Two Alternative Promoters Direct Neuron-Specific Expression of the Rat Microtubule-Associated Protein 1B Gene

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Microtubule-associated protein 1B (MAP1B) is a major constituent of the neuronal cytoskeleton that is expressed at high levels during early brain development and plays a role in axonal growth and neuronal plasticity. Previous studies suggested that the regulation of its gene expression is primarily at the transcriptional level. Thus, the characterization of the promoter region should help to define regulatory elements that control neuron-specific and developmental expression of the MAP1B gene. We have isolated genomic clones containing up to 11 kb of the upstream region of the rat MAP1B gene, sequenced ~1.8 kb upstream from the translation start codon, and identified several consensus sequences. These sequences include a consensus element common to several neuronal genes, a TCC repeat, a cAMP response element, and two TATA boxes that were 134 nucleotides apart from each other. S1 nuclease

and RNase protection assays identified two corresponding groups of transcription initiation sites that were used selectively in distinct regions of the nervous system and during different stages of development. Transient transfection assays with neuronal and non-neuronal cell lines demonstrated that each TATA sequence and its corresponding adjacent region could independently direct neuron-specific expression of a reporter gene. Furthermore, the transcription of the reporter gene was initiated from the same sites as those of the MAP1B gene *in vivo*. These results suggest that two alternative and overlapping promoters, one inducible and the other constitutive, regulate the temporal and tissue-specific expression of the rat MAP1B gene.

Key words: gene expression; alternative promoters; MAP1B; transcription; TATA box; transient transfection; cAMP-response element

Microtubule-associated protein 1B (MAP1B) is an abundant, high molecular weight (320,000) neuronal protein that is developmentally regulated (Bloom et al., 1984, 1985). Structural analysis of MAP1B indicates that the protein has an extended filamentous structure (Sato-Yoshitake et al., 1989) with an amino-terminal microtubule-binding domain (Noble et al., 1989). The phosphorylated isoform of MAP1B is highly enriched in growing axons of the developing nervous system but is usually present at relatively low levels in mature axons (Mansfield et al., 1992; Black et al., 1994; Boyne et al., 1994). In regions of adult brain that retain a capacity for synaptic remodeling, such as the olfactory bulb and retina, the levels of MAP1B remain relatively high and are not downregulated (Safaei and Fischer, 1989; Tucker and Matus, 1988). Furthermore, adult sensory neurons in dorsal root ganglia (DRG) and motor neurons in spinal cord express MAP1B at relatively high levels (Fawcett et al., 1994; Nothias et al., 1995), reflecting their potential to regenerate. Recent experiments with antisense oligonucleotides in PC12 cells (Brugg et al., 1993) and hippocampal neurons (L. Boyne and I. Fischer, unpublished data) resulted in the inhibition of neurite outgrowth. Taken together, these studies strongly support a role for MAP1B in axonal outgrowth.

MAP1B is the earliest MAP expressed during nervous system development. In general, the protein is abundant early in devel-

opment and at low levels in the adult, although the precise profile of MAP1B expression is region-specific (Safaei and Fischer, 1989; Schoenfeld et al., 1989). The parallel decrease in the levels of MAP1B and its mRNA during development suggests that the expression of MAP1B is regulated mainly at the level of transcription (Safaei and Fischer, 1989; Perrone-Bizzozero et al., 1991). In cell culture systems such as PC12 cells, both protein and mRNA levels of the MAP1B are induced in parallel after NGF treatment (Noble et al., 1989; Brugg and Matus, 1988); however, little information is available on the mechanism or regulatory elements involved in the regulation of MAP1B gene expression.

In this report, we describe the isolation and characterization of the MAP1B promoter region. In contrast to several other neuron-specific genes in which neuronal specificity is conferred by distant silencer elements acting on a relatively promiscuous promoter, we show that 127 bp of the MAP1B proximal promoter are sufficient to confer strong neuron-specific expression of a reporter gene in transient transfection assays. We also describe two TATA box sequences that are 134 nucleotides (nt) apart from each other in the promoter region, each of which in association with its adjacent *cis*-regulatory elements is sufficient for initiating transcription of a reporter gene at the same sites at which transcription of the MAP1B gene is initiated *in vivo* and for conferring neuron-specific expression. *In vivo*, these two adjacent promoters are used selectively in different regions of the nervous system and at different stages of development, making MAP1B a distinct example of a neuronal gene that can be controlled by positive regulatory elements and alternative promoters.

MATERIALS AND METHODS

PCR amplification of rat MAP1B cDNA clones. Three primers, designated as p1 (5'-AGAGGAACACTTCTCTCAGGCTTG-3', sense, nt 15–38),

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p2 (5'-CACCAGCAAGTAGAACT TGCTGTC-3', antisense, nt 177–154), and p3 (5'-TGAGCTCGCCAGTGTCTCAAAGC-3', antisense, nt 504–481), respectively, derived from the 5' region of the mouse MAP1B cDNA (Noble et al., 1989) were synthesized. Reverse transcription was performed using total RNA from rat brain. The resulting cDNA product was used subsequently for PCR amplification. Two overlapping cDNA fragments of 162 and 490 bp were amplified using p1/p2 and p1/p3, respectively, and subcloned into pBluescript II (Stratagene, La Jolla, CA). These clones were designated as pBS162 and pBS490, respectively.

Screening of a genomic DNA library. A Lambda DASH II library prepared from Sprague–Dawley male rat testis (Stratagene) was screened with the 490 bp cDNA probe under high stringency hybridization conditions. Approximately 500,000 independent clones were screened, and 12 positive clones were isolated. Phage DNA was prepared from these clones as described (Sambrook et al., 1989), digested with different restriction enzymes (Promega, Madison, WI), and analyzed by Southern blots using the 162 and 490 bp fragments. Three fragments positive to both probes, a 2.3 kb *EcoRI*, a 2.5 kb *HindIII*, and a 2.3 kb *XbaI* fragment, were identified and subcloned into pBluescript II or pUC18 vectors. The three resulting clones, pBS-2.3R, pUC-2.5 Hr, and pUC-2.3X, were used for further analysis.

