Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1389–1393, March 1988
Biochemistry

A 40-base-pair sequence in the 3′ end of the β-actin gene regulates β-actin mRNA transcription during myogenesis

(actins/gene regulation/transcriptional control)

LILIANA DEPONTI-ZILLI, ANNE SEILER-TUYNS*, AND BRUCE M. PATERSON

Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Communicated by Maxine F. Singer, October 26, 1987 (received for review August 21, 1987)

ABSTRACT **In an earlier report, evidence was presented that the down-regulation of β-actin mRNA during myogenesis was controlled by a region 3′ to the promoter of the gene. In this paper we report the location of this regulatory sequence, determined by deletion analysis and the use of chimeric genes, transfected stably into the mouse myogenic cell line C2C12. The domain responsible for the reduction in β-actin mRNA levels is at most 40 base pairs long and is located just 5′ to the canonical polyadenylylation signal in the gene. Placement of this sequence in the corresponding 3′ position both in the α-cardiac-actin gene and in the neomycin-resistance gene in pSV2-neo confers the β-actin mRNA regulatory pattern when these constructs are stably introduced into C2C12 cells. Nuclear run-on experiments indicate that transcriptional control can account for the decrease observed in β-actin mRNA levels during myogenesis for both the endogenous as well as the transfected β-actin gene constructs. This 3′ transcriptional control sequence is conserved in all of the vertebrate β-actin genes sequenced and is not similar to any of the 3′ processing–adenylylation or termination sequences described previously. This mode of gene regulation may reflect a more general mechanism involved in the process of gene suppression during development.**

During the process of myogenesis (or muscle formation), the dividing myoblasts withdraw from the cell cycle in the G_0 state and fuse to form a multinucleated syncitia (or myotube), which then begins to elaborate all the contractile proteins, enzymes, and surface markers characteristic of skeletal muscle (1). We have shown (2) that there is a reduction by a factor of 5–6 in the total number of genes expressed as muscle formation takes place. However, little is known about the mechanisms that suppress gene expression in a particular cell or tissue. An earlier paper from this laboratory described the regulation of the chicken β-actin gene transfected into the mouse C2C12 myogenic cell line (3). As the cells fused, expression from both the endogenous mouse and transfected chicken β-actin genes decreased in parallel. In our preliminary attempts to distinguish between the transcriptional or posttranscriptional control of this decrease in the steady-state levels of β-actin mRNA, we constructed a chimeric gene containing the chicken β-actin gene promoter and the human α-globin gene body. When this construct was transfected into C2C12 cells α-globin mRNA was produced constitutively at all stages of myogenesis (4). From these observations we concluded that the steady-state level of β-actin mRNA was not controlled through the promoter *per se* but was regulated by posttranscriptional processes instead. To confirm this interpretation, we performed nuclear run-on studies with C2C12 cells transfected with the complete chicken β-actin gene (unpublished observations). Unexpectedly, the decrease in transcription observed from the exogenous gene could account entirely for the decrease in the steady-state level of β-actin mRNA. No decrease in transcription was observed from the colinear selectable marker gene *gpt* (data not presented here). These collective results led us to conclude that the decrease in β-actin mRNA associated with myogenesis was transcriptionally controlled by a region 3′ to the promoter of the gene.

We now describe the location and some of the properties of this transcriptional control region in the β-actin gene. Deletion analysis and the use of various chimeric gene constructs places this transcriptional control sequence within the 40 base pairs (bp) 5′ to the canonical polyadenylylation signal in the gene, a region corresponding to the 3′ untranslated region (UTR) of the β-actin mRNA. This sequence can impart the β-actin transcriptional pattern to completely heterologous genes as demonstrated here with the chicken α-cardiac-actin gene and the neomycin-resistance gene *neo* from the pSV2-neo vector. The core sequence and its location are preserved in all of the vertebrate β-actin genes sequenced to date.

MATERIALS AND METHODS

Cell Culture and Transfection. The C2C12 myogenic cell line (5) was transfected and grown as described (3); however, all constructs were derivatives of pSV2-neo (6) and were selected with 250–400 μg of G418 per ml. Fused cultures were treated with cytosine arabinoside (araC, 100 μg/ml) in midfusion to minimize the number of undifferentiated cells from the myotube cultures (5). C2C12 cells are available from the American Type Culture Collection (no. CRL 1772).

