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The Chicken Skeletal Muscle α-Actin Promoter Is Tissue Specific in Transgenic Mice

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We have generated transgenic mouse lines that carry the promoter region of the chicken skeletal muscle α (α_{sk}) actin gene linked to the bacterial chloramphenicol acetyltransferase (CAT) gene. In adult mice, the pattern of transgene expression resembled that of the endogenous α_{sk} actin gene. In most of the transgenic lines, high levels of CAT activity were detected in striated muscle (skeletal and cardiac) but not in the other tissues tested. In striated muscle, transcription of the transgene was initiated at the normal transcriptional start site of the chicken α_{sk} actin gene. The region from nucleotides −191 to +27 of the chicken α_{sk} actin gene was sufficient to direct the expression of CAT in striated muscle of transgenic mice. These observations suggest that the mechanism of tissue-specific actin gene expression is well conserved in higher vertebrate species.

The development of methods to introduce foreign genes into the germ lines of higher eucaryotic organisms has contributed significantly to the understanding of gene regulation, cell differentiation, development, and cancer (for reviews, see references 5, 17, and 32). This technology can be used to investigate protein function, to trace cell lineages during development, to engineer genetic improvements in agriculturally important crops and livestock, and to establish in vivo model systems for the study of human disease. The success of many of these studies requires that the expression of transgenic sequences be confined to a specific cell or tissue type. The ability to direct, or to restrict, the expression of a heterologous gene product to a desired eucaryotic cell or tissue type and/or to a particular period of development is becoming increasingly more important.

We have chosen the actin gene family to study the transcriptional regulation of tissue-specific gene expression. Actins are a family of highly conserved proteins that are present in the microfilaments of nonmuscle cells and in the myofibrils of muscle cells (for reviews, see references 1, 34, 35). Members of the actin family are structurally distinct, and their expression is tissue specific and developmentally regulated (7, 40, 42, 43). At least six different actins have been described in higher vertebrates. Striated muscle (skeletal and heart muscle) coexpresses α skeletal (α_{sk}) and α cardiac (α_c) actins (12, 14, 29, 33, 39). The principal actins expressed in vascular and visceral smooth muscles are α smooth and γ smooth, respectively (4, 6, 40, 43). Nonmuscle cells contain β and γ cytoplasmic actins (38, 41).

The expression of actin in striated muscle has been characterized in the developmental and adult stages of several species (2, 11, 12, 14, 19, 23, 30, 33, 39). In adult skeletal muscle, the levels of α_{sk} actin are much greater than the levels of α_c actin. However, the reverse is true in mature cardiac muscle and embryonic skeletal muscle, in which the levels of α_c-actin are much higher. The regulation of α_{sk} and α_c actin transcription has been studied in vitro by fusing a reporter gene to various α_{sk} and α_c actin promoter sequences and measuring the level of reporter gene activity after the fusions have been transiently or stably introduced into various cultured muscle and nonmuscle cells. This analysis has identified *cis*-acting transcriptional control elements within the 5′ flanking sequences immediately adjacent to the transcriptional start sites of the α_{sk} and α_c actin genes (3, 10, 15, 20–22, 25, 26, 28, 36). More recent studies suggest that these elements interact with *trans*-acting factors to regulate transcription in a tissue-specific manner (13, 24, 27, 44, 45).

Several studies indicate that primary muscle cell cultures and established myogenic cell lines, when induced to differentiate in vitro, do not acquire the fully differentiated state of adult skeletal muscle (2, 14, 15, 21, 36). Therefore, to determine whether the α_{sk} actin sequences that display *cis*-acting transcriptional regulatory activity in vitro can direct the expression of a heterologous gene in striated muscle in vivo, we have created mouse lines that carry hybrid α_{sk} actin-chloramphenicol acetyltransferase (CAT) transgenes. In the majority of the transgenic mouse strains, the patterns of transgene expression closely resemble that of the endogenous α_{sk} actin gene. High levels of CAT activity can be detected in the skeletal muscle and heart of transgenic mice but not in nonmuscle tissues. The α_{sk} actin-CAT transcripts initiate at the correct transcriptional start site in the chicken α_{sk} actin promoter. Our findings in vivo support previous in vitro studies that have localized *cis*-acting transcriptional control elements within the 5′ flanking region and suggest that the mechanism responsible for regulating tissue-specific actin gene expression is highly conserved among vertebrates.

MATERIALS AND METHODS

α_{sk} actin-CAT constructs. A *Sau*3AI fragment containing the bacterial CAT gene was cloned into the *Bam*HI site of the adaptor plasmid CLA12 in both orientations to generate two plasmids, CLA12CAT1 and CLA12CAT3, that differ only with respect to the orientation of the CAT insert within the polylinker array (Fig. 1A). A 218-base-pair (bp) fragment of the α_{sk} actin gene that extends from position −191 to position +27 (relative to the transcriptional start site) was inserted into the *Eco*RI site upstream of the CAT gene in CLA12CAT1 to generate plasmid CLA12-191αACTCAT1

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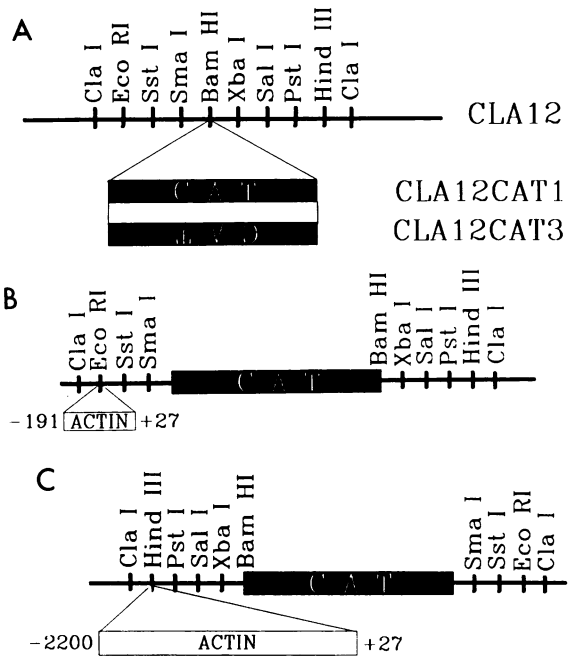

