The Neuronal Microtubule-Associated Protein 1B Is under Homeoprotein Transcriptional Control

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To identify genes regulated by homeoprotein transcription factors in postnatal neurons, the DNA-binding domain (homeodomain) of Engrailed homeoprotein was internalized into rat cerebellum neurons. The internalized homeodomain (EnHD) acts as a competitive inhibitor of Engrailed and of several homeoproteins (Mainguy et al., 2000). Analysis by differential display revealed that microtubule-associated protein 1B (MAP1B) mRNA is upregulated by EnHD. This upregulation does not require protein synthesis, suggesting a direct effect of the homeodomain on MAP1B transcription. The promoter region of MAP1B was cut into several subdomains, and each subdomain was tested for its ability to bind Engrailed and EnHD and to associate with Engrailed-containing cerebellum nuclear extracts. In addition, the activity, and regulation by Engrailed, of each subdomain and of the entire promoter were evaluated in vivo by electroporation in the chick embryo neural tube. These experiments demonstrate that MAP1B promoter is regulated by Engrailed in vivo. Moreover, they show that one promoter domain that contains all ATTA homeoprotein cognate binding sites common to the rat and human genes is an essential element of this regulation. It is thus proposed that MAP1B, a cytoskeleton protein involved in neuronal growth and regeneration, is under homeoprotein transcriptional regulation.

Key words: neuronal morphogenesis; cytoskeleton; MAP1B; transcriptional targets; homeoproteins; engrailed; in vivo electroporation; differential display

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associated protein 1B (MAP1B) gene encoding a cytoskeletal protein is a homeoprotein-regulated target.

MATERIALS AND METHODS

Cell cultures. Primary neurons were prepared as follows. Fragments from posterior mesencephalon and cerebellum (rat postnatal day 1) were incubated (5 min, room temperature) in trypsin-EDTA, washed in phosphate buffer plus 33 mM glucose (PBS) and 10% fetal calf serum (FCS), and incubated (10 min, 37°C) with 30 μg/ml DNase I (Sigma, St. Louis, MO). The cells were dissociated mechanically, washed three times with PBS, and plated at a density of 200,000 cells/cm² on dishes (35 mm diameter) coated with 3 μg/ml fibronectin (Sigma). Culture medium (MS) consisted of DMEM/F12 (1:1; Life Technologies, Cergy, France) with 33 mM glucose, 2 mM glutamine, 10 mM HEPES, pH 7.4, 9 mM NaHCO₃, 5 U/ml penicillin, 5 μg/ml streptomycin. MS was complemented with 0.1% ovalbumin, 25 μg/ml insulin, 100 μg/ml transferrin, 20 μg/ml progesterone, 60 μM putrescine, and 30 mM selenium. CHP-100 cells (Schlesinger et al., 1976) were grown in RPMI 1640 (Life Technologies, Guthersburg, MD) plus 15% FCS.

Differential display. Differential display was performed as described by Liang and Pardee (1992) with minor modifications. In brief, 500 ng of total RNA (RNase kit; Qiagen, Courtaboeuf, France) from cells treated or not with EnHD (300 ng/10² cells) in the presence of cycloheximide (1 μg/ml) and DNase I (15 μg/ml) were reverse transcribed using Superscript II (Life Technologies), following the supplier’s protocol. Before reverse transcription, RNA was systematically treated with RNase-free DNase I (Promega, Charbonni`res, France) to eliminate DNA contaminations and repurified (RNase kit). Anchored oligo-dT primers used for reverse transcription were 5’-AAG CTT TTT TTT TTT C-3’ (HTC primer) or 5’-AAG CTT TTT TTT TTT A-3’ (HTA primer). PCR reactions were performed in the presence of 18°C dATP (>1000 Ci/mmole; Amersham Pharmacia Biotech, Orsay, France) with the following cycles: 93°C for 30 sec, 39°C for 1 min, and 72°C for 1 min (3 cycles); 93°C for 30 sec, 43°C for 1 min, and 72°C for 1 min (37 cycles); 72°C for 5 min. PCR products were separated on 6% denaturing sequencing gels that were dried on Whatman 3MM paper under vacuum and subjected to autoradiography. Bands of interest were excised, eluted, reamplified, and cloned in pBluescript (KS) vector (Stratagene, La Jolla, CA). Partial sequencing was performed by standard techniques. Primers leading to PCR amplification of clone 31 were 5’-AAG CTT ATT GGT C-3’ and HTT.

