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Making Sense of the Multiple MAP-2 Transcripts and Their Role in the Neuron

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Abstract

Microtubule-associated protein-2 (MAP-2) is a family of heat-stable, phosphoproteins expressed predominantly in the cell body and dendrites of neurons. Three major MAP-2 isoforms, (MAP-2a, MAP-2b, MAP-2c) are differentially expressed during the development of the nervous system and have an important role in microtubule dynamics. Several MAP-2 cDNA clones that correspond to the major MAP-2 transcripts and additional, novel MAP-2 transcripts expressed in the CNS and PNS have been characterized. The transcripts result from the alternative splicing of a single MAP-2 gene consisting of 20 exons. Studies are now being directed toward understanding the role of the multiple MAP-2 forms that contain novel exons in the nervous system. The expression, localization, and possible functions of the newly identified spliced forms are the focus of this review.

Index Entries: Microtubule-associated protein-2; MAP-2; MAP-2 gene structure; MAP-2 transcripts.

Introduction

Neurons are highly specialized cells that extend polarized processes known as axons and dendrites. The neuronal cytoskeleton, rich in microtubules and their associated proteins, contribute to the functionally differentiated domains of these structures. Axons, responsible for propagating signals away from the cell body, are uniform in diameter and can extend many centimeters from the cell body. The major structural component of axons are microtubules that are oriented with their plus ends extending away from the cell body. By contrast, the dendrite, responsible for receiving electrical information and relaying it back to the cell body, tapers and extends less than 500 μm from the cell body. The microtubules in dendrites are of mixed polarity with the plus ends pointing away and toward the cell body

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(Bass et al., 1988; Burton, 1988). During the life of the neuron, microtubules undergo extensive growth and rearrangement providing a balance between rigidity and plasticity. Posttranslational modifications of tubulin subunits are correlated with differentially stable subsets of microtubules (reviewed by Greer and Rosenbaum, 1989; Wordeman and Mitchison, 1994). Detyrosinated and acetylated tubulin subunits are enriched in stable populations of microtubules (Gundersen et al., 1987; Piperno et al., 1987; Schulze and Kirschner, 1987). Since detyrosinated alpha-tubulin does not affect microtubule stability or assembly (Wordeman and Mitchison, 1994), it has been proposed that the posttranslational modifications of tubulin subunits promote the binding of microtubule-associated proteins (MAPs). MAPs are a diverse group of proteins that copurify with tubulin and promote the assembly of microtubules (Gaskin et al., 1974; Keates and Hall, 1975; Murphy and Borisy, 1975; Sloboda et al., 1975). In vitro, MAPs have been shown to bind to and stabilize microtubules (Cleveland et al., 1977; Mandelkow et al., 1988; Takemura et al., 1992).

Microtubule-associated protein-2 (MAP-2), tau, and microtubule-associated protein-4 (MAP-4) belong to the family of structural MAPs. Whereas MAP-4 is found in many cell types, tau and MAP-2 are highly enriched in the neuron. Tau is the major MAP found in axons and MAP-2 is the major MAP in dendrites. The structural MAPs bind to tubulin via either three or four imperfect repeats of the microtubule-binding domain (MTBD) located in the carboxy region of the molecule. Each repeat is composed of 18 amino acids separated by 13–14 amino acids. By virtue of these repeats, MAPs bind to tubulin and reduce the critical concentration of tubulin required to polymerize into microtubules (Murphy and Borisy, 1975; Sloboda et al., 1975). A number of excellent reviews have been written on the function of the structural MAPs during neuronal morphogenesis (Vallee and Bloom, 1984; Olmsted, 1986; Matus, 1988, 1994; Tucker, 1990). The intent of this review is to concentrate on the molecular and biochemical characterization of MAP-2 and discuss the multiple forms expressed in the nervous system.

