We have studied the transcriptional activity of the mouse MyoD1 gene promoter in vivo and in vitro using mouse G8 myoblasts and muscle cell nuclear extracts. 5′ deletion analysis of the promoter and transcription-competition analysis using oligonucleotides corresponding to several cis-acting elements revealed that the basal activity of the MyoD1 promoter is conferred by two SP1 boxes, an AP-2 box, and a CAAT box. We have identified a negative regulatory sequence located between nucleotide position −342 to −322 with respect to the cap site. The negative regulatory element shows sequence homology with cAMP-responsive element (CRE) and AP-1 binding site (5′-GAGCAGTGGTACAG-3′). As determined by gel mobility shift competition analysis, oligonucleotides containing AP-1 binding sites inhibit protein interactions with the MyoD1 CRE-like element. We also show that binding to this element is down-regulated during myogenic differentiation and can be reinduced by the addition of serum. Furthermore, mutation of the CRE-like element induces MyoD promoter activity in dividing myoblasts. By using anti-c-Fos antibodies we show that AP-1 is binding to the MyoD1 CRE-like element. Our results indicate that AP-1 negatively modulates MyoD1 expression in growing myoblasts and strongly suggest that c-Fos and c-Jun inhibit myogenesis and MyoD1 expression by direct binding to a negative cis-acting element in the MyoD1 promoter.

Muscle cell differentiation is presumably the end-point of a cascade of intracellular events involving progenitor cell determination to the myogenic lineage, multiplication and withdrawal from the cell cycle of the myogenic precursor cells, and terminal differentiation and modulation of the terminally differentiated state by developmental and physiological signals. This complex and multistep process appear to be directed by a hierarchy of regulatory genes. Several genes whose protein products are potentially responsible for the determination of the myogenic phenotype have been characterized, including MyoD1, MRF4, Myogenin, and Myf5 (Davis et al., 1987; Pinney et al., 1988; Edmonson and Olson, 1989; Wright et al., 1989). Each of these muscle-specific regulatory factors was shown to convert 10T1/2 fibroblasts to the muscle phenotype. Interestingly, all of these proteins share a putative helix-loop-helix domain, which is also present in the myc proto-oncogene family (Davis et al., 1987; Caouy et al., 1988). The best characterized member of the myogenic factors is MyoD1, which is a nuclear phosphoprotein (Tapscott et al., 1988), binds as a homo- or heterodimer to the consensus sequence CANNTG (Davis et al., 1990), and trans-activates muscle-specific promoters (Lassar et al., 1989b; Piente et al., 1990; Lin et al., 1991; Sartorelli et al., 1990; Wentworth et al., 1991). The dimerization with other regulatory proteins like E12 and E47 (Murre et al., 1989; Baldwin and Burden, 1989) or with its inhibitor ID (Benezra et al., 1990) takes place by means of its helix-loop-helix domain. Skeletal muscle differentiation is blocked by serum (Peterson et al., 1989) and growth factors, like fibroblast growth factor and transforming growth factor-β; these peptides inhibit both fusion and MyoD1 expression (Olson et al., 1986; Wic et al., 1987; Vaidya et al., 1989). Several oncogenes have also been shown to inhibit muscle differentiation and MyoD1 expression, like ras (Olson et al., 1987), myc (Melder and Wold, 1991), fos and jun (Lassar et al., 1989a). Jun can form heterodimers with MyoD1 through the leucine zipper and the helix-loop-helix motives and inhibits trans-activation of the MyoD1 promoter and the muscle creatine kinase enhancer (Bengal et al., 1992). All of the jun and fos genes are immediate early genes, the transcription of which is rapidly induced in response to cell stimulation with growth factors, cytokines, and other agents (Lamph et al., 1988; Angel et al., 1987). The fos and jun gene products constitute the transcription factor AP-1, which trans-activates several promoters in response to serum and growth factors (Brenner et al., 1989; Kim et al., 1990). AP-1 can also inhibit transcription (Schule et al., 1990a, 1990b); moreover, a single DNA sequence might mediate both AP-1 positive and negative effects on transcription (Diamond et al., 1990). Recently, it was shown that okadaic acid (OA) blocks myogenesis by inhibiting MyoD1 expression and MyoD1 binding activity. Inhibition of MyoD1 expression by OA correlates with induction of mRNA expression for the c-fos family, to a lesser extent for the jun family, and the consequent formation of active AP-1 complexes (Kim et al., 1992; Park et al., 1992). Taken together, these observations suggest that AP-1 might bind to the MyoD1 promoter and negatively regulate MyoD1 expression. The results presented here provide evidence that a Fos-related protein can bind specifically to a negative cis-acting element in the MyoD1 promoter. This element shows sequence homology with a cAMP-responsive element (CRE) and with AP-1 recognition sequences. We also show that binding to the MyoD1 CRE-like element is regulated during myoblast differentiation.

