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Sp1 and ETS family transcription factors regulate the mouse Mta2 gene expression

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Abstract

Dynamic changes in chromatin structure through nucleosome remodelling and core histone tail acetylation play important roles in transcriptional regulation. The purification and functional characterization of a nucleosome remodelling and histone deacetylase complex, NuRD, has suggested that nucleosome remodelling and core histone tail modification are potentially linked processes. MTA2, a component of the NuRD complex, plays an important role in regulating histone deacetylase activity of the NuRD complex. Similar to the candidate metastasis associated protein MTA1, an elevated level of MTA2 correlates with cellular proliferation. To understand the regulation of *Mta2* transcription, we characterized the mouse *Mta2* gene and its transcriptional regulatory elements. We found that MTA2 is encoded by 18 exons that span 10 kb. Primer extension analysis identified a major transcriptional start site locates 259 base pairs upstream of the ATG translational start codon. Transient transfection studies localized its promoter, lacking a canonical TATA box, to within 60 base pairs upstream of the transcriptional start site. Gel-mobility-shift and mutagenesis studies revealed that Sp1 and ETS elements play important roles in regulating *Mta2* transcription. Information concerning the regulation of the *Mta2* gene expression will be useful in understanding the regulation of NuRD histone deacetylase activity, which in turn will help in our general understanding of the transcriptional repression mechanism.

Keywords: Gene expression; Histone deacetylase; Nucleosome remodelling; NuRD

1. Introduction

DNA in a eukaryotic cell is packaged with core histones and other nucleosomal proteins in the form of chromatin. Dynamic changes in chromatin structure play important roles in transcriptional regulation. Studies in the past several years have identified at least two types of protein complexes that are capable of altering chromatin structure to allow protein factors access to nucleosomal DNA. One involves multiprotein complexes that utilize energy derived from ATP hydrolysis to 'remodel' nucleosomes (Vignali et al., 2000); the other involves covalent modification, in particular acetylation, of core histone tails (Spencer and Davie, 1999). Until recently, it was not known whether these two types of chromatin modification processes were linked. The purification and functional characterization of a protein complex, NuRD, possessing both nucleosome remodelling and histone deacetylase activities, suggests that the two chromatin modification processes are potentially linked (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998a; Wade et al., 1999).

NuRD is a multi-subunit protein complex that possesses both nucleosome remodelling and histone deacetylase activities (Zhang et al., 1998a). In addition to the four-subunit histone deacetylase core, HDAC1/2 and RbAp46/48, also present in the Sin3 histone deacetylase complex (Zhang et al., 1997, 1998b), NuRD contains at least three more subunits: Mi2, MBD3 and MTA2 (Zhang et al., 1998a, 1999). Mi2 is a SWI2/SNF2 type helicase/ATPase domain containing protein that was first identified as a dermatomyositis-specific autoantigen (Seelig et al., 1995), and has been postulated to be responsible for the chromatin remodelling activity of the NuRD complex (Zhang et al., 1998a). MBD3 is a methyl-CpG-binding domain containing protein, similar to MBD2 (Hendrich and Bird, 1998; Zhang et al., 1999). However, the function of MBD3 in the NuRD complex is not known. MTA2 is a zinc-finger protein that is highly similar (65% identical) to the candidate metastasis asso-

Abbreviations: NuRD, nucleosome remodelling and histone deacetylase; MTA2, metastasis associated protein 2; PCR, polymerase chain reaction

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ciated protein MTA1 (Toh et al., 1994; Zhang et al., 1999). The expression level of MTA1 has been correlated with metastatic status in cancer cell lines and primary tumor tissues (Toh et al., 1997, 1999). A similar observation for MTA2 has also been made in cervical cancer tissues (L.X. and Y.Z., unpublished observation). Biochemical characterization of MTA2 indicates that it plays an important role in modulating the histone deacetylase activity of the NuRD complex (Zhang et al., 1999). Consistent with its role in stimulating histone deacetylase activity and its potential involvement in cancer is the finding that histone deacetylase inhibitors are potent anticancer drugs (Marks et al., 2000).

Given the important roles of MTA2 in the NuRD complex, and the potential link between *MTA2* expression and cancer metastasis, it is important to determine how the expression level of MTA2 is regulated. As a first step toward understanding the regulation of *Mta2* gene expression, we studied *Mta2* at the transcriptional level. Here we report the isolation and detailed analysis of the mouse *Mta2* gene. Our study revealed that *Mta2* gene transcription is driven by a TATA-less promoter located within 60 bp upstream of its transcription initiation site. In addition, we identified Sp1 and ETS elements to be critical elements in regulating *Mta2* transcription.

2. Materials and methods

2.1. Isolation and sequencing of the mouse Mta2 genomic clones

DNA primers 5′-CCGGGTGGGAGATTACGTC-3′ and 5′-CCACCACGAGAAACTGATC-3′, corresponding to nucleotides 328–346 and 881–990 of mouse MTA2 cDNA (GenBank accession no. AF159259), respectively, were used to generate a 663-bp polymerase chain reaction (PCR) product which was ^{32}P-labelled to screen a bacterial artificial chromosome 129/SvJ genomic library (Shizuya et al., 1992). Plasmid DNA was purified from positive clones and was digested with different restriction enzymes and analyzed by Southern hybridization. Selected genomic DNA fragments were subcloned into the pBluescript cloning vector (Stratagene) and sequenced by the di-deoxy chain-termination method (Sanger et al., 1977). The entire mouse *Mta2* gene was sequenced and the sequences have been deposited in GenBank (accession no. AF348083).

