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The down-regulation of the chicken cytoplasmic β actin during myogenic differentiation does not require the gene promoter but involves the 3' end of the gene

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ABSTRACT

The chicken cytoplasmic ß actin gene is ubiquitously expressed in all cell types. In terminally differentiated muscle cells, however, the concentration of ß actin specific mRNA is down-regulated to scarcely detectable levels. To test for gene regions which are involved in the muscle specific reduction of ß actin specific mRNA, the isolated complete chicken ß actin gene or chimeric gene constructs containing parts of the gene were stably transfected into the myogenic mouse cell line C2C12 and their transcriptional activity was compared in proliferating myoblasts and postmitotic myotubes. A hybrid construct containing the ß actin promoter fused to the bacterial CAT gene showed high and constitutive expression during myocyte differentiation. In contrast, constructs containing the SV40 early promoter linked to the 3'end of the ß actin gene led to a marked reduction of ß actin transcripts in differentiated C2C12 myotubes. The stability of ß actin mRNA was analyzed in actinomycin D treated cells and found to be virtually unchanged in myotubes as compared to myoblasts. These results suggest that a sequence element located in the 3'end or 3'flanking region of the ß actin gene confers the myotube specific down-regulation that is not primarily due to destabilization of mRNA.

INTRODUCTION

The cytoskeletal isoforms of actins are among the most abundant cellular proteins in almost all eucaryotic cells. In contrast to the muscle specific isoforms, e.g. the alpha-skeletal actin, the alpha-cardiac, and the alpha- and gamma-smooth muscle actins (1-3), the cytoplasmic beta- and gamma-actins constitute components of the microfilament network and are involved in cell architecture and cell motility as well as in organelle movement and cytokinesis (4,5). All actins have a very similar protein structure and belong to a multigene family in vertebrate organisms (6-12). Despite their structural similarity and presumably similar physiological role, the synthesis of the individual actins is distinctly regulated in a tissue- and developmental stage-specific manner (13). It has been documented in mammalian and avian organisms that the muscle specific actins are only synthesized during the terminal stages of myogenic differentiation after proliferating myoblasts

have fused into multinucleated myotubes (14-20). The nonmuscle actin isoforms, however, are already present in the myogenic precursor cells and gradually disappear after the formation of functional muscle cells (15,21). Proliferating myoblasts can be propagated in culture and also triggered to differentiate by withdrawal of serum. Therefore, it is possible to study the process of differential gene expression during muscle cell fusion in primary cultures or in established myogenic cell lines in vitro.

Earlier studies with muscle cells of various organisms have demonstrated that the differentiation of myoblasts to form myotubes is accompanied by a large change in gene expression (22,23), presumably due to altered transcription of muscle specific genes (23). As muscle specific proteins and their corresponding mRNAs begin to accumulate, the concentration of transcripts for the nonmuscle contractile protein ß actin gradually declines (15,21,24). As a result of this down-regulation process, the fully matured muscle fiber retains only the sarcomeric actin isoforms. Relatively little is known about the mechanisms and the elements involved in the down-regulation of ß actin transcripts during myogenesis. In this report we used the isolated chicken ß actin gene or chimeric constructs containing only parts of the gene to stably transfect the mouse muscle cell line C2C12 and follow the mRNA accumulation from the transfected genes during cell differentiation. We found that the ß actin promoter is constitutively expressed in myotubes and does not contribute to the phenomenon of down-regulation. In contrast, a sequence located in the 3'end region of the ß actin gene confers down-regulation to transcripts even when their production is regulated by the heterologous SV40 early promoter.

MATERIALS AND METHODS

Construction of chimeric ß actin genes

We and others have previously reported the isolation of the cytoplasmic ß actin gene from chicken (25,26). A genomic Bam HI fragment containing the ß actin gene plus flanking sequences was subcloned into plasmid pSV2-neo (27) yielding a selectable ß actin construct. To generate plasmids P1-CAT and P2-CAT a 1277 bp XhoI/NcoI fragment containing the promoter, the complete first exon and intron was joined to Hind III linkers and cloned into plasmid pSV0-CAT (28). The orientation of the promoter fragment was tested in the resulting clones by single and double digestion with appropriate restriction endonucleases. P1-CAT contains the promoter in the correct orientation to the CAT gene, P2-CAT designates the reverse orientation.

Hybrid genes containing the SV40 early promoter in exchange for the ß actin promoter were obtained by subcloning the 4 Kb NcoI ß actin gene fragment into the Hinc II site of pUC19 (29). This fragment spans the structural gene from the AUG start codon to the poly (A^+) addition site plus approximately 800 nucleotides of 3'flanking sequence. The SV40 early promoter was isolated from pSV2-CAT (28) by digestion with AccI and Hind III. The promoter fragment was then subcloned after blunt end formation into the SmaI site. The correct orientation of the resulting construct pSV-ß actin was examined by DNA sequence analysis (30).

