BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **237,** 170–176 (1997)
ARTICLE NO. RC976980

Distal Regulatory Regions of the Rat *MRF4* Gene

Carol M. Kerkvliet and Timothy J. Hinterberger[1]
Biomedical Program and Department of Biological Sciences, University of Alaska Anchorage, 3211 Providence Drive, Anchorage, Alaska 99508

Received June 9, 1997

MRF4 is a muscle-specific transcription factor that is expressed both in embryonic somites and later in fetal and adult muscle fibers. *Cis*-regulatory elements of the *MRF4* gene responsible for its complex expression pattern have not yet been identified, although previous studies of the rat *MRF4* gene have demonstrated the presence of enhancer activity located several kilobases 5′ to the transcription start site. Using cell transfection assays in vitro, we have now localized one of the regulatory regions of *MRF4* to a 590-base-pair sequence between 4 and 5 kilobases upstream from the start site. This sequence region functioned as an enhancer in combination either with the *MRF4* promoter or with the viral *thymidine kinase (tk)* promoter. Deletion analysis of *MRF4* indicated the existence of another regulatory region, closer to the promoter, which functioned as an enhancer in combination with the *MRF4* promoter but not with the *tk* promoter.

The muscle regulatory factor (MRF) gene family encodes the basic/helix-loop-helix (bHLH) transcription factors MyoD, myogenin, Myf-5 and MRF4, which are expressed exclusively in skeletal muscle cells (1-3). Like all bHLH factors, MRFs regulate transcription by binding via their basic domains to DNA sequence elements containing an E-box motif, CANNTG. The HLH domain interacts with ubiquitous bHLH proteins such as E12, forming heterodimers essential for efficient DNA binding. MRFs also function cooperatively with transcription factors of the MEF2 family, which are highly enriched in muscle and certain other tissues. Besides binding to E boxes to activate transcription, MyoD and myogenin have been shown to interact directly with MEF2 proteins in their DNA-binding domains, enabling either the MEF2 or the MRF protein to activate transcription through the other's binding site (4). Much evidence now supports that idea that protein-protein interactions involving the MRFs (5-7), or post-translational modifications of MRFs (3, 8), are the key mechanisms by which a wide variety of growth and differentiation signaling pathways converge to regulate the expression of skeletal muscle-specific genes.

Myf-5 is the first MRF protein detectable immunohistochemically in the mouse embryo, in dorsal anterior somite cells prior to formation of the dermamyotome. Myogenin protein is detected next, in myotome cells only, and MRF4 appears about 12 hours later, followed by MyoD. The distinct expression patterns of the four MRFs seen in dorsal and ventral subdomains of the somites are consistent with the idea that skeletal muscle cells originate and differentiate via multiple molecular pathways utilizing the MRFs' distinct transcriptional roles (9). As in the somites, *Myf-5* is the first MRF mRNA detectable in limb myoblasts and its expression is transient (10). *Myogenin* and *MyoD* are expressed together in limb, in contrast to their pattern in the somite where they appear sequentially (11).

While the other MRF genes show peak expression in limb muscle at embryonic and early fetal stages, *MRF4* transcripts accumulate to their highest levels in late fetal and adult muscle (12-15). In cell cultures of primary limb myoblasts or muscle cell lines, *MRF4* transcripts accumulate only after myoblast fusion (12, 15). Although MRF4 activates expression of some muscle fiber-specific genes, it may also repress some early, transiently expressed muscle genes (16). Transgenic embryos in which *MRF4* expression is driven by the *myogenin* promoter display altered patterns of muscle gene expression, indicating that precocious production of MRF4 accelerates myocyte development in the most rostral somites (17).

In order to understand how the particular patterns of MRF gene expression are initiated and maintained, attention has begun to be directed toward their transcriptional regulatory elements. A complex network of auto- and cross-activation appears to contribute to regulation of the MRF genes (1-3, 8). Whether this activa-

[1] To whom correspondence should be addressed. Fax: (907) 786-4607. E-mail: aftjh@uaa.alaska.edu.

0006-291X/97 $25.00

tion is direct, through binding of MRF proteins to E boxes in the regulatory regions of their own and each other's genes, or indirect, through induction of other factors such as MEF2, or both direct and indirect, remains a major question in the molecular control of muscle development.

Of the four members of the MRF family, *MyoD* gene regulation has been the subject of the most study. Three regulatory regions have been identified in the 5′ flanking sequence of *MyoD* genes from mice and humans: the promoter (18-20); the so-called distal regulatory region (DRR) at approximately −5 kilobases (kb) (19, 21); and an enhancer located at −20 kb (18, 22, 23). Quail *MyoD* is regulated by a complex array of *cis*-acting control sequences, including the proximal promoter, a distal region at −11.5 kb, and a relatively weak enhancer region between −3.3 and −5 kb from the transcription start site (24). A *Xenopus MyoD* promoter has been shown to utilize both an E box and a MEF2 binding site (25), while the chicken *MyoD* promoter, though it contains numerous E boxes and MEF2 binding sites, apparently depends on none of these sites for muscle-specific transcriptional activity (26).