RNA Isolation and Nuclease S1 Analysis. Total RNA was isolated from cultured cells by using the guanidine thiocyanate procedure described earlier and modified by Okayama *et al.* (7). Nuclease S1 analysis was carried out with end-labeled double-stranded DNA fragments as described (8). Nuclease S1 probes were prepared from the following fragments: *neo* probes [pSV2-neo (6) 5′-end-labeled at the *Bgl* II site and then cut with *Nde* I, giving a fragment of 890 nucleotides (nt) that protects 390 nt; pRSV-neo (6) 5′-end-labeled at the *Bgl* II site and then cut with *Eco*RI, giving a fragment of 411 nt that protects 322 nt], chicken β-actin probe [cDNA (9) recloned in pUC8, 5′-end-labeled at the *Nco* I site, and then cut with *Pvu* II, giving a fragment of 315 nt that protects 90 nt], and mouse β-actin probe [cDNA (10) recloned in pUC18, 5′-end-labeled at the *Bgl* II site, and then cut with *Nde* I, giving a fragment of 430 nt that protects 210 nt].

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: nt, nucleotide(s); UTR, untranslated region; SV40, simian virus 40.

*Present address: Institute de Biologie Animale, Batiment de Biologie, Universitae de Lausanne, CH-1015 Lausanne, Switzerland.

Construction of β-Actin Gene Deletions and Chimeric Genes. The actin genes utilized in this study have been described (3). The vector shown in Fig. 1*B*, pSV2-neo-FX, was constructed as a recipient for the various 3′ β-actin constructs presented here. The unique *Bam*HI site in pSV2-neo was converted to a *Sal* I site with linkers. The *Xho* I–*Eco*RI fragment containing the entire β-actin gene was then cloned into *Sal* I/*Eco*RI-cut pSV2-neo-FX. The unique *Fsp* I site in this β-actin gene fragment had been converted to a *Sal* I site with linkers to subclone the complete 3′ end of the β-actin gene [*Sal* I(*Fsp* I)–*Eco*RI fragment] in pUC18 or pUC19 for *Exo* III/*Exo* VII (11) or BAL-31 (8) deletion analysis. Appropriate deletions were sequenced by the dideoxy chain-termination method (12) and used to replace the 3′ deletion (3′Δ) shown in Fig. 1*B*. The double-stranded oligonucleotide used to construct "oligo" (Fig. 1*A*, line 4) was based on the sequence of chicken cytoplasmic β-actin (see Fig. 5, sequence 1) and contained 5′ *Sal* I and 3′ *Bgl* II sticky ends. pSV2-neo-3′β was prepared by replacing the *Sma* I–*Eco*RI fragment of pSV2-neo (6) with the *Sal* I (flush)–*Eco*RI fragment of the β-actin Δ12 deletion construct (Fig. 1*A*, line 2). The remaining constructs are either explained in the text or the legend to Fig. 1.

Nuclear Transcription Studies. Nuclei isolated from unfused and fused C2C12 cultures transformed with the various β-actin gene constructs were incubated *in vitro* to produce run-on RNA transcripts (13). Newly synthesized RNA was hybridized to target DNA transferred to Zeta Probe (Bio-Rad) membranes as published (14). Target DNA fragments are described in the legend to Fig. 4.

RESULTS

The Steady-State Level of β-Actin mRNA Is Regulated by a Sequence in the 3′-End of the Gene. Although our earlier studies indicated that the β-actin promoter was active in myofibrils when joined to a heterologous gene (4), we had not shown that regions of the β-actin gene 3′ to the promoter would impart the β-actin regulatory pattern to an unrelated promoter. We have shown previously that the chicken β-actin gene behaves like the endogenous gene when stably transfected into C2C12 cells. In contrast, the gene for chicken α-cardiac-actin is expressed equally in myoblasts and myofibrils, even though the endogenous gene is expressed only in the differentiated cells (3). The chicken α-cardiac-actin and β-actin gene are diagrammed in Fig. 1*A*. Each gene has a *Bgl* II site in exon III (amino acid 85) and either a *Hpa* I or a *Stu* I site in the last intron. These sites were used to construct chimeric α/β-actin genes through exchanges at either the *Bgl* II or the *Hpa* I/*Stu* I cuts in order to locate the β-actin mRNA regulatory region more precisely. These chimeric genes were transfected stably into C2C12 cells, and the mRNA transcripts were analyzed by nuclease S1 digestion (data not presented here). In every case the 3′ fragments from the β-actin gene imparted the normal β regulatory pattern to mRNA transcripts produced from the α-actin gene promoter (Fig. 1*A*). It should be noted that the α/β chimera shown in Fig. 1*A* [diagram designated α–β (Exon VI)] has essentially none of the β-actin gene intronic regions; thus intronic sequences do not appear to play a substantial role in β-actin mRNA regulation as proposed (15). When 5′ β-actin gene regions with the β-actin promoter were joined to the appropriate α-actin gene segments, constitutive α-actin mRNA expression was observed during myogenesis (data not presented here). These results were taken to indicate that sequences contained in or flanking exon VI of the β-actin gene modulate β-actin mRNA levels during myogenesis.