RNA isolation and Northern blot analysis. Total RNA (15–20 μg, RNase kit) was run in formaldehyde agarose gels and transferred by capillarity to Hybond-N+ membranes (Amersham Pharmacia Biotech), and Northern blot hybridizations were performed according to the manufacturer’s instructions. Probes were labeled with α-32P-dCTP (3000 Ci/mmole; Amersham Pharmacia Biotech, Orsay, France) with the following cycles: 93°C for 30 sec, 39°C for 1 min, and 72°C for 1 min (3 cycles); 93°C for 30 sec, 43°C for 1 min, and 72°C for 1 min (37 cycles); 72°C for 5 min. PCR products were separated on 6% denaturing sequencing gels that were dried on Whatman 3MM paper under vacuum and subjected to autoradiography. Bands of interest were excised, eluted, reamplified, and cloned in pBluescript (KS) vector (Stratagene, La Jolla, CA). Partial sequencing was performed by standard techniques. Primers leading to PCR amplification of clone 31 were 5’-AAG CTT ATT GGT C-3’ and HTT.

DNA fragments were end labeled by filling with Klenow fragment and α-32P-dCTP. Binding reactions were performed in a final volume of 20 μl (15 mM HEPES, pH 8, 50 mM dithiothreitol, 25% (v/v) glycerol). Complete 1× (Roche Diagnostics). Nuclear debris were removed by centrifugation at 14,000 × g for 30 min at 4°C, and supernatants were stored in aliquots at –80°C. Protein concentration was determined by a modified Bradford assay (Bio-Rad, Irvine, France), using bovine serum albumin (BSA) as a standard.

Gel shift assays. DNA fragments were end labeled by filling with Klenow fragment and α-32P-dCTP. Binding reactions were performed in a final volume of 20 μl (15 mM HEPES, pH 8.0, 0.6 mM dithiothreitol, 6 mM MgCl₂, 18% glycerol, 1 μg of salmon sperm DNA, and 10 μg of BSA). Salt concentrations varied with conditions: 100 mM KCl (see Fig. 6A), 80 mM KCl (see Fig. 6B), 135 mM NaCl (see Fig. 5A), or 80 mM KCl plus 40 mM NaCl (see Figs. 5B, 7). After incubation on ice for 30 min, DNA-protein complexes were analyzed on 4% polyacrylamide gels (acrylamide/bisacrylamide, 80:1) in 0.25× TBE buffer (TBE 1× is 100 mM Trizma base, 95 mM borate acid, and 2 mM EDTA) and 2.5% glycerol. For supershift experiments, probes were first incubated with the nuclear extracts for 30 min on ice and then for an additional 30 min with a polyclonal antibody that recognizes both En1 and En2 proteins (Plaza et al., 1997) (a gift from Dr. S. Saule, Institut Curie, Orsay). In that case DNA–protein complexes were analyzed on 4% polyacrylamide gels (acrylamide/bisacrylamide, 80:1) in 0.25× TBE buffer and 2.5% glycerol. For supershift experiments, probes were first incubated with the nuclear extracts for 30 min on ice and then for an additional 30 min with a polyclonal antibody that recognizes both En1 and En2 proteins (Plaza et al., 1997) (a gift from Dr. S. Saule, Institut Curie, Orsay). In that case DNA–protein complexes were analyzed on 4% polyacrylamide gels (acrylamide/bisacrylamide, 80:1) in 0.25× TBE buffer and 2.5% glycerol. For supershift experiments, probes were first incubated with the nuclear extracts for 30 min on ice and then for an additional 30 min with a polyclonal antibody that recognizes both En1 and En2 proteins (Plaza et al., 1997) (a gift from Dr. S. Saule, Institut Curie, Orsay). In that case DNA–protein complexes were analyzed on 4% polyacrylamide gels (acrylamide/bisacrylamide, 80:1) in 0.25× TBE buffer and 2.5% glycerol. For supershift experiments, probes were first incubated with the nuclear extracts for 30 min on ice and then for an additional 30 min with a polyclonal antibody that recognizes both En1 and En2 proteins (Plaza et al., 1997) (a gift from Dr. S. Saule, Institut Curie, Orsay). In that case DNA–protein complexes were analyzed on 4% polyacrylamide gels (acrylamide/bisacrylamide, 80:1) in 0.25× TBE buffer and 2.5% glycerol. For supershift experiments, probes were first incubated with the nuclear extracts for 30 min on ice and then for an additional 30 min with a polyclonal antibody that recognizes both En1 and En2 proteins (Plaza et al., 1997) (a gift from Dr. S. Saule, Institut Curie, Orsay). In that case DNA–protein complexes were analyzed on 4% polyacrylamide gels (acrylamide/bisacrylamide, 80:1) in 0.25× TBE buffer and 2.5% glycerol. For supershift experiments, probes were first incubated with the nuclear extracts for 30 min on ice and then for an additional 30 min with a polyclonal antibody that recognizes both En1 and En2 proteins (Plaza et al., 1997) (a gift from Dr. S. Saule, Institut Curie, Orsay). In that case DNA–protein complexes were analyzed on 4% polyacrylamide gels (acrylamide/bisacrylamide, 80:1) in 0.25× TBE buffer and 2.5% glycerol.
RESULTS