Linker-dependent genomic walking. An alternative method, the PCR-based linker-dependent genomic walking procedure, was used simultaneously to isolate the MAP1B promoter region (Fors et al., 1990). Primers used in the genomic walking experiment were p4 (5'-TAGGAAGCGGTGCGACAGGCTGG-3', antisense, nt 159–137), p5 (5'-GGTGGCGATGCTGCCGATGGCTC-3', antisense, nt 118–94), and p6 (5'-ATGGTCCGGCTCGGTGGCTTCCAC-3', antisense, nt 100–76). Genomic DNA from rat brain first was sheared mechanically using a 22 gauge needle and then digested with a mixture of *BglII*, *XbaI*, and *BamHI* (Promega) overnight. First-strand DNA was synthesized by annealing p4 with digested genomic DNA and extended with Vent DNA polymerase (New England Biolabs, Beverly, MA) at 95°C for 2 min, at 60°C for 30 min, and at 76°C for 10 min using a thermocycler. The resulting products were ligated with a unique linker DNA fragment (LMPCR1, 5'-GCGGTGACCCGGGAGATCTGAATTC-3'; LMPCR2, 5'-GAATTCAGATC-3'). The annealed product contained one blunt end and a single-stranded region at the other end. The ligated products were used for a first round PCR amplification with primers p5 and LMPCR1. PCR products were separated on a 1% agarose gel, transferred onto a nylon membrane, and hybridized with the radiolabeled 490 bp MAP1B cDNA probe. Positive bands were purified and used as templates for a second round of PCR amplification. PCR reaction conditions were the same as above except that p6 was used instead of p5.

Preparation of plasmid constructs. PCR was performed to generate the promoter sequence construct in plasmid p(–549/+60)chloramphenicol acetyltransferase (CAT), using DNA from plasmid pBS-2.3R as a template. The 5' primer corresponded to the T7 promoter sequence in the pBluescript II vector. The 3' primer was a 24-mer oligonucleotide (5'-GCTCTAGACCTGCCGGCTCTGCTA-3') located immediately upstream from the translation start codon ATG of the MAP1B gene containing an *XbaI* site at its 5' end for convenient subsequent manipulation. The amplified DNA fragment was digested with *HindIII* (at the polylinker region of pBluescript II) and *XbaI* and ligated into pOCAT (pCAT/basic, Promega). Plasmid p(–383/+60)CAT was prepared by digesting p(–549/+60)CAT with *HindIII* and *AvaI*, blunt-ending with the Klenow fragment, and self-ligating. Plasmids p(–283/+55)CAT and p(+55/–283)CAT were prepared by ligating a 338 bp *NaeI* fragment, in both orientations, into pOCAT digested with *PstI* and blunt-ends generated with the Klenow fragment. Plasmid p(–1610/+60)CAT was prepared by ligating a 1.1 kb *HindIII/BstXI* fragment from pUC-2.3X with p(–549/+60)CAT digested with *HindIII* and *BstXI*. Plasmid p(–1717/+60)CAT was prepared by ligating a 1.2 kb *HindIII/BstXI* fragment from pUC-2.5 Hr into p(–549/+60)CAT digested with *HindIII* and *BstXI*. Plasmids p(–1610/–284)CAT and p(–549/–284)CAT were prepared by deleting the 338 bp *NaeI* fragment from p(–1610/+60)CAT and p(–549/+60)CAT, respectively. p(–549/–68)CAT and p(–283/–68)CAT were prepared by deleting a *BssH II/XbaI* fragment from p(–549/+60)CAT and p(–283/+55)CAT, respectively. Plasmid p(–72/+55)CAT was prepared by deleting a *HindIII/BssH II* fragment from p(–283/+55)CAT. Plasmid p(+55/–72)CAT was prepared by deleting a *HindIII/BssH II* fragment from p(+55/–283)CAT. Plasmid p(–68/–283)CAT was prepared by deleting a *BssH II/XbaI* fragment from p(+55/–283)CAT. Plasmids p(–116/+60)CAT, p(–163/+60)CAT, p(–817/+60)CAT, p(–1165/+60)CAT, and p(–1356/+60)CAT were prepared by *ExoIII*

deletion of p(–1610/+60)CAT. Briefly, p(–1610/+60)CAT was first digested with *BbuI* (*SphI*) and *SalI* and treated with *ExoIII* (Promega) at 30°C. Aliquots were taken at different time points after deletion with *ExoIII*, treated with S1 nuclease for 30 min at room temperature, blunt-ended with the Klenow fragment, and ligated with T4 DNA ligase. All constructs were verified by restriction enzyme digestion and sequence analysis.

Analysis and sequencing of genomic clones. Both strands of the promoter region were sequenced by dideoxynucleotide chain termination method (Sanger et al., 1977) using a sequenase II kit (U.S. Biochemical, Cleveland, OH). First, the coding strand was sequenced by using the sequential deletion products of pCAT constructs as templates and the reverse primer (5'-CAGGAAACAGCTATGAC-3'), whose sequence is present in the pCAT/basic vector. Then, primers complementary to the coding strand were synthesized and used to obtain the second-strand sequence. Nucleotide sequence alignments and analyses were performed with the software package of Genepro (Riverside Scientific Enterprises, Bainbridge Island, WA).

RNA preparation and S1 nuclease and RNase protection assays. Total RNA from cells or tissues was purified by the guanidinium isothiocyanate method (Chomczynski and Sacchi, 1987), except those RNAs from transfected NB2A cells, which were prepared by the CsCl centrifugation method (Glisin et al., 1973) after lysis with guanidinium isothiocyanate solution. For S1 nuclease protection assay, 10 pmol of pBS-2.3R DNA was denatured with NaOH, mixed with 5 pmol of primer p6 (5'-ATGGCTCCGGCTCGGTGGCTTCCAC-3', antisense, nt +100 to +76), and end-labeled with α -³²P-ATP. The mixture was heated at 85°C for 5 min, annealed by cooling slowly to 40°C, and extended by four units of the Klenow fragment for 50 min at 37°C. After *PstI* digestion, the resulting probe was purified by electrophoresis on a 8.3 M urea/6% polyacrylamide gel. The single-strand end-labeled probe that was generated spanned residue –264 to +100. Approximately 1×10^4 cpm of single-stranded probe was mixed with 50 μ g of total RNA from postnatal day 16 rat brain or 50 μ g of yeast tRNA. After hybridization, 300 μ l of S1 nuclease buffer (0.28 M NaCl/50 mM NaOAc, pH 4.5/4.5 mM ZnSO₄ with salmon sperm DNA at 20 mg/ml) and 50 units of S1 nuclease (Promega) were added, and the mixture was incubated at 37°C for 30 min. The protected fragments were electrophoresed on an 8.3 M urea/6% polyacrylamide gel.

