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Displacement of BrdUrd-induced YY1 by serum response factor activates skeletal α-actin transcription in embryonic myoblasts

TE-CHUNG LEE*, YANG SHI†, AND ROBERT J. SCHWARTZ*

*Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030; and †Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA 02115

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ABSTRACT **Muscle-restricted transcription of the skeletal α-actin gene is controlled in part by a positive regulator, serum response factor (SRF), and a negative regulator, F-ACT1, which bind competitively to the most proximal serum response element (SRE1). We show here that F-ACT1 is identical to a transcription factor recently cloned and described as YY1, NF-E1, δ, or UCRBP. We found that although the DNA-binding activity of SRF accumulates during myogenesis, that of YY1 diminishes simultaneously. Myoblasts rendered incapable of differentiation by BrdUrd treatment exhibited the highest level of YY1 and the lowest level of SRF activities. Transfected SRF could directly transactivate the skeletal α-actin promoter by overcoming the inhibitory effect of BrdUrd-induced YY1. The transactivation depends on intact SRE DNA elements and requires the DNA-binding/dimerization domain of SRF as well as its C-terminal half rich in serines and threonines. Since the functions of YY1 and SRF appear to be developmentally regulated, the convergence of their binding sites upon the SRE constitutes an integrated mechanism whereby temporal and spatial muscle gene expression may be accomplished.**

Transcription of sarcomeric actin genes is developmentally regulated during myogenesis through fine-tuned control mechanisms involving multiple cooperative and antagonistic transcription factors (1–3). Among the cis-acting DNA elements recognized by these factors is the sequence CC(A/T)$_6$GG of the serum response element (SRE), which is present in a number of growth factor-inducible and myogenic specified genes (4, 5). Intact SREs are required for skeletal α-actin gene activity (3). We proposed that the skeletal α-actin promoter can be repressed or activated by two functionally opposite SRE-binding proteins, F-ACT1 and serum response factor (SRF), depending on the outcome of their competitive interactions with the most proximal SRE (6). It is not clear, however, to what extent the two seemingly ubiquitous DNA-binding factors may contribute to muscle-specific expression of the actin genes.

SRF is highly conserved throughout evolution (5, 7). Its DNA-binding and dimerization domain of 90 amino acids, termed the MADS box, bears striking homology to yeast transcription factors MCM1 and ARG80 (8). In the mammalian c-*fos* and the yeast mating-type control genes, SRF and MCM1 are thought to function by recruiting accessory factors to their DNA target sites (9, 10). Using a DNA probe spanning the conserved DNA-binding domain, Pollock and Treisman (8) have isolated several SRF-related proteins (RSRFs), which bind to the regulatory regions of nonmuscle and muscle-specific genes as MEF-2 sites (11). In spite of the efforts in identifying these potential gene regulators, their precise roles in muscle-specific gene expression remain largely undefined. In particular, a direct demonstration that SRF or RSRF may act as transcriptional activators of muscle-specific genes has not been documented.

SRF can be detected in a wide variety of cell types and in most cases its DNA-binding activity following serum stimulation remains unchanged (5). However, using an improved binding assay condition here, we are able to show that the DNA-binding activities of SRF and its competitor F-ACT1 are modulated differentially during normal myogenesis. The SRF-binding activity increased as myogenesis proceeded with the ending of myoblast replication and the onset of fusion, whereas the F-ACT1-binding activity was reduced with terminal differentiation. F-ACT1 is identical to a multifunctional transcription factor recently cloned and described variously as YY1, NF-E1, δ, or UCRBP (12–15). We found that the factor can be induced by the differentiation inhibitor BrdUrd. BrdUrd has the effect of blocking the expression of the differentiated phenotype without significantly affecting the general functions of a cell (16). In particular, transcription of the skeletal α-actin gene was profoundly inhibited by the nucleotide analogue (17). It is shown here that BrdUrd simultaneously enhanced the F-ACT1 and diminished the SRF DNA-binding activities. The differential effects of BrdUrd therefore favor occupation of the SRE1 by F-ACT1, coincident with the repression of muscle actin gene transcription. Transfection of SRF cDNA, however, is able to overcome the inhibitory effect of F-ACT1, which allowed us to map the transactivation domain of SRF involved in activating the skeletal α-actin transcription.

MATERIALS AND METHODS

Primary Embryonic Myoblast Culture. Primary 11-day chicken embryonic myoblast cultures were established as described (3) except that 6×10^5 cells were seeded per 90-mm dish and 30 μM BrdUrd was included in the medium. Calcium phosphate-mediated DNA transfection and chloramphenicol acetyltransferase (CAT) assay were as described (3, 6) and cells were harvested 48 hr after transfection.

