Myotonic dystrophy is the most common inherited adult neuromuscular disorder with a global frequency of 1/8000. The genetic defect is an expanding CTG trinucleotide repeat in the 3'-untranslated region of the myotonic dystrophy protein kinase gene. We present the in vitro characterization of cis regulatory elements controlling transcription of the myotonic dystrophy protein kinase gene in myoblasts and fibroblasts. The region 5' to the initiating ATG contains no consensus TATA or CCAAT box. We have mapped two transcriptional start sites by primer extension. Deletion constructs from this region fused to the bacterial chloramphenicol acetyltransferase reporter gene revealed only subtle muscle specific cis elements. The strongest promoter activity mapped to a 189-base pair fragment. This sequence contains a conserved GC box to which the transcription factor Sp1 binds. Reporter gene constructs containing a 2-kilobase pair first intron fragment of the myotonic dystrophy protein kinase gene enhances reporter activity up to 6-fold in the human rhabdomyosarcoma myoblast cell line TE32 but not in NIH 3T3 fibroblasts. Cotransfection of a MyoD expression vector with reporter constructs containing the first intron into 10 T1/2 fibroblasts resulted in a 10–20-fold enhancement of expression. Deletion analysis of four E-box elements within the first intron reveal that these elements contribute to enhancer activity similarly in TE32 myoblasts and 10 T1/2 fibroblasts. These data suggest that E-boxes within the myotonic dystrophy protein kinase first intron mediate interactions with upstream promoter elements to upregulate transcription of this gene in myoblasts.

Myotonic dystrophy (DM)\(^1\) is a dominant multisystemic disease, which in the adult form shows myotonia, progressive muscle weakness and wasting, and heart conduction defects (1). In males, testicular atrophy and premature balding are also observed (1). Major features of congenital DM include hypotonia, respiratory distress, mental retardation, and a delay in terminal muscle differentiation (1). The genetic defect for DM is an expanding CTG trinucleotide repeat found in the 3'-untranslated region of a serine threonine protein kinase (DMPK) (2–4). An increase in the severity of the disease correlates with an increase in the number of trinucleotide repeats, which provides a molecular basis for genetic anticipation (4–6).

The consequences of CTG repeat expansion remains uncertain. Conflicting results have been reported as to the levels of total DMPK transcript and protein in patients (7–14). The mutant message can be detected by Northern blot analysis (12, 15, 16) and appears to accumulate in the nucleus of DM patient myoblasts and fibroblasts (16, 17). Reduction of mutant (18) and both mutant and normal (19) DMPK mRNA in the polyadenylated fraction has also been reported. Mouse nullizygous for DMPK are virtually asymptomatic (20, 21), indicating that DMPK is not an essential protein in this species.

DMPK is expressed mainly in heart, lens, skeletal muscle, testes, and brain (2, 22, 23). Delineation of the biological role of DMPK in these tissues will allow a greater understanding of the pathological process of DM. Accordingly, we have begun analysis of the DMPK gene for cis elements that direct its expression in various cultured cell lines. Transgenic mice containing a 15-kb human genomic DNA fragment encompassing the entire DMPK gene express the human mRNA and protein in the appropriate tissues, suggesting at least a subset of tissue-specific cis elements are contained within the transgene sequence\(^2\) (21).

Here we attempt identification of transcriptional regulatory elements required for increased DMPK mRNA production in cultured myoblasts versus fibroblasts. A 2-kb fragment of the DMPK upstream sequence driving the chloramphenicol acetyltransferase (CAT) gene in NIH 3T3 fibroblasts and TE32 myoblasts indicates a ubiquitous, housekeeping-type promoter region with only minor muscle-specific regulatory activity. We also investigated various deletions of the DMPK first intron for transcriptional regulatory sequences, which may enhance expression of CAT driven by endogenous and exogenous promoters in fibroblasts and muscle cells. Results presented indicate that the DMPK gene is regulated by a low level promoter that operates in conjunction with an enhancer element in the first intron. This first intron enhancer element is responsive to

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\(^3\)/EBI Data Bank with accession number(s) L08835 (for DMPK) and Z21503–Z21506.

