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Demonstration of a transcription element in vitro between the capping site and translation initiation site of the mouse myelin basic protein gene

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A transcription element was identified, by in vitro analyses, just downstream from the capping site of the mouse myelin basic protein (MBP) gene. Deletion of this element caused a dramatic drop of transcription efficiency in mouse brain, rat liver and HeLa cell nuclear extracts, regardless of the form of DNA being closed circular or linear form. DNase I footprint analysis demonstrated the presence of a ubiquitous *trans*-acting factor for this region. This element functioned even when it is located in the normal direction downstream from the adenovirus major late promoter. Mutation analysis suggested that an essential part of the downstream element was located between +25 and +45.

Transcription in vitro; Transcriptional control; Myelin basic protein

1. INTRODUCTION

Initiation of transcription is directed by the core promoter, which includes TATA [1] and initiator elements [2]. For maximal and tissue-specific transcription, activating elements and enhancers, which are located far upstream or downstream from the transcription start site, are required [3,4]. Certain gene need *cis*-elements which are located just downstream from transcription start sites [5–10]. Previous reports from our laboratory have described the transcriptional regulation of the mouse myelin basic protein (MBP) gene [11–14]. Since the MBP gene is strongly expressed exclusively in the nervous system [15], this gene is a good tool for studying brain-specific gene expression. Studies using cell-free transcription [11–14], transfection [16,17] and transgenic mice [18,19] have identified various upstream elements that are required for efficient and brain-specific transcription. Strong MBP promoters, which have been reported in the literature, contain a considerable length of the DNA stretch downstream from the transcription start site [11–17]. However, effects of this downstream sequence have not been investigated. In this paper, we report on the existence of a *cis*-element within the MBP gene, just downstream from the transcription start site, and demonstrate its function in an in vitro system.

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Abbreviations: MBP, myelin basic protein; MLP, adenovirus major late promoter

2. MATERIALS AND METHODS

2.1. *Plasmid DNAs*

BP1318/60 DNA contains the mouse MBP sequences spanning bases at −1318 through +60 [12], and other DNAs of BP-series are denoted in the same way. In these DNAs, the initiator methionine codon is located at +48. pML is a plasmid of the minimal adenovirus major late promoter (MLP) at −34 (*Eco*RI) through +33 (*Bam*HI) inserted into pBR322 [11]. For construction of pML/DE1, DE1 oligonucleotide (oligo) which spans from +22 to +47 of the MBP gene (Fig. 1), was flanked by *Bam*HI linkers and inserted into the pML *Bam*HI site just downstream of the MLP sequence in the normal direction. Structures of pML/DE1M, pML/DE2 and pML/DE3 are similar to pML/DE1 but mutated or shorter oligos were inserted into pML instead of DE1 oligo (see Fig. 1). In pML/DE1i, DE1 oligo was placed inversely against the MLP DNA.

2.2. *Preparation of extracts and in vitro transcription*

Mouse brain and rat liver nuclear extracts [12], and HeLa whole cell extracts [20] were prepared as previously described. In vitro transcription was carried out at 30°C for 45 min using 6 μl of extract (10 mg/ml) and 300–450 ng of closed circular DNA [12]. Transcripts were detected by a modified S1-nuclease mapping procedure and gel electrophoresis [12].

2.3. *DNase I footprint*

DNase I footprint was carried out as previously described [11]. BP253/60 DNA was linearlized by an *Aval* cut at +56 and labeled with ^{32}P by kination. Specific activity of the probe was 10 000 cpm/2 ng, and 1 ng of the probe DNA was used in each assay.

3. RESULTS

Transcription of the mouse MBP promoter in BP1318/60 DNA, in mouse brain nuclear extracts, resulted in a heavy MBP transcription signal (Fig. 2A, lane 1). BP1318/25 DNA with 3′-truncation was also transcribed. Interestingly, the signal was very weak as compared to BP1318/60 (about 7%) (Fig. 2A, lane 2). The use of BP53/60 and BP53/25 DNAs carrying much

