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Structure and expression of major histocompatibility complex-binding protein 2, a 275-kDa zinc finger protein that binds to an enhancer of major histocompatibility complex class I genes

(MBP-2 major histocompatibility complex enhancer binding protein)

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ABSTRACT **We have isolated a cDNA encoding a transcription factor that binds to the enhancer of major histocompatibility complex (MHC) class I genes. MHC-binding protein 2 (MBP-2) is a 275-kDa protein, containing two sets of widely separated zinc fingers and a stretch of highly acidic amino acids, a putative transactivation domain. The two zinc finger regions, when expressed individually as bacterial fusion proteins, bind with highest affinity to the MHC class I gene enhancer. Several proteins found in mammalian nuclear extracts bind the MHC class I enhancer in an electrophoresis mobility shift assay. Only one of these, a ubiquitously expressed factor, forming a slow-migrating retarded complex, can be supershifted by a MBP-2 antiserum. The same antiserum also precipitates a protein of >250 kDa from COS cells transfected with a MBP-2 expression vector. Our data indicate that MBP-2 is a transcription factor involved in the regulation of MHC class I gene expression.**

The transcription of major histocompatibility complex (MHC) class I genes is regulated by several enhancer elements in its promoter (1). The palindromic motif 5′-GGGGATTCCCC-3′, also known as enhancer A-region 1, is a strong enhancer element found in the promoters of most MHC class I genes (2–5). We have shown previously that in neuroblastoma, N-*myc* suppresses the expression of MHC class I molecules by reducing the binding of a transcription factor to this MHC enhancer motif (6). We were therefore interested in cloning transcription factors that bind to this MHC enhancer motif.

Several transcription factors have been described that bind this palindromic MHC enhancer: H2TF1, NF-κB, KBF-1, MBP-1/PRDII-BF1, and MBP-2 (MBP = MHC-binding protein; refs. 7–12). H2TF1 has only been identified as a protein–DNA complex in total cellular protein extracts of mammalian cells. The structure of the other factors has been partially (MBP-2) or completely (NF-κB, KBF-1, MBP-1) elucidated. These DNA-binding factors can be subdivided into two classes: either they consist of dimers of *rel*-related subunits (NF-κB and KBF-1) (13–15) or they are zinc finger polypeptides (MBP-1 and MBP-2) (11, 12). These transcription factors bind to the MHC class I enhancer and to several related enhancers, although the affinity for the different enhancers varies. These related enhancer motifs are found in the regulatory regions of the immunoglobulin κ gene, the β_2-microglobulin gene, the interferon β gene, the interleukin 2 receptor gene, the long terminal repeat of the human immunodeficiency virus type 1, the angiotensinogen gene, the α1-antitrypsin gene, and the αA-crystallin gene (16–18).

We have reported that there is a family of at least three closely related zinc finger proteins (MBP-1, MBP-2, and KBP-1) that share a homology of >90% in one of their DNA-binding regions (12). Full-length and partial clones of MBP-1 from different species were isolated by five different groups, who named them PRDII-BF1 (11), MBP-1 (10), HIV-EP1 (19), αACRYBP1 (18), and AT-BP2 (17). We and others have reported human or rat partial cDNA clones for MBP-2, isolated using either oligonucleotide or low stringency hybridization, and these were named MBP-2 (12), AT-BP1 (17), AGIE-BP1 (20), and HIV-EP2 (21). Here we describe the complete nucleotide sequence of MBP-2 [9175 base pairs (bp)].† We show that the MBP-2 cDNA specifies a protein of 275 kDa that binds to the palindromic enhancer A-region 1 of MHC class I genes.

MATERIALS AND METHODS

Isolation of MBP-2 cDNA Clones and DNA Sequence Analysis. cDNA libraries of Nalm-6 {human pre-B cells [λZAP, oligo(dT) primed, gift of A. Bernards, Massachusetts General Hospital Cancer Center]}, of HOS [human osteosarcoma cells (λZAPII, constructed with a MBP-2-specific primer: nucleotides 2227–2248)], and of RCA [adenovirus 12-transformed retinal cells (λZAPII, random primed, gift of K. LeClair, Massachusetts Institute of Technology Cancer Center)] were initially screened with the most 5′ fragment of the original MBP-2 partial cDNA (12) and subsequently with the most 5′ fragment of each additional clone by standard procedures. Positive λZAP/pBSK subclones were obtained by the *in vivo* excision method (Stratagene). Four overlapping cDNA clones of 6, 5, 0.9, and 1.7 kilobases (kb) were isolated. A series of overlapping exonuclease III deletion mutants was made in both directions of each cDNA clone; these were sequenced with the T7 sequencing kit (Pharmacia) using T3 or T7 sequencing primers or using MBP-2-specific primers. Both strands were sequenced for >98% of the DNA.