2.2. Primer extension assays

Primer extension reactions were performed as previously described (Coats et al., 1994). The antisense primer 5′-GACGACGACACTGCTATAGCCTCACGCC-3′, corresponding to bases 107 to 134 of the mouse MTA2 cDNA sequence, was end-labelled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The labelled primers (10^6 CPM) were mixed with 20 μg of total RNA isolated from mouse liver, NIH3T3, or NMuLi cells. The mixture was first incubated at 65°C for 1.5 h in hybridization buffer containing 0.25 M KCl, 10 mM Tris (pH 8.0), and 1 mM EDTA before gradually cooling to 37°C. The annealed hybridization mixture was then extended at 37°C for 1 h with ten units of AMV reverse transcriptase in buffer containing 20 mM Tris (pH 8.0), 75 mM KCl, 10 mM $MgCl_2$, 1 mM of each of dNTPs, 10 mM DTT, 1 unit/μl RNasin and 50 mg/ml actinomycin D. After phenol extraction and ethanol precipitation, the extension products were resolved on a 6% polyacrylamide/7 M urea gel.

2.3. Northern blot analyzes

Multiple mouse tissue Northern blots were purchased from Clontech. The random prime labelled cDNA probe was the same as that used in the genomic clone isolation. Hybridization with ExpressHyb solution was performed as suggested by the manufacturer (Clontech). To control for loading variability, the blot was stripped and reprobed with ^{32}P-labelled mouse β-actin cDNA. Fifteen micrograms of total RNA isolated from $Sp1^{+/+}$ and $Sp1^{-/-}$ mouse ES cells were used in the Northern blot in Fig. 6B.

2.4. Plasmids and mutagenesis

pGL2-Basic, which contains a luciferase open reading frame in a promoterless background, was obtained from Promega. To generate the p-60Luc reporter construct, forward primer 5′-TAAGATCTGACGTCTGCTGGGACTCGGTG-3′ and reverse primer 5′- CAAAGCTTAGCTCCAGACGAAGCC-3′ were used to amplify the promoter region between −60 and +200 and cloned into *Bgl*II and *Hind*III sites of pGL2-basic. The *Aat*II site adjacent to the *Bgl*II site (−55) in the p-60Luc construct was used to clone other reporter constructs as follows: Reporter p-4kLuc, which contains about 4 kb mouse MTA2 sequence upstream of the transcription start site and 200 bp downstream of the transcription start site, was constructed by digesting the genomic clone with *Bam*HI and *Aat*II and cloned into the *Bgl*II and *Aat*II sites of p-60Luc; Construct p-1072Luc was generated by digesting the genomic clone with *Xho*I and *Aat*II and cloned into the *Xho*I and *Aat*II sites of p-60Luc; Constructs p-710Luc, p-354Luc, and p-133Luc were similarly generated by digesting the genomic clone with *Msc*I/*Aat*II, *Bss*HI/*Aat*II, *Eag*I followed by Klenow fill in and *Aat*II, and cloned into appropriate sites in p-60Luc construct, respectively. The p-266Luc was generated by PCR using forward primer 5′-ATCTCGAGCGAAAGGAGCCGAG-3′ and reverse primer 5′-GCTCCAGACGAAGCC-3′, and cloned into *Xho*I and *Aat*II sites of p-60Luc. For the Sp1 and Ets binding site mutagenesis, the p-354Luc was used as parent plasmid. PCR was performed using forward primer 5′-ATAGCGCGCCTTTCGACGCC-3′ and reverse primer 5′-CCAGCAGACGTCGTGCG-3′. The three pairs of primers for mutagenesis were 5′-GAGTTAATTTTGGCTCC-3′ and 5′-GTGAGGAGCCAAAATTAACTC-3′ for the Ets binding site upstream the Sp1

site; 5′-CCTCAGGAACGGGGTCGAG-3′ and 5′-GCTCGACCCCGTT CCTGAGG-3′ for Sp1 binding site; 5′-GAGCTGCAACGCGTATAACCGAG-3′ and 5′-CTCGGTTATACGCGTTGCAGCTC-3′ for the Ets site downstream the Sp1 site. All the mutants are generated by PCR and the sequences of the constructs were confirmed by DNA sequencing.

2.5. Transfection and luciferase assays

All the cell lines used were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 2 mM L-glutamine, and 100 mg/ml penicillin-streptomycin. For transfection, about 3×10^5 cells were seeded in each 60 mm culture dish for 16–24 h. Four micrograms of pGL2-Basic or pGL2-Basic-derived plasmids were co-transfected with 1 μg of pCH110 (Pharmacia) encoding β-gal using the Superfect transfection kit (Qiagen). Twenty-four to thirty hours after transfections, cells were harvested, and luciferase and β-galactosidase activities were determined using the luciferase and β-gal assay systems (Promega).