FIG. 1. Construction of α_{sk} actin-CAT genes. (A) A *Sau*3AI fragment containing the bacterial CAT gene was cloned into the *Bam*HI site of the adaptor plasmid CLA12 in both orientations to generate plasmids CLA12CAT1 and CLA12CAT3. (B) A 218-bp fragment of the chicken α_{sk} actin gene from positions −191 to +27 was inserted into the *Eco*RI site of CLA12CAT1 to generate plasmid CLA12-191αACTCAT1. (C) A 2.2-kbp fragment of the chicken α_{sk} actin gene from a *Hin*dIII site at approximately position −2200 to position +27 was inserted into the *Hin*dIII site of CLA12CAT3 to generate plasmid CLA12-2200αACTCAT3. For microinjection into mouse embryo pronuclei, α_{sk} actin-CAT genes were isolated as *Cla*I fragments.

(Fig. 1B). A 2.2-kbp fragment of the chicken α_{sk} actin gene that extends from a *Hin*dIII site approximately 2,200 bp upstream of the transcription start site to position +27 was inserted into the *Hin*dIII site upstream of the CAT gene in CLA12CAT3 to generate plasmid CLA-2200αACTCAT3 (Fig. 1C). The sequence of the chicken α_{sk} actin promoter region was determined by standard dideoxy-chain termination methods, using the Sequenase enzyme (U.S. Biochemical Corp.). Sequence information across the region where α_{sk} actin sequences were joined to CAT sequences was obtained by using two oligonucleotide primers that are complementary to sequences at the 5′ end of the CAT-coding sequence.

Transgenic mice. Hybrid genes containing either 2.2 kbp or 191 bp of the 5′ flanking sequence and 27 bp of 5′ untranslated sequence from the chicken α_{sk} actin gene linked to the CAT gene were recovered as *Cla*I fragments from plasmid CLA12-2200αACTCAT3 or CLA12-191αACTCAT1, respectively (Fig. 1B and C). α_{sk} actin-CAT DNA inserts were isolated and microinjected into fertilized mouse zygotes by previously published methods (31). Zygotes were generated from C57BL/6NCr matings (founders 710 and 2896) or by mating C57BL/6C3F_1 females to C57BL/6NCr males (founders 2854, 2875, 3071, 3072, 3074, and 3085). C57BL/6C3F_1 females were derived from crosses between C57BL/6NCr females and C3H/HeNCr males.

The transgenic founder mice and their transgenic progeny were identified by restriction endonuclease digestion and Southern transfer analysis of DNA isolated from mouse tails. A 20-μg sample of mouse tail DNA was digested overnight with the restriction endonuclease *Pvu*II or *Nco*I. The DNA fragments were separated by electrophoresis in 1.0% agarose gels and transferred to nitrocellulose filters in 10× SSC (SSC is 0.15 M NaCl plus 0.15 M sodium citrate) or to BioTrace nylon filters (Gelman Sciences, Inc.) in 0.4 N NaOH. Filters were hybridized overnight in 50% formamide at 42°C to a nick-translated *Acc*I-*Sst*I fragment of CLA12 CAT3 containing the entire CAT sequence (Fig. 1A). Filters were washed in 0.1× SSC–0.1% sodium dodecyl sulfate at 55°C for 2 h.

CAT assays. Mouse tissues were homogenized in 2.5 to 10.0 ml of cold 0.25 M Tris chloride (pH 7.8), using a Polytron tissue homogenizer (Brinkmann Instruments, Inc.). Tissue homogenates were cleared by an initial low-speed spin, and 1.0 ml of the resulting supernatants was spun for an additional 5 min in a microcentrifuge. Protein concentrations of each homogenate were determined by using the Bio-Rad protein assay system. For each set of assays, CAT activity was measured by using an equivalent amount of protein from each tissue homogenate according to standard methods (9).

RNase protection. Mouse spleen, heart, stomach, leg muscle, brain, lung, liver, and kidney were excised and immediately frozen in liquid nitrogen. Frozen tissues from three to five mice were pooled and homogenized in the presence of guanidinium thiocyanate, and total RNA was pelleted through CsCl as described previously (18). Riboprobes were prepared and RNase protection experiments were performed by the methods described by Gilman (8). A 516-bp *Cla*I-*Eco*RI fragment containing the α_{sk} actin sequences and the 5′ 250 bp of the CAT gene from plasmid CLA12-191α ACTCAT1 was subcloned into pBLUESCRIPT KS$^+$ (Stratagene, Inc.; see Fig. 5). This plasmid was linearized upstream of the α_{sk} actin sequences by digestion with the restriction endonuclease *Asp* 718 (an isoschizomer of *Kpn*I; Boehringer Mannheim Biochemicals), and uniformly labeled riboprobes were prepared by using T7 RNA polymerase in the presence of [α-^{32}P]UTP (specific activity, 10^9 cpm/μg). The riboprobe (5×10^5 cpm) was incubated overnight at 45°C with 20 μg of mouse tissue total RNA. After annealing, the reactions were treated with RNase T_1 (2 μg/ml) for 60 min at 30°C. The RNase T_1 was removed by extraction with phenol-chloroform and ethanol precipitation. The samples were then heat denatured, and the protected fragments were fractionated on a 6% polyacrylamide–7.6 M urea gel.

RESULTS

Transgenic mice. To determine whether the sequences that flank the transcriptional start site of the chicken α_{sk} actin gene can appropriately direct the expression of a heterologous reporter gene to skeletal muscle in vivo, mouse lines were created that carry hybrid α_{sk} actin-CAT transgenes. Either 191 bp or 2.2 kbp of 5′ flanking sequence plus 27 bp of the 5′ untranslated region of the chicken α_{sk} actin sequence was joined to the CAT gene to generate the hybrid α_{sk} actin promoter-CAT gene plasmids CLA12-191αACTCAT1 and CLA12-2200αACTCAT3 (Fig. 1). To generate transgenic mice, the α_{sk} actin-CAT genes were recovered as *Cla*I fragments and microinjected into fertilized mouse zygotes.

