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Two novel HMW MAP2 variants with four microtubule-binding repeats and different projection domains

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Abstract The brain microtubule-associated protein MAP2 is composed of two high molecular (MAP2a and b) and one low molecular (MAP2c) weight isoforms. All these forms were thought to contain three repeated microtubule-binding domains in their C-terminal region but a MAP2c variant containing four repeats has recently been identified. We report here the existence of two high molecular weight MAP2 isoforms with four microtubule-binding domains in the sensory neuronal cell line ND 7/23. A stretch of 135 bp is missing in one of these forms suggesting that several HMW MAP2 variants can be produced by alternative splicing.

Key words: Neuron; Cytoskeleton; Microtubule; MAP2a/b; Alternative splicing

1. Introduction

Two of the major microtubule-associated proteins, tau and MAP2, are believed to play an important role during neuritogenesis and acquisition of neuronal polarity [1–3]. Tau is essentially axonal whereas MAP2a/b is present only in dendrites. The composition and activity of these proteins changes markedly during development [4]. Two or three LMW tau forms of 48 kDa are expressed in the brain at early postnatal stages and five or six variants of 50–65 kDa in adulthood. Several variants of much higher molecular weight (HMW taus) have been identified in the peripheral nervous system [5–9]. All these LMW and HMW tau species are produced from a single gene by alternative splicing [8]. Five out of 15 of the exons of this gene can be differentially alternatively spliced depending on the developmental stage and the region of the nervous system [9].

The structure of the MAP2 gene is unknown and the number of encoded entities described so far is much lower. Two HMW species of 270 kDa (MAP2a and MAP2b) are expressed in adulthood whereas MAP2b and a much smaller variant of 70 kDa (MAP2c) have been identified at early developmental stages [10–14]. MAP2a/b is only present in dendrites, whereas MAP2c seems to be distributed in all the neuronal domains and in astroglial cells [1,15,16]. The cDNAs for MAP2b and MAP2c have been cloned [17,18]. They are encoded by different mRNAs of 9 and 6 kb, respectively, which are produced from a single gene by alternative splicing [13]. The difference between MAP2a and MAP2b is not known.

The higher tubulin polymerization activity of the mature tau forms [19] has been ascribed to the presence of four homologous repeats [20] in their C-terminal domain compared to three repeats in the immature forms [21]. In contrast, MAP2b and MAP2c seemed to contain always three repeats, whatever the developmental stage. Recently, MAP2c forms with four repeats have been identified [22–24].

In this work we report for the first time the existence of two high molecular weight MAP2 isoforms containing four repeats in a neuronal cell line (ND 7/23) obtained by fusion of primary sensory neurons with neuroblastoma cells [25]. In one of these transcripts a stretch of 135 bp coding for 45 amino acids is missing, indicating a shorter projection domain of that protein.

2. Materials and methods

ND 7/23 cells [25] were cultured in L15-medium (Gibco) with 10% FCS (Gibco), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine in 5% CO_2 at 37°C. Differentiation was done for three days in L15 medium complemented with 0.5% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 1 mM DBcAMP and 50 ng/ml NGF.

Total RNA was isolated as described [26]. Poly(A)$^+$ RNA was purified by chromatography on oligo(dT) cellulose (Pharmacia).

Total RNA from P6 and P52 rat brain and poly(A)$^+$ RNAs from non-differentiated and differentiated ND 7/23 cells were reverse transcribed from an oligo(dT) primer using a first strand synthesis kit (Stratagene).

Amplification of the first strand products was carried out in a DNA thermal cycler from Perkin Elmer-Cetus Instruments. Samples were subjected to 32 cycles of amplification using 50 ng first strand product, 200 ng of each primer and 2.5 units of Taq DNA polymerase in a 100 μl volume. Each cycle consists of a 1-min denaturation step at 94°C, a 2-min annealing step at 58°C and a 3-min elongation step at 72°C. After the last cycle samples were incubated for an additional 5 min at 72°C to ensure completion of the final extension step. The oligonucleotides used as primers in PCR experiments based on the rat sequence of Kindler et al. [18] were the following: primer 1 (nt 187–206) 5′-TCACAGGGCACCTATTCAGA-3′, primer 2 (nt 4,162–4,181) 5′-ATTATGGATGCCGACAGCCT-3′, primer 3 (nt 5,196–5,215) 5′-CCTTCTCCTTGAAATCCAGC-3′.