In *myogenin* transgenic mice, a well-conserved 180-base-pair (bp) region immediately upstream from the start site of both human and mouse genes is sufficient to recapitulate its expression pattern (27, 28). This region contains two E boxes and a MEF2 binding site that are required for faithful recapitulation of normal embryonic expression. However, a transgene containing 1.6 kb of flanking sequence shows even higher activity, implying the presence of an enhancer between −0.2 kb and −1.6 kb (27). Interestingly, the 1.6-kb transgene is expressed normally in *myogenin*-knockout mice, demonstrating the absence of an obligatory autoregulatory loop (29).

For *MRF4*, only a proximal regulatory region has been described in detail. The promoters of mouse (30) and rat (31) *MRF4* genes *cis*-activate myofiber-specific expression in cultured myogenic cells, and they are strongly *trans*-activated by MyoD, myogenin, and Myf-5, acting synergistically with MEF2, but MRF4 does not activate its own promoter. The synergism of MRF proteins with MEF2 requires an intact MEF2 site in the rat minimal *MRF4* promoter but does not require an intact E box, suggesting that MRF-MEF2 protein interaction is sufficient to permit the MRF to participate in activating transcription without MRF protein binding directly to DNA (31).

Although the *MRF4* promoter by itself drives myotube-specific expression of reporter genes in transient transfection assays, other evidence has pointed to the importance of upstream enhancers for producing normal in vivo *MRF4* expression patterns. Myogenic cells stably transfected with rat *MRF4* gene clones of various length were shown to up-regulate the expression of all these genes upon differentiation, but the amount of up-regulation was diminished when a region located between about −1.5 kb and −5.0 kb from the protein-coding region was deleted (32). In transiently transfected cells, 8.5 kb of *MRF4* flanking sequence activates expression more strongly than does a 0.4-kb promoter (31), and transgenic mice reveal that these two constructs function quite differently in vivo. While both constructs exhibit myofiber-specific expression in several transgenic mouse lines, only the 8.5-kb transgene is activated in the embryonic myotomes. Even more strikingly, the 0.4-kb transgene is active in fewer than 1% of fetal myofibers, whereas all fibers express the 8.5-kb transgene (33).

Here, we describe the localization of at least some of the upstream enhancer activity of rat *MRF4* to a 590-bp region between about 4.4 kb and 5.0 kb from the transcription start site. The sequence of this region includes potential binding sites for both MRF and MEF2 proteins. Although this fragment was unique within the 8.5 kb of *MRF4* 5′ flanking sequence in its ability to function as an enhancer when linked to an exogenous promoter, deletion studies showed that a second region between 3.5 kb and 0.4 kb from the *MRF4* start site also enhances activity of the *MRF4* promoter.

MATERIALS AND METHODS

Gene constructions. Characterization of a rat genomic clone containing the *MRF4* gene was reported previously (32). Restriction enzyme fragments of the 8.5-kb *MRF4* 5′ flanking region, labeled ① through ⑤ in Figure 1, were inserted 5′ of the herpes simplex *thymidine kinase* promoter in plasmid *tk*CAT (34) with *Bam*H I linkers. The fragment labeled ③ was used to generate the construct shown as AR3.5 in Fig. 1. Subfragments of AR3.5 were obtained from complete or partial digests at the restriction enzyme sites indicated, followed by addition of *Hin*d III linkers and insertion into *tk*CAT. Correct insertion and orientation of the constructs were confirmed by sequencing with Sequenase version 2.0 (Amersham), as was the complete sequence of the fragment AV855. Subcloning of 5′ flanking sequence fragments from the original *MRF4* gene clone into a nuclear-localized β-galactosidase reporter plasmid to make constructs −8500*nlacZ* and −336*nlacZ* also was described previously (31). The constructs −5000*nlacZ* and −3500*nlacZ* were prepared by cutting the 8.5-kb fragment at the corresponding *Apa* I and *Hin*d III restriction enzyme sites indicated in Fig. 1 and at a unique *Sal* I site contained within the −336 promoter region, then ligating to *Sal* I-digested −336*nlacZ* to recreate the genomic sequence. The AV855 fragment was subcloned into pBluescript (Stratagene) to facilitate sequencing prior to ligating it into a unique *Hin*d III site at the 5′ end of −336*nlacZ*, thus the AV855− 336*nlacZ* construct includes some pBluescript polylinker sequence between the two *MRF4* fragments.

