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Analysis of transcription control elements of the mouse myelin basic protein gene in HeLa cell extracts: demonstration of a strong NFI-binding motif in the upstream region

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ABSTRACT

Promoter elements of the mouse myelin basic protein (MBP) gene were analyzed by in vitro transcription using HeLa cell extracts. We demonstrated the MBTE (MBP transcription element), GC-box core and TATA-box elements, at -130, -93 and -34, respectively. The TATA-box was indispensable for the promoter function. The GC-box was suggested to function co-operatively with far upstream sequences including the MBTE. The MBTE was crucial to direct maximal transcription, and also functioned with a heterologous promoter irrespective of its orientation. We identified a ubiquitous binding factor which interacted specifically with the MBTE and activated transcription. Intensive footprinting studies demonstrated that the MBTE had a NFI-binding sequence. The MBTE was considered to be one of the strongest NFI-binding motif among known cellular genes. Interestingly, similar strong NFI-binding motifs were suggested to be present in the enhancer of JC virus whose gene is expressed like the MBP gene, in the nervous system.

INTRODUCTION

It is now well documented that the central nervous system is composed of heterogenous cells, and complex cellular interactions are very important for brain function. Myelinogenesis is a typical example of neuron and glia interaction. Myelin surrounds the neuronal axons and plays an essential role for nerve function by facilitating conduction of electrical impulses as an insulator. Myelin basic protein (MBP) is a major myelin structural protein, about 30% of the total myelin protein (1), and is essential to form fully functional myelins (2-6). We are studying the transcription of the MBP gene to understand molecular mechanisms of myelination. Since accumulation of the MBP in brain is synchronized with the mRNA level (5), the MBP expression is considered to be transcriptionally regulated.

We have demonstrated that the MBP promoter is transcribed efficiently in neural cells (7). Our aim is to define cis -acting elements and their trans-acting factors that govern MBP gene expression. One approach is to analyze MBP gene transcription in a cell-free system. The use of a cell-free system has proven powerful in studying transcription factors (8-10). As a first approach to understand molecular mechanisms of MBP gene expression, we applied a HeLa cell extract transcription system to analyze general and fundamental promoter elements. In this study, using a variety of deletion and base substitution mutants, we demonstrate that the MBP promoter is composed of multiple sequence elements including TATA-box, GC-box and the MBTE (MBP transcription element). In particular, the MBTE is a critical cis-acting element, which by extensive binding experiments is shown to be a strong NFI-binding motif.

MATERIALS AND METHODS

Plasmids

Protocols for recombinant DNA experiments are standard methods (11). The original 3.6 kb HindIII fragment mouse MBP genomic clone including the 5'-flanking region and the first exon, was a kind gift from Dr. L. Hood (12). We created a HindIII site at +25 or +60 by site-specific mutagenesis(13), and the 1.3kb HindIII fragment was subcloned into pBR322 as pBP-DH(+25) or pBP-H(+60). We used the pBP-H DNA as a standard MBP template in this study except for reference promoters in pdBP-series DNAs. As for construction of the pdBP-series plasmids carrying two MBP promoters (Fig. 3), we first made 5'-deletions from EcoRI to downstream appropriate restriction sites in the pBP-H using an EcoRI linker. The BglII(-1297)-BamHI fragment of the pBP-DH was then introduced into the BamHI site in each 5'-deletion mutant in a head-to-tail orientation. For templates of in vitro transcription, we used EcoRV sites to cut, so that 218 and 183 bases-long transcripts are recognized as experimental and reference run-off signals, respectively.

BAL 31 Deletion Mutants

Structure of the BPΔ-series plasmid is shown in Fig. 3. They contain one reference MBP promoter from -256 in the SalI site, and another experimental promoter between EcoRI and HindIII sites, of pBR322. The experimental promoter has a 5'-end deletion from the StuI site (-256) directed by BAL 31 nuclease digestion. The NruI site in pBR322 was changed to a XhoI site, so that cleavage of the BPΔ-series plasmids with dual promoters by XhoI and BamHI directs 550 (reference) and 411 (experimental) nucleotides-long transcripts, respectively.

In Vitro Transcription

HeLa whole cell extracts were prepared according to the method of Manley et al. (14). The standard in vitro transcription run-off assay using HeLa extracts was done at 25 ^{o}C as

described by Miyamoto et al. (15), except that 1.7 mM spermidine was added. For competition transcription assays, competitor DNA fragment and the extract were added to the pre-incubation mixture. After 10 min at 25 °C, the template DNA was added to the mixture to allow incubation for another 10 min. RNA synthesis was performed for 45 min. Run-off transcripts were purified and analyzed on 5% acrylamide-8.3 M urea gels.