Plasmid Construction. The expression vector used for constructing pMSV-SRF was modified from pEMSV (18) as described below. The *Eco*RI–*Kpn* I fragment of pEMSV was replaced by a 50-base-pair (bp) synthetic polylinker containing *Eco*RI, *Sal* I, *Sph* I, *Apa* I, *Bam*HI, *Pst* I, *Xba* I, and *Kpn* I, creating pTC20. A 250-bp *Bam*HI–*Xba* I fragment isolated from pEMSV was inserted into pTC20 cut with *Bam*HI and *Xba* I, generating pTC21. The *Nde* I (filled)–*Bam*HI SRF DNA fragment isolated from pAR-SRF (19) was inserted into the *Sal* I (filled) and *Bam*HI sites of pTC21, creating pMSV-SRF. DM1 was constructed by digestion of pAR-SRF with *Apa* I, removing amino acids 54–114 of SRF. DM2 was constructed by digestion of pAR-SRF with *Sac* II, removing

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Abbreviations: SRE, serum response element; SRF, serum response factor; RSRF, SRF-related protein; CAT, chloramphenicol acetyltransferase.

amino acids 10–72. DM3 was generated by digestion of DM2 with *Sma* I and *Stu* II, further deleting amino acids 141–172. To construct DM4, pMSV-SRF was first cut with *Sph* I and trimmed by nuclease S1. The linearized DNA was further digested with *Bgl* II and blunted with Klenow. An in-frame deletion removing amino acids 246–414 was selected following DNA ligation. To construct DM5, pMSV-SRF was cut with *Bgl* II and blunted with Klenow. An in-frame TGA termination codon at amino acid 246 was created following DNA ligation. The pSK110-CAT construct was generated by replacing a *Bgl* II–*Hin*dIII fragment of pOVA-CAT (20) with a *Bgl* II–*Hin*dIII fragment isolated from M19-CAT (3).

Crude Protein Extracts and Gel Shift Assays. Myoblasts grown on 90-mm dishes were scraped off in 1 ml of ice-cold phosphate-buffered saline. Cells were spun and resuspended in 0.1 ml of 10 mM Hepes, pH 7.9/0.5 M KCl/0.5 mM EDTA/1 mM dithiothreitol/0.5 mM phenylmethylsulfonyl fluoride/5% glycerol. Cells were lysed by three freeze-thaw cycles and lysates were incubated on ice for 30 min with occasional shaking. Crude protein extracts were then clarified at 4°C in a Microfuge for 10 min, aliquoted, and stored at −70°C. An improved gel shift assay using poly(dG-dC) as nonspecific DNA competitors was described recently (21), which allowed for simultaneous detection of the SRF and F-ACT1 DNA-binding complexes directly from crude protein extracts. In brief, a cloned SRE1 fragment (3) was used as a probe and the DNA-binding assay was evaluated by electrophoresis in 6% polyacrylamide gels cast in 0.5× Tris-glycine buffer.

Purification of F-ACT1. Heparin agarose-enriched F-ACT1 as described previously (6) was further purified through phosphocellulose and SRE1 DNA-affinity columns. The phosphocellulose column was washed with 0.3 M KCl and F-ACT1 was eluted with 0.5 M KCl. The eluted fractions were diluted 10-fold with column buffer (6) and applied to the SRE1 affinity column. After wash at 0.1 M KCl, F-ACT1 was eluted at 0.4 M KCl. For zinc removal, purified F-ACT1 was first dialyzed in the presence of 10 mM EDTA for 2 hr followed by 2 hr in 0.1 mM EDTA.

RESULTS

F-ACT1 Is Indistinguishable from the Common Factor YY1. A common factor capable of recognizing sites in several diverse promoters (22), including the skeletal α-actin promoter, has recently been cloned by several groups (12–15). We compared the DNA sequences recognized by the common factor (referred to as YY1) and F-ACT1 in Fig. 1*A*. This comparison coupled with our previous mutational analysis of the F-ACT1-binding site (3, 6) generates a consensus, AANATGGNC/G. We have taken several approaches to verify that F-ACT1 is indeed identical to YY1. Fig. 1*B* shows that the *in vitro* translated YY1 had the same gel shift mobility as purified F-ACT1. Faster-migrating complexes, which were shown to be derived from proteolytic cleavages of F-ACT1 (23), were similarly present for both factors. Mutant SRE1 DNA sequences (M14.5 and M15) previously shown to eliminate F-ACT1 binding were then used to examine the binding specificity of the *in vitro* translated YY1. Fig. 1*B*