Cell cultures and transfections. Mouse C2C12 myoblasts were grown in DMEM plus 20% fetal bovine serum (Hyclone or Summit Biotechnology). For analysis of gene expression in myotubes, calcium phosphate precipitates (35) of equimolar amounts of plasmids were added to myoblast cultures of approximately 80% to 100% confluence on gelatin-coated dishes. In the case of *tk*CAT gene constructs, the amounts of plasmids were equivalent to 5μg of the negative control *tk*CAT. After 6 hours, myoblasts were shocked with 20% glycerol in

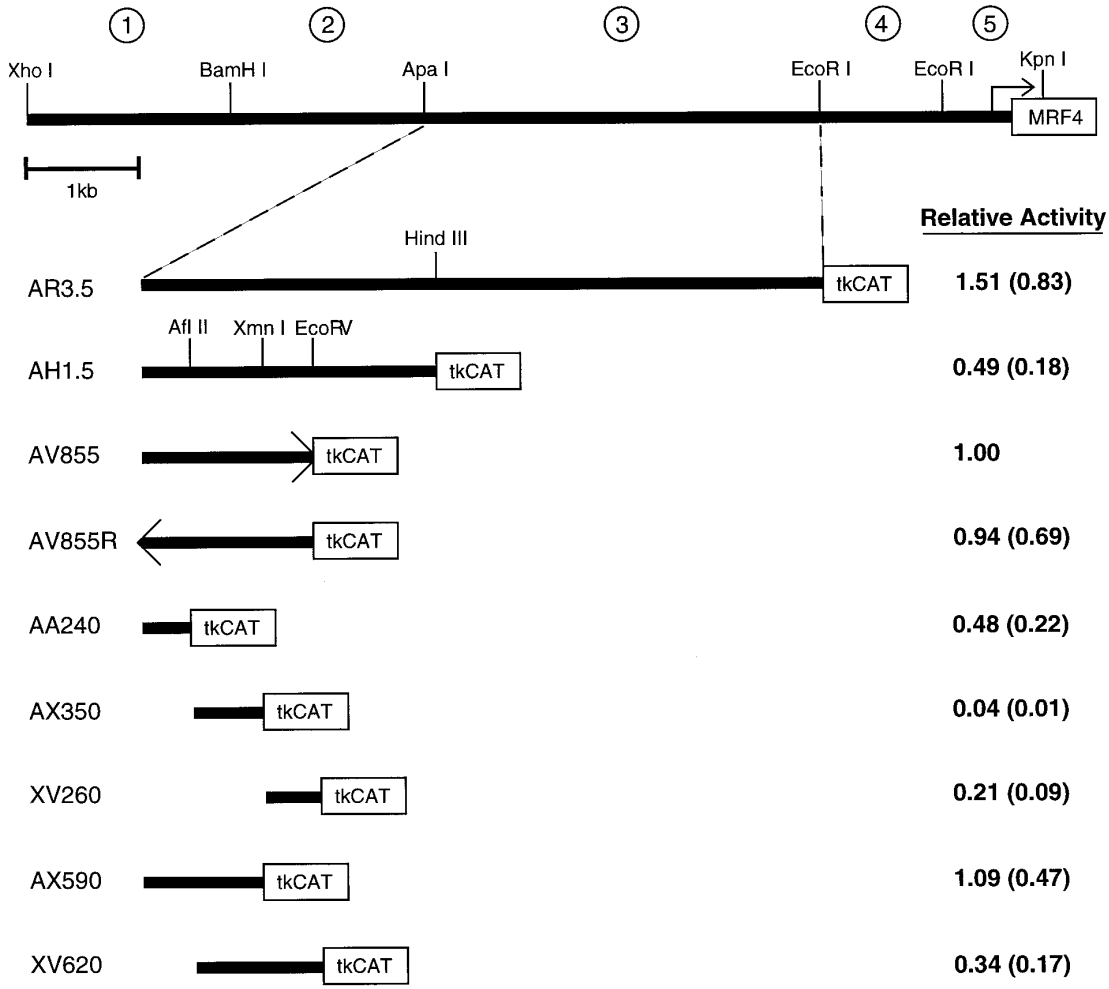

FIG. 1. Deletion analysis of *MRF4* gene fragments in the *tk*CAT reporter. Top line represents the *MRF4* gene with 8.5 kb of 5′ flanking sequence; arrow indicates the *MRF4* transcription start site. Fragments ① through ⑤ were isolated by cutting at the indicated restriction enzyme sites. The AR3.5 fragment displayed enhancer activity and was analyzed by additional deletions as shown. In AV855*tk*CAT the *MRF4* fragment was ligated into the reporter plasmid in the same orientation as it occurs in the intact *MRF4* gene, while in AV855R*tk*CAT the same fragment is in the reverse orientation. Relative activity of each construct, in terms of percent chloramphenicol conversion per microgram of protein of cell extract normalized to the percent conversion obtained with the AV855 construct, is the average of two to five independent experiments (standard error). The relative activity of negative control *tk*CAT transfections was 0.18 (0.06).

DMEM for 2 minutes and then were induced to differentiate in DMEM containing 2% horse serum and insulin-transferrin-selenium supplement (Sigma) (ITS medium, ref. 12) for 4 days. Myotube lysates were prepared by sonication in 0.25 M Tris-HCl, pH 7.5, and their protein content measured (Bio-Rad Protein Assay). For each experiment, CAT reactions were done from a positive control plasmid transfection (RSV-CAT, TnI-CAT1, or TnI-CAT23, ref. 36) to determine the amount of protein needed to obtain results in the linear range of the assay, then this amount of protein, usually between 5 μg and 50 μg, from each transfected cell culture was assayed for CAT activity by thin-layer chromatography (35), after which the chromatography plates were cut up for scintillation counting. When *nlacZ* gene constructs were used, equimolar amounts of the plasmids each corresponded to 7 μg of −336*nlacZ*. Transfected C2C12 myoblasts were induced to differentiate for 4 days and either fixed in situ for β-galactosidase histochemistry with X-gal as the substrate (35) or quantitated colorimetrically using O-nitrophenyl β-D-galactopyranoside as the substrate (36).