Background on the Traditional MAP-2 Forms Expressed in the Brain

MAP-2 is a heat-stable, phosphoprotein that is expressed predominantly in the cell body and dendrites of neurons of the CNS (Fellous et al., 1977; Matus et al., 1981). It is also expressed in dorsal root ganglion (DRG) of the PNS (Forleo et al., 1996), reactive glia (Papasozomenos and Binder, 1986; Tucker et al., 1988), and a number of cell types in the testis (Loveland et al., 1996). Historically, MAP-2 was isolated from bovine brain and three main isoforms were identified based upon their electrophoretic mobility on SDS poylacrylamide gel electrophoresis. These isoforms, designated as MAP-2a, MAP-2b, and MAP-2c, migrate as two high-molecular-weight (HMW) forms, at approx 280 kDa (MAP-2a) and 270 kDa (MAP-2b), respectively (Kim et al., 1979; Johnson and Jope, 1992), and a low-molecular-weight (LMW) form migrating at 70 kDa (MAP-2c; Burgoyne and Cumming, 1984; Binder et al., 1984; Garner et al., 1988; Vallee, 1980). MAP-2a and MAP-2b consist of a large projection arm and a short MTBD (Murphy and Borisy, 1975; Kim et al., 1979; Vallee, 1980). The projection arm is postulated to form a 40–90-nm projection from the microtubule wall, which serves as a strut, maintaining the regular spacing of microtubules within neuronal processes (Kim et al., 1979; Voter and Erickson, 1982; Chen et al., 1992). It is believed that the projection arm interacts with other cellular proteins and further stabilize the cytoskeleton (Matus, 1994).

Multiple MAP-2 forms are expressed during development. In rodents, MAP-2b and MAP-2c are expressed during fetal CNS development during which MAP-2c functions to maintain the cytoskeleton in a more flexible state. MAP-2c contains the first 151 amino

acids of MAP-2b joined to the last 321 amino acids, thus removing a large central portion of the projection arm (Garner and Matus, 1988; Doll et al., 1990; Albala et al., 1993). The continued expression of MAP-2c in the photosensitive cells of the adult retina and in the olfactory system suggests that MAP-2c is involved in maintaining a more immature role in the neuron (Tucker and Matus, 1988). When neuronal circuitry is forming, MAP-2c expression allows for flexibility and rearrangement in the neuron (Tucker et al., 1988; Viereck et al., 1989). MAP-2c has been shown to be a poor promoter of microtubule polymerization, perhaps allowing for neuronal plasticity (Tucker, 1990). Time-lapse recordings of live, nonneuronal cells transfected with green fluorescent protein tagged-MAP-2c demonstrated that MAP-2c did not significantly affect the rates of microtubule growth and shrinkage. Over time, the MAP-2c-induced microtubules disappeared as a result of cell-shape changes (Kaech et al., 1996). The overexpression of epitope-tagged-MAP-$2c_{myc}$ in transgenic mice did not have an adverse effect on neuronal morphology or brain maturation (Marsden et al., 1996). In the adult mice brain, MAP-$2c_{myc}$ was detected in the cell bodies and in the dendrites but not in the axon demonstrating the correct expression of the protein. Chloramphenicol acetyltransferase (CAT) expression from the same vector did not exclude CAT from the axon or dendrites suggesting that the selective exclusion of MAP-$2c_{myc}$ from the axon occurs by a sorting mechanism whereby specific mRNAs or proteins are targeted to select sites in the cell. This will be discussed in more detail below.

In the developing rat brain, MAP-2b levels remain fairly constant. During synaptogenesis, there is a downregulation of MAP-2c and an upregulation of MAP-2a (Burgoyne and Cumming, 1984; Binder et al., 1984; Riederer and Matus, 1985). It was postulated that MAP-2a and MAP-2b were the result of different phosphorylation states of the same protein; however, it was not possible to generate MAP-2b from MAP-2a by phosphatase treatment (Binder et al., 1984; Kindler et al., 1990). Recent data demonstrate that the difference between MAP-2b and MAP-2a is the result of the translation of two alternatively spliced transcripts (Kalcheva et al., 1995, 1998; Chung et al., 1996; Shafit-Zagardo et al., 1997).

Molecular analysis of MAP-2 began with the identification of several cDNA clones isolated from brain libraries (Kindler et al., 1990; Lewis et al., 1988; Dammerman et al., 1989). Northern blot analysis determined that these clones detect 9.5-kb and 6 kb transcripts that encode MAP-2b and MAP-2c respectively, (Lewis et al., 1998; Dammerman et al., 1989; Kosik et al., 1988; Neve et al., 1986). However, recent studies have shown that multiple MAP-2 transcripts hybridize to the 9.5 kb and 6-kb transcripts. Studies are now being directed toward understanding the role of the multiple MAP-2 forms that contain novel exons in the nervous system. The identification of these additional MAP-2 transcripts expands the repertoire of the traditional MAP-2a, MAP-2b, and MAP-2c transcripts. The expression, localization, and possible functions of the newly identified spliced forms are the focus of this review.