MBP-2 Expression Constructs. A DNA fragment encompassing the upstream zinc finger region of the MBP-2 cDNA (nucleotides 611–1804) was expressed as a β-galactosidase fusion protein using the inducible bacterial expression vector pBSK.

A full-length mammalian expression construct was made by inserting the cDNA fragment from nucleotide 1 to nucleotide 7576 into the vector pSVoriCMV (gift of M. Timmers, Massachusetts Institute of Technology Cancer Center). An antisense construct was made with the insert in the reverse orientation.

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Abbreviations: MHC, major histocompatibility complex; MBP, MHC-binding protein; EMSA, electrophoresis mobility shift assay; ORF, open reading frame.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. X65644).

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   1 MLFLNLATAHQEIEYISPPLEHGSNQALRSTALETLHESALLSGELALKTLCNKMDTGDTALGQKATSRSGETDKASGRWRQEQSAVIKMSTFGSHEGQR  100
 101 QPQIEPEQIGNTASAQLFGSGKLASPSEVVQQVAEKQYPPHRPSPYSCQHSLSFPQHSLPQGVMHSTKPHQSLEGPPWLFPGPLPSVASEDLFPFPIHGH  200
 201 SGGYPRKKISSLNPAYSQYSQKSIEQAEEAHKKEHKPKKPGKYICPYCSRACAKPSVLKKHIRSHTGERPYPCIPCGFSFKTKSNLYKHRKSHAHAIKAG  300
 301 LVPFTESAVSKLDLEAGFIDVEAEIHSDGEQSTDTDEESSLFAEASDKMSPGPPIPLDIASRGGYHGSLEESLGGPMKVPILIIPKSGIPLPNESSQYIG  400
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 501 RAAMGRKGIMEPLPHVNTRLDVKMFEDPVSQLIPSKGDVDPSQTSMLKSTKFNSESRQPQIIPSSIRNEGKLYPANFQGSNPVLLEAPVDSSPLIRSNSV  600
 601 PTSSATNLTIPPSLRGSHSFDERMTGSDDVFYPGTVGIPPQRMLRRQAAFELPSVQEGHVEVEHHGRMLKGISSSSLKEKKLSPGDRVGYDYDVCRKPYK  700
 701 KWEDSETPKQNYRDISCLSSLKHGGEYFMDPVVPLQGVPSMFGTTCENRKRRKEKSVGDEEDTPMICSSIVSTPVGIMASDYDPKLQMQEGVRSGFAMAG  800
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 901 SPEWAPPGDGAESGGKPSPSQQVQQQSYHTQPRLVRQHNIQVPEIRVTEEPDKPEKEKEAQSKEPEKPVEEFQWPQRSETLSQLPAEKLPPKKKRLRLAD 1000
