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A Large Protein Containing Zinc Finger Domains Binds to Related Sequence Elements in the Enhancers of the Class I Major Histocompatibility Complex and Kappa Immunoglobulin Genes

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A cDNA from a B-cell library was previously isolated that encodes a sequence-specific DNA-binding protein with affinities for related sites in a class I major histocompatibility complex (MHC) and κ immunoglobulin gene enhancers. We report here approximately 6.5 kilobases of sequence of the MBP-1 (MHC enhancer binding protein 1) cDNA. MBP-1 protein has a molecular weight predicted to be greater than 200,000. A DNA-binding domain with high affinity for the MHC enhancer sequence TGGGGATTCCCCA was localized to an 118-amino-acid protein fragment containing two zinc fingers of the class Cys_2-X_{12}-His_2. Analysis of expression of MBP-1 mRNA revealed relatively high expression in HeLa cells and in a human retinal cell line, with lower levels in Jurkat T cells and in two B-cell lines. Interestingly, expression of MBP-1 mRNA was inducible by mitogen and phorbol ester treatment of Jurkat T cells and by serum treatment of confluent serum-deprived human fibroblasts.

A strategy was recently described for the molecular cloning of sequence-specific DNA-binding proteins by using recognition site probes (42). The first recombinant clone isolated by this technique, λH3, encoded a β-galactosidase fusion protein that binds a symmetrical sequence element $\substack{\text{TGGGGATTCCCCA}\\\text{ACCCCTAAGGGGT}}$ in the enhancer of a class I major histocompatibility (MHC) gene. Furthermore, the protein encoded by this bacteriophage also binds a related site $\substack{\text{GGGGACTTTCC}\\\text{CCCCTGAAAGG}}$ in the κ immunoglobulin gene enhancer (42). This latter site is recognized by a B-cell-specific factor called NF-κB and is a B-cell-specific enhancer element (39). The cDNA insert of λH3 hybridizes under stringent conditions to a single-copy human gene and to an approximately 9.5-kilobase (kb) mRNA detectable in HeLa and human B-cell lines.

The symmetrical MHC element is located 165 base pairs (bp) upstream of the *H-2K*^{b} class I MHC gene transcription initiation site and functions to stimulate gene expression approximately 7- to 10-fold in fibroblasts (1, 19). Multiple DNA-binding factors, including H2TF1 (1), KBF1 (15), NF-κB (2), and EBP-1 (10), recognize this 13-bp sequence. H2TF1 was originally identified as an MHC enhancer-binding protein that is present in HeLa and MEL nuclear extracts. KBF1 has been defined as a 48-kilodalton (kDa) protein in thymoma cell extracts that binds with approximately equal affinity to the symmetrical sequence in the MHC enhancer and to a related sequence in the promoter of the β_2-microglobulin gene (15). H2TF1 binds with 5- to 10-fold-lower affinity to the β_2-microglobulin site than to the MHC enhancer site, which suggests that H2TF1 and KBF1 are distinct factors (A. Baldwin, unpublished data). NF-κB was initially characterized in B-cell extracts by its interaction with a site in the immunoglobulin κ gene enhancer (39). NF-κB binds with approximately equal affinity to the MHC enhancer and to the κ enhancer (2) but can be distinguished from H2TF1 and KBF1 by its inducibility and cell type distribution (40). The NF-κB-binding site in the κ enhancer is highly related to sequence elements in the 72-bp repeat enhancer element of simian virus 40 (SV40), the interleukin-2 receptor (IL-2R) α-chain promoter, the human immunodeficiency virus (HIV) long terminal repeat, the β_2-microglobulin promoter, and the beta interferon promoter (Table 1). In fact, factors with properties of NF-κB have been shown to bind these elements and are presumed to play a role in the regulation of the expression of several of these genes (6, 22, 31, 46). EBP-1, a factor probably distinct from those described above, is a 60-kDa protein purified from HeLa cells that binds the SV40 and MHC enhancer sites (10).

We describe here the nucleotide sequence of the ~1-kb cDNA segment of λH3 as well as that of overlapping cDNA clones that together delineate a large open reading frame that predicts a protein with a molecular weight of greater than 200,000. We discuss features of the predicted MHC gene-binding protein (MBP-1) and show by in vitro translation that two tandem Cys_2-X_{12}-His_2 class zinc finger motifs contained in the carboxy-terminal portion of MBP-1 constitute a specific DNA-binding domain. A comparison of the levels of MBP-1 mRNA expressed in cell lines before and after mitogenic or serum stimulations suggests that the MBP-1 protein is a regulatory component of a growth control circuit operative in distinct cell types.

MATERIALS AND METHODS

Cells. The human cervical carcinoma cell line HeLa was grown in minimal essential medium and 5% horse serum, the adenovirus 12 E1A-transformed human retinal cell line (gift of R. Bernards) was grown in Dulbecco modified Eagle medium (DMEM) and 10% fetal calf serum, and the human fibroblast line GM0010 (obtained from the National Institute of General Medical Sciences Human Genetic Cell Repository, Camden, N.J.) was grown in DMEM and 10% calf

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TABLE 1. Related nuclear factor-binding sites

Promoter/enhancer	Sequence
MHC	TGGGGATTCCCCA
β_2-Microglobulin	AAGGGACTTTCCC
κ	AGGGGACTTTCCG
SV40	TGGGGACTTTCCA
HIV(1)	AAGGGACTTTCCG
HIV(2)	TGGGGACTTTCCA
Beta interferon	GTGGGAAATTCCT
Eα class II MHC	GCGGGACTTCCCA
IL-2Rα	AGGGGAATCTCCC
MHC double point mutant	TGCGGATTCCCGA

serum. The Jurkat human T-cell line and the B-cell lines BJA-B and X50-7 were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM L-glutamine. For mitogen stimulation studies, 1 μg of phytohemagglutinin P per ml (PHA-P) and 50 ng of phorbol myristate acetate per ml (PMA) (Sigma Chemical Co., St. Louis, Mo.) were added to the Jurkat cell cultures. For the serum stimulation studies, GM0010 fibroblasts were grown to confluence in DMEM with 10% calf serum and then maintained for 48 h in DMEM with 0.5% serum. Cells were then collected or cultured for an additional 3 h in DMEM with 20% serum before RNA isolation.