DNase I Footprinting

The standard DNase I footprinting assay consists of a 20 µl reaction mixture containinig variable amounts of HeLa cell extract, about 2 ng (1 x 10^4 cpm as Cerenkov radiation) of the labelled DNA probe prepared by kination, 200 ng of linearized pBR322, buffer C (20 mM HEPES-KOH (pH 7.8), 20 mM KCl, 2 mM DTT, 0.2 mM EDTA and 15% glycerol) and 2 mM spermidine. After a pre-incubation period for 10 min at 25 °C, 2 µl of a mixture containing 80 ng of DNase I including 33 mM $CaCl_2$ in the same buffer was added to start digestion. The standard competition footprinting assay was as described above excepted that the 5'-end labelled DNA probe was added after the initial preincubation period in the presence of variable amounts of competitor DNA and the reaction mixture was incubated for an additional 10 min prior to DNase I addition. Reactions were terminated at appropriate times to obtain "balanced" DNase I digestion patterns. DNA digests were analyzed on 8% acrylamide-8.3 M urea gels together with G+A size markers (16).

S1 Nuclease Analysis

A 32-mer single-stranded DNA identical to the lower strand from +20 to -12 was chemically synthesized, and the 5'-end (+20) was labelled by kination to get a specific activity of one milion cpm/pmole. The S1 mapping procedure was as described (17). S1 nuclease-protected DNAs were analyzed by electrophoresis through 15% sequencing gels.

RESULTS

Promoter Elements of the Mouse MBP Gene Recognized by HeLa Cell Transcription Factors In Vitro Lie Downstream from -139

We first wished to establish that the *in vitro* transcription of the MBP gene occurred at the same position as the initiation site *in vivo*. The structure of the MBP 5'-flanking region up to -1319(HindIII) is shown in Fig. 1. We analyzed *in vitro* transcription start sites by S1 mapping (Fig. 2A). One major (indicated by an arrow head) and several minor protected bands were identified in *in vitro* transcripts (lane 4) as α-amanitin sensitive signals, and the same major bands also appeared in brain poly(A)$^+$-RNA (lane 2). Hence, the initiation of MBP gene transcription in the HeLa extracts was demonstrated to be equivalent to that observed *in vivo*. The first base of the transcript was determined to be an adenine from mobilities of G+A ladders with a -1.5 base correction.

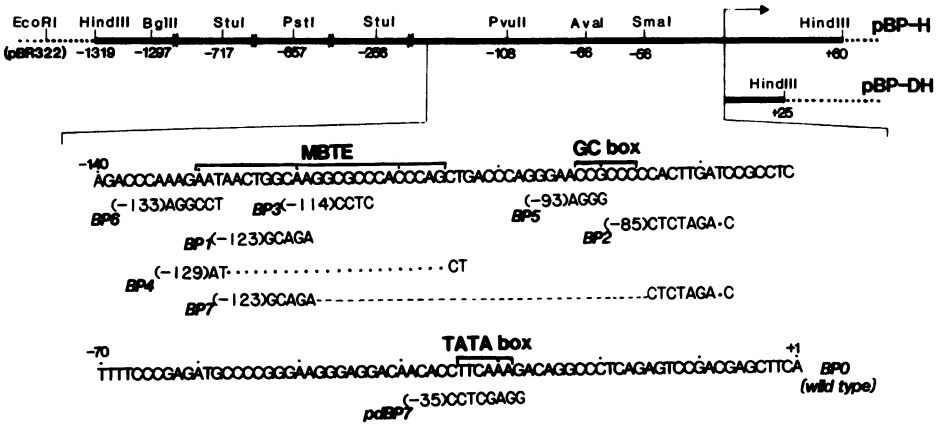

Fig. 1 Structure of the mouse MBP promoter. The pBP-DH is similar with the pBP-H, but the 3'-flanking region expands to +25 instead of +60. Three promoter elements characterized in this study, MBTE, GC-box, and TATA-box elements are indicated. Mutated bases in an experimental template of the BP-series DNAs, (Fig. 3) and pdBP7 (Fig. 4) are indicated. Dots and bars in each sequence of mutants demonstrate deleted and native bases, respectively.

To delineate the 5'-border of the MBP promoter, we constructed the pdBP-series of plasmids each of which has a common reference promoter and an experimental promoter in one plasmid (Fig. 3). A schematic representation of experimental MBP promoters having various 5'-deletions are shown in Fig. 4. As shown in Fig. 2B, deletion of the sequence upstream from the -256 StuI site (lanes 2 to 4) did not affect the promoter activity. However, when the upstream sequence was further deleted to the -56 SmaI site (lane 5), almost all transcription activity was abolished. These results indicate that there is a positive transcription element(s) between -256 and -56. Neither insertion of an EcoRI linker between -54 and -53 (lane 6) nor elimination of the internal StuI fragment between -717 and -256 from pdBP5 (lane 7) affected MBP promoter activity.