*Pvu*II digestion and Southern transfer analysis on mouse tail DNA using a CAT sequence hybridization probe were used to identify eight founder mice (data not shown) that were subsequently bred to establish transgenic mouse lines. Each transgenic line bears the number of the original founder mouse (2854, 2875, 2896, 3071, 3072, 3074, or 3085). Lines

2854, 2875, 3071, 3072, and 3074 carry the -191αACTCAT1 gene, and lines 2896 and 3085 carry the -2200αACTCAT3 gene. An additional founder mouse (710) also carried the -191αACTCAT1 gene but failed to reproduce, and we were unable to produce a 710 line. Eventually mouse 710 was sacrificed, and its tissues were used for CAT analysis. Based on the size of the major bands generated by *Pvu*II or *Nco*I digestion and Southern transfer analysis of mouse tail DNA, all of the transgenic lines appeared to have multiple, tandemly integrated copies of the α_{sk} actin-CAT transgene (Fig. 2). Although the actual number of copies varied widely between transgenic lines, in every case, as expected, the overall organization was head-to-tail arrays. Careful inspection of the junction fragments generated by *Pvu*II digestion and Southern transfer analysis of tail DNA from N_1 generation mice indicated to us that three founder mice (2854, 2875, and 3085) may have acquired copies of the transgene at two independent positions in the genome (Fig. 2). This was confirmed by examining the junction fragments generated by *Nco*I digestion (Fig. 2). In all three cases, when the founder mice were bred to establish transgenic lines, the transgenes had segregated independently to generate three additional mouse lines. Transgenic lines generated from the same founder mouse are distinguished by the letter A or B following the original founder number (i.e., 2854A, 2854B, 2875A, 2875B, 3085A, and 3085B).

CAT activity in transgenic mouse tissues. To examine whether the α_{sk} actin-CAT transgenes were expressed, CAT assays were performed on tissue homogenates prepared from the spleen, heart, stomach, leg muscle, brain, lung, liver, kidneys, and occasionally testes of mice from each transgenic line (Fig. 3). CAT activity was detected in tissue homogenates from all 10 transgenic lines (2854A, 2854B, 2875A, 2875B, 2896, 3071, 3072, 3074, 3085A, and 3085B) and in tissue homogenates of founder 710. In all but one line (3085B), CAT activity was detected almost exclusively in the striated muscle (skeletal and heart muscle) homogenates. In lines 2854A, 2854B, 3071, 3072, 3074, and 3085A and in the founder mouse 710, the principle site of CAT expression was skeletal muscle. In these animals, significantly lower levels of activity were detected in cardiac muscle, and little or no activity could be found in other tissues, including stomach, which contains visceral smooth muscle. In line 2875B, the transgene was expressed at relatively low levels in both skeletal and cardiac muscle but could not be detected in other tissues. In two lines (2875A and 2896), CAT activity was present at significantly higher levels in heart than in skeletal muscle but, again, little or no activity could be detected in the other tissues tested. In line 3085B, the level of α_{sk} actin-CAT transgene expression was unexpectedly higher in brain than in skeletal muscle. However, it should be noted that the levels of CAT activity in 3085B brain homogenates were lower than the levels of expression in the striated muscle in several other lines.

The levels of α_{sk} actin-CAT transgene expression varied over a relatively wide range among mouse lines (Fig. 3). However, because there were significant differences in the amounts of protein assayed, the incubation times of the assays, and the film exposure times shown in Fig. 3, direct comparison of these data is not possible. To compare directly the relative levels of transgene expression among all of the transgenic lines, equivalent amounts of protein from each skeletal muscle and cardiac muscle homogenate were assayed for CAT activity (Fig. 4). These data confirmed that the levels of α_{sk} actin-CAT transgene expression varied substantially from one line to the next. From these data and

