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Core promoter of the mouse myelin basic protein gene governs brain-specific transcription *in vitro*

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The core promoter of the mouse myelin basic protein (MBP) gene from −36 to +12 was preferentially transcribed in brain nuclear extracts. Both the TATA at −34 and downstream elements to +12 were required for efficient, accurate and brain-specific transcription. From brain and liver nuclear extracts, we have partially purified the general transcription factor TFIID. The partially purified fractions contained TATA element binding factors of the MBP promoter as well as adenovirus major late promoter (MLP). The tissue-derived TFIID was functionally exchangeable for the HeLa TFIID, and directed transcription from the MLP. Surprisingly, the brain TFIID activated transcription from the MBP core promoter while the liver TFIID did to a much lesser extent. Exchange of the TATA-containing short DNA stretch to the MBP core promoter for a corresponding region of the mouse albumin promoter or MLP abolished the brain specificity. We found that several tissue-specific promoters other than MBP, such as mouse neurofilament and human α-1-antitrypsin promoters were also transcribed much more efficiently by the brain and liver TFIID, respectively. We suggest that different tissues contain functionally non-equivalent TFIID or TFIID-like activities.

Key words: core promoter/myelin basic protein/TATA element/tissue-specific transcription/TFIID

Introduction

In eukaryotes, promoters of protein coding genes transcribed by RNA polymerase II (B) (pol II) can be divided into two regions: the proximal and distal elements. The proximal promoter contains sequence elements required for basal transcription from a correct start site: the TATA element located between 25 and 35 bp upstream of the RNA start site is the most essential regulatory element in the core promoter (Breathnach and Chambon, 1981); sequences around the start site (initiation site elements) are also required for efficient and accurate transcription (Corden *et al.*, 1980; Concino *et al.*, 1984; Tokunaga *et al.*, 1984; Smale and Baltimore, 1989). In yeast, where transcription does not initiate at a strictly defined distance from the TATA element, sequences near the start site have been shown to be important for accurate initiation (Chen and Struhl, 1985; Hahn *et al.*, 1985). Purified pol II alone will not accurately initiate transcription *in vitro*, without addition of general transcription factors (GTFs) purified from HeLa cells and designated TFIIB, -D and -E (Matsui *et al.*, 1980; Dignam *et al.*, 1983). This combination of GTF and pol II will accurately initiate from a minimum adenovirus major late promoter (MLP). Among GTFs, TFIID [also known as BTF1 (Cavallini *et al.*, 1988) or DB (Fire *et al.*, 1984)] is interesting since it contains a protein that specifically binds to the TATA element, and functions in transcription commitment (Davison *et al.*, 1983; Fire *et al.*, 1984; Sawadogo and Roeder, 1985; Cavallini *et al.*, 1988). One of the first steps in transcription initiation is correlated with TFIID binding to the TATA element (Fire *et al.*, 1984; Hawley and Roeder, 1985). The distal promoter is composed of various *cis*-elements such as activating sequences, enhancers and silencers which function upstream or downstream from the proximal promoter (Khoury and Gruss, 1983; Yaniv, 1984; Dynan and Tjian, 1985; Hatzopoulos *et al.*, 1988; Nicolas *et al.*, 1988). In many tissue-specific genes, tissue-specific *cis*-elements in distal promoters and their cognate factors have been identified. These elements may contribute to the control of tissue-specific patterns of their target genes.

Myelin basic protein (MBP) is one of the major components of myelin which functions as an insulator of neuronal axons of vertebrates (Ritchie, 1984). MBP gene expression is restricted to the nervous system and is regulated at transcription level (Okano *et al.*, 1987). Transgenic mice studies (Katsuki *et al.*, 1988) and *in vivo* transfection (Miura *et al.*, 1989) suggested the presence of tissue-specific *cis*-elements within the MBP upstream region. We have established tissue-specific *in vitro* transcription (Tamura *et al.*, 1989b, 1990), and suggested that the mouse MBP promoter from −253 to +62 is responsible for efficient and tissue-specific transcription (Tamura *et al.*, 1989a). We have demonstrated that the MBP distal promoter from −253 to −54 is responsible for brain specificity (Tamura *et al.*, 1989a; Aoyama *et al.*, 1990) whereas the MBP proximal region from −53 to +62, which contains a TATA element at −34, is required for basal transcription (Tamura *et al.*, 1989a). Surprisingly, the MBP proximal promoter appears to govern brain-specific *in vitro* transcription to some extent (Tamura *et al.*, 1989a), implying the presence of a brain-specific *cis*-element within this region.

In this study, we have delineated sequences absolutely required for substantial MBP proximal promoter function, and have specified the core promoter between −36 and +12 which directs brain-specific transcription. Using a brain and liver reconstituted transcription system depleted of TFIID activity, we have shown that efficient *in vitro* transcription of brain-specific promoters such as MBP and neurofilaments or liver-specific α-1-antitrypsin promoter is dependent on the tissue-specific origin of TFIID used to reconstitute the transcription system. These results suggest that multiple

tissue-specific forms of mammalian TFIID or TFIID-like activity play a role in the tissue-specific control of gene expression.