2.6. Gel-mobility-shift assays

Two pairs of primers 5′-GCCCTCAGGGGCGGGGTCGAGCTG-3′ and 5′-GCAGCTCGACCCCGCCCCTGAGGG-3′, 5′-GTCGAGCTGCAACGGAAATAACCGAG-3′ and 5′-GCTCGGTTATTTCCGTTGCAGCTCGA-3′ were annealed and end labelled with Klenow and [α-^{32}P]dCTP. Prior to use, probes were purified on a 15% polyacrylamide gel. Two to ten micrograms of nuclear protein, prepared from NIH3T3 or mouse embryonic stem (ES) cells (Dignam et al., 1983), and 20 to 40 fmol of labelled probes were mixed in buffers containing 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM DTT, 1 mM EDTA, 5% glycerol, 3 μg poly(dI-dC). Reactions were incubated at room temperature for 30 min before resolving on a 4% non-denaturing polyacrylamide gel in buffer containing 190 mM glycine, 1 mM EDTA, and 25 mM Tris. Dried gels were subjected to autoradiography. Where indicated, 10 pmole of the competitors were added in the binding reaction. The mutant competitors used in the competition assays were generated by annealing two pairs of primers used in promoter mutagenesis studies described above.

3. Results

3.1. Mta2 is ubiquitously expressed

Previously we have shown that MTA2 plays an important role in regulating the HDAC activity of the NuRD histone deacetylase core complex, HDAC1/2 and RbAp46/48 (Zhang et al., 1999). One interesting question is whether MTA2 functions in a tissue or cell-specific manner. To answer this question, we determined *Mta2* expression pattern in major mouse organs by Northern blot analysis using a mouse MTA2 cDNA probe. Results shown in Fig. 1A indicate that a single MTA2-specific RNA species of 3.5 kb is present in all tissues examined. This widespread expression pattern, similar to the ubiquitous expression patterns of HDAC1 and HDAC2 (Yang et al., 1997), indicates that MTA2 is likely to play a general role in the NuRD complex and is consistent with the predicted broad function of the NuRD complex. Quantification by using β-actin as a control indicates that the levels of MTA2 mRNA vary in different organs with the highest level in liver and lowest level in skeletal muscle (Fig. 1B).

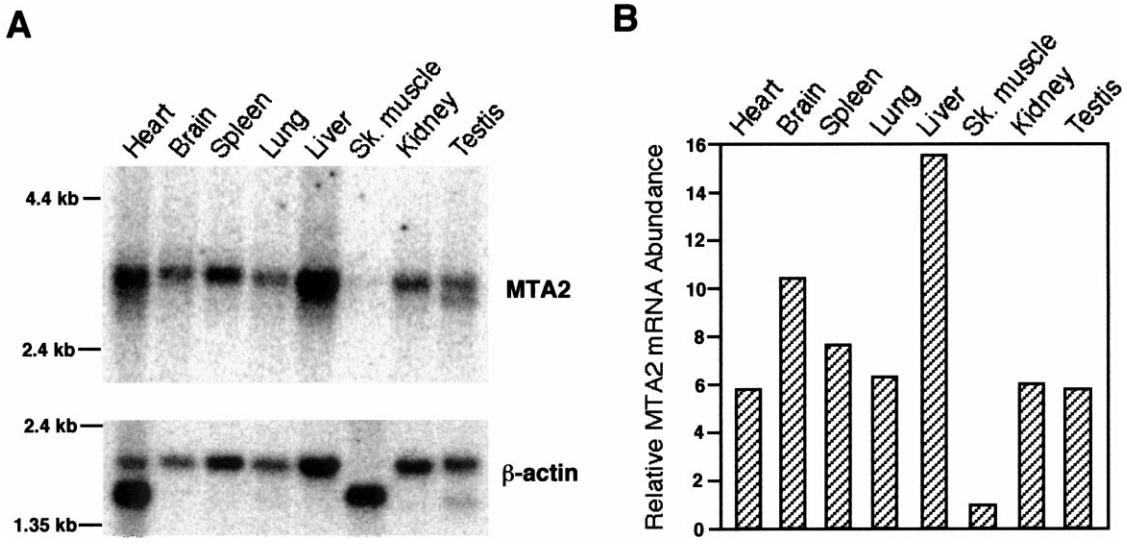

Fig. 1. Northern blot analysis of MTA2 from different mouse organs. (A). Total RNA isolated from different mouse organs was analyzed by Northern blot using MTA2 cDNA as a probe. A single band of about 3.5 kb was detected in all tissues analyzed. Hybridization to a mouse β-actin probe is shown as control for equal loading. The sizes of molecular mass markers are indicated on the left. (B). Relative MTA2 mRNA abundance normalized using β-actin as a control.

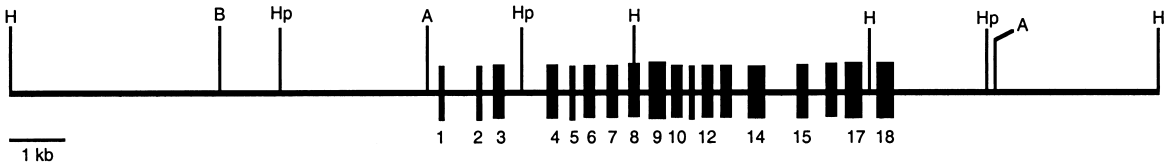

Fig. 2. Organization of the mouse *Mta2* gene. Exons (1–18) are shown as filled boxes; introns and flanking sequences are shown as lines. Restriction sites were *Hind*III (H), *Bam*HI (B), *Hpa*I (Hp), and *Aat*II (A).