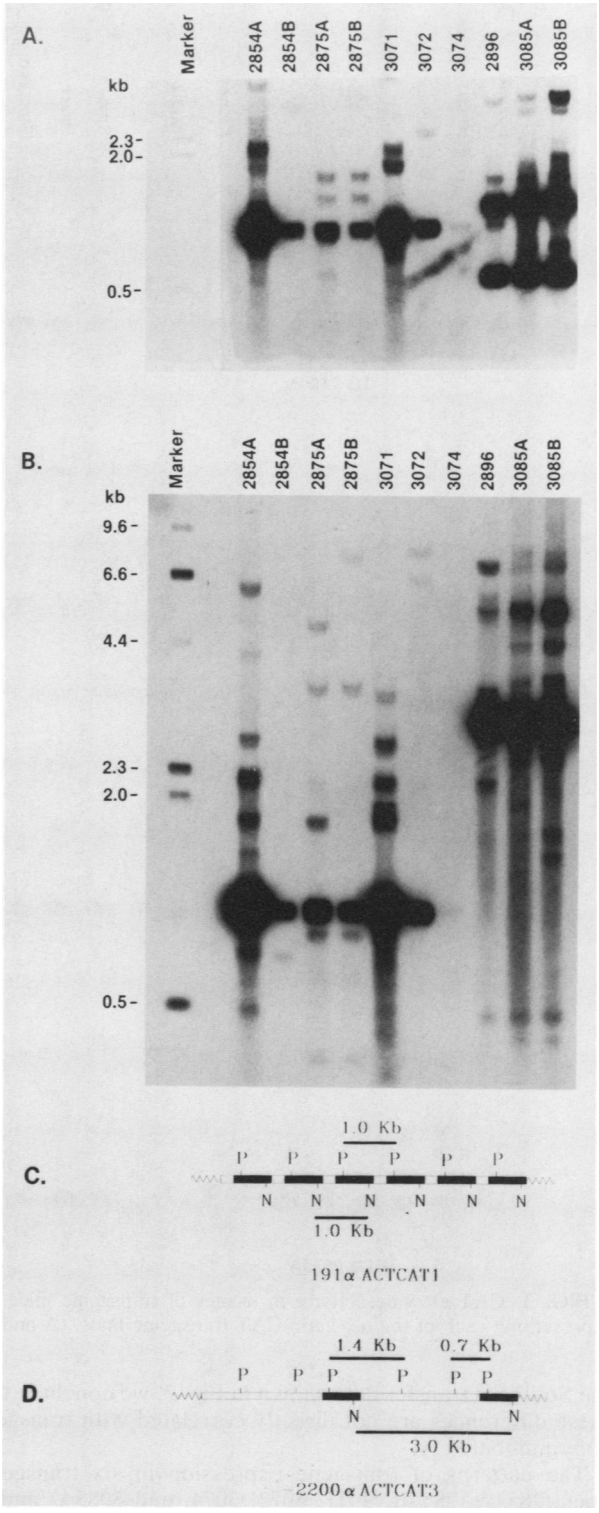

FIG. 2. Southern transfer analysis of transgenic mouse tail DNA after *Pvu*II (A) or *Nco*I (B) digestion. (C and D) Predicted size fragments generated from *Nco*I (N) and *Pvu*II (P) digestion of -191αACTCAT1 (C) and -2200αACTCAT3 (D) genes that are organized in tandem, head-to-tail arrays. Symbols: ■, CAT sequences; □, chicken α_{sk} actin sequences. Tail DNAs were from mice representing each of the α_{sk} actin-CAT transgenic lines. Films were deliberately overexposed to show hybridization of the CAT sequence probe to 3074 DNA.

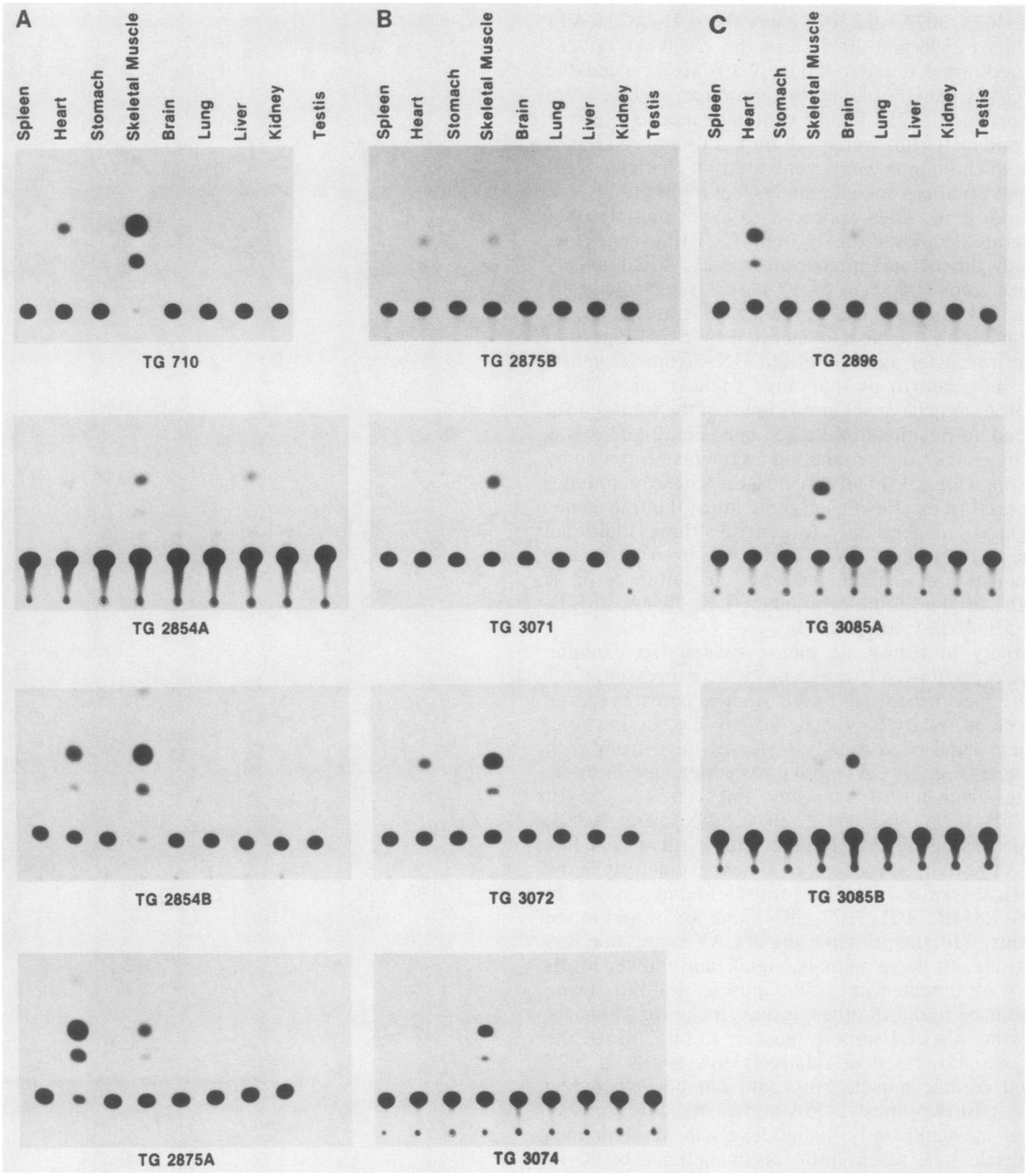

FIG. 3. CAT enzyme activity in tissues of transgenic mice. CAT assays were performed on tissue homogenates prepared from mice representing each of the α_{sk} actin-CAT transgenic lines. (A and B) -191αACTCAT1 lines; (C) -2200αACTCAT3 lines.

the Southern transfer data shown in Fig. 2, we conclude that these differences are not directly correlated with transgene copy number.