Multiple MAP-2 Isoforms Encoded from a Single Gene

Studies have determined that the multiple MAP-2 transcripts expressed in neurons result from the alternative splicing of a single MAP-2 gene (Garner and Matus, 1988; Kalcheva et al., 1995). In humans, MAP-2 resides on chromosome 2 (Neve et al., 1986). The human MAP2 gene has been cloned and 20 exons have been identified (Fig. 1; Kalcheva et al., 1995). Based on the consensus sequence reported for the mouse MAP-2b cDNA (Lewis et al., 1988), the sequences encoding MAP-2b (1830 amino acids) are within exons 5–7, 9–12, 14, 15, and 17–19 (Kalcheva et al., 1995). Whereas the addition and exclusion of exons encode other MAP-2 forms, all transcripts identified to-date utilize the same AUG translational start site.

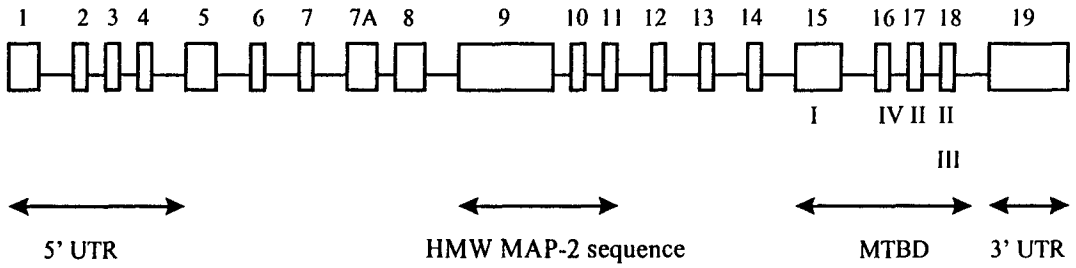

Fig. 1. Schematic representation of the human MAP-2 gene. The exons are shown as boxes separated by the introns (solid lines). Although not drawn to scale, the largest exons, exons 9 and 19, are >3.5 kb. The double arrows denote some of the important regions of the MAP-2 gene. For detailed information on the gene structure see Kalcheva et al. (1995).

The translational start site is located in exon 5 with a number of in frame stop codons located in the 5′ untranslated region (5′ UTR) that spans exons 1–5. Multiple 5′UTRs of MAP-2 transcripts have been identified by the technique of 5′ rapid amplification of cDNA ends (RACE). Studies performed with mRNA from a human neuroblastoma cell line (MSN) and human fetal spinal cord determined that MAP-2 transcripts contain three unique 5′ UTRs (Kalcheva and Shafit-Zagardo, 1995). The MAP-2 transcripts share a common domain consisting of 171 nucleotides (nt) spanning exons 4 and 5. The common domain is expressed as part of all MAP-2 transcripts identified and 81% homology is shared between the human and rodent sequence. Upstream of the common domain, there is sequence divergence as a result of alternative usage of exons 1, 2, or 3, referred to as the unique UTRs. The size of the unique 5′ UTRs in human is 281 nt (exon 1), 146 nt (exon 2), or 104 nt (exon 3). Whereas full-length 5′ UTRs have not been isolated from brain cDNA libraries, a number of clones containing partial 5′ UTRs have been reported. Human and mouse brain partial cDNA clones (Accession numbers U01828 and M21041) contained homology with exons 3–5, whereas rat cDNA clones, isolated from a rat-brain cDNA library and a C6 glioma library (Kindler et al., 1996), had homology with the 5′ UTR sequences in exon 1, 4, and 5. Northern blot analysis of steady-state mRNA demonstrated that the 281 nt UTR was the most abundant of the three unique UTRs expressed in MSN cells. Reverse transcription PCR (RT-PCR) performed with mRNA isolated from MSN cell polysome preparations demonstrated that each unique UTR is contained in HMW and LMW MAP-2 transcripts and hence the mRNAs are translated. RT-PCR performed with mRNA isolated from either fetal or adult tissue demonstrated that all three of the 5′ UTRs are expressed at the same developmental time points (Kalcheva and Shafit-Zagardo, 1995). Whereas the function of the independent 5′ UTRs is uncertain, the likelihood that these sequences serve as targeting signals for the MAP-2 transcripts is diminished by the fact that MAP-2b and MAP-2c transcripts can use each of the 5′ UTRs and only MAP-2b mRNA localizes to the dendrite (Garner et al., 1988; Papandrikopoulou et al., 1989; Tucker et al., 1989). These studies suggest that the signal for targeting MAP-2b mRNA to the dendrite resides either in the projection domain of MAP-2b mRNA not retained by MAP-2c or the signal for targeting resides on the protein (*see below*). Perhaps in response to different cellular or extracellular signals, the unique 5′ UTRs play a role in either translational control or mRNA stability (Kalcheva and Shafit-Zagardo, 1995).