RESULTS

Using restriction enzyme sites identified previously (32) and some additional ones (Fig. 1), 8.5 kb of *MRF4* 5′ flanking sequence was divided into five fragments which were each inserted into an enhancer trap plasmid containing the herpes simplex *thymidine kinase (tk)* promoter and the chloramphenicol acetyltransferase (CAT) reporter. When activity was tested in mouse C2C12 myotubes by transient transfection, one *MRF4* gene fragment located between approximately −5.0 kb and −1.5 kb from the transcription start site was found to function as an enhancer with the *tk* promoter (Fig. 2A). The level of enhancement was modest, yielding CAT activity which was on average only 8 times greater

than that which was obtained with the *tk* promoter alone. This 3.5-kb *Apa* I-*Eco*R I fragment which we termed AR3.5 corresponded to the region whose deletion previously was found to cause a decrease in *MRF4* gene expression in stable transfection assays (32).

Deletions of AR3.5 were constructed in *tk*CAT and tested in C2C12 cells to localize its enhancer activity (Fig. 2B). An 855-bp region (AV855, Fig. 1) appeared to contain essentially all of the activity of the parent fragment AR3.5, and it functioned as a classical enhancer in orientation-independent fashion. AV855*tk*-CAT was active both in differentiated C2C12 myotubes and in myoblasts (data not shown). To further localize the active elements of AV855, this fragment was divided into three non-overlapping subfragments, each of which was inserted into *tk*CAT. One of these subfragments (AA240, Fig. 1) consistently displayed the highest enhancer activity, but the level was less than that of AV855. To determine whether larger segments of AV855 might contain its full activity, we examined two overlapping subfragments each including about 600 bp of the *MRF4* gene. The subfragment (AX590, Fig. 1) derived from the 5′ end of AV855 retained its full activity and the other overlapping subfragment (XV620) retained partial activity, although the region of overlap between these two subfragments by itself (AX350) displayed no enhancer activity. Sequencing AV855 revealed the presence of three E boxes and a MEF2 site, all within the AX590 region (Fig. 3).