An aliquot of the amplified DNA (5 μl for brain, 25 μl for cells) was separated in a 1.6% agarose gel and transferred to a nylon membrane (Hybond, Amersham) as described [27]. The blots were hybridized with a MAP2c-specific cDNA [28]. This probe was labeled using the megaprime technique (Amersham) with [α-^{32}P]dCTP. Blots were washed for 10 min at room temperature in 2× SSC (600 mM NaCl, 60 mM Na-citrate), 0.1% SDS and 30 min in 2× SSC, 0.1% SDS at 65°C and exposed to Hyperfilm MP (Amersham).

PCR-fragments subcloned in the plasmid pGEM-T (Promega) were sequenced using a kit from USB according to the manufacturer's instructions.

3. Results

To establish the existence of a HMW MAP2 form we performed PCR experiments with oligonucleotides specific for MAP2b as indicated in Fig. 1. More than one band seems to be present on the blots of the P6 and P52 brain preparations

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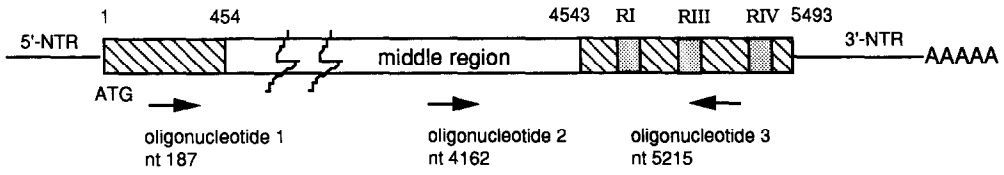

Fig. 1. Schematic representation of the MAP2b sequence (numbering refers to the rat sequence of Kindler et al. [18]) showing the position of the oligonucleotides used as primers to produce a HMW MAP2 fragment (MAP2b; oligonucleotides 2 and 3) and a LMW MAP2 fragment (MAP2c; oligonucleotides 1 and 3). The localization of the C-terminal microtubule-binding domains is represented as shadowed boxes. The sequence of MAP2c is shown by the N- and C-terminal hatched regions.

whereas three bands were revealed with non-differentiated and differentiated ND cells (Fig. 2A). In contrast, a single PCR fragment was obtained (Fig. 2B) with oligonucleotides specific for MAP2c (Fig. 1). In both cases, weaker signals were observed with differentiated ND cells compared to non-differentiated cells.

The MAP2b-specific PCR fragments obtained from ND cells were cloned and the sequence of six clones was determined (Fig. 3). All these clones had a 93 bp insertion between repeat I and II of the MAP2b sequence (Figs. 3 and 4). This means that in ND cells there exist HMW MAP2 isoforms containing four microtubule-binding domains. The sequence of this additional repeat is identical to that published by Ferhat et al. [23] for a four repeat MAP2c variant. In one set of three clones with an insert size of 1,144 bp the rest of the sequence was identical to the rat MAP2b sequence [18]. The other three clones with an insert size of 1,009 bp had a deletion of 135 bp (Figs. 3 and 4) directly upstream the junction between MAP2b and MAP2c. This deletion does not alter the reading frame and the sequences at the 5′ and 3′ frontiers are consistent with the presence of splicing consensus sites at both ends. An additional difference to the rat MAP2b form is the absence of three nucleotides at position 187 in all clones.

Cloning and sequencing of the PCR products obtained with brain RNAs revealed only HMW transcripts containing three repeats.

The PCR fragment specific for MAP2c (Fig. 1) had the length of 1,029 bp calculated for a MAP2c species containing four repeats. Sequencing of this fragment confirmed that it contained the same fourth repeat identified recently [23].

4. Discussion

The data reported in this work demonstrate for the first time the existence of two HMW MAP2 variants containing four repeats in their C-terminal domain (Fig. 4). In one of these new variants, the sequence of the amplified projection domain was identical to that found for MAP2b whereas the second variant was shorter by 135 bp (i.e. 45 amino acids). Although the polymerization activity of the fourth repeat identified in these new HMW entities and in the recently discovered MAP2c variant have not been tested one may assume, by analogy with the situation described for tau proteins [20,21], that microtubules assembled in the presence of these entities are more stable than those produced in the presence of three repeat forms. Contrarily, Doll et al. [22] have shown that, when transfected to non-neuronal cells, the four-repeat MAP2c variant behaved indistinguishly compared to the three repeat form in stabilizing and rearranging cellular microtubules.

It is not clear whether this new HMW MAP2 species corresponds to MAP2a which appears in the brain late during development and which has never been cloned. Indirect results have been obtained by Kindler et al. [24] on the presence of HMW MAP2 transcripts containing four repeats in the brain: Northern blot analysis with an oligonucleotide complementary to repeat II of MAP2c revealed both the MAP2a/b specific 9 kb transcript and the MAP2c specific 6 kb transcript. In contrast, Doll et al. [22] detected by the same method only the 6 kb message in rat brain. In this work, we have been unable to obtain brain PCR products derived from four-repeat HMW transcripts.

