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Genomic structure of human microtubule-associated protein 2 (MAP-2) and characterization of additional MAP-2 isoforms

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ABSTRACT **We have determined that the gene for human microtubule-associated protein 2 (MAP-2) spans 19 exons, including 6 exons identified in this study, 1–4, 8, and 13; all six of these exons are transcribed. The alternative splicing of coding exons generates a greater diversity of MAP-2 transcripts and isoforms. The first three exons encode alternate 5′ untranslated regions that can be spliced to additional untranslated sequences contained in exons 4 and 5. Exons 8 and 13 are transcribed in human fetal spinal cord, adult brain, MSN cells, and rat brain, and each exon maintains an open reading frame with both high and low molecular weight MAP-2 isoforms. Antibodies generated to synthetic peptides of exons 8 and 13 demonstrate that these exons are translated and MAP-2 isoforms containing these exons are generated.**

Microtubule-associated protein 2 (MAP-2) isoforms are found predominately in neurons (1). The principal functions of MAP-2 are to reduce the critical concentration of tubulin required to polymerize microtubules and to maintain neuronal morphology by regulating the spacing of microtubules (2–4). Multiple high molecular weight (HMW) and low molecular weight (LMW) MAP-2 isoforms are expressed within axons, dendrites, and cell bodies. The human MAP-2 isoforms are encoded on 9.5- and 6-kb mRNAs. These transcripts are alternatively spliced from a single gene localized to chromosome 2 (5). While the coding regions of the HMW and LMW MAP-2 transcripts are 5595 bp and 1419 bp, respectively, the distribution of the 5′ and 3′ untranslated sequences is not known. In this report, we have isolated and characterized a series of genomic and cDNA clones encompassing the human MAP-2 gene. This analysis not only has led to an understanding of the organization of the MAP-2 gene and its 5′ and 3′ untranslated regions (UTRs) but also has led to the identification of several additional exons.§

MATERIALS AND METHODS

Characterization of Human Genomic MAP-2 Clones. Four human genomic libraries were screened with MAP-2 cDNAs (American Type Culture Collection). Several positive clones were identified and further characterized by subcloning and sequencing in both directions (Sequenase; United States Biochemical).

PCR of Human Genomic DNA. To determine the intron–exon boundaries, 500 ng to 1 μg of the appropriate genomic clone or genomic DNA isolated from human fibroblasts (6) was used in a single PCR. For amplification of large DNA fragments, XL-PCR kit (Perkin–Elmer) was used in combination with the hot-start technique. Amplifications were performed on a DNA thermocycler (Perkin–Elmer). To determine whether the entire 3′ UTR was contained within a single exon, PCR was performed with a sense primer located approximately 2.5 kb from the translational stop site and an antisense primer to the end of the rat 3′ UTR (18). The PCR product was cloned and sequenced.

Generation of Polyclonal Antibodies to Exons 8 and 13 and Western Blot Analysis. Synthetic peptides were conjugated by the *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) method (Pierce) to keyhole limpet hemocyanin. The synthetic peptide for exon 8 was a 15-mer (MAEEEKPAALPEKEC) while the synthetic peptide for exon 13 was a 20-mer (LSKIPALQGSTKSPRYSSAC). Each peptide was individually injected into two female New Zealand rabbits (Charles River Breeding Laboratories). The antibodies were affinity-purified by using SulfoLink coupling columns according to the exact protocol of the manufacturer (Pierce). Heat-stable proteins from rat brain or MSN cells (7) were isolated and immunoblot analysis was performed as described (8). AP-18 (9) and tau 46 (10) were generously provided by Lester Binder at Molecular Geriatrics, Inc., Lake Bluff, IL, and Virginia Lee at University of Pennsylvania, respectively.

Isolation of RNA from Cell Lines and Human Tissue. Total RNA was isolated from cells by hot phenol extraction (11–12) or from tissue by using Tri-Reagent (Molecular Research Center; Cincinnati). Poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography and Northern blot analysis was performed (13). Tissue was obtained from the Pathology Department at Albert Einstein College and affiliated hospitals.