1001 MEHSSGESSFESTGTGLSRSPSQESNLSHSSSFSMSFEREETSKLSALPKQDEFGKHSEFLTVPAGSYSLSVPGHHHQKEMRRCSSEQMPCPHPAEVPEV 1100
1101 RSKSFDYGNLSHAPVSGAAASTVSPSRERKKCFLVRQASFSGSPEISQGEVGMDQSVKQEQLEHLHAGLRSGWHHGPPAVLPPLQQEDPGKQVAGPCPPL 1200
1201 SSGPLHLAQPQIMHMDSQESLRNPLIQPTSYMTSKHLPEQPHLFPHQETIPFSPIQNALFQFQYPTVCMVHLPAQQPPWWQAHFPHPFAQHPQKSYGKPS 1300
1301 FQTEIHSSYPLEHVAEHTGKKPAEYAHTKEQTYPCYSGASGLHPKNLLPKFPSDQSSKSTETPSEQVLQEDFASANAGSLQSLPGTVVPVRIQTHVPSYG 1400
1401 SVMYTSISQILGQNSPAIVICKVDENMTQRTLVTNAAMQGIGFNIAQVLGQHAGLEKYPIWKAPQTLPLGLESSIPLCLPSTSDSVATLGGSKRMLSPAS 1500
1501 SLELFMETKQQKRVKEEKMYGQIVEELSAVELTNSDIKKDLSRPQKPQLVRQGCASEPKDGLQSGSSSFSSLSPSSSQDYPSVSPSSREPFPPSKEMLSG 1600
1601 SRAPLPGQKSSGPSESKESSDELDIDETASDMSMSPQSSSLPAGDGQLEEEGKGHKRPVGMLVRMASAPSGNVADSTLLLTDMADFQQILQFPSLRTTTT 1700
1701 VSWCFLNYTKPNYVQQATFKSSVYASWCISSCNPNPSGLNTKTTLALLRSKQKITAEIYTLAAMHRPGTGKLTSSSAWKQFTQMKPDASFLFGSKLERKL 1800
1801 VGNILKERGKGDIHGDKDIGSKQTEPIRIKIFEGGYKSNEDYVYVRGRGRGKYICEECGIRCKKPSMLKKHIRTHTDVRPYVCKLCNFAFKTKGNLTKHM 1900
1901 KSKAHMKKCLELGVSMTSVDDTETEEAENLEDLHKAAEKHSMSSISTDHQFSDAEESDGEDGDDNDDDEDEDDFDDQGDLTPKTRSRSTSPQPPRFSSL 2000
2001 PVNVGAVPHGVPSDSSLGHSSLISYLVTLPSIRVTQLMTPSDSCEDTQMTEYQRLFQSKSTDSEPDKDRLDIPSCMDEECMLPSEPSSSPRDFSPSSHHS 2100
2101 SPGYDSSPCRDNSPKRYLIPKGDLSPRRHLSPRRDLSPMRHLSPTKEAALRREMSQRDVSPRRHLSPRRPVSPGKDITARRDLSPRRERRYMTTIRAPSP 2200
2201 RRALYHNPPLSMGQYLQAEPIVLGPPNLRRGLPQVPYFSLYGDQEGAYEHPGSSLFPEGPNDYVFSHLPLHSQQQVRAPIPMVPVGGIQMVHSMPPALSS 2300
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2401 GPDQPARVQEPHQNPLGSAHVSIRHFSRPEPGQPCTSATHPDLHDGEKDNFGTSQTPLAHSTFYSKSCVDDKQLDFHSSKELSSSTEESKDPSSEKSQLH 2500
2501 *
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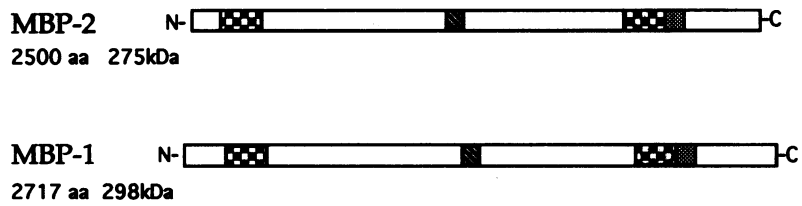