No More Than 40 bp in the 3′ UTR Region of the Gene Regulate β-Actin mRNA Levels. The unique *Fsp* I site in exon VI of the β-actin gene (Fig. 1*A*) was used as a starting point to generate 5′ deletions toward the polyadenylylation signal. A comparison of the 3′ UTRs for the mouse, rat, and chicken β-actin genes indicated substantial sequence homology throughout this region for all of the genes (not presented here); therefore, deletion analysis of the entire region was performed. Stable transformants with these deletions showed that ≈400 bp 3′ to the *Fsp* I site could be removed with no change in either the mRNA expression level or regulatory pattern (Fig. 1*A*, line NS), as judged by nuclease S1 analysis. 3′ deletions from the *Pst* I site further reduced the region to the 142 bp spanning the polyadenylylation signal [Fig. 1*A*, line 2 (Δ12 construct) and Fig. 2, lanes 2]. The smallest 5′ deletion placed the boundary 39 bp upstream of the last adenosine in the β-actin gene polyadenylylation signal [Fig. 1*A*, line 3 (Δ9 construct) and Fig. 2, lanes 3]. We synthesized a double-stranded oligonucleotide containing these 39 bp with additional 5′ *Sal* I/3′ *Bgl* II sticky ends. The polyadenylylation signal in the chicken cardiac α-actin gene contains the first adenosine in an adjacent *Bgl* II site, and this latter site is 5′ to an *Eco*RI site in the 3′ end of the genomic clone originally described (16). This *Bgl* II/*Eco*RI fragment was joined to the 39-mer oligonucleotide through the *Bgl* II site and was then inserted into pSV2-neo-FX [Fig. 1*A*, line 4 ("oligo") and Fig. 2, lanes 4]. The α-actin gene fragment was used to supply any adenylylation signals 3′ to the canonical site essential for normal poly(A) addition (17–19). These 39 bp alone are sufficient to maintain the normal β-actin mRNA expression levels and regulation pattern when inserted into pSV2-neo-FX (Fig. 1*B*).

The 3′ Sequence Element in the β-Actin Gene Can Regulate the Simian Virus 40 (SV40) Enhancer–Promoter. The SV40 enhancer–promoter element has been well characterized as a relatively strong promoter capable of directing transcription from numerous nonviral genes in a variety of cell backgrounds (20). In the presence of the enhancer sequence alone, gene promoters that are transcriptionally silent are often transcribed at high levels (20). We constructed chimeric β-actin genes containing either the SV40 enhancer–promoter element (Fig. 2 *Left*, lane 6) joined at the initiator methionine (*Nco* I site) or the SV40 3′ UTR sequence with the early splice and polyadenylylation signal (Fig. 2 *Left*, lane 5) joined at the *Fsp* I site, respectively. As in the case of the β-actin gene chimera with exon VII from the cardiac α-actin gene (Fig. 1*A*), the 3′ SV40 sequence element did not regulate β-actin mRNA appropriately, and equivalent levels of β-actin mRNA were expressed in myoblasts and myofibrils (Fig. 2, lanes 5). In contrast, transcription from the SV40 promoter was regulated by the 3′ β-actin sequence element, even though overall levels of β-actin mRNA expression were measurably lower in this case (Fig. 2, lanes 6). To confirm this observation directly, we substituted deletion Δ12 (Fig. 1*A*, line 2), the 142-bp fragment containing the 39-bp regulatory sequence and the polyadenylylation signal, in place of the SV40 sequences 3′ to the *neo* coding region in pSV2-neo (*Sma* I–*Eco*RI fragment). Although steady-state levels of neo mRNA from pSV2-neo were unaffected during myogenesis (Figs. 2 and 3), neo transcripts from pSV2-neo3′β behave like β-actin mRNA when deletion Δ12 is 3′ to the *neo* gene (Fig. 3).