We then focused our attention on the promoter region downstream from the -256 StuI site, and analyzed it in more detail using BAL 31 nuclease-driven deletion plasmids (Fig. 3, BPΔ-series). Deletion to -139 had little or no effect on MBP promoter function (lanes 1-5 of Fig. 2C). However, a con-

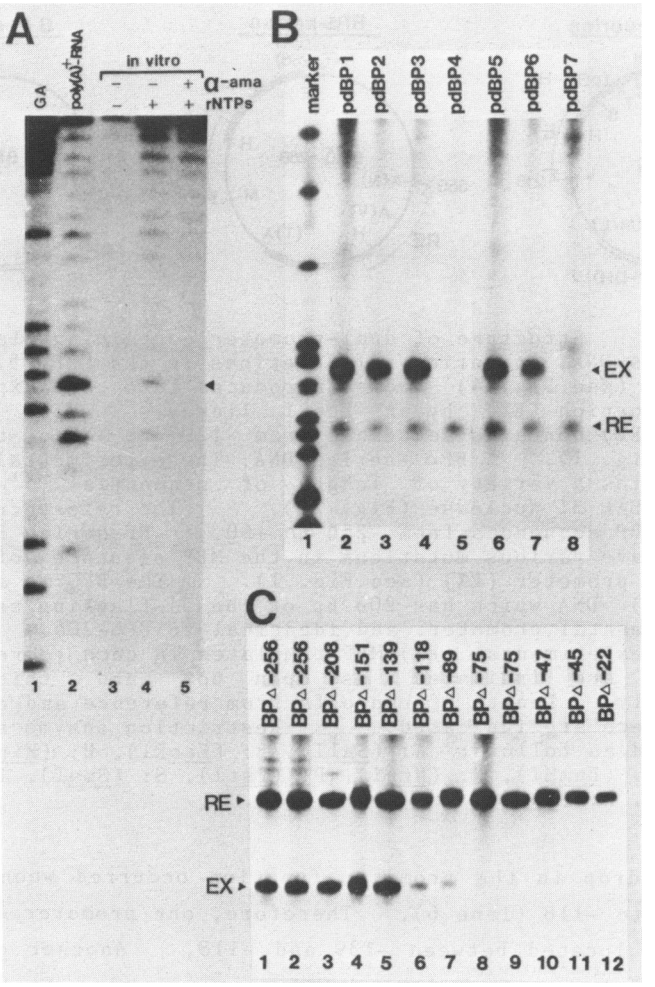

Fig. 2 In vitro transcription of the MBP gene in HeLa cell extracts. **A**: Analysis of the transcription start site of in vitro transcripts by S1 mapping using pBP-H template and oligonucleotide probe (see **MATERIALS AND METHODS**). Poly(A)$^+$-RNA from the mouse brain (0.25 μg) was also analyzed. Lane 1 shows G+A ladders of the probe. The arrow head indicates the +1 position. **B**: Run-off transcription from the MBP promoter of the pdBP-series DNA (see Figs. 3 and 4) truncated with EcoRV. MspI-digested pBR322 DNA is a size marker. **C**: Transcription from the MBP promoter of the BPΔ-series DNA (see Fig. 3) truncated with BamHI and XhoI, and subjected to in vitro transcription. Numbers of each plasmid indicate the 5' deletion end point for the experimental promoter. Run-off signals from the experimental (**EX**) and reference (**RE**) promoters in **B** and **C** are indicated by arrow heads.

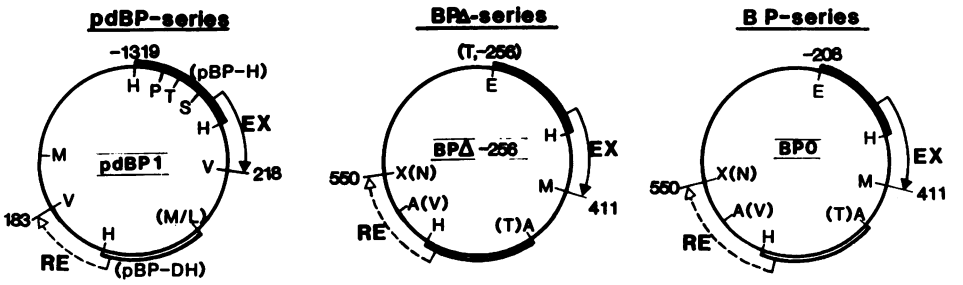

Fig. 3 Structure of dual-promoter containing-plasmid DNA. pdBP-series DNA; A variety of deletions in the MBP 5'-flanking sequences (see Fig. 4) were introduced into the experimental promoter portion (**EX**) by an EcoRI linker. The reference promoter (**RE**) contains sequences from -1297 to +25 of the pBP-DH DNA (see Fig. 1). BPΔ -series DNA; The experimental promoter (**EX**) contains a variety of length of sequential 5'-deletions driven by BAL 31 nuclease (Fig. 2C). The reference promoter (**RE**) has MBP sequences from -256 to +60. BP-series DNA; These plasmids have various mutations in the MBP sequence of the experimental promoter (**EX**) (see Fig. 1). The BPO is a parental (wild type) DNA which has 208 bp of the 5'-flanking sequence in the experimental promoter, and identical to BPΔ-206. Reference (**RE**) and experimental (**EX**) MBP templates in each representative construct are indicated as open box and thick line, respectively. Length of run-offs from reference and experimental promoters are indicated. Restriction enzyme sites are abbreviated as follows; A: (SalI), E: (EcoRI), H: (HindIII), L: (BglII), M: (BamHI), N: (NruI), P: (PstI), S: (SmaI), T: (StuI), V: (EcoRV), X: (XhoI).

siderable drop in the promoter function occurred when deletion proceeded to -118 (lane 6). Therefore, one promoter element is apparently located between -139 and -118. Another cis-acting element which was weak but significant was also found between -89 and -75 (lanes 7 and 8).