The patterns of transgene expression in six transgenic lines (2854A, 2854B, 3071, 3072, 3074, and 3085A) and in founder mouse 710 are qualitatively similar and much like that of the endogenous α_{sk} actin gene (23). The expression is restricted to striated muscle, and significantly higher levels of expression are found in skeletal muscle than in cardiac muscle. To estimate the difference in the levels of expression in skeletal muscle and heart, CAT assays were performed on serially diluted samples of skeletal muscle and cardiac muscle homogenates prepared from the founder mouse 710 (data not shown). A 1:16 dilution of the skeletal muscle sample contained approximately the same CAT activity as did the undiluted sample from cardiac muscle. Thus, the levels of transgene expression in cardiac muscle were approximately 6% of the levels found in skeletal muscle.

Transcription of α_{sk} actin-CAT transgenes. We performed RNase protection assays to measure the steady-state levels of α_{sk} actin-CAT RNA in various tissues and to identify the transcriptional start site(s) of the α_{sk} actin-CAT transgenes. Uniformly labeled riboprobes were transcribed with T7 RNA polymerase from α_{sk} actin-CAT sequences that had been subcloned into the pBLUESCRIPT KS$^+$ vector. RNAs prepared from the spleen, heart, stomach, leg muscle, brain, lung, liver, and kidney of line 2854B and from the heart, leg muscle, brain, liver, and kidney of lines 3072 and 2875A

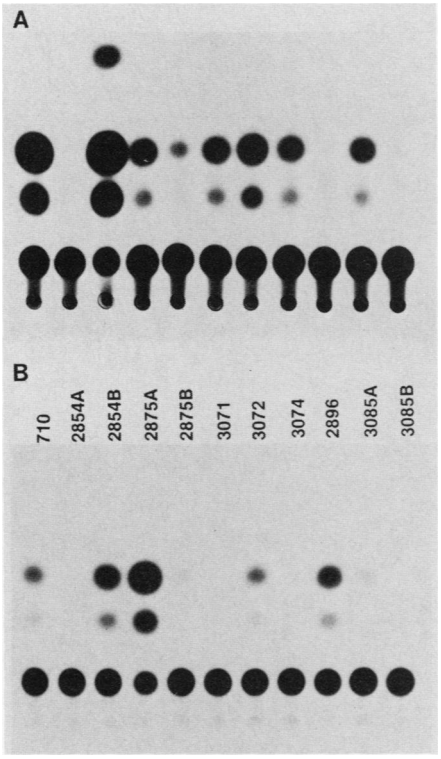

FIG. 4. Relative levels of α_{sk} actin-CAT transgene expression. CAT assays were performed by using equivalent amounts of protein from the skeletal muscle (A) and cardiac muscle (B) homogenates prepared from a mouse of each transgenic line.

were hybridized to riboprobes and then digested with RNase T_1.

A 611-bp probe that contains chicken α_{sk} actin sequences from positions −191 to +27 linked to the first 250 bp of CAT sequence up to the *Eco*RI site was used to protect the 5′ end of α_{sk} actin-CAT transcripts in order to map the transcriptional start site(s) of the transgenes (Fig. 5). A 305-base fragment was well protected in all of the RNAs prepared from skeletal muscle and heart but not in tissues in which CAT activity was not detected. This fragment is the expected length for α_{sk} actin-CAT transcripts that initiate at the authentic transcriptional initiation site of the chicken α_{sk} actin gene. The relative amounts of this fragment protected by various striated muscle RNA samples correlated well with the levels of functional CAT protein detected. These data imply that *trans*-acting striated muscle-specific transcription factors in the mouse correctly interact with the chicken α_{sk} actin sequences to initiate transcription at the proper start site.

In addition to the 305-base fragment protected by striated muscle RNAs that represented properly initiated transcripts, RNAs isolated from skeletal muscle, heart, and some but not all brain samples also protected a fragment of 516 bases, which is the predicted length of the fully protected α_{sk} actin-CAT sequence (Fig. 5). Because no polyadenylation signals were introduced into the α_{sk} actin-CAT constructs, we believe that in striated muscle this larger fragment is protected by readthrough transcripts that extend through adjoining tandem copies of the α_{sk} actin-CAT transgene. The protection of this 516-base fragment by some brain RNA samples was unexpected, since brain does not produce significant levels of CAT protein. The presence of CAT sequences in the brain RNAs of lines 2854B and 3072 has been confirmed by Northern (RNA) transfer analysis.

DISCUSSION

Several laboratories have studied the tissue specificity of striated muscle actins by measuring the expression of a reporter gene that has been fused to various actin sequences and introduced into primary muscle cell cultures or established myogenic cell lines. Together, these in vitro studies have localized tissue-specific transcriptional regulatory elements within the 5′ flanking sequences of the α_{sk} and α_c actin genes (3, 10, 15, 20, 22, 23, 25, 26, 28, 36). Except for a study by Shani (37), analogous in vivo studies in transgenic mice have not been reported. That report described the expression of a chimeric gene consisting of two-thirds of the rat α_{sk} actin gene plus 730 bp of its 5′ flanking sequences fused to the 3′ end of the human embryonic globin gene. Although the rat α_{sk} actin-globin transgene was appropriately expressed in the striated muscle of transgenic mice, the possible presence of regulatory elements in exon and intron sequences cannot be eliminated.

Our studies using mice that carry hybrid α_{sk} actin-CAT transgenes indicate that the 5′ flanking sequences upstream of the transcription start site and the first 27 bp of the 5′ untranslated region of the chicken α_{sk} actin gene can specifically direct the expression of a heterologous, reporter protein (CAT) to striated muscle. We have shown that the expression of CAT transgenes linked to either 2.2 kb or 191 bp of 5′ flanking sequence is restricted to striated muscle. In general, CAT activity was not detected in the other tissues tested. In a majority of the transgenic mice tested, the pattern of transgene expression is very similar to that of the endogenous murine α_{sk} actin gene. The principal site of transgene expression is skeletal muscle; expression in cardiac muscle is approximately 20-fold lower. We find that the expression of α_{sk} actin-CAT transgenes that contain 191 bp of 5′ flanking sequence (710, 2854, 2875, 3071, 3072, and 3074) does not differ significantly from the expression of transgenes that contain 2.2 kb of 5′ flanking sequence (2896 and 3085). These findings define the location of tissue-specific, *cis*-acting transcriptional regulatory elements within a 218-bp region that spans from positions −191 to +27 immediately surrounding the transcription start site of the gene. This same region may also regulate lower levels of expression of α_{sk} actin in cardiac muscle. The data suggest that it may be possible to direct or restrict the expression of any protein of interest to the skeletal muscle of transgenic animals by simply fusing its coding sequence to the α_{sk} actin promoter.