RESULTS

The MAP-2 Gene Spans 19 Exons. The isolated genomic MAP-2 clones and primer pairs used for the characterization of the MAP-2 gene are illustrated in Fig. 1. To determine whether there were additional coding exons for MAP-2, two approaches were used: reverse transcription-coupled PCR (RT–PCR) and Northern blot analysis. MSN, human fetal spinal cord, or adult brain mRNA were examined for the expression of additional exons by performing RT–PCR with primers to coding sequences that flanked introns. In addition, some introns were used as radiolabeled probes to determine whether transcripts would be detected in MSN mRNA by Northern blot analysis. By using these strategies two exons,

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Abbreviations: MAP-2, microtubule-associated protein 2; HMW, high molecular weight; LMW, low molecular weight; UTR, untranslated region; RT–PCR, reverse transcription-coupled PCR; P, postnatal day; MTBD, microtubule-binding domain.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. exon 1, U32993; exon 2, U32994; exon 3, U32999; exons 4 and 5, U32995; exon 6, U34059; exon 7, U34060; exon 8, U32997; exons 9–12, U34061–U34064; exon 13, U32998; exons 14–18, U34065–U34069; exon 19, U32996).

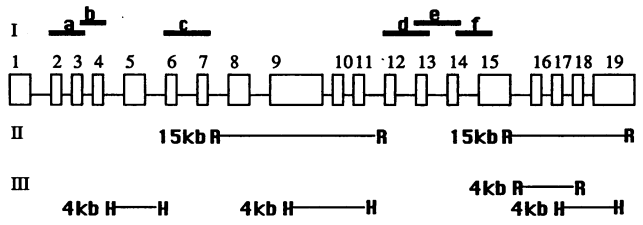

FIG. 1. Diagrammatic representation of the clones spanning the MAP-2 gene. The exon organization of the MAP-2 gene is illustrated by boxes and the lines denote the introns. Gene organization is not drawn to scale. (*I*) Clones obtained by PCR. The primers utilized were as follows: a, sense (GATAGGCTGATAGACTCGACT) and antisense (CAGCGCATATGCAGCAAT); b, sense (CACAGACACGAACTGGTGGCT) and antisense (CTCGGTTAGAGACAAGCTGAAGAA); c, sense (GTAGTCACTGCTGAGGCTGTAGCA) and antisense (ACAGTCTGTTCTGAGGCAGGTGAT); d, sense (GGGGAATCAGCTCTGGCTCCCAGTGTATTT) and antisense (CTTGTTCCCTGATTTTGAGTATGG); e, sense (AATTCTACCTTGTCAAAGATTCCT) and antisense (AAGGTCTTGGGAGAGAAGAGCGCT); f, sense (GACGGAGTAACCAAGAGCCCA) and antisense (GTGTGCGTGAAGAATAACTTGGTG). (*II* and *III*) Genomic clones isolated from a partial *Eco*RI library (*II*) or from human chromosome 2 libraries (*III*).

exon 8 and 13, were isolated and are detailed below. Table 1 shows the nucleic acid sequence at the intron–exon junctions; this was based on a comparison of the MAP-2 cDNA sequences and the genomic clones. Almost every splice junction conforms to the GT/AG rule, which denotes the 5′ donor and the 3′ acceptor splice sites of the introns.

The first five MAP-2 exons contain the 5′ untranslated sequences that vary in length from 275 bp to 452 bp, depending upon the exons being utilized. The first three exons are unique and are independently used with exons 4 and 5, which are referred to as the common region of the 5′ UTR. The common region of the UTR is 171 bp with 142 bp contained within exon 4 and 29 bp within exon 5. The expression of this region is complex and is discussed elsewhere (14). Exon 5 also contains the AUG initiation start site and 259 bp of coding sequence. The sequence encoding the binding site for the type II regulatory subunit of cAMP-dependent protein kinase (15) is split between exons 5 and 6. The splice junction after exon 7 is an important splice site that generates multiple MAP-2 transcripts including MAP-2c.