Isolation of MBP-1 DNAs. The 1.1-kb *Eco*RI cDNA fragment of phage λH3 (42) was subcloned into pUC18. The cDNA insert was isolated by agarose gel electrophoresis and labeled to approximately 10^9 cpm/μg specific activity by a random priming procedure (Pharmacia, Inc., Piscataway, N.J.). HepG2 (gift of M. Shia) and retinal cell (gift of S. Friend) λgt11 cDNA libraries were probed with ^{32}P-labeled λH3 insert by standard procedures (25). A single positive phage (λHep1 and λRet1) was isolated from each library screen of approximately 50,000 phage. A random primed retinal cell cDNA library was constructed with a commercially obtained cDNA synthesis kit (Invitrogen) and the λZAP vector (Stratagene, La Jolla, Calif.) and screened with a radiolabeled 400-bp *Eco*RI-*Bam*HI cDNA fragment derived from the extreme 5′ end of the λRet1 cDNA (Fig. 1). A single phage isolate (λRet2) was identified. For λgt11 phage clones, DNA was isolated by the LambdaSorb Phage Adsorbent method (Promega Biotec, Madison, Wis.) and then *Eco*RI digested and subcloned into the *Eco*RI site of pUC18. The λZAP subclones were obtained by an in vivo excision method as recommended (Stratagene).

DNA sequencing. Plasmid DNAs were sequenced either by the dideoxy method (37) or by the chemical degradation method (26). Primers used for dideoxy sequencing were commercially obtained or synthesized at the Massachusetts Institute of Technology DNA synthesis facility (S. Scaringe). More than 95% of the nucleotide sequence presented was confirmed either by sequencing the opposite strand or by sequencing the same strand from different initiation sites. The complete nucleotide and deduced amino acid sequences are available on request and have been submitted to the GenBank sequence data base.

In vitro transcription and translation. Complementary synthetic oligonucleotides designed to contain an ATG codon flanked by consensus nucleotides for optimal eukaryotic translation initiation (CACCATGGCCATCGATATC) (21) were inserted into *Pst*I-*Bam*HI-digested pBS(+/−) vector (Stratagene). This vector construct (pBS-ATG) is suitable for in vitro transcription and in-frame translation of λgt11-derived *Eco*RI cDNA fragments. The *Eco*RI ends of the cDNA fragment of λH3 were filled in with Klenow DNA polymerase and then ligated into *Sma*I-digested pBS-ATG to obtain the plasmid pBS-ATG-H3. In vitro transcription from the pBS-ATG-H3 plasmid DNA template with T3 polymerase was performed with a Riboprobe system (Promega) as described previously (27). A wheat germ extract system (Promega) was used for in vitro translation of MBP-1 proteins.

DNA-binding assays. Interactions of MBP-1 protein with DNA were monitored by the gel mobility shift procedure (43). Wheat germ extract translations (1 μl) of RNA derived from either *Aha*II or *Eco*RI digests of pBS-ATG-H3 were incubated for 30 min at 30°C with 10,000 cpm of DNA probe, 2 μg of poly (dI-dC), and variable amounts of unlabeled competitor DNA fragments as described previously (1). Binding site and competitor DNAs were synthesized with *Bam*HI ends and cloned into pUC18. Binding sites, including the MHC double-point mutant (mhc) (TGCGGATTCC CGA), are shown in Table 1. Electrophoresis was in 4% polyacrylamide–Tris–glycine–EDTA gels (43). Gels were

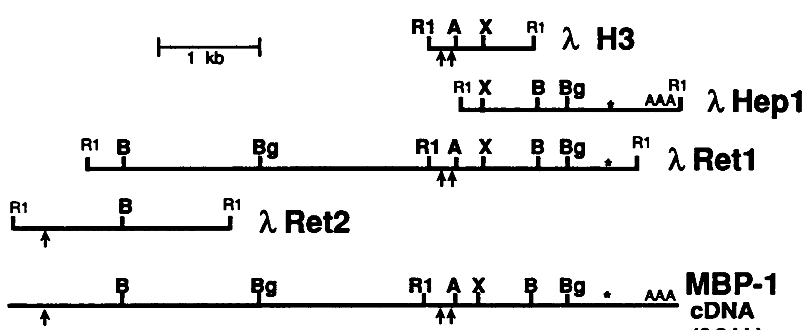

FIG. 1. Restriction maps of MBP-1 cDNAs. The partial restriction map of λH3, isolated as described previously (42), is shown at the top. λHep1 represents an overlapping clone isolated from a HepG2 cDNA library. λRet1 represents a 6.3-kb cDNA isolated from a retinal cDNA library. λRet2 represents an extended cDNA isolated from a random primed human retinal library generated as described in Materials and Methods. Restriction enzyme sites: A, *Aha*II; B, *Bam*HI; Bg, *Bgl*II; R1, *Eco*RI; X, *Xba*I. Natural *Eco*RI sites are shown in boldface letters, and linker-derived *Eco*RI sites are indicated with smaller, lightface type. Except for *Aha*II, all occurrences of indicated sites have been shown. At the bottom is a composite MBP-1 cDNA map derived from the above cDNAs. The asterisk denotes the position of the first in-frame translation termination codon (TGA). A poly(A) sequence detected in λHep1 is shown as AAA. Arrows show the location of the three zinc finger motifs found in the MBP-1 cDNAs.

vacuum dried and exposed to XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) for autoradiography.