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1 The abbreviations used are: DM(PK), myotonic dystrophy (protein kinase); HFSF, human foreskin fibroblasts; CAT, chloramphenicol acetyltransferase; TK, thymidine kinase; kb, kilobase pair(s); bp, base pair(s).

2 M. Narang and R. G. Korneluk, submitted for publication.
MyoD, requires the presence of conserved E-boxes, and in the presence of the DMPK promoter, confers increased expression of CAT in myoblasts.

MATERIALS AND METHODS

Cell Culture, DNA Transfections, CAT, and β-Galactosidase Assays—Cell lines were grown in α-minimal essential medium containing penicillin and streptomycin supplemented with 10% fetal bovine serum and 2 mM glutamine (Life Technologies, Inc.). In the case of C2C12 differentiation medium, 10% horse serum was used in place of 10% fetal bovine serum. Mammalian cells were cultured at 37 °C in a 5% CO₂ atmosphere. Primary human foreskin fibroblasts (HFSF) were a gift from Dr. Chaim Birnboim (Ottawa Regional Cancer Center, Ottawa, Canada), C2C12 mouse muscle cells, TE32 human myoblasts (24), NIH 3T3 mouse fibroblasts, human HeLa cells, and C3H10T1/2 mouse fibroblasts were obtained from the ATCC. Transfections were performed with 5 μg of CsCl-purified test plasmid, 5 μg of β-galactosidase expression construct (pSV2-gal-Prokamega) by the calcium phosphate co-precipitate method with a glycerol shock essentially as described (25). β-Galactosidase assays were carried out by adding 45 μl of cell extract to 155 μl of Z-buffer (60 mM NaHPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, 50 mM 2-mercaptoethanol, pH 7) and 40 μl of O-Nitrophenyl-β-pyranogalactoside (Sigma) (4 mg/ml in 0.1 mM phosphate buffer, pH 7, 7.73 g of Na₂HPO₄, 29.1 g of NaH₂PO₄, H₂O in 500 ml of H₂O) and incubating at 30 °C for 2 to 15 h. The reactions were stopped by adding 100 μl of 1 M Na₂CO₃ and the optical density measured at 420 nm on a spectrophotometer. CAT assays were performed essentially as described (25).

Plasmid DNA Constructions—A series of deletions of the DMPK 5′ region were generated by standard methods and sub-cloned into pBS or pBlu/HindIII digestion promoterless pCAT-Basic vector (Promega). All 5′ region of the CAT deletion constructs (Fig. 4) are named according to the position of the 5′ end of the inserted sequence relative to the DMPK translation initiation codon, and all share the same 3′ terminus, which is 42 bp upstream of the ATG initiation codon. Promoter/first intron combinations —722 CAT IVS, —722 CAT SVI, TK CAT IVS, and TK CAT SVI were constructed by subcloning a 2-kb EcoRI/HindIII fragment into EcoRI/HindIII digested pCAT-Basic vector downstream of the CAT gene. The —722, —940 DMPK promoter and TK promoter fragments were inserted into HindIII/PstI, HindIII/XhoI, and HindIII sites, respectively, upstream of the CAT gene. The TK promoter was generated by polymerase chain reaction amplification of the herpes simplex thymidine kinase (TK) promoter from a plasmid (4R TKCAT). To construct TK CAT, this polymerase chain reaction product was subcloned into the HindIII site upstream of the CAT gene in pCAT-Basic. IVS TK CAT and SVI TK CAT were constructed by fusing the 2-kb intron fragment into the PstI site in both orientations upstream of the TK promoter in TK CAT. First intron deletions were generated by polymerase chain reaction, cloned into pCR 2.1, excised with EcoRI, and cloned into pCAT-Basic. Subsequently, TK and DMPK promoter elements were cloned into these constructions as described above. To ensure all clones were properly constructed, all plasmid DNA constructions were analyzed by restriction digest and/or DNA sequencing.