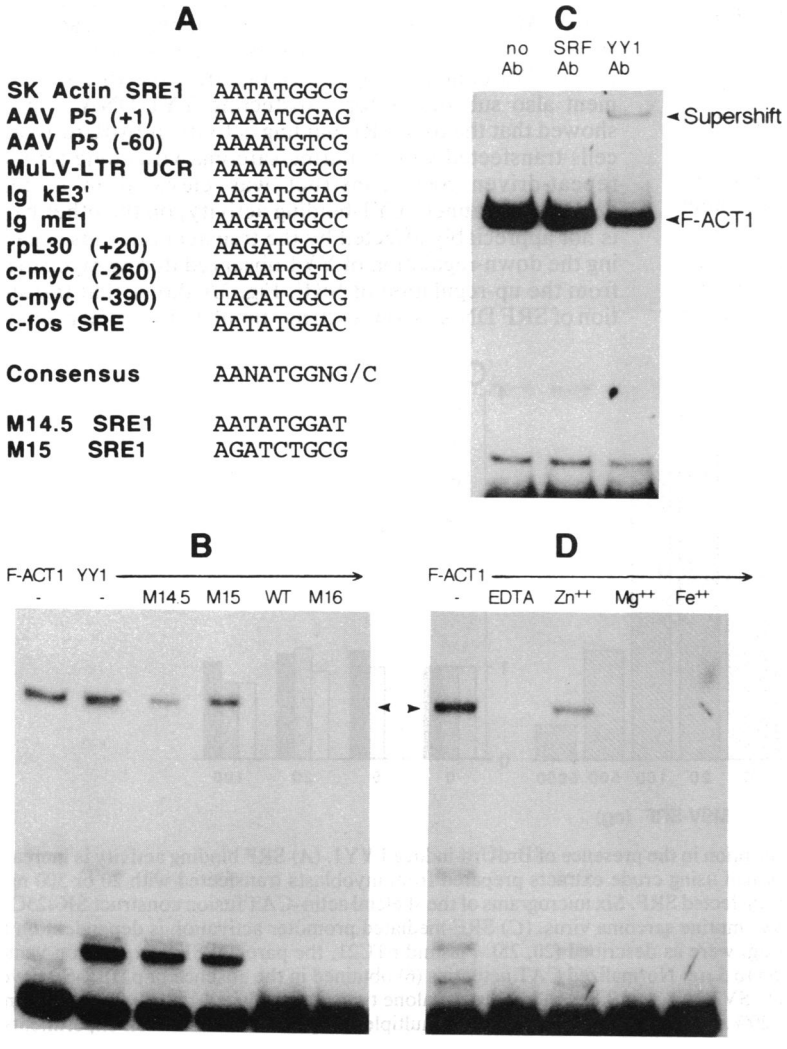

FIG. 1. F-ACT1 is indistinguishable from YY1. (*A*) DNA sequence comparison of documented binding sites for F-ACT1 (6), YY1 (12), NF-E1 (13), δ (14), UCRBP (15), and CF1 (22). DNA sequences for two skeletal α-actin SRE1 mutations that abolish F-ACT1 binding partially (M14.5) or completely (M15) are shown on the bottom (3, 6). Mutation M16 changed DNA sequences outside the nonanucleotide motif and does not affect F-ACT1 binding. MuLV-LTR, murine leukemia virus long terminal repeat. (*B*) F-ACT1 and YY1 have identical DNA-binding sequence specificity. Purified F-ACT1 was used in the left lane. *In vitro* translated YY1 was used for binding competition analysis. Duplex oligonucleotides corresponding to M14.5, M15, and M16 SRE1 mutations were used at a competitor/probe molar ratio of 100 in the indicated lanes. WT, wild type. (*C*) F-ACT1 and YY1 share common antigenicity. A gel shift assay using purified F-ACT1 was probed with 2 μl of a polyclonal anti-SRF antibody (kindly provided by R. Prywes; see ref. 19) and 0.5 μl of a monoclonal anti-YY1 antibody. Detailed information regarding generation of the monoclonal antibody will be reported elsewhere. The supershift caused by anti-YY1 antibody is indicated. (*D*) F-ACT1 and YY1 are zinc fingers. Purified F-ACT1 dialyzed in EDTA was used except in the left lane. Divalent cations were added to the binding reaction mixtures at a final concentration of 0.25 mM in the indicated lanes.