3.2. Genomic organization of the mouse Mta2 gene

The above studies indicate that the mRNA level of MTA2 in different mouse organs are differentially regulated. To understand how this regulation is achieved, we decided to investigate its regulation at the transcriptional level. Toward this end, we isolated three mouse BAC clones from a bacterial artificial chromosome 129/SvJ genomic library with a 663 bp MTA2 cDNA fragment as a probe. Restriction fragment mapping and Southern blot analysis indicate that all the three BAC clones contained the *Mta2* gene (data not shown). After subcloning the smallest *Mta2* containing BAC clone (about 28 kb), we sequenced the gene and determined its genomic organization (Fig. 2 and Table 1). Comparison of genomic DNA sequences with the MTA2 cDNA sequences revealed that MTA2 is encoded by 18 exons that span approximately 10 kb (Fig. 2). All the exons are quite small, with sizes ranging from 28 to 229 bp (Table 1). All the introns are also relatively small. Only three of the seventeen introns are over 1 kb (Table 1). The DNA sequences of all splice donor and acceptor sites comply with the invariant GT and AG rule (Table 1).

3.3. Determination of the mouse Mta2 gene transcriptional initiation site

Having obtained the mouse *Mta2* gene and determined its organization, we sought to determine its transcriptional initiation site so that reporter constructs containing different upstream sequences could be generated. To this end, several primers were used in reverse transcriptase primer extension assays using total RNA isolated from mouse liver, NIH3T3 and NMuLi cells. Parallel reactions were carried out with yeast tRNA as a negative control. Results from one primer consistently generated specific doublet signals indicating two major transcriptional initiation sites. Alignment of this primer extension product with a dideoxynucleotide sequence ladder from the same primer revealed that the two strong bands correspond to two As (Fig. 3). We suspect that transcription is initiated at the first A (259 bp upstream of the ATG translational start codon) and that the cap structure of the mouse MTA2 mRNA accounts for the staggered ends.

3.4. Localization of the mouse Mta2 promoter

To locate the DNA elements responsible for *Mta2* gene transcription, a series of luciferase reporter constructs containing different lengths of promoter sequences were generated (Fig. 4A). The relative luciferase activities of these constructs were determined by transiently transfecting these constructs into different human and mouse cell lines. Consistent with the differential expression observed by Northern blot analysis (Fig. 1), *Mta2* expression in the

Table 1
Organization of the mouse MTA2 gene

Exon/Intron	Exon size (bp)	5′ Splicing junction	3′ Splicing junction	Intron size (bp)
1/1	28	GGA G/gtgag	tacag/AT TAC	1081
2/2	68	AAT AAG/gtgag	tttag/ACT GCA	480
3/3	94	GCT C/gtgag	tctag/GG GAG	1647
4/4	118	ATA AG/gtata	cttag/G GGG	132
5/5	64	AAG GAG/gtgag	ttcag/GAC TGC	197
6/6	118	GAG G/gtaag	ctcag/GA GAA	263
7/7	103	GCG CG/gtgag	tttag/A GCT	286
8/8	100	ACT CTG/gtaag	ggtag/TTC CAT	115
9/9	189	GAC TTT/gtaag	tttag/CTA CCC	84
10/10	75	CAG CAG/gtatg	tacag/AAA AGA	127
11/11	59	ACT TA/gtaag	attag/T ACC	239
12/12	98	CAC A/gtgag	ggtag/CC ACA	137
13/13	140	ACC ACA/gtaag	tctag/GAG CCA	555
14/14	229	GAG T/gtaag	tccag/GC TCG	1121
15/15	90	CTG G/gtatg	gacag/TG GCT	522
16/16	119	GAG ACT/gtgag	tccag/ATG GCA	105
17/17	149	ACC AG/gtagg	gccag/G GCT	205
18	163			

mouse muscle cells (C2C12) was observed to be substantially lower than that in the mouse liver cells (NMuLi) for each of the reporter construct (Fig. 4B) indicating that muscle cells are likely to lack some of the transcriptional factors involved in *Mta2* transcription. In addition, the results shown in Fig. 4B indicate that, in all four cell lines tested, a promoter construct that contains 354 bp upstream from the transcription initiation site (p-354Luc) correlated with higher luciferase activity (as much as 3100-fold in NIH3T3 and NMuLi cells) relative to a promoterless reporter construct (pGL2-Basic). However, deletion of the DNA sequences to 226 bp upstream from the transcription initiation site dramatically reduced the reporter activity in all cell lines tested. These results indicate that a 130 bp region between −226 and −354 contains major transcriptional elements that control the expression of the *Mta2* gene. In addition, the shortest promoter sequence construct tested (p-60Luc) still retains 33 to 110-fold higher transcriptional activity over the background (pGL2-Basic), indicating that the core promoter sequence resides between −60 to +200. No canonical TATA box was found in the core promoter region (Fig. 5A) indicating that the *Mta2* gene is driven by a TATA-less promoter.