RNA extraction and Northern (RNA) blot analysis. Total cellular RNA was isolated from cells by the guanidinium thiocyanate lysis technique (7), and poly(A)$^+$ RNA was selected by two cycles of binding to oligo(dT)-cellulose. Alternatively, some poly(A)$^+$ RNA samples (HeLa and retinal) were prepared by the Fast Track lysis and oligo(dT) selection procedure (Invitrogen). No qualitative differences have been noted when blotting RNAs prepared by either of these methods (K. LeClair, unpublished data). For Northern blot analysis, poly(A)$^+$ RNA samples were denatured for 1 h at 50°C in 1 M glyoxal and separated by electrophoresis on a 1% agarose gel in 10 mM sodium phosphate, pH 6.8. To enhance transfer of high-molecular-weight RNA, the gel was soaked for 30 min at room temperature in 50 mM NaOH–10 mM NaCl, neutralized for 30 min at room temperature in 100 mM Tris hydrochloride (pH 7.3), and then soaked for an additional 30 min in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The RNA samples in the gel were transferred overnight in 20× SSC to a Zetabind nylon filter (AMF Cuno, Meriden, Conn.). The membrane was dried and baked for 2 h at 80°C and then soaked in 0.5% sodium dodecyl sulfate–0.1× SSC for 60 min at 65°C. The filter was prehybridized and hybridized as described previously (9) with 5×10^6 cpm of the cDNA insert of λH3 per ml labeled to a specific activity of $\sim 1 \times 10^9$ cpm/μg by a random priming method (Pharmacia). After being washed (9), the membrane was dried and exposed to XAR-5 film at −70°C. For rehybridization, the blot was first rinsed three times in 0.1× SSC–0.1% sodium dodecyl sulfate for 15 min at 95°C. A human β-actin cDNA, pHFβHuA-1 (13) (gift of M. Brown), was radiolabeled and hybridized as described above.

RESULTS

Nucleotide and predicted amino acid sequence of MBP-1. Previously, we described the isolation of a λgt11 recombinant phage (λH3) from a human B-cell cDNA library that encodes a protein that binds a symmetrical site in the MHC enhancer (42). The 1.1-kb cDNA segment in λH3 was subcloned into pUC18, and a restriction map was prepared. To obtain larger and overlapping cDNAs, we used the cDNA segment from λH3 to probe a cDNA library prepared from the human liver cell line HepG2 (gift of M. Shia) and a human retinal cell library (gift of S. Friend). Two recombinant phage, λHep1 and λRet1 (Fig. 1), with large overlapping cDNA inserts were isolated. Restriction endonuclease analysis demonstrated an identical map in the regions overlapping the three cDNA segments in λH3, λHep1, and λRet1. A probe derived from the 5′ end of λRet1 was then used to isolate λRet2 (see Fig. 1 and Materials and Methods).

DNA sequencing was undertaken with the cDNA segments in λH3, λHep1, and λRet1 and -2. The λH3 and λRet1 inserts were sequenced completely. The λHep1 segment was sequenced extensively and was found to be identical to the corresponding regions of λH3 and λRet1. λRet2 was sequenced to its 5′ boundary from the region of overlap with λRet1. The combined sequence of these clones predicts a single open reading extending 5,752 nucleotides that is terminated by several in-frame stop codons. The 3′ untranslated region is 535 nucleotides long and terminates with a poly(A) tract detected in λHep1. Preceding the polyA tract by 18 bp is a potential poly(A) addition sequence of AGT AAA. The 3′ untranslated region is noticeably A+T-rich and contains several blocks of sequences similar to the A+T-rich sequence described by Shaw and Kamen (41) which appears to confer instability to an mRNA.

The nucleotide sequence of the open reading frame was translated into its corresponding 1,917-amino-acid sequence, which is presented in Fig. 2. The molecular weight of the MHC gene-binding protein 1 (MBP-1) encoded by the full-length mRNA therefore must be greater than 200,000. The amino acid sequence encoded in λH3 was examined for a segment related to a known DNA-binding domain. Two zinc fingers of the class Cys_2-X_{12}-His_2 (Fig. 2 and 3), originally described in the transcription factor TFIIIA (29) and since found in several DNA-binding protein domains, are present in this region of MBP-1. Immediately carboxy terminal to the zinc finger region is a highly acidic segment (23 of 42 positions, 55% acidic), which may be a transcriptional activating domain related to those first described in yeast activator proteins GCN4 (14) and GAL4 (24).

Examination of the remaining protein sequence revealed a third zinc finger motif in the amino-terminal region which was separated from the previously described pair by 1,107 amino acids (Fig. 2 and 3). This finger is of the type Cys_2-X_{12}-His-Cys, a rarer type, similar to ones found in Xfin, SWI5, Evi-1, and su(HW) (20, 30, 34, 36).

Other features of potential interest in the MBP-1 sequence include a region rich in serine and threonine residues (18 of 30 Ser or Thr) approximately 500 amino acids from the amino terminus of the sequence (Fig. 2). Regions rich in serine and threonine have been described in Sp1, another zinc finger-containing protein (16) and may constitute sites of phosphorylation. A sequence, Pro-Lys-Lys-Lys-Arg-Leu-Arg, that is very similar to the nuclear localization sequence in SV40 large T antigen (17) is located just amino terminal of the Ser-Thr-rich region (Fig. 2). Interestingly, the nuclear localization sequence of large T antigen is located just carboxy terminal of a region known to be phosphorylated on Ser and Thr residues (38). A computer search of several data bases revealed that the protein encoded by the MBP-1 cDNA has no extended homology with any previously characterized protein. However, very short regions of amino acid sequence were related to those of other proteins.