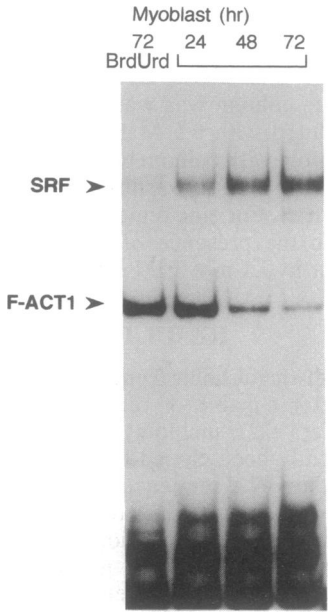

FIG. 2. SRF and F-ACT1 DNA-binding activities are differentially regulated during normal myogenesis. Crude protein extracts were prepared from primary myoblast cultures 24, 48, and 72 hr after plating; myoblasts grown in medium containing 30 μM BrdUrd were harvested at 72 hr after plating. Four micrograms of protein extracts and 0.2 ng of labeled cloned SRE1 fragment were used in each lane.

showed that the two mutant SRE1 sequences failed to competitively inhibit the YY1 binding, thus demonstrating an identical DNA-binding specificity for F-ACT1 and YY1. Furthermore, a monoclonal antibody directed against YY1 was used as a structural probe in Fig. 1*C*. The anti-YY1 monoclonal antibody specifically recognized purified F-ACT1, converting the F-ACT1 binding complex to a supershift, while a polyclonal anti-SRF antibody had no effect on the F-ACT1 complex. Since YY1 is a zinc finger protein belonging to the Kruppel gene family (12), we tested the zinc dependence of the F-ACT1-binding activity in Fig. 1*D*, which shows that Zn^{2+} but not other divalent cations restored the binding activity of F-ACT1 dialyzed in the presence of EDTA. We therefore conclude that F-ACT1 is indistinguishable from YY1.

SRE1 Is Differentially Occupied by YY1/F-ACT1 and SRF During Normal Myogenesis. We proposed a model that expression of the skeletal α-actin gene is restricted by F-ACT1, which can be displaced from the SRE1 site by SRF upon myogenic differentiation (6). Recently, we have modified gel shift assay conditions by using poly(dG-dC) as nonspecific DNA competitors, which enabled us to detect simultaneously the two competitive SRE-binding activities directly from crude muscle cell extracts (21). Fig. 2 demonstrates a dynamic and contrasting DNA-binding profile for YY1 and SRF present in cultured embryonic myoblasts. During the transition from prefusion myoblasts (24 hr), fusion myoblasts (48 hr), to myotubes (72 hr), YY1-binding activity gradually diminished while that of SRF accumulated severalfold toward terminal differentiation. This switch in trans-factor binding activities coincides with the ending of myoblast replication and precedes the appearance of skeletal α-actin mRNA (24). Fig. 2 further reveals that treatment of myoblasts with BrdUrd, which blocks myogenic differentiation and represses α-actin gene activity (17), dramatically diminished SRF content and enhanced F-ACT1-binding activity (compare cells at 72 hr with and without BrdUrd). These observations indicate that the SRF–SRE interaction is favored during myogenesis and that the actin SRE1 site can be differentially occupied at different stages of myogenesis.

Transfected SRF Activates the Actin Promoter by Overcoming BrdUrd-Induced YY1. Our initial DNA transfection study performed in fusion myoblasts failed to reveal significant transactivation function of SRF on the skeletal α-actin promoter. We therefore chose to use myoblasts treated with BrdUrd, which reduced endogenous SRF activities and induced YY1 contents (Fig. 2). In practice, the BrdUrd treatment also substitutes for transfecting YY1 DNA. Fig. 3*A* showed that the total SRF binding activity in BrdUrd-treated cells transfected with a murine sarcoma virus long terminal repeat-driven SRF construct was elevated in a dose-dependent manner. YY1-binding activity, on the other hand, is not appreciably affected by the transfection, thus separating the down-regulation of YY1 observed during myogenesis from the up-regulation of SRF. Fig. 3*B* shows that transfection of SRF DNA as low as 20 ng stimulated skeletal promoter