Primer Extension Analysis—Approximately 50 ng of DMPK primer 2151 (5′-TGGACACAGCGCCATCTAG-3′) was end-labeled with [γ-32P]ATP (Amersham Pharmacia Biotech) using T4 polynucleotide kinase. Primer extension reactions were set up using 60 μl of TE32 myoblast or HFSF RNA essentially as described (25), except Superscript II reverse transcriptase (Life Technologies, Inc.) was used in place of murine reverse transcriptase. Standards for sizing primer extension products were generated from the control sequence of the Sequenase 2 kit (U.S. Biochemical Corp.). These were synthesized according to manufacturers instructions with [γ-32P]dATP (Amersham Pharmacia Biotech) using single-stranded M13mp18 DNA and the −40 primer provided.

Northern Blot Analysis—RNA was extracted from confluent plates of cultured mammalian cells according to Birnboim (26) and a Northern blot performed as described (27). A 1.2-kb XhoI DNA fragment from the 5′ end of the DMPK cDNA (pBS DMK) was labeled with [γ-32P]dCTP using the Redi-prime labeling system (Amersham Pharmacia Biotech). The membrane was prehybridized at 65 °C for 2–6 h in 6 × SSC, 0.5 × Denhardt’s, 0.25% SDS, and 2.5 mg of herring sperm DNA and then hybridized overnight with the probe in 4 parts prehybridization buffer, 1 part 50% dextran sulfate. Following the incubation, the membrane was washed twice for 15 min at room temperature with 2 × SSC, 0.1% SDS then twice at 50 °C for 15 min in 0.2% SSC, 0.1% SDS and then once at 65 °C in 0.1 × SSC, 0.1% SDS for 15 min. The membrane was covered with plastic wrap, exposed to film overnight at ~80 °C, and developed the following day. The blot was then stripped by washing twice for 15 min in hot water and hybridized with a fragment of the phosphoglycerate kinase gene (Pgk-1) probe by performing labeling, prehybridization, hybridization, and washing steps as described above. Nuclear extracts were obtained, using the procedure of Hoppe-Seyler et al. (28), which were quantitated for total protein concentration using a Pierce Micro-BCA kit according to manufacturer’s instructions. Nuclear extract (5 μg) was incubated with 2.5 × 10⁶ cpm of labeled oligonucleotide, 1 μg of poly(dI-dC), 1 μl of bovine serum albumin (10 mg/ml), 2 μl of gel shift buffer (250 mM HEPES, pH 7.6, 50 mM MgCl₂, 340 mM KCl), and competitor for 20 min on ice. The complexes were then mixed with 2 μl of preimmune or anti-Pgk-1 (20% Ficol 400, 0.1% bromphenol blue), and electrophoresed on a 5% nondenaturing polyacrylamide gel at 150 V for 1.3 h. Gels were then exposed to film overnight at ~80 °C.

Oligonucleotides—Sequences of oligonucleotides used in electrophoretic mobility shift assay and competition analysis (shown in Fig. 5) were synthesized using an ABI 394 DNA/RNA synthesizer and are as follows: thymidine kinase oligonucleotide, 5′-ATGCCAACAAACCCCGG-CAGGTTCTTGC-3′ (29); AP-2 oligonucleotide, 5′-AGTCCCGGGCTCC-CCACGAG-3′ (30); wild type DMPK oligonucleotide, 5′-TAAGGCT-GGGAGCGGGAAGGGGCTGG-3′; mutant DMPK oligonucleotide, 5′-TAAGGCTGGAGGGTTAGGGGGGCTGG-3′ (mutated nucleotides are shown in boldface italics).

RESULTS

The focus of this study was on DMPK transcriptional regulation in muscle cells versus fibroblasts. Initially, we characterized expression of the DMPK mRNA in a number of human and mouse cell lines in an effort to identify cell lines suitable for the study of DM protein kinase gene myoblast specific cis elements. Northern blot analysis (Fig. 1) using a probe from the DMPK cDNA was performed on RNA isolated from a number of human and murine cell lines. Relative to a Pgk-1 control, DMPK expression was strongest in TE32 myoblasts, followed by C2C12 myotubes and C2C12 myoblasts. Weak DMPK expression was observed in NIH 3T3, primary HFSF, and C3H10T1/2 cells, while the message was barely detectable in HeLa cells. Given the high expression level of DMPK mRNA in TE32 myoblasts and the lower level in NIH 3T3 fibroblasts, compar
human and mouse genes is also evident upstream of the transcription start site located at position −421.