Binding specificity of carboxy-terminal zinc fingers. We previously demonstrated that the β-galactosidase fusion protein encoded by λH3 had relatively high affinity for related sites in the MHC and κ immunoglobulin gene enhancers (42). Related sites exist in regulatory regions of several other genes and viral genomes (Table 1). The relative affinity of the DNA-binding segment of MBP-1 for several of these sites was compared by using MBP-1 protein generated by translation in vitro of RNA transcribed from the 1.1-kb λH3 segment (Fig. 4A). The λH3 cDNA segment was subcloned into an in vitro RNA expression vector which had been modified by the insertion of a synthetic oligomer containing an ATG codon flanked by the Kozak consensus nucleotides required for efficient initiation (21) (see Materials and Methods).

The binding of the in vitro translation product to a probe containing the symmetrical MHC site was analyzed by the gel mobility shift assay. Relative affinities were determined by using cold DNA competitors encompassing sites from the MHC, β_2-microglobulin, κ immunoglobulin, and HIV enhancer site 1 [HIV(1)] (Table 1). The complexes formed with the MHC probe were virtually abolished by inclusion of 10 ng (approximately 20- to 30-fold molar excess) of the MHC competitor oligonucleotide. In contrast, even 40 ng of double-point-mutant MHC (mhc) DNA did not detectably compete for MBP-1 binding to the MHC probe. Thus, all these