Exons 9–11 encode the large portion of the projection arm of HMW MAP-2, which is spliced from the LMW MAP-2 transcripts. Exon 9 is the second largest MAP-2 exon (3.7 kb) and exons 10 and 11 are 207 bp and 135 bp, respectively. In the genomic clone, exon 9 begins with 12 bp (ATTTACTTACAG) encoding 4 amino acids, which were not present in our original MAP-2 cDNA clone II4 but are present in the published rodent and human fetal cDNA clones listed in GenBank (accession nos. M21041 and U01828). II4 was isolated from an adult human brain cDNA expression library (16) and the missing 12 bp were internal to the 1.7-kb II4 clone. While exons 9–11 are contained only within HMW MAP-2, exons 12 and 14–19 are common to HMW and LMW MAP-2 transcripts. Exon 15 contains the first repeat of the microtubule-binding domain (MTBD) and 286 bp of upstream sequence. The fourth repeat of the MTBD is contained in a single exon, exon 16, with no other MAP-2 sequences. This is expected since the fourth repeat is developmentally regulated and not expressed as part of all MAP-2 transcripts. Sequences encoding the second repeat of the MTBD are split between exons 17 and 18. Exon 18 is 113 bp containing 8 bp from the second repeat and the entire third repeat of the MTBD. Exon 19 contains the remaining 213 bp of the MAP-2 coding sequence, the translational stop site, and the entire 3′ UTR. The human 3′ UTR has extensive sequence homology with that of the rat. Both 3′ UTRs are greater than 3.5 kb and contain many polyadenylylation consensus signals. Usage of these individual polyadenylylation signals may add additional diversity to the MAP-2 transcripts.

Exon 8 and Exon 13. Exon 8 was isolated by RT–PCR using MSN mRNA, a sense primer from exon 7 (ATCACCTGCCTCAGAACAGACTGTCACAGT), and an antisense primer from exon 9 (CTGGTCTGAAGGCTCAGCTGT). With these primers, two PCR products were obtained: the expected 103-bp product and a 349-bp product that was cloned and sequenced. The clones contained an exon of 246 bp designated as exon 8. Exon 8 sequence is shown in Fig. 2. Exon 8 has 82% sequence homology with a MAP-2 cDNA clone isolated from an adult rat brain cDNA library (C.G., unpublished data). The translated

Table 1. Intron–exon junctions of the MAP-2 gene

Exon	3′ acceptor site [(u/c(n$_{11}$)cag/G)]	Exon size, kbp	5′ donor site [AG/gu(a)agu]	Intron size, kbp
1	*ND*/GAGTCT	0.282	AG/*ND*	>5.0
2	*ND*/*GATAGG*	0.104	TC/gtaagt	0.171
3	ccctttccccggaca/CACAGA	0.146	TG/gtaatg	0.280
4	gttttgtttcggtag/ATTCTT	0.141	AG/*ND*	>3.5
5	ttttttctctttcag/TTGCAG	0.291	AG/gtaact	>1.4
6	*ND*/AGGAGG	0.114	AG/gtaaat	2.0
7	ttgtttgatctttag/CAGCTG	0.074	AG/*ND*	>2.0
8	tttttcatccctaag/AAGAAG	0.245	AG/gtgtgt	>0.65
9	acccgattacttcag/ATTTAC	3.714	AG/gtgtg	≈0.60
10	ttgctttgtccacag/ATGATG	0.207	AG/gttcat	0.168
11	ttttcttattcatag/CAGTTT	0.135	AG/gtgact	*ND*
12	*ND*/CAGCAG	0.062	CT/gtgaga	≈4.2
13	ttctttcatggctag/AATTCT	0.171	AG/gtaagg	≈1.1
14	gttttatccaagcag/GACGGA	0.149	AG/ttaggt	≈4.3
15	ttgcttgttatacag/GTCAGA	0.401	AG/*ND*	*ND*
16	tccaattccttttag/GTTAGG	0.093	TG/taagta	*ND*
17	tgtggttcactatag/GTACAA	0.082	AG/gtaaat	*ND*
18	tttcttccctcatag/GTGGCG	0.113	AG/gtaaga	*ND*
19	gcttgtcttaaacag/ATTGAC	>3.5	AG/*ND*	*ND*