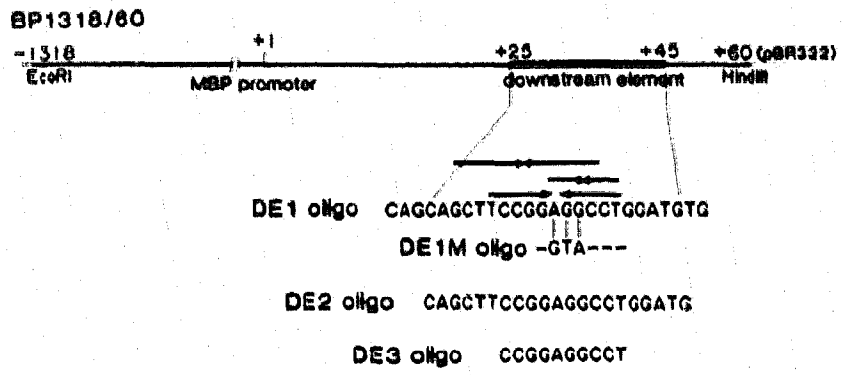

Fig. 1. Structure of BP1318/60. The mouse MBP DNA from −1318 to +60 relative to the transcription start site was inserted into pBR322 [12]. Inverted or complementary inverted repeats observed in the downstream element are schematically shown by arrows. DNA sequences in DE1, DE1M (similar to DE1 oligo but includes mutations between +35 and +37), DE2 and DE3 oligos are shown.

shorter 5'-sequences also led to strong and weak signals, respectively (Fig. 2A, lanes 3 and 4). These DNAs lack upstream activating sequences such as an NFI-site, M1-site, GC-box and CCAAT-box [11,13]. These results demonstrated that the sequence downstream from the transcription start site contains a potent *cis*-element, and it can function independently from the uptream *cis*-elements. We further transcribed BP53/60 and BP53/25 DNAs, in rat liver nuclear extracts and obtained results similar to those obtained with mouse brain nuclear extracts (Fig. 2A, lanes 5 and 6). It was highly likely that the action of the downstream sequence was modulated by a ubiquitous factor. Since closed circular DNA template of the MBP promoter is not transcribed well in HeLa cell extracts

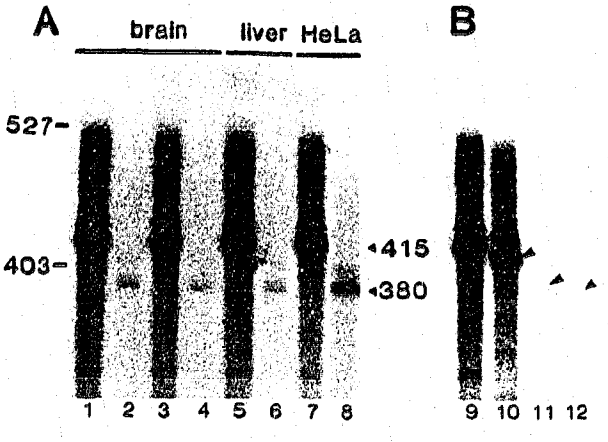

Fig. 2. In vitro transcription from various templates of the mouse MBP promoter. 450 ng of closed circular DNA was transcribed in the mouse brain, rat liver of HeLa cell extract. Probe for S1-mapping was derived from each template. A: BP1318/60 (lane 1), BP1318/25 (lane 2), BP53/60 (lanes 3, 5 and 7) and BP53/25 (lanes 4, 6 and 8). In lanes 7 and 8, DNA (300 ng) was linearlized with *Sal*I. Positions of 415 (for BP1318/60 and BP53/60) and 380 (for BP1318/25 and BP53/25) bases-long transcripts, and 403 and 527 bases-long size markers are shown. B: BP253/60 (lane 9), BP253/45 (lane 10), BP253/25 (lane 11) and BP253/22 (lane 12) DNAs were transcribed in the brain nuclear extract. Specific signals are marked by arrowheads.

[12], we transcribed linearlized BP53/60 and BP53/25 DNAs in HeLa cell extracts (Fig. 2A, lanes 7 and 8). Similar results were obtained indicating that the downstream element seemed to function in such a 'general' cell-free transcription system regardless of the form of DNA. The decrease in transcription efficiency in HeLa cell extracts was markedly less significant than those in brain and liver extracts. Two further templates were constructed, which have 3'-deletion up to +45 (BP253/45) and +22 (BP253/22), and they were transcribed in the brain nuclear extracts (Fig. 2B). Transcription signals, observed with BP253/45 and BP253/60, were similar to each other. Transcription using BP253/22 was dramatically reduced, as in the case of BP253/25. This is consistent with the presence of a positive *cis*-element upstream from +45.