Isolation of MAP1B cDNA by differential display

To find genes controlled by Engrailed we looked for modifications in expression profiles of postnatal rat cerebellum neurons on treatment by EnHD. Postnatal day 1 cerebellums were dissociated, plated for 4 hr, and incubated for another 12 hr with or without EnHD (in duplicate), in the presence of cycloheximide to prevent protein synthesis and regulation of indirect targets (Mainguy et al., 1999). Cycloheximide was not toxic because its removal allowed all neurons to resume differentiation (data not shown). In these conditions EnHD was internalized by the cells and could be visualized in the nucleus by immunocytochemistry as reported earlier (Mainguy et al., 1999). Expression profiles were then analyzed by differential display (Liang and Pardee, 1992).

Total RNA samples were reverse transcribed in duplicate with one of two different anchored oligo-dT primers (HTA or HTC primers). The subsets of mRNAs defined by each of these primers were amplified by PCR using 12 different 13-mers with arbitrary sequences in combination with the corresponding oligo-dT primer. Samples derived from EnHD-treated and control cells showed a small number of differentially expressed bands (Fig. 1A). Primer. Samples derived from EnHD-treated and control cells were amplified by PCR using 12 different 13-mers with arbitrary primers). The subsets of mRNAs defined by each of these primers were then analyzed by differential display (Liang and Pardee, 1992).

Identification of Engrailed-binding sites within the MAP1B promoter region

The MAP1B promoter region of the rat MAP1B gene (Liu and Fischer, 1996, 1997) contains two independent TATA boxes and several regulatory motifs, including two cAMP-responsive elements, an Sp1 site, a “neuronal motif,” and a TCC repeat motif (Fig. 3A). The MAP1B promoter region (nucleotide positions −1626 to +60) was cloned in front of a luciferase reporter gene (pGL2bas promoter-less vector). This construct (pMAP-luc) was tested by transient transfection of a human neuroepithelial cell line (CHP-100) (Mainguy et al., 1999) (Fig. 2). Differential expression of MAP1B was confirmed by Northern blot analysis using RNA isolated from rat cerebellum neurons, incubated or not with EnHD in the presence of cycloheximide. A representative Northern blot showing that EnHD increases the expression level of MAP1B by twofold is presented in Figure 1B.