Lowercase type represents intron sequence; uppercase type represents exon sequence. *ND*, not determined.

Exon 8: 246 nucleotides and 83 amino acids

```
G (from exon 7)/

AAGAAGAAACGCTAGAGAGTCGGATGGCTGAGGAAGAGAAACCTGCTGCTCTTCCTGAGA
  E  E  T  L  E  S  R  M  A  E  E  E  K  P  A  A  L  P  E  K
AAGAGTGTGGGGCTGCTAAGTCCTCAGACCAACCCAAGGGCCTCAGTAAGGGCCAAATGG
  E  C  G  A  A  K  S  S  D  Q  P  K  G  L  S  K  G  Q  M  E
AGCCTAGTGCGGAGGCCCAAATAGTTCCCGAAGAGAGTGCCCCAGCAGGGGCCCCACATG
  P  S  A  E  A  Q  I  V  P  E  E  S  A  P  A  G  A  P  H  E
AGAAAAGTGTAAAAGAGGTCAAGGAGGTGGCTCCAGAAGTAAAAACCCCTTCCTCTGCTG
  K  S  V  K  E  V  K  E  V  A  P  E  V  K  T  P  S  S  A  G
GGGAAG
  E  N (exon 9)
     A (exon 12)
```

Exon 13: 171 nucleotides and 57 amino acids

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AATTCTACCTTGTCAAAGATTCCTGCTTTACAGGGTAGCACAAAGTCCCCAAGATACAGC
N  S  T  L  S  K  I  P  A  L  Q  G  S  T  K  S  P  R  Y  S
TCAGCCTGCCCTAGCACGACTAAAAGGGCTACATTTTCTGACAGTTTATTAATACAGCCC
S  A  C  P  S  T  T  K  R  A  T  F  S  D  S  L  L  I  Q  P
ACCTCAGCAGGCTCCACAGACCGTTTGCCATACTCAAAATCAGGGAACAAG
T  S  A  G  S  T  D  R  L  P  Y  S  K  S  G  N  K
```

FIG. 2. Sequences of human MAP-2 exons 8 and 13.

exon maintains an open reading frame of 83 amino acids with exons 7 and 9 or exons 7 and 12.

To determine whether exon 8 is expressed in other cell types and tissues, RT–PCR was performed with mRNA from rat C6 glioma cells, human fetal spinal cord (23 weeks of gestation), and human adult brain. The primers used were a sense primer (AAGAAGAAACGCTAGAGAGTCGGA) and an antisense primer (TTTTACTTCTGGAGCCACCTCCTT), both from exon 8. As shown in Fig. 3*A*, lanes 1–4, a 224-bp product was obtained from all mRNAs.

The PCR with which exon 8 was initially obtained demonstrated that exon 8 is transcribed as part of a HMW MAP-2 transcript. Northern blot analysis was performed to determine whether exon 8 is also transcribed as part of the LMW MAP-2 (Fig. 3*B*). Poly(A)$^+$ mRNA was isolated from MSN cells and probed with random-primed exon 8 insert (lane 1) or the first 265 bp of coding sequence that are common to all MAP-2 transcripts (lane 2). Surprisingly, exon 8 recognized a 6-kb transcript (lane 1) but a HMW MAP-2 transcript was not detected even upon prolonged exposure. Only by the sensitive technique of RT–PCR, using a sense primer from exon 8 and an antisense primer from exon 9, were we able to demonstrate that exon 8 is part of a HMW MAP-2 transcript (Fig. 3*A*, lane 5). Additionally, RT–PCR confirmed the Northern blot data demonstrating that exon 8 can be part of a LMW MAP-2 transcript (data not shown). These data indicate that in MSN cells HMW and LMW MAP-2 transcripts containing exon 8 exist, but the LMW transcript is expressed in higher abundance. When a Northern blot containing mRNA from adult rat brain was probed with random-primed exon 8, only a 9.5-kb transcript was observed (Fig. 3*C*, lane 1). This suggests that exon 8 is transcribed as both HMW and LMW transcripts at different times during development.