The chimeric plasmids pSV2-neo 600 and pSV2-neo 1400 were derived from pSV2-neo (27) from which the SV40 polyadenylation cassette had been deleted by NruI/Eco RI digestion. The remaining 3.8 Kb pSV2-neo fragment was blunt ended and either ligated to the 600 bp cDNA insert of clone pA2, kindly provided by D. Cleveland (11), or to the 1.4 Kb MstI/NcoI genomic fragment that includes the 3' untranslated sequence and 800 bp of 3'flanking sequence. Alternatively, the 600 bp Hind III cDNA fragment of clone pA2 was cloned into the NruI site of pSV2-neo to retain the SV40 polyadenylation cassette. This construct was designated as pSV2-neo 600*.

Transfection of C2C12 mouse cells

C2C12 cells, originally isolated by D. Yaffe (31) and subcloned by H. Blau (32) were generously provided to us by B. Paterson, NIH. The cells were grown in DMEM medium (Gibco Labs, Grand Island, N.Y.), supplemented with 20% FCS and antibiotics (32). For myotube formation nearly confluent cultures were changed to DMEM medium plus 2% horse serum for several days. Supercoiled plasmid DNA was prepared for transfection as described elsewhere (33). Cells were transfected either with the selectable pSV-neo-ß actin construct or with chimeric actin genes plus unlinked pSV2-neo DNA using the calcium phosphate precipitation procedure (34). Approximately 1 ug of pSV2-neo DNA was cotransfected with 20 ug of chimeric ß actin plasmid DNA on 5x 10^4 cells per 10 cm dish. 24 hours later the selection for growing cells was started with 400 ug G 418 /ml medium (G418-geneticin; Gibco Labs). Between 50 and 200 colonies per plate were grown for 10 to 14 days under G 418 selection. Colonies on 1 plate were pooled and analyzed for expression of neomycine phosphotransferase- and ß actin mRNA.

Determination of RNA expression by S1 nuclease or Northern blot analysis

Total cellular RNA was prepared by the guanidinium /cesium chloride method (35) and analyzed with nuclease S1 as described (36). For the determination of ß actin mRNA, the Hind III fragment of clone pA2 (11) containing the 3'

untranslated sequence was subcloned into bacteriophage M13 mp8 to generate a single strand probe that was homogenously labeled as described (37). A neo-specific probe was generated by SphI/BglII digestion of the pSV2-neo plasmid as described by Stafford and Queen (38). The ß actin-CAT transcripts were measured with the 1584 bp Eco RI/Nde I fragment isolated from P1-CAT and labeled at the 5'end with T4 polynucleotide kinase (39). RNA for Northern blot analysis was either separated on agarose gels and transferred to nitrocellulose or directly spotted onto the filter for dot blots as described by Thomas (40). The ß actin hybridization probe was labeled by nick translation and used as described (40).

Nuclear run-on experiments with unfused and fused C2C12 mouse cells stably transfected with pSV-ß-actin and pSV2-neo

Nuclei were isolated from differentiated and undifferentiated C2C12 cell pools that had been stably transfected with pSV-neo and pSV-ß-actin by the method described by Konieczny and Emerson (54). For each experiment approximately $1x10^8$ nuclei were incubated with 200 uCi of (^{32}P)-UTP (specific activity: 600 Ci/mmmol) for 30 min at 37°C and radioactively labeled RNA was isolated. To determine the rate of synthesis for pSV-ß-actin and pSV2-neo transcripts, about $1-2x10^7$ cpm (Cerenkov) of total nuclear RNA were hybridized to nitrocellulose filters containing pSV-ß-actin DNA (digested with XbaI and Eco RI) and pSV2-neo HindIII/NruI fragment. Filters were hybridized and washed as described for Northern blot analysis.

RESULTS

Down-regulation of cytoplasmic actin mRNA and the concomittant increase of sarcomeric actin mRNAs has been demonstrated for muscle cells in culture (15,24). To investigate and reassess this phenomenon for the *in vivo* situation, we analyzed the steady state concentrations of actin mRNAs during chicken development. As shown in figure 1, total RNA was isolated from whole embryos at day 5 and shown to contain almost exclusively the larger mRNA species coding for the cytoplasmic ß actin isoform. RNA from leg muscle of embryos between days 12 and 20 *in ovo* contained increasing concentrations of the smaller mRNA species coding for the sarcomeric alpha-actin, whereas the ß actin mRNA concentration decreased to a nearly undetectable level at day 20. This result confirmed that the down-regulation of ß actin mRNA, originally observed in myogenic tissue culture cells, occurs also during the development of chicken *in ovo*.