The β-Actin Gene Is Transcriptionally Controlled by the 3′ Sequence Element. As mentioned previously, the stably transfected chicken β-actin gene was transcriptionally controlled in C2C12 cells [i.e., the nuclear transcription rates decrease during myogenesis in parallel with the steady-state levels of the mRNA (data not presented here)], as judged by nuclear run-on experiments and nuclease S1 assays. Furthermore, transcription rates (not presented here) and mRNA levels from the colinear selectable marker genes controlled by the SV40 promoter, either *neo* (Figs. 2 and 3)

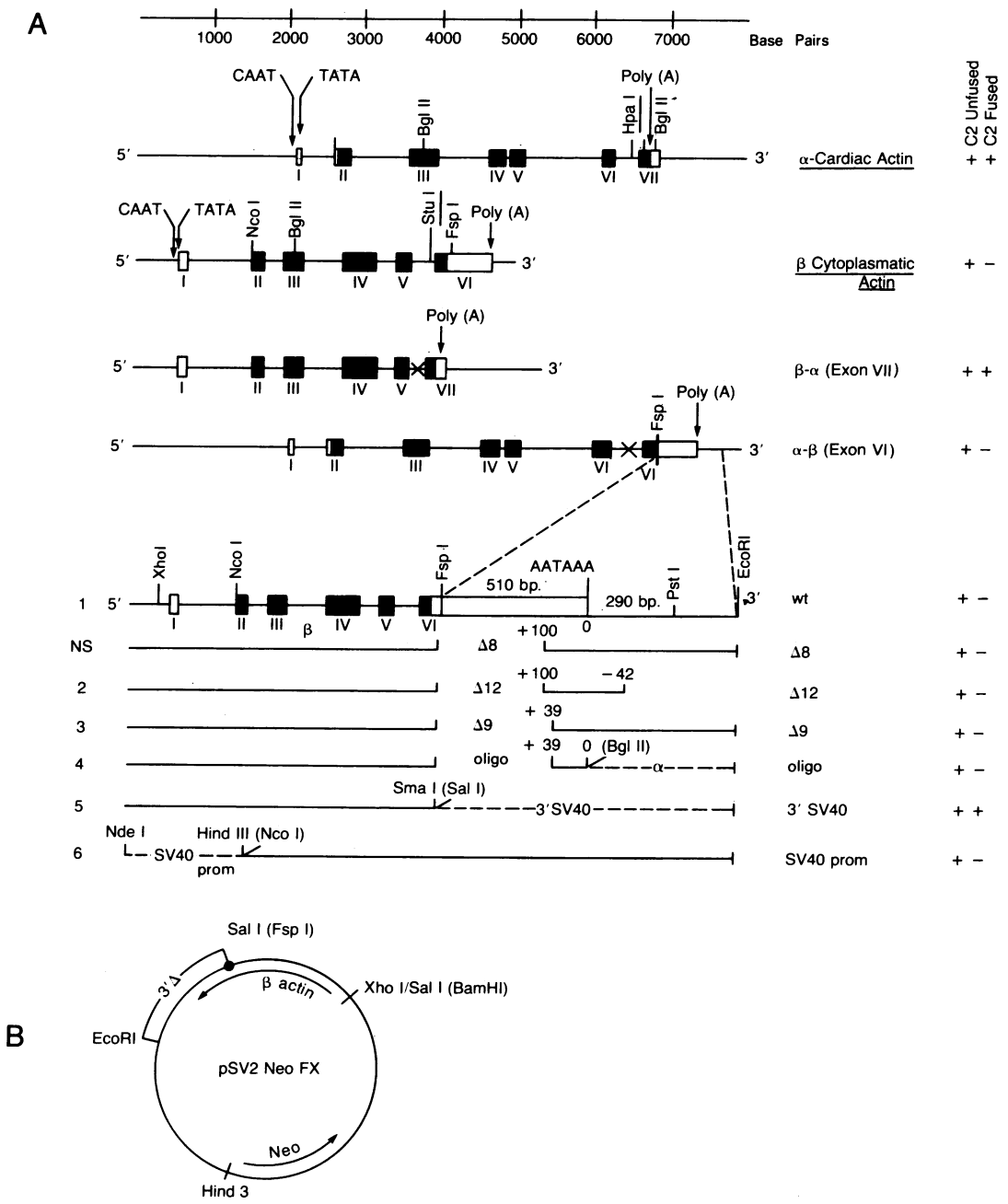

FIG. 1. β-Actin gene constructs used to locate and analyze the 3′ mRNA regulatory region. (*A*) The first two diagrams give the structure of the α-cardiac-actin gene and the β-actin gene, respectively, and indicate the restriction sites used to construct the α/β-actin chimeric genes. The third and fourth diagrams, β/α (exon VII) and α/β (exon VI), are the chimeric β- and α-actin genes in which we have exchanged the last exons throughout the *Hpa* I/*Stu* I sites. The exchange site is indicated by the × in the last intron of each construct. The chimeric α/β-actin genes exchanged through the common *Bgl* II sites are not shown. The numbered and lettered lines in the lower portion of *A* show the detailed deletion and exchange constructs with exon VI. The last adenosine in the canonical polyadenylylation signal was arbitrarily set at zero, with positions 5′ designated plus and 3′ designated minus. Line 1 is an expanded view of β-actin exon VI. The nuclease S1 analysis of the β-actin mRNA produced from these constructs (lines 