Results

Tissue-specific in vitro transcription from the MBP proximal promoter

Transcription of the various MBP proximal promoters was performed using brain or liver nuclear extracts, and *in vitro* transcripts were analyzed by a modified S1 nuclease mapping procedure (Figures 1 and 2) (Tamura *et al.*, 1989b). As previously reported (Tamura *et al.*, 1989a), BP53/62 was transcribed more efficiently by brain nuclear extract than liver nuclear extract; under similar conditions the minimum MLP (−34 to +33) promoter was transcribed equally in both extracts (Figure 2A). We did not include an internal promoter as a control in our assays to avoid interference with internally added sequences (Tamura *et al.*, 1989a). Further upstream deletion of BP53/62, to give BP45/62, resulted in a slight decrease of promoter activity compared with BP53/62 in both brain and liver extracts. A 3′ terminus deletion (BP53/24) caused a dramatic loss of transcription efficiency (as compared with BP53/62) suggesting the existence of a downstream sequence element located around position +35 (unpublished observations). Triple point mutations from TTCAAA to CTCGAG at −34 (BP53TA/62) abolished almost all transcription activity of the mutant template in both extracts, thus suggesting that this AT-rich sequence functions as a TATA element even though it diverges from consensus TATAAA.

The BP53/62 promoter was further 5′ and/or 3′-end deleted and transcribed *in vitro* (Figure 2B). BP36/1 (lacking downstream sequences) and BP20/12 (lacking the TATA element) were not transcribed from the correct site in brain extracts, and not transcribed in liver extract, although BP36/1 and BP20/12 yielded significant amounts of incorrectly initiated transcripts in the brain nuclear extract (marked by solid circles in Figure 2B). However, BP36/12 was accurately and efficiently transcribed in the brain extract which demonstrated that both the TATA element and sequences up to +12 were necessary for the efficient and accurate initiation of the MBP gene transcription. In addition, we found that sequences from +2 to +12 contained an initiation/downstream element of the MBP promoter. It is worthwhile mentioning that transcription of the mouse albumin promoter (from −35 to +1: AL35/1) was also observed using both liver and brain nuclear extracts.

Delineation of the MBP core promoter

To accurately map the 5′ and 3′ limits of the MBP core promoter we constructed nested 5′ and 3′ deletions of a modified BP36/12 template, McBP, and tested them in an *in vitro* transcription system. The BP36/12 promoter, in

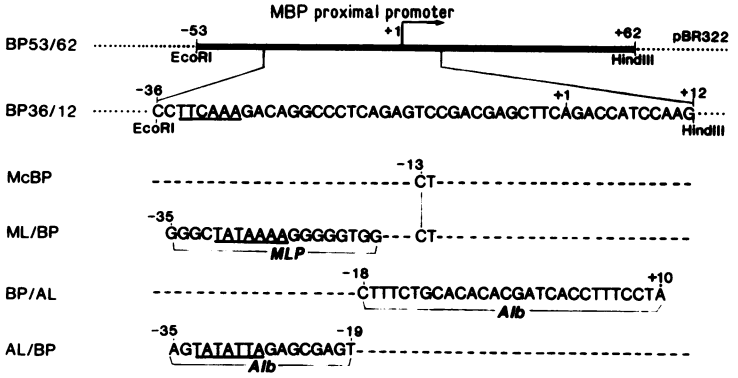

Fig. 1. Structure of the MBP proximal promoter and its derivatives. Promoter sequences were inserted between the *Eco*RI and *Hin*dIII sites of pBR322. Short bars indicate bases identical to those in BP36/12. *MLP*, adenovirus major later promoter sequence; *Alb*, mouse albumin promoter sequence; underlines, TATA element.

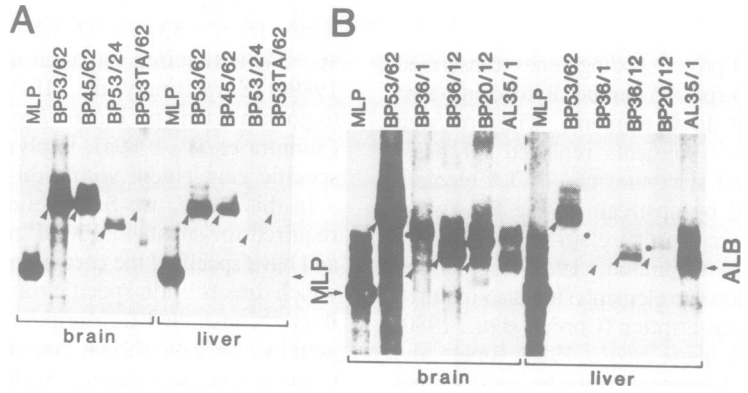

Fig. 2. *In vitro* transcription from the proximal MBP promoters. Each template was transcribed in brain and liver nuclear extracts and specific transcripts (indicated by arrowheads) were detected by S1 nuclease analysis. MLP, pML DNA; (●), unfaithful transcription. Positions of MLP and albumin transcripts are shown.

which TC at −13 was replaced by CT (McBP, see Figure 1), was stronger in transcription efficiency than BP36/12 (data not shown) and preferentially transcribed by brain nuclear extract as the wild-type promoter (Figure 9, compare BP36/12 and McBP transcriptions). Consequently we have used McBP in this study. McBP containing an intact TATA element produced substantial amounts of specific transcripts. However, only a 31 bp deletion from either the 5′ or 3′ ends severely affected the promoter activity (Figure 3, upper and lower panels). Deletion of the entire TATA element abolished almost all transcription. Abundant non-specific transcripts are observed ~403 nucleotides in size. Since the amount of specific transcription decreases as a function of the deletion, it is possible that non-specific transcripts are initiated from some upstream sequences (Figure 3). These results demonstrated that the sequence from −36 to +12 was necessary and sufficient for the MBP promoter function. Consequently we specified sequences from −36 to +12 as an MBP core promoter.