FIG. 1. (*Upper*) Complete amino acid sequence of MBP-2. The ORF begins at nucleotide 16 and ends at nucleotide 7515 of the cDNA. The Cys_2His_2 zinc finger regions are boxed (the cysteines and histidines of each finger are indicated); the nuclear localization signal and the domain of acidic amino acids are underlined. (*Lower*) Comparison of MBP-2 and MBP-1. Conserved regions (>85% homology) are indicated: upstream and downstream zinc fingers (blocked), nuclear localization signal and serine/threonine-rich region (dashed), and acidic domain (stippled).

MBP-2 Antiserum. A polyclonal anti-MBP-2 antiserum was made by immunizing rabbits with a purified MBP-2 bacterial fusion protein, consisting of glutathione-*S*-transferase linked to MBP-2 amino acids 605–1044 in the bacterial expression vector pGEX3X (Pharmacia) (22).

Electrophoresis Mobility Shift Assay (EMSA). Bacterial protein extracts and whole cell protein extracts from mammalian cell lines were made as described (12, 23). The sequences and labeling procedure of the different EMSA probes as well as the electrophoresis in Tris-glycine buffer were carried out as described (12, 23).

Cell Culture and Transfections. COS-7 cells were transfected by electroporation. For each transfection 3×10^6 cells were pulsed (1.2 kV, 25 μF) in a volume of 0.8 ml of

phosphate-buffered saline with 30 μg of plasmid DNA. Forty-eight hours after transfection cells were metabolically labeled for 3 hr with [^{35}S]methionine, cells were lysed, and proteins were immunoprecipitated with the appropriate antibodies and separated on a 6% polyacrylamide/sodium dodecyl sulfate gel by standard procedures (22).

RESULTS

Cloning of the Full-Length MBP-2 cDNA. We have reported previously the isolation of a 3.7-kb partial MBP-2 cDNA clone from a cDNA library made from adenovirus-transformed human retinal cells (12). Analysis of RNA from different tumor cell lines showed that MBP-2 is expressed as an ≈10-kb mRNA (12). To obtain a full-length MBP-2 cDNA, human cDNA libraries were screened with the most 5′ fragment of the original, partial MBP-2 cDNA, and subsequently with the most 5′ fragment of each additional clone. In total, four overlapping cDNA clones of 6, 5, 1.7, and 0.9 kb were isolated and the complete nucleotide sequence of the MBP-2 cDNA was determined. The DNA sequence reveals that a 5′ untranslated region of 15 bp is followed by an open reading frame (ORF) of 7500 bp, which is followed by a 3′ untranslated region of 1660 bp. An in-frame stop codon is present nine nucleotides upstream of the first ATG codon, indicating that the ORF did not extend beyond our cDNA. Only the second methionine codon at amino acid position 55 lies within a perfect Kozak consensus sequence. We therefore cannot assign the start of translation unambiguously. The translation of the MBP-2 ORF, shown in Fig. 1 *Upper*, reveals the presence of two sets of two Cys_2His_2 zinc fingers (designated here upstream and downstream zinc fingers), a nuclear localization signal, followed by a serine/threonine-rich region that constitutes a potential target for phosphorylation, and, 3′ of the downstream zinc fingers, a putative transactivation domain of acidic amino acids.

We have reported that the downstream zinc finger region of MBP-2 has homology with two closely related factors MBP-1/PRDII-BF1 and KBP-1 (12). Comparison with the published sequence of MBP-1/PRDII-BF1 (11) shows that the homology between the MBP-2 and MBP-1 sequences extends outside this region (Fig. 1 *Lower*). The overall similarity between MBP-2 and MBP-1 is 51% (33% identity), though in some regions (indicated in Fig. 1 *Lower*), the percentage of homology is much higher: 92% (upstream zinc fingers), 85% (nuclear localization signal, serine/threonine-rich region), and 92% (downstream zinc fingers). It is also noteworthy that the spacing of these different regions of the two proteins is the same.

DNA-Binding Specificity. We determined the DNA-binding specificity of the upstream zinc fingers of MBP-2 by making a bacterial expression construct encompassing this region. Protein extracts from induced bacteria were then tested in an EMSA with the MHC enhancer and related sequences as probes. Fig. 2 *Upper* shows that the upstream zinc finger region exclusively binds to the MHC enhancer. This is in contrast to the downstream zinc finger region, which also binds to the β_2-microglobulin- and NF-κB-enhancer motifs, albeit with lower affinity (12) (Fig. 2 *Lower*).

The *in vitro* DNA-binding results suggest that MBP-2 mRNA-expressing mammalian cells should contain a protein that has the ability to bind to the MHC class I gene enhancer. To address this question, protein extracts from the human pre-B-cell line Nalm-6 were tested in an EMSA assay. Fig. 3, lane 6, shows that Nalm-6 cell extracts contain two proteins that specifically bind an oligonucleotide that specifies the wild-type palindromic MHC class I gene enhancer. One comigrates with the H2TF1 retarded complex (data not shown); the second is a slower migrating complex. To ask whether this slower complex contains MBP-2, we made a