To identify the location of promoter elements, we constructed promoter deletion mutants of the DMPK 5′ region. A 1963-bp fragment of the DMPK 5′ region was fused to the bacterial CAT reporter gene and a series of deletion constructs from this parental plasmid were constructed (Fig. 4). To localize possible myoblast specific transcriptional control elements within this sequence, these constructs were transfected into TE32 myoblasts and NIH 3T3 mouse fibroblasts. Relative CAT activities were normalized to promoterless CAT-Basic to compare the strengths of each construct between the two cell lines (Fig. 4). Most myoblast-specific expression of CAT was observed for constructs −1963 CAT, −537 CAT, and −403 CAT (Fig. 4); however, most deletion fragments produced similar relative CAT activities in both cell lines, suggesting that only weak myoblast-specific regulatory elements are present in this region of the DMPK gene. A 189-bp deletion fragment of DMPK 5′ sequence driving CAT exhibited the highest level of CAT expression in TE32, NIH 3T3, and several other cell lines tested, including HeLa and monkey kidney cells (data not shown). The strength of this promoter fragment was about half the strength of the phosphoglycerate kinase promoter in NIH 3T3 cells and 1/10 the strength of this promoter in TE32 cells (data not shown). Removal of 66 bp from −232 CAT, which includes a conserved GC box (Fig. 3), resulted in near-background CAT expression in all cell lines tested, suggesting important promoter elements reside within this sequence (Fig. 4).

To investigate the possibility that Sp1 may bind this region of the DMK promoter and activate transcription, electrophoretic mobility shift assays were performed using a probe to the putative Sp1 binding site. Four apparent protein-DNA complexes were observed (a-d) (Fig. 5). Extracts from several cell lines, including TE32 myoblasts, yield identical banding patterns (data not shown) consistent with the CAT assay data, suggesting that this region of DMPK 5′ sequence is part of a ubiquitous promoter element. Complex d appears to be probe as it is present in the lane containing only probe (Fig. 5, lane 1). Complex c can be competed for by wild type DMPK oligonucleotide, an oligonucleotide corresponding to a medium affinity Sp1 site (29) from the thymidine kinase promoter (TK), an oligonucleotide corresponding to GC-rich paired AP-2 sites from the SV40 early promoter (30) and DMPK oligonucleotides mutated in two positions in the GC box (lanes 3–10). This suggests that complex c represents a protein binding GC-rich sequences, but not specifically to the Sp1 binding site. The binding of the protein to the DNA in complexes a and b was specific as a molar excess of both the unlabeled DMPK probe (Fig. 5, lanes 3 and 4) and TK oligonucleotide (Fig. 5, lane 5 and 6) could compete for both complexes. Furthermore, only very weak competition was observed when oligonucleotides corresponding to the GC-rich binding site of the unrelated transcription factor AP-2 were used as competitor (Fig. 5, lanes 7 and 8). In addition, the mutant DMPK oligonucleotide could only compete weakly for complexes a and b, suggesting the NIH 3T3 nuclear protein(s) are binding the GC box specifically (Fig. 5, lane 9 and 10). Also, this mutant oligonucleotide could not form complexes with NIH 3T3 nuclear extracts (data not shown). Purified Sp1 protein was able to form a complex with the DMPK oligonucleotide of identical mobility to complex a seen in lane 2 (lane 11), but not with mutant oligonucleotide (lane 12), suggesting that complex a is Sp1-bound to the DMPK oligonucleotide. Taken together, these results demonstrate that Sp1 binds specifically to a GC box located within a minimal region of the DMPK 5′ sequence, which behaves as a minimal promoter, suggesting a key role for Sp1 in the basal

![Image](https://example.com/image.png)