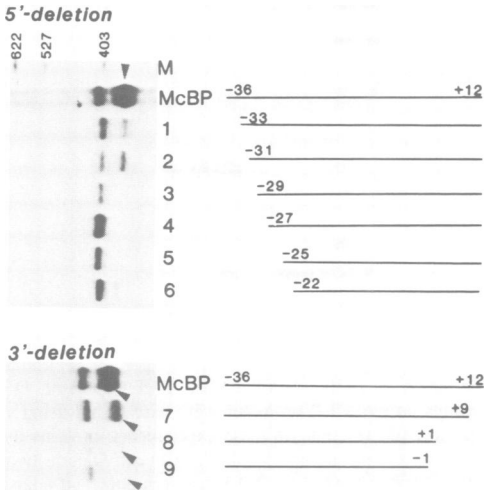

Fig. 3. Deletion analysis of the MBP core promoter. A derivative of BP36/12, McBP, was used as an original plasmid for deletion. 5′ deletion (lanes 1−6) and 3′ deletion (lanes 7−9) mutants were transcribed in the brain nuclear extract, and the specific transcript (indicated by arrowhead) was analyzed. M, pBR322/*Msp*I size marker.

Preparation of tissue-derived TFIID

The results shown above raised the possibility that factors interacting with the MBP core promoter were functionally non-equivalent in different tissues, which suggests that some of the GTFs generated the brain-specific transcription. Accumulated results have shown that the TFIID is a unique GTF that binds to the proximal promoter and commits transcription (Cavallini *et al.*, 1988; Nakajima *et al.*, 1988; Buratowski *et al.*, 1989). For this reason we hypothesized that the TFIID activity of brain and liver may be functionally non-equivalent. Brain nuclear extracts were resolved by DEAE−cellulose chromatography (Figure 4). As previously reported (Nakajima *et al.*, 1988), TFIID activity was detected by a transcription complementation assay using heat-treated crude extracts in which TFIID is selectively inactivated. TFIID activity (Figure 4, left panel) was eluted from DEAE−cellulose by gradient elution of KCl from 0.05 to 1 M. As shown in the superimposed figure, fractions 21 and 22 which contained high TFIID activity were pooled and applied on a phosphocellulose column (right panel). Almost all TFIID activity was detected in the P100 fraction (5% of the initial material). Purification of liver TFIID was carried out using the same procedure, and liver TFIID was also recovered in the same fractions as brain TFIID (data not shown).

When heated under the same conditions reported for HeLa nuclear extract, brain nuclear extract was not able to restore MLP transcription unless the P100 fraction derived from brain extract was added. HeLa GTFs and pol II were also partially purified, and added to the heated extract (Figure 5). Although amounts of each GTF were enough to transcribe MPL, neither HeLa TFIIB, TFIIE nor pol II were able to restore transcription. However, when the brain heat treated extract was supplemented with partially purified HeLa TFIID, MLP transcripts were restored. We thus concluded that the heat treated brain nuclear extract was TFIID dependent for transcription activity. The P100 fraction was