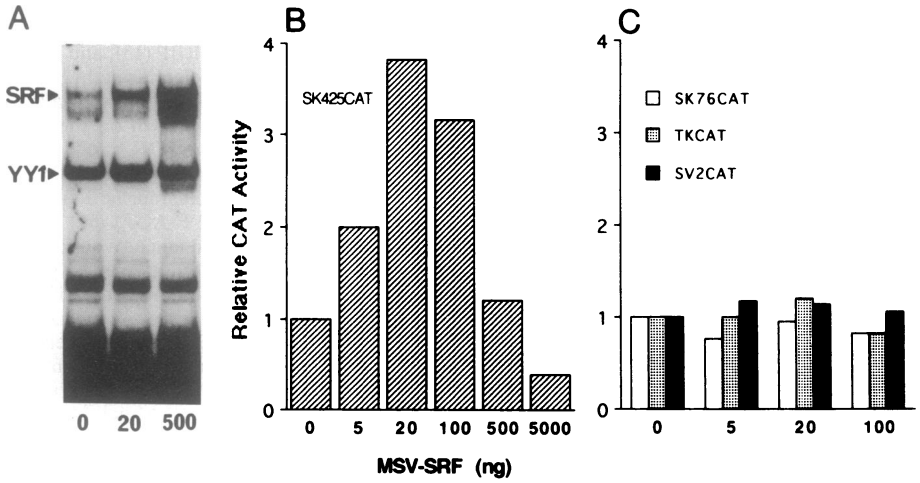

FIG. 3. Transfected SRF activates skeletal α-actin transcription in the presence of BrdUrd-induced YY1. (*A*) SRF binding activity is increased by transfection of pMSV-SRF. A gel shift assay was performed using crude extracts prepared from myoblasts transfected with 20 or 500 ng of pMSV-SRF. (*B*) Activation of skeletal α-actin promoter by transfected SRF. Six micrograms of the skeletal actin–CAT fusion construct SK425CAT described previously (3) was used in each transfection. MSV, murine sarcoma virus. (*C*) SRF-mediated promoter activation is dependent on the SRE. SK76CAT (12 μg), TKCAT (3 μg), and SV2CAT (3 μg) were as described (20, 25). Plasmid pTC21, the parental SRF expression vector, was used to adjust the expression vector DNA concentration to 5 μg. Normalized CAT activities (6) obtained in the absence of pMSV-SRF were arbitrarily set at 1. Under the BrdUrd condition, SK425CAT, SV2CAT, and TKCAT constructs alone typically produced 150, 40,000, and 5 units of CAT activity, respectively. Experimental errors were <20%. Values are representatives of multiple independent transfection experiments.

activity near 4-fold. A significant transfection-mediated promoter activation can typically be observed with <100 ng of SRF DNA under the BrdUrd conditions. Inhibition of skeletal promoter activity observed with higher doses of SRF DNA is most likely due to its nonspecific inhibitory effect, as also noticed in *in vitro* transcription assays (26). The stimulation was SRE dependent since removal of the three actin SREs from the skeletal promoter abolished the activation (SK76CAT in Fig. 3*C*) and the three SREs are the only demonstrable positive cis-acting elements within the deleted promoter region (3). Furthermore, no activation could be seen with the thymidine kinase and simian virus 40 promoters, neither of which binds SRF.

The Transactivation Domain of SRF Resides Within Its C-Terminal Half. Although the DNA-binding domain of SRF has been determined, its transactivation domain has only been preliminarily indicated by an *in vitro* study (26). The ability to demonstrate a SRF-mediated promoter activation in muscle cells allowed us to define its protein domain required for myogenic transactivation. A series of SRF deletion mutants (DM) were generated as outlined in Fig. 4*A*. Among the five DM mutants, only DM3 (Δ10–72/Δ141–172) lost its ability to bind DNA due to an internal deletion within the DNA-binding domain (data not shown; also see refs. 5 and 8). Fig. 4*B* compares the functional activity of the DM mutants to transactivate the skeletal α-actin promoter. Two N-terminal deletion mutants, DM1 (Δ54–114) and DM2 (Δ10–72), were as effective as the wild-type SRF in activating skeletal promoter function. Notably, the casein kinase II phosphorylation site previously shown to enhance the DNA-binding affinity of SRF (19) was deleted in DM1 without significantly affecting the transactivation function of SRF. In addition, DM2 appeared to fully retain its stimulatory activity at the higher DNA inputs, which might be caused by the deletion of a potential inhibitory domain, a glycine- and alanine-rich domain (27). On the other hand, no activation could be observed with the other three SRF deletion mutants. DM3, which failed to bind DNA, also failed to transactivate, consistent with our conclusion that an intact SRE DNA is required for the function of SRF. This finding indicates that the DNA-binding domain of SRF is essential but insufficient for dictating its role as a transcriptional activator. DM4 (Δ246–414) and DM5 (Δ246–508), which removed the C-terminal half partially and completely, were both inactive. A C-terminal activation domain for SRF is also consistent with the presence of a large number of potential protein phosphorylation sites and glutamine and proline residues, which were shown to be associated with other transcriptional activators (28).