RNase protection assays were performed according to standard procedure (Chamberlin et al., 1982). The pGEM-T582 plasmid, containing 5' flanking region and part of the first exon of MAP1B, was linearized with *AvaI* and used as template for riboprobe synthesis. The resulting riboprobe spanned residue –383 to +100 of the MAP1B promoter region and exon 1, as well as 52 nt from the pGEM-T vector sequence. The specific activity of the labeled riboprobes was estimated to be $0.5\text{--}1 \times 10^8$ cpm/ μ g. Approximately 1×10^5 cpm of the radiolabeled riboprobe was used for each hybridization reaction. After hybridization, 300 μ l of RNase digestion buffer [10 mM Tris-HCl, pH 7.5, 5 mM EDTA, 300 mM NaCl, 4 μ g of RNase A, and 80 units of RNase T1 (Ambion, Austin, TX)] were added, and the samples were incubated at 37°C for 30 min. Then, 35 μ l of 10% SDS and 2.5 μ l of proteinase K (80 mg/ml) were added and incubated for 30 more min. The protected fragments were electrophoresed in 8.3 M urea/6% polyacrylamide gel.

Cell culture and DNA transfections. PC12 cells (Greene and Tischler, 1976) were grown in DMEM supplemented with 5% fetal calf serum and 5% horse serum. NB2A and L6 cells were grown in DMEM containing 10% fetal calf serum; 3T3 cells were grown in DMEM containing 10% calf serum. All cells were maintained in a humidified 37°C incubator with a 5% CO₂/95% air atmosphere. Tissue culture serum, antibiotics, and medium were purchased from Gibco Life Technologies (Gaithersburg, MD), and tissue culture dishes and plates were from Falcon. All tissue culture medium contained 100 U/ml penicillin G and 100 μ g/ml streptomycin.

For transfections, PC12 cells were plated on polylysine-coated 12-well tissue culture plates at a density of 2×10^5 cells per well and grown for 16–18 hr. NB2A and 3T3 cells were treated similarly except that they were plated at a density of 5×10^4 cells per well. L6 cells were plated on a 6-well plate at a density of 5×10^4 per well. Transfections were carried out in a 12-well plate using 4 μ l of Lipofectamine (2 mg/ml; Gibco Life Technologies), 0.5 μ g of pOCAT DNA, or an equimolar amount of other plasmid constructs and pSVCAT in 0.6 ml Opti-Mem medium (Gibco Life Technologies). In addition, 0.5 μ g of pSV- β -gal DNA were cotransfected in each well. Duplicate wells were used for each sample. Four hours later, 0.6 ml of complete medium with double concentrations of

serum was added in each well. Cells were harvested for CAT and β -galactosidase assays 48 hr later.

CAT assays and quantitation. CAT activity was determined by the liquid scintillation counting method (Seed and Sheen, 1988). Briefly, cell extracts were prepared by rapid freezing/thawing cycles on dry ice. Depending on cell types, from one eighth to half of the cell extracts were used for CAT assay. Cell extracts were incubated with 0.25 μ Ci of 3 H-chloramphenicol and *n*-butyryl coenzyme A in a total volume of 125 μ l at 37°C for 60 min. After incubation, samples were extracted with 300 μ l of mixed xylenes. Because each pCAT construct was cotransfected with pSV- β -gal, β -galactosidase activity was used to normalize any transfection variations. CAT activity results, normalized against β -galactosidase activity, were expressed as the percentage of control pSVCAT (pCAT/control, Promega) activity.

RESULTS

Isolation and sequencing of the 5' end of rat MAP1B cDNA

Two overlapping cDNA fragments of 162 and 490 bp were amplified using p1/p2 and p1/p3, respectively, subcloned into pBlue-script II, and sequenced. These clones were designated as pBS162 and pBS490, respectively. The sequence of these clones (Liu and Fischer, 1996; Genbank accession number U55276) corresponds to the missing 5' end of rat cDNA reported previously (Zauner et al., 1992; Genbank accession number X60550). The overlapping combination of these cDNA sequences represents the entire rat MAP1B cDNA coding sequence. This sequence information, together with the determination of the MAP1B transcription initiation sites (see below), confirms that the first ATG codon identified by Noble et al. (1989) is the actual MAP1B translation start site, because no other potential upstream site could be identified. A comparison of the MAP1B 5' end cDNA sequence among different species shows that this region is highly conserved. At the amino acid level, the rat MAP1B sequence exhibits 96% and 95% identity, respectively, to its mouse and human counterparts.

Selection and restriction mapping of the rat MAP1B genomic clones

Screening of the genomic phage library with the 490 bp cDNA probe under high stringency conditions (see Materials and Methods) yielded 12 positive clones. Southern blot analysis confirmed that all of these clones contained restriction fragment(s) that hybridized with the 162 or 490 bp cDNA probes (data not shown). Three of these positive clones (10a, 1a, and 8a) were used to obtain a partial restriction map of the genomic DNA (Fig. 1). The results indicated that the 10a clone contained up to 11 kb of the 5' end of the gene, including the first exon and part of the first intron. Phage clone 8a contained the first exon, the first intron, and at least part of the second exon. The first exon/intron junction was determined by comparing the genomic DNA sequence with the cDNA sequence. The consensus exon/intron junction sequence (A/C)AG/GT(A/G)AGT (Breathnach and Chambon, 1981) matched at six out of nine nt in the region from +242 to +250, including a conserved G at residue 244 and the invariable GT at positions +245 and +246. The first MAP1B exon/intron junction in rat was located at the same site as it is in humans (Lien et al., 1994). The size of the first intron was determined to be >5.3 kb but <10.5 kb, which was comparable with 6.6 kb of the first intron in human MAP1B. The three restriction fragments, 2.3 kb *Eco*RI, 2.5 kb *Hind*III, and 2.3 kb *Xba*I, which contained exon 1 and up to 1.8 kb of the 5' end of the gene (Fig. 1), were subcloned into the pBlue-script II or pUC18 vectors to generate plasmids pBS-2.3R, pUC-2.5 Hr, and pUC-2.3X.