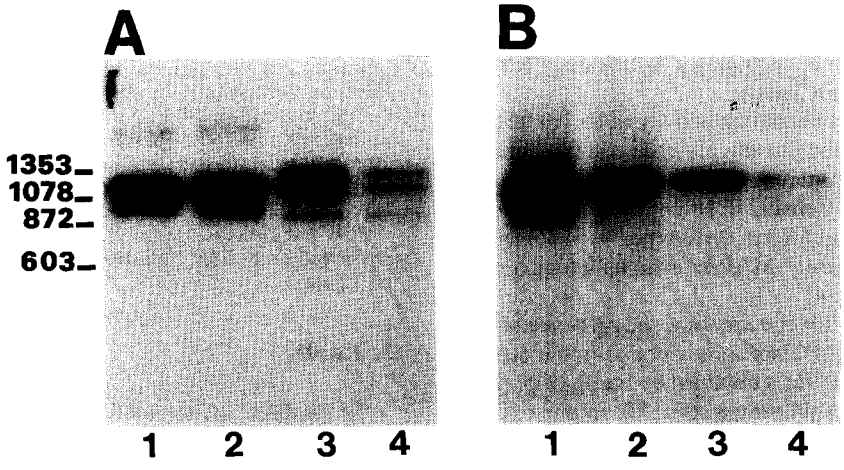

Fig. 2. Southern blot analysis of the HMW (A) and LMW (B) MAP2 fragments obtained by PCR amplification. Lane 1, P6 rat brain; lane 2, P52 rat brain; lane 3, non-differentiated ND cells; lane 4, differentiated ND cells. The blots were hybridized with a MAP2c-specific probe [28].