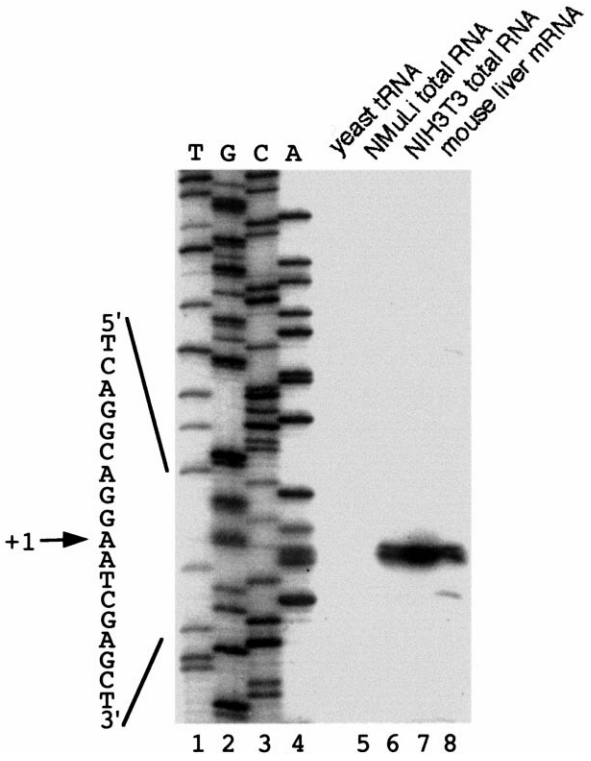

Fig. 3. Determination of the 5′-end of mouse *Mta2* transcripts. Purified total RNA from different cell lines or mouse tissues, as indicated, was used as templates. Primer extension was performed using a ^{32}P-labelled oligodeoxynucleotide with AMV reverse transcriptase. A genomic sequencing ladder was run in parallel and is shown on the left. The arrow indicates the most likely transcription start site.

3.5. Sp1 and ETS family proteins are involved in Mta2 transcription

Since deletion of the DNA sequences between −226 and −354 resulted in a significant loss of transcriptional activity in all cell lines tested (Fig. 4B), this region is likely to contain major enhancer elements responsible for the ubiquitous expression of *Mta2*. Analysis of the sequences in this region identified several potential transcription factor binding sites, including those for Sp1 and the ETS family proteins (Fig. 5A). To determine which of these potential transcription factor binding sites play a role in *Mta2* transcription, the three potential binding sites between −226 and −354 were mutagenized individually or in combination (Fig. 5B). The resulting mutant reporter plasmids were transfected into NIH3T3 cells and their luciferase activities were compared with that of the wild-type reporter. Results shown in Fig. 5C indicate that mutation of the putative Sp1 family protein binding site significantly reduced the reporter activity (compare pM2Luc with p-354Luc). However, mutations of neither of the two ETS binding sites alone affected reporter activity significantly (compare pM1Luc and pM3Luc with p-354Luc). Interestingly, mutation of the Sp1 site and its downstream ETS site in combination synergistically inhibited the reporter activity (compare pM2 + 3Luc with pM2Luc and pM3Luc) indicating ETS and Sp1 family proteins may co-operate in regulating *Mta2* gene activity. In summary, the mutational studies revealed that Sp1 family proteins play an important role in regulating *Mta2* transcription. In addition, the ETS family transcription factors are likely to function together with the Sp1 family proteins through the element downstream of the Sp1 binding site. The putative ETS site upstream of the Sp1 site does not seem to contribute to *Mta2* transcription.

Having established that two DNA elements play important roles in regulating *Mta2* gene expression, we predict transcription factors involved in *Mta2* transcription should bind specifically to the two elements. To reveal the nature of these binding factors, gel-mobility shift assays were performed using probes corresponding to the two DNA elements and nuclear extracts from NIH3T3 cells. As shown in Fig. 5D, two shifted bands were observed when the Sp1 probe was used (lanes 2 and 4). Both shifts are specific as they are competitively inhibited by wild-type non-labelled competitor but not by a mutant competitor (compare lanes 3 and 4). Previous studies have demonstrated that both Sp1 and Sp3 recognize the same DNA sequences and they exhibit a specific binding pattern when both proteins present in the binding assay (Suske, 1999). Comparison of the binding pattern with the established binding pattern for Sp-family proteins (Suske, 1999), the top and the bottom bands are likely to represent Sp1 and Sp3 binding, respectively. Specific protein binding was also observed when a DNA element containing the putative ETS binding site was used as a probe (lanes 5–8). However, at least five bands were observed, indicative of binding by