**MATERIALS AND METHODS**

**Plasmid Construction**—The template used for in vitro transcription was the plasmid pM050 (Fig. 1), which contains the MyoD1 promoter.

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1 The abbreviations used are: OA, okadaic acid; CRE, cAMP response element; CREB, cAMP response element binding protein; ERE, estrogen response element; CAF, chloramphenicol acetyltransferase; PCR, polymerase chain reaction; DM, differentiation medium; GM, growing medium; TPA, 12-O-tetradecanoylphorbol-13-acetate.
linked to a synthetic oligonucleotide from the cauliflower mosaic virus genome, from nucleotide 1643 to nucleotide 1671 (Franck et al., 1980). This sequence is unique and therefore suitable for specific detection. The deletion mutants (Fig. 1) were performed taking advantage of the presence of specific restriction sites. As an internal control in the in vitro transcription assay we use the plasmid pCH110 (Pharmacia LKB Biotechnology AB, Uppsala, Sweden), which contains the globin gene and the SV40 promoter. For DNA transfection, the plasmid pMO54 containing the MyoD1 promoter linked to the luciferase gene was used (Zingg et al., 1991), and the deletion mutants were those described above. The CRE-like and the F oligonucleotides (see below) were created by blunt-end ligation and the plasmid pCATP (Promega, Madison, WI). Mutation of the CRE-like element was done using PCR according to Higuchi et al., 1988. Primary PCRs were performed in pairs containing the mismatched oligonucleotide 5'-GAG-CATGTCCTCCCGGACCGCCCCTTCT-3' (the mutated bases are shown in bold) and the corresponding 3' or 5' oligonucleotides. The primer used at the 5' end was the standard M13 universal primer and the primer at the 3' end was (5'-ACGCCCCTAGTCTGGCAGGGGCCCCCCT-3') expanding sequences of the MyoD1 promoter located between nucleotide positions (−47) to (−32). The target DNA was pMO, which contains the genomic clone of the MyoD1 gene (Zingg et al., 1991). Reactions were performed with Tag polymerase following the manufacturer's instructions. Each cycle consisted of denaturation at 95 °C for 30 s, annealing at 42 °C for 40 s, and extension at 72 °C for 1 min. Twenty-five cycles were performed. Extension time at the last cycle was 10 min. After the primary PCRs were completed 20 μl of the reaction mix were separated on a 3% agarose gel. Each pair fragment of the expected size was cut out and purified by agarose gel electrophoresis. The fragments were then used as target DNAs for the secondary PCR, and the 5' and 3' oligonucleotides mentioned above were used as primers. The conditions for the secondary PCR were the same as for the primary reactions. The final mutated PCR product was cloned in vector pCR-1 (Invitrogen). The mutation was confirmed by chain termination sequencing. The sequences between the HindIII and PvuII restriction sites of the wild type MyoD1 promoter were substituted in the plasmid pM054 (Somogyi). The mutation was confirmed by chain termination sequencing. The sequences between the HindIII and PvuII restriction sites of the wild type MyoD1 promoter were substituted in the plasmid pM054 (Somogyi), and the deletion mutants were those described previously (Zingg et al., 1991). Transfection of the plasmids containing the CAT gene as reporter was performed by the CaPO4 method. Cells were transfected with 5 μg of reporter plasmid, 3 μg of expression vector, and 1 μg of pCMV internal control plasmid. Expression vectors c-Jun and c-Fos have been previously described (Schöntal et al., 1988; Hirai et al., 1990), and the CAMP-response element binding protein (CREB) expression vector (Gonzalez et al., 1989) was kindly provided by Dr. Y. Nagamine at the Friedrich Miescher Institut. CAT assay was performed according to Gorman et al. (1983). For these experiments the internal control plasmid was pRSV-β-GAL (Gynberg et al., 1982) to avoid competition with the SV40 promoter. β-Galactosidase assay was performed according to Lucibello and Mueller (1989).