We detected several DNA stretches in the MBP promoter homologous to well known transcription elements such as an NFI-binding site (18, 19) at -124, a GC-box core (20) at -93, a CCAAT box-like structure (21) at -86 and a TATA box-like sequence at -34 (22) (Fig. 1). Among these deletion analyses demonstrated one apparent cis-acting element between -139 and -118 overlapping the NFI-binding motif. Another weak elememt was located at -89 to -75, interrupting a GC-box and a CCAAT-box-like CCACT sequence.

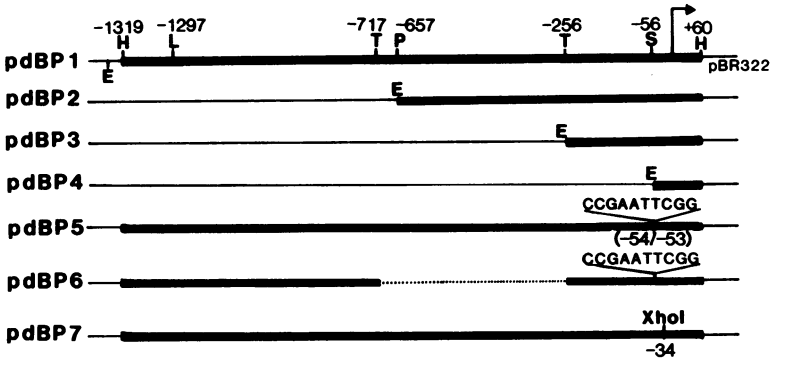

Fig. 4 Structure of the experimental MBP promoter in the pdBP-series DNA. The dBP5 contains an EcoRI site-carrying 10-mer oligonucleotide between -54 and -53. A XhoI site is generated at -34 in the pdBP7. Abbreviations of restriction enzyme sites are as in Fig. 3. Thick line; MBP promoter, thin line; vector sequence, dotted line; deleted portion.

Mutation Analysis of the MBP Promoter

We generated various template DNAs containing base substitution mutations to identify functional cis-acting elements and, to map them precisely. Substituted bases in each DNA are indicated in Fig. 1. First, the effects of the pdBP7 mutation around the TATA box-like sequence are shown in Fig. 2B, lane 8. Base substitutions at -34, -31 and -29 (see Fig. 1) abolished almost all run-off transcripts initiated from the correct start site, even though the 1.3 kb upstream sequences remained intact. Instead, larger and faint non-specific signals were seen, suggesting inaccurate transcription initiation from around -20. This result strongly suggests that the AT-rich sequence at -34 functions as the TATA-box element.

Figure 5A shows the results of transcription assays for the BP-series plasmids (see Figs. 1 and 3). Mutations in the CCAAT-box-like sequence (BP2 and BP7), CCACT at -86, did not have an appreciable effect on MBP transcription. The contribution of the CCAAT homologue to the whole promoter function appeared to be only about 20%, in an additive manner. The BP5 mutation lies in the GC-box core, and the first two C-residues are replaced by Gs. Experimental run-offs dropped significantly to

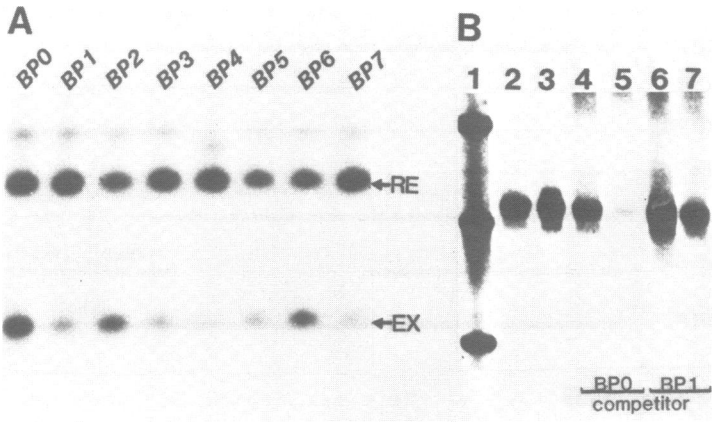

Fig. 5 Effect of the base substitution mutations on transcription activity. **A**: Run-off assay of the BP-series DNA (see the legends of Fig. 1 and Fig. 3). DNA was cut with BamHI and XhoI, and reference (**RE**) and experimental (**EX**) transcripts were analyzed. **B**: Transcription competition of the MBP promoter function by the MBTE sequence. We used 36 ng of EcoRI-BamHI fragment of the pBP-H DNA (Fig. 1) as a template, and analyzed 411-bases long transcripts. Lane 1: pBR322/MspI marker, lanes 2 and 3: run-off assay without competitor, lanes 4 and 6: with 25 ng competitor, lanes 5 and 7: with 40 ng competitor. Competitor is a MBP segment from the EcoRI(-256) to the SmaI(-56) site, of the native (BP0) or mutated (BP1) MBP sequence, as indicated.

about 30% of the wild type promoter. Thus, the GC-box core sequence appears to be a positive promoter element.