DISCUSSION

We conclude that F-ACT1, responsible for repressing the skeletal α-actin promoter, is identical to YY1 based on their gel mobility, DNA-binding sequence specificity, zinc-mediated protein structure, and immunological cross-reactivity. YY1 has been shown to mediate the function of the adenovirus E1A protein and can act either as a transcriptional activator or as a repressor (12–15). Interestingly, YY1 appears to activate c-*myc* expression (K. Calame, personal communication), which has been shown to down-regulate myogenic events (29). E1A, another potent myogenic inhibitor as well (30), stimulates c-*myc* activity. We therefore speculate that YY1, aside from being a direct repressor of the α-actin gene, might suppress myogenic events through its ability to activate the expression of c-*myc*. Consistent with this hypothesis, inhibition of the α-actin promoter function caused by transfected YY1 DNA could only be partially relieved by cotransfected SRF (T.-C.L., unpublished results), suggesting a dominant negative effect of YY1.

Presumably, the functional diversity of YY1 might be related to its structural plasticity (14). In this aspect, we showed that the YY1 DNA-binding activity can apparently be

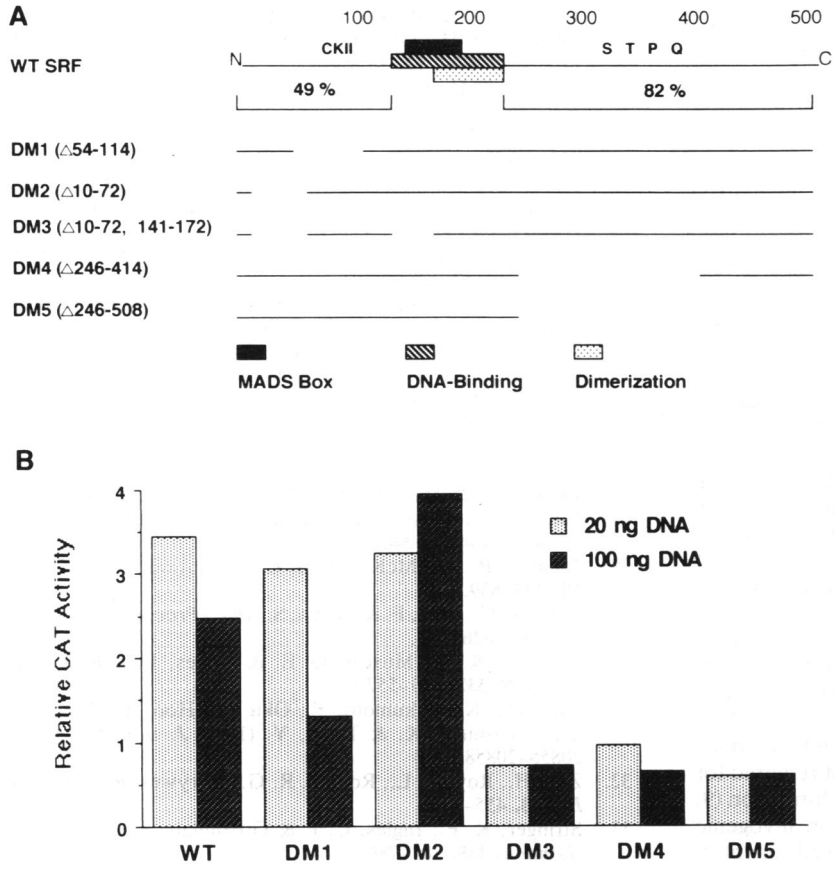

FIG. 4. Transactivation domain of SRF resides within its C-terminal half. (*A*) SRF map showing its functional domains and deletion mutants. The DNA-binding/dimerization domain and the MADS box have been described (8). The homology between the human and *Xenopus* SRF is 49% at the N-terminal portion and 82% at the C-terminal half. Also shown are the casein kinase II phosphorylation site and the C-terminal domain rich in serine, threonine, proline, and glutamine. Amino acids deleted in each mutant (DM) are indicated by numbers. WT, wild type. (*B*) The C-terminal half of SRF contains an activation domain. Transfections were as described in the legend to Fig. 3 except that 12 μg of SK110CAT was used, which typically produced 15 units of CAT activity in the absence of pMSV-SRF. Activities obtained without pMSV-SRF were again set at 1. Experimental errors were <20%. Each SRF DNA construct was tested at 20 ng and 100 ng.