Cyto-immunofluorescence—Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline for 10 min at room temperature. Permeabilization was done with 15% Nonidet P-40 in phosphate-buffered saline for 5 min at room temperature. Cells were then incubated for 10 min at 37 °C with 10 ng/ml goat anti-rabbit antibody (Sigma). The anti-c-Jun antibody was used at a 1:100 dilution in 10% goat serum, followed by staining with rhodamine-conjugated goat anti-rabbit antibody (Sigma). For c-Jun detection, the anti-c-Jun antibody was used at 1:25 dilution in 10% goat serum.

RESULTS

MyoD1 5' Flanking Region Sequences Are Required for Efficient Transcription Both in Vivo and in Vitro—Recently we reported a genomic clone of the MyoD1 gene and 653 base pairs of its 5' sequence. We showed that the promoter is active both in vivo and in vitro (Zingg et al., 1991). To study the relationship between cis-acting elements and trans-acting factors and their effect on MyoD1 transcription, several deletion mutants were constructed (Fig. 1A) and tested for their transcriptional activity in vivo. As shown in Fig. 1B, deletion to the XbaI site (−470, relative to the mRNA start site) had no effect on the luciferase activity when compared with the wild type. However, an increase in the activity was observed by further deletion to the Accl site (−314). Deletion to the PvuII site (−222) reduced the luciferase activity to wild type levels. As expected, further deletion to the SmaI site (−90) dramatically reduced luciferase activity, because this deletion removes all putative binding sites for transcription factors.
sites for the positive ubiquitous trans-acting factors (see Fig. 1A). By using the same deletion mutants in the in vitro transcription system (Fig. 1C), we observed basically the same effect of the deletions on MyoD1 transcription. Deletion mutant to the AccI site increased transcription (2–3-fold), whereas deletion to the PvuII site decreased transcription to the wild type levels. On the other hand, deletion to the Smal site did not decrease transcriptional activity as for the in vivo expression. These in vivo and in vitro experiments suggest that negative regulating sequences are situated between nucleotide positions −470 and −314 (AccI deletion mutant) and, furthermore, that 222 nucleotides (PvuII mutant) can confer the basal promoter activity of the MyoD1 gene.

Functional Analysis of cis-Acting Elements by in Vitro Transcription Competition Assay—As an alternative approach to confirm the above results, we used the in vitro transcription competition assay of the MyoD1 wild type construct, pM050 (Fig. 1A), with a series of synthetic oligonucleotides covering the upstream region of the MyoD1 promoter. Table I shows the oligonucleotides tested in the competition assays. The amount of oligonucleotide needed to compete all of the specific factors binding to the DNA template was determined by gel mobility shift competition assays under the same conditions as for the in vitro transcription assay. 100-fold molar excess of unlabeled oligonucleotide was sufficient to completely displace the specific protein-DNA complexes formed in the presence of 10 μg of nuclear protein (data not shown). Three different concentrations of each oligonucleotide were used for the in vitro transcription competition experiments, but only the results obtained with 100 x molar excess of competitor are shown in Fig. 2. As expected, all of the oligonucleotides containing binding sites for ubiquitous transcription factors like CAAT box, SP1, and AP-2 decreased the transcriptional activity to some extent. CAAT box oligonucleotide, which does not contain the M-CAAT sequences, decreased the activity to 40% of the wild type (Fig. 2B). In addition, GC1, GC2, and AP-2 oligonucleotides all decreased the transcription activity by 30–50%, suggesting that these sequences confer the basal activity of the MyoD1 promoter.