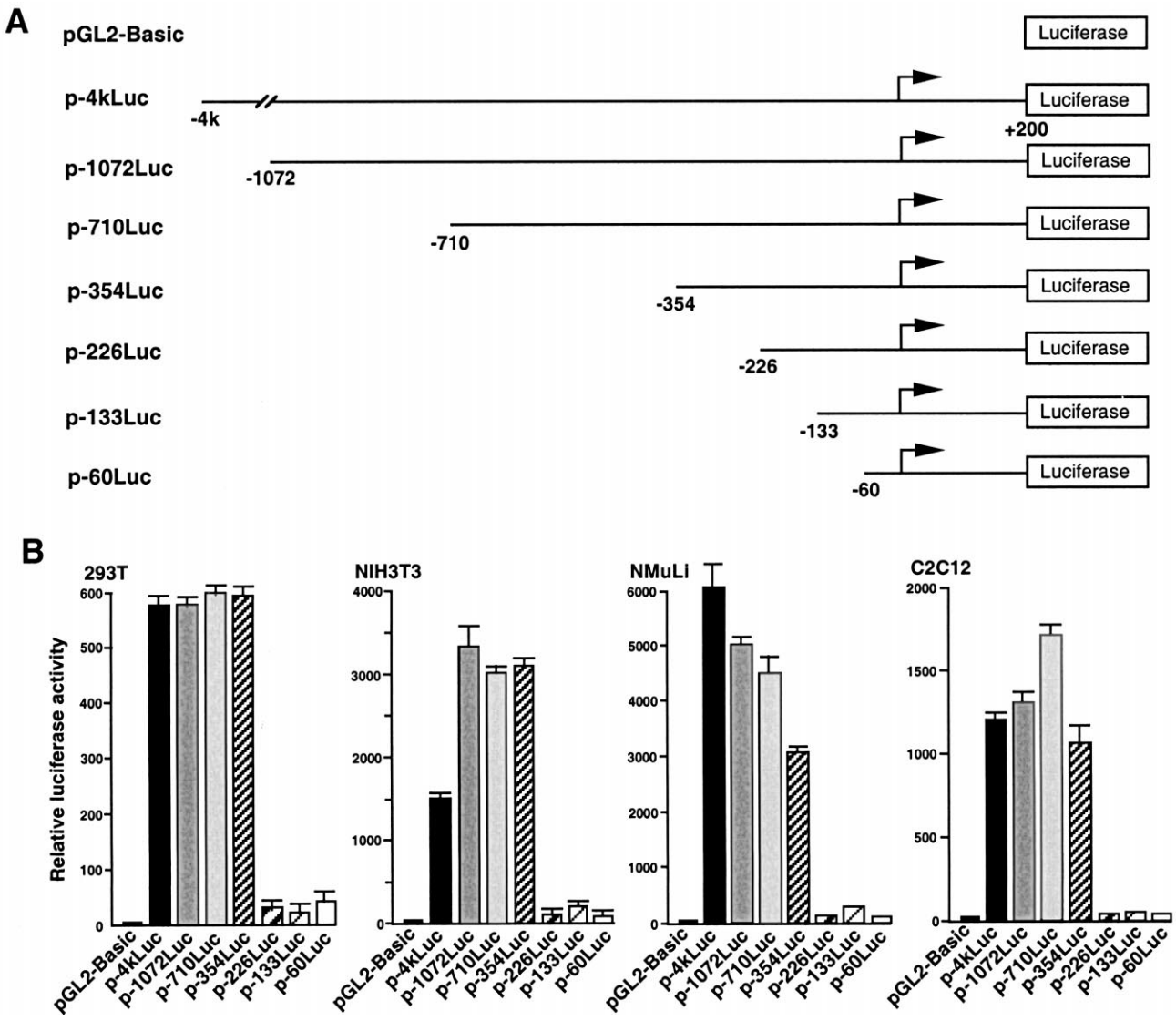

Fig. 4. Identification of regulatory elements for mouse *Mta2* transcription. (A). Schematic diagram of different *Mta2* promoter fragments subcloned into the luciferase reporter plasmid pGL2-Basic. Hooked arrows indicate the transcription initiation site and direction. (B). Different reporter plasmids were transfected into different cell lines as indicated. All the luciferase activity was normalized to the promoter-less pGL2-Basic plasmid. Transfection efficiencies were normalized using a β-galactosidase assay. Transfections were repeated at least two times in duplicate. Variations between experiments are indicated by error bars.

multiple proteins of the ETS family (Graves and Petersen, 1998). Competition experiments indicate that all shifts are specific (compare lanes 7 and 8). Therefore, both DNA elements that contribute to *Mta2* transcription can serve as specific transcription factor binding sites.

3.6. Disruption of the Sp1 gene does not affect Mta2 expression

The above studies indicate that the Sp1 family proteins play important roles in regulating *Mta2* expression. However, since all the Sp1 family proteins (Sp1, Sp2, Sp3, and Sp4) recognize the same DNA sequences (Suske, 1999), it is not clear which of the family members is involved in *Mta2* transcription. Since *Mta2* has a ubiquitous expression pattern (Fig. 1), the Sp1 family members, Sp2 and Sp4, that only express in restricted tissues, are not likely to be involved in *Mta2* transcription. To determine whether Sp1 is involved in *Mta2* transcription, we examined the effect of Sp1 gene deletion on *Mta2* expression. Total protein and RNA were isolated from $Sp1^{+/+}$ and $Sp1^{-/-}$ ES cells and analyzed by Western and Northern blots (Marin et al., 1997). Alpha-tubulin and beta-actin were analyzed as controls of equal loading. Results shown in Fig. 6 indicate disruption of *Sp1* does not affect *Mta2* expression on either the RNA or the protein level. Although this result does not support the involvement of Sp1 in *Mta2* transcription, we can not rule out its involvement either because it is possible that Sp1 and Sp3 may regulate *Mta2* transcription in a redundant manner.