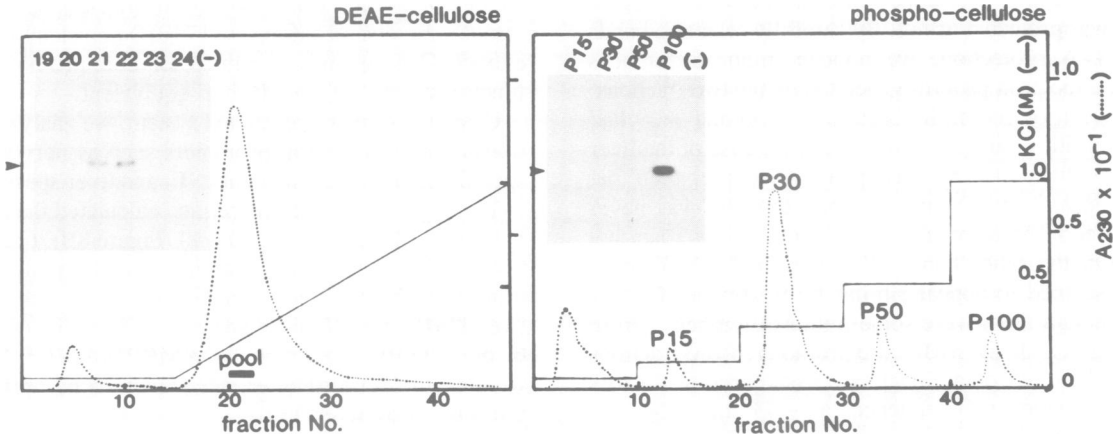

Fig. 4. Purification of TFIID of mouse brain. Chromatographic profiles of brain TFIID on DEAE−cellulose (**left panel**) and phosphocellulose (**right panel**) are shown. In each panel, the vertical axes indicate KCl concentration (solid line) or optical density at 230 nm (dotted lines). One fraction was collected by 1 ml. In the DEAE−cellulose column chromatography, fractions 21 and 22 were pooled for TFIID activity, and applied to a phosphocellulose column. For assaying TFIID activity, 4 μl (DEAE−cellulose) or 1 μl (phosphocellulose) of each fraction was used for the reconstituted transcription containing heated brain nuclear extract and pML DNA. Results are indicated by the superimposed figures. Transcription signals are indicated by arrowheads. (−), without TFIID fraction.

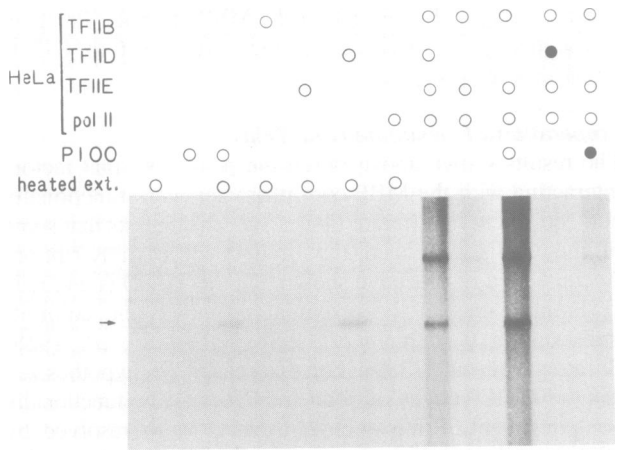

Fig. 5. Characterization of the heated nuclear extract and P100 fraction on transcription reaction. HeLa GTFs and pol II were purified as described in Materials and methods. Each GTF (1.5 μl), pol II (1.0 μl), the P100 fraction (1 μl) or heated brain nuclear extract (6 μl) was used as indicated in the figure (shown by open circles) and pML DNA was transcribed. The position of the specific signal is indicated (arrowhead). Solid circle, heated at 47°C for 15 min.

demonstrated to be exchangeable for the HeLa TFIID. We used brain and liver P100 fractions in DNase I footprint experiments (Figure 6). We found that both fractions contained a DNA binding factor of the MLP TATA element. Interestingly, protected sequences extended from the TATA element to about the +20 region. These observations are consistent with the previous reports on HeLa TFIID preparations (Sawadogo and Roeder, 1985; Nakajima *et al.*, 1988).

A DNase I footprint experiment was carried out using the MBP promoter (Figure 7). Unexpectedly, many bands in the MBP promoter became DNase I hypersensitive upon addition of brain and liver P100 fractions. However, we were able to see one partial but significant protection within the TATA element. At least three bands of −31, −30 and −29 were weakened by both fractions. This footprint driven by the brain fraction seemed to be slightly stronger than the liver fraction. Since DNase I does not make bands from −34 to −32, we could not judge whether the whole TATA sequence was protected or not. By carefully scanning bands in the TATA protection, we noticed minor differences between the footprint patterns generated by the two fractions. Bands induced by the brain fraction at −30 and −29 had similar intensities (lane 5). However, in the case of the liver fraction, the band at −30 was apparently darker than that at −29 (lanes 6 and 7), and this phenomenon was never altered by addition of much more liver fractions (data not shown). On the other hand, all bands in the MBP core promoter seemed to appear similar by treatment of either fraction, though there were some minor differences. From footprinting outside the MBP core promoter, P100 fractions were found to be contaminated by tissue-specific factors which can interact with sequences at −43 and +24.

TFIID from brain and liver is functionally non-equivalent

We analyzed the function of tissue-derived TFIID on the MBP core promoter using heat-treated nuclear extracts and either brain (B) or liver (L) TFIID (Figure 8A). Addition of brain TFIID to the heated (TFIID-inactivated) brain nuclear extract directed transcription from the MLP. The

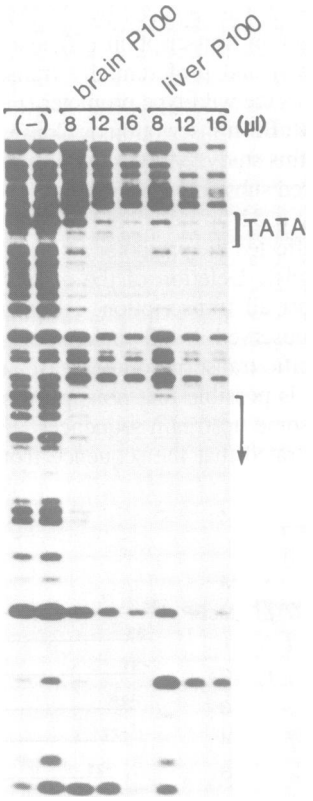

Fig. 6. Interaction of TFIID with the MLP TATA element. DNase I footprint assays on the MLP promoter were carried out using various amounts of the P100 fraction. In these experiments, proteins in the P100 fraction were concentrated 5-fold. TFIID activities in two P100 fractions were titrated toward the MLP transcription, and adjusted to the equivalent activity. (−), no protein fraction. For 'a' probes, a base at +33 was labeled by kination. Positions of the TATA element and transcription initiation sites are indicated.