1–6) is shown in Fig. 2. Line NS is not shown in Fig. 2. Line 4 ("oligo") describes the construct made with the 39-mer regulatory region joined to the cardiac α-actin gene fragment 3′ to the polyadenylylation signal. Line 5 is the β-actin/SV40 chimera with the 3′ SV40 early splice and polyadenylylation signal joined as a *Sma* I/*Eco*RI fragment from pSV2-neo to *Sal* I (flush)/*Eco*RI-digested pSV2-neo-FX (see below). Line 6 is the SV40 enhancer–promoter/β-actin chimera joined as an *Nde* I (flush)/*Hin*dIII (flush) fragment from pSV2-neo to *Xho* I (flush)/*Nco* I (flush)-digested pSV2-neo-3′β containing the entire *Eco*RI β-actin gene fragment (3). High or low expression levels of β-actin mRNA are noted as plus or minus, respectively. (*B*) Diagram of the β-actin vector used to analyze most of the 3′ β-actin deletions. Restriction sites in parentheses were converted to *Sal* I sites in the vector and gene separately. The *Xho* I site in the β-actin gene was used to clone the *Xho* I/*Eco*RI gene fragment into the *Sal* I-modified pSV2-neo vector.

or *gpt* (not presented here), remained unchanged with differentiation. Nuclear run-on analysis of the pSV2-neo-3′β construct, along with the endogenous mouse skeletal muscle α-actin and β-actin genes as controls, clearly showed that this 3′ sequence element can transcriptionally control heterologous genes in a muscle cell background (Fig. 4). In contrast to the myoblast cultures (Fig. 4*B*, unfused lanes), run-on neo transcripts from pSV2-neo-3′β were not detectable in fused cultures (Fig. 4*B*, fused lanes). The control transcripts for mouse skeletal muscle α-actin increased (≈5