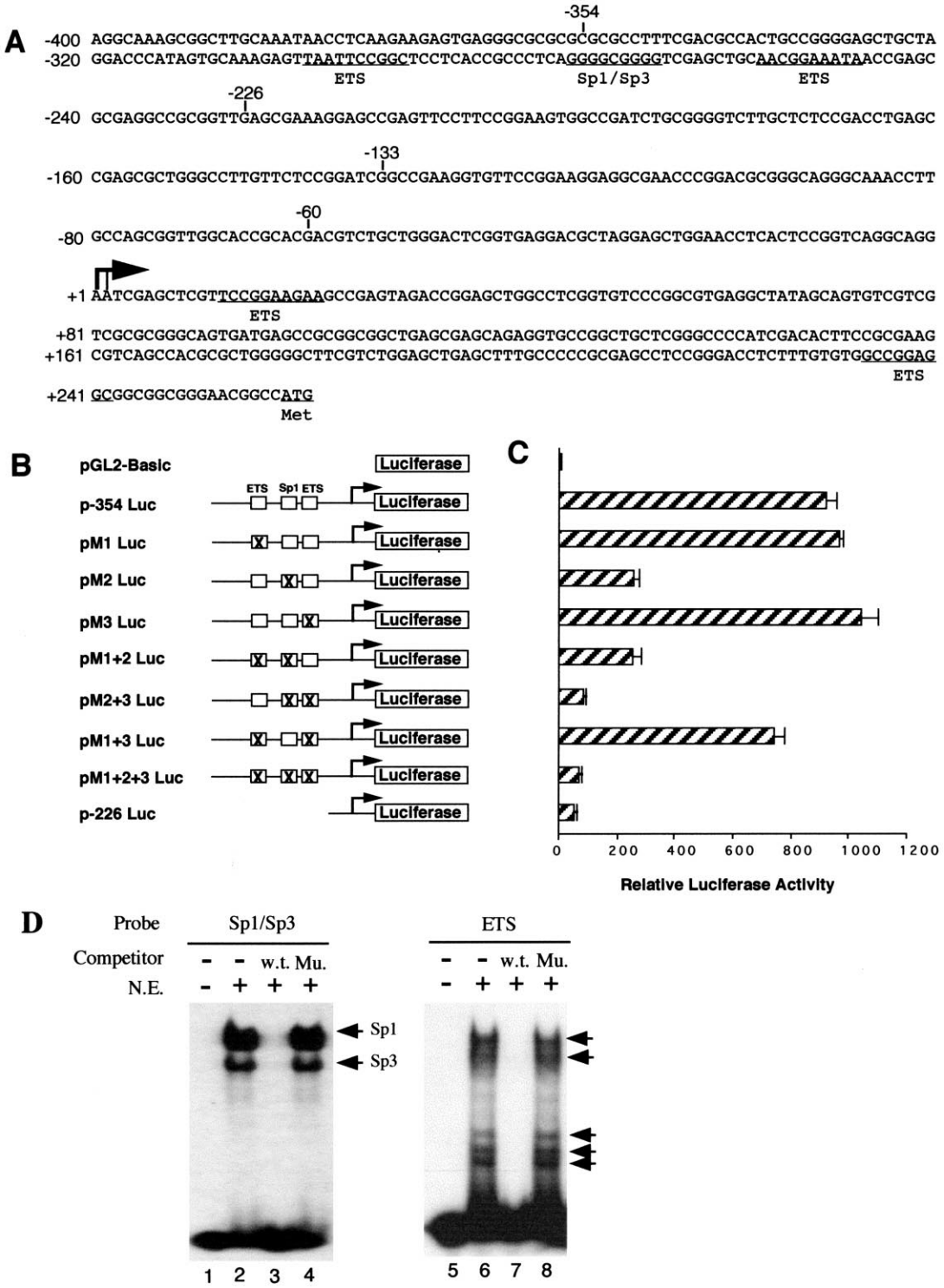

Fig. 5. Sp1 and ETS family transcription factors are involved in mouse *Mta2* gene transcription. (A). DNA sequences upstream of the translational start codon of the mouse *Mta2* gene. The hooked arrow indicates the major transcriptional initiation site. An adjacent initiation site is also indicated. Some potentially relevant transcription factor binding sites are underlined. (B). Schematic diagram of different mutant and wild-type reporter constructs. The three potential transcription factor binding sites analyzed are boxed. 'x' indicates mutated binding site. (C). Relative luciferase activities of the different reporter constructs shown in B. All the luciferase activity was normalized relative to the promoter-less pGL2-Basic plasmid. Transfection efficiencies were normalized using a β-galactosidase assay. Transfections were repeated at least two times in duplicate. Variations between experiments are indicated by error bars. (D). Gel-mobility shift assays demonstrate specific protein binding to the two DNA elements involved in *Mta2* transcription. Arrows point to specific shift bands. Probes used are indicated. The presence and absence of nuclear extracts, or competitors are indicated by ' + ' or ' − '. Wild-type and mutant competitors are indicated by ' w.t. ' and ' Mu. ', respectively.