Exon 8 is 83 amino acids and would add an additional 10 kDa to MAP-2. To determine whether exon 8 is translated, a 15-mer synthetic peptide (MAEEEKPAALPEKEC) was synthesized for antibody production and the affinity-purified antibody was used to examine the expression of exon 8-encoded protein in MSN and postnatal day 11 (P11) heat-stable rat-brain homogenates. The monoclonal antibody AP-18, which recognizes the N termini of HMW and LMW MAP-2, served as the control (Fig. 3*D*, lane 1). In MSN protein homogenates, both HMW and LMW MAP-2 were recognized with the antibody raised to exon 8 synthetic peptide, hereafter referred to as antibody 8 (lane 3, see arrows). While a HMW isoform was observed with antibody 8, its expression was low relative to the total amount of HMW MAP-2 expressed. Three LMW bands ranging in size from 76 to 90 kDa were consistently detected with antibody 8 (lane 3). The two higher bands probably represent degradation products of the HMW MAP-2 since these fragments are not observed with tau 46. Tau 46 recognizes a nonphosphorylated epitope on the C-terminal portion of MAP-2 (11), suggesting that cleavage of the C-terminal of MAP-2 yielded these fragments. In heat-stable homogenates from P11 rat brain, only HMW MAP-2 was detected with antibody 8 (lane 5). In contrast, both HMW and LMW MAP-2 were detected with AP-18 (lane 6). This suggests that HMW and LMW MAP-2 isoforms recognized by antibody 8 may be expressed at different times of development.

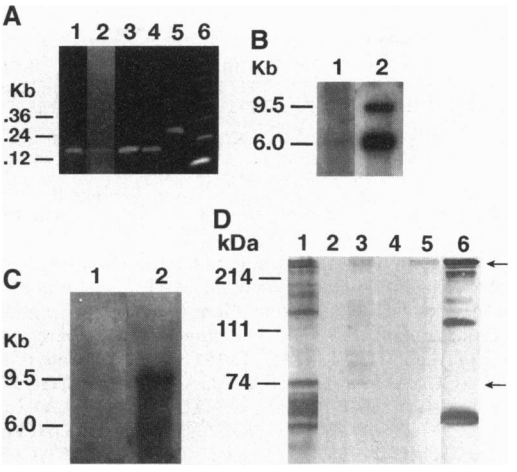

FIG. 3. Exon 8 is transcribed and translated. (*A*) RT–PCR demonstrates that exon 8 is transcribed in mRNAs from a number of central nervous system cell types and tissues. RT–PCR using sense and antisense primers from exon 8 demonstrated that a 224-bp product is obtained in mRNA from MSN cells (lane 1), rat C6 glioma (lane 2), human fetal spinal cord (lane 3), and human adult brain (lane 4). Lane 5 demonstrates that exon 8 is transcribed as part of HMW MAP-2 in MSN mRNA. The sense primer was from exon 8 (AAGAAGAAACGCTAGAGAGTCGGA) and the antisense primer was from exon 9 (CTGGTCTGAAGGCTCAGCTGT). The DNA marker is a 123-bp ladder (lane 6; GIBCO/BRL). Samples were electrophoresed on a 0.8% agarose gel and stained with ethidium bromide. (*B*) Northern blot probed with exon 8 detects a 6-kb transcript in MSN mRNA. Poly(A)$^+$ mRNA (5 μg) was loaded per lane. Lane 1 was probed with exon 8. Lane 2 was probed with the first 265 bp of MAP-2 coding sequence. There were 10^8 counts per lane and final wash stringency was 0.1× SSC/0.1% SDS. The exposure time was 8 days for lane 1 and 2.5 days for lane 2. (*C*) A 9.5-kb transcript is detected in adult rat brain with the human exon 8 probe. Rat poly(A)$^+$ mRNA (5 μg) was loaded per lane and probed with either human exon 8 (lane 1) or the first 265 bp of the human MAP-2 coding region (lane 2). Equal counts per lane were used. Exposure time was 6 days for lane 1 and 16 h for lane 2. Final wash stringency was 1× SSC/0.1% SDS. (*D*) Antibody 8 is immunoreactive in human MSN and rat brain homogenates. Heat-stable protein homogenates from MSN cells (lanes 1–4) or from P11 rat brain (lanes 5 and 6) were loaded on a 10% SDS/PAGE gel at 50 μg per lane and subsequently transferred to nitrocellulose support. Blots were incubated with either AP-18 (1:10 dilution, lanes 1 and 6) or antibody 8 (1:50 dilution, lanes 3 and 5). Absorption of the antibody with the synthetic peptide resulted in loss of immunoreactivity (lane 4). The preimmune serum was negative (1:50 dilution, lane 2). Strips were visualized by diaminobenzidene.