Transfection and in vitro transcription with the deletion mutant AccI (see Fig. 1, B and C) strongly suggest that the sequences located between the two restriction sites XbaI and AccI (nucleotide positions −470 to −314) contain a negative regulatory element. As shown previously (Zingg et al., 1991), a half palindrome of the estrogen response element (ERE) and a putative CRE can be found in this region. Two oligonucleotides covering this sequence were used in the transcription competition experiments. Competition with the oligonucleotide ERE (nucleotide positions −364 to −347) had no effect on transcription (Fig. 2, A and IB), whereas competition with the oligonucleotide containing the CRE-like element (nucleotide positions −342 to −322) resulted in a 2.8-fold increase in the transcriptional activity. These results clearly show that the negative effect of this sequence on MyoD1 promoter activity is mediated mainly by a CRE-like element.

To test whether in vivo the CRE-like element is playing a role in the negative regulation of the MyoD1 promoter, we transfected G8 myoblast with the plasmid PM054mut, in which the CRE-like element has been mutated, and the rest of the MyoD1 promoter remains intact. Fig. 3 shows that mutation of the CRE-like element induces luciferase activity by 1.5–2-fold when compared with the wild type construct, thus providing direct evidence for a functional role of this element in the negative regulation of the MyoD1 promoter in dividing myoblasts.

| Potential regulatory elements | Position* | Sequence* |
|-----------------------------|------------|-----------|
| GC1                         | −120 to −105 | 5′-CTCCCTTCGACCTTTTCCTTA-3′ |
| CAAT                        | −137 to −123 | 5′-CTCTATTGGCTTGTATG-3′ |
| GC2                         | −155 to −142 | 5′-AAGCCCTCAGCTTACCA-3′ |
| AP2                         | −180 to −165 | 5′-CTTCACTGTTCTCCCGGATACCC-3′ |
| CRE                         | −342 to −322 | 5′-GTCCGAGATCTTGTACAG-3′ |
| ERE                         | −364 to −347 | 5′-GTCCGAGATCTTGTACAG-3′ |

*Position is relative to the start size of transcription (Zingg et al., 1991).
*Only the upper strand is shown. The recognition sequence is shown in bold letters.
MyoD1 Promoter Regulation

A

![Image of gel mobility shift competition assay](image)

**Fig. 2. In vitro transcription competition assay.** A, in vitro transcription was performed as described under "Materials and Methods" in the presence of 100 molar excess of the indicated competitor oligonucleotide (see Fig. 1 and Table I). The transcripts of the MyoD1 and SV40 promoters are indicated. B, graphic representation of the average of three independent experiments. Values are shown as the percent of the MyoD1 promoter activity relative to the activity of the SV40 promoter. Dried gels were quantified as indicated under "Materials and Methods."

B

![Graph](image)

were able to compete for binding (lanes 5 and 6). Lee et al. (1993) have recently characterized an AP-1 element from the porcine urokinase plasminogen activator promoter. Interestingly, this element shows perfect sequence homology with the MyoD1 CRE-like element (Fig. 4A). Oligonucleotides containing this AP-1 element and various mutants, which do not affect TPA responsiveness, were able to abolish binding (Fig. 4B, lanes 7-9). On the other hand, a mutant that lacks TPA responsiveness competed to a lesser extent the binding to the MyoD1 CRE-like element (Fig. 4B, lane 11). Finally, an oligonucleotide containing the SV40 TPA responsive element (Angel et al., 1987) competed the binding (lane 10). These results suggest that AP-1 may bind to the MyoD1 CRE-like element.