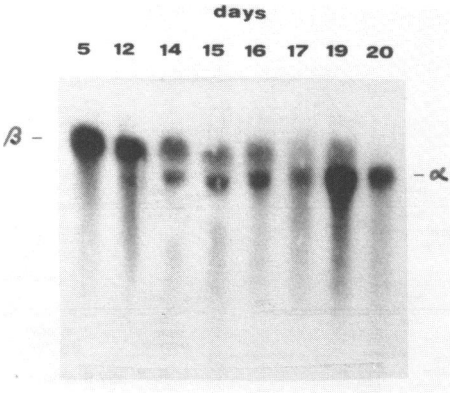


Fig. 1
The expression of alpha- and ß actin mRNAs in skeletal muscle of developing chicken embryos.
Twenty micrograms of RNA isolated from total embryos (day 5) or leg muscle tissue (days 12 to 20) were used for the Northern blot analysis. The blot was hybridized to $3x10^6$cpm of (^{32}P)-labelled, cloned chicken ß actin cDNA (spec. activity $5x10^7$cpm/ug) and washed as described under Materials and Methods. Exposure was overnight at -80°C, on X-ray film (Kodak) with intensifying screen.

Construction of chimeric genes containing either the ß-actin promoter or other parts of the ß actin gene.

Transfection of cloned genes into a wide variety of host cells has been a generally accepted strategy to study gene activity and gene regulation. It has been previously shown that actin genes from chicken transfected into the permanent muscle cell lines C2C12 derived from mouse (32) or L6 cells derived from rat muscle (47) were expressed in a regulated fashion that in many respects resembled the expression of these genes in vivo (24,41-43). The muscle-specific modulation of the promoter activity of skeletal alpha-actin genes from chicken (43), rat (44) and human (45,46) has been demonstrated to require cis-regulatory elements located in the 5' flanking sequences of these genes. In order to analyze whether the modulation of ß actin gene activity in muscle cells would also depend on the gene promoter plus 5' upstream sequences, we constructed the hybrid genes P1-CAT and P2-CAT that contained the 5' end sequence of the ß actin gene as control elements for the bacterial gene for chloramphenicol acetyl transferase. Details of the constructions are shown in fig.2A. Alternatively, a ß actin hybrid gene was constructed that had its own promoter replaced by the SV40 early promoter and was designated pSV-ß actin. To generate these constructs, the gene body

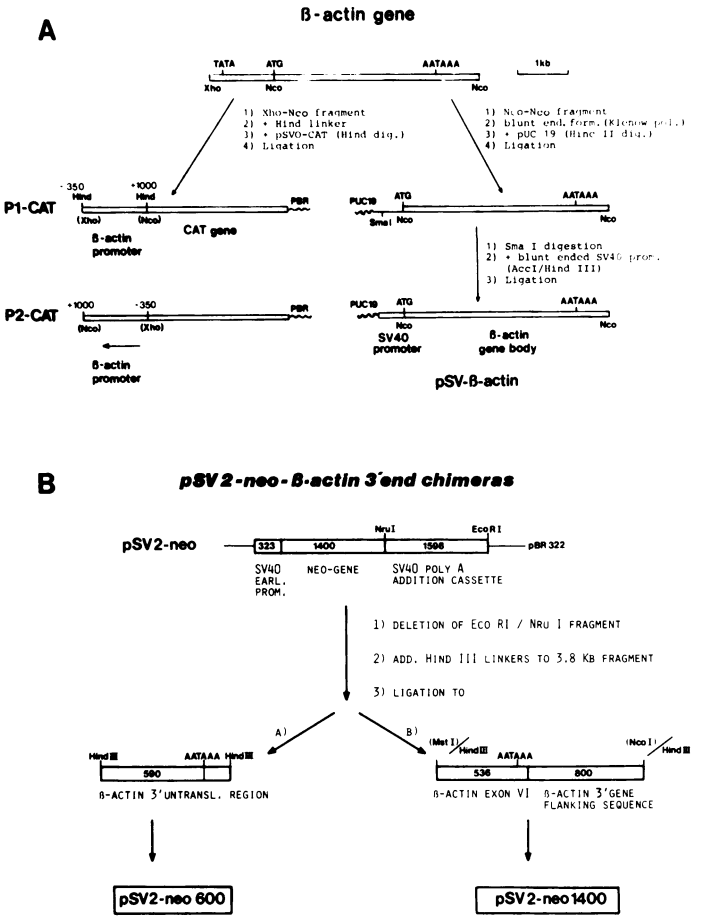


Fig. 2

Chicken ß actin gene constructs used to stably transfect C2C12 mouse myoblasts.