An RT–PCR using mRNA from MSN cells and sense and antisense primers from exons 12 and 14 demonstrated that an additional MAP-2 exon existed. Exon 13 is a 171-bp exon that, to our knowledge, has not been characterized as part of MAP-2 transcripts (see Fig. 2). By RT–PCR, we determined that exon 13 is transcribed in C6 glioma, human fetal spinal cord, and human adult brain mRNA (Fig. 4*A*, lanes 1–4). The translated sequence encodes 57 amino acids that are in-frame with HMW and LMW MAP-2 isoforms.

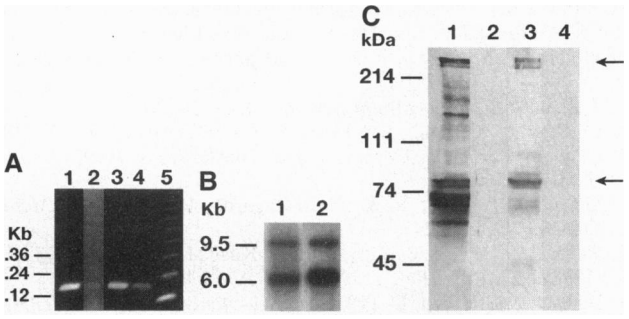

FIG. 4. Exon 13 is transcribed and translated. (*A*) RT–PCR demonstrates that exon 13 is transcribed in mRNAs from a number of central nervous system cell types and tissues. RT–PCR using sense and antisense primers from exon 13 (AATTCTACCTTGTCAAAGATTCCT and CTTGTTCCCTGATTTTGAGTATGG, respectively) amplified a 171-bp product in mRNA from MSN cells (lane 1), C6 glioma (lane 2), human fetal spinal cord (lane 3), and human adult brain (lane 4). Samples were loaded on a 0.8% agarose gel and products were visualized by ethidium bromide. Lane 5 is a 123-bp DNA marker. (*B*) Northern blot probed with exon 13 detects 9.5- and 6-kb transcripts in MSN mRNA. Poly(A)$^+$ mRNA (5 μg) was loaded per lane. Lane 1 was probed with exon 13. Lane 2 was probed with the first 265 bp of MAP-2 coding sequence. There were 10^8 counts per lane and final wash stringency was 0.1× SSC/0.1% SDS. The exposure time was 2.5 days. (*C*) Affinity-purified antibody to a 20-mer synthetic peptide of exon 13 is immunoreactive in MSN protein homogenates. Heat-stable protein homogenates (50 μg per lane) were loaded on a 10% SDS/PAGE gel and subsequently transferred to nitrocellulose support. Individual lanes of the blot were incubated with either AP-18, the monoclonal antibody that recognizes HMW and LMW MAP-2 isoforms (1:10 dilution, lane 1), or the exon 13-specific antibody (1:500 dilution, lane 3). Absorption of the antibody with the synthetic peptide resulted in loss of immunoreactivity (lane 4). The preimmune serum was negative (1:500 dilution; lane 2). Strips were visualized by diaminobenzidene.

As shown in Fig. 4*B*, the random-primed exon 13 isolated insert detected 9.5-kb and 6-kb transcripts on a representative Northern blot containing MSN mRNA (lane 1). Lane 2 was probed with the first 265 bp of human MAP-2 coding sequence. Both 9.5-kb and 6-kb transcripts were observed.