The sequences flanking the transcription initiation site of the chicken α_{sk} actin gene have been well studied. The nucleotide sequence of the 218-bp region that is contained in the -191αACTCAT1 transgene is highly conserved in the corresponding rat and mouse α_{sk} actin genes (16, 46). We have sequenced this region in the chicken gene and note several discrepancies between our sequence and that of a previous report (16). Our sequence data are identical to those of Bergsma et al. (3) except at nucleotide position −119. This region of the gene contains a perfectly matched copy of the CArG box consensus sequence $CC(A/T)_6GG$ at positions −82 to −91. Two single-base-pair mismatched copies of this element are also present at positions −127 to −136 and positions −172 to −181. Gel retardation studies have shown that CArG boxes in the chicken α_{sk} and human α_{sk} and α_c actin genes comprise the binding sites for *trans*-

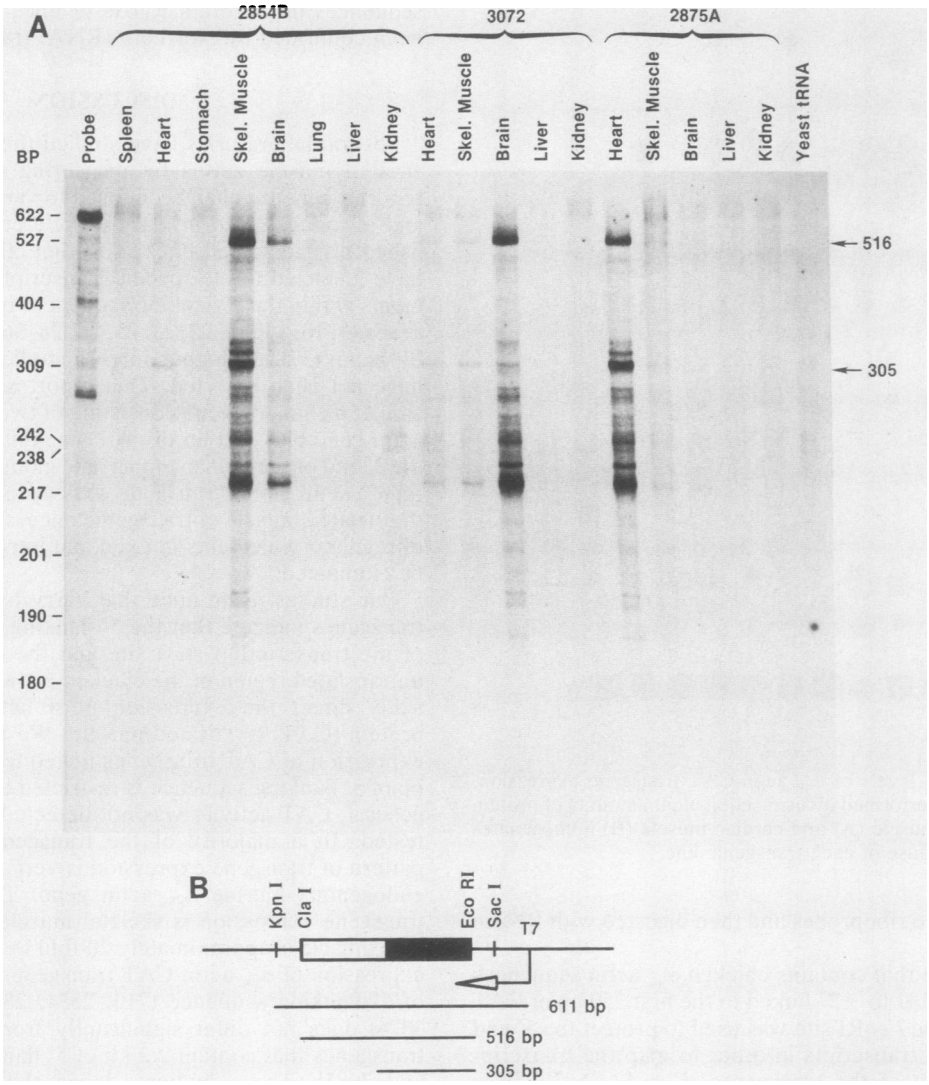

FIG. 5. RNase protection assays of transgenic mouse tissue RNAs. (A) Autoradiogram of a polyacrylamide gel used to separate the riboprobe fragments that were protected in a RNase protection experiment using 20 μg of total RNA. In the diagram (B), the riboprobe was labeled with [α-^{32}P]UTP and contained the complement of the α_{sk} actin promoter sequence (positions −191 to +27 [□]) joined to the first 250 bp of CAT sequence (■). The arrow designates the direction of riboprobe transcription from the T7 RNA polymerase promoter within the pBLUESCRIPT KS$^+$ vector. The expected sizes of the full-length riboprobe (611 bases) and the riboprobe fragments protected by transgene transcripts initiated at the chicken α_{sk} actin transcription start site (305 bases) or by readthrough transcripts extending into tandem, downstream copies of α_{sk} actin-CAT sequences (516 bases) are shown. A 220-base fragment that may have been generated by RNase digestion at an A+T-rich stretch within the CAT sequence is also evident.

acting regulators of transcription (13, 24, 27, 44, 45). Recent data from several laboratories suggest that the expression of α_{sk} actin genes is dependent on the formation of a complex involving muscle-specific binding proteins and this binding site (27, 45).

By analyzing the RNA isolated from the tissues of several mouse lines that express the -191αACTCAT1 transgene, we have shown that CAT transcripts accumulate in striated muscle but not in other tissues in which CAT activity is not detected. An exception is brain, which contains significant levels of CAT RNA but does not produce detectable levels of functional CAT protein. In general, we have found that the levels of CAT RNA in striated muscle samples correlate well with the levels of CAT activity detected. These findings are consistent with a tissue-specific transcriptional activation model.