R T S S S S D I P K S P F T P T E K S K Q V F L L S V P S L D C L P I T R S N S M P T T G Y S A V P 50

A N I I P P P H P L R G S Q S F D D K I G A F Y D D V F V S G P N A P V P Q S G H P R T L V R Q A A 100

I E D S S A N E S H V L G T G Q S L D E S H Q G C H A A G E A M S V R S K A L A Q G P H I E K K K S 150

H Q G R G T M F E C E T C R N R Y R K L E N F E N H K K F Y C S E L H G P K T K V A M R E P E H S P 200

V P G G L Q P Q I L H Y R V A G S S G I W E Q T P Q I R K R R K M K S V G D D E E L Q Q N E S G T S 250

P K S S E G L Q F Q N A L G C N P S L P K H N V T I R S D Q Q H K N I Q L Q N S H I H L V A R G P E 300

Q T M D P K L S T I M E Q Q I S S A A Q D K I E L Q R H G T G I S V I Q H T N S L S R P N S F D K P 350

E P F E R A S P V S F Q E L N R T G K S G S L K V I G I S Q E E S H P S R D G S H P H Q L A L S D A 400

L R G E L Q E S S R K S P S E R H V L G Q P S R L V R Q H N I Q V P E I L V T E E P D R D L E A Q C 450

H D Q E K S E K F S W P Q R S E T L S K L P T E K L P P K K K R L R L A E I E H S S T E S S F D S T 500

L S R S L S R E S S L S H T S S F S A S L D I E D V S K T E A S P K I D F L N K A E F L M I P A G L 550

N T L N V P G C H R E M R R T A S E Q I N C T Q T S M E V S D L R S K S F D C G S I T P P Q T T P L 600

T E L Q P P S S P S R V G V T G H V P L L E R R R G P L V R Q I S L N I A P D S H L S P V H P T S F 650

Q N T A L P S V N A V P Y Q G P Q L T S T S L A E F S A N T L H S Q T Q V K D L Q A E T S N S S S T 700

N V F P V Q Q L C D I N L L N Q I H A P P S H Q S T Q L S L Q V S T Q G S K P D K N S V L S G S S K 750

S E D C F A P K Y Q L H C Q V F T S G P S C S S N P V H S L P N Q V I S D P V G T D H C V T S A T L 800

P T K L I D S I S N S H P L L P P E L R P L G S Q V Q K V P S S F M L P I R L Q S S V P A Y C F A T 850

L T S L P Q I L E T Q D L P N Q P I C Q T N H S V V P I S E E Q N S V P T L Q K G H Q N A L P N P E 900

K E F L C E N V F S E M S Q N S S L S E S L P I T Q K I S V G R L S P Q Q E S S A S S K R M L S P A 950

N S L D I A M E K H Q K R A K D E N G A V C A T D V R P L E A L S S R V N E A S K Q K K P I L V R Q 1000

V C T T E P L D G V M L E K D V F S Q P E I S N E A V N L T N V L P A D N S S T G C S K F V V I E P 1050

I S E L Q E F E N I K S S T S L T L T V R S S P A P S E N T H I S P L K C T D N N Q E R K S P G V K 1100

N Q G D K V N I Q E Q S Q Q P V T S L S L F N I K D T Q Q L A F P S L K T T T N F T W C Y L L R Q K 1150

S L H L P Q K D Q K T S A Y T D W T V S A S N P N P L G L P T K V A L A L L N S K Q N T G K S L Y C 1200

Q A I T T H S K S D L L V Y S S K W K S S L S K R A L G N Q K S T V V E F S N K D A S E I N S E Q D 1250

K E N S L I K S E P R R I K I F D G G Y K S N E E Y V Y V R G R G R G K Y I C E E C G I R C K K P S 1300

M L K K H I R T H T D V R P Y H C T Y C N F S F K T K G N L T K H M K S K A H S K K C V D L G V S V 1350

G L I D E Q D T E E S D E K Q R F S Y E R S G Y D L E E S D G P D E D D N E N E D D D E D S Q A E S 1400

V L S A T P S V T A S P Q H L P S R S S L Q D P V S T D E D V R I T D C F S G V H T D P M D V L P R 1450

A L L T R M T V L S T A Q S D Y N R K T L S P G K A R Q R A A R D E N D T I P S V D T S R S P C H Q 1500

M S V D Y P E S E E I L R S S M A G K A V A I T Q S P S S V R L P P A A A E H S P Q T A A G M P S V 1550

A S P H P D P Q E Q K Q Q I T L Q P T P G L P S P H T H L F S H L P L H S Q Q Q S R T P Y N M V P V 1600

G G I H V V P A G L T Y S T F V P L Q A G P V Q L T I P A V S V V H R T L G T H R N T V T E V S G T 1650

T N P A G V A E L S S V V P C I P I G Q I R V P G L Q N L S T P G L Q S L P S L S M E T V N I V G L 1700

A N T N M A P Q V H P P G L A L N A V G L Q V L T A N P S S Q S S P A P Q A H I P G L Q I L N I A L 1750

P T L I P S V S Q V A V D A Q G A P E M P A S Q S K A C E T Q P K Q T S V A S A N Q V S R T E S P Q 1800

G L P T V Q R E N A K K V L N P P A P A G D H A R L D G L S K M D T E K A A S A N H V K P K P E L T 1850

S I Q G Q P A S T S Q P L L K A H S E V F T K P S G Q Q T L S P D R Q V P R P T G L P R R Q P T V H 1900

F S D V S S D D D E D R L V I A T * 1917

FIG. 2. Partial amino acid sequence of MBP-1 cDNA. The nucleotide sequence of the composite MBP-1 cDNA was determined, is available upon request, and has been submitted to the GenBank data base (accession number M32019). For illustration purposes, only the 1,917 amino acids encoded by the large open reading frame of the MBP-1 cDNA are presented here with several structural features noted. The three zinc finger motif homologies contained within the MBP-1 protein are indicated by heavy underlines. The potential nuclear localization signal (PKKKRLR), denoted by asterisks, occurs just amino terminal to the Ser-Thr-rich region, which is shown with a thin underline. The highly acidic segment which follows the third zinc finger motif is delineated by double underlines.

F E C E T C R N R Y R K L E N F E N H K K F Y C

Y I C E E C G I R C K K P S M L K K H I R T H T D V R P

Y H C T Y C N F S F K T K G N L T K H M K S K A H

$F/Y - X - C - X_{2-4} - C - X_3 - F - X_5 - L - X_2 - H - X_{3-4} - H$ T G E R/K P

FIG. 3. Amino acid sequences of three zinc finger motifs of MBP-1 protein aligned to a zinc finger motif consensus. The top line shows the amino-terminal Cys_2-X_{12}-His-Cys zinc finger motif, and the middle two lines present the amino acid sequence of the two tandem Cys_2-X_{12}-His_2 zinc fingers of the MBP-1 protein. At the bottom is the zinc finger structural motif consensus sequence (12), together with an interfinger linker consensus (TGER/KPF/YX) of TFIIIA (20). Invariant cysteines and histidines are shown boxed. The amino acids that occur in the MBP-1 zinc fingers at other key positions in the consensus are shown underlined.

complexes represent sequence-specific interactions. The multiple specific complexes are likely due to prematurely terminated translation products of MBP-1 RNA. DNA containing the related β_2-microglobulin sequence competed slightly less effectively than the MHC competitor, as 20 ng of the latter DNA competes approximately the same as 10 ng of the former DNA. Competitor DNAs derived from the κ and HIV(1) enhancers were significantly less effective in binding, as 40 ng of these DNAs competed less well than 10 ng of MHC enhancer DNA. Thus, the region of the MBP-1 protein containing the two carboxy-terminal Cys_2-X_{12}-His_2 zinc fingers has the highest affinity for MHC and β_2-microglobulin sites and approximately 5- to 10-fold-lower affinity for the related κ and HIV enhancer sites.

Interestingly, a cDNA segment encoding the MBP-1 DNA-binding domain has also been isolated by screening a λgt11 expression library with a regulatory motif of the beta interferon gene promoter (C.-M. Fan and T. Maniatis, Genes Dev., in press). This motif is structurally related to known MBP-1 binding sites (Table 1).