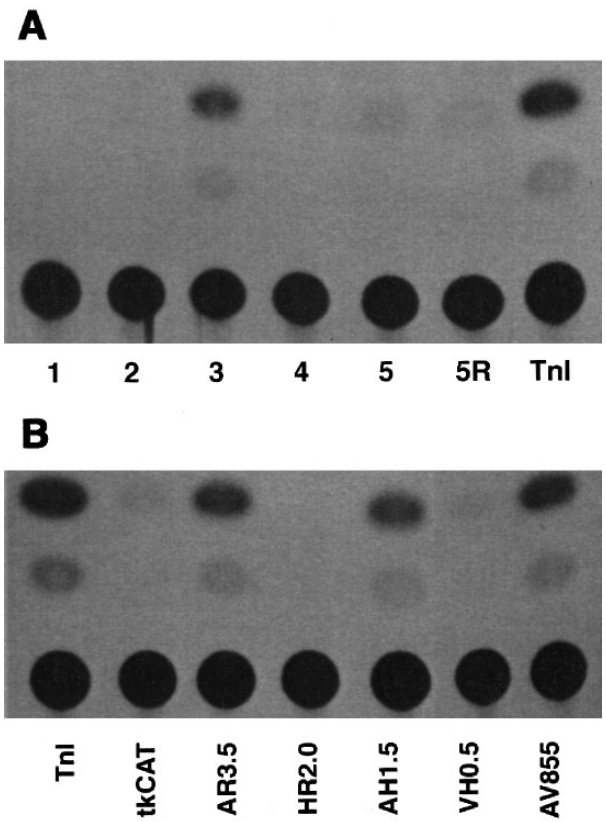

FIG. 2. Autoradiograms of CAT assay results. (A) Activities of fragments ① through ⑤ of the *MRF4* gene shown in Figure 1 inserted into *tk*CAT. The gene construct labeled 5 contains fragment ⑤ including the *MRF4* promoter in its normal orientation, while construct 5R contains the same fragment inserted in reverse orientation. The construct labeled TnI contains the muscle-specific enhancer from the quail troponin I gene (36) as a positive control. (B) Activities of fragment AR3.5 and its subfragments inserted into *tk*CAT. AH1.5 and AV855 fragments are indicated in Fig. 1, HR2.0 is the approximately 2.0-kb region between the *Hin*d III site and the *Eco*R I site of AR3.5, and VH0.5 is the approximately 0.5-kb region between the *Eco*R V site and the *Hin*d III site of AH1.5.

```
GGGCCCTTCCTCTGGGCTTCATGACTACAGTGTCCCCTATCTAGGTCCTACCCCATTTCT   60
TTGCATAACTTTGGAAGGGTAGAGCTGTGGATTTTCATATCTTTCCTTTCCTCTGCTTGG  120
TTGAAGCCCTTCCCCCCATGTCCCCAGAAGTGACTCAAATGTTCTCTCAGTGTGTAAGTG  180
CAAGCTATTTTTAACCATCGTGTTTGGGAATCTCTGAGCACACATTGCTGGCAGCTTAAG  240
TAAGCTCCGGGTGCTCAGGGTTCATATTTCTCAGTGTCCGCAAGTTTGAGGCAAGGCTGA  300
TTCTGAGTATTGCCTTTTACTCACAGACCCGCTGCTTTCCCTTTCTTCCCCAGGGCCAAT  360
CATCCATGTTTCTCCAGATTAGTCTTAACGTTCTGTCCATTTTTCTGCTCCATTCTACTC  420
AAGTTTCCTTTCATTTTGTTCCATCGTTAGCCCTGAGGTCTATCTTCTCAGCTAGTAATT  480
TCTCTGAATCCAGTTACATAATAATGTAGCCCCAACCCCAAGTGGTAGCCCACGGAAACT  540
TCTTTAACTGACTCTTCATCTGTTCTCTGTGGCAACCGCTTCTTTGAACTTCTTCCTACA  600
CTCACTGCCGTGCCAGAACAGTTTTCCAAAATCCTCAACTTCTCTTCTCAGAAATATCAA  660
CCTAACATCTTAAAACCTCTGTCCCTAAATCATTCCTCCTCATATTTCCTGCTCTGAGAG  720
TCTTTACTGTTGCATACCTGAAGGGATGGTATTCTCCTTCAAAGTTCTTAGCTATTCGCT  780
TCTGTGTGTTTTAGCCACTGTCACTGTCCCCAGTAAACTGAAATTTACTACGTATAATAC  840
TTTCACTTTGATATC                                               855
```

FIG. 3. Nucleotide sequence of the AV855 region. Numbering begins at the 5′ end of the *Apa* I restriction enzyme site and ends at the 3′ end of the *Eco*R V site. E boxes (CANNTG elements) are single-underlined and a consensus MEF2 binding site is double-underlined. This sequence has been deposited in Genbank under accession number U85650.

In order to examine enhancer activity of AV855 in combination with the *MRF4* promoter, which by itself is more highly expressed in myotubes than in myoblasts (31), AV855 was inserted upstream of the promoter in a reporter construct containing the bacterial *lacZ* gene with a viral nuclear localization signal (−336*nlacZ*, ref. 31). The resulting gene construct, AV855−336*nlacZ*, was compared with −336*nlacZ* and two other *MRF4* constructs: −5000*nlacZ*, which contains approximately 5.0 kb of *MRF4* 5′ flanking sequence including the AV855 region; and −3500*nlacZ*, which contains approximately 3.5 kb of *MRF4* 5′ flanking sequence and thus lacks the AV855 region. While both −336*nlacZ* and AV855−336*nlacZ* genes were expressed predominantly in myotubes, AV855−336*nlacZ* was activated in many more myotubes per culture (Fig. 4). As expected, β-galactosidase activity was greater in cell extracts from differentiated cultures transfected with either of the gene constructs which contained the AV855 region (i.e., AV855−336*nlacZ* and −5.0*nlacZ*) than from those transfected with −336*nlacZ* (Fig. 5). However, β-galactosidase expression from