RNase protection experiments have been used to characterize the 5′ ends of the α_{sk} actin-CAT transcripts generated in several transgenic lines. These results indicate that the site of transcription initiation of the transgene in striated muscle corresponds to the transcription initiation site of the endogenous chicken α_{sk} actin gene. This observation implies that *trans*-acting factors in the muscle cells of the mouse can interact appropriately with the *cis*-acting regulatory sequences of the chicken α_{sk} actin gene to correctly initiate transcription.

Although expression of CAT was, in almost every case, appropriately directed to striated muscle by the α_{sk} actin promoter, the levels of expression (measured both at the RNA and protein levels) varied significantly among the transgenic lines. We have been able to show that higher levels of α_{sk} actin-CAT transgene expression are not the

direct result of increased copy number. However, we cannot rule out the possibility that the levels of expression do not reflect the number of transcriptionally active transgene copies in the genome. Another possible explanation for this quantitative variation is that sequences at or near the site of transgene integration influence the levels of transgene expression or that a *cis*-acting transcriptional regulatory element is absent from our constructs.

It is not clear why two transgenic lines produce significantly higher levels of CAT protein and CAT RNA in heart than in skeletal muscle. We have observed this pattern in mice that carry the -2200αACTCAT3 transgene (line 2896) as well as the -191αACTCAT1 transgene (line 2875A). Northern transfer analyses of RNAs isolated from the tissues of 2875A mice demonstrate that the endogenous α_{sk} actin gene is appropriately expressed. In contrast to the transgene, the endogenous gene is expressed at high levels in skeletal muscle but at significantly lower levels in heart (data not shown).

A previous study reported the presence of α_{sk} actin RNA in chicken embryonic brain (30); however, a more recent study has not confirmed this initial observation (33). Although CAT RNA was detected in brain in some of our transgenic lines, transcripts starting at the site of α_{sk} actin transcription initiation in muscle were not present or were present at low levels and CAT activity could not be detected. We suggest that these RNAs are produced as a consequence of inappropriate transcription of the transgene from a cryptic transcriptional regulatory element somewhere within or upstream of the α_{sk} actin-CAT sequence.

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LITERATURE CITED

1. **Amos, L. A.** 1985. Structure of muscle filaments studied by electron microscopy. Annu. Rev. Biophys. Chem. **14**:291–313.
2. **Bains, W., P. Ponte, H. Blau, and L. Kedes.** 1984. Cardiac actin is the major actin gene product in skeletal muscle cell differentiation in vitro. Mol. Cell. Biol. **4**:1449–1453.
3. **Bergsma, D. J., J. M. Grichnik, L. M. A. Gossett, and R. J. Schwartz.** 1986. Delimitation and characterization of *cis*-acting DNA sequences required for the regulated expression and transcriptional control of the chicken skeletal α-actin gene. Mol. Cell. Biol. **6**:2462–2475.
4. **Carroll, S. L., D. J. Bergsma, and R. J. Schwartz.** 1986. Structure and complete nucleotide sequence of the chicken α-smooth muscle (aortic) actin gene. J. Biol. Chem. **261**:8965–8976.
5. **Cory, S., and J. M. Adams.** 1988. Transgenic mice and oncogenesis. Annu. Rev. Immunol. **6**:25–48.
6. **Gabbiani, G., E. Schmid, S. Winter, C. Chaponnier, C. de Chastoncey, J. Vandekerckhove, K. Weber, and W. N. Franke.** 1981. Vascular smooth muscle cells differ from other smooth muscle cells: predominance of vimentin filaments and a specific α-type actin. Proc. Natl. Acad. Sci. **78**:298–302.
7. **Garrels, J. I., and W. Gibson.** 1976. Identification and characterization of multiple forms of actin. Cell **9**:793–805.
8. **Gilman, M.** 1987. Ribonuclease protection assay. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman, and K. Struhl (ed.), Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
9. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
10. **Grichnik, J. M., D. J. Bergsma, and R. J. Schwartz.** 1986. Tissue restricted and stage-specific transcription is maintained within 411 nucleotides flanking the 5′ end of the chicken α-skeletal actin gene. Nucleic Acids Res. **14**:1683–1701.
11. **Gunning, P., E. Hardeman, R. Wade, P. Ponte, W. Bains, H. M. Blau, and L. Kedes.** 1987. Differential patterns of transcript accumulation during human myogenesis. Mol. Cell. Biol. **7**: 4100–4114.
12. **Gunning, P., P. Ponte, H. Blau, and L. Kedes.** 1983. α-Skeletal and α-cardiac actin genes are coexpressed in adult human skeletal muscle and heart. Mol. Cell. Biol. **3**:1985–1995.
13. **Gustafson, T. A., T. Miwa, L. M. Boxer, and L. Kedes.** 1988. Interaction of nuclear proteins with muscle-specific regulatory sequences of the human cardiac α-actin promoter. Mol. Cell. Biol. **8**:4110–4119.
14. **Hayward, L. J., and R. J. Schwartz.** 1986. Sequential expression of chicken actin genes during myogenesis. J. Cell. Biol. **102**: 1485–1493.
15. **Hickey, R., A. Skoultchi, P. Gunning, and L. Kedes.** 1986. Regulation of a human cardiac actin gene introduced into rat L6 myoblasts suggests a defect in their myogenic program. Mol. Cell. Biol. **6**:3287–3290.
16. **Hu, M. C.-T., S. B. Sharp, and N. Davidson.** 1986. The complete sequence of the mouse skeletal α-actin gene reveals several conserved and inverted repeat sequences outside of the protein-coding region. Mol. Cell. Biol. **6**:15–25.
17. **Jaenisch, R.** 1988. Transgenic animals. Science **240**:1468–1474.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Mayer, Y., H. Czosnek, P. Zeelon, D. Yaffe, and U. Nudel.** 1984. Expression of the genes coding for the skeletal and cardiac actin in the heart. Nucleic Acids Res. **12**:1087–1100.
20. **Melloul, D., B. Aloni, J. Calvo, D. Yaffe, and U. Nudel.** 1984. Developmentally regulated expression of chimeric genes containing muscle actin DNA sequences in transfected myogenic cells. EMBO J. **3**:983–990.
21. **Minty, A., H. Blau, and L. Kedes.** 1986. Two-level regulation of cardiac actin gene transcription: muscle-specific modulating factors can accumulate before gene activation. Mol. Cell. Biol. **6**:2137–2148.
22. **Minty, A., and L. Kedes.** 1986. Upstream regions of the human cardiac actin gene that modulate its transcription in muscle cells: presence of an evolutionary conserved repeated motif. Mol. Cell. Biol. **6**:2125–2136.
23. **Minty, A. J., S. Alonso, M. Caravatti, and M. E. Buckingham.** 1982. A fetal skeletal muscle actin mRNA in the mouse and its identity with cardiac actin mRNA. Cell **30**:185–192.
24. **Miwa, T., L. Boxer, and L. Kedes.** 1987. CArG boxes in the human cardiac α-actin gene are core binding sites for positive trans-acting regulatory factors. Proc. Natl. Acad. Sci. USA **84**:6702–6706.
25. **Miwa, T., and L. Kedes.** 1987. Duplicated CArG box domains have positive and mutually dependent regulatory roles in expression of the human α-cardiac actin gene. Mol. Cell. Biol. **7**:2803–2813.
26. **Mohun, T., N. Garret, and J. B. Gurdon.** 1987. Upstream sequences required for tissue specific activation of the cardiac actin gene in *Xenopus laevis* embryos. EMBO J. **5**:3185–3193.
27. **Muscat, E. O., T. A. Gustafson, and L. Kedes.** 1988. A common factor regulates skeletal and cardiac α-actin gene transcription in muscle. Mol. Cell. Biol. **8**:4120–4133.
28. **Muscat, E. O., and L. Kedes.** 1987. Multiple 5′-flanking regions of the human α-skeletal actin gene synergistically modulate muscle-specific expression. Mol. Cell. Biol. **7**:4089–4099.
29. **Ordahl, C. P.** 1986. The skeletal and cardiac α-actin genes are coexpressed in early embryonic striated muscle. Dev. Biol. **117**:488–492.
30. **Ordahl, C. P., S. M. Tilghman, C. Ovitt, J. Fornwald, and M. T.**