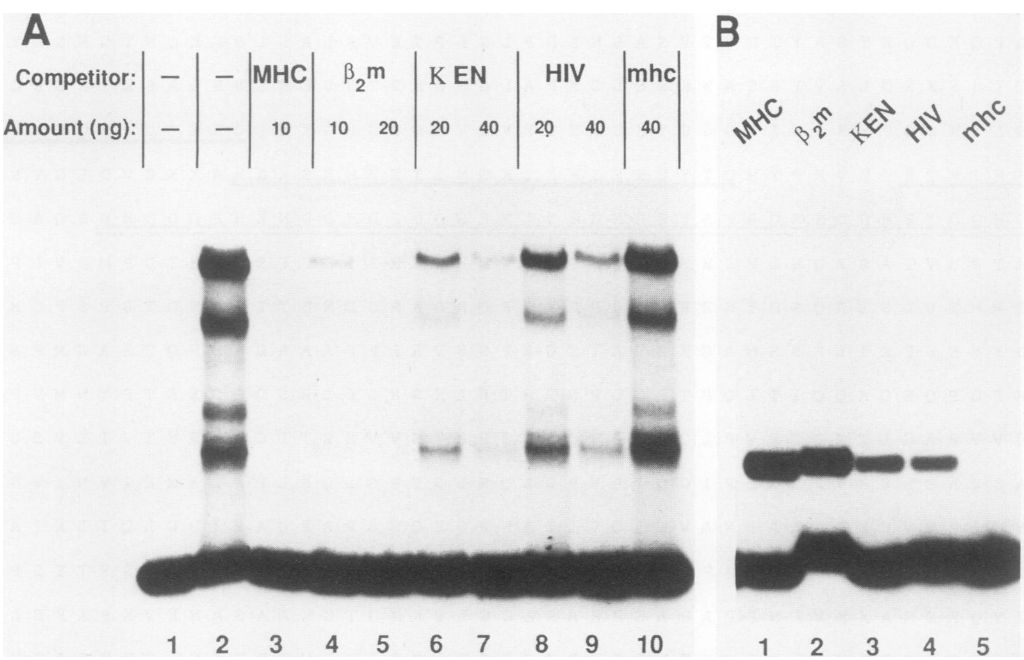

FIG. 4. Binding of in vivo-translated MBP-1 protein. (A) Competition analysis. Protein translated from ~1.1-kb RNA transcribed from the pBS-ATG-H3 construct (Fig. 1) was incubated with MHC wild-type enhancer probe, electrophoresed, and exposed as described in Materials and Methods. Lane 1, Wheat germ extract with no added MBP-1 RNA; lane 2, MBP-1 in vitro-translated protein with no competitor DNA; lanes 3 to 10, Competitor DNA included, with amounts shown at the top. MHC, Wild-type MHC competitor; β_2m, β_2-microglobulin competitor; κ EN, competitor from κ enhancer; HIV, HIV element 1; mhc, double-point-mutant MHC enhancer. (B) Direct binding analysis. Protein translated from RNA transcribed from an *Aha*II digest of pBS-ATG-H3 was used in a binding reaction with MHC (lane 1), β_2-microglobulin (lane 2), κ enhancer (lane 3), HIV (lane 4), or mhc double point mutant (lane 5) probes.

The specific binding of shorter fragments of the protein generated from the 1.1-kb cDNA of λH3 suggested that the DNA-binding domain could be further localized to a discrete region. Based on the restriction map of the cDNA, restriction enzyme sites within λH3 were chosen at which the recombinant template was linearized before transcription in vitro. T3 RNA polymerase was used to generate RNAs of shorter lengths corresponding to these truncated DNA templates. Translation in vitro of these RNAs generated a series of carboxy-terminal-truncated proteins that all bound specifically to the MHC enhancer probe (data not shown). Further binding analyses were done with protein generated from the *Aha*II-linearized template (Fig. 4B). This truncated protein, containing 118 amino acids and including two zinc fingers, bound with a specificity similar to the protein derived from the 1.1-kb segment of λH3. The MHC and β_2-microglobulin probes were bound with high affinity, and the κ and HIV sites were recognized with significantly lower affinity. The double-point-mutant mhc site was not effectively recognized by the truncated protein (Fig. 4).

Expression of MBP-1 mRNA. To study the expression pattern of the MBP-1 gene, we isolated RNA from various tissue culture cell lines. Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography and analyzed by Northern blotting with the 1.1-kb segment of λH3 as a probe (Fig. 5). As demonstrated previously (42), only a single hybridizing RNA species was detected, with an estimated size of ~9.5 kb. Relatively high levels of MBP-1 mRNA were detected in HeLa cells and in a human retinal cell line, but considerably lower levels were found in two human B-cell lines and the Jurkat T-cell line. When the blot was stripped and rehybridized with a radiolabeled 800-bp *Bam*HI-*Xho*I fragment from the 5′ end of the total cDNA sequence (Fig. 1), an identical pattern of bands was observed (data not shown).

The Jurkat T-cell line was chosen to study the potential induction of MBP-1 gene expression. Several groups have reported that stimulation of this cell line with PHA and PMA leads to increases in the levels of transcription factors that bind related sites in the regulatory regions of the HIV genome and in the IL-2Rα gene (Table 1) (6, 31). RNA isolated from Jurkat cells 2 or 6 h after PHA and PMA costimulation was analyzed in parallel with RNA from unstimulated Jurkat cells. Hybridization with the λH3 cDNA probe revealed a marked increase in levels of MBP-1 RNA following stimulation (Fig. 5, top). To control for potential variation in the levels of RNA electrophoresed, the blot was rehybridized with a human fibroblast β-actin probe (13). Because comparable levels of hybridizing RNA were observed with the actin probe (Fig. 5, bottom), the relative increase in MBP-1 RNA following stimulation of Jurkat cells appears to be significant.

The expression of the MBP-1 gene is also growth regulated (12a). We therefore examined its expression in a dividing human fibroblast cell line, in a culture grown to confluence in 10% calf serum and then maintained for 48 h in 0.5% serum, and in the confluent culture stimulated with 20% serum for 3 h. Northern analysis of RNA derived from these cells demonstrated a basal level of MBP-1 mRNA in confluent cells that was very similar to the level of expression in dividing cells (Fig. 5). However, addition of serum for 3 h resulted in an approximately fourfold increase in MBP-1 mRNA levels.