Figure 2 shows the results of the genomic walking analysis. After a first-round PCR amplification, no distinct bands were

Figure 1. Restriction map and exon/intron organization of MAP1B genomic clones. *A*, Phage clones 10a, 1a, and 8a were isolated by screening a phage λ Dash II genomic library. *B*, Restriction map and exon/intron organization of MAP1B genomic clones. The first exon/intron junction was determined by comparing the genomic DNA sequence with the cDNA sequence. The exact location and size of exon 2 were not determined. Exons are indicated by thick lines. *C*, Phage DNA was digested with *Eco*RI (*R*), *Hind*III (*H*), and *Xba*I (*X*), and the fragments were cloned in pUC18 or pBS II SK (–) plasmids.

detected by ethidium bromide staining (Fig. 2*B*), probably attributable to the low degree of specificity of this first step. After Southern blot analysis, however, bands were observed (Fig. 2*C*), suggesting that specific products containing the MAP1B promoter region had been amplified. Indeed, after purifying these bands from the gel and using them as templates for a second round of PCR amplification, two distinct PCR products of 495 and 582 bp were generated (Fig. 2*D*), which also hybridized with the 490 bp probe used in the library screening (Fig. 2*E*). These PCR products were subcloned into the pGEM-T vector (Promega) and designated as pGEM-T495 and pGEM-T582. These fragments corresponded to residues –395 to +100 and –482 to +100, respectively. Therefore, both the genomic library screening and the genomic walking methods yielded similar results and successfully generated the 5' flanking region of the MAP1B gene.

Sequence analysis of the 5' flanking region of the rat MAP1B gene

Approximately 1.8 kb of genomic DNA, upstream from the translation start codon, were sequenced (Fig. 3). The numbering system used was based on a proposed transcription initiation site (see below) defined as +1. Several consensus regulatory sequences were found in this region, including a cAMP responsive element sequence (CREB) located at –471, two TATA boxes at –18 and –152, an Sp1 element at –46, a fat-specific element 2 (FSE2) at +7, and a Pu-box at +23. No CAAT box was found on either strand. A sequence comparison between the promoter region of rat and human MAP1B (Fig. 4) showed high homology and suggested the presence of similar regulatory elements for the expression of this protein.

Sequence analysis revealed several other motifs commonly found in promoters (Fig. 3), including a “neuronal element” at position –238 to –212. This sequence motif is shared by several other genes specifically expressed in neurons, including GAP-43 (Nedivi et al., 1992), type II sodium channel (Maue et al., 1990), peripherin (Thompson and Ziff, 1989), rat SCG10 (Mori et al., 1992), and mouse neurofilament (Lewis and Cowan, 1986) (Table 1). A second common motif was a stretch of imperfect TCC repeats located at position –197 to –175 (Fig. 3). This motif has

Figure 2. Isolation of MAP1B promoter region using the linker-dependent genomic walking method. Primers and their relative positions are shown schematically in *A*. Rat genomic DNA was digested with a mixture of *Bgl*II, *Xba*I, and *Bam*HI, annealed with p4, extended with Klenow fragment, and then ligated with the unique linkers LMPCR1 and LMPCR2. First-round PCR amplification with primers p5 and LMPCR1 (*B*) and the corresponding Southern blot (*C*) show a smear with no clearly distinct bands in lane *S*. Second-round PCR amplification was performed using p6 and LMPCR1 as primers and first-round PCR products as templates (*D*) with the corresponding Southern blot (*E*). Lanes 1 and 2 in *D* and *E* show two distinct PCR products of 495 and 582 bp. *M* is a 100 bp DNA ladder.

AAGCTTATG CAGACCCCA GTGTGATAAG TCAGACATTG TCTTTAGTCA -1671
 CCACCACTGA TCACACTAGT GTAGTCTTTG GCTGCTCACC GAGTATCGCC -1621
 TATCCAGGCT TCTAGAAAGA ATGGGAGAAA AGGCCTCCAA GTAAATCATG -1571
 GGGGTCAACC CCTCAGACAC TGGGGTTGGG TTGGTTGGTT GGCTGGTTT -1521
 GTTTTAAATCG GGGTCTTACA TATTCAGGCT GATCCTGAAC TCATCAGCTA -1471
 AGAATGGCTT TGGACTTCTG ATCCCTCCITC TGCACGGCCT GAGTGTCTGG -1421
 ACTGCACTGG CCAGGGGTTT TGGTGTCTGG ATGGGCATGA AGTCTATCAG -1371
 CTGAGCCACA ACGCCTCCAG TTGGTATTTT AAAACCGGTA TATATCCAG -1321
 TGTAAAGGT ATATGGACTA ACATATACTA AACTATTATT TAGTATGTTA -1271
 TATTTAGCAT AAATTAAGAG TAGTATATAC CCCAATATTA GTTATATCAG -1221
 ATGAAGATAG AGGGCTATTG TTCCCTATTCC ATCTATTTT CTCCTTAAAA -1171
 TGACCCGTTT TTTATGATGA CGAGGTGTTT GCCGTCTCTC CTGCGCTTAA -1121
 TACAGAAATG TATTCATTAG ATTTGTAGGG AATGAATGTT TACGAGGGCC -1071
 TTTAGAAACA TTATTATGAG TTCTTCTAAA TATAGACAAA GGCGATAAAA -1021
 ACTGGCCAGC CTACGGGTAT CTTAAAGAAC ATCATTTGTA CGCAAAATAA -971
 TTTTGCCTGT GTGTTCCTCA GGAAGCTGAA AAGCACATTA TAGTCACTGT -921
 AATCCTGCAG GGCAGAGACA GTATCTTCTC TATCTTTTGT TATGGCTGAT -871
 GCTATCTGTT CCGCATATTA AGAAAAGAAA TCTGTATCTA GCATAATATG -821
 TGTGCTCTT CTATCCATT TCCCATGAG ACCAAAGGCC AGTTCTTTTC -771
 AGCAGTACTC CTTCAGACT AGAATCCAAA GTCAGGACAA ATTAGCGGTA -721
 GGTCTTGCAA AGTCTCCTTT AAGCAAAGGT GATAAATATT CCTGTTTTTT -671
 TTTTTTTAA TGGACAAATA CGCCTTTCTA TATTTACAA GTCAAAAAGA -621
 CAGGTTTATG TCTTTGGGTG GGTGACCTC AACCACGCGT CCACCGTGAC -571
 TGCTGCAGCT ACTCAATTC TGAATTCACG TGGATGGGAT ACAGGCACAG -521
 GCCTGAAGAT GTGCAATCGG ACTCAGGACA TTCTCTGCT CTGAAGCAGG -471
 AGCTCATPCC CATCTCCGG CACAGCCAG TTGATGGTTT GGGGCAAGGA -421
 CREB
 AGCGTGCGT ATGTAAGGT GGTGGTGCAG AAGCCCTCG GGGCTGCAGC -371
 AGAGTCTTTT GGGTCCCCA GAGCCTGGCT CCTGGCCCTG CCCAGACCAT -321
 TGTCACACA CCTCGTCTT CCAGCTTCCG GGCCGCCGGC GAAATGATTT -271
 GCTGCAGATG ACATCAGCCC TGGGCTAAAG CTGGAGGAG TGGAGGACG -221
 neuronal motif
CACCACGAG ACGTGCCGGG TGGTCTCTTC CTCTCTCTCT TCCTCCAGG -171
 TCC repeat motif
 CTCTAGCCT AGTCTCCATA TAAACGGCAG CGCCTCCCG GCCTCTCACT -121
 TATA1
CCCCGCTCCT CTCTCCGCG CAGGAGAGAG GCGGAGGGGG AGGCGACGCG -71
 CGCCGGGAGG AGGGGGGACG CACTGGGCGG AGCGAGAGCA GCACCTTCGG -21
 Sp1
 AGATAATCTT TTCTCCTGCC CGAGGACGAG GGAAGCGCGG GAGAGGATCA +30
 TATA2 cap2 cap3 FSE2 Pu-box
 CTCTCCAG GCTTTAGCAG AGCCGGCAGG ATGGCGACCG TGGTGGTGA +80
 M A T V V V E
 AGCCACCGAG CCGGAGGCAT CGGGCAGCAT CGGCAACCCG GCGGCGATCA +130
 A T E P E P S G S I G N P A A T T
 CCTCGCCAG CCTGTGCGAC CGCTTCCTAG ACAGCAAGTT CTAATGCTG +180
 S P S L S H R F L D S K F Y L L
 GTGGTGGTCG GCGAGACGGT GACCGAAGAG CACCTGAGGC GTGCCATCGG +230
 V V V G E T V T E E H L R R A I G
 CAACATCGAG CTGGgttaagc cgtcccca +258
 N I E L