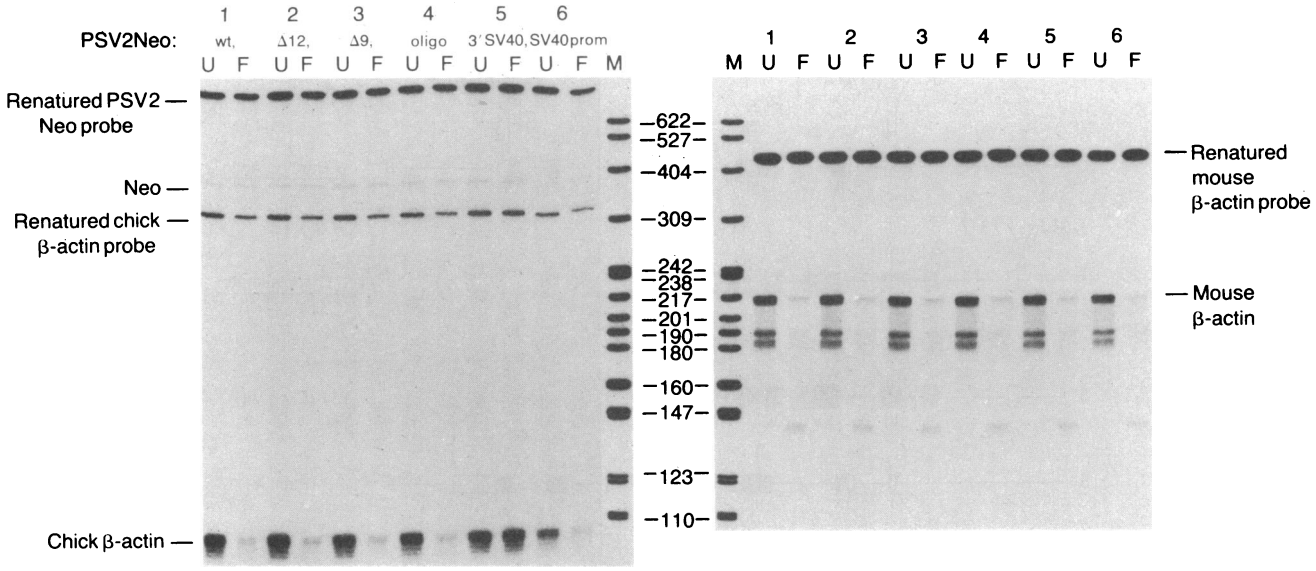

FIG. 2. Nuclease S1 analysis of the 3′ deletions and exchanges shown in Fig. 1. (*Left*) Nuclease S1 analysis of the constructs diagrammed in Fig. 1*A*, lines 1–6 except line NS. Results are normalized to neo mRNA levels because the *neo* gene, under the control of the early SV40 enhancer–promoter, is constitutively expressed in unfused (lanes U) and fused (lanes F) C2C12 cultures. (*Right*) Nuclease S1 analysis of the endogenous mouse β-actin gene regulation with the same RNA samples shown in *Left*. Probes are described in *Materials and Methods*.

times), and those for mouse β-actin decreased (≈3 times), reflecting the normal developmental expression pattern for the endogenous genes.

DISCUSSION

The β-actin gene is transcribed in essentially all dividing cells but is inactive in myofibrils (3, 23). The chicken β-actin gene, when introduced into the C2C12 mouse myogenic cell line on pSV2 vectors, is regulated in parallel to the endogenous gene; thus, the developmental control of the exogenous gene can be analyzed in detail (3). We have utilized this fact to locate those sequences within the β-actin gene responsible for the decrease in β-actin mRNA levels during myogenesis.

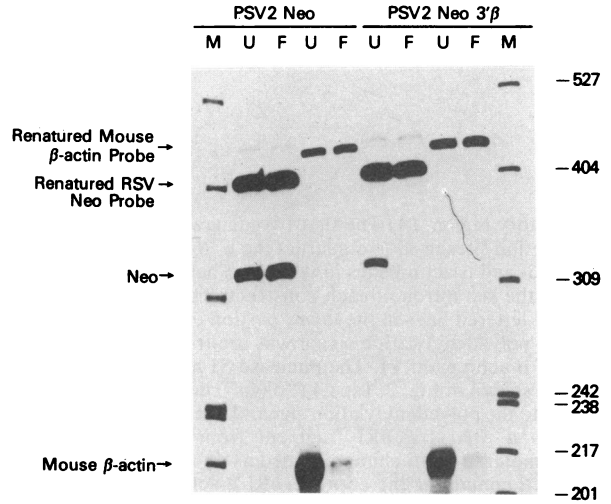

FIG. 3. Nuclease S1 analysis of pSV2-neo and pSV2-neo-3′β (with the 3′ chicken β-actin sequence) mRNA levels during myogenesis. RNA samples from unfused (lanes U) and fused (lanes F) C2C12 cultures transfected with pSV2-neo or pSV2-neo-3′β were analyzed for both neo (the left two samples in each set) and mouse β-actin (the right two samples in each set) mRNA levels. In this instance the *neo* probe was prepared from pRSV-neo in order to eliminate the double transcriptional start seen with the SV40 promoter in pSV2-neo (3).

Chimeric gene constructs between the cardiac muscle α-actin and β-actin genes localized the regulatory region in or adjacent to exon VI of the β-actin gene. A detailed deletion analysis of exon VI further defined this region to the 40 bp 5′ to the canonical polyadenylylation signal, an area corresponding to the 3′ UTR in the β-actin mRNA. A double-stranded oligonucleotide containing these 40 bp was sufficient to maintain the normal expression level and regulatory pattern of β-actin mRNA when reintroduced into the deleted gene. Furthermore, a DNA fragment containing this sequence element, when substituted for the 3′ end of the *neo* gene in pSV2-neo, transferred the β-actin regulatory pattern to neo mRNA in myogenic cells even though the standard *neo* gene is expressed constitutively during myogenesis.