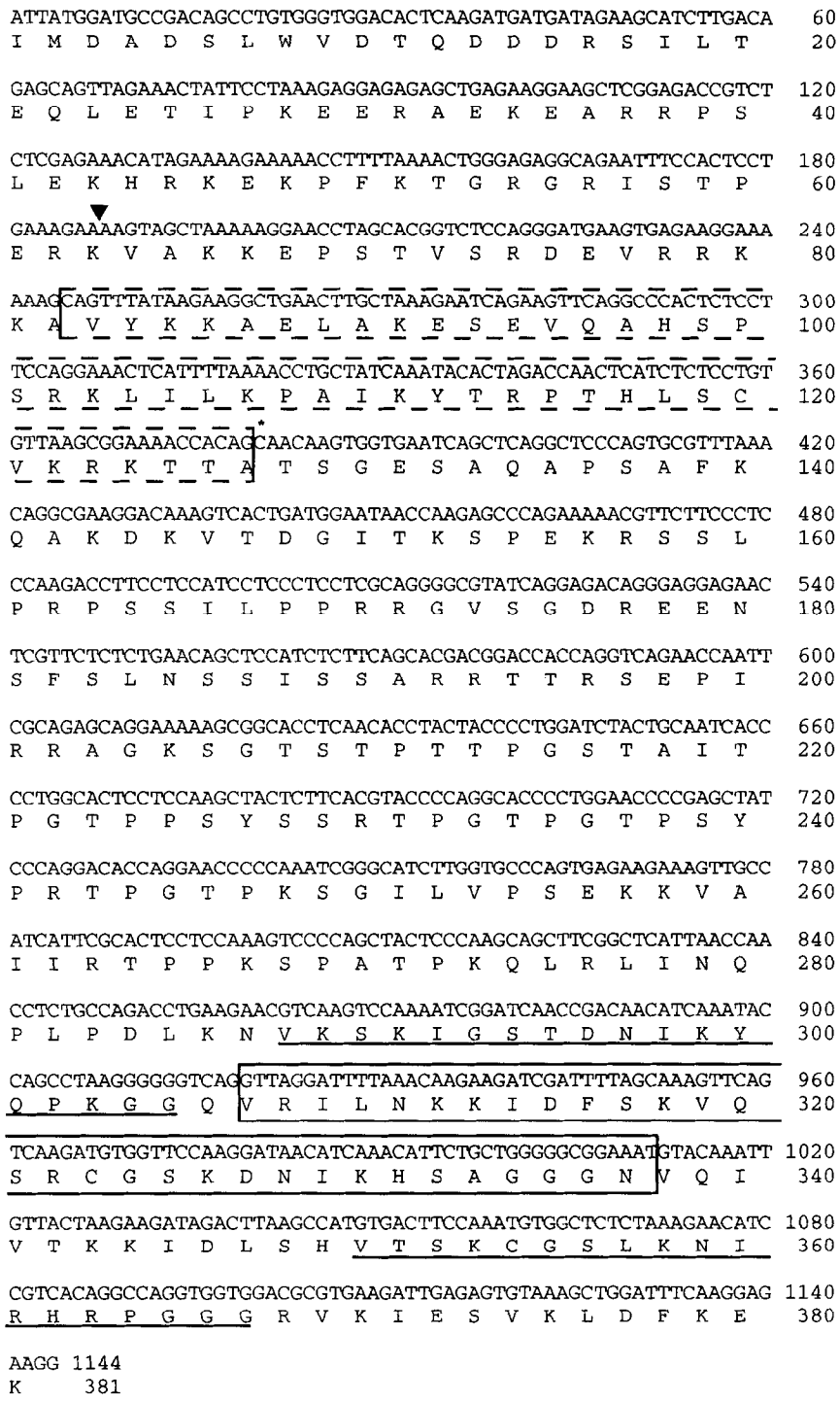

Fig. 3. Nucleotide and deduced amino acid sequence of the four repeat HMW MAP2 PCR fragments. The boxed region corresponds to the fourth repeat (repeat II) and the dashed region to the 135 bp domain which is absent in one set of the PCR clones. The other homologous repeats (repeats I and III) are underlined. *Marks the splice junction between MAP2b and MAP2c, and (▼) the position of the three nucleotides absent in sequences established in ND cells.

Since ND cells have a peripheral origin [25] it might be that the HMW MAP2 variants identified in this work are expressed specifically in neurons of the peripheral nervous system. The diversity of MAP2 proteins in neurons could influence their function during development of the nervous system and acquisition of the neuronal polarity.

ND cells also express the same MAP2c variant which has been discovered recently in rat brain and which contains four repeats [22,23]. MAP2b differs from MAP2c by the presence of a long middle region of approximately 200 kDa apparent molecular weight [18]. Early work on MAP2a/b suggested that a C-terminal fragment of 36 kDa, which retains the polymeriza-

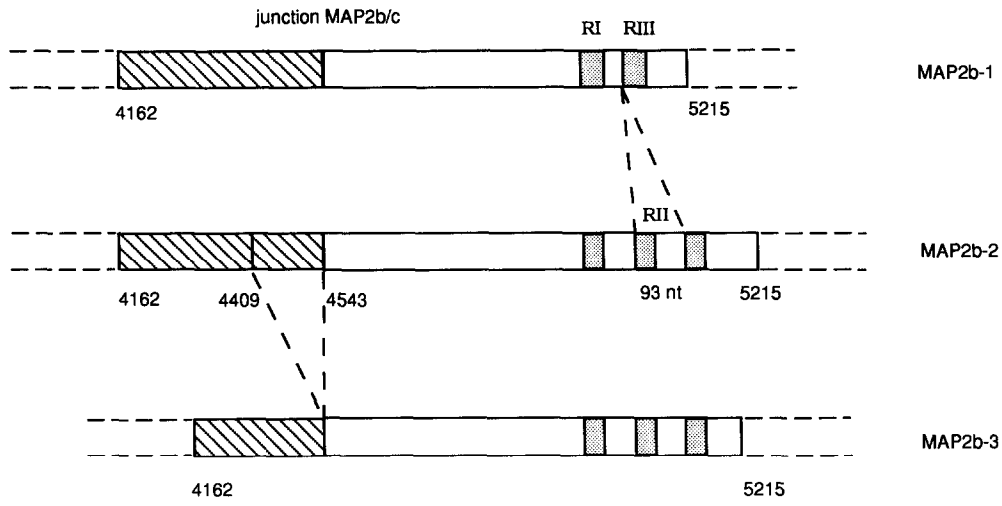

Fig. 4. Schematic representation of MAP2b from rat brain (MAP2b-1, [18]), compared to the isoforms identified in this work in ND cells, i.e. MAP2b-2, four repeat form, and MAP2b-3, four repeat form missing 135 bp.

tion activity of MAP2, can be cleaved and binds to the microtubule lattice [29]. It has been proposed that the rest of the molecule, i.e. the middle and N-terminal regions, that projects from the microtubule wall [30], determines the spacing between microtubules in the dendrites and is responsible for the interaction of MAP2 with itself [31] or other components of the cytoskeleton [32,33]. The functional significance of a HMW MAP2 variant containing four repeats and a shorter projection domain compared to MAP2b remains to be determined. One may assume that a shorter projection domain may alter either the distance between microtubules and/or their interaction with other cytoskeletal elements. Another possibility is that the long middle region within the HMW MAP2 transcript might contain the signal for targeting MAP2b mRNA into the dendrites [34], but the precise location of this signal has never been determined.

