfection experiments implicating sequences between positions −115 and +44 for lens expression of the murine αB-crystallin gene (26), coupled with the present DNase I footprinting and transient transfection results, indicate that the LSR (−147/−118) interacts with elements further downstream, as well as with αBE-1, αBE-2 and αBE-3 in the −427/−259 enhancer (25), for the expression of the murine αB-crystallin gene in the lens. Although the LSR is not sufficient for expression in the skeletal muscle and heart, we cannot eliminate the possibility that it is used for expression in these tissues along with other control elements.

It is interesting that the sequences between positions −124 and −111 (CTGCCAAATCCCTG), which includes the 3′ border of the footprinted region (position −118) and the 5′ border of the region implicated earlier for lens-expression (position −115) (26), differs by only the underlined C from the αCE1 consensus sequence, C(T/A)GGN$_6$CC(A/T)G, thought to be important for lens expression of crystallin genes (36). Transfection experiments indicated that 3 copies of αCE1 needed to interact with 3 copies of αCE2 (TGCTGACC) for full enhancer activity (35). The sequence between positions −146 to −139 of the αB-crystallin promoter footprinted here (TGAGTACC) shows some resemblance to αCE2, although it differs at the underlined

Table 1. Expression of αB-crystallin promoter-CAT genes in F1 generation of FVB/N transgenic mice

Construct	Line[a]	Mouse	Sex	Copy #	Age[b]	CAT Activity[c] Lung	Heart	Liver	Spleen	Kidney	Brain	Muscle	Lens
α B426-CAT	4	34	M	11-20	2.25	247	20260	382	10	63	246	47642	40313
	4	35	M	6-10	2.25	266	22048	157	55	87	509	46699	44444
	4	40	M	6-10	1.75	2514	40963	348	45	84	918	40491	50088
	4	42	F	11-20	1.75	643	12567	90	0	66	111	42830	39486
	4	44	F	6-10	2.25	63	20038	27	39	0	74	46203	48358
	4	45	F	21-30	2.25	284	11939	53	12	49	309	35951	46474
	26	55	M	5-6	2.00	74	2013	0	0	29	136	9728	7934
	26	60	F	21-30	2.00	857	5836	109	29	119	416	10538	7298
	26	64	F	21-30	2.25	379	9474	60	17	105	390	51112	53917
	26	50	M	11-20	2.25	432	20522	57	33	90	887	48569	43671
	19	67	M	31-40	2.00	193	4104	90	503	400	138	11868	85736
	19	68	F	31-40	2.00	126	14278	15	1213	462	305	11451	111909
					Avg. Activity:	506	15336	115	163	129	369	29739	48302
α B164-CAT	22	62	M	11-20	2.25	13.3	0.0	41.0	8.3	17.4	0.0	2.2	8900
	22	65	F	5-6	2.25	55.7	0.0	36.6	0.0	0.0	0.0	0.0	7503
	7	49	M	11-20	2.25	4.2	5.2	9.8	1.7	2.8	0.0	11.3	2452
	28	69	M	6-10	2.25	0.0	16.5	29.8	0.0	26.9	1.4	0.0	3126
	41	78	M	6-10	2.00	0.0	0.0	0.0	0.2	9.0	0.0	0.0	1976
	41	80	F	1-5	2.00	0.0	0.0	2.0	0.1	7.8	0.0	0.0	2293
	37	86	M	5-10	2.75	6.6	0.0	8.4	6.9	13.0	19.6	9.9	19171
	37	89	F	5-6	2.75	3.6	7.7	28.3	10.6	20.6	6.1	6.8	12812
					Avg. Activity:	10.4	3.6	19.4	3.4	12.8	3.3	3.7	7290
Non-Transg.	26	58	M	-	5.25	1.95	13.32	17.27	2.15	22.57	3.05	3.82	1.30
	22	67	F	-	5.25	3.12	19.97	16.05	0.25	14.65	0.00	0.00	0.00
					Avg. Activity:	2.57	16.65	16.65	1.20	18.61	1.52	1.52	0.65

[a]Separate lines of transgenic mice were derived from different founder mice; all examined were F1 transgenic mice containing only one integration site; the non-transgenic controls were sibling mice of the transgenic animals.
[b]Age in months.
[c]CAT activity: expressed as cpm[^{3}H] acetylchloramphenicol produced/minute/μg total protein.

nucleotides (see Fig. 1). No other obvious similarities with sequences previously implicated in lens-specific expression of other crystallin genes were noted in the LSR sequence of the αB-crystallin gene (36, 37).

Previous studies on transgenic mice (16) have shown that the pattern of expression of the αB-crystallin intronless minigene containing 666 bp of 5′ and approximately 2400 bp of 3′-flanking sequence and lacking exon 2 and portions of exon 1 and 3, was similar to that of the endogenous gene. In subsequent studies (26) a transgene comprising sequences from −661 to +44 of αB-crystallin gene fused to CAT gene was expressed preferentially in the lens and skeletal muscle in transgenic mice. When present in multiple copies, the αB-crystallin promoter−CAT transgene was expressed at high levels in the lens, heart and skeletal muscle, at low levels in the kidney, spleen, lung and liver and in trace amounts in the brain. Together these experiments demonstrated that the 5′ flanking sequence (−661 to +44) of the αB-crystallin gene is sufficient for expression in lens and skeletal muscle and suggested that the 3′ flanking region may contain regulatory elements for expression in other tissues. The present transgenic mouse experiments establish that sequences between positions −426 and −164 are essential for expression in skeletal muscle and heart, and sequences between −164 and +44 are sufficient for expression in lens. Our results also indicate that the expression of the murine αB-crystallin gene in the lens, skeletal muscle and heart involves a combination of shared and tissue-specific control elements.

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