MBP core promoter (BP36/12) was also transcribed when brain TFIID was added. However, complementation of the TFIID depleted brain nuclear extract with liver TFIID yielded only trace amounts of MBP transcripts. Equivalent results were obtained if we used TFIID-depleted liver nuclear extract. Consequently, for the MBP core promoter, TFIID functions were apparently non-equivalent.

Using the same reconstituted system, we analyzed *in vitro* transcription of several promoters such as nervous system specific mouse neurofilament (NF) and liver specific human α-1-antitrypsin (AT) promoter and calculated the specificity index (see Materials and methods) (Figure 8B). Interestingly, under reaction conditions yielding similar amounts of MLP transcripts from extracts supplemented with either brain or liver TFIID, the NF promoter was preferentially transcribed by brain TFIID in addition to the MBP promoter (BP36/12), whereas the AT promoter was shown to be preferentially transcribed by liver TFIID.

Sequence requirement for TFIID-directed tissue-specific transcription

We have demonstrated that the TATA element is indispensable for MBP promoter function (Figures 2A and 3) (Tamura *et al.*, 1988). To investigate if the MBP TATA element was involved in tissue-specific transcription, two

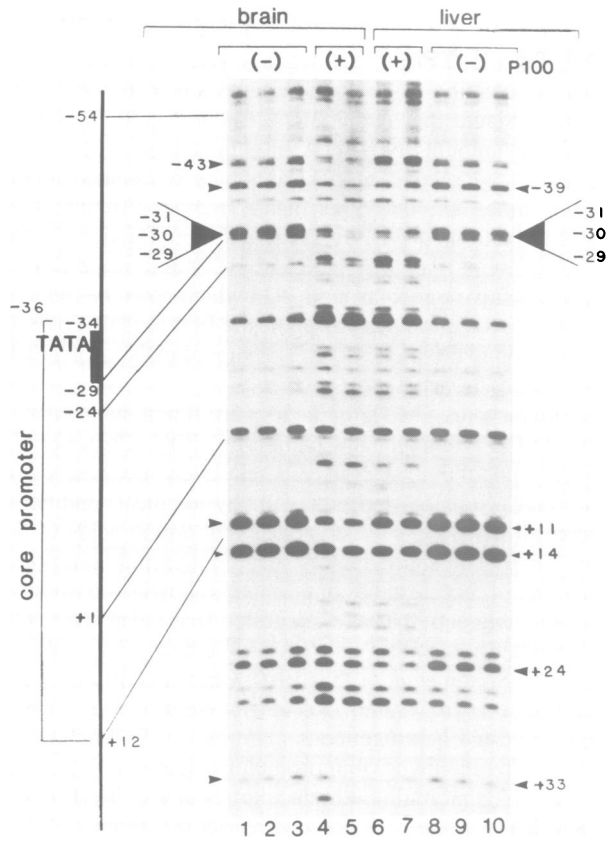

Fig. 7. P100 fractions contain a binding factor of the MBP TATA element. DNase I footprint experiments were carried out as described in Figure 6. The probe was labeled at +62 by kination and cut at −73. 6 μl (lanes 4 and 7) or 12 μl (lanes 5 and 6) of each P100 fraction was added. (−), no fraction. Positions and extents of the protections are indicated by arrowheads. Beside the autoradiogram, map positions of the core promoter and TATA element in the MBP promoter are shown schematically.

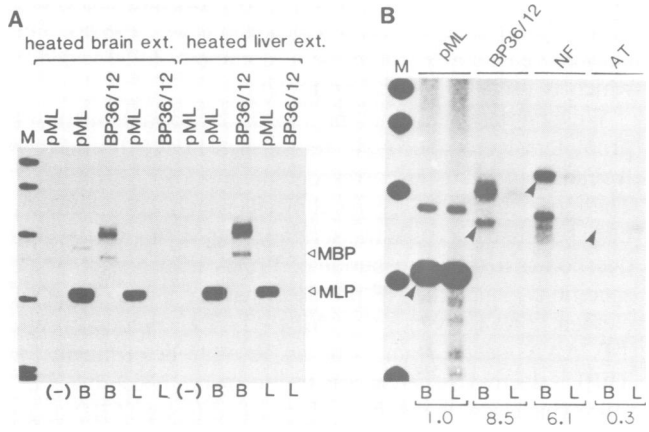

Fig. 8. Effect of partially purified TFIID on the MBP core promoter. (**A**) Heated brain or liver nuclear extract was used to transcribe pML and BP36/12 supplemented with brain (B) or liver (L) TFIID. M, pBR322/*Msp*I marker; (−), without TFIID fraction. (**B**) Experiment carried out as described above using the heated brain nuclear extract. NF331/72 (NF) and AT640 (AT) DNA (see Materials and methods) were also transcribed. The position of the specific signal is indicated by an arrowhead. The specificity index is shown under the panel.