Cyclic AMP (cAMP)-dependent protein kinase is composed of two regulatory subunits and two catalytic subunits to form the inactive

holoenzyme. Upon activation by cAMP, the catalytic subunit is released and is free to phosphorylate cellular proteins including MAP-2. cAMP-dependent protein kinase binds to MAP-2 via the regulatory subunit (RII) of the kinase (Vallee et al., 1981). The RII binding domain on MAP-2 is found in the amino terminus of the MAP-2 molecule (Rubino et al., 1989; Obar et al., 1989). The 31-amino-acid region encoding the binding site for RII is encoded by sequences within exons 5 and 6 (Kalcheva et al., 1995; Rubino et al., 1989). This region is highly conserved in all mammalian species examined and is retained as part of all MAP-2 isoforms.

The sequences encoding the large projection arm region of MAP-2b and MAP-2a that is removed from MAP-2c is contained within exons 9–11. The individual repeats of the MTBD are encoded by sequences within exons 15–18. Exon 15 encodes the first repeat of the MTBD and 286 bp of upstream sequence. The fourth repeat of the MTBD found to be alternatively spliced into both HMW and LMW MAP-2 transcripts (Kalcheva and Shafit-Zagardo, 1995; Doll et al., 1993; Couchie et al., 1996) is contained in exon 16. Sequences encoding the second and third repeats of the MTBD are contained within exons 17 and 18. The consensus stop codon is in exon 19 which also contains over 3.7 kb of the 3′ untranslated region (3′ UTR). In the human 3′ UTR there are six consensus polyadenylation signals and numerous dinucleotide repeats whose significance is unknown. Although the rat 3′ UTR contains four putative polyadenylation sites, two cDNA clones containing a poly (A) tail used the most 3′ polyadenylation signal, suggesting that in adult brain only one polyadenylation signal is used (Kindler et al., 1996).

The exons encoding MAP-2c protein include exons 5–7, 12, 14, 15, and 17–19. MAP-2c does not contain the fourth repeat of the MTBD encoded within exon 16. However, when the fourth repeat is spliced into MAP-2c, an additional LMW MAP-2 transcript known as MAP-2d is generated and is expressed in cultured glial cells in higher amounts than in neurons. Unlike MAP-2c, MAP-2d is expressed later in development (Doll et al., 1993). Ferhat et al. (1994), also determined that MAP-2d is expressed at high levels in the adult rat CNS and in cultured cerebellar neurons and at low levels in glial cultures. The relative abundance of MAP-2d in the cultures may be reflected by the different culture conditions. Olesen (1994) expressed recombinant MAP-2c and MAP-2d and, by in vitro microtubule-assembling assays, determined that both recombinant proteins were biologically active. When tubulin polymerization rates were measured, tubulin polymers were formed twice as fast in the presence of MAP-2d when compared to MAP-2c. These results suggest that like mature tau with four repeats, MAP-2d contributes to the rapid rate of microtubule polymerization (Langkopf et al., 1994). The significance of this finding in the mature CNS is not known however, Ferhat determined that in vitro MAP-2d, but not MAP-2c, was capable of binding to actin (Ferhat et al., 1996). This is in contrast to the results of Cunningham et al. (1997) who determined that MAP-2c, but not MAP-2b or tau, was able to bind to actin in actin-binding protein 280-deficient melanoma cells.