Chimeric promoters, derived from pML were constructed to investigate whether the downstream elements function within a heterologous promoter. DE1 oligo, containing the downstream sequences from +22 to +47 (see Fig. 1), was inserted into the *Bam*HI site of pML, in normal and inverted directions, just downstream from the MLP to give pML/DE1 and pML/DE1i, respectively. These DNAs were transcribed in the mouse brain nuclear extract. pML/DE1 yielded 3-times more transcripts than pML (Fig. 3, lanes 1 and 2), and pML/DE1i transcripts were fewer than those of pML/DE1 (Fig. 3, lanes 2 and 3). Inverse orientation of the DE1 oligo inhibited transcription from MLP (Fig. 3, compare lanes 1 and 3). Transcription was performed using the pML/DE2 and pML/DE3 chimeric promoters which contain inserts of the DE2 and DE3 oligos as described in Fig. 1. These are oriented in the normal direction, and are shorter than the DE1 component present in the gene. When pML/DE2 was used, there was approximately 2-fold more transcription compared with pML (Fig. 3, lanes 1 and 4). pML/DE3 yielded apparently fewer transcripts than pML (Fig. 3, lanes 1 and 5). These results demonstrated that DE1 and DE2 oligos can activate transcription from the MLP,

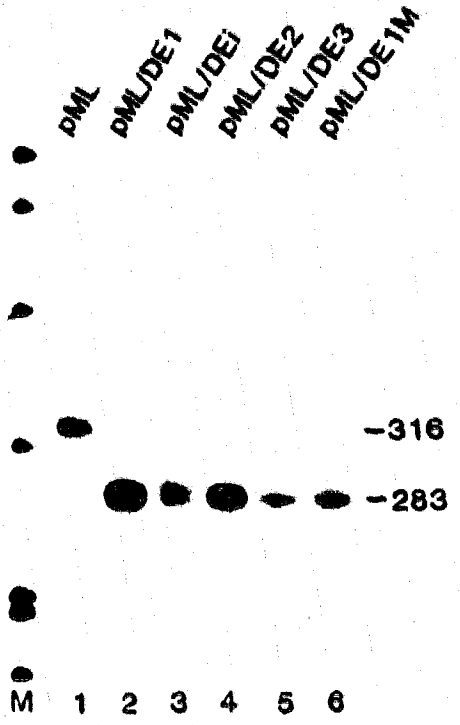

Fig. 3. Transcription of chimeric promoters containing the minimal MLP and MBP downstream element. M, pBR322/*Msp*I size marker. Oligonucleotides were flanked with *Bam*HI linkers and inserted into the *Bam*HI site of pML DNA just at the 5'-terminus of the MLP sequence. DNA (300 ng) was transcribed in the brain nuclear extract and transcripts were detected with a probe for pML. Thus, transcripts of pML and its chimeric constructs are to be observed at 316 and 283 bases in size, respectively.

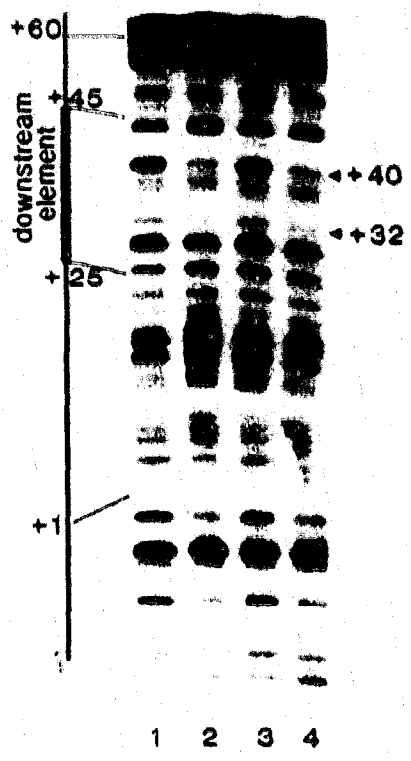

Fig. 4. DNase I footprint on the MBP downstream element. Lanes 1 and 3, no extract; lanes 2 and 4, 4 μl of the mouse brain and rat liver nuclear extract, respectively. Protected bases at +32 and +40 are indicated. Positions for representative nucleotides and the downstream element are schematically illustrated.

whereas a much shorter DE3 oligo used in this study, does not. The AGG at +35 in the DE1M oligo was mutated to GTA to give the construct pML/DE1M DNA (see Fig. 1). pML/DEM1 was not transcribed as efficiently as pML/DE1, suggesting that the AGG at +35 was important for the function of the downstream element. From these experiments, the MBP downstream element was localized betweeen +25 and +45.

The results are consistent with these of a *trans*-acting factor interacting with sequence downstream from the transcription start site. DNase I footprinting was performed to detect such a nuclear factor (Fig. 4). Clear DNase I protection, on bands at +32 and +40, was observed in the presence of brain and liver nuclear extracts (Fig. 4, lanes 2 and 4). Protection patterns on the downstream element generated by both extracts were indistinguishable. These results revealed the existence of a ubiquitous binding factor(s) for the downstream element.