Sequences around -120 were extensively characterized by using five mutants including BP1, BP3, BP4, BP6 and BP7 (see Fig. 1). Base substitutions around -120 (BP1 and BP7), -115 (BP3), and a short internal deletion from -127 to -106 (BP4) resulted in a drastic drop in the transcription efficiencies. The BP4 mutation most severely affected the promoter activity (about 5% of the wild type). These results demonstrate that sequence around -120 includes a critical MBP promoter upstream element. The BP6 mutation which lay just upstream from the BP1 mutation did not affect the transcription activity. Consequently, mutation analyses combined with deletion assays revealed at least three important transcription elements which

included sequences from -127 to -106, the GC-box core motif at -93, and the TATA-box element at -34.

A Trans-Acting Factor Interacts with the Critical MBP Promoter Element: MBTE

The fact that mutations in a particular promoter sequence cause loss of *in vitro* transcription activity of the MBP gene suggests a direct interaction of protein in the HeLa cell extracts with specific sequences. Footprinting experiment is one of the best methods to study such DNA-protein interactions. We carried out DNase I footprinting experiments on the wild type (BP0), and mutant (BP1, BP3, BP6 and BP7) MBP templates ^{32}P-labelled on the non-coding (upper) strand (Fig. 6A). Incubation of the BP0 DNA with HeLa extracts yielded a DNase I-protected "footprinting" region around -120. Experiments using the BP6 template that had mutations at -132 through -129 (see Fig. 1) and exhibited an intact transcription activity (Fig. 5A), demonstrated strong protections in the same portion as the BP0 DNA. On the other hand, three mutated templates, BP1, BP3 and BP7, which had poor transcription activities, yielded little apparent footprinting in the corresponding region. We next tested the coding (lower) strand probe, ^{32}P-labelled at the AvaII site (-66) (Fig. 6B). Again, sequences around -120 on the wild type (BP0), but not the mutant (BP1), promoter were strongly protected by the HeLa extracts. Consequently, from an exact correlation between transcription efficiency and binding activity, this footprinting region was demonstrated to be essential for the maximal MBP transcription in HeLa extracts. We referred to the footprinting region from -130 to -106 as "MBTE" (MBP transcription element) (Fig. 6C). Other binding assays such as methylation protection and gel retardation also proved the binding of a cellular factor to the MBTE sequence (data not shown).

To demonstrate "stable" interaction of a cellular transcription factor with the MBTE sequence, we carried out competition transcription analyses using pBP-H DNA (see Fig. 1) as a template (Fig. 5B). Increasing the dose of a competitor DNA fragment carrying the wild type MBTE sequence in BP0 resulted in

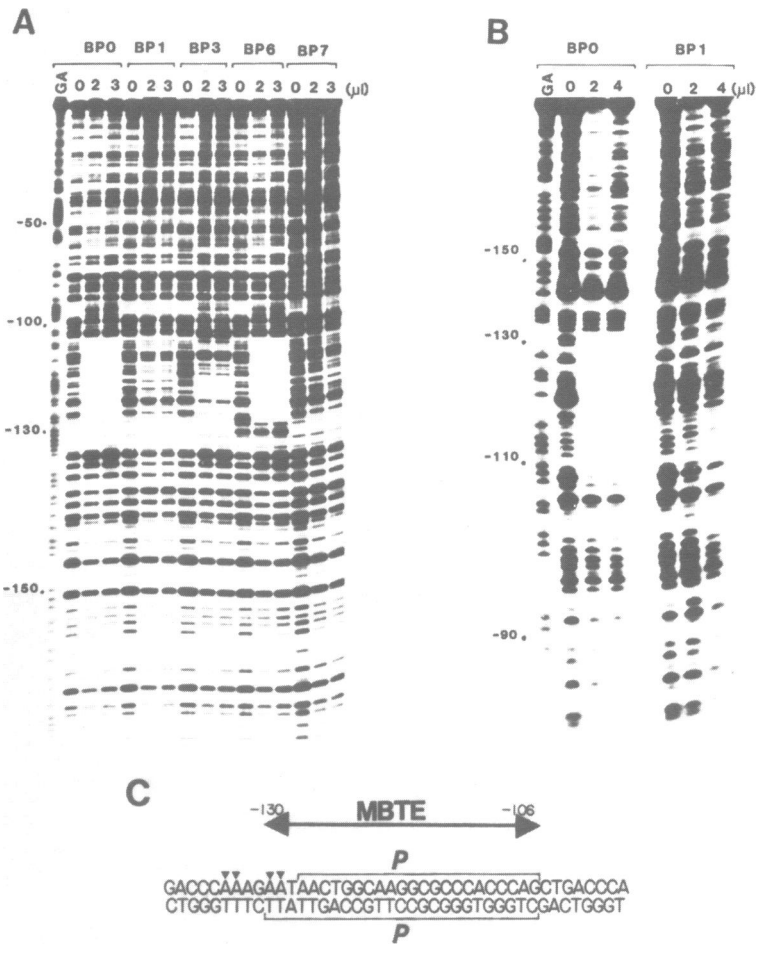

Fig. 6 DNase I footprinting on the MBP promoter. **A;** Footprinting experiments were carried out using the non-coding (upper) strand probes 5' end-labelled at the -208 EcoRI site. **B:** Footprinting experiments using the coding (lower) strand probes labelled at the -66 AvaII site. Various amounts of HeLa extracts (0, 2, or 3 µl) were used. **C:** Schematic representation of the footprinting profile and the MBTE structure. Protected (**P**) and hyper-digested (▼) regions are shown.