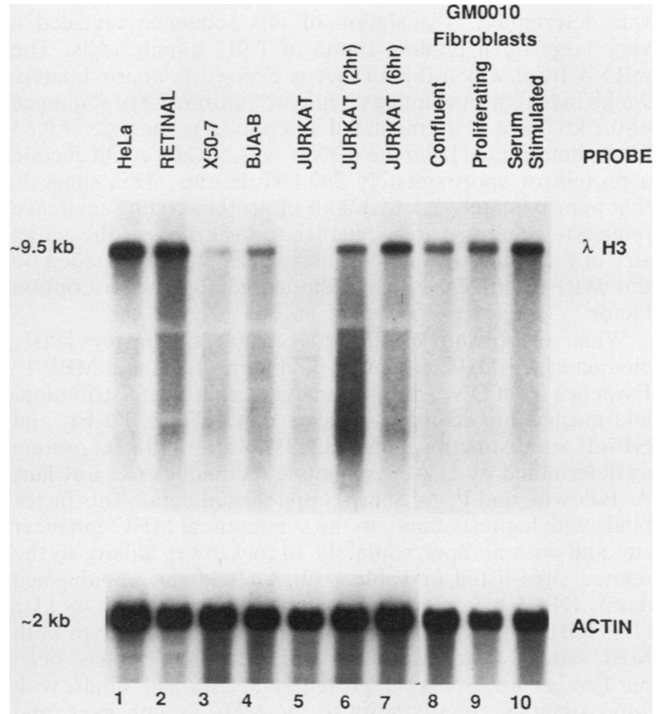

FIG. 5. Northern analysis of human cell line poly(A)$^+$ RNAs. Tissue culture cells were grown and harvested for RNA isolation, and approximately 4 μg of poly(A)$^+$ RNA was electrophoresed, blotted, and probed as described in Materials and Methods. The cell source of the RNA sample in each lane is indicated at the top of the figure. Jurkat cells were treated with PHA-P (1 μg/ml) and PMA (50 ng/ml) for 2 h (lane 6) or for 6 h (lane 7) before collection. GM0010 fibroblasts were grown and collected while still subconfluent (proliferating, lane 9); grown to confluence and maintained in 0.5% serum for 48 h (confluent, lane 8); or grown to confluence as above and then stimulated for an additional 3 h with 20% serum (serum stimulated, lane 10). The results of hybridizing with the λH3 probe are shown in the top panel. Following this hybridization, the blot was stripped and reprobed with a human β-actin cDNA (13). The molecular weights of the hybridizing bands, as determined by mobility relative to molecular weight standards (data not shown), are given at the left of the figure.

DISCUSSION

One of the emerging themes in the study of eukaryotic transcriptional control mechanisms is that a given regulatory motif is recognized by multiple distinct transcription factors. Examples of such motifs include CCAAT (8), octamer (44), and glucocorticoid hormone receptor response elements (45). The symmetrical sequence TGGGGATTCCCCA located in an MHC class I gene enhancer is another example of this phenomenon. This site binds distinct transcription factors including H2TF1 (1), KBF1 (15), NF-κB (2), and EBP-1 (10).

We describe here a cDNA sequence of 6.5 kb encoding a zinc finger protein (MBP-1) that has high affinity for the symmetrical MHC enhancer element. A 1.1-kb cDNA segment was originally identified by screening a λgt11 expression library prepared from B-cell mRNA with radiolabeled MHC-binding site DNA (42). This cDNA hybridized to a 9.5-kb mRNA present in a variety of cell types. Other cDNA segments containing overlapping sequences were isolated from libraries prepared from liver and retinal cells. The nucleotide sequence of a composite cDNA segment of 6.5 kb was determined. Translation of this sequence revealed a very large open reading frame of 1,917 amino acids. The mRNA from which this cDNA is derived is approximately 9.5 kb in length. Assuming a typical 5′ untranslated sequence of 0.2 kb and a 3′ untranslated and poly(A) sequence of 0.65 kb, as determined from the cDNA, this mRNA could encode a protein of approximately 290,000 daltons. This suggests that approximately 2.5 to 3.0 kb of protein-coding sequence remain to be cloned and sequenced. Regardless of the actual size of the 5′ untranslated sequence, the protein encoded by the MBP-1 mRNA is atypically large for a transcription factor.

What relationship exists between any of the previously characterized MHC enhancer-binding proteins and MBP-1? Evidence from DNA-binding analysis, cell-type distribution, and purification strongly suggests that H2TF1, KBF1, and NF-κB are distinct proteins. H2TF1 is an ~110-kDa protein as determined by UV cross-linking techniques (K. LeClair, A. Baldwin, and P. A. Sharp, unpublished data). This factor binds with highest affinity to the symmetrical MHC enhancer site and with an approximately 10-fold-lower affinity to the related sites listed in Table 1 (2; A. Baldwin, unpublished data). KBF1 has been purified and shown to be ~48 kDa (47). KBF1 binds with approximately equal affinity to both MHC and β_2-microglobulin sites (15). NF-κB has been purified as an ~50-kDa protein (18, 23) and binds with approximately equal affinity to the MHC, κ enhancer, and β_2-microglobulin sites (2; A. Baldwin, unpublished data). NF-κB also recognizes with comparable affinity the SV40 (39), the IL-2Rα (6), Eα class II MHC (5), beta interferon (22, 46), and both HIV sites (6, 31) (Table 1). The factors H2TF1 and KBF1 can be distinguished from NF-κB on the basis of their pattern of activities in different cell types. The former two activities are detectable as constitutive DNA-binding factors in a variety of cell types. In contrast, NF-κB is constitutively active only in mature B cells and is inducible in a variety of cell types by treatment with phorbol esters. This induction does not require new protein synthesis, and thus mRNA encoding NF-κB is probably synthesized in most cell types. Given the differences in binding specificity and levels of constitutive activity in different cell types, it is probable that at least three different proteins bind with high affinity to the symmetrical site in the MHC enhancer. The molecular masses of the proteins characterized as H2TF1, KBF1, and NF-κB are significantly smaller than the 290,000-Da estimated size of MBP-1. This suggests that one or more of the three characterized proteins represents a proteolytic fragment of MBP-1. Alternatively, H2TF1, KBF1, NF-κB, and MBP-1 may be distinct proteins. Given their overlapping binding specificities, these proteins could be members of a gene family, each with a related DNA-binding domain.