Figure 3. Sequence of the rat MAP1B promoter region, the first exon, and the 5' end of the first intron. The amino acid sequence encoded by the first exon is shown under the nt sequence in *single-letter code*. The 5' end of the first intron of the MAP1B gene is shown in *small letters*. The conserved initiator at nt -126 and the conserved nucleotides in the exon/intron junction are *underlined*, and the invariable gt sequence is *bold* and *italic*. Three transcription initiation sites (cap sites) determined by RNase and S1 nuclease protection assays are *bold* and labeled as *cap 1*, *cap 2*, and *cap 3*, respectively. The second transcription initiation site G is also *underlined* and designated as +1. The translation start codon ATG is *underlined*. Two TATA boxes are shown in *bold* and *boxed in dotted lines* and designated as *TATA1* and *TATA2*, respectively. An Sp1 (GGGCGG) element at nt -46, an FSE2 (CAGAGGA) at nt +7, a Pu-box (GAG-GAA) at nt +23, and a cAMP responsive element (CREB) (GACGTCA) at nt -471 are *boxed in dotted lines*. Two motifs, shown in *boxes*, are designated as neuronal element and TCC repeat motif, respectively. The Genbank accession number for the sequence is U55276.

been found in promoter regions of many growth factor and hormone receptor genes, such as human EGF receptor, human IGF-I receptor, human progesterone receptor, and human androgen receptor, as well as in promoter regions of many proto-oncogenes, such as chicken myb, human c-erbB-2, human c-k-ras, mouse c-jun, and mouse c-myc proto-oncogenes. In the promoter region of human epidermal growth factor receptor, the motif

RAT	AACGGCACCG	CCTCCCCGGC	--CTCTCACT	CCCCGCTCCT	-111
HUMAN	AAGCGCGCCG	CCTCCCCGGC	CTCTCTCACT	CCCCGCTCCT	-126
CON	AA--GC-CCG	CCTCCCCG-C	--CTCTCACT	CCCCGCTCCT	
RAT	CTCTTCCGCG	CA-----	-----GGGAG	AGAGCGGAGG	-84
HUMAN	CTCCGCGCCG	CACCTCTCGG	GCGCTGGGAG	AGGGCGGAGG	-86
CON	CTC--CCGCG	CA-----	-----GGGAG	AG-GCGGAGG	
RAT	GGGAGGCGAC	GC---GCGCC	GGGAGGAGGG	GGGACGCGAGT	-47
HUMAN	GGGAGGCGGC	GCGCGCGGCC	--AGAGGAGGG	GGGACGCGAGG	-47
CON	GGGAGGCG-C	GC---GCGCC	--GAGGAGGG	GGGACGCGAG-	
			β-A-globin.4		
RAT	GGGCGGAGCG	GAGACAGCAC	CTTCGGAGAT	AATCCTTTCT	-7
HUMAN	GGGCGGAGCG	GAGACAGTAC	CTTCGGAGAT	AATCCTTTCT	-7
CON	GGGCGGAGCG	GAGACAG-AC	CTTCGGAGAT	AATCCTTTCT	
	Sp1 TATA				
RAT	CCTGCCGCGAG	AGCAGAGGAG	CGGCGGGAGA	GGAACACTTC	+34
HUMAN	CCTGCCGCGAG	TGGAGAGGAG	CGGCGGGAGC	GAGACACTTC	+34
CON	CCTGCCGCGAG	-G-AGAGGAG	CGGC-GGAG-	G--ACACTTC	
	+1 FSE2				
RAT	TCCAGGCTT	TAGCAG-AGC	CGGCAGG		+61
HUMAN	GCCGAGGC--	-A-CAGAGC	CGGCAGG		+57
CON	-CC-AGGC--	-A-CAG-AGC	CGGCAGG		

Figure 4. Sequence comparison of rat and human MAP1B promoter region. Rat MAP1B promoter region is aligned with the published human MAP1B promoter region (Lien et al., 1994). CON represents the consensus sequence between these two species. The overall homology in this region is 81%. The second transcription initiation site in rat and the transcription initiation site in human MAP1B are *bold* and designated as +1. Also *bold* are the TATA box, a β-A-globin.4 element, an Sp1 site, and an FSE2 element.

TCCTCCTCC has been found to be sensitive to S1 nuclease and to bind two specific factors. In addition, mutations in this motif decrease promoter activity by three- to fivefold, indicating its functional importance in regulating EGF receptor gene expression (Johnson et al., 1988).

Mapping of the MAP1B transcription initiation sites

S1 nuclease protection assay (see Materials and Methods) was carried out with 50 μg of total brain RNA (postnatal day 16) and 50 μg of yeast tRNA as negative control. Two strong bands of 100 and 97 nt were clearly observed after a relatively short exposure (Fig. 5A); however, a much weaker band of 224 nt was detected after a longer exposure (Fig. 5B), suggesting that transcription of the MAP1B gene might be initiated from a second site.