a marked reduction of run-off transcripts. Whereas the fragment containing the BP1 mutation exhibited little inhibitory effect. Taken together with results of competition footprinting studies (see Fig. 8A, lane 5) and gel retardation assays (data

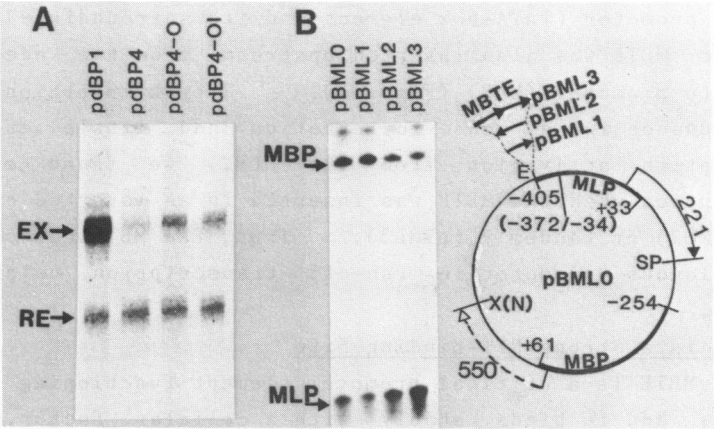

Fig. 7 Transcription activation by the isolated MBTE sequence. Chemically synthesized MBTE from -135 to -103 flanked with an EcoRI adaptor was transferred into the EcoRI site of pdBP4 (**A**) or pBML0 (**B**). **A**; The MBTE sequence was inserted into the pdBP4 in a sense (pdBP4-O) or an anti-sense (pdBP4-OI) orientation. Experimental (**EX**) and reference (**RE**) run-off signals are shown. **B**: Activation of transcription from the adenovirus type 2 major late promoter (MLP) by the MBTE sequence. The MLP was derived from the PM34 (15), and lacking most sequences upstream from -34. The MBTE segment was inserted at EcoRI site of pBML0. Numbers and directions of the inserted MBTE are shown by arrows. Template DNA was cut with XhoI and SphI (**SP**), and run-off products from the MBP and the MLP were analyzed.

not shown), the stable interaction of a cellular transcription factor to the MBTE was proven to be necessary for efficient transcription of the MBP gene.

The MBTE Can Function as a General Transcription Element

To provide more solid evidence for MBTE-driven transcription activation, we synthesized an oligonucleotide from -135 to -103 containing an entire MBTE, and inserted it into the EcoRI site at -53 of the MBTE-less pdBP4 DNA (see Figs. 3 and 4). In vitro run-off assays demonstrated MBTE-containing templates, in which the MBTE was located in a sense (pdBP4-O) or in an anti-sense (pdBP4-OI) orientation, (see the legend of Fig. 7), showed activation of transcription compared to the parental pdBP4 DNA (Fig. 7A). Both reconstituted promoters, however, yielded partial promoter activities as compared to the wild type pdBP1 DNA. These phenomena may be due to the absence of sequences from -102 to -54, or due to the position effect of the MBTE relative to

the core promoter (TATA-box element and its surroundings). The synthetic MBTE was also inserted upstream from the adenovirus major late promoter (MLP) (Fig. 7B). Though insertion of one MBTE sequence in a sense orientation had little effect on transcription activation from the MLP, we found enhanced transcription when the MBTE was inserted in an inverted orientation (pBML2) or tandemly (pBML3). Thus, the MBTE can activate a heterologous promoter in run-off transcription using HeLa extracts.

The MBTE is a Strong NFI-Binding Site

The MBTE is a critical promoter element functioning in HeLa extracts, and it binds stably with a cellular factor. To characterize this MBTE-binding factor, we carried out footprinting competition using various promoter sequences. In our experimental conditions that the MBP promoter competed out the footprinting, neither viral promoters (SV40, polyomavirus, herpes simplex virus-thymidine kinase, adenovirus major late, and mouse mammary tumor virus LTR) nor cellular promoters (β-globin and albumin) affected the footprinting (data not shown). It was noteworthy that human papova JC virus (JCV) enhancer competed the footprinting as strongly as the MBTE (data not shown), as this result may have implications for tissue-specific MBP gene expression (see discussion).

Since the MBTE contains a sequence homologous to the NFI-binding motif [TGG(A/C)N_5GCCAA] (19), we conducted footprinting competition experiments using a synthetic oligonucleotide of the adenovirus type 2 replication origin [ADori] (Fig. 8). The MBTE-footprinting by HeLa extracts was competed by the homologous MBP (lanes 5-8) or heterologous ADori (lanes 9-12). We performed similar footprinting experiments on the ADori template (lanes 13 to 24). When the MBTE or the ADori competitor DNA was added, a similar competition profile was obtained. Therefore, the binding of a cellular factor to these two binding motifs appears to be roughly equivalent. These results suggest that the MBTE and the ADori bind to the same cellular factor.