Regulation of MAP1B promoter activity by homeoproteins

The promoter region of the MAP1B gene (Liu and Fischer, 1996, 1997) contains two independent TATA boxes and several regulatory motifs, including two cAMP-responsive elements, an Sp1 site, a “neuronal motif,” and a TCC repeat motif (Fig. 3A). The MAP1B promoter region (nucleotide positions −1626 to +60) was cloned in front of a luciferase reporter gene (pGL2bas promoter-less vector). This construct (pMAP-luc) was tested by transient transfection of a human neuroepithelial cell line (CHP-100) (Mainguy et al., 1999). Figure 4A illustrates that transfecting increasing amounts of a plasmid expressing En2 leads to a dose-dependent activation of the MAP1B promoter.

The En2-mediated activation of MAP1B promoter was not observed when an En2ΔH2D1-expressing plasmid was used (Fig. 4B). En2ΔH2D1 (Joliot et al., 1998) is an En2 mutant lacking amino acids 36–46 in the homeodomain; it is still addressed to the nucleus but does not bind DNA (data not shown). To test whether other homeoproteins were also active, pMAP-luc and Hoxa5 coding plasmid were cotransfected in CHP-100 cells. Figure 4B shows that Hoxa5 also regulates MAP1B promoter activity ex vivo.

Identification of Engrailed-binding sites within the MAP1B promoter region

The MAP1B promoter contains several putative homeoprotein binding sites (ATTA or TAAT sites) (Fig. 3). Five DNA fragments (from A to E) covering the entire MAP1B promoter region were generated (Fig. 3A), and each fragment was used in gel-shift experiments. Fragments A and E (417 and 138 bp, respectively) contain one ATTA site (overlapping the TATA-2 box in the case of fragment E); fragments B (421 bp) and C (547 bp) contain 10 and 7 ATTA sites, respectively; fragment D (398 bp) contains no ATTA sequence. Purified EnHD (Fig. 5A) and GST-En2 protein (Fig. 5B) retarded probes A, B, C, and D (the latter to a lesser extent) but failed to retard probe E even when a sevenfold excess of GST-En2 protein was used in the binding reaction (data not shown).

Gel-shift assays were then performed with nuclear extracts from P0 mouse cerebella. Probes A, B, C, and D (although to a lesser degree), but not E, were retarded (Fig. 6A), indicating the existence, in the nuclear extract, of one or more factors that specifically recognize the DNA fragments. That Engrailed is one of these factors was confirmed by a supershift experiment using a polyclonal anti-Engrailed antibody (Fig. 6B). Finally, to test whether Engrailed forms an active complex with factors in the nuclear extract, a supershift experiment was achieved by adding GST-En2. Figure 7 illustrates that GST-En2 (Fig. 7A), but not GST alone (Fig. 7B), interacts with one or several factors. Taken
together, these results demonstrate that Engrailed is present in the extracts and recognizes different fragments of the MAP1B promoter in an appropriate molecular context.

Repression of MAP1B promoter activity by Engrailed in the neural tube of chick embryos

The results described above demonstrate that Engrailed binds different fragments in the MAP1B promoter and regulates the expression of this promoter in transfected neuroepithelial cells. To better sustain the idea that MAP1B is regulated by Engrailed and to identify which domains in the promoter are involved, several lacZ-reporter constructions (Fig. 8) were expressed in the neural tube of chick embryos at stage HH9–11 (Hamburger and Hamilton, 1951), and their expression was analyzed 1 d later.

