The Myogenic Regulatory Factor MRF4 Represses the Cardiac \( \alpha \)-Actin Promoter through a Negative-acting N-terminal Protein Domain*

(Received for publication, June 25, 1996, and in revised form, August 20, 1996)

Jennifer Barnett Moss‡, Eric N. Olson§, and Robert J. Schwartz

From the Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030 and the \( \dagger \)Hamon Center for Basic Cancer Research, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Cardiac \( \alpha \)-actin is activated early during the development of embryonic skeletal muscle and cardiac myocytes. The gene product remains highly expressed in adult striated cardiac muscle yet is dramatically reduced in skeletal muscle. Activation and repression of cardiac \( \alpha \)-actin gene activity in developing skeletal muscle correlates with changes in the relative content of the four myogenic regulatory factors. Cardiac \( \alpha \)-actin promoter activity, assessed in primary chick myogenic cultures, was activated by endogenous myogenic regulatory factors but was inhibited in the presence of co-expressed MRF4. By exchanging N- and C-terminal domains of MRF4 and MyoD, the N terminus of MRF4 was identified as the mediator of repressive activity, revealing a novel negative regulatory role for MRF4. The relative ratios of myogenic regulatory factors may have fundamental roles in selecting specific muscle genes for activation and/or repression.

Vertebrate cardiac and skeletal \( \alpha \)-actin gene expression is distributed in distinct muscle-specific patterns during development. In birds and small mammals, the ratios of cardiac to skeletal \( \alpha \)-actin mRNA and protein gradually decrease from 80% in embryonic skeletal muscle to 50% in the neonate and finally to about 5% in the adult limb (1). Thus, cardiac \( \alpha \)-actin can be considered an embryonic skeletal muscle isoform that is down-regulated during skeletal muscle maturation while retaining high level expression in the heart. This switch in striated actin isoforms is mediated during development by control regions present in transcriptional regulatory sequences (2). When linked to a \( \beta \)-galactosidase reporter, the proximal promoter of the mouse cardiac actin gene produces low level but specific in vivo expression that mimics the developmental down-regulation of the endogenous cardiac actin gene (3).

E-boxes (DNA consensus sequence CANNNTG) are found in the regulatory regions of many genes including those expressed only in muscle. For example, the avian cardiac actin promoter depends upon an intact E-box for expression in primary embryonic skeletal muscle cultures (4). The skeletal muscle-specific basic helix-loop-helix (bHLH) factors MyoD, Myf5, myogenin, and MRF4 can bind and activate E-boxes both in vitro and in vivo (reviewed in Ref. 5). These four myogenic regulatory factors (MRFs) share extensive homology within the bHLH motif that mediates DNA binding and heterodimerization with ubiquitously expressed bHLH proteins such as E12/E47, E2-2, and HEB (6). Transcriptional activation relies upon protein-DNA contacts made by two residues in the conserved basic region, creating a permissive protein conformation (7). The core DNA-protein complex is modulated by non-homologous transcriptional activation domains, which confer upon each factor distinct functional abilities (8–11). Interestingly, MRF4 and MyoD contain N-terminal activation domains, yet MRF4 fails to induce the expression of many muscle-specific genes despite its ability to bind E-box sequences. The differential activities of MRFs may be one of the mechanisms whereby diverse myogenic phenotypes are achieved.

The developmental expression of a MRF has often been correlated with that of its target gene. Cardiac actin transcripts appear in the first skeletal muscle cells to develop in the somitic myotome of the mouse, concurrent with the expression of Myf5 (12). The expression of MRFs in discrete myotomal compartments may serve as an address system for distinct myoblast populations (13). Myoblasts require MyoD and Myf5 expression since mice containing deletions in both genes lack all myoblast populations (reviewed in Ref. 5). Myogenin-null mice fail to form functional skeletal muscle in vivo yet contain myoblasts that differentiate in vitro (14). Three different MRF4-negative mouse lines with skeletal rib defects of varying severity appear to reflect alterations in expression of Myf5 that is linked to MRF4 (15). The only viable MRF4-null mouse expresses normal levels of Myf5 and MyoD and increased levels of myogenin mRNA (16). The generation of double knockouts of MRF4 and MyoD or myogenin should further define the role of MRF4 during development.