chimeric promoters consisting of sequences of the MBP proximal promoter element and albumin promoter either from −35 to −19 (AL/BP) or from −18 to +10 (BP/AL)

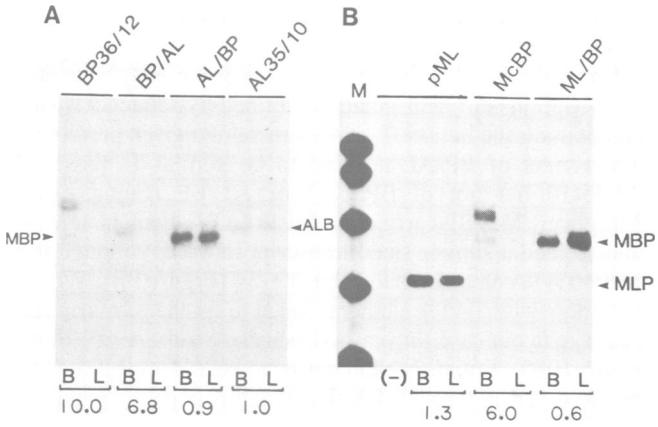

Fig. 9. Transcription of chimeric promoters by the reconstituted reaction system with brain (B) or liver (L) TFIID in the presence of heated brain nuclear extract. Specificity index is indicated under the panels. (**A**) Experiments were carried out as described in Figure 8. Configuration of the chimeric templates is indicated in Figure 1. Hybridization probes used for AL53/10 and BP36/12 were also used for BP/AL and AL/BP, respectively. (**B**) For McBP and ML/BP, transcripts from the MBP +1 site were analyzed. M, pBR322/*Msp*I size marker; (−), without TFIID fraction.

were constructed and transcribed in the reconstituted transcription system using both TFIID fractions (Figure 9A). BP/AL, an albumin promoter where the TATA element is substituted by the MBP TATA element, was preferentially transcribed by brain TFIID but not by liver TFIID. However, AL/BP, an MBP promoter (carrying the albumin TATA element instead of the MBP element) was transcribed by both TFIIDs, in a similar fashion to wild-type albumin core promoter AL35/10. We then constructed another chimeric promoter, ML/BP, that contained the MBP sequence from −19 to +12, and MLP TATA-carrying sequences from −36 to −20 (Figure 1). McBP was preferentially transcribed by brain TFIID, suggesting that the 2 bp substitution in the MBP core promoter did not affect the TFIID-directed tissue-specific transcription. ML/BP was equally transcribed by either brain or liver TFIID, indicating that the tissue specificity of this promoter was abolished. These results showed that tissue-specific transcription of the MBP core promoter was mainly regulated by a short DNA stretch carrying the TATA element.

Discussion

We previously demonstrated that the MBP proximal promoter from −53 to +62 was preferentially transcribed in brain nuclear extracts (Tamura *et al.*, 1989a). In the present study, we delimited the minimal MBP (which can be transcribed tissue specifically *in vitro*) core promoter from −36 to +12. Results of our present study suggest a mechanism in which the core promoter itself is involved in tissue-specific transcription. Our *in vitro* studies could demonstrate tissue-specific transcription from the mouse NF and human AT genes as well (Figure 8B) which also suggests that core promoter mediated tissue-specific transcription is not an unusual event. A previous report (Dierich *et al.*, 1987) indicated that core promoters such as chicken conalbumin and ovalbumin containing a TATA element but no upstream sequence are transcribed in a tissue-specific manner. Recently, Takiya *et al.* (1990) also reported core promoter

mediated tissue-specific *in vitro* transcription of the fibroin gene.

From the results shown in Figure 9 we supposed that transcription start sites could be defined by sequences with an initiation site but not TATA element of a given promoter. Transcripts of BP/AL seemed to be similar to those of AL35/10 but not of BP36/12 or AL/BP (Figure 9A). Moreover, ML/BP carried the MLP TATA element at −31 and yielded a similar sized transcript to McBP, though the MBP TATA element is located at −34 (Figure 9B). We do not know why TATA elements failed to fix the transcription start site in our case. In yeasts (Chen and Struhl, 1985; Hahn *et al.*, 1985), initiation elements have been demonstrated to be more critical than TATA elements for fixing the initiation site. Furthermore, it has been observed that TATA mutations did not always alter RNA start sites (Hen *et al.*, 1982; Dierks *et al.*, 1983); also, there are many TATA-less promoters. Thus we suggest that the MBP TATA element alone is not sufficient for fixing the transcription start site, and a region around the transcription start site may also participate in fixing the transcription start site. It is possible that the MBP core promoter possesses an initiation element 'initiator' as suggested by Smale and Baltimore (1989). The MBP downstream element may differ from a typical initiator since BP20/12 in the absence of a TATA element was not capable of accurately initiating transcription (Figures 2 and 3). However, we considered that the MBP initiation element within −20 to +12 appears to facilitate transcription directed by homologous (Figures 2 and 3) and heterologous (Figure 9A, AL/BP) TATA elements.