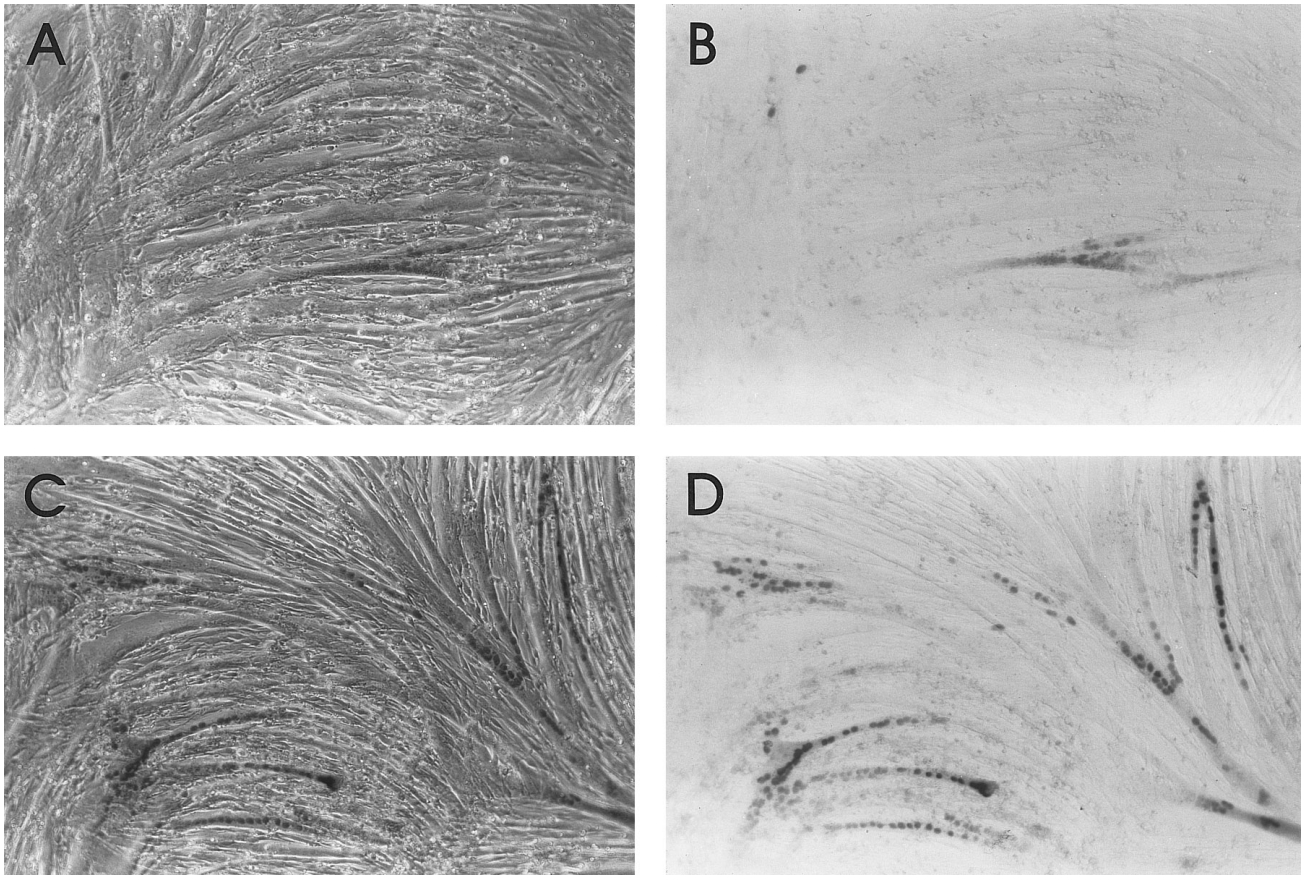

FIG. 4. C2C12 myotube cultures transfected with either −336*nlacZ* (A, B) or AV855−336*nlacZ* (C, D). Cultures were processed for β-galactosidase histochemistry, revealing localization of the enzyme to the nuclei; all the nuclei in a myotube may display the reporter as a result of gene expression in a single nucleus. Representative fields were photographed with phase-contrast (A, C) or brightfield (B, D) illumination.

−3500*nlacZ*, which lacks the AV855 region, was also greater than that from −336*nlacZ*, suggesting the presence of an additional positive regulatory region located between AV855 and the −336 promoter in the *MRF4* gene.

DISCUSSION

In this paper we report the identification of a regulatory region distant from the promoter of the *MRF4* gene, in a region between −4 kb and −5kb from the transcription start site. Although a minimal *MRF4* promoter approximately 130 bp in length has been shown to activate myotube-specific expression in transiently transfected cells (31), the existence of an enhancer at a position several kb 5′ from the promoter is consistent with studies of both *MRF4* and *MyoD* transgenes.

When rat myogenic cells stably transfected with cloned rat *MRF4* genes of various length were induced to differentiate, transcript levels from genes containing either 8.5 kb or 5 kb of 5′ flanking sequence increased by a factor that was nearly double the increase from genes containing either 1.5 kb or 0.4 kb of flanking sequence (32). In a recent study of *MRF4* linked to a *lacZ* reporter gene in mice (33), transgenic lines containing 8.5 kb of *MRF4* 5′ flanking sequence recapitulated the normal biphasic pattern of *MRF4* expression. β-galactosidase activity was first seen in the thoracic somites at precisely the time that endogenous *MRF4* transcripts are detected, and a second wave of transgene expression occurred in all skeletal muscles at the normal time. In sharp contrast, transgenic lines containing 0.4 kb of flanking sequence lacked all somitic β-galactosidase activity, and expression in skeletal muscle at later stages was severely limited. *MRF4* transgenic mice have also been made using an intermediate length of mouse 5′ flanking sequence (approximately 6.5 kb, up to a *Bam*H I site probably identical to the one shown in Fig. 1) linked to a *lacZ* reporter (37). This gene construct was expressed in skeletal myofibers at about the normal time; however, the early phase of expression in somites was not seen. Considered together these results suggest that sequence ele-

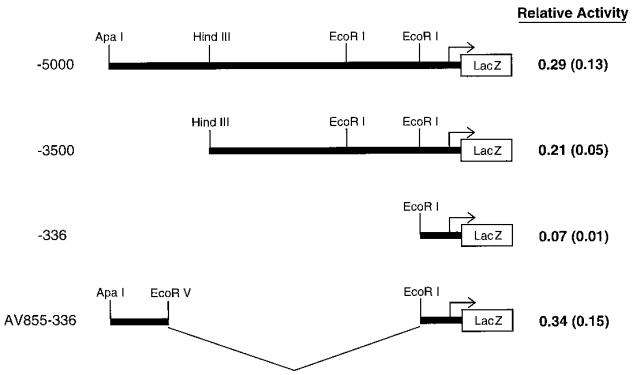

FIG. 5. Deletion analysis of *MRF4* 5′ flanking sequence linked to the *nlacZ* reporter. Relative activity of each construct, expressed in terms of β-galactosidase activity per microgram of protein of cell extract normalized to a value of 1.00 for the positive control RSV*lacZ* (34), is the average of two to three independent experiments (standard error). The relative activity of negative control *nlacZ* vector transfections was 0.05 (0.00).

ments in the region between −6.5 kb and −8.5 kb contribute to somitic *MRF4* expression, and elements in the region between −6.5 kb and −0.4 kb, including the AV855 region described here, contribute to myofiber expression.