Two additional exons were identified during the cloning of the human gene. These were designated as exon 8 and exon 13 based upon their position in the gene. Both exons are expressed in MSN cells, human brain, and spinal cord (Kalcheva et al., 1995). Northern blot analysis detected a 9.5-kb transcript in adult rat brain mRNA probed with exon 8. RT-PCR confirmed that exon 8 is transcribed in brain as a HMW MAP-2 form (Kalcheva et al., 1995; Guilleminot et al., 1995). Exon 8 is 246 nt and, when translated, adds 83 amino acids (10 kDa) to MAP-2. This could account for the difference in the observed molecular weight of MAP-2a and MAP-2b. Antibodies generated against either exon 8-expressed protein or synthetic peptides corresponding to exon 8-encoded sequences showed that exon 8 is expressed in a manner that parallels the expression of MAP-2a during rodent postnatal development (Chung et al., 1996; Shafit-

Zagardo et al., 1997). A rat cDNA clone spanning exons 7, 8, and 9 was isolated from an adult cDNA library (Chung et al., 1996) and antibodies generated to the expressed exon 8 revealed that the newly spliced form containing exon 8 is MAP-2a. This further supported the concept that MAP-2a is a splice variant and not a phosphorylation variant of MAP-2b.

Although studies demonstrate that a HMW MAP-2 form expressing exon 8 exists in mature brain and appears to be MAP-2a, the cDNA clone did not extend far enough 3′ to determine whether exon 13 or the fourth repeat of the MTBD are also part of MAP-2a (Chung et al., 1996). In human fetal spinal cord and in MSN cells, exon 13 is transcribed as part of both HMW and LMW transcripts and a HMW MAP-2 transcript consisting of MAP-2b+8+13 has been identified (Kalcheva et al., 1997). Unfortunately, it has not been possible to obtain intact HMW MAP-2 from autopsied human brain to verify whether MAP-2a consists only of MAP-2b and exon 8 or exon 13 is also present. However, in human brain, immunostaining with antibodies generated to exon 8- or exon 13-encoded sequences did not overlap, suggesting that multiple MAP-2 forms containing 8 and 13 exist (Kalcheva et al., 1997; Shafit-Zagardo et al., 1997, and unpublished observations). In rat brain, MAP-2a has been shown to consist of MAP-2b with three repeats of the MTBD and exon-8 but not exon-13 (Kalcheva et al., 1998).

In human fetal spinal cord, MAP-2c and MAP-2b transcripts are expressed during early gestation. In addition, six other MAP-2 transcripts were detected by RT-PCR at 23 wk gestation. Of the six alternatively spliced transcripts, MAP-2b+8, MAP-2b+13, MAP-2b+8+13, MAP-2c+8, MAP-2c+8+13, and MAP-2c+13, three were translated. The translated proteins, MAP-2b+8, MAP-2b+13, and MAP-2c+8+13, were detected by Western blot analysis using polyclonal antibodies generated to synthetic peptides of sequences encoded by exon 8 and exon 13. Immunocytochemical studies showed that staining was detected in independent and overlapping populations of neurons (Kalcheva et al., 1997).

A novel MAP-2 exon, exon 7A was identified in rat spinal cord (Forieo et al., 1996). RT-PCR performed with cDNA prepared from spinal cords of 6- and 36-d-old rats generated multiple HMW MAP-2 products. These products consisted of sequences that encode for MAP-2b, and additional products that contained exon 8 as well as a novel exon designated 7A. Exon 7A (237 nt) was inserted in frame after exon 7 and prior to exon 8. Products containing MAP-2b sequences plus exon 7A and 8 (MAP-2b+7A+8), MAP-2b+7A, and MAP-2b+8 were all expressed in the mature spinal cord as additional splice variants (Couchie et al., 1996). Northern blot analysis determined that exon 7A is most abundant in the adult spinal cord with negligible amounts detected in immature spinal cord and brain. Sequence data confirmed that exon 7A is expressed in human spinal cord RNA and that the exon is approx 90% homologous to the rat sequence. The human sequence has an additional 6 nt that are in-frame with the existing open reading frame for rat exon 7A (BSZ, unpublished results). However, in the absence of an antibody to exon 7A-encoded sequences the presence of the protein and its distribution in the spinal cord are unknown.

The expression of MAP-2 mRNAs in the peripheral nervous system has been examined in rat dorsal root ganglia. RT-PCR amplification of MAP-2 using primers from the middle of the projection arm and the 3′ end determined that MAP-2b exists with either 3 or 4 repeats of the MTBD at day 36. Exon 13 is not expressed at any developmental time in DRG. Exon 7A is expressed in very low amounts by RT-PCR, but is not detected by Northern analysis. Other forms of MAP-2 that have been detected by RT-PCR in DRG at 36-d postnatal development are MAP-2b minus exon 10 plus 3 or 4 repeats of the MTBD; MAP-2b minus exon 11 plus 4 repeats of the MTBD; and MAP-2b plus 7A and 8 with 3 repeats of the MTBD (Forleo et al., 1996).