MRF4 is the predominant myogenic bHLH protein in neonatal mouse limb, where cardiac \( \alpha \)-actin expression is dramatically reduced (17). In neonatal chick pectoralis muscle, MyoD levels approach those of MRF4 while myogenin mRNA declines at birth (18). Because commitment to a muscle cell fate can be achieved by increasing the ratio of activating over inhibiting factors such that a threshold is overcome, the absolute level of any given myogenic factor may be less important than the relative levels of all the activating myogenic factors. If MRF4 suppresses the expression of some muscle genes and not others, then the relatively high levels of MRF4 late in development may restrict the levels of muscle gene expression in some muscle fiberotypes but not others. In support of this hypothesis,

SRF; serum response factor; SRE, serum response element; EMSV, Murine sarcoma virus promoter CAT expression vector.
the myogenin promoter binds MRF4 but is not activated (16). Furthermore, myogenin overexpressed in mouse fast isoform-specific muscles causes high neonatal lethality and the upregulation of acetylcholine receptor genes. The result is the formation of increased numbers of receptors at extrasynaptic surfaces (19). Supersensitivity to acetylcholine leads to a denervation phenotype and subsequent muscle atrophy. Thus, the balance of myogenic factors may be critical for the establishment and maintenance of the muscle phenotype.

We asked whether one function of MRF4 was to suppress cardiac α-actin gene expression in developing skeletal muscle. Our experiments addressed the potential of rat MRF4, as well as its human homolog Myf6, to repress cardiac α-actin promoter activity in embryonic day 11.5 chick pectoralis primary muscle cultures. We detected repression by MRF4 in the primary myotube cultures since myogenesis had already been initiated in the presence of endogenous MRFs. Repression by MRF4 was compared with that of Id, a dominant negative inhibitor of bHLH function (20). We engineered two mutations in the DNA binding domain and showed the necessity for occupation of the E-box in repression. Unlike Id, MRF4 mediated repression partially through binding to the target E-box. The production of chimeric MyoD and MRF4 proteins demonstrated that the N terminus of MRF4 is unique among the four MRFs in its ability to negatively regulate cardiac α-actin promoter activity in skeletal muscle cell cultures, despite the presence of an intact transcriptional activation domain.

MATERIALS AND METHODS

Cell Culture—10T1/2 mouse fibroblast cell lines expressing MRF4 and MyoD were maintained in 400 μg/ml G418 (21). 11.5-day embryonic chick breast myoblasts were plated at a density of 10^6/60-mm dish. After 16 h, the medium was replaced with fresh medium, and then calcium phosphate was transfected with 2.5 μg of purified plasmid. CAT assays were performed after 72 h with 20 μg of total protein (4). The conversion of [14C]chloramphenicol to acetylated forms was monitored by TLC analysis and quantitated on a Molecular Dynamics scanner.

In Vitro Transcription and DNA Binding Assays—The MyoD, myogenin, MRF4, and Myf5 EMSV expression vectors were linearized with BamHI. E12R (22) was linearized with EcoRI. Templates were transcribed in vitro with T3 RNA polymerase (Stratagene). RNA was then added (either alone or at a 1:1 ratio of E12 to myogenic factor) to rabbit reticulocyte lysate (Promega) with or without [35S]methionine. Labeled translation products were quantitated on a 10% denaturing polyacrylamide gel and used to estimate protein concentration.

The proximal cardiac actin E-box probe (50 ng, 5'CGCGCTGCTCCG-CACCTGCCCTTAGATGGCC3') was end-labeled and then annealed with a 2-fold excess of complement. The duplex was gel purified and then eluted in 500 μl of 50 mM NaCl. 125 ng of sonicated salmon sperm DNA and 0.2 pmol of each translated protein were incubated in binding buffer (20 mM HEPES, pH 7.9, 60 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol) for 15 min at room temperature. 5 × 10^6 cpm/μg of labeled probe was added and incubated for another 15 min. Products were analyzed on a 5% non-denaturing polyacrylamide gel.
Mutations in the MRF4 Basic Region and MyoD/MRF4 Chimeras—
The MRF4 DNA binding region and the N- and C-terminal domains were constructed using a polymerase chain reaction protocol (23). The MRF4 EcoRI fragment and two separate basic region mutations (BS2, GCCCCCACAGATGGTGGGGGCGAGCTACCCTG (R 94R95K96 to GGG) and BS3, CTGCGCGAAATGCTGGGGCTTAAGAA (R 103R104R105 to MLG) were ligated into KS2 (Stratagene). The polymerase chain reaction product was sequenced, cut with HindIII and PpuMI, and then reintroduced into the EMSV expression vector. Transformants were screened using radiolabeled BS oligonucleotides (24).