A) The restriction sites used to isolate the promoter fragment (XhoI/XcoI) and the gene body (NcoI/NcoI) are indicated. The location of the promoter is shown by the TATA motif, the AATAAA sequence indicates the polyadenylation signal sequence at the 3'end of the gene. The chimeric gene P1-CAT contains the ß actin promoter linked to the bacterial CAT gene in the correct orientation. The fragment extends from nucleotide -350 to +1000 when the transcriptional start site is counted as +1. P2-CAT contains the same promoter fragment in opposite orientation. The 4 kb NcoI/NcoI gene body fragment was subcloned into plasmid pUC 19 and joined to the SV40 early promoter in correct orientation to obtain the hybrid construct pSV-ß-actin.

B) pSV2-neo-ß actin 3'end chimeras were constructed as shown. The SV40 polyadenylation cassette (1598 nucleotides) of pSV2-neo was deleted by digestion with EcoRI and NruI and the remaining 3.8 kb fragement was joined with Hind III linkers and recombined with either the 3'untranslated region of the cDNA clone pA2 (590 nucleotide HindIII insert) or with the MstI/NcoI fragment of

the 3'end of the ß actin gene (1400 nucleotides). The resulting constructs were designated as pSV2-neo 600 and pSV2-neo 1400, respectively. The hybrid pSV2-neo 600* was obtained by insertion of the pA2 cDNA (590 nucleotides) into the NruI site of pSV2-neo thereby retaining the SV40 polyadenylation cassette (not shown).

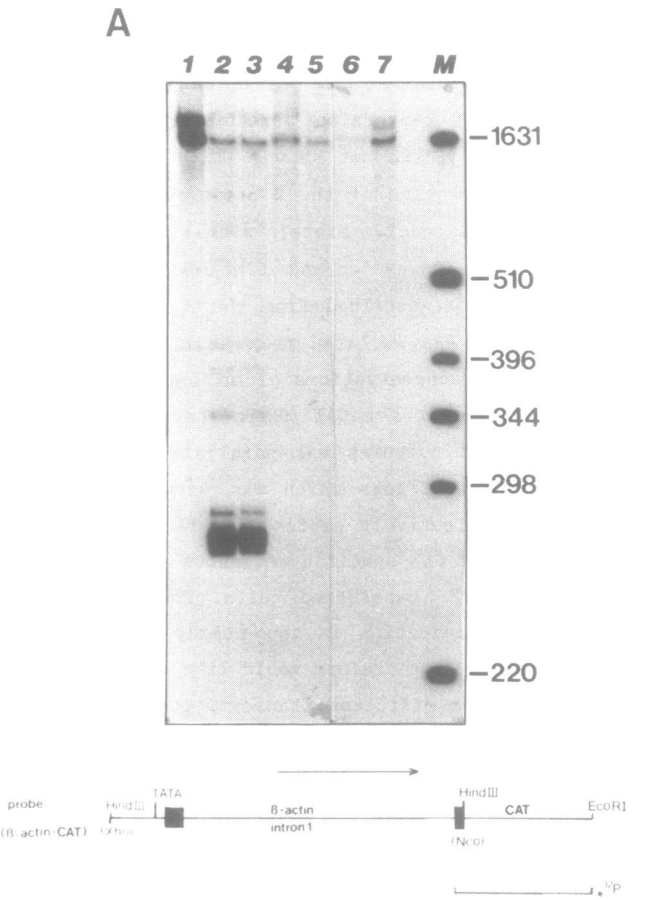
including the 3'end plus flanking sequence was separated from the promoter containing fragment by the NcoI site which is conveniently located at the AUG translational start codon (figure 2 and Material and Methods).

The ß actin promoter is constitutively expressed in undifferentiated and differentiated C2C12 muscle cells.

The hybrid plasmids P1-CAT and P2-CAT were transfected into mouse C2C12 cells together with plasmid pSV2-neo containing the selectable marker gene coding for G 418 (geneticin) resistance. 100 - 200 resistant colonies of stably transfected cells were generally recovered from one plate and pooled for further analysis. When extracts of C2C12 cells containing the integrated P1-CAT plasmid were examined for CAT enzyme activity, approximately equal levels of chloramphenicol conversion were observed in proliferating myoblasts and fused myotubes (data not shown). This enzyme activity was dependent on the correct orientation of the ß actin promoter. In order to avoid the possibility that the extensive protein stability of the CAT enzyme would make a reduced gene expression and down-regulation of mRNA, we directly analyzed the CAT mRNA transcribed from the ß actin promoter by S1-analysis. As shown in fig.3A, RNA isolated from undifferentiated or fused C2C12 cells, exhibited similar concentrations of an approximately 250 bp protected fragment which was diagnostic for CAT mRNA. The slightly decreased concentration in differentiated cultures was paralleled by the protected signals for the neomycine-resistance gene which was used as an internal standard that is presumably constitutively expressed during myogenic differentiation (fig.3B). No protection of CAT specific sequences was observed with RNA from mock-transfected or P2-CAT transfected cells. These results taken together suggested that no gross-reduction in the steady state concentration of ß actin CAT mRNA occurred. We therefore would like to conclude that the chicken ß actin promoter allows efficient transcription in a myogenic mouse cell but this transcription appears not to be down-regulated in differentiated muscle cells. The ß actin promoter including the entire first intron is constitutive in muscle cells and is not involved in the muscle specific regulation of the gene.