Fig. 2. Primer extension analysis mapping two major DMPK transcription start sites with mRNA from TE32 myoblasts and HFSF fibroblasts at positions −71 and −421 relative to the translation initiation codon. A, upstream primer extension product reverse transcribed from 60 μg of TE32 RNA (lane 1) or from 60 μg of HFSF RNA (lane 2). B, downstream primer extension product also using primer 2151 reverse-transcribed from 60 μg of TE32 RNA (lane 1). The DNA size standard in both A and B is a sequencing ladder generated from single-stranded M13mp18 DNA using the −40 primer (see "Materials and Methods").
Evidence of only minor increases in CAT transcription in myoblasts over fibroblasts found with control elements in the DMPK 5'-9 region prompted us to look within the first intron for other sequences that may increase CAT expression in myoblasts. Mouse and human DMPK sequences were aligned (Fig. 6) and complete conservation of five potential E-boxes, a C(A/T)CCC box, a BEE-1 element (40), a CArG box (41, 42), a GC box (29), and two thyroid hormone response elements (43) was revealed. To explore the possibility that functional muscle-specific regulatory elements are present in the first intron, a 2-kb fragment (Fig. 9A) containing most of the first intron was cloned downstream of the CAT gene in both orientations in the presence of a 679-bp fragment of DMPK 5'-9 sequence (Fig. 7A).

These constructs were then transfected into TE32 and NIH 3T3 cells. Fig. 7 shows that the presence of the first intron, irrespective of orientation, in combination with DMPK 5'-9 sequence, prompted us to look within the first intron for other sequences that may increase CAT expression in myoblasts. Mouse and human DMPK sequences were aligned (Fig. 6) and complete conservation of five potential E-boxes, a C(A/T)CCC box, a BEE-1 element (40), a CArG box (41, 42), a GC box (29), and two thyroid hormone response elements (43) was revealed. To explore the possibility that functional muscle-specific regulatory elements are present in the first intron, a 2-kb fragment (Fig. 9A) containing most of the first intron was cloned downstream of the CAT gene in both orientations in the presence of a 679-bp fragment of DMPK 5'-9 sequence (Fig. 7A).

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can activate transcription of the CAT gene up to 6-fold in TE32 myoblasts, whereas no activation was observed in NIH 3T3 fibroblasts. This demonstrates the existence of an orientation-independent myoblast-specific transcriptional enhancing element within the first intron of the DMK gene.

The muscle-specific transcription factor MyoD has the capability of activating the myogenic program by inducing the expression of muscle-specific genes via E-boxes located in their transcriptional regulatory regions (44, 45). To further define a myoblast-specific enhancer role for the first intron, we investigated its responsiveness to MyoD and whether it could function in the context of a heterologous promoter. Constructs in which the first intron was cloned upstream or downstream of the TK promoter driving the CAT gene were generated (Fig. 8A) and co-transfected with a MyoD expression vector (EMSV-MyoD) or with parental vector (EMSV) into C3H10T1/2 fibroblasts. This cell line can be readily differentiated into myoblasts by transfection of members of the MyoD family of myogenic regulators, and this system is used to indicate MyoD responsiveness of co-transfected cis sequences (44). Also, a construct containing the ninth intron (TK CAT IN-1), which is similar in size to the first intron and also contains a similar number of possible E-box elements, was included in the transfections as a control (Fig. 8A). Expression of CAT from constructs containing the first intron was activated up to 20-fold over the activity of TK CAT G) and deletion of a further 15 bp completely abrogated expression (compare 940 CAT C to 940 CAT H) (Fig. 8B). The enhancer deletion mutants failed to activate CAT expression in NIH 3T3 fibroblasts (Fig. 9B). These data indicate that a 51-bp region within the DMK first intron containing two conserved E-box elements is required for enhancer function in TE32 myoblasts.