**MyoD1 CRE-like Element binds c-Fos and c-Jun Oncoproteins**—It is well known that myoblasts in culture fuse and differentiate several days after removal of the serum from the medium. Fusion is preceded by an increase in MyoD1 mRNA levels (Bengal et al., 1992). We therefore tested the ability of the MyoD1 CRE-like element to bind c-Fos and c-Jun. G8 myoblasts were transfected with the MyoD1 promoter wild type plasmid (pM054wt) or with the plasmid carrying the mutated CRE-like element (pM054mut). Luciferase and β-galactosidase assay were performed 48 h after transfection. The data represent the average of 4 independent experiments. Standard deviation was around 10%.

**Fig. 3. Mutation of the CRE-like element induces MyoD1 transcriptional activity in dividing myoblasts.** G8 myoblasts were transfected with the MyoD1 promoter wild type plasmid (pM054wt) or with the plasmid carrying the mutated CRE-like element (pM054mut). Luciferase and β-galactosidase assay were performed 48 h after transfection. The data represent the average of 4 independent experiments. Standard deviation was around 10%.

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TGAGCAGCTGACTGACTAC CRE wt (3)
TGAGCAGCTGAGTGTAC CRE mut1 (4)
TGAGCAGCTGAGGAGGTAC CRE mut2 (5)
TGAGCAGCTGAGGTCAGAC CRE mut3 (6)
GAGAAATGAGGTACCC AP1 wt (7)
GAGAAATGAGGAGTCC AP1 mut1 (8)
GAGAAATGAGGTACCC AP1 mut2 (9)
ATTAGTCAGTCGAG SV40 AP1 (10)
GAGAAAGattccATCC AP1 mut3 (11)
binding activity of nuclear extracts to the CRE-like oligonucleotide from G8 myoblast grown in the presence of serum and nuclear extracts from G8 myoblasts grown in low serum medium. As shown in Fig. 5A, nuclear extracts from G8 myoblasts (GM) show high binding activity to the CRE-like element (lane 2), whereas, nuclear extracts from G8 myoblasts grown in DM for 48 h show reduced binding to the CRE element (lane 3).

Because expression of the jun and fos gene families can be induced upon cell stimulation with serum, we tested whether the binding to the CRE-like element could be induced in cells grown 48 h in differentiation medium (low serum levels) and then restimulated for 4 h with growing medium (high serum levels). Fig. 5A (lane 4) shows clearly that serum can reinduce the binding of a factor to the CRE-like element. Binding is induced up to 3-fold upon addition of serum (compare lanes 3 and 4). Fig. 5B shows that the DNA-protein complex with the CRE probe and nuclear extracts from myoblasts restimulated with serum can be competed by AP-1 oligonucleotides. Thus, AP-1 binding activity to the negative cis-acting element of the MyoD1 promoter is down-regulated during myogenensis and can be restored by serum stimulation. Binding activity to an oligonucleotide, which contains the MyoD1 binding site of the muscle creatine kinase enhancer (Lassar et al., 1989b), does not change under these conditions (Fig. 5D).

It has been shown that the addition of anti-Fos antibodies to nuclear extracts from several cell types blocks the formation of AP-1-DNA complexes (Distel et al., 1987; Rauscher et al., 1988). As a control we used anti-Mos antibodies, which had no effect on protein-DNA complex formation (lane 1). The same results were obtained with extracts from dividing myoblasts (data not shown). As determined by indirect immunofluorescence using anti-c-Fos and anti-c-Jun antibodies serum levels regulate c-fos and c-jun expression in G8 myoblasts (Fig. 6). Altogether, these results clearly show a direct relationship between c-fos and c-jun expression and DNA binding activity to the MyoD1 CRE-like element. The results strongly suggest that the increase in MyoD1 mRNA levels after serum removal is in part due to the down-regulation of the AP-1 binding activity to the negative element (CRE) of the MyoD1 promoter.