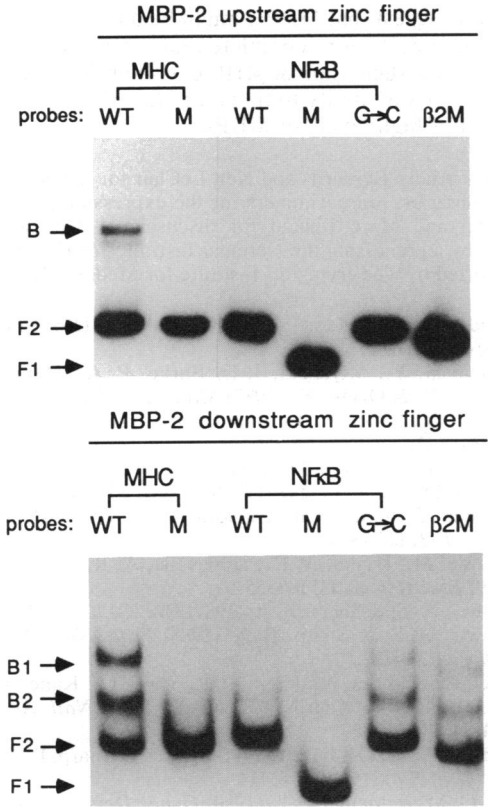

FIG. 2. EMSA with bacterial fusion proteins of the MBP-2 zinc finger regions (*Upper*, upstream zinc finger; *Lower*, downstream zinc finger region). Protein extract (*Upper*, 0.2 μg; *Lower*, 5 μg) from induced bacterial cultures was incubated with the following end-labeled oligonucleotides: MHC wild type (WT), 5′-GGGGATTCCCC-3′; MHC mutant (M), 5′-GCGGATTCCCG-3′; NF-κB wild type (WT), 5′-GGGGACTTTCCG-3′; NF-κB mutant (M), 5′-ATTCACTTTCCG-3′; NF-κB (G→C) mutant, 5′-GGGGACTTTCCC-3′; β_2-microglobulin (β2M), 5′-AGGGACTTTCCC-3′. F1 and F2, positions of free oligonucleotides; B, B1, and B2, positions of retarded complexes.

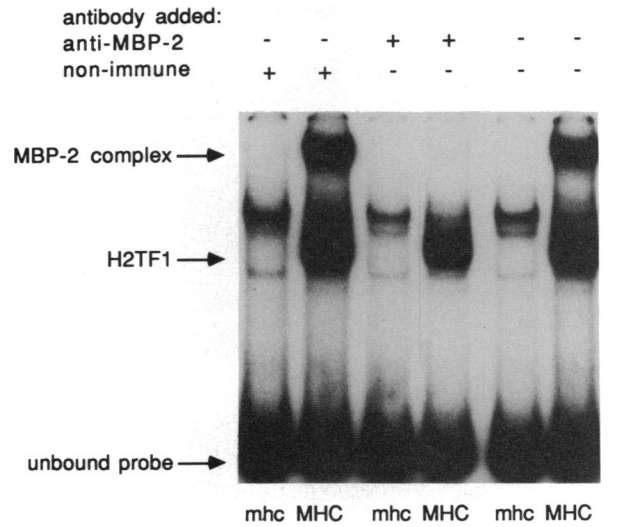

FIG. 3. EMSA with proteins from the human pre-B-cell line Nalm-6. Ten micrograms of whole cell protein extract was incubated with the end-labeled MHC wild-type (MHC) or mutant (mhc) oligonucleotide, after preincubation of 2 hr at 4°C in the presence (+) or absence (−) of 5 μl of whole serum as indicated.

polyclonal antiserum against a part of MBP-2 (amino acids 605–1044). Fig. 3, lane 4, shows that the migration of the slower complex is perturbed by the MBP-2 antiserum, whereas the nonimmune serum did not alter the migration of this retarded complex. The same result was obtained when extracts derived from other mammalian cells were used (data not shown). As can be seen in Fig. 3, the MBP-2 serum did not perturb the migration of the H2TF1 factor. We conclude that MBP-2 is present in mammalian cells, binds the MHC enhancer, and is distinct from the H2TF1 retarded complex.

MBP-2 Expression in Transfected COS Cells. The sequence of the MBP-2 cDNA predicts an ORF of 2500 amino acids, encoding a protein of 275 kDa. To investigate whether MBP-2 is indeed expressed as a 275-kDa protein in mammalian cells, we made a full-length expression construct of MBP-2 under the control of the cytomegalovirus promoter-enhancer and a simian virus 40 origin of replication. This expression vector was transiently transfected in COS-7 cells. After 2 days the cells were labeled with [^{35}S]methionine, lysed, and immunoprecipitated with either the MBP-2 antiserum or a control serum. As can be seen in Fig. 4, the MBP-2 serum precipitates a protein with a molecular mass of >250 kDa only when a MBP-2 sense expression vector is transfected into the COS cells (lane 4 from the left).