Two of the P/MI sites flanking the MyoD bHLH domain were used as unique cloning sites to form chimeras. The NsiI (GTGCAAGCCTCTGAT- GCAATGCCCAGAGCC, L105R105 to MH) and SpeI (CGTGCAAGC- GACTGACTACCAAGGCTGAT, K104T105 to TS) oligonucleotides were introduced via a polymerase chain reaction into sites that would be compatible with those previously introduced into MRF4 (25). The final product yielded a 566-base pair fragment that was cut with P/MI and reintroduced into a MyoD-EMSV vector from which the wild type P/MI insert had been removed. Site-directed MyoD and MRF4 mutations were generated using EcoRI/NsiI (44D), EcoRI/SpeI (D44), or SpeI/NsiI (D4D). Chimeras were double cesium chloride gradient-purified, sequenced, and used in transfections or as templates for in vitro transcription.

RESULTS

MRF4 Repressed Cardiac α-Actin Promoter Activity in Primary Chick Myotube Cultures and in Stable Cell Lines—We observed that of the four MRFs transfected into 10T1/2 fibroblasts, MyoD stimulated chick cardiac actin CAT expression to the greatest extent whereas MRF4 was virtually inactive (4). These results are consistent with the activity of MRF4 upon other complex muscle regulatory regions, such as the troponin I enhancer (26). We evaluated the relative activities of the four MRFs on cardiac actin promoter expression in primary myotubes (Fig. 1A). These cultures expressed high levels of endogenous cardiac actin (27) as well as MRFs (18). Introduced MyoD, Myf5, and myogenin stimulated cardiac actin activity at levels 2-fold above that mediated by endogenous MRFs. In contrast, MRF4 inhibited promoter expression. We carefully titrated the amount of transfected plasmid to optimize for MyoD and MRF4 activities and to avoid nonspecific inhibition by transcriptional squelching (Fig. 1B). Our data indicated that a muscle regulatory factor can function as a repressor of muscle.
gene expression and that the relative ratios of the MRFs might have differential effects on cardiac actin promoter function.

We examined cardiac actin CAT expression in stably transduced 10T1/2 cell lines that overexpressed MRF4 or MyoD (21). The clonal cell line 7 expressed relatively large amounts of MRF4 that activated myogenin and MyoD, indicating the ability of the MRFs to regulate one another’s expression. In the MyoD clonal cell line 8, low levels of myogenin were present while MRF4 expression was absent. We observed large differences in cardiac actin CAT expression within these stable cell lines (Fig. 2A). 10T1/2 fibroblasts as well as the MyoD stable cell line 8 sustained high cardiac actin promoter activity in the presence of co-transfected MyoD. However, 4-fold lower levels of cardiac actin activity were obtained in the MRF4 stable cells when co-transfected with MyoD. The residual level of cardiac actin CAT expression in the MRF4 stable cell line may have been the result of the stimulation by MRF4 of endogenous myogenin or MyoD expression, which sustained cardiac actin CAT activity above baseline. Rat MRF4 and human Myf6 (28) had the same inhibitory effect on cardiac actin CAT expression suggesting the regions of the protein that mediated repression have been conserved across species (Fig. 2B).