To investigate the role of the E-boxes in the observed activation of CAT expression in 10T1/2 cells by MyoD, the same first intron deletion fragments were cloned downstream of the CAT gene driven by the thymidine kinase promoter. Maximal responsive enhancer element in myoblasts. However, it is unknown if MyoD interacts directly with first intron elements or has a trans effect through the activation of another transcription factor gene. We investigated this question by performing deletion analysis of the entire first intron, particularly of the first four conserved E-boxes. For these experiments, a 897-bp fragment of the DMK promoter was used to drive the CAT gene, because it contains all possible transcription start sites. Deletion of 653 bp from the 3' end of the entire 2.4-kb first intron (IN-1) had no effect on CAT activity, indicating this region is dispensable for enhancer function (Fig. 9, A and B, compare −940 CAT IN-1 to −940 CAT A). Conversely, deletion from the 5' end appeared to relieve a slight inhibition (compare −940 CAT A, B, and C). Deletion of 113 bp, which includes the first four conserved E-boxes from the 5' end of −940 CAT C resulted in complete loss of enhancer activity, implicating these elements in enhancer function (compare −940 CAT C and −940 CAT D). Further deletions within the 113-bp region revealed that loss of 51 bp from the 5' end of −940 CAT C, which corresponds to deletion of two of the four E-boxes, severely compromised enhancer activity (compare −940 CAT C to −940 CAT G) and deletion of a further 15 bp completely abrogated enhancer activity (compare −940 CAT C to −940 CAT H) (Fig. 9B). The enhancer deletion mutants failed to activate CAT expression in NIH 3T3 fibroblasts (Fig. 9B). These data indicate that a 51-bp region within the DMK first intron containing two conserved E-box elements is required for enhancer function in TE32 myoblasts.

To investigate the role of the E-boxes in the observed activation of CAT expression in 10T1/2 cells by MyoD, the same first intron deletion fragments were cloned downstream of the CAT gene driven by the thymidine kinase promoter. Maximal
activation is observed with TKCAT F but is reduced dramatically with each E-box deletion (compare TKCAT F, G, and H), although some residual activity remains in deletions TKCAT H and I. Therefore, the first three E-boxes are required for maximal stimulation of CAT activity when MyoD is co-transfected with the first intron deletion constructs into 10T1/2 cells, implicating these elements as key mediators of the DMPK first intron enhancer function.

**DISCUSSION**

This is the first report describing regulatory elements controlling mRNA expression of the DMPK gene. Initially we identified transcription start sites within the 5' region of the DMPK gene and also confirmed one start site previously reported at nucleotide position 1394 (2773 relative to ATG translation initiation codon) (31). This site was first mapped using primer extension in a cell-free extract system that utilized a cloned fragment of DMPK 5'-untranslated region from 2700 to 21550 relative to the ATG codon and therefore may have missed transcription start sites downstream from 2700.

Primer extension analysis of human TE32 myoblast and HFSF fibroblast RNA mapped two major transcriptional start sites at position 271 and 2421 and also several minor sites. The relative importance of each of these start sites in various tissues is not clear. The varied transcriptional start sites may be due to

**FIG. 6.** Sequence comparison between mouse and human DMPK first introns. Shown in the figure are: positions and nucleotide comparisons for thyroid hormone response elements and the first four conserved E-boxes (A). B, position and nucleotide conservation of the fifth conserved (downstream) E-box. C, location and conservation of a CA/T/CCC box, BEE-1 element, GC box, and CArG box.

**FIG. 7.** Ability of DMPK first intron element to activate muscle specific expression of CAT with a fragment of the DMPK promoter. A, the DMPK first intron was positioned downstream of CAT in both orientations (IVS = orientation orientation, SVI = orientation) to test for enhancer activity with a 679-bp fragment of DMPK 5'-upstream region containing promoter sequence (−722 CAT). B, these constructs, as well as the parental construct (−722 CAT), were co-transfected with pSV β-gal into NIH 3T3 fibroblasts and TE32 myoblasts, CAT and β-galactosidase activities were assayed, and relative CAT activities determined. The value of 1 was arbitrarily assigned to the relative activities of −722 CAT in both cell lines, and activities of all other constructs were determined relative to these constructs. Three separate experiments were performed with at least three samples for each construct. Data from a representative experiment are shown. The DMPK first intron, independent of orientation, activates expression of CAT via the DMPK promoter specifically in myoblasts.