Finally, we carried out footprinting experiments using purified NFI protein using the MBTE and the ADori probes (Fig.

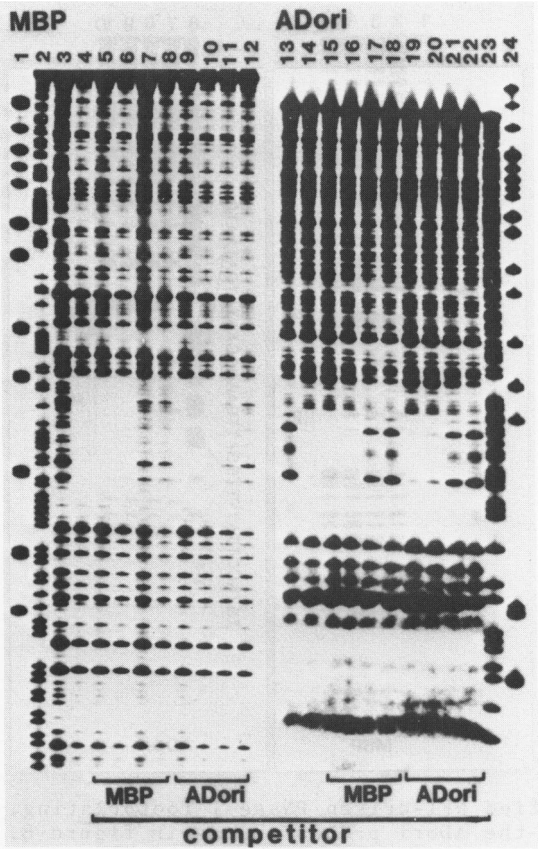

Fig. 8 Footprinting competition by the MBP promoter and the adenovirus replication origin. The MBP upper strand probe labeled at -208 (1 to 12) was prepared. The replication origin of the adenovirus type 2/5 [ADori] (48) flanked with adopter sequences for HindIII(at -18) and BamHI(at +17) was inserted into pBR322. The ADori probe DNA has a ^{32}P at the EcoRI site (13 through 24). Lanes 1 and 24: pBR322/MspI marker, 2 and 23: G+A ladders of corresponding probes. Footprinting experiment was carried out with 3 µl HeLa extracts. Lanes 3 and 13: DNase I digestion without extract. Competitor DNA fragment of MBP (-208 to -56) or ADori (-18 to +17) was added as indicated in the figure. Amounts of competitor are 0 (lanes 4 and 14), 0.04 (lanes 5, 9, 15 and 19), 0.12 (lanes 6, 10, 16 and 20), 0.36 (lanes 7, 11, 17 and 21) and 1.4 (lanes 8, 12, 18 and 22) pmole.

9, lanes 1-5 and 6-10, respectively). It is quite apparent that purified NFI binds to the MBTE motif. Adding 0.8 µl of the protein fraction to the reaction mixture gave strong

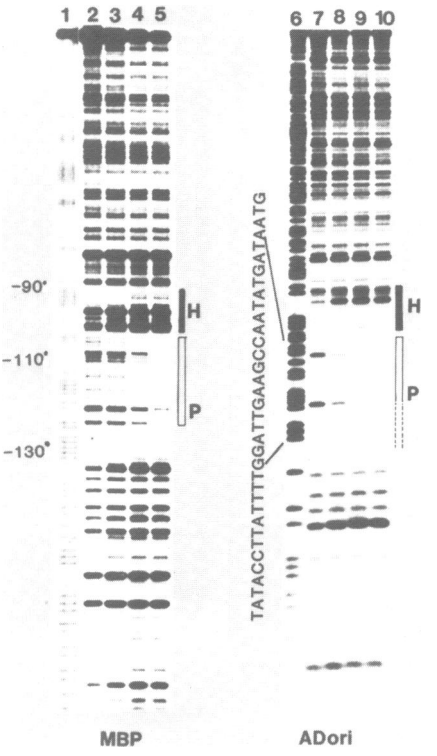

Fig. 9 Purified NFI-driven DNase I footprinting. Structures of the MBP and the ADori probes are as in figure 8. Lanes 1 and 6: A+G ladders of the probes, 2 and 7: DNase I digestion without protein fraction, 3 and 8: with 0.1 µl of NFI (1.3 ng/µl), 4 and 9: with 0.3 µl of NFI, 5 and 10: with 0.8 µl of NFI. Protected (**P**) and hyper-digested (**H**) regions are illustrated.

protection, and the protected sequences were the same as those using crude extracts. This indicates that the MBTE is a strong NFI-binding site. As expected, the ADori sequence had a substantial affinity for the NFI, and only 0.3 µl of protein fraction gave maximal protection. Competition footprinting using purified NFI also showed slightly stronger binding of NFI to the ADori sequence than to the MBTE (our unpublished results).