MRF4 Repressed Cardiac a-Actin Promoter Activity

MRF4 Required DNA Binding to Mediate Full Repression—Forced expression of Id, which lacks a DNA binding domain, blocked MyoD-dependent activity by competing for binding to E proteins (20). Id repressed cardiac actin promoter activity in our assay similar to levels observed for MRF4 (Fig. 2B). MRF4 could similarly repress cardiac actin expression by titrating out an obligate E protein heterodimerization partner. Alternatively, because MRF4 contained a basic domain capable of binding the cardiac actin proximal E-box (4), MRF4 may have competed with other myogenic regulatory factors for binding to the E-box. To begin to distinguish between these two possibilities, we engineered MRF4 binding site mutations that extinguished DNA binding when introduced into myogenin (29) or MyoD (30). Three amino acids in two sites (BS2 and BS3) within the basic domain of MRF4 were replaced with neutral amino acids (Fig. 3). [35S]Methionine-labeled, in vitro synthesized MyoD, MRF4, BS2, and BS3 proteins were co-translated with E12 and analyzed by SDS-polyacrylamide gel electrophoresis. The 42-kDa MyoD protein, as well as the 27-kDa MRF4 BS2 and BS3 proteins, migrated as major bands (data not shown). We also tested binding to the cardiac actin proximal E-box probe in a gel shift assay. Both MyoD and MRF4 were bound as an E12 heterodimer whereas neither BS2 nor BS3 bound to this site (data not shown). When the mutated constructs were co-transfected with the cardiac actin promoter CAT vector in chick primary myobute cultures (Fig. 3), the levels of activity were greater when compared with MRF4 alone. Thus, the BS2 and BS3 mutations were not as efficient as MRF4 at suppressing cardiac actin CAT activity. When the reporter gene and the DNA binding mutations BS2 or BS3 were co-transfected with a MyoD expression vector, we observed higher cardiac actin CAT activity than was observed with

---

**Fig. 3.** DNA binding was required for MRF4 directed transrepression of the cardiac actin promoter. A, diagram of the MRF4 basic region and site-directed mutations. The BS2 and BS3 mutants are N- or C-terminal to the conserved alanine/threonine residues (underlined). B, co-transfections of binding site mutations into primary myobute cultures. CAT activity is expressed relative to co-transfection of cardiac actin CAT with 2.5 µg of EMSV (None). 2.5 µg of MyoD transfected alone or in the presence of an additional 2.5 µg of MyoD transactivated cardiac actin to 210%. With 2.5 µg of added MRF4, transactivation was reduced. The BS2 or BS3 binding site mutations reduced co-transfected MyoD activity to 175 and 150%, respectively.

---

**Factor Expressed**

- None
- MyoD (2.5 ug)
- MRF4 (2.5 ug)
- MyoD (2.5 ug) and MRF4 (2.5 ug)
- BS2 (2.5 ug)
- MyoD (2.5 ug) and BS2 (2.5 ug)
- BS3 (2.5 ug)
- MyoD (2.5 ug) and BS3 (2.5 ug)
MyoD/MRF4 co-transfections. An intact DNA binding domain was necessary for facilitating full repression of the cardiac actin promoter by MRF4. However, complete repression also required heterodimerization with an E protein partner present in the primary myotube cultures resulting in the formation of inactive MRF4-containing complexes.

The N-terminal Domain of MRF4 Mediated Repression—To locate the MRF4 domain(s) responsible for repression, two unique restriction sites (SpeI and NsiI) were introduced on either side of the bHLH region of MyoD. These MyoD sites were recipients for existing MRF4 mutations (25). The introduction of the SpeI (K104T105 to T104S105) and NsiI (L164R165 to M164H165) sites did not alter protein function since co-transfection into primary myotube cultures yielded the same cardiac actin promoter-driven CAT activity as unmodified MyoD (Fig. 4A). The three-part chimeras contained both the N and C termini of MyoD (D4D), the N terminus of MyoD (D44), or the C terminus of MyoD (44D). In vitro translation was monitored by SDS-polyacrylamide gel electrophoresis, and each chimera was capable of binding to a chick cardiac actin E-box probe in the presence of co-translated E12 (data not shown). When the chimeras were co-transfected with cardiac actin CAT into chick primary cultures, the MRF4 N-terminal construct (44D) singularly repressed the activity of the cardiac actin promoter below wild type levels (Fig. 4A). The D4D and D44 constructs produced levels of activity comparable with MyoD. Similarly, both the N- and C-terminal domains of myogenin were required to rescue the failure of the MRF4 protein to transactivate the muscle creatine kinase enhancer in 10T1/2 cells (25). We transfected myogenin/MRF4 chimeras into chick primary myotube cultures (Fig. 4B). Only the 448 (MRF4:MRF4:myogenin) chimera produced repression at levels similar to those of MRF4 in cardiac actin CAT co-transfections. Therefore, the N-terminal domain of MRF4 was required for repression of the cardiac actin promoter, in the context of either the MyoD or myogenin C-terminal domains.