The location of this regulatory sequence element within the 3′ UTR of the gene suggested mRNA stability was the likely mechanism controlling β-actin mRNA levels. Nuclear

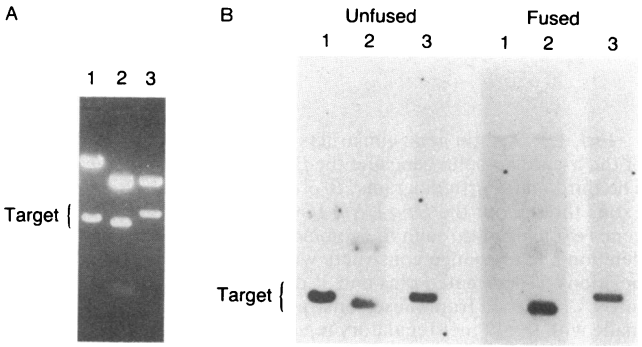

FIG. 4. Transcriptional analysis of pSV2-neo-3′β during myogenesis. Nuclei were prepared from unfused and fused C2C12 cultures stably transfected with pSV2-neo-3′β and were incubated *in vitro* as described (13). (*A*) Nascent RNA transcripts were hybridized to the target DNA shown in the ethidium-stained gel. Lanes: 1, pSV2-neo (6) digested with *Nco* I to release the 1023-bp fragment (pSV2-neo-3′ β-chicken) containing the *neo* gene; 2, mouse skeletal α-actin cDNA clone (21) digested with *Pst* I to release the 925-bp insert; 3, mouse β-actin cDNA clone (22) digested with *Pst* I to release the 1140-bp insert. (*B*) Nascent transcript levels from the pSV2-neo-3′β (lane 1), mouse skeletal α-actin gene (lane 2), and mouse β-actin gene (lane 3).

FIG. 5. Sequence comparison and location of the 3′ β-actin transcriptional control region in vertebrate β-actin genes sequenced to date. The sequence data are taken from the following references for each gene: line 1, ref. 22; line 2, ref. 24; line 3, ref. 25; and line 4, ref. 26. The gap is used to maximize homology, and the lowercase letters indicate a mismatch.

run-on experiments measuring transcription from the chimeric constructs and endogenous genes forced us to conclude changes in β-actin mRNA levels were controlled primarily by transcriptional processes and not by changes in mRNA stability. Although we have not presented the data here, *in vitro* RNA transcripts produced with the T7 promoter, using wild-type and 3′ β-actin gene–*neo* constructs, were equally stable in nuclear extracts from unfused and fused C2C12 cells. Thus, this 3′ sequence would not appear to be an endonuclease cleavage site under the *in vitro* reaction conditions used here (13). The importance of this transcriptional control sequence is further emphasized when one compares the position and conservation of this element (Fig. 5). The evolutionary distance between the chicken and human genes is more than 200 million years (10), yet this sequence (22, 24–26) and its position are conserved in the 3′ noncoding regions of all β-actin genes cited.

The β-actin 3′ regulatory sequence is similar in action to the "silencer" element described for the *MAT* locus in yeast (27). However, unlike the yeast element, the β-actin "silencer" does not function at a distance outside the gene: the 3′ end of the β-actin gene is ≈2 kb from the pSV2-neo promoter in all of our constructs, yet the viral promoter is active and β-actin transcription is repressed. Preliminary results indicate the β-actin gene 3′ transcriptional control sequence does not behave in enhancer fashion. We have positioned this element in both orientations 5′ to the SV40 promoter–enhancer and 3′ to the *neo* coding sequence in pSV2-neo, displacing the normal 3′ SV40 processing–adenylylation signals, yet only the normal orientation and position give the β-actin mRNA regulatory pattern to neo mRNA (unpublished observations). Furthermore, the action of this element does not appear to be restricted to any particular gene or promoter, if one considers the different constructs presented here, but depends only on the expression of the gene in question in a muscle cell background. Transcription attenuation-type mechanisms do not seem to be involved in the regulation of β-actin mRNA levels because nuclear run-on experiments (not presented here) with the transfected chicken β-actin gene indicate in fused cells that no transcription is occurring through the first intron of the β-actin gene. In this instance the 3′ control sequence is >5 kb away.

Although there is a large body of work on transcription termination (17), none of the events correlated with this process appear to be involved in β-actin mRNA regulation for the following reasons. Nuclear run-on data excludes 3′ cleavage and polyadenylylation as regulatory points in the control of β-actin mRNA levels. In addition, the location and composition of this transcriptional control sequence have none of the hallmarks characteristic of sequence elements involved in mRNA stability, the 3′ cleavage/adenylylation reactions or transcription termination that have been described so far (17–19). Two examples of 3′ sequence elements modulating gene expression have been reported for the human gastrin (28) and transferrin receptor genes (29). In the former case, the oligothymidylate-rich sequence involved appears to modulate transcription termination and possibly mRNA stability (30); in the latter case, transcriptional control was never demonstrated with the 2.3-kb 3′ UTR implicated in the iron regulation of receptor expression.

Note Added in Proof. Preliminary reports by S. Sharp and N. Davidson (15) and H. Arnold (16) report similar results for the steady-state regulation of β-actin mRNA during myogenesis.