The TFIID has been characterized as one of the GTFs that bind to the TATA-containing proximal promoter (Buratowski *et al.*, 1988, 1989; Cavallini *et al.*, 1988; Nakajima *et al.*, 1988; Horikoshi *et al.*, 1989). We concluded that the phosphocellulose P100 fraction contained a TFIID factor for the following reasons. First, the chromatographic profiles of tissue-derived TFIID were quite similar to those of HeLa TFIID (Dignam *et al.*, 1983; Fire *et al.*, 1984; Moncollin *et al.*, 1986). Second, the P100 fraction contains a DNA binding factor for the MLP TATA element. Third, by using a HeLa GTF-directed reconstituted transcription system, the P100 fraction was interchangeable with HeLa TFIID. Fourth, TFIID in the P100 fraction was also heat sensitive like the HeLa factor (Figure 5).

Results shown in Figure 8A demonstrated that brain and liver TFIID were functionally non-equivalent in their capacity to stimulate transcription from the MBP core promoter, which suggests first that transcription from the MBP core promoter is tissue specific and second the presence of a tissue-specific factor for the MBP core promoter. Experiments using chimeric promoters (Figure 9) suggested that a tissue-specific element of the MBP core promoter was included in sequences between −35 and −19. Footprint experiments showed that only the TATA element was significantly protected within the core promoter. As described above, tissue-specific transcription was directed by the TFIID fraction. Furthermore, we found that TFIID-like activity was also heat labile in transcription of the MBP core promoter as a conventional TFIID (data not shown). Consequently, we may conclude that tissue-specific transcription from the MBP core promoter is governed via TFIID or TFIID-like activity. This idea will raise questions of how the TFIID molecule(s) exists in tissues and exhibits tissue specificity. We can imagine two possibilities. First, there are multiple/tissue-specific TFIID-like factors, and the MBP core promoter preferentially uses a brain-type TFIID-like factor. Second, TFIID is homogeneous in tissues, but some TFIID associating tissue-specific factors modify the TFIID activity. Since the liver P100 fraction still contained significant amounts of TATA binding factor of the MBP promoter, the second model cannot be eliminated at the present time. On the other hand, minor differences in footprint patterns driven by brain and liver fractions in the TATA element (bands at −30 and −29) and in the DNase I hypersensitive region around −24 which could be induced by TATA protection, may support the first model. To draw a conclusion to these questions, isolation of cDNAs of TFIID from tissues is necessary.

In prokaryotes, the sigma factors are functionally related to the TFIID (Helman and Chamberlin, 1988; Chater *et al.*, 1989). Importantly, bacterial cells selectively use these sigma factors for promoters depending on physiological conditions. Horikoshi *et al.* (1989b) suggested that amino acids of the yeast TFIID and sigma factors sheared a partial homology. A number of recent studies of promoters showing constitutive and inducible activity have suggested that eukaryotic cells may contain more than one TATA element factor (Wu *et al.*, 1987; Simon *et al.*, 1988, 1990; Chen and Struhl, 1988; Giangrande *et al.*, 1989). Recently, Wefald *et al.* (1990) have suggested heterogeneous mammalian TATA binding factors using the myoglobin promoter/enhancer. Therefore, it is possible that multiple molecular species of TFIID exist which differ in their capacity to recognize various TATA elements and their tissue-specific distribution. Our footprint results also support multiple TFIID-like factors since footprint patterns on the MBP and MLP promoter were different (Figures 6 and 7) as reported by Nakajima *et al.* (1988). Although cloning studies of the yeast TFIID demonstrated only one cDNA sequence (Cavallini *et al.*, 1989; Hahn *et al.*, 1989; Horikoshi *et al.*, 1989b), it must be noted that the yeast TFIID factor has been isolated on the basis of its activity towards the MLP (Buratowski *et al.*, 1988; Cavallini *et al.*, 1988; Horikoshi *et al.*, 1989a). The isolated yeast TFIID might be a 'common' TFIID species which is used by several promoters.

We demonstrated that the mouse NF and human AT promoters were selectively transcribed by TFIID derived from tissues where the corresponding promoter is expressed. These results indicate a general mechanism of core promoter mediated tissue-specific transcription, but do not address the question as to the contribution of TATA sequences to tissue specificity, since the NF promoter has a consensus TATA element (TATAAA). The MBP TATA element itself, TTCAAA, seemed not to be an absolute determinant for TFIID selective tissue-specific transcription. Nakajima *et al.* (1988) have shown that partially purified HeLa TFIID using MLP TATA element exhibited different DNase I footprinting patterns on the MLP and *hsp70* promoters, both of which have an identical TATA element but different TATA element flanking sequences. This indicates that TATA element flanking sequences are also important for tissue specificity.