Full-length MAP1B promoter is expressed in vivo and repressed by Engrailed (Fig. 9A). On the basis of cell transfection assays, Liu and Fischer (1996) have proposed that the two MAP1B TATA boxes, in association with their adjacent regulatory cis-elements, function independently and confer neuron-specific expression. To test whether each TATA box is repressed by Engrailed in the developing chick neural tube, pD-lacZ and pE-lacZ were electroporated with or without Engrailed. Plasmid pD-lacZ contains TATA box1 (TATA-1) and associated upstream motifs (Fig. 3A), and plasmid pE-lacZ contains TATA box2 (TATA-2) and the upstream Sp1 motif (Fig. 3A). As illustrated in Figure 9, only pD-lacZ is active (Fig. 9B), whereas pE-lacZ shows no basal neural activity (Fig. 9C) and neither construct expression is regulated by Engrailed. Because some activity was observed in adjacent non-neural tissue in several embryos, it is likely that TATA-2 can drive lacZ expression but lacks the motifs necessary for neural expression.

Fragment D contains no ATTAG sequence and is not repressed by Engrailed. We thus tested whether fragment ABC, which contains all ATTAG sequences, confers regulation. Figure 9D illustrates the expression of pABCD-lacZ in the neural tube and its repression by En2. Little difference was observed between pABCD-lacZ and the full-length promoter (Fig. 9, compare A and D). Aligning human and rat sequences highlights two homologous MAP1B promoter regions and, in particular, shows that only ATTAG sites at positions 9, 10, 12, and 13 of the rat promoter (Fig. 3B) are conserved between rat and human. Because fragment C contains all of these conserved sites (Fig. 3), pCD-lacZ was electroporated in the chick neural tube with or without Engrailed. Figure 9E confirms that motifs in fragment C mediate the regulation of MAP1B promoter by Engrailed.

DISCUSSION

This study demonstrates that MAP1B is a homeoprotein target and, most probably, an Engrailed target. This conclusion is based on the following series of experiments. First, EnHD internalization in primary cerebellum neurons provokes an accumulation of MAP1B mRNA in the presence of cycloheximide. Second, cerebellum nuclear extracts retard several fragments of the MAP1B promoter, and Engrailed is present in the retarded complex. Finally, the promoter is active and regulated by Engrailed in vivo.
A differential display approach was associated with the internalization of EnHD by cerebellum primary neurons. The molecular basis of homeodomain internalization has been well established, and it is now clear that specific properties of the third helix are involved (Prochiantz, 1996; Derossi et al., 1998). The absence of chiral receptors and the translocation across artificial lipid layers (Thoren et al., 2000) explains why homeodomains gain direct access to the cytoplasm and thereafter, through the nuclear pores, to the nucleus.

Internalized homeodomains compete with endogenous homeoproteins for cognate binding sites in the genome. This was shown by the use of the homeodomain of Antennapedia (AntpHD) and by its mutated version where glutamine in position 50 has been replaced by an alanine (AntpHD-50A). Indeed, AntpHD-50A is internalized and conveyed to the nucleus but does not bind DNA with high affinity (Le Roux et al., 1993) and, contrary to AntpHD, does not regulate gene expression (Le Roux et al., 1995; Mainguy et al., 1999).

In vitro promoter analysis

The MAP1B promoter region (Liu and Fischer, 1996) shows 16 putative homeoprotein binding sites. Gel-shift experiments demonstrating Engrailed binding to four promoter fragments (fragments A, B, C, and D) support a direct regulation of MAP1B expression by Engrailed. In fact, the use of a protein synthesis inhibitor (cycloheximide) in the differential display protocols...
prevents cascade effects and favors the isolation of targets directly regulated by EnHD.

The ATTA motif present in fragment E (which is not retarded) overlaps the second TATA box of the \textit{MAP1B} promoter (Liu and Fischer, 1996) and probably does not represent a physiological homeoprotein-binding site. In contrast, fragment D does not contain any ATTA site but forms a complex with EnHD or En2. However, a comparison of bound versus free probe in gel-shift experiments (same amounts of probe and protein for all fragments) suggests that the relative affinity of Engrailed for the probes is C>B>A>D (Fig. 5B) and that the affinity between En2 and D is low.