**FIG. 8.** Responsiveness of DMPK first intron element to MyoD. A, the DMPK first intron was positioned in both orientations either downstream of the CAT gene driven by the TK promoter or upstream of TK driving CAT. As a control, the DMPK ninth intron was positioned in the reverse orientation downstream of the CAT gene driven by the TK promoter. B, these constructs were co-transfected with pEMV-MyoD or the pEMV parental vector. CAT and β-galactosidase activities were assayed and relative CAT activities determined. The relative activity of TK CAT in EMSV-transfected cells was assigned the value of 1, and all other CAT activities were adjusted relative to this value. Three separate experiments were performed with at least three samples of each construct. Data from a representative experiment are shown. MyoD overexpression can activate expression of a CAT reporter driven by the TK promoter up to 20-fold in the presence of the DMPK first intron, but not the ninth intron.
the lack of a canonical CCAAT and TATA box in the DMPK promoter region, resulting in multiple sites of transcription initiation often observed with promoters lacking these consensus sequences (46–48). However, most housekeeping and growth control gene promoters have their many initiation sites scattered within a 15–20-bp region (49). Exceptions to this include the Ha-ras gene, which has multiple start sites located within a 90-bp region (50), and the 3.7-kb mRNA for testicular inhibin/activin β B-subunit, which initiates transcription from multiple sites over 150 nucleotides (51). We believe that tran-

**FIG. 9.** Deletion mapping of DMPK first intron enhancer. **A,** schematic map of DMPK first intron showing putative binding sites for various transcription factors. **IN-1,** entire 2.4-kb first intron; **IVS,** 2-kb first intron fragment; **A–I,** various first intron deletions; **E,** E-box. **Numbers** indicate 5' and 3' ends of first intron fragments relative to ATG initiation codon (see Fig. 3). **B,** mapping of first intron sequences required for maximal enhancer activity in TE32 myoblasts. Constructs were prepared and transfected as outlined in Fig. 7 and under "Materials and Methods." The experiment was performed three times with similar results obtained in each. Shown is a representative experiment. **C,** mapping of sequences required for full activity of the DMPK first intron enhancer in 10T1/2 cells co-transfected with MyoD. Constructs were prepared and transfected as described in the legend to Fig. 8 and under "Materials and Methods." The experiment was performed three times with similar results obtained in each. Shown is a representative experiment. Deletion of three of the four E-boxes in the DMPK first intron almost completely abolishes enhancer activity in both cell types.
scription initiation from the mapped start site locations (−71, −421, −773) in the DMPK 5′ region are controlled by separate promoter elements, and we are currently investigating this possibility. Housekeeping style promoters have been found for such protein kinase genes as casein kinase II subunit β (47), the murine hck gene (48), choline kinase R (52), and the β-adrenergic receptor kinase (37).

This study sought to identify cis elements that may activate expression of DMPK preferentially in myoblasts over fibroblasts. It is likely that distinct cis elements located elsewhere in the gene impact transcription of DMPK in other tissues. Increased levels of DMPK mRNA in myoblasts versus fibroblasts may be due either to an increase in DMPK transcription in myoblasts or to differential mRNA stability. In an effort to identify myoblast-specific cis elements, a series of CAT deletion mutants of DMPK 5′ sequence were constructed and tested for CAT activity in TE32 myoblasts and NIH 3T3 fibroblasts (Fig. 4). However, only minor myoblast-specific expression of CAT (2–3-fold) was observed for deletion clones −1963 CAT, −537 CAT, and −403 CAT (Fig. 4), and this activity alone cannot account for the increased levels of DMPK mRNA observed in myoblasts over fibroblasts.

Two peaks of promoter activity mapped within the DMPK 5′ region by transfection of CAT deletion constructs into NIH 3T3 cells (Fig. 4) was consistent with the mapped transcription start sites at position −421 and −71. Of all the DMPK 5′ region deletions, maximal promoter activity was observed with the 189-bp −232 CAT deletion mutant in all cell lines tested (Fig. 4). Low level DMPK mRNA expression was detected in many cell lines by RNase protection (data not shown) and Northern blot analysis (Fig. 1), suggesting a nearly ubiquitous low level presence of DMPK mRNA in several tissue types. We believe that transcription initiating from the −71 start site contributes to this basal level of DMPK mRNA.