4. DISCUSSION

The existence of downstream *cis*-elements is not usual in RNA polymerase II-driven genes. However, certain [illegible] genes [5,7,8] and genes from other sources [illegible] been demonstrated to have downstream [illegible]. Recently, certain nervous system-specific ge[illegible]e human glial fibrillary acidic protein [10] and neuron-specific enolase (R. Kuwano, personal communication), have been shown to have *cis*-elements in their downstream regions. These observations, together with our results, may be of interest because nervous system-related promoters often contain downstream elements.

We do not know whether the MBP downstream element functions in vivo. However, Miura et al. [16] found a strong MBP promoter activity in transfection experiments using MBP promoters with downstream sequences up to +60. Devine-Boach et al. [17] also demonstrated efficient MBP promoter function spanning from −1816 to +105. Therefore, the MBP downstream element might function in vivo. Transgenic mice studies demonstrated that the MBP promoter spanning from −1318 to +22 directs tissue-specific transcription [18,19]. In their study [19], the rate of transcription from a single transgene was markedly low in comparison to the native MBP gene. These observations are consistent with the existence of a *cis*-element being lost from these constructs, and the downstream

element reported in this study might be one of these elements.

In this study, we identified a downstream element in the MBP gene which functions in vitro. Essential portion of the element was localized between +25 and +45. It is interesting that inverted and complementary-inverted repeats are observed in this region as shown in Fig. 1. The ubiquitous factor interacting with this region as observed in this report, is a potential candidate of a cognate *trans*-acting factor of the downstream element.

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REFERENCES

[1] Breathnach, R. and Chambon, P. (1981) Annu. Rev. Biochem. 50, 349–383.
[2] Smale, S.T. and Baltimore, D. (1989) Cell 57, 103–113.
[3] Nicholas, C.J., Rigby, P.W.J. and Ziff, E. (1988) Genes Dev. 2, 267–281.
[4] Hatzopoulos, A.K., Schlokat, U. and Gruss, P. (1988) In: Transcription and Splicing (Hames, B.D. and Glover, D.M. eds) pp. 43–96, IRL Press, Oxford.
[5] Hultmark, D., Klemenz, R. and Gehring, W.J. (1986) Cell 44, 429–438.
[6] Cohen, R.B., Yang, L., Thompson, J.A. and Safer, B. (1988) J. Biol. Chem. 263, 10377–10385.
[7] Perkins, K.K., Dailey, G.M. and Tjian, R. (1988) Genes Dev. 2, 1615–1626.
[8] Soeller, W.C., Poole, S.J. and Kornberg, T. (1988) Genes Dev. 2, 68–81.
[9] Chen, R., Ingraham, H.A., Treacy, M.N., Albert, V.R., Wilson, L. and Rosenfeld, M.G. (1990) Nature 346, 583–586.
[10] Nakatani, Y., Brenner, M. and Freese, E. (1990) Proc. Natl. Acad. Sci. USA 87, 4289–4293.
[11] Tamura, T., Miura, M., Ikenaka, K. and Mikoshiba, K. (1988) Nucleic Acids Res. 16, 11441–11459.
[12] Tamura, T., Aoyama, A., Inoue, T., Miura, M., Okano, H. and Mikoshiba, K. (1989) Mol. Cell. Biol. 9, 3122–3126.
[13] Aoyama, A., Tamura, T. and Mikoshiba, K. (1990) Biochem. Biophys. Res. Commun. 167, 648–653.
[14] Tamura, T., Sumita, K., Hirose, S. and Mikoshiba, K. (1990) EMBO J. 9, 3101–3108.
[15] Okano, H., Miura, M., Moriguchi, A., Ikenaka, K., Tsukada, Y. and Mikoshiba, K. (1987) J. Neurochem. 48, 470–476.
[16] Miura, M., Tamura, T., Aoyama, A. and Mikoshiba, K. (1989) Gene 75, 31–38.
[17] Devine-Beach, K., Lashgari, M.S. and Khalili, K. (1990) J. Biol. Chem. 256, 13830–13835.
[18] Katsuki, M., Sato, M., Kimura, M., Yokoyama, M., Kobayashi, M. and Nomura, T. (1988) Science 241, 593–595.
[19] Kimura, M., Sato, M., Akatsuka, A., Nozawa-Kimura, S., Takahashi, R., Yokoyama, M., Nomura, T. and Katsuki, M. (1989) Proc. Natl. Acad. Sci. USA 86, 5661–5665.
[20] Manley, J.L., Fire, A., Cano, A., Sharp, P.A. and Gefter, M.L. (1980) Proc. Natl. Acad. Sci. USA 77, 3855–3859.