Functional Significance of the Multiple MAP-2 Isoforms

MAP-2 may play a role in dendritic polarity, outgrowth, branching, plasticity, spacing, and stability of microtubules during different stages of neuronal development. This dynamic and multistage process may be orchestrated by different MAP-2 isoforms expressed at different times of development and as a result of posttranslational modifications on the MAP-2 molecule and on microtubules. MAP-2 expression coincides with neuritic outgrowth in several cell lines and primary cultures (Chamak et al., 1987; Ferreira et al., 1989, 1990; Fischer et al., 1991) and treatment of neuronal cultures with MAP-2 antisense oligonucleotides result in neurons with shorter and less branched neurites. In addition, microtubule spacing is disrupted in antisense-treated cultures indicating that MAP-2 is required for elongation of dendrites and for the spacing of microtubules (Dinsmore and Solomon, 1991; Caceres et al., 1992; Sharma et al., 1994). Whereas Friedrich and Aszodi (1991) suggested that changes in the length of the projection arm of MAP-2 may mediate the branching of dendrites, a specific MAP-2 form has not been found to localize to branch points or to be expressed in higher amounts in neurons with extensively branched processes.

It is generally accepted that MAP-2b is required for neurite outgrowth, whereas the expression of MAP-2c in processes allows for greater neurite flexibility and rearrangement during early development. Known to correlate with synaptogenesis, the developmental switch in MAP-2 protein expression from MAP-2c to MAP-2a occurs during maturation of neuronal processes and is believed to be related to the increase in thickness of dendrites that takes place during their growth (Morest, 1962). The varying length of the projection arm of MAP-2 serves as a spacer between microtubules and in Sf9 cells, the expression of MAP-2b or MAP-2c resulted in cells containing microtubules with different spacing (Chen et al., 1992). Microtubules in cells expressing MAP-2b were approx 50 nm apart, whereas MAP-2c-expressing cells had microtubules with spacing of approx 20 nm apart. The microtubule spacing in the transfected cells was consistent with the presence of MAP-2b observed in dendrites in brain and MAP-2c in spinal cord. If the length of the projection arm of MAP-2 determines the spacing of the microtubules, then changes in the length of the projection arm of additional MAP-2 isoforms should result in microtubules with varied spacing. Indeed, Voter and Erickson (1982) demonstrated that taxol-stabilized microtubules decorated with MAP-2 isolated from adult hog brain could extend from the microtubule wall up to 90 nm in length. Although taking that data into account, the additional 57 amino acids from expressed exon 13, or the additional 83 amino acids from expressed exon 8, would lengthen the MAP-2 projection arm, but this would not significantly alter the spacing of microtubules. Perhaps interactions with other cellular proteins or phosphorylation of the MAP-2 molecule can alter the rigidity of the protein or affect the spacing of the microtubules and their dynamic state in the neuron. It has been reported (Guilleminot et al., 1995; Chung et al., 1996) that sequences encoded in exon 8 of MAP-2 are similar to sequences in the MARCKS protein (amino acids 103–135) known to interact with the actin cytoskeleton. However, only 35% of the amino acids in these two sequences are identical, and this identity does not include the basic region of the MARCKS protein known to be phosphorylated by protein kinase C and to regulate calmodulin binding (Stumpo et al., 1989).

An approach to address the role of the additional MAP-2 isoforms is to examine their ability to promote microtubule assembly and tubulin polymerization relative to the known MAP-2b and MAP-2c. As noted above, MAP-2d was shown to polymerize microtubules more readily than MAP-2c thereby increasing the rate of microtubule assembly (Olesen, 1994). In COS cells, MAP-2c allows the forma-

tion of long parallel arrays of stable microtubules that as in brain and in other tissues, have been shown to be detyrosinated or acetylated (Piperno et al., 1987; Gurland and Gundersen, 1993; Bulinski and Gundersen, 1991; Cambry-Deakin and Burgoyne, 1987; Baas and Black, 1990). Transfection of the individual MAP-2 constructs into nonneuronal or neuronal cells would address this question. Whether the newly identified MAP-2 forms can alter microtubule dynamics or the encoded sequences interact with cellular proteins that impact on either thickening or stabilizing dendrites remains to be determined. Transfection experiments and experiments designed to examine protein–protein interactions are ongoing.