Promoter activity of the 189-bp −232 CAT was lost upon the deletion of 66 bp from this sequence (Fig. 4). Our data clearly demonstrate that the ubiquitous transcription factor Sp1 binds to an oligonucleotide probe containing a conserved GC box located within this deleted sequence. Furthermore, this binding is specific and purified Sp1 incubated with this oligonucleotide forms an identical complex (complex a, Fig. 5) to the slowest migrating complex observed with NIH 3T3 nuclear extracts. This evidence suggests that Sp1 binds to the GC box contained in this sequence, thus contributing to a ubiquitous basal level of transcription in several cell types.

The promoter analysis data indicated that cis elements controlling the majority of DMPK transcription in myoblasts were most likely located elsewhere in the gene (Fig. 4). In support of this, transgenic mice expressing β-galactosidase from a 3.7-kb fragment of the DMPK promoter region exhibit mainly neural-specific expression (53). Several muscle-specific genes have tissue-specific regulatory elements contained within their first introns (40, 54–56). Therefore we analyzed the DMPK first intron for conserved muscle-specific transcription factor consensus binding sites and found five E-boxes and a CArG element (Fig. 6) representing MyoD (57) and muscle actin promoter factor 1 and 2 (41) binding sites, respectively. We tested the ability of the DMPK first intron to trans-activate CAT gene expression in the context of a 679-bp fragment of its homologous promoter (Fig. 7A). A 4–6-fold orientation independent up-regulation was observed in TE32 myoblasts, but not in NIH 3T3 fibroblasts, demonstrating that the DMPK first intron contains a myoblast-specific enhancer element (Fig. 7B).

The MyoD family of basic helix loop helix myogenic regulators bind the sequence CANNTG to activate muscle-specific gene transcription (44, 57). Existing evidence observed with P19 embryonal carcinoma cells and DM patient fibroblasts, both overexpressing a stably integrated MyoD gene, suggests that DMPK mRNA expression may be regulated either directly or indirectly by one of these transcription factors (15, 16, 58). We tested the responsiveness of the DMPK first intron to MyoD. This myogenic regulator could trans-activate expression of CAT in the presence of the DMPK first intron, but not the similarly sized ninth intron in 10T1/2 cells. Regardless of position or orientation around the TK promoter (Fig. 8) or the DMPK promoter (data not shown), CAT expression was trans-activated through the DMPK first intron by MyoD.

Deletion analysis of the first intron revealed that a 51-bp sequence containing two conserved E-boxes is required for full enhancer function in TE32 myoblasts. Deletion of three of the four E-boxes was required for near-complete abrogation of enhancer function in 10T1/2 cells co-transfected with MyoD and CAT first intron deletions driven by the TK promoter. Since E-boxes are the only known cis elements present within this sequence, and given their complete conservation between mouse and human sequence (Fig. 6), it is very likely that factors binding these elements mediate enhancer function. It is apparent that the first three E-boxes each contribute in an additive fashion to enhancer activity as the sequential removal of each results in a decrease in activity in both TE32 and 10T1/2 cells co-transfected with MyoD and CAT. Mutating each of the four E-boxes within the 113-bp segment of first intron sequence will allow assessment of their relative importance for enhancer function.

Interestingly, it has been shown that certain rhabdomyosarcoma cell lines, including TE32, are deficient in a MyoD co-factor that prevents them from fusing and forming multinucleate myotubes (59). It is possible that in this cell line MyoD may be able to up-regulate a subset of genes not involved in the fusion process in the absence of this co-factor. An alternative explanation is that one of the other myogenic factors or another transcription factor may activate DMPK expression through the 51-bp element in the first intron. In any case, the first three E-boxes in the DMPK first intron are clearly required for maximal DMPK enhancer function and likely play an important role in expression of the gene in muscle.

In summary, DMPK transcription in myoblasts is at least partially regulated by a 51-bp MyoD-responsive element located in the first intron of the gene. This sequence cooperates with DMPK promoter elements, one of which binds Sp1, to up-regulate transcription in myoblasts. These data, in conjunction with other studies showing MyoD responsiveness of the DMPK transcript (15, 16, 58), implicate this myogenic factor in regulating transcription of this gene in muscle. Experiments aimed at understanding the interaction between the promoter and enhancer and which individual E-boxes within the 51 bp element are required for enhancer function are underway.

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