Table 1. The "neuronal motif" in promoter regions of neuronal genes

Rat MAP1B	-238 to -234	TGGAG
Rat MAP1B	-230 to -226	TGGAG
Rat MAP1B	-224 to -212	CAGCCACCACGAG
Rat GAP-43	-308 to -312	TGGAG
Rat GAP-43	-271 to -283	CAGCTGCCAGGAG
Rat type II Na ⁺ channel	-52 to -50	CCAGGAGATGGAG
Rat peripherin	-167 to -155	AGC CCAGGAGA
Rat peripherin	-72 to -68	TGGAG
Rat SCG10	-327 to -333	CCAGGAG
Mouse neurofilament	-78 to -62	AGC CCAGG TGGAG

The "neuronal motif" noted previously (Thompson and Ziff, 1989; Maue et al., 1990) is also present in rat MAP1B promoter region on the coding strand. This region is compared with the promoter regions of other neuron-specific genes. The numbers refer to the nt positions within the promoter region of each gene. This motif occurs on the noncoding strand of GAP-43 and SCG10 genes and therefore is inverted when compared with other genes. Nonconsecutive sequences from the same gene are set on different lines. Spaces represent unmatched bases.

Figure 5. Determination of MAP1B transcription initiation sites by S1 nuclease protection assay. Polyacrylamide gels (*A*, *B*) show the results of S1 nuclease protection assay. Approximately 50 μ g of rat brain total RNA (*S*) or control yeast tRNA (*C*) was hybridized with the 32 P-end-labeled, single-stranded DNA probe and then treated with S1 nuclease. Three protected fragments and their size (nt) are indicated by arrowheads, and the corresponding TATA boxes are shown above. The size of molecular weight markers (nt) is indicated to the left. *P*, Untreated probe; *M*, molecular weight marker; *L*, DNA sequence ladder obtained by using the same primer and template as for S1 probe preparation. A diagram (*D*) shows the structure of the single-stranded DNA probe used for S1 nuclease protection assay and the three protected fragments.

Figure 6. Determination of MAP1B gene transcription initiation sites by RNase protection assay. In the RNase protection assay (*A*), 20 μ g of total RNA from adult and postnatal day 4 rat DRG (*lanes 1, 2*), adult and postnatal day 4 rat brain cortex (*lanes 3, 4*), adult and postnatal day 4 rat liver (*lanes 5, 6*), PC12 (*lane 7*), NB2A (*lane 8*), 3T3 (*lane 9*), and L6 (*lane 10*) cells were hybridized with 32 P-labeled riboprobe spanning nt -383 to $+100$ in the MAP1B gene and 52 nt of the pGEM-T vector sequence, and then treated with a mixture of RNase A and RNase T1. The protected fragments and their size (nt) are indicated by arrowheads. The size of molecular weight markers (nt) is indicated to the left. A diagram (*B*) shows the structure of the MAP1B gene antisense riboprobe and the three protected fragments.

To examine whether these potential initiation sites could be used selectively in different regions of the nervous system or at different stages of development, we carried out additional RNase protection assays. Twenty micrograms of total RNA from DRG, brain cortex, and liver of both postnatal day 4 and adult rat as well as 20 μ g of total RNA from PC12, NB2A, 3T3, and L6 cells were used. A schematic representation of the riboprobe is shown in Figure 6*B*. Three protected bands with sizes of 224, 101, and 98 nt were detected (Fig. 6*A*) in neuronal tissues (DRG and brain cortex) and neuronal (PC12) cells, but not in liver or non-neuronal (3T3 and L6) cells. The two shorter bands of 54 and 52 nt, instead of the fully protected bands observed in NB2A mouse neuroblastoma cells, were probably attributable to an incomplete

protection of the rat probe by the mouse MAP1B transcripts. The exact transcription initiation sites were tentatively determined to be at an A residue and two G residues at 183, 60, and 57 nt upstream from the translation start codon ATG, respectively, because single-stranded RNA moves slower than the single-stranded DNA ladder, and G or A residues are used more commonly as a transcription initiation site (Bucher and Trifonov, 1986). The second transcription initiation site (G residue) was designated arbitrarily as $+1$ (Fig. 3). The upstream transcription initiation site (A residue) was located 29 nt downstream from the first TATA sequence, and the functional initiator CTCANTCT (Smale et al., 1990) was conserved at seven out of eight nt. The downstream transcription initiation sites were located 19 and 22

Figure 7. Rat MAP1B-CAT chimeric constructs. Diagrams of construct structure are aligned with a map of ~1.8 kb promoter region of the MAP1B gene and the first exon. Two TATA boxes, the translation start codon ATG, and a few restriction sites are shown, and the first exon is indicated as a solid, thicker line. The scale is shown below the map. The locations of the 5' and 3' end of each construct are indicated relative to the second transcription initiation site (+1). Each promoter construct is constructed in front of the reporter gene CAT in the orientation indicated by an arrowhead as shown for p(-1720/+60)CAT.

nt downstream from the second TATA sequence, respectively (Fig. 3). MAP1B transcripts were found only in neuronal tissues and cell lines consistent with previous observations on the protein and mRNA distributions (Rienitz et al., 1989; Safaei and Fischer, 1989); however, the transcription initiation sites were used selectively during development in different regions of the nervous system. For example, in brain cortex, the upstream initiation site was much stronger in neonate rat than in adult rat. This observation correlates well with the decreased expression of MAP1B during brain development. In contrast, in DRG sensory neurons, the upstream initiation site was much weaker in neonate rat than in adult rat, consistent with the relatively high levels of MAP1B seen in adult DRG. Little or no change was observed in the usage of the downstream transcription initiation sites during development.