induced by BrdUrd in myoblasts and is enriched in nonmuscle cell types (6). If YY1 transcription itself is constitutive, as suggested by its relatively constant mRNA level during differentiation of F9EC cells (15), translational or posttranslational mechanisms may be involved in modulating its DNA-binding activity. Given the finding that E1A can modify the function of YY1, there may exist other YY1 accessory factors, which may alter or neutralize its DNA-binding activity under various conditions. It is possible that BrdUrd treatment of myoblasts might inactivate a YY1 accessory factor, thereby increasing the concentration of free YY1 capable of binding DNA. If true, the accessibility of its interacting protein partners under different cell growth conditions may provide another level of regulatory cues. We also noticed that YY1 is prone to degradation during myogenesis (23), raising the possibility that protease-mediated degradation events may also contribute to diminished YY1 DNA-binding activity.

Our results indicate that SRF is the primary activator for the skeletal α-actin gene. It should be mentioned that the single E box (CACCTG centered at -155) of the actin promoter can be mutated without significantly affecting promoter activity, although the E box binds MyoD/E12 heterodimer with high affinity (T.-C.L., unpublished results). How does a seemingly ubiquitous factor, such as SRF, regulate myogenic specific gene expression? (*i*) We note that transcription of the SRF gene is itself transiently increased upon serum stimulation (5). We have found that SRF transcripts accumulate and coincide with SRF binding activity during embryonic myogenesis (J. Croissant, T.-C.L., and R.J.S., unpublished result). Similar to our finding, Mohun *et al.* (7) observed a rapid accumulation of *Xenopus* SRF transcripts following gastrulation. The increased SRF transcription following serum stimulation and during gastrulation and myogenesis can thus be mediated through a conserved signaling mechanism, which could be overridden by BrdUrd treatment. (*ii*) The function of SRF might be temporally regulated by SRF accessory factors, as has been shown for the yeast MCM1 (9). It is possible that the activities of SRF and its accessory factors are down-regulated in BrdUrd-treated myoblasts. This might account for the observed promoter activation of <5-fold by transfected SRF alone. (*iii*) The c-*fos* SRE has been shown to be the target of the activated c-*raf-1* kinase (31). Growth factor-mediated phosphorylation events of SRF might therefore contribute to its myogenic regulated transcriptional activity.

Our *in vivo* study demonstrating activation of the actin SRE by SRF is quite consistent with that reported by Prywes and Zhu (26) using an *in vitro* transcription approach to show activation of the c-*fos* SRE. In both cases, up to a 5-fold transcriptional activation could be observed only with low amounts of SRF, whereas high amounts of SRF nonspecifically inhibited transcription. Both studies indicated that the N-terminal domain of SRF is dispensable for its transactivation function. This finding may not be unexpected considering the fact that the N-terminal domain is not highly conserved between the human and *Xenopus* SRFs. However, given the documented role of casein kinase II in enhancing the binding activity of SRF, it is somewhat surprising to learn that deleting the phosphorylation site for casein kinase II from SRF has little effect on its transactivation function. We note that the N-terminal domain of SRF contains a glycine- and alanine-rich domain, which could act as a negative-acting domain in several other transcription factors (27). Thus, deleting the potential negative-acting domain of SRF may compensate for the loss of the casein kinase II site. Like transactivation domains found in other transactivators, the C-terminal domain of SRF is particularly rich in serine, threonine, proline, and glutamine residues (28). Since the C-terminal domain is not required for directing either SRF or MCM1 ternary complex formation (9, 10) and $p62^{TCF}$ complexes were not observed in myogenic extracts (Fig. 2), we speculate that it may be used for interacting with basal transcriptional machinery. This view is supported by the finding that the TFIID preinitiation complex can be stabilized by SRF (32). Whether SRF can contact through its C-terminal domain TFIID or TFIID-associated components, as found for VP16 (33), remains to be determined. In conclusion, the DNA-binding/dimerization domain and the C-terminal half of SRF can act together to partially overcome the myogenic inhibitory effect of YY1/F-ACT1.