The complete ß actin gene or hybrids containing the 3'end region are down-regulated in fused myotubes.

The entire chicken ß actin gene was transfected into C2C12 mouse cells. Beta-actin mRNA accumulation from the transfected gene was measured by S1 analysis in proliferating C2C12 cells and 3, 5, and 7 days after onset of differentiation. As shown in figure 4A, the concentration of ß actin mRNA clearly decreased in cells that had been shifted to differentiation medium. A similar result was obtained with the construct pSV-ß actin in which the ß actin gene was placed under the control of the SV40 early promoter (fig.4). We consistently found a decrease of ß actin transcripts in myotubes by a factor of 8 to 10 relative to myoblasts. The variation of neo-mRNA used as internal standard in these experiments was at most twofold (fig.4B). It is



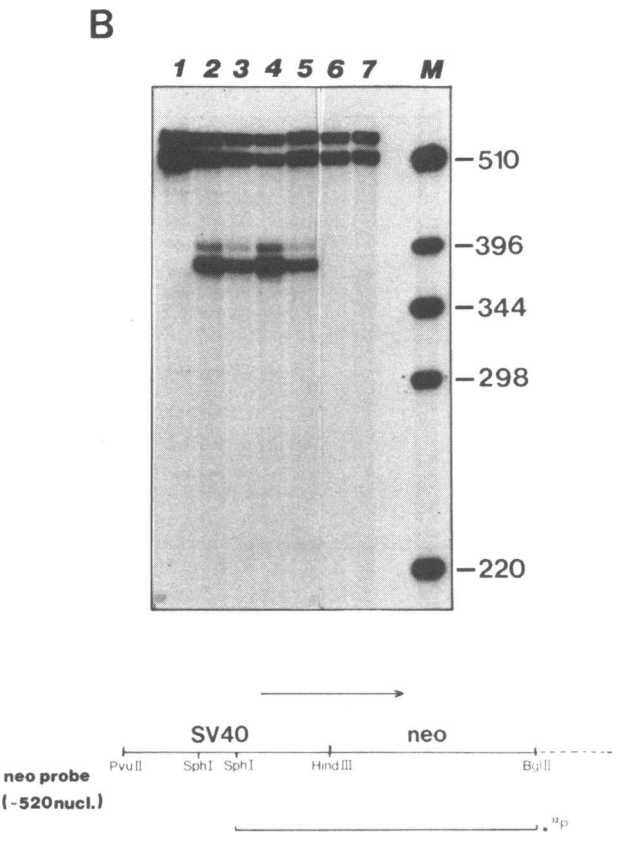


Fig. 3
S1 analysis of P1-CAT and pSV2-neo mRNA levels transcribed from stably transfected genes in differentiating C2C12 cells.
RNA samples of C2C12 cells cotransfected with pSV2-neo plus P1-CAT (lanes 2 and 3) or pSV2-neo plus P2-CAT (lanes 4 and 5) or RNAs of mock-tansfected cells (lanes 6 and 7) were analyzed for the level of CAT transcripts (A) or Neo transcripts (B) with the hybridization probes indicated below the gels and described in Materials and Methods. Lanes 2, 4 and 6 contain RNA from unfused myoblasts; lanes 3, 5 and 7 contain RNA from well differentiated fused myotubes. Lane 1 shows undigested probe. Molecular weight standards are indicated.

evident then that ß actin gene sequences other than the promoter region or the first intron were required for muscle specific down-regulation of cytoplasmic actin.