LacZ transgenes driven by the *MyoD* promoter are ectopically expressed with no consistent pattern (18), but a 258-bp sequence located at about −20 kb from the start site, when linked to either the *MyoD* promoter or the *tk* promoter, is sufficient to activate expression in all muscle-forming regions of transgenic mice. Although there are three E boxes in this enhancer that are conserved in both position and nucleotide sequence between mouse and human, mutation of all three sites affects neither the timing nor the pattern of expression (23). A role for the −20-kb enhancer in normal embryonic expression is supported by the finding that *MyoD* transcription in somitic cells of mouse embryos is preceded by of demethylation of this genomic region (38). Unlike the −20-kb enhancer, expression driven by the *MyoD* DRR (a 720-bp region located at approximately −5 kb) linked to the *MyoD* promoter is delayed in limb and craniofacial muscles of transgenic mice relative to endogenous *MyoD* (21). However, the promoter-and-DRR transgene reproduces the persistent expression of the endogenous *MyoD* gene in fast glycolytic fibers of adult mice (21), whereas expression mediated only by the −20-kb enhancer disappears at postembryonic stages (23). These results have led to the speculation that the −20-kb enhancer may have been added during the evolution of tetrapod vertebrates from their prochordate ancestors to regulate early *MyoD* expression in the head and limbs (21).

Functionally modular gene organization has been observed also for *myogenin*, but in this case the functional units are individual transcription factor-binding sites in the promoter, where mutation of an E box and a MEF2 site together results in complete loss of *myogenin* promoter-*lacZ* transgene expression. Mutation of the E box alone has no effect on expression in somites of transgenic mice but leads to delayed expression in limb bud and visceral arches. When the MEF2 site is mutated, limb bud expression is delayed and the pattern of transgene expression is altered in the somites (27, 28). Verification of specific protein binding sites within the *MRF4* AV855 region must await further mutagenesis and testing, and assignment of specific embryonic or later roles to these elements must await their individual analysis in transgenic animals.

The results of the present study also point to another upstream regulatory region of *MRF4* that remains to be isolated. Identification of AV855 as an enhancer region relied on its ability to function in combination with a heterologous promoter, but 5′ deletions of *MRF4* linked to the *lacZ* reporter showed that, even in the absence of the AV855 region, a 3.5-kb proximal sequence region was more active than was the −336 promoter alone in C2C12 myotubes. This finding was unexpected, since a construct linking most of this 3.5-kb sequence region to the *tk* promoter (i.e., HR2.0 in Fig. 2B) produced no enhancement. It may also be significant that the average activity of AR3.5*tk*CAT was greater than that of AH1.5*tk*CAT, which in turn was less than that of AV855*tk*CAT. These results may indicate that the HR2.0 region contains an element which can act in combination with the AV855 region to enhance activity of the *tk* promoter as well as act alone to enhance the activity of the *MRF4* promoter, and that the VH0.5 region contains a suppresser whose effect is abolished in the presence of the HR2.0 elements. Models of *MRF4* gene regulation involving such specific interactions among proteins bound to multiple upstream regions will become testable with the isolation of these additional regulatory elements.

ACKNOWLEDGMENTS

This work was supported by a grant from the American Heart Association, Alaska Affiliate, and by the Biomedical Program at the University of Alaska Anchorage.

REFERENCES

1. Venuti, J. M., and Cserjesi, P. (1996) *Curr. Topics Dev. Biol.* **34,** 169–206.
2. Rudnicki, M. A., and Jaenisch, R. (1995) *Bioessays* **17,** 203–209.
3. Ludolph, D. C., and Konieczny, S. F. (1995) *FASEB* **9,** 1595–1604.
4. Molkentin, J. D., Black, B. L., Martin, J. F., and Olson, E. N. (1995) *Cell* **83,** 1125–1136.
5. Gu, W., Schneider, J. W., Condorelli, G., Kaushal, S., Mahdavi, V., and Nadal-Ginard, B. (1993) *Cell* **72,** 309–324.
6. Sartorelli, V., Huang, J., Hamamori, Y., and Kedes, L. (1997) *Mol. Cell. Biol.* **17,** 1010–1026.