Gel-shift experiments gave similar results when purified EnHD, GST-En2, or cerebellum nuclear extracts were used. Engrailed is abundant in the cerebellum at P0 and in the nuclear extracts (data not shown). As demonstrated by the supershift experiments, Engrailed is part of the retarded complexes formed between the probes and proteins in the nuclear extract. Although in these retarded complexes Engrailed might not bind directly to the promoter, the fact that purified En2 binds directly and that nuclear extracts and purified En2 show the same order of relative affinities for the five fragments is very much in favor of a direct binding.

Enhanced retardation of \textit{MAP1B} promoter probes when En2 is added to cerebellum nuclear extracts (Fig. 7) suggests the presence of cofactors. Groucho/TLE and Exd/Pbx are recognized cofactors of Engrailed in invertebrates and vertebrates. Groucho/TLE proteins do not bind DNA but are co-repressors of several transcription factors, including Hairy-related, Runt domain, and Engrailed proteins (for review, see Fisher and Caudy, 1998; Chen and Courey, 2000). In contrast, Pbx homeoproteins have the ability to modulate the binding activity of Hox and Engrailed through cooperative DNA binding (Chang et al., 1995; van Dijk et al., 1995). In rat embryos, Pbx1 and Engrailed show overlapping expression patterns (Roberts et al., 1995), and Pbx1 is detected by Western blot in cerebellum nuclear extracts (data not shown). However, the possibility that added purified En2 binds Pbx1 or other cofactors present in the extract has not been investigated.

\textbf{Ex vivo and in ovo promoter activity}

Transfections in the neuroepithelial cell line CHP-100 provided some interesting information. First, Engrailed regulates \textit{MAP1B} promoter activity, and this regulation is lost when 11 amino acids are deleted in the homeodomain. It was indeed verified that the mutated protein is synthesized and accumulates in the nucleus. Second, HoxA5, another member of the Q50 homeoprotein family, also regulates \textit{MAP1B} promoter expression. This supports the
idea that different Q50 homeoproteins may regulate the same target genes (Biggin and McGinnis, 1997), including MAP1B. This is not a surprise because homeoproteins are region-specific transcription factors, whereas MAP1B is expressed in all neurons and not specifically in the mid-hindbrain.

En2 represses the MAP1B promoter after electroporation in the chick neural tube, a gain of function protocol that permits the study of gene activity in vivo (in ovo, rather), thus in a physiological context. This repression is in agreement with the differential display results because internalized EnHD antagonizes Engrailed activity (Mainguy et al., 2000) and leads to an upregulation of the MAP1B transcript in cultured midbrain and cerebellum neurons. The in ovo experiments show that overexpressed Engrailed completely downregulates MAP1B promoter activity. This does not mean that Engrailed-expressing neurons do not express MAP1B but that Engrailed acts in conjunction with other transcription factors to regulate the appropriate levels of the protein. In fact, it must be kept in mind that the activity of a transcription factor is both dose and context dependent. For example, in Drosophila, Engrailed either activates or represses polyhomeotic, and the two opposite effects depend both on Engrailed concentration (high concentrations are inhibitory) and on the presence of Exd as a cofactor (necessary for activation) (Serrano and Maschat, 1998). Another example is given in this study because Engrailed is a repressor of MAP1B promoter activity in neuronal cells and an activator in CHP-100 cells.

Expression in the chick embryo of the different reporter constructs demonstrates that only TATA box1 (and not TATA box2, in fragment E) is active in the nervous system. These experiments also show that fragment D is not regulated by Engrailed in vivo and suggest that the lower in vitro affinity of Engrailed for fragment D (in comparison with the other fragments) is physiologically relevant. This has allowed us to use the TATA box of fragment D to test C domain activity by electroporating pCD-lacZ and to show that this domain, which contains all ATTA sequences conserved between rat and human, permits regulation by Engrailed. pMAP-lacZ, pABCD-lacZ, and pCD-lacZ constructions are active in the mid-hindbrain region (Fig. 9) where Engrailed is normally expressed, and repression follows Engrailed electroporation. As already discussed in the case of polyhomeotic, this illustrates that MAP1B regulation by Engrailed is probably dose dependent and that the presence of physiological amounts of Engrailed is compatible with MAP1B expression.