In an attempt to further delineate the essential regulatory part of the gene, constructs were made that either contained only the 3'untranslated sequence of the ß actin cDNA clone pA2 linked to the pSV2-neo gene or alter-

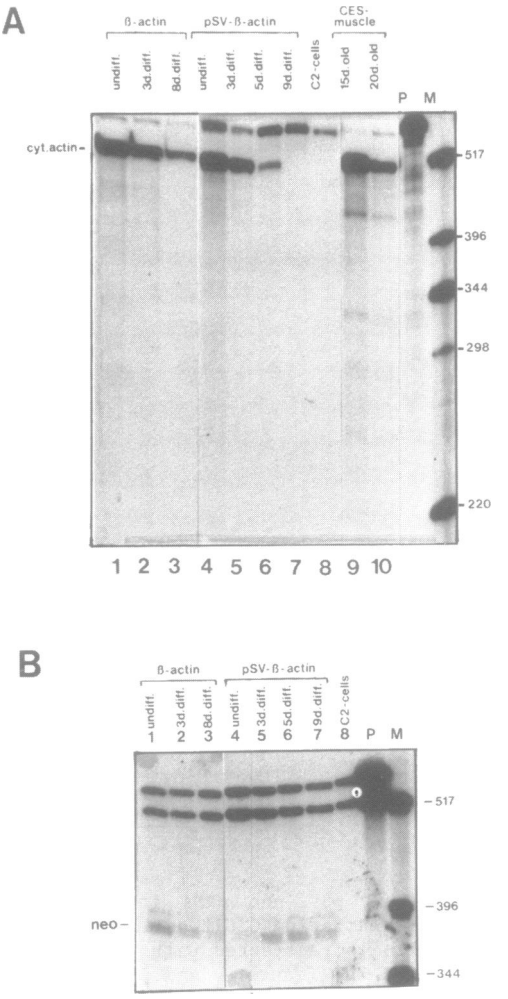


Fig. 4
Steady state concentrations of ß actin RNA transcribed from transfected gene chimeras and pSV2-neo in differentiating C2C12 muscle cells.
A) RNA samples isolated from C2C12 mouse cells stably transfected with the chicken ß actin gene (lanes 1 - 3), the pSV-ß actin hybrid gene (lanes 4 - 7), or from untransfected C2 myotubes (lane 8), or 12 and 20 day old embryonic chicken muscle (CES; lanes 9 and 10) were subjected to S1 analysis with a single stranded chicken ß actin specific probe (see Materials and Methods). 15 ug of RNA were used for each lane. P designates the homogenously labeled ss-probe. M shows the size standards in nucleotides.
B) S1 analysis of parallel RNA samples as shown in fig. 4A with pSV2-neo specific probe. Details are given in legend to fig. 3B.

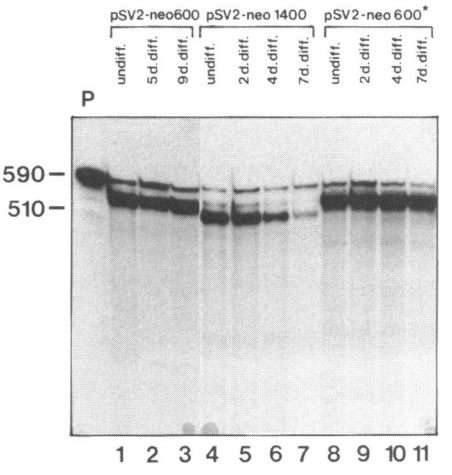


Fig. 5
S1 analysis of pSV2neo-ß actin 3'end chimeras transfected into C2C12 Mouse muscle cells.
The plasmid constructs used for transfection are described in Materials and Methods. RNA samples (15 ug) of cells with stably integrated pSV2-neo 600 (lanes 1 - 3), pSV2-neo 1400 (lanes 4 - 7) and pSV2-neo 600* were analyzed with S1 nuclease using the ß actin specific single-stranded probe (P = 590 nucleotides). The days given in the superscript indicate the time for which the cultures were grown in differentiation medium. The protected doublet in pSV-neo 600 indicates that two sites within the poly(A) stretch are used for termination.

natively contained this same part of the coding region plus 800 bp of 3' flanking DNA (fig.1B). Both plasmids, pSV2-neo 600 and pSV2-neo 1400, were individually transfected and S1 analysis of RNA accumulated from these genes was performed as described before. The results are shown in fig.5. Myotube-specific down-regulation was clearly demonstrated with pSV2-neo 1400 by the progressively reduced level of gene transcripts in cells that had been differentiated during one week.