DNA binding analysis with truncated MBP-1 protein synthesized in vitro showed that segments containing the carboxy-terminal zinc finger domains have high affinity for MHC and β_2-microglobulin sites and a lower affinity for the κ enhancer and HIV sites. In terms of binding specificity, the domain of MBP-1 containing the two zinc fingers recognizes probes with a specificity more similar to KBF1 than either H2TF1 or NF-κB. However, this preference is slight, and given the incomplete nature of the MBP-1 protein, no identification can be made. Methylation interference analysis indicates that the MBP-1 protein binds to the symmetrical MHC site in a somewhat symmetrical fashion since methylation of the N-7 position of any of the four guanine residues on either strand interferes with its binding (42). It is interesting in this regard that the β_2-microglobulin site is recognized at a higher affinity than the HIV(1) site. These two sites differ in the regions of comparison by the 3′-most nucleotide (C→G), suggesting that the protein contacts extend to this terminus.

A high-affinity DNA-binding domain for MBP-1 has been localized to a 118-amino-acid protein fragment that contains two zinc finger domains of the class Cys_2-X_{12}-His_2. This conserved motif, found in many DNA-binding proteins (3, 12, 20), was initially described in the transcription factor TFIIIA (29). Atomic absorption spectroscopy has shown that there is a ratio of one zinc atom per finger structure (29). Consistent with this, chelation of metal ions by EDTA or by 1,10-phenanthroline inactivates the binding of MBP-1 to DNA (H. Singh, unpublished data).

The tandem zinc finger domains of MBP-1 share many of the conserved residues found in other Cys_2-X_{12}-His_2 fingers. Both have a tyrosine residue two positions amino terminal to the first cysteine. This tyrosine is conserved as part of a consensus in TFIIIA, the sex-determining gene (33), and in a nerve growth factor-induced gene (28). Both have a leucine residue at position 10 in the finger, which is also highly conserved among the above genes. The first finger domain in this pair does not have the conserved phenylalanine at position 4 but has a relatively similar amino acid, cysteine. These two finger domains are joined by a prototype H-C link (HTDVRPYHC) which is found in the zinc finger domains of TFIIIA and the Kruppel protein (35).

A number of transcription factors in addition to TFIIIA have now been shown to have Cys_2-X_{12}-His_2 zinc finger domains. The proteins Spl, SWI5, and ADR1 are most similar to MBP-1 in containing a small number of tandem domains (three, three, and two, respectively). High-affinity binding sites for each of these proteins have been characterized. Similar to SWI5 (20, 32) and ADR1 (see below) (11), MBP-1 recognizes a sequence of approximately 5.5 bp per finger domain (assuming a monomer binds to the site). This conforms nicely to the suggestion from analysis of TFIIIA binding to the 5S gene where each of the nine domains were modeled as contacting a 5.5-bp region on one face of the helix (29). In this regard, the Spl factor is an exception in that it is a protein with three finger domains and recognizes a 10-bp sequence, although it is possible that a domain does not contact DNA. The puzzling feature of the MHC-binding site is its palindromic structure. This could simply reflect a coincidence where different amino acids in the two zinc finger domains make similar contacts with the symmetrical guanine residues in the two halves of the site. Alternatively, MBP-1 may bind as a dimer to this site. The only Cys_2-X_{12}-His_2 protein thought to bind as a dimer is ADR1, which protects a symmetrical 22-bp region from nuclease cleavage (11). At this point, it is apparent that the Cys_2-X_{12}-His_2 class of protein-DNA interactions is more varied than the strict dimer protein-DNA interactions of the Cys_4-class finger domains of the steroid receptor proteins (4).

The amino-terminal zinc finger domain (Fig. 2 and 3) is of the rare class, Cys_2-X_{12}-His-Cys. This class has been detected as a single finger in Xfin (36) SWI5 (20), Evi-1 (30), and su(HW) (34). However, in no case has it been shown that this finger contributes to DNA binding. This MBP-1 domain has several conserved features of a zinc finger domain: a loop length of 12 residues, a phenylalanine residue two positions amino terminal to the first cysteine, a not uncommon tyrosine residue at position 4 within the finger, and a less common phenylalanine residue at position 10 within the finger. These features support the contention that this domain is involved in DNA binding. If a single domain can only contact 5.5 bp (based on the model for TFIIIA), then it is likely this structure would need to bind in conjunction with another protein domain to constitute a very high affinity complex.

The level of MBP-1 mRNA is variable in several cell types analyzed. We detected relatively high levels of the 9.5-kb mRNA in HeLa and retinal cell poly(A)$^+$ RNA. Two B-cell lines, BJA-B and X50-7, expressed detectable, but considerably lower, levels of MBP-1 mRNA. The Jurkat T-cell line has very low levels of MBP-1 mRNA, but the mRNA was significantly induced over a 6-h period by treatment of these cells with mitogen (PHA) and phorbol ester (PMA). These results suggest that MBP-1 plays a role in T-cell activation, presumably in controlling the expression of other genes. MBP-1 binding sites, which are also recognized by NF-κB, are critically important for induction of transcription of the IL-2Rα gene and of a latent HIV genome during T-cell activation (6, 31). It is interesting to speculate that induction of MBP-1 mRNA might produce adequate protein levels to influence transcription of the IL-2Rα gene and the HIV genome in activated T cells. Furthermore, we confirmed that the level of MBP-1 mRNA is induced by serum treatment of quiescent human fibroblasts. These results suggest that MBP-1 is a regulator of cell proliferation.

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