MRF4 Repression Did Not Occur by Interference with Factors Bound to an Adjacent Site—MRFs and serum response factor (SRF) share overlapping binding sites in the cardiac actin promoter composed of the proximal E-box and the juxtaposed serum response element (SRE3). One explanation for repression by MRF4 could be occlusion of SRF binding. We asked if
cardiac actin promoter activity became resistant to MRF4 repression by eliminating competitive factor interactions. A cardiac actin promoter mutation M1, in which the 3' SRF contact sites on the SRE3 were altered to a BglII site, blocked DNA binding by the SRF but not binding by the MRFs (31). Co-transfections of MyoD or MRF4 with either the wild type cardiac actin promoter or the SRE3 mutation were evaluated in primary chick myotube cultures (Fig. 5). We observed no significant change in the transactivation potential of MyoD or in the transrepression activity of MRF4 on either the wild type reporter or the SRE3 mutation. These results were consistent with co-transfections of myogenin and the cardiac actin CAT reporter gene in 10T1/2 fibroblasts in which the SRE3 mutation did not eliminate promoter activity (31). Therefore, the mechanism of MRF4-directed repression did not occur through mutually exclusive interactions over the E-box and the SRE.

**MRF4 Repressed Some Promoters but Not Others—**To further investigate the inhibitory activity of MRF4, we examined its effect on 4RTKCAT. This reporter contained four copies of the muscle creatine kinase enhancer right E-box attached to the thymidine kinase basal promoter (32). Co-transfected MRF4 reduced but did not abolish expression from the 4RTKCAT reporter in 10T1/2 cells (25). When this construct was co-transfected with MyoD or MRF4 into primary myotube cultures, we observed repression of CAT activity by MRF4 (Fig. 5). Therefore, the binding site requirement for MRF4 repression in primary cultures relied only upon the presence of the muscle creatine kinase right E-box. In contrast, a muscle-restricted desmin promoter-CAT construct was activated by MRF4 in 10T1/2 cells (33). MRF4 was also capable of activating desmin promoter-CAT activity in chick primary myotube cultures (Fig. 5).

**DISCUSSION**

Many regulatory factors in a variety of cell types belong to families that share DNA binding and/or heterodimerization motifs. Some of the family members activate while others repress transcription. Because these factors can act in combinations, a small change in the stoichiometry of a particular family member can alter cell phenotype. Relative protein concentrations change due to an altered rate of mRNA or protein synthesis and degradation. As development proceeds, threshold levels of different factors could shift a critical balance, initiating a series of subsequent events.

MRF4 is predominantly expressed in adult skeletal muscle. We hypothesized that MRF4 had a functional role distinct from MyoD, Myf5, or myogenin in regulating cardiac actin and other muscle genes that are not expressed in all adult muscles. By altering the effective concentrations of the myogenic bHLH transcription factors in a primary myoblast culture system where myogenesis had been initiated, we determined that MRF4 suppressed expression from the introduced cardiac actin promoter yet activated the desmin promoter. We also tested 10T1/2 cells engineered to stably express MyoD or MRF4. Cardiac actin was expressed at high levels in the presence of MyoD. However, in the presence of stably expressed MRF4, co-transfected MyoD activated cardiac actin expression 10-fold less efficiently than in the MyoD-expressing cells. These experiments suggested a mechanism whereby MyoD was in competition with MRF4. Increasing levels of MRF4 relative to other myogenic factors during development such that certain genes are repressed while others remain activated may ultimately contribute to the restriction of muscle cell phenotype.