Materials and methods

Plasmid DNAs

The mouse MBP gene (Takahashi *et al.*, 1985; Tamura *et al.*, 1988) with a proximal promoter from −53 to +62, BP53/62, was inserted into the *Eco*RI and *Hin*dIII sites of pBR322. BP53AT/62 contains base substitutions

at −34 (T to C), −31 (A to G) and −29 (A to G). McBP is also a base substitution mutant derived from BP36/12 (Figure 1). Oligonucleotide-directed *in vitro* mutagenesis was carried out as described previously (Tamura *et al.*, 1988). AL35/1 and AL35/10 are mouse albumin promoters (Gorski *et al.*, 1986) carrying sequences from −35 to +1 and from −35 to +10, respectively. These promoter sequences are flanked by the *Eco*RI (upstream) and *Hin*dIII (downstream) sites of pBR322. The adenovirus major later promoter (pML) from −34 (*Eco*RI) to +33 (*Bam*HI) was inserted into pBR322. The mouse NF promoter (NF331/72) was inserted into the *Hin*dIII site of pBR322. The human AT (AT640) DNA (Monaci *et al.*, 1988) (a kind gift from Dr R.Cortese) carrying the G-free cassette was also used as a template. Deletion mutants shown in Figure 3 and chimeric promoters shown in Figure 9 were generated by using synthetic oligonucleotides.

Preparation of nuclear extracts and in vitro transcription

Nuclear extracts from mouse whole brain and rat liver were prepared as described previously (Tamura *et al.*, 1989b). *In vitro* transcription was carried out for 45 min at 30°C. The preincubation mixture contained 8 μl of extract (10 mg of protein per ml), 4 μl of dialysis buffer (Tamura *et al.*, 1989b) and 1.5 μl of template DNA (300 ng/μl). After preincubation for 10 min on ice, 6.5 μl of a mixture containing 50 mM HEPES−KOH, pH 7.6, 9% glycerol, 80 mM KCl, 18 mM $MgCl_2$, 20 U RNase inhibitor, 1.18 mM CTP, 2 mM ATP, 2 mM UTP, 2 mM GTP and 8 μCi of [α-^{32}P]CTP was added to start transcription. The RNA transcripts were then purified by phenol−chloroform extraction and ethanol precipitation. Transcripts were analyzed by a modified S1 nuclease mapping procedure (Tamura *et al.*, 1989b) using single-stranded DNA probe. S1 resistant RNA was analyzed through a 5% sequencing gel. Probe DNAs for the MBP, albumin and their derivatives contain sequences from the *Eco*RI to *Bam*HI of a corresponding template. For pML and NF331/72, the probe sequences contain bases from *Eco*RI to *Sal*I and from *Eco*RI to *Bam*HI, respectively. G-free cassette transcription was carried out as described previously (Tamura *et al.*, 1989b).

Purification of tissue-derived TFIID

Nuclear extract (15 ml) was fractionated by chromatography on DEAE−cellulose (DE52, Whatman). Nuclear extracts were dialyzed against buffer A (20 mM Tris−HCl, pH 7.6, 5% glyerol, 0.2 mM EDTA and 1 mM DTT) plus 50 mM KCl. The DEAE−cellulose column (bed volume, 25 ml) was also equilibrated with the same buffer. Dialyzed nuclear extract was loaded onto the column, which was then washed with buffer A containing 50 mM KCl, and eluted with a linear 0.05−1 M KCl gradient in buffer A (100 ml). Each fraction was assayed for TFIID activity, and peak fractions of the activity were pooled and dialyzed against buffer A containing 50 mM KCl. The dialyzed sample was loaded onto a phosphocellulose column (P11, Whatman; bed column, 20 ml) equilibrated with buffer A containing 50 mM KCl. After loading the sample and washing the column, proteins were eluted with 150 mM, 300 mM, 500 mM and 1 M KCl in buffer A. Peak fractions of each salt elution were pooled, dialyzed with buffer A containing 50 mM KCl and stored at −80°C.

Purification of HeLa general transcription factors and pol II

HeLa GTFs were obtained as previously described (Dignam *et al.*, 1983; Watanabe *et al.*, 1988). Briefly, HeLa cell nuclear extracts were fractionated by a heparin−Sepharose column and collected in a 50−600 mM KCl portion, which was then applied to a DEAE−cellulose column to obtain 50−250 mM KCl fractions. The DEAE−cellulose fractions were used for purification of TFIID, -E and -B. The pol II was prepared from nuclear pellets of HeLa cells as reported previously (Reinberg and Roeder, 1987). Each fraction was confirmed not to be detectably contaminated by other GTFs.

DNase I footprint

A DNase I footprint experiment was carried out as previously described (Tamura *et al.*, 1988). 20 μl of the reaction mixture contains 2 ng of ^{32}P-labeled probe (2 × 10^4 c.p.m. by kination), buffer C (Tamura *et al.*, 1988), 200 ng of *Escherichia coli* DNA and various amounts of the P100 fraction. Binding reaction was carried out at 30°C for 15 min. DNA digests were analyzed on 8% polyacrylamide−8.3 M urea gels.

Reconstituted in vitro transcription

In vitro transcription using heated and TFIID-depleted nuclear extracts and partially purified TFIID was carried out as previously reported (Nakajima *et al.*, 1988). Heat treatment of nuclear extracts was performed by incubating 300 μl portions of extract in 1.5 ml Eppendorf tubes at 47°C for 15 min. The extract was then frozen and stored at −80°C. The transcription competent reconstituted reaction mixture consisted of 6 μl of heated extract and 1−4 μl of TFIID fraction. Other conditions for transcription and S1 nuclease mapping were standard protocols as described above. Specificity index was designated as a ratio of brain TFIID directed transcript to liver TFIID directed transcript. Specific transcripts were determined by densitometry.

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