To further characterize exon 13, RT–PCR and cloning were performed with mRNA isolated from human fetal spinal cord and adult brain. Sense and antisense primers were from exons 9 and 13, respectively. These studies will be reported elsewhere (N.K. and B.S.-Z., unpublished results), however, cDNA clones containing full-length exon 13 and clones lacking the first 33 bp of exon 13 were isolated. The elimination of exonic sequences was similar to that observed with the II4 cDNA clone and prompted us to examine carefully the deleted sequences. The sequences deleted from exon 9 (12 bp) and exon 13 (33 bp) revealed a potential splice site that might generate these transcripts. Fig. 5 illustrates the splice junction and the beginning of exons 9 and 13. The thin line spans the actual intron–exon splice site (u/cN$_{11}$cag/G) used by canonical MAP-2 transcripts. The sequences in boldface type represent the nucleotides deleted from some of the cDNA clones. The arrows point to the potential splice sites and the thick underlined nucleotides represent a sequence that resembles

FIG. 5. Isolation of cDNA clones containing unusual splice junctions at exons 9 and 13.

the conserved 3′ donor sequence essential for splicing. While these shortened transcripts represent additional MAP-2 splice variants, the significance of these spliced transcripts or their translational status is not known.

Exon 13 is 57 amino acids and would add approximately 7 kDa to MAP-2. To examine whether exon 13 is translated, a 20-mer synthetic peptide (LSKIPALQGSTKSPRYSSAC) was generated and affinity-purified antibody was produced. The purified antibody was incubated with Western blots containing heat-stable protein homogenates from MSN cells (Fig. 4*C*). Lane 1 shows HMW and LMW MAP-2 observed with monoclonal antibody AP-18, which served as the control. Lane 3 shows both HMW and LMW MAP-2 with the exon 13-generated antibody, hereafter referred to as antibody 13 (see arrows). The preimmune serum served as the negative control (lane 2). After preincubation with the synthetic peptide, the antibody did not recognize any bands on the immunoblot (lane 4).

DISCUSSION

MAP-2 is regulated in both a tissue- and developmentally specific manner and alternative splicing of MAP-2 transcripts is an efficient mechanism to generate protein diversity. The MAP-2 gene is divided among 19 exons and potentially can be spliced into at least 39 different transcripts. As illustrated in Fig. 6, 13 HMW and LMW transcripts with three and four repeats of the MTBD, plus and minus exons 8 or 13, could be transcribed from the MAP-2 gene. Furthermore, HMW MAP-2 transcripts lacking exon 11 have been reported (17). Each of these transcripts can exist with three different 5′ UTRs (14) and this would triple the possible repertoire of expressed transcripts. These 39 transcripts do not include the transcripts generated by splicing within exons as was observed for the cDNAs containing shorter regions of exons 9 and 13. In addition, LMW and HMW MAP-2 transcripts containing exons 8 and 13 together could exist.

While we have no direct evidence that either exon 8 or 13 is encoded as part of MAP-2a, exon 13 can be excluded since exon 13 is not translated in adult rat brain, a time when MAP-2a is optimally expressed. Antibody 8 recognized a HMW MAP-2 isoform whose expression increased during rat postnatal development (data not shown); however, whether exon 8 is sufficient to create MAP-2a or whether exon 16 is also required is not yet known. W.J. Chung and C.G. (unpublished results) have isolated a MAP-2 clone from an adult rat brain cDNA library containing sequences from exons 7–9, demonstrating that a HMW MAP-2 transcript containing exon 8 is expressed in adult brain. With a polyclonal antibody generated to the fusion protein encoded by rat exon 8, they determined that the antibody recognized a HMW MAP-2 isoform that migrated above MAP-2b and paralleled the expression of MAP-2a during postnatal rat development (W.J. Chung and C.G., unpublished results).