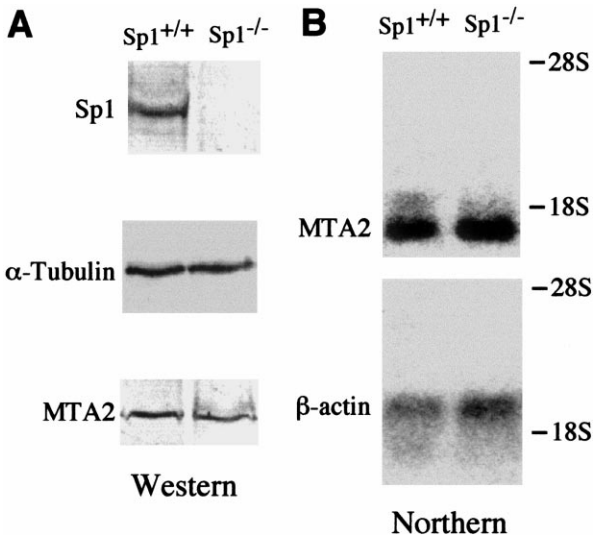

Fig. 6. Disruption of the mouse Sp1 gene does not affect Mta2 expression in mouse ES cells. (A). Western blot analysis demonstrating that MTA2 protein level is not affected by Sp1 gene deletion. Thirty micrograms of total protein extracted from $Sp1^{+/+}$ and $Sp1^{-/-}$ mouse ES cells were used in the assay. (B). Northern blot analysis demonstrating that MTA2 mRNA levels are not affected by *Sp1* gene deletion. Fifteen micrograms of total RNA isolated from $Sp1^{+/+}$ and $Sp1^{-/-}$ mouse ES cells were used in the Northern blot analysis. The blot was first hybridized with a ^{32}P-labelled MTA2 probe and exposed to X-ray film. The blot was then stripped and hybridized to a β-actin probe for quantification. No significant difference in the MTA2 levels were detected. The migration positions of 28S and 18S rRNAs are indicated.

4. Discussion

Dynamic changes in chromatin structure through nucleosome remodelling and core histone acetylation/deacetylation play important roles in the regulation of eukaryotic transcription (Spencer and Davie, 1999; Vignali et al., 2000). Almost all the nucleosome remodelling factors and histone acetyltransferases/deacetylases characterized so far function in large protein complexes. One such protein complex, NuRD, possesses both nucleosome remodelling and histone deacetylase activities, and has the potential to link histone tail modification and nucleosome remodelling (Zhang et al., 1998a). One component of the NuRD complex, named MTA2, plays an important role in modulating the histone deacetylase activity of the NuRD complex (Zhang et al., 1999). Consistent with the observation that inhibition of histone deacetylase activity induces differentiation of transformed cells (Marks et al., 2000), high expression levels of MTA1 and MTA2 have been correlated with cellular proliferation (Toh et al., 1997, 1999). To understand the transciptional regulation of *Mta2* gene expression, we have isolated and characterized the mouse *Mta2* gene promoter.

Mouse *Mta2* is expressed in all the major organs tested. However, the expression level varies as much as 16-fold with the highest mRNA level observed in liver and lowest in skeletal muscle (Fig. 1). To find a molecular explanation for the differential expression level, we cloned the mouse *Mta2* gene and analyzed its genomic organization. We found that mouse *Mta2* is encoded by 18 exons scattered over 10 kb (Fig. 2). The transcription initiation site has been mapped to 259 bp upstream of the ATG translational start codon (Fig. 3). Promoter deletion and transient transfection studies have revealed that the core promoter of mouse *Mta2* resides within 60 bp upstream of its transcriptional initiation site, which lacks a TATA box, a feature common to many housekeeping genes.

One of the most intriguing findings in this study was the identification of a 130 bp region between −226 and −354 that contains a major enhancer element for *Mta2* transcription (Fig. 4). Using gel-mobility-shift, mutagenesis, and reporter assays (Fig. 5), we further demonstrated that Sp1 and ETS family proteins play important roles in *Mta2* transcription. Based on the fact that disruption of the *Sp1* gene does not affect *Mta2* expression (Fig. 6), we conclude that Sp3 likely to have a role in *Mta2* transcription. However, the role of Sp1 in MTA2 could not completely ruled out due to its potential redundant function with Sp3. In addition, we identified that an ETS binding site functions synergistically with its upstream Sp1 binding site in regulating *Mta2* transcription (Fig. 5). Thus far, about twenty ETS family members have been identified in mammals. They share a highly conserved DNA binding domain, termed the ETS domain, which recognizes the core motif, 5′-GGA(A/T)-3′ (Graves and Petersen, 1998). Genetic studies have clearly demonstrated that different ETS proteins perform distinct biological functions despite their overlapping cellular distribution and similar DNA-binding properties (Graves and Petersen, 1998). Based on the ubiquitous expression pattern of *Mta2* (Fig. 1), we believe that the onco-protein ETS-2 is the most likely candidate transcription factor that controls *Mta2* expression because ETS-2 is the only member in the ETS family that is ubiquitously expressed (Graves and Petersen, 1998). Consistent with ETS-2 as a potential activator for *Mta2* gene and our observation that MTA2 levels are up regulated in cervical carcinomas (L.X. and Y.Z., unpublished data), increased expression of ETS-2 has been found in 60% of carcinoma cell lines (Simpson et al., 1997).

Our results also suggest that the Sp1 and ETS family proteins may function together in regulating *Mta2* expression (Fig. 5 B and C). Several studies have demonstrated synergistic activation of transcription by the Sp1 and ETS family transcription factors (Gegonne et al., 1993; Gory et al., 1998; Shirasaki et al., 1999; Yan et al., 2000). It worth noting that the target genes controlled coordinately by Sp1 and ETS family transcription factors are all up regulated in tumors, and the proteins encoded by these genes are in some form linked to cancer metastasis. These genes include cathepsin B (Hackel et al., 2000), tenascin-C (Jahkola et al., 1998), and vascular endothelial cadherin (Cai et al., 1999). The fact that *Mta2* transcription is also coordinately regulated by Sp1 and ETS family proteins is consistent with

its potential roles in cancer. It will be important to determine whether up regulation of *Mta2* plays a direct role in cellular proliferation and cancer. Identification and characterization of the *Mta2* gene will certainly facilitate this endeavor. In addition, with the now fully characterized mouse *Mta2* gene, we should be able to begin to address its biological function through targeted gene deletion in mice.

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