In Drosophila, Engrailed, and Futsch (a MAP1B-like gene) (Hummel et al., 2000; Roos et al., 2000) are ectopically expressed in Tramtrack mutants (Xiong and Montell, 1993; Giesen et al., 1997). Because Tramtrack (a zinc-finger transcription factor) binds the Engrailed promoter (Read and Manley, 1992), an appealing possibility is that a direct genetic interaction between Engrailed and MAP1B has been conserved in several species, including human, rat, chick, and possibly Drosophila.

**Physiological significance**

MAP1B, a major component of the neuronal cytoskeleton (Bloom et al., 1984, 1985), is the earliest MAP expressed during brain development (Riederer et al., 1986), reaching highest expression levels 2–3 d after birth. In the adult, MAP1B is downregulated (Saafai and Fischer, 1989; Garner et al., 1990), except in regions that retain high levels of axonal growth and synaptic

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**Figure 7.** Enhanced retardation of MAP1B promoter by GST-En2 and cerebellum nuclear extract. A, Band-shift assays with MAP1B probes A to E (0.4 ng) with (+) or without (−) 2 μg of cerebellum nuclear extract and/or 1.25 μg of GST-En2. B, Band-shift assays with fragments A to E (0.4 ng) with (+) or without (−) 2 μg of cerebellum nuclear extract and/or 10 μg of GST. Designation of MAP1B probes refers to promoter regions indicated in Figure 3A. Note that the five probes are of different sizes and thus migrate with different velocities.

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**Figure 8.** MAP1B reporter plasmids for in ovo electroporations. The indicated regions of the MAP1B promoter were fused to a lacZ-reporter gene. ATTA sites are indicated with filled circles. Letters refer to regions indicated in Figure 3A.
plasticity, for example, the olfactory bulb, the hippocampus, and Purkinje cells in the cerebellum (Sato-Yoshitake et al., 1989; Schoenfeld et al., 1989). Although the precise function of MAP1B remains unclear, the phenotype of MAP1B knock-out mice suggests a role in neuronal differentiation (Edelmann et al., 1996; Takei et al., 1997; Gonzalez-Billault et al., 2000). This view is confirmed by antisense experiments showing that MAP1B downregulation in PC12 cells or cultured cerebellum macro-neurons reduces neurite outgrowth (Brugg et al., 1993; DiTella et al., 1996).

Figure 9. Activity of MAP1B promoter fragments in the developing chick neural tube. Chick embryos (HH9–11) were co-electroporated with empty (control) or En2-expressing (En2) plasmids, together with the following lacZ-reporter plasmids: A, pMAP-lacZ; B, pD-lacZ; C, pE-lacZ; D, pABCD-lacZ; E, pCD-lacZ. Embryos were stained for β-galactosidase activity 24 hr after electroporation. Histograms show the percentage of electroporated embryos for each condition showing strong (+ + + / + +), weak (+), or no (−) β-galactosidase activity. The number of embryos for each condition is indicated on the top of the corresponding bar. Examples of β-galactosidase expression in each experimental condition are shown on the right side of the Figure.
In conclusion, this report adds MAP1B to the list of established cytoskeleton genes that interact with one or several homeogenes. This list contains β3-tubulin (Serrano et al., 1997), centrosomin (Li and Kaufman, 1996), MAP2 (Ding et al., 1997), calponin (Morgan et al., 1999), NF68 (Biagioli et al., 2000), and BPA1 (Mainguy et al., 1999, 2000). The fact that homeoprotein transcription factors regulate the expression of several components of the cytoskeleton, and probably of adhesion molecules (Edelman and Jones, 1993), provides a molecular basis for their well established functions in tissue and cell morphogenesis (Joliot et al., 1991; Bloch-Gallego et al., 1993; Le Roux et al., 1993).

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