MyoD1 CRE-like Element Confers an Additive Effect to the SV40 Minimal Promoter in Response to TPA Stimulation and to c-fos and c-jun Overexpression—To determine whether the CRE-like element of the MyoD1 promoter is able to negatively modulate a heterologous promoter, we cloned this element into the pCAT reporter plasmid containing the CAT gene under the control of the minimal SV40 promoter. As shown in Fig. 7A the CRE-like element showed no effect on the basal activity of the SV40 promoter when transfected into G8 myoblasts. In contrast, the CRE-like element induced the SV40 promoter activity in response to TPA stimulation and to c-fos and c-jun expression (2-fold). No such effect was observed by cloning an oligonucleotide containing a half-palindrome of the ERE from the chicken vitellogenin promoter (oligonucleotide F). Similar results were obtained by transfecting HeLa cells (data not shown).

Because an oligonucleotide containing the CRE from the rat somatostatin promoter can compete for binding to the MyoD1 CRE-like element (data not shown), we also tested whether this element is able to respond to the cAMP signal transduction pathway. As shown in Fig. 7B, neither treatment of the cells with forskolin nor cotransfection with a CREB expression vec-
In cultured cells, serum levels control myoblast proliferation and differentiation into myotubes. Upon serum removal, myoblasts withdraw from the cell cycle and fuse. Fusion is preceded by a 2–3-fold induction in MyoD1 mRNA levels in C2C12 (Bengoa et al., 1992) and in G8 myoblasts. c-fos and c-jun are expressed in dividing myoblasts, but transcription is turned off when myoblasts are induced to differentiate. Here we show that the binding activity to the CRE-like element is downregulated during myogenic differentiation. Moreover, we show that this binding activity can be recovered by addition of serum to the cultures. We have also shown that the binding activity to the CRE-like element can be competed by oligonucleotides containing AP-1 recognition sequences. Direct evidence that the serum-dependent binding is due to AP-1 complexes was obtained with anti-c-Fos antibodies. Anti-c-Fos antibodies inhibited DNA-protein complex formation with extracts prepared from cells induced to differentiate and restimulated with serum. These data clearly show that AP-1 is binding to the MyoD1 CRE-like element and strongly suggest that AP-1 is negatively modulating MyoD1 promoter activity in growing myoblasts by direct binding to a negative cis-acting element. Recently it was shown that OA can inhibit MyoD1 expression and MyoD1 promoter activity (Kim et al., 1992). OA induces the expression of c-fos and c-jun family members and activates AP-1 complexes (Park et al., 1992). Thus the effect of OA on MyoD1 transcription could possibly be explained by the induction of jun and, therefore, the inhibition of the MyoD1 autoregulatory feedback loop. Interestingly, an internal deletion that removes the negative regulatory sequences of the MyoD1 promoter is less sensitive to OA treatment (Kim et al., 1992) indicating that the effect of OA on MyoD1 promoter activity is in part due to AP-1 interactions with these sequences. Despite the fact that myogenin is not expressed until myoblasts enter the differentiation pathway in response to serum withdrawal (Edmonson and Olson, 1989), it has been shown that high serum levels inhibit the expression and the autoregulatory loop of myogenin. This negative effect was found to be directed to the myogenin promoter as well as to the myogenin promoter (Edmonson et al., 1991). Furthermore, OA also inhibits myogenin expression (Kim et al., 1992). Moreover, sequence comparison shows that the myogenin promoter contains an element like the CRE from the MyoD1 promoter and a consensus AP-1 binding site, suggesting that AP-1 may bind to these elements and negatively modulate myogenin expression. However, recently it has been reported that in cardiac myocytes fos and jun repress transcription of the atrium natriuretic factor gene. Repression does not require a typical AP-1 binding site but is targeted to the cardiac-specific element of the atrium natriuretic factor promoter (McBride et al., 1993). It is likely therefore that c-fos and c-jun exert the negative effect on the expression of skeletal and cardiac myogenic factors by distinct control mechanisms.