MAP-2 isoforms are heat-stable, phosphoproteins that contain PEST sequences found in proteins that undergo rapid proteolytic degradation (Friedrick and Aszedi, 1991). The sequences are repeats of proline, glutamic acid, serine, and threonine and most likely account for the fact that MAP-2 is highly susceptible to proteolysis. The ability of MAP-2 to be rapidly degraded may influence microtubule dynamics. Changes in phosphorylation may differentially regulate the MAP-2 forms and indeed there are numerous phosphorylation sites in the projection arm of the HMW MAP-2 forms that are not present in the LMW MAP-2 forms (Theurkauf and Vallee, 1983; Hernandez et al., 1987; Tsuyama et al., 1986; Hoshi et al., 1992). In addition, there are phosphorylation sites in the microtubule-binding domain (Ainsztein and Purich, 1984). It has been observed that depending upon the state of phosphorylation, MAP-2 in living cells can be bound to microtubules or remain soluble in the cytoplasm (Brugg and Matus, 1991). In vitro, phosphorylation has been shown to alter the ability of MAP-2 to promote tubulin assembly, and increased phosphorylation blocks the binding of MAP-2 to microtubules (Chen et al., 1992; Burns et al., 1984). MAP-2 has many consensus phosphorylation sites that, in vivo, may regulate the rigidity of the projection arm and interactions with cellular proteins. It is possible that within the MAP-2 projection arm there is electrostatic repulsion between the phosphate moieties that results in an elongated and possibly stiffer MAP-2 molecule (Tsuyama et al., 1987). Again, the presence of additional encoded sequences from exons 7A, 8, or 13 or the deletion of the sequences encoded by exons 10 or 11 may impact on the microtubule dynamics in the CNS and the PNS.

The generation of antibodies to sequences encoded by the specific exons will yield insight into the neuronal subpopulations and the subcellular localization of these novel MAP-2 forms. The data available indicate that different MAP-2 forms are expressed in distinct subpopulations of neurons. Exon 8-encoded sequences are found in subpopulations of neurons in adult brain and spinal cord (Chung et al., 1996; Shafit-Zagardo et al., 1997). In addition, it was determined that antibodies recognizing sequences within the projection arm and to sequences in exon 8 stain mitochondria and postsynaptic densities (Shafit-Zagardo et al., 1997). The significance of these finding requires further study. Antibodies generated to sequences encoded by exon 8, exon 13, and the joiner region of MAP-2c have determined that in human fetal spinal cord, subpopulations of neurons express these forms at different developmental times (Kalcheva et al., 1997; Albala et al., 1995).

Localization of HMW MAP-2 mRNA to the Dendrite with the Exclusion of MAP-2c mRNA

In situ hybridization using oligonucleotides to the MAP-2c joiner sequence (Garner et al., 1988) or a myc probe for MAP-2c$_{myc}$ in transgenic animals (Marsden et al., 1996) demonstrated that MAP-2c mRNA is expressed solely in the cell body and does not enter the dendrite or the axon. By contrast, a probe specific to HMW MAP-2 demonstrated the presence of