Repression would be inefficient unless the level of inhibitor was very high, binding by the repressor occurred with greater affinity than binding by activators, or if the repressor failed to interact with surrounding factors required for the formation of an active transcription initiation complex. Our experiments suggested that high levels of MRF4 relative to other MRFs had the effect of selecting targets for repression. This result is supported by the observation that in adult mouse skeletal muscle, levels of MRF4 are at least 10-fold greater than MyoD, and cardiac actin expression is absent. In addition, factors binding to the SRE did not affect MRF4 activity, suggesting that the primary cause of repression was the interaction among MRF4, its heterodimerization partner, and the E-box. We also found that the 4RTKCAT construct, containing a multimerized E-box, was inhibited by MRF4 and activated by MyoD. Our primary muscle cell culture system detected the inhibitory effect of MRF4 on 4RTKCAT whereas this construct was activated at low levels by MRF4 in 10T1/2 fibroblasts (25).

The repression we observed might occur by means of a model suggested by the crystal structure of a MyoD homodimer bound to a synthetic E-box. A highly conserved alanine and threonine in the MyoD basic region exerted indirect effects on the protein-DNA complex that was relayed to the protein surface (7). Thr-115 contacted a nucleic acid in the E-box while the small size of the adjacent Ala-114 allowed a nearby Arg-111 to be buried inside the major groove. An additional nucleic acid contact, which may be required for transcriptional activation, became possible. A homologous arginine (Arg-95) in MRF4 was
changed to a glycine in the BS2 mutation. An inactive complex was formed when the critical contacts with the DNA major groove could not be made. BS2 was unable to bind DNA yet continued to form E protein heterodimers, titrating available partners that would otherwise be available for the production of MyoD activator complexes.

Further consideration of structure-function relationships elucidates the role of the N terminus in repression. The MRF4 N-terminal domain was crucial for negative regulation in chick primary myotube cultures. MRF4 amino acids 1–88 were capable of conferring repressive activity onto the MyoD or myogenin C terminus. Potential structural conformations adopted by MRFs in contact with different E-boxes may also rely on amino acid sequences outside the conserved bHLH domains. Examination of the N-terminal MRF sequences reveals that 5 out of 10 amino acids near the MyoD basic region (amino acids 65–74) are prolines whereas 1 out of 10 for MyoD and Myf5 and 2 out of 10 for myogenin are found in this location. Prolines have the capacity to twist the shape of the protein away from a helical configuration or out of a β sheet. MRF4 may adopt different conformations that still allow for binding to an E-box yet do not form transcriptionally active complexes because the adopted conformation fails to produce the correct protein-DNA contacts. Other examples of bHLH proteins that act as repressors in the Drosophila peripheral nervous system (34) or in the rat central nervous system (35) rely on prolines within the basic region. Their function is to restrict neuroblast lineage by interacting with ubiquitous E protein partners that otherwise would be expected to overexpress cardiac actin in adult skeletal muscle, since the relative levels of MRF4 had been reduced. Levels of cardiac actin have not been compared in wild type and MRF4-null adult muscle. Continued examination of the expression of cardiac actin and other embryonic isoforms in MRF4-null neonates should lend insight into repression by MRF4.

Acknowledgments—We thank Tushar Chakraborty, Semie Capetanaki, Steve Konieczny, and Hans Henning Arnold for reagents.