7. Lenormand., J. L., Benayoun, B., Guillier, M., Vandromme, M., Leibovitch, M., and Leibovitch, S. A. (1997) *Mol. Cell. Biol.* **17,** 584–593.
8. Olson, E. N. (1993) *Genes Dev.* **4,** 1454–1461.
9. Smith, T. H., Kachinsky, A. M., and Miller, J. B. (1994). *J. Cell Biol.* **127,** 95–105.
10. Ott, M.-O., Bober, E., Lyons, G., Arnold, H. H., and Buckingham, M. (1991) *Development* **111,** 1097–1107.
11. Sassoon, D., Lyons, G., Wright, W. E., Lin, V., Lassar, A., Weintraub, H., and Buckingham, M. (1989) *Nature* **341,** 303–307.
12. Hinterberger, T. J., Sasson, D. A., Rhodes, S. J., and Konieczny, S. F. (1991) *Dev. Biol.* **147,** 144–156.
13. Bober, E., Lyons, G., Braun, T., Cossu, G., Buckingham, M., and Arnold, H. H. (1991) *J. Cell Biol.* **113,** 1255–1265.
14. Miner, J. H., and Wold, B. (1990) *Proc. Natl. Acad. Sci.* **87,** 1089–1093.
15. Montarras, D., Chelly, J., Bober, E., Arnold, H. H., Ott, M.-O., Gros, F., and Pinset, C. (1991) *New Biol.* **3,** 592–600.
16. Moss, J. B., Olson, E. N., and Schwartz, R. J. (1996) *J. Biol. Chem.* **271,** 31688–31694.
17. Block, N. E., Zhu, Z., Kachinsky, A. M., Dominov, J. A., and Miller, J. B. (1996) *Dev. Dynamics.* **207,** 382–394.
18. Goldhamer, D. J., Faerman, A., Shani, M., and Emerson, C. P. (1992). *Science* **256,** 538–542.
19. Tapscott, S. J., Lassar, A. B., and Weintraub, H. (1992) *Mol. Cell. Biol.* **12,** 4994–5003.
20. Zingg, J-M., Alva, G. P., and Jost, J-P. (1994) *Nucleic Acids Res.* **22,** 2234–2241.
21. Asakura, A., Lyons, G. E., and Tapscott, S. J. (1995) *Dev. Biol.* **171,** 386–398.
22. Faerman, A., Goldhamer, D. J., Puzis, R., Emerson, C. P., and Shani, M. (1995) *Dev. Biol.* **171,** 27–38.
23. Goldhamer, D. J., Brunk, B. P., Faerman, A., King, A., Shani, M., and Emerson, C. P. (1995) *Development* **121,** 637–649.
24. Pinney, D. F., De La Brousse, F. C., Faerman, A., Shani, M., Maruyama, K., and Emerson, C. P. (1995) *Dev. Biol.* **170,** 21–38.
25. Leibham, D., Wong, M-W., Cheng, T.-C., Scrhoeder, S., Weil, P. A., Olson, E. N., and Perry, M. (1994) *Mol. Cell. Biol.* **14,** 686–699.
26. Dechesne, C. A., Wei, Q., Eldridge, J., Gannoun-Zaki, L., Millasseau, P., Bougue, L., Caterina, D., and Paterson, B. M. (1994) *Mol. Cell. Biol.* **14,** 5474–5486.
27. Cheng, T-C., Wallace, M. C., Merlie, J. P., and Olson, E. N. (1993) *Science* **261,** 215–218.
28. Yee, S.-P., and Rigby, W. (1993) *Genes Dev.* **7,** 1277–1289.
29. Cheng, T-C., Tseng, B. S., Merlie, J. P., Klein, W. H., and Olson, E. N. (1995) *Proc. Natl. Acad. Sci. U.S.A.* **92,** 561–565.
30. Black, B. L., Martin, J. F., and Olson, E. N. (1995) *J. Biol. Chem.* **270,** 2889–2892.
31. Naidu, P. S., Ludolph, D. C., To, R. Q., Hinterberger, T. J., and Konieczny, S. F. (1995) *Mol. Cell Biol.* **15,** 1885–1894.
32. Hinterberger, T. J., Mays, J. L., and Konieczny, S. F. (1992) *Gene* **117,** 201–207.
33. Pin, C. L., Ludolph, D. C., Cooper, S. T., Klocke, B. J., Merlie, J. P., and Konieczny, S. F. (1997) *Dev. Dynamics* **208,** 299–312.
34. Jaynes, J. B., Chamberlain, J. S., Buskin, J. N., Johnson, J. E., and Hauschka, S. D. (1986) *Mol. Cell. Biol.* **6,** 2855–2864.
35. Ausubel, F. M., Brent, R., Kingston, E., Moore, D. D., Seidman, G., Smith, J. A., and Struhl, K. (1991) Current Protocols in Molecular Biology, Wiley, New York.
36. Yutzey, K. E., Kline, R. L., and Konieczny, S. F. (1989) *Mol. Cell. Biol.* **9,** 1397–1405.
37. Patapoutian, A., Miner, J. H., Lyons, G. E., and Wold, B. (1993) *Development* **118,** 61–69.
38. Brunk, B. P., Goldhamer, D. J., and Emerson, C. P., Jr. (1996) *Dev. Biol.* **177,** 490–503.