DISCUSSION

We have examined in detail sequences required for efficient *in vitro* transcription of the mouse MBP gene. Transcripts of

this gene were correctly initiated in HeLa extracts, even though the extract is not a brain-specific transcription system for the MBP gene. Apparently the MBP promoter elements are recognized by the HeLa cell transcription factors. Deletion (Fig. 2) and point (Fig. 5) mutational analyses of these promoter elements demonstrated that the MBP promoter is complex, consisting of several discrete sequence motifs. Sequences recognized by HeLa extracts were locating between -130 and -106; MBTE, at -93; GC-box core, and at -34; TATA-box (Fig. 1).

The TATA-box was indispensable for the MBP promoter function, since point mutations in this element completely abolished specific transcription. A sequence CCACTT at -86 which had been suggested to be a CCAAT-box homologue by Takahashi et al. (12) did not play a major role in the MBP promoter function (about 20% of the whole promoter activity). On the other hand, the GC-box core motif appeares to function with far-upstream sequences. We did not detect the GC-box function in the deletion studies (Fig. 2), it might be possible that pBR322 sequences subsitute the GC-box core motif. The region between -130 and -106 in which sequences were protected from DNase I digestion by HeLa extracts, was referred to as the "MBTE". It contained the most critical transcription element of the MBP promoter. Direct evidence for MBTE-mediated transcription activation came from experiments in which oligonucleotides were inserted into MBTE-less homologous (MBP) and heterologous (MLP) promoters (Fig. 7). These observations demonstrate that the MBTE sequence can function as a general transcription element irrespective of its orientation.

In the present study, we demonstrated an interaction between the MBTE and a cellular trans-acting factor. Competition studies of transcription and binding assays, such as DNase I footprinting (Figs. 5 and 8), gel retardation and methylation protection assays (data not shown), have proven that the stable interaction between the MBTE and its trans-acting factor is required for transcription activation. Critical nucleotides for the MBTE function seem to be located between -124 and -111, and the MBTE consists of a dyad symmetry of two core motifs [TGGCA(-124) and CGCCCA(-116)].

The MBTE has a sequence homologous to the NFI-binding motif (19, 23). Conclusive results were obtained by footprinting studies using purified NFI in which footprinting patterns were the same as those provided by crude extracts. NFI was originally found to stimulate replication of adenovirus DNA *in vitro* in HeLa cells (23). Since then, many promoters have been documented to contain NFI-binding sites as transcription elements, including chicken lysozyme (24), human c-*myc* (25), human IgM (26), albumin (27, 28), mouse mammary tumor virus (29) and cytomegalovirus (30). NFI and CTF have been identified as the same polypeptide (31). The TGGCA-binding proteins are also included in the NFI (32). Recent studies further demonstrate that several distinct NFI/CTF proteins are present in eukaryotic cells (33-37). Gronostajski *et al.* (38) demonstrated that NFI functioned in the MLP *in vitro*, and their results are well coincident with ours. Competition footprinting demonstrated that the affinity of the MBTE to its binding factor in the HeLa extracts was at least 6 times stronger than that to the NFI-binding sites in the MMTV, albumin and β-globin promoters (data not shown).

In this communication, we do not address tissue-restricted MBP gene transcription. However, Katsuki *et al*. (39) recently demonstrated by transgenic mouse analyses that the 1.3 kb 5'-flanking region of the MBP gene is sufficient for brain-specific MBP gene expression. Moreover, our transfection studies suggest that sequences between -139 and -108 have a *cis*-acting element governing efficient gene expression in neural cells (7). The MBTE and its surrounding sequences can be a candidate of determinants for tissue-specific transcription of the MBP gene. A few molecular mechanisms for tissue-restricted MBP gene expression can be hypothesized as follows.

Although we found that the MBTE-binding factor is a ubiquitous protein among the vertebrates, a nervous system-specific NFI-like protein may interact with the MBTE (see above). Santoro *et al.* (40) isolated cDNA clones for NFI/CTF expressed in HeLa cells. It would be interesting to isolate cDNA of the NFI-like protein in the brain. We found that the JCV enhancer competed the MBTE-footprinting (data not shown). JCV is

known to cause a variety of diseases in human and animal brains (41, 42). Tissue-restricted gene expression of JCV is directed by the viral enhancer/promoter (43). Khalili et al. (44) demonstrated that a DNA sequence [GGATGGCTGCCAGCCAAGCATGAGGTC] in the viral enhancer bound to cellular protein(s) in a tissue-specific manner. It is noteworthy that underlined sequence is a consensus NFI-motif (19), and we have proved recently that JCV sequence shown above has a strong NFI-binding motif (45).

Alternatively, Lemke et al. (46) have described enhanced transcription of the MBP gene in forskolin-treated Schwann cells which are also MBP producers in the peripheral nervous system. If such a change is caused at the transcription initiation level, phosphorylation of a MBTE-binding protein or other unknown transcription factors by cAMP-dependent protein kinase A (47) may elevate MBP gene expression. To clarify cis-elements and their trans-acting factors for tissue-restricted MBP gene expression, it will be necessary to establish a specific in vitro transcription system for the MBP promoter.

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