Selective activity of the MAP1B promoter in neuron-derived cell lines

To better understand the function of the MAP1B promoter, we used a transient transfection system with CAT as a reporter gene. Different fragments of the MAP1B promoter region were ligated to a promoterless vector pCAT/basic (Promega) in front of the reporter gene. For some promoter fragments, both orientations were tested (Fig. 7). As a positive control, pSVCAT (pCAT/control, Promega) containing the SV40 promoter and enhancer sequences was used. Two neuron-derived cell lines (PC12, NB2A), which express MAP1B, and two non-neuronal cell lines

Table 2. CAT activity in transiently transfected cell lines

CAT gene constructs	CAT activity (%)			
	PC12	NB2A	3T3	L6
SV40 early promoter	100.0	100.0	100.0	100.0
No promoter (p0CAT)	2.9	2.8	4.8	0
MAP1B (-1610/+60)	180.1	83.3	5.6	18.0
MAP1B (-1610/-284)	0	0.5	2.7	0
MAP1B (-1356/+60)	218.4	ND	7.0	ND
MAP1B (-1165/+60)	238.9	84.5	7.4	10.0
MAP1B (-817/+60)	253.9	ND	7.2	ND
MAP1B (-549/+60)	167.1	162.0	6.2	14.0
MAP1B (-549/-68)	53.7	194.1	9.1	29.0
MAP1B (-549/-284)	32.8	9.7	4.3	4.7
MAP1B (-383/+60)	283.9	213.1	8.0	ND
MAP1B (-283/+55)	410.5	281.9	24.0	22.2
MAP1B (+55/-283)	ND	121.1	6.6	2.5
MAP1B (-163/+60)	232.6	80.8	5.0	6.2
MAP1B (-116/+60)	182.4	77.3	8.0	4.6
MAP1B (-72/+55)	210.4	144.8	12.6	7.0
MAP1B (+55/-72)	ND	31.8	3.9	ND
MAP1B (-283/-68)	215.2	442.5	16.4	52.0
MAP1B (-68/-283)	ND	99.6	5.3	ND

CAT activity was measured as described in Materials and Methods. The 5' and 3' nucleotides of each MAP1B-CAT construct as well as the orientation are indicated in parentheses (see Fig. 7). CAT activity in each cell line transfected with pSVCAT is assigned as 100%. All other values for CAT activity are expressed relative to this value. Several independent transfections with different DNA plasmid preparations were performed for most constructs in different cell lines. Results of a representative experiment are shown. All transfections were performed in duplicate; most duplicate values after normalization with β -galactosidase activity varied by <20%. The conversion rate of ^3H -chloramphenicol to ^3H -*n*-butyryl-chloramphenicol in all cell lines transfected by pSVCAT ranged from 8.8 to 46.7%. Same DNA preparations were used for transfection in all cell lines. ND, Not determined.

(L6 and 3T3), which do not express MAP1B (Fig. 6), were used for transfection experiments. Table 2 shows CAT activities in different cell lines transiently transfected with various CAT constructs. All constructs derived from 5' deletions had strong promoter activity in the neuron-derived cell lines but very weak, if any, promoter activity in the non-neuronal cells. The shortest construct tested, p(-72/+55)CAT, still retained a promoter activity comparable to that of the longest promoter construct p(-1610/+60)CAT. Surprisingly, the short construct also conferred significant neuron specificity.

Two TATA boxes and their adjacent *cis*-acting elements function independently in conferring neuron-selective expression of the reporter gene

Sequence analysis of the rat MAP1B promoter region indicated the presence of two TATA boxes that were 134 nt apart from each other. Furthermore, S1 nuclease and RNase protection assays identified one upstream and two downstream transcription initiation sites that were located within a consensus distance from the TATA sequences (Fig. 5 and 6), suggesting that both TATA boxes could function *in vivo* in regulating the initiation of MAP1B gene transcription by RNA polymerase II. To test this hypothesis, several MAP1B promoter-CAT fusion constructs were prepared by deleting one or both TATA boxes. For some constructs, the promoter sequence was subcloned in both orientations relative to the reporter gene. Transient transfections were performed with these constructs, and the results of the CAT activity experiments are shown in Table 2. For example the p(-283/+55)CAT construct that contained both TATA boxes showed high promoter

activity and neuronal specificity (Table 2). When both TATA boxes were removed by deletion of this fragment, most of the promoter activity was lost, as shown with p(–1610/–284)CAT and p(–549/–284)CAT. When only the upstream TATA box was deleted, most of the promoter activity and the neuronal specificity remained, as shown with p(–116/+60)CAT and p(–72/+55)CAT. Similar results were obtained when the second TATA box was deleted, as shown with p(–549/–68)CAT and p(–283/–68)CAT. Furthermore, the promoter activity was orientation-dependent for both TATA boxes, because most of the promoter activity was lost when the promoter sequences were positioned in the reverse orientation in front of the reporter gene, as shown with p(+55/–72)CAT and p(–68/–283)CAT. These results demonstrated that each TATA box and its associated *cis*-elements were able to function independently in regulating the expression of the reporter gene in a transient transfection assay.

MAP1B promoter initiates transcription of the reporter gene from the same MAP1B transcription initiation sites used *in vivo*

To obtain further evidence that the two TATA boxes in the MAP1B promoter region and their adjacent *cis*-elements functioned as independent promoters, RNase protection assays were performed using the same probe shown in Figure 6B, with RNA isolated from NB2A cells transfected with various MAP1B promoter-CAT constructs. This mouse neuronal cell line was chosen because its endogenous MAP1B transcripts only partially protected the riboprobe (Fig. 6A) and therefore could be distinguished from those transcripts of the reporter gene.

Four MAP1B promoter-CAT constructs were used to transfect NB2A cells (Fig. 7). Plasmids p(–1610/+60)CAT and p(–283/+55)CAT contained both TATA boxes, but the 3' ends of these two constructs differ from each other by 5 bp. Plasmids p(–72/+55)CAT and p(–283/–68)CAT contained only the downstream or upstream TATA box, respectively. Total RNA was isolated from NB2A cells 24 hr after transfection. RNAs isolated from NB2A cells transfected with p0CAT and untransfected NB2A cells were included as controls. The RNase protection assay results are shown in Figure 8. As expected, two major short fragments of 54 and 52 nt were observed in untransfected NB2A cells and in NB2A cells transfected with p0CAT (lanes 1 and 2, Fig. 8), representing endogenous mouse MAP1B transcripts. In NB2A cells transfected with p(–1610/+60)CAT, three additional protected fragments of 183, 60, and 57 nt in size were observed (lane 3, Fig. 8). Transfection with p(–283/+55)CAT resulted in three protected fragments of 178, 55, and 52 nt, owing to the fact that this plasmid was 5 bp shorter at its 3' end than p(–1610/+60)CAT (lane 4, Fig. 8). In contrast, NB2A cells transfected with p(–72/+55)CAT showed only the two shorter protected fragments (lane 5, Fig. 8A). The 52 nt protected band overlapped with the endogenous band in lanes 4 and 5 and was therefore more intense than the 54 nt protected fragment. In NB2A cells transfected with p(–283/–68), a construct containing only the upstream TATA box, a protected fragment of 55 nt was observed in addition to the endogenous transcripts (lane 6, Fig. 8). In lanes 3, 4, 5, and 6, the top bands of 443, 338, 127, and 215 nt, respectively, correspond to the riboprobe protected by trace amounts of contaminated plasmid DNA used for transfection in RNA samples. For example, the p(–1610/+60)CAT plasmid protected the riboprobe, which spanned residue –383 to +100, generating a fragment of 443 nt from nt –383 to +60, as observed in lane 3 (top band). Some extra minor bands could be background