REFERENCES
1. Vandekerckhove, J., Bugaisky, G., and Buckingham, M. (1986) J. Biol. Chem. 261, 1838–1843
2. Cox, R. D., and Buckingham, M. E. (1992) Dev. Biol. 149, 228–234
3. Biben, C., Hadjouzel, J., Tajbakhsh, S., and Buckingham, M. (1996) Dev. Biol. 175, 200–212
4. Moss, J. B., McQuinn, T. C., and Schwartz, R. J. (1994) J. Biol. Chem. 269, 12731–12740
5. Weintraub, H. (1993) Cell 75, 1241–1244
6. Lassar, A. B., Davis, R. L., Wright, W. E., Kadesch, T., Murre, C., Voronova, A., Baltimore, D., and Weintraub, H. (1991) Cell 66, 305–315
7. Ma, P. C. M., Roulid, M. A., Weintraub, H., and Pabo, O. C. (1994) Cell 77, 451–459
8. Weintraub, H., Dwariki, V. J., Verma, I., Davis, R., Hollenberg, S., Snider, L., Lassar, W., and Tappscott, S. J. (1991) Genes Dev. 5, 1377–1386
9. Schwartz, J. J., Chakraborty, T., Martin, J., Zhou, J., and Olson, E. N. (1992) Mol. Cell. Biol. 12, 266–275
10. Mak, K.-L., To, R. Q., Kong, Y., and Konieczny, S. F. (1992) Mol. Cell. Biol. 12, 4334–4346
11. Braun, T., Winter, B., Bober, E., and Arnold, H. H. (1990) Nature 346, 663–665
12. Ott, M. O., Bober, E., Lyons, G., Arnold, H. H., and Buckingham, M. E. (1991) Development 111, 1097–1107
13. Smith, T. H., Kachinsky, A. M., and Miller, J. B. (1994) J. Cell. Biol. 127, 85–100
14. Venuti, J. M., Morris, J. H., Vivian, J. L., Olson, E. N., Klein, W. H. (1995) J. Cell Biol. 128, 563–576
15. Olson, E. N., Arnold, H. H., Righy, P. W. J., and Wold, B. J. (1996) Cell 85, 1–4
16. Zhang, W., Behringer, R. R., and Olson, E. N. (1996) Genes Dev. 9, 1388–1399
17. Hinterberger, T., Sassoon, D., Rhodes, S., and Konieczny, S. F. (1991) Dev. Biol. 147, 144–156
18. Saitoh, O., Fujisawa-Sehara, A., Nabeshima, T. I., and Periasamy, M. (1993) Nucleic Acids Res. 21, 2563–2569
19. Gundersen, K., Rabben, I., Klöcke, B. J., and Merlie, J. P. (1995) Mol. Cell. Biol. 15, 7127–7134
20. Pesce, I., and Benezra, R. (1995) Mol. Cell. Biol. 15, 7874–7880
21. Rhodes, S. J., and Konieczny, S. F. (1989) Genes Dev. 3, 2050–2261
22. Murre, C., Voronova, A., and Baltimore, D. (1990) Mol. Cell. Biol. 11, 1156–1160
23. Higuchi, R. (1990) Reconstituent PCR in PCR Protocols (Innis, M., Gelfand, D., Sninsky, J., and White, T., eds) Academic Press, New York
24. Sambrook, J., Fritsch, E., and Maniatis, T. (eds) (1989) Molecular Cloning: A Laboratory Manual, pp. 5.4.1 Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
25. Chakraborty, T., and Olson, E. N. (1991) Mol. Cell. Biol. 11, 6103–6108
26. Banerjee-Basu, S., and Buonanno, A. (1993) Mol. Cell. Biol. 13, 7019–7028
27. Hayward, L. J., and Schwartz, R. J. (1986) J. Cell Biol. 102, 1445–1493
28. Braun, T., Buschhausen-Denyser, G., Bober, E., Tunnich, E., and Arnold, H. H. (1988) EMBO J. 7, 571–579
29. Brennan, T. J., Chakraborty, T., and Olson, E. N. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5675–5679
30. Davis, R. L., Cheng, P., Lassar, A. B., and Weintraub, H. (1990) Cell 60, 723–746
31. French, B. A., Chow, K., Olson, E. N., and Schwartz, R. J. (1991) Mol. Cell. Biol. 11, 2439–2450
32. Weintraub, H., Bober, E., Lockshon, D., and Lassar, A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5625–5629
33. Li, H., and Capetanaki, Y. (1993) Nucleic Acids Res. 21, 335–343
34. van den Dore, M., Ellis, H. M., and Posakony, J. W. (1991) Development 113, 2345–2355
35. Sasai, Y., Kaigeyama, R., Tagawa, Y., Shigemoto, R., and Nakashima, S. (1992) Genes Dev. 6, 2620–2634
36. Sartorelli, V., Webster, K. A., and Kedes, L. (1990) Genes Dev. 4, 1811–1822
37. Skerjanc, I. S., and McBurney, M. W. (1994) Dev. Biol. 163, 125–132