In contrast, when the pSV2-neo 600 or pSV-neo 600* construct, in which the ß actin fragment was inserted into the NruI site of pSV2-neo thereby retaining the SV40 polyadenylation cassette, were transfected and analyzed no appreciable reduction of RNA concentrations were obtained in differentiated myotubes. It is interesting to note, however, that in both constructs termination occurred at different places in comparison to the ß actin gene. This is evident from the varied lengths of the protected fragments. This experiment clearly indicates that the 3'end of the gene is sufficient for ß actin down-regulation in muscle cells and none of the protein coding sequence or 5' up-

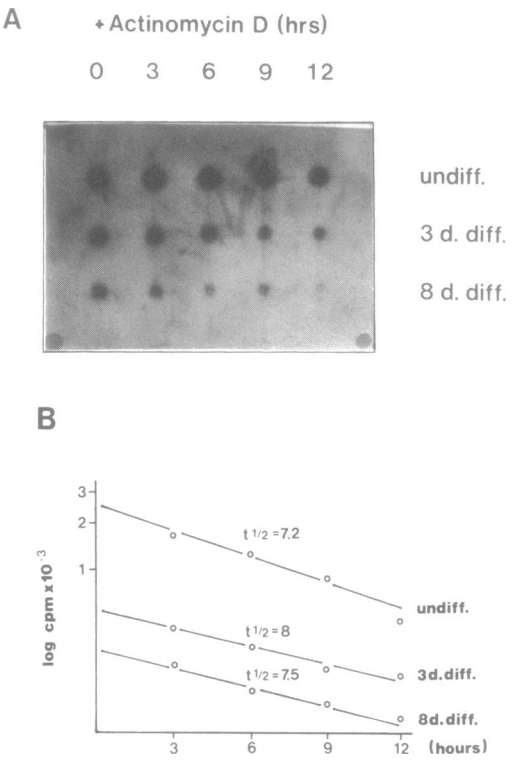


Fig. 6
Determination of the ß actin mRNA stability in the presence of actinomycin D. A) C2C12 cells with stably integrated chicken ß actin gene were grown in the presence of actinomycin D (5 ug/ml medium) for the indicated times. Total cellular RNA was isolated and 20 ug (undiff.), 40 ug (3 days diff.) and 80 ug (8 days diff.) of RNA were spotted for each time point on nitrocellulose. Hybridisation was carried out with $5x10^6$cpm of (^{32}P) labeled chicken ß actin specific probe (spec.activity $7x10^6$ cpm/ug) and the filter was exposed on X-ray film over night at -80°C.
B) To determine the half-life of ß actin mRNA in undifferentiated and 3 or 8 days differentiated myotubes, the dot area of each time point was cut out and counted in a scintillation counter. The log cpm was plotted versus the time after actinomycin addition; t 1/2 indicates the half-life of ß actin mRNA in hours.

stream sequences seem to be required. It furthermore indicates that elements outside the transcribed sequence are needed for the regulatory effect, since the 3'end cDNA sequence alone did not produce this effect. Whether in fact only 3'flanking DNA is required, or whether essential sequence elements overlapping the end of the mRNA sequence are necessary, remains to be determined by a more detailed analysis. At present we cannot completely rule out

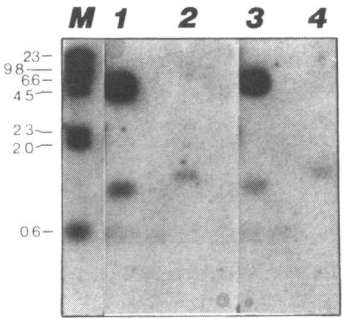


Fig. 7
Run-on transcription of the transfected pSV-ß-actin gene in nuclei from differentiated and undifferentiated C2 muscle cells.
Approximately $1x10^8$ nuclei from C2 myoblasts (lanes 1 and 2) or C2 myotubes (lanes 3 and 4) were isolated to generate nuclear RNA by run-on transcription in the presence of 200 uCi (^{32}P)UTP (54). For each hybridization $1-2x10^7$ cpm (Cerenkov) of labeled transcripts were used. 1.5 ug of pSV-ß-actin DNA digested with XbaI and Eco RI (lanes 1 and 3), and 0.5 ug of the isolated HindIII/NruI neo-fragment (lanes 2 and 4) were separated on gels and blotted onto nitrocellulose filters. The conditions for hybridization and washing were as described under Materials and Methods.

the possibility that in pSV-neo 600, and even in pSV-neo 600*, the lack of the authentic ß actin poly (A) addition site prevents the myogenic down-regulation rather than any other missing sequence element.

The stability of ß actin mRNA appears unaltered in fused C2C12 myotubes.

A possible explanation for reduced steady-state concentrations of specific mRNAs in differentiated cells could be decreased mRNA stability and thus increased degradation. To test this possibility, C2C12 cells, which had been stably transfected with the complete ß actin gene, were grown in the presence of actinomycin D at concentrations that completely inhibit gene transcription by RNA polymerase II (48) RNA was isolated from undifferentiated C2C12 cells as well as from cells that had been shifted to differentiation medium for 3 and 8 days. The relative concentration of chicken ß actin specific mRNA was measured at various times after addition of actinomycin D by blot analysis as demonstrated in fig.6A. To compensate for the considerably lower concentrations of ß actin mRNA in fused myotubes, 2 to 4 fold more of total RNA was spotted as compared to proliferating myoblasts. As shown in fig.6B, the stability of chicken ß actin mRNA could be determined in undifferentiated and 3 or 8 day differentiated mouse muscle cells based on the gradual loss of ß actin specific signals in the presence of actinomycin. Within the limits of experimental variations the half-life of ß actin

specific mRNA was found to be approximately 8 hours and no substantial change of this value was observed. Particularly, no destabilization of ß actin mRNA in well differentiated myotubes could be demonstrated under our experimental conditions. We therefore conclude that myotube-specific mRNA degradation does not essentially contribute to the down-regulation of the ß actin mRNA in differentiated muscle cells.