DISCUSSION

We report here the cloning of a full-length cDNA encoding the MHC enhancer binding protein MBP-2. This protein is a member of a family of large Cys_2His_2 zinc finger-containing transcription factors (12). MBP-2 contains two widely separated zinc fingers. Surprisingly, both zinc finger regions, when expressed independently as bacterial fusion proteins, were found to bind with high affinity to the palindromic MHC enhancer site. MBP-1 resembles MBP-2 in that it also contains two widely spaced zinc fingers that bind the MHC enhancer with high affinity (11). The zinc finger regions of MBP-1 and MBP-2 are the most highly conserved regions between the two proteins. The finding that the region between the DNA-binding motifs is more divergent indicates that although the proteins may interact with similar DNA motifs *in vivo*, they are likely to have different effects when bound to DNA.

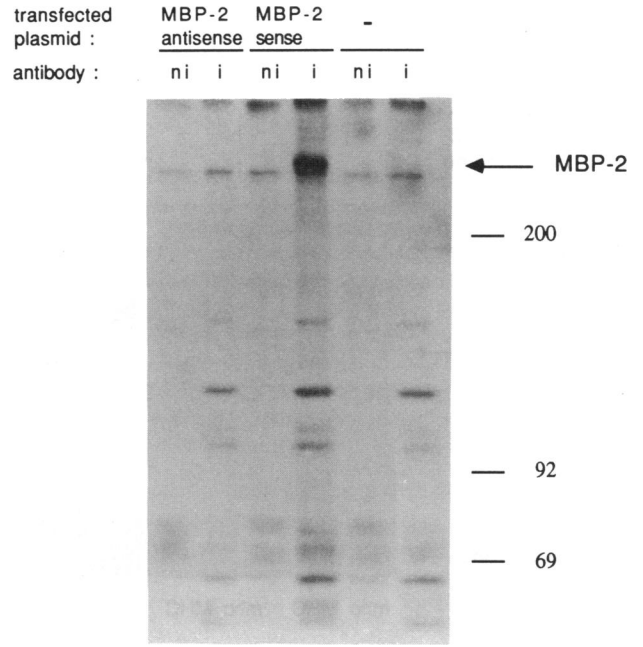

FIG. 4. MBP-2 expression in transfected COS cells. [^{35}S]Methionine-labeled proteins of transfected COS cells were immunoprecipitated with 10 μl of nonimmune serum (ni) or MBP-2 antiserum (i). Molecular masses are indicated in kDa.

Several proteins found in mammalian cell extracts can bind the palindromic MHC enhancer in an EMSA; they include H2TF1, KBF1, and NF-κB. Binding of H2TF1 to the MHC I enhancer induces MHC class I expression by ≈10-fold (7). Furthermore, activation of NF-κB by phorbol ester also increases expression of MHC class I antigens (24). Our finding that an anti-MBP-2 antibody recognizes a protein in mammalian cell extracts that binds the palindromic MHC site suggests that MBP-2 may also be involved in regulation of expression of MHC class I antigens. We attempted to investigate the involvement of MBP-2 in the regulation of MHC class I antigen expression by transfecting two different mammalian cell lines with a MBP-2 expression vector. Unfortunately, of the >100 transfectants tested, not one expressed full-length MBP-2 protein, whereas high levels of MBP-2 protein could be readily obtained in a transient transfection assay (Fig. 4). These results probably indicate that high expression of MBP-2 is not tolerated in mammalian cells. When we cotransfected the MBP-2 expression plasmid with a palindromic MHC enhancer chloramphenicol acetyltransferase (CAT) reporter construct in COS-7 cells, we found a slight (2- to 2.5-fold) reduction in (CAT) activity by MBP-2 (data not shown). In agreement with this is the finding that MBP-2 mRNA expression is very high in brain (17, 20), which expresses only very low levels of MHC class I antigens (1). These data suggest that MBP-2 might be a negative regulator of transcription. A more complete understanding of the role of MBP-2 in the regulation of MHC class I antigen expression will depend on our ability to find a cell line that allows stable expression of high levels of MBP-2.

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