The fact that the MyoD1 CRE-like element did not show a negative effect on the basal activity of the SV40 promoter indicates that this element may work only in the context of its own promoter and may be modulating specific interactions with the transcriptional machinery. The other possibility is that MyoD1 sequences flanking the CRE-like element are necessary to mediate negative modulation. It is interesting to note that there is an inverted repeat of the sequence TGGAGT located upstream of the half-palindromic ERE. Moreover, the CRE-like element contains a half-palindrome of the ERE (GGTCA) (see Fig. 8), therefore it is possible that these sequences are also required for negative modulation of the MyoD1 promoter. The fact that the deletion mutant AccI shows higher activity than the CRE-like mutant favor the second possibility. Several elements with homology to TRE or CRE containing the sequences GTCA are known to induce (Gaub et al., 1990) or to repress transcription (Schule et al., 1990b). The one-genes jun and fos and steroid hormone receptors have been
**Fig. 7. Functional characterization of the MyoD1 CRE-like element.** G8 myoblasts were transfected with a vector containing the minimal SV40 promoter (pCATP), pCATP modified by the addition of a copy of the CRE-like element (CRE-pCATP), or by a copy of the oligo(F) from the chicken vitellogenin gene (see "Materials and Methods") (OF-pCATP). A, the CRE-like element confers an additive effect on SV40 promoter activity upon stimulation of cells with TPA and cotransfection with c-fos and c-jun expression vectors. Cells were transfected with 5 µg of the above mentioned reporter plasmids and 1 µg of the indicated expression vector. TPA (30 ng/ml) was added to the cells 8 h before harvesting. B, CREB does not trans-activate CAT activity from the CRE-pCATP construct. Cells were transfected with the reporter vector shown on the left side and with the CREB expression vector. Treatment with forskolin (10 µM) was done for 16 h. CAT assay was performed as described under "Materials and Methods." The average of four independent experiments is shown with a standard deviation below 15%.

**Fig. 8. Sequence comparison between the mouse and the rat MyoD1 5' sequences.** The sequences between the XbaI and the AccI sites are shown. The CRE-like element and the half-palindrome of the ERE are indicated by bold letters.

It has previously been shown that multiple synthetic copies of the consensus AP-1 binding site can act as TPA-inducible enhancer (Lee et al., 1987). Additionally, in the case of the human β-globin gene, naturally occurring tandem AP-1 sites constitute an inducible enhancer (Ney et al., 1990). When the MyoD1 CRE-like element is placed upstream of the SV40 promoter, it can confer an additive effect upon TPA stimulation and c-fos and c-jun expression. This suggests that AP-1 complexes can bind the MyoD1 CRE-like element in vivo and act cooperatively with the SV40 AP-1 site. However, the CRE-like element did not show any effect on the SV40 promoter in response to cAMP signaling. The CRE-like element is identical to an element from the major histocompatibility complex class implicated in both instances. Recently, it has been shown that direct protein-protein interaction between Jun and/or Fos and members of the steroid hormone receptor family is required for biological function (Yang-Yen et al., 1990; Schule et al., 1991; Doucas et al., 1991). Furthermore, a single DNA sequence may mediate both the positive and negative effects of AP-1 (Diamond et al., 1990) and confers opposite regulation between mitogenic and differentiation pathways (Schule et al., 1990a). Therefore, it is possible that the upstream half-ERE and the CRE-like element are required to form a higher order DNA-protein complex with AP-1 and a steroid hormone receptor to mediate negative modulation. Currently we are testing these possibilities.
I promoter, which can respond to TPA and cAMP stimulation (Israel et al., 1989). Thus, despite the fact that the MyoD1 CRE-like element is unable to respond to cAMP levels or to CREB expression in actively dividing myoblast, it does not rule out a role of this element within the myogenic differentiation pathway in response to a cAMP-specific signaling cascade.

The CRE-like element is conserved in the rat MyoD1 promoter (Vaidya et al., 1992) and only one base pair substitution is observed (Fig. 8). The sequences located between the XbaI and AccI sites (negative regulatory sequences) show 90% homology with the rat sequences suggesting a similar functional role in the MyoD1 promoter regulation in rodents. Whether or not the CRE-like element is also involved in the regulation of the of MyoD1 expression in other species remains to be determined.

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