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References

[1] Bernhardt, R. and Matus, A. (1984) J. Comp. Neurol. 226, 203–221.
[2] Binder, L.I., Frankfurter, A. and Rebhun, L.I. (1985) J. Cell Biol. 101, 1371–1378.
[3] Brion, J.P., Guilleminot, J., Couchie, D., Flament-Durand, J. and Nunez, J. (1988) Neuroscience 25, 139–146.
[4] Mareck, A., Fellous, A., Francon, J. and Nunez, J. (1980) Nature 284, 353–355.
[5] Couchie, D., Mavilia, C., Georgieff, I.S., Liem, R.K.H., Shelanski, M.L. and Nunez, J. (1992) Proc. Natl. Acad. Sci. USA 89, 4378–4381.
[6] Goedert, M., Spillantini, M.G. and Crowther, R.A. (1992) Proc. Natl. Acad. Sci. USA 89, 1983–1987.
[7] Georgieff, I.S., Couchie, D., Mavilia, C., Liem, R.K.H., Nunez, J. and Shelanski, M.L. (1993) J. Cell Sci. 105, 729–737.
[8] Mavilia, C., Couchie, D., Mattei, M.G., Nivez, M.P. and Nunez, J. (1993) J. Neurochem. 61, 1073–1081.
[9] Mavilia, C., Couchie, D. and Nunez, J. (1994) J. Neurochem., in press.
[10] Couchie, D. and Nunez, J. (1985) FEBS Lett. 118, 331–335.
[11] Riederer, B. and Matus, A. (1985) Proc. Natl. Acad. Sci. USA 82, 6006–6009.
[13] Garner, C.C. and Matus, A. (1988) J. Cell Biol. 106, 779–783.
[14] Garner, C.C., Brugg, B. and Matus, A. (1988) J. Neurochem. 50, 609–615.
[15] Tucker, R.P. (1990) Brain Res. Rev. 15, 101–120.
[16] Charrière-Bertrand, C., Garner, C.C., Tardy, M. and Nunez, J. (1991) J. Neurochem. 56, 385–391.
[17] Wang, D., Lewis, S.A. and Cowan, N.J. (1988) Nucleic Acids Res. 16, 11369–11370.
[18] Kindler, S., Schulz, B., Schwanke, B. and Garner, C.C. (1990) Nucleic Acids Res. 18, 2822.
[19] Francon, J., Lennon, A.M., Fellous, A., Mareck, A., Pierre, M. and Nunez, J. (1982) Eur. J. Biochem. 129, 465–471.
[20] Kosik, K.S., Orecchio, L.D., Bakalis, S. and Neve, R.L. (1989) Neuron 2, 1389–1397.
[21] Lee, G., Cowan, N. and Kirschner, M.W. (1988) Science 239, 285–288.
[22] Doll, T., Meichsner, M., Riederer, B.M., Honneger, P. and Matus, A. (1993) J. Cell Sci. 106, 633–640.
[23] Ferhat, L., Ben-Ari, Y. and Khrestchatisky, M. (1994) C.R. Acad. Sci. Paris 317, 304–309.
[24] Kindler, S. and Garner, C.C. (1994) Mol. Brain Res., in press.
[25] Wood, J.N., Bevan, S.J., Coote, P.R., Dunn, P.M., Harmar, A., Hogan, P., Latchman, D.S., Morrison, C., Rougon, G., Theveniau, M. and Wheatley, S. (1990) Proc. R. Soc. Lond. 241, 187–194.
[26] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[27] Southern, M. (1975) J. Mol. Biol. 98, 503–517.
[28] Langkopf, A., Guilleminot, J. and Nunez, J. (1994) J. Neurochem., in press.
[29] Vallee, R.B. and Borisy, G.G. (1977) J. Biol. Chem. 77, 3206–3210.
[30] Voter, W.A. and Erickson, H.P. (1982) J. Ultrastruct. Res. 80, 374–382.
[31] Furtner, R. and Wiche, G. (1987) Eur. J. Cell Biol. 45, 1–8.
[32] Miyata, Y., Hoshi, M., Nishida, Y., Minami, Y. and Sakai, H. (1986) J. Biol. Chem. 261, 13026–13030.
[33] Hirokawa, N., Hisanaga, S.-I. and Shiomara, Y. (1988) J. Neurosci. 8, 2769–2779.
[34] Papandrikopoulou, A., Doll, T., Tucker, R.P., Garner, C.C. and Matus, A. (1989) Nature 340, 650–652.