Largen. 1980. Structure and developmental expression of the chick α-actin gene. Nucleic Acids Res. **8**:4989–5005.
31. **Osborn, L., M. P. Rosenberg, S. A. Keller, and M. H. Meisler.** 1987. Tissue-specific and insulin-dependent expression of a pancreatic amylase gene in transgenic mice. Mol. Cell. Biol. **7**:326–334, 1987.
32. **Palmiter, R. D., and R. L. Brinster.** 1986. Germ-line transformation of mice. Annu. Rev. Genet. **20**:465–499.
33. **Paterson, B. M., and J. D. Eldridge.** 1984. α-Cardiac actin is the major sarcomeric isoform expressed in embryonic avian skeletal muscle. Science **224**:1436–1438.
34. **Pollard, T. D., and J. A. Cooper.** 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. Ann. Rev. Biochem. **55**:987–1035.
35. **Pollard, T. D., and R. R. Weihing.** 1974. Actin and myosin and cell movement. Crit. Rev. Biochem. **2**:1–65.
36. **Seiler-Tuyns, A., J. D. Eldridge, and B. M. Paterson.** 1984. Expression and regulation of chicken actin genes introduced into mouse myogenic and nonmyogenic cells. Proc. Natl. Acad. Sci. USA **81**:2980–2984.
37. **Shani, M.** 1986. Tissue-specific and developmentally regulated expression of a chimeric actin-globin gene in transgenic mice. Mol. Cell. Biol. **6**:2624–2631.
38. **Tokunaga, K., K. Takeda, K. Kamiyama, H. Kageyama, K. Takenaga, and S. Sakiyama.** 1988. Isolation of cDNA clones for mouse cytoskeletal γ-actin and differential expression of cytoskeletal actin mRNAs in mouse cells. Mol. Cell. Biol. **8**: 3929–3933.
39. **Vandekerckhove, J., G. Bugaisky, and M. Buckingham.** 1986. Simultaneous expression of skeletal muscle and heart actin proteins in various striated muscle tissues and cells. J. Biol. Chem. **261**:1838–1843.
40. **Vandekerckhove, J., and K. Weber.** 1978. Mammalian cytoplasmic actins are the products of at least two genes and differ in primary structure in at least 25 identified positions from skeletal muscle actins. Proc. Natl. Acad. Sci. USA **75**:1106–1110.
41. **Vandekerckhove, J., and K. Weber.** 1978. At least six different actins are expressed in a higher mammal: an analysis based on the amino acid sequence of the amino-terminal tryptic peptide. J. Mol. Biol. **126**:783–802.
42. **Vandekerckhove, J., and K. Weber.** 1981. Acting typing of total cellular extracts: a highly sensitive protein-chemical procedure able to distinguish different actins. Eur. J. Biochem. **113**: 595–603.
43. **Vandekerckhove, J., and K. Weber.** 1984. Chordate muscle actins differ distinctly from invertebrate muscle actins: the evolution of the different vertebrate muscle actins. J. Mol. Biol. **179**:391–413.
44. **Walsh, K., and P. Schimmel.** 1987. Two nuclear factors compete for the skeletal muscle actin promoter. J. Biol. Chem. **262**: 9429–9432.
45. **Walsh, K., and P. Schimmel.** 1988. DNA-binding site for two skeletal actin promoter factors is important for expression in muscle cells. Mol. Cell. Biol. **8**:1800–1802.
46. **Zakut, R., M. Shani, D. Givol, S. Neuman, D. Yaffe, and U. Nudel.** 1982. Nucleotide sequence of the rat skeletal muscle actin gene. Nature (London) **298**:857–859.