From the multiple MAP-2 transcripts, at least eight MAP-2 isoforms can be translated. These isoforms include

HMW MAP-2 containing the designated exons with either exon 1, 2 or 3.
MAP-2 with 3R: Exons 4 5 6 7 9 10 11 12 14 15 17 18 19
MAP-2 with 4R: Exons 4 5 6 7 9 10 11 12 14 15 16 17 18 19
MAP-2 plus exon 8, +/- exon 16: Exons 4 5 6 7 8 9 10 11 12 14 15 16 17 18 19
MAP-2 plus exon 13, +/- exon 16: Exons 4 5 6 7 9 10 11 12 13 14 15 16 17 18 19
MAP-2 minus exon 11: Exons 4 5 6 7 9 10 12 14 15 16 17 18 19

LMW MAP-2 containing the designated exons with either exon 1, 2 or 3.
MAP-2 with 3R: Exons 4 5 6 7 12 14 16 17 18 19
MAP-2 with 4R: Exons 4 5 6 7 12 14 15 16 17 18 19
MAP-2 plus exon 8, +/- exon 16: Exons 4 5 6 7 8 12 14 15 16 17 18 19
MAP-2 plus exon 13, +/- exon 16: Exons 4 5 6 7 12 13 14 15 16 17 18 19

FIG. 6. MAP-2 gene organization and 39 possible mRNA.

MAP-2b with three and four repeats, MAP-2c with three and four repeats, HMW MAP-2 with exons 8 or 13, and LMW MAP-2 with exons 8 or 13. We have established that HMW and LMW MAP-2 isoforms containing exon 8 or exon 13-encoded sequences exist in MSN homogenates. While RT–PCR detects exons 8 and 13 in C6 glioma mRNA, no protein was detected by immunoblot analysis.

Our data suggest that exon 8- and exon 13-encoded proteins are differentially expressed during development. Western blot analysis of P11 and adult rat brain homogenates showed that exon 8-encoded protein was synthesized only as a HMW isoform. Exon 13-encoded sequences were not detected in adult rat brain but HMW and LMW MAP-2 isoforms were expressed at P11 (data not shown). Interestingly, while we observe HMW and LMW MAP-2 isoforms expressed in MSN homogenates, we have not observed the LMW MAP-2 isoform containing exon 8 in heat-stable homogenates from postnatal rat brain, supporting the notion that the LMW isoform is expressed early in development. The expression of these isoforms in protein homogenates from embryonic and postnatal rat brain development should permit the examination of their abundance during development. Further, an extensive examination of neuronal populations should define subpopulations that express different MAP-2 isoforms. In support of this hypothesis, we have determined that in human fetal spinal cord, MAP-2c was expressed only in anterior motor neurons at 22–24 weeks of gestation (8). Also, in adult human midfrontal cortex sections, antibody 8 detects antigen in a subpopulation of neurons (B.S.-Z., unpublished results). Thus, in the central nervous system, different MAP-2 isoforms may function to regulate microtubule spacing or neurite outgrowth within subpopulations of neurons.

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