Nuclear run-on experiments in differentiating C2C12 cells do not reveal an altered rate of transcription for the stably integrated chicken pSV-ß-actin gene.

Isolated nuclei from C2 myoblasts and C2 myotubes were used to generate nuclear RNA in run-on experiments and the rate of transcription was determined for the stably integrated chicken pSV-ß-actin and the pSV2-neo gene. As shown in fig.7 by blot analysis, the relative level of pSV-ß-actin transcripts as compared to the level of the presumably constitutive pSV2-neo transcripts appeared virtually unchanged. A slight decrease of the pSV2-neo RNA was sometimes observed in the fused myotubes probably due to the fact that differentiated myotubes were cultured in the absense of the drug G 418. These results were reproduced in seven independent experiments which, in two cases, involved the complete chicken ß-actin gene (transcription from its own promoter). From these data we like to conclude that the down-regulation of the ß-actin gene in differentiated skeletal muscle cells is not primarily the result of a transcriptional control mechanism.

DISCUSSION

Cytoplasmic ß actin constitutes one of the major cellular proteins in most eucaryotic cells but is not expressed in differentiated myofibrils. To analyze the molecular events of muscle specific regulation of the ß actin gene, we utilized the fact that the chicken ß actin gene, when stably introduced into the C2C12 mouse muscle cell line, is regulated in a differentiation specific manner (24,25). To determine those segments of the gene that are responsible for the myotube specific down-regulation of ß actin mRNA, chimeric genes were constructed containing either the promoter, the gene body without its own promoter, or the 3'end region. Upon transfection into myogenic mouse cells the hybrid genes containing the 3'end sequence of the chicken ß actin gene exhibited the same muscle specific down-regulation as the endogenous gene or the complete chicken ß actin gene.

In contrast, hybrids between the ß actin promoter and other reporter genes did not show the typical down-regulation but rather behaved like constitu-

tively expressed "house keeping" genes. This indicated that the promoter of the cytoplasmic ß actin gene by itself was constitutively transcribed in all cellular backgrounds including the well differentiated myofibrils of C2C12 cells in culture. Although it was evident from our results that the 3'end segment was also capable of mediating down-regulation when the SV40 enhancer-promoter was included in the construct, and neither the ß actin promoter nor the coding part of the gene body was required for the appropriate regulation of the ß actin mRNA level in muscle cells, we have not determined the precise length and location of the essential sequence element. It is interesting to note that large blocks of sequence homology have been conserved in the 3'untranslated region of vertebrate ß actin genes during evolution (26,49, 50). This strong conservation of a noncoding sequence suggests the functional significance of these elements. According to our results, however, it appeared that 3'end mRNA sequences alone were not sufficient for the regulatory effect, but the inclusion of 3'flanking sequences seemed to be required. An alternative explanation of the results could be that the removal of parts of the authentic poly A addition site in plasmid pSV2-neo 600 interferes with the proper down-regulation rather than a required regulation sequence element that is located in the 3'flanking sequence downstream of the polyadenylation site. Detailed mutational experiments are necessary to further delineate the minimal essential sequence for myogenic down-regulation.

Posttransciptional regulation of eucaryotic mRNA expression has been shown to involve sequence elements located in the 3'untranslated region of lymphokine mRNAs, e.g. GM-CSF (48), c-fos mRNA (51), human gastrin mRNA (52) and the transferrin receptor mRNA (53). At least in the first three cases AU-rich sequence elements appear to modulate the stability of the mRNAs. The involvement of the 3'end region of the ß actin gene in the tissue-specific down-regulation suggested a similar mechanism of increased mRNA degradation for the ß actin. The determination of the decay of ß actin mRNA in actinomycin D treated myoblasts and myotubes did not reveal any significant destabilization.

Several experiments with nuclear run-on transcripts in myoblasts and myotubes did not indicate gross variations in transcriptional rates of the ß actin gene. Taking together these results, we would like to hypothesize that, as a possible mechanism for ß-actin down-regulation, interference with transcriptional termination might lead to post-transcriptional events such as altered processing or transport resulting in a diminished level of ß actin mRNA.

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