Figure 8. Determination of transcription initiation sites of the reporter gene directed by different MAP1B promoter fragments. RNase protection assay was performed as described in Materials and Methods and in Figure 6 using the same riboprobe, 20 μ g of total RNA from untransfected NB2A cells (lane 1) and NB2A cells transfected with p0CAT (lane 2), p(–1610/+60)CAT (lane 3), p(–283/+55)CAT (lane 4), p(–72/+55)CAT (lane 5), and p(–283/–68)CAT (lane 6). The size of the riboprobe protected by endogenous MAP1B transcripts as well as new transcripts encoded by transfected DNA were determined by comparing with the adjacent sequencing ladder (A, G, C, T). Arrowheads indicate protected fragments in different lanes. The size of molecular weight markers (nt) is indicated to the left. M, Molecular weight marker.

from endogenous transcripts, as seen in lane 1. These results demonstrated that each TATA box and its associated *cis*-elements was able to direct initiation of the reporter gene transcription independently from the same transcription initiation site(s) as those used by MAP1B gene *in vivo*.

DISCUSSION

We have described the isolation of clones containing up to 11 kb of the upstream sequence of the rat MAP1B gene. Approximately 1.8 kb of the 5' flanking region from the MAP1B gene was sequenced and analyzed. This region is GC rich and contains several consensus elements common to promoters transcribed by RNA polymerase II. These motifs include two TATA boxes separated from each other by 134 bp. A short fragment of 127 bp of the promoter DNA containing the downstream TATA box is sufficient to direct neuron-specific transcription. Another unusual feature of this promoter is that two distinct groups of transcription initiation sites have been identified by S1 and RNase protection assays. Furthermore, each TATA box and its adjacent sequences can function independently in regulating the transcription of a reporter gene from the same sites used *in vivo*. These two alternative promoters are used differentially in distinct regions of the nervous system and during different stages of development.

Neuron specificity of the MAP1B promoter

Two major mechanisms for conferring neuron specificity have been observed through functional studies of neuron-specific promoters. The first mechanism includes a "silencer" protein that imposes neuron-selective expression on a relatively nonspecific core promoter, as exemplified by the recent identification of the neuron-restrictive silencer factor NRSF (Schoenherr and Anderson, 1995). This NRSF is expressed in non-neuronal cells and binds to the neuron-restrictive silencer element (NRSE) present within the promoter regions of at least 18 neuronal genes. Functional NRSEs have been identified in four of these neuronal genes: SCG10 (Mori et al., 1992), Na II channel (Kraner et al., 1992), synapsin I (Li et al., 1993), and neuronal Na⁺, K⁺-ATPase subunit (Pathak et al., 1994). The second mechanism is that a relatively short region (a few hundred base pairs) of the promoter contains the regulatory elements sufficient to confer neuron specificity. This has been reported for the GAP-43 gene, in which a small, 386 bp fragment containing the core GAP-43 promoter exhibits considerable tissue specificity (Nedivi et al., 1992). Similarly, our results indicate that a 127 bp fragment of the MAP1B promoter can confer neuron-specific expression, and this regulation can be accomplished by a relatively short positive element. It is clear, however, that because of the complexity of the nervous system, the expression of specific genes in different regions and in different types of neurons has to be modulated by the complex interactions of many regulatory elements to ensure neuron-specific expression. It has been proposed that the regulation of neuron-specific expression of a particular gene is related to the evolutionary origin of its tissue-specificity requirements (Nedivi et al., 1992). Thus, the "silencer" mode may have evolved in genes belonging to a multigene family, such as the SCG10, Na II channel, synapsin I, and Na⁺, K⁺-ATPase subunit genes that presumably evolved through duplication of an ancestral common gene, which had developed neuronal specificity at later stages by the addition of an upstream silencer to its promoter. In contrast, the mode of neuron specificity characterized by a short region of the individual gene promoter may apply to single genes such as GAP-43 (LaBate and Skene, 1989) and MAP1B (Lewis et al., 1986).

Significance of alternative promoters in regulating MAP1B gene expression during development

The synthesis of distinct mRNAs from a single gene by the alternative usage of promoters was first described in bacterio-

phage I by Reichardt and Kaiser (1971). A few examples have also been found in eukaryotes, including the α -amylase-1^A gene of the mouse (Hagenbuchle et al., 1981; Young et al., 1981; Schibler et al., 1983), the Discoidin-Ia gene of *Dictyostelium* (Jellinghaus et al., 1982), the alcohol dehydrogenase gene of *Drosophila melanogaster* (Benyajati et al., 1983), the rat T-kininogen gene (Sierra et al., 1989), the rat growth hormone-releasing hormone gene (Gonzalez-Crespo and Boronat, 1991), the human aminopeptidase N gene (Shapiro et al., 1991), and the rat brain-derived neurotrophic factor gene (Timmusk et al., 1993). It has been proposed that alternative promoters are commonly used to regulate the expression of a gene at different stages of development or in different cell types (Schibler and Sierra, 1987). The following observations support the notion that two alternative promoters regulate the transcription of MAP1B gene *in vivo*. (1) Two TATA sequences separated by 134 bp are present in the promoter region and located within the expected distance from two distinct groups of transcription initiation sites; (2) *in vivo*, these two groups of transcription initiation sites are used differentially in different regions of the nervous system during development; (3) *in vitro*, each TATA sequence and its adjacent promoter region can independently regulate the expression of a reporter gene in a neuron-specific manner, as determined by transient transfection assay; and (4) the DNA fragments from the MAP1B promoter region regulate the initiation of CAT gene transcription from the same sites used *in vivo*.

These observations emphasize the importance of the selective usage of alternative promoters in different developmental stages and in various regions of the nervous system. In the case of the MAP1B gene, this mechanism may relate to tissue-specific requirements for regulatory factors. One way differentiated cells could regulate the level of the MAP1B gene expression is by selecting the site, and therefore the rate, at which transcription and/or translation are initiated. It is also possible that the 5' nontranslated sequences may influence mRNA stability or may be involved in differential processing. We therefore propose that two alternative and overlapping promoters located adjacently regulate MAP1B gene expression during development. The upstream promoter is inducible during development, whereas the downstream promoter is constitutive and allows low levels of MAP1B gene expression in the adult nervous system.

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