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1. Grichnik, J. M., Bergsma, D. J. & Schwartz, R. J. (1986) *Nucleic Acids Res.* **14**, 1683–1701.
2. Minty, A. & Kedes, L. (1986) *Mol. Cell. Biol.* **6**, 2125–2136.
3. Chow, K. L. & Schwartz, R. J. (1990) *Mol. Cell. Biol.* **10**, 528–538.
4. Petropoulous, C. J., Rosenberg, M. P., Jenkins, N. A., Copeland, N. G. & Hughes, S. H. (1989) *Mol. Cell. Biol.* **9**, 3785–3792.
5. Norman, C., Runswick, M., Pollock, R. & Treisman, R. (1988) *Cell* **55**, 989–1003.
6. Lee, T. C., Chow, K. L., Fang, P. & Schwartz, R. J. (1991) *Mol. Cell. Biol.* **11**, 5090–5100.
7. Mohun, T. J., Chambers, A. E., Towers, N. & Taylor, M. V. (1991) *EMBO J.* **10**, 933–940.
8. Pollock, R. & Treisman, R. (1991) *Genes Dev.* **5**, 2327–2341.
9. Dolan, J. W. & Fields, S. (1991) *Biochim. Biophys. Acta* **1088**, 155–169.
10. Shaw, P. E., Frasch, S. & Nordheim, A. (1989) *EMBO J.* **8**, 2567–2574.
11. Gossett, L. A., Kelvin, D. J., Sternberg, E. A. & Olson, E. N. (1989) *Mol. Cell. Biol.* **9**, 5022–5033.
12. Shi, Y., Seto, E., Chang, L. S. & Shenk, T. (1991) *Cell* **67**, 377–388.
13. Park, K. & Atchison, M. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9804–9808.
14. Hariharan, N., Kelley, D. E. & Perry, R. P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9799–9803.
15. Flanagan, J. R., Becker, K. G., Ennist, D. L., Gleason, S. L., Driggers, P. H., Levi, B. Z., Appella, E. & Ozato, K. (1992) *Mol. Cell. Biol.* **12**, 38–44.
16. Weintraub, H., Campbell, G. L. & Holtzer, H. (1972) *J. Mol. Biol.* **70**, 337–343.
17. Schwartz, R. J. & Rothblum, K. N. (1981) *Biochemistry* **20**, 4122–4129.
18. Harland, R. & Weintraub, H. (1985) *J. Cell Biol.* **101**, 1094–1099.
19. Manak, J. R., de Bisschop, N., Kris, R. M. & Prywes, R. (1990) *Genes Dev.* **4**, 955–967.
20. Bradshaw, M. S., Tsai, M. J. & O'Malley, B. W. (1988) *Mol. Endocrinol.* **2**, 1286–1293.
21. Lee, T. C. & Schwartz, R. J. (1992) *Nucleic Acids Res.* **20**, 140.
22. Riggs, K. J., Merrell, K. T., Wilson, G. & Calame, K. (1991) *Mol. Cell. Biol.* **11**, 1765–1769.
23. Lee, T. C. & Schwartz, R. J. (1992) *BioTechniques* **12**, 486–490.
24. Hayward, L. J., Zhu, Y. Y. & Schwartz, R. J. (1988) *J. Cell Biol.* **106**, 2077–2086.
25. Bergsma, D. J., Grichnik, J. M., Gossett, L. M. A. & Schwartz, R. J. (1986) *Mol. Cell. Biol.* **6**, 2462–2475.
26. Prywes, R. & Zhu, H. (1992) *Nucleic Acids Res.* **20**, 513–520.
27. Licht, J. D., Grossel, M. J., Figge, J. & Hansen, U. M. (1990) *Nature (London)* **346**, 76–79.
28. Johnson, P. F. & McKnight, S. L. (1989) *Annu. Rev. Biochem.* **58**, 799–839.
29. Falcone, G., Tato, F. & Alema, S. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 426–430.
30. Webster, K. A., Muscat, G. E. & Kedes, L. (1988) *Nature (London)* **332**, 553–557.
31. Kaibuchi, K., Fukumoto, Y., Oku, N., Hori, Y., Yamanoto, T., Toyoshima, K. & Takai, Y. (1989) *J. Biol. Chem.* **264**, 20855–20858.
32. Zhu, H., Roy, A. L., Roeder, R. G. & Prywes, R. (1991) *New Biol.* **3**, 455–464.
33. Stringer, K. F., Ingles, C. J. & Greenblatt, J. (1990) *Nature (London)* **345**, 783–786.