1. Buckingham, M. E. & Minty, A. J. (1983) in *Eukaryotic Genes: Their Structure, Activity and Regulation*, eds. Maclean, N., Gregory, S. P. & Flavell, R. A. (Butterworth, London), Vol. 2, pp. 365–395.
2. Paterson, B. M. & Bishop, J. O. (1977) *Cell* **12**, 751–765.
3. Seiler-Tuyns, A., Eldridge, J. D. & Paterson, B. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2980–2984.
4. Paterson, B. M., Eldridge, J. & Seiler-Tuyns, A. (1986) in *Molecular Biology of Muscle Development*, eds. Emerson, C., Fischman, D., Nadal-Ginard, B. & Siddiqui, M. A. Q. (Liss, New York), Vol. 29, pp. 521–529.
5. Blau, H. M., Pavlath, G. K., Hardeman, E. C., Chiu, C.-P., Silverstein, L., Webster, S. G., Miller, S. C. & Webster, C. (1985) *Science* **230**, 758–766.
6. Gorman, C. (1985) in *DNA Cloning*, ed. Glover, D. M. (IRL, Washington, DC), Vol. 2, pp. 143–190.
7. Okayama, H., Kawaichi, M., Brownstein, M., Lee, F., Yokota, T. & Arai, K. (1988) *Methods Enzymol.*, in press.
8. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
9. Cleveland, D. W., LoFata, M. A., MacDonald, R. T., Counn, N. J., Rutter, W. J. & Kirschner, M. (1980) *Cell* **20**, 95–105.
10. Alonso, S., Minty, A., Bourlet, Y. & Buckingham, M. (1986) *J. Mol. Evol.* **23**, in press.
11. Yanish-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33**, 103–119.
12. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
13. Konieczny, S. F. & Emerson, C. P. (1985) *Mol. Cell. Biol.* **5**, 2423–2432.
14. Reed, K. C. & Mann, D. A. (1985) *Nucleic Acids Res.* **13**, 7207–7221.
15. Ng, S.-Y., Gunning, P., Eddy, R., Ponte, P., Leavitt, J., Shows, T. & Kedes, L. (1985) *Mol. Cell. Biol.* **5**, 2720–2732.
16. Eldridge, J., Zehner, Z. & Paterson, B. M. (1985) *Gene* **36**, 55–63.
17. Birnstiel, M. L., Buzzlinger, M. & Strub, K. (1985) *Cell* **41**, 349–359.
18. McDevitt, M. A., Hart, R. P., Wong, W. W. & Nevins, J. R. (1986) *EMBO J.* **5**, 2907–2913.
19. Gil, A. & Proudfoot, N. J. (1987) *Cell* **49**, 399–406.
20. Serfling, E., Jasin, M. & Schaffner, W. (1985) *Trends Genet.* **1**, 224–230.
21. Lasar, B. A., Paterson, B. M. & Weintraub, H. (1986) *Cell* **47**, 649–656.
22. Kost, T. A., Theodorakis, N. & Hughes, S. (1983) *Nucleic Acids Res.* **11**, 8287–8301.
23. Paterson, B. M. & Eldridge, J. D. (1984) *Science* **224**, 1436–1438.
24. Tokunaga, K., Taniguchi, H., Shimizu, M. & Sakiyama, S. (1986) *Nucleic Acids Res.* **14**, 2829.
25. Nudel, U., Zakut, R., Shani, M., Neuman, S., Levi, F. & Yaffe, D. (1983) *Nucleic Acids Res.* **11**, 1759–1771.
26. Ponte, P., Ng, S. Y., Engle, P., Gunning, P. & Kedes, L. (1984) *Nucleic Acids Res.* **12**, 1687–1696.
27. Brand, A. H., Breeden, L., Abraham, J., Sternglarz, R. & Nasmyth, K. (1985) *Cell* **41**, 41–48.
28. Sato, K., Ito, R., Kwang-Hee, B. & Agarwal, K. (1986) *Mol. Cell. Biol.* **6**, 1032–1043.
29. Owen, D. & Kuhn, L. C. (1987) *EMBO J.* **6**, 1287–1293.
30. Reeves, R., Elton, T. S., Nisen, M. S., Lehn, D. & Johnson, K. R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6531–6535.
31. Sharp, S. & Davidson, N. (1986) *J. Cell. Biochem. Suppl.* **10**, 68 (abstr.).
32. Arnold, H. (1987) *J. Cell. Biochem. Suppl.* **11**, 66 (abstr.).