native HMW MAP-2 mRNA throughout the dendrite (Marsden et al., 1996; Kindler et al., 1996; Tucker et al., 1989; Bruckenstein et al., 1990; Kleiman et al., 1990). The reason for the exclusion of MAP-2c mRNA from the dendrite is not clear since the protein is found in the dendrite. Therefore, RNA targeting is not obligatory for protein targeting. Since it is known that the HMW MAP-2 forms are involved in dendritic outgrowth, it is possible that the majority of the MAP-2c mRNA is synthesized prior to process outgrowth and therefore is retained in the cell body. How the mRNA is retained in a cellular locale or what permits HMW MAP-2 mRNA to enter the dendritic subcellular domain is not known. How neurons presort mRNAs targeted to dendrites or other cellular locales is presently under study. Messenger RNAs in neurons have been reported to localize on granules and to travel via microtubules, suggesting that the dendritic localization of MAP-2b mRNA relies on a mechanism whereby cellular mRNAs are first presorted (Knowles et al., 1996). The signal for granule transport of the select mRNAs found in the dendrite may reside within the HMW MAP-2 mRNA and occur via RNA binding proteins. Once in the dendrite, the MAP-2 mRNA and the other messages known to reside in the distal portion of the dendrite can be translated on the abundant polysomes; growth cones and dendrites have the necessary machinery for protein synthesis (Crino and Eberwine, 1996). Another possibility is that the HMW MAP-2 mRNA enters the dendrite associated with polysomes and the final translation of the protein occurs in the dendrite. This mechanism, which would rely on the partial synthesis of HMW MAP-2, seems unlikely since it was determined that treatment of neurons with the protein-synthesis inhibitor puromycin did not inhibit dendritic transport of RNA (Kleiman et al., 1993). It is interesting to note that proteins having mRNAs larger than HMW MAP-2, such as MAP-1b, are excluded from the dendrite, further discounting the idea that HMW MAP-2 is too large a protein to be fully translated in the cell body. One might also invoke the "what came first, the chicken or the egg?" approach to address why HMW MAP-2 mRNA is at the distal portion of the dendrite. If HMW MAP-2 is responsible for dendritic outgrowth, then the microtubules are slowly becoming elongated within the dendrite at the same time that MAP-2 is being synthesized. As demonstrated by antisense studies, the elongation of the dendrite may be totally dependent upon the continued synthesis of HMW MAP-2 and the addition of the protein to growing and extending microtubules. The dendrite is highly specialized in its ability to relay signals back to the cell body and this may require microtubules, hence the presence of oppositely oriented microtubules in the dendrite. HMW MAP-2 mRNA may have a very long half-life and continue to survive in the dendrite for continuous MAP-2 protein synthesis required for microtubule dynamics within the dendrite. Perhaps the different UTRs are responsible for regulating the stability of the individual MAP-2 mRNAs.

Multiple Sorting Mechanisms for MAP-2 in Neurons: MAP-2 and Neuronal Polarity

The polarized distribution of MAP-2 in neurons is well established and believed to be important for neuronal morphogenesis (Bernhardt and Matus, 1984; Caceres et al., 1984; Cummings et al., 1984; De Camilli et al., 1984). Multiple mechanisms are proposed for the compartmentalization of LMW and HMW MAP-2 to the dendrite. These include the differential localization of MAP-2 mRNAs, differential turnover of MAP-2, suppressed transit of HMW MAP-2 into the axon by its N-terminal projection domain, and differential phosphorylation of MAP-2 by a number of kinases (Marsden et al., 1996; Okabe and Hirokawa, 1989; Kanai and Hirokawa et al., 1996). MAP-2

is differentially phosphorylated throughout development and in response to external stimuli such as glutamate activation (Tucker, 1990; Quinlin and Halpain, 1996a,b). The phosphorylation of MAP-2 also regulates its association with microtubules (Tucker, 1990). Transfection studies with various MAP-2 constructs into primary cultured neurons or microinjection of biotinylated MAP-2b, MAP-2c, or tau into mature spinal cord neurons determined that MAP-2b and MAP-2c proteins preferentially localize to the cell body and dendrites following extended times in culture (Kanai and Hirokawa, 1995; Hirokawa et al., 1996). Neuronal polarity examined in MAP-2c transgenic mice determined that MAP-2c protein localized to the dendrite and not the axon. As noted above, the MAP-2c mRNA does not enter the dendrite, therefore, the signal for MAP-2c localization to the dendrite appears to reside on the protein (Marsden et al., 1996). If the putative signal resides on the MAP-2c protein, then the dendritic localization signal must also reside on HMW MAP-2 proteins because of shared amino acid sequences. Whether this signal is on the native protein or a result of posttranslation modification such as phosphorylation remains to be determined.

Summary

The recent identification of additional MAP-2 transcripts has expanded the number of possible MAP-2 forms expressed in the nervous system, complicating our understanding of the coordinated regulation of the multiple MAP-2 forms. The MAP-2 promoter has not been identified and it is not known whether there is a single promoter or multiple promoters that regulate the multiple MAP-2 transcripts. The isolation and characterization of putative regulatory sequences located in the promoter, the 5′ UTR, the 3′UTR, and possibly within introns will help to sort out the complex regulation, expression, and localization of MAP-2. Native MAP-2 sequences and post-translational modifications also will impact on MAP-2 protein localization and neuronal polarity. Various sites on MAP-2 are phosphorylated by a number of kinases that regulate MAP-2 interactions with microtubules and other regulatory proteins (i.e., calmodulin, cAMP-dependent protein kinase). Some of these kinases may be developmentally expressed or activated by signal-regulated events resulting in the finely tuned regulation of MAP-2 that is important for neuronal polarity and cytoskeletal interactions.

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