Participation of structural microtubule-associated proteins (MAPs) in the development of neuronal polarity

ARTICLE *in* JOURNAL OF NEUROSCIENCE RESEARCH · APRIL 2002

Impact Factor: 2.59 · DOI: 10.1002/jnr.10161 · Source: PubMed

6 AUTHORS, INCLUDING:

Christian González-Billault
University of Chile
60 PUBLICATIONS **1,502** CITATIONS
SEE PROFILE

Eva Jimenez
Royal College of Surgeons in Ireland
41 PUBLICATIONS **1,070** CITATIONS
SEE PROFILE

Francisco Wandosell
Universidad Autónoma de Madrid
128 PUBLICATIONS **3,459** CITATIONS
SEE PROFILE

Journal of Neuroscience Research 67:713–719 (2002)
DOI 10.1002/jnr.10161

Participation of Structural Microtubule-Associated Proteins (MAPs) in the Development of Neuronal Polarity

C. González-Billault,[1] M. Engelke,[1] E.M. Jiménez-Mateos,[1] F. Wandosell,[1] A. Cáceres,[1,2] and J. Avila[1]*

[1]Centro de Biología Molecular Severo Ochoa, CSIC, Universidad Autónoma de Madrid, Campus Cantoblanco, Madrid, Spain
[2]Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET), Córdoba, Argentina

Several lines of evidence have indicated that changes in the structure of neuronal cytoskeleton provide the support for the dramatic morphological changes that occur during neuronal differentiation. It has been proposed that microtubule-associated proteins can contribute to the development of this phenomenon by controlling the dynamic properties of microtubules. In this report we have characterized the effect of the combined suppression of MAP1B and tau, and MAP1B and MAP2 on neuronal polarization in cultured hippocampal cells grown on a laminin-containing substrate. We have taken advantage of the use of a mouse line deficient in MAP1B expression obtained by the gene trapping approach. In addition to this engineered mice line we used the antisense oligonucleotide approach to induce the suppression of tau or MAP2, in wild type and MAP1B-deficient neurons. Together these results show a synergistic role for MAP1B/MAP2 and MAP1B/TAU.

Key words: microtubule-associated protein; neuronal polarity; antisense oligonucleotides; knock out; gene trapping

Neuronal development involves dramatic morphological changes. In a pioneer study Kirschner and Mitchison (1986) suggested that microtubule proteins contribute to transform the precursor spherical neuroblast into the mature neuron displaying many cytoplasmic extensions. They further suggested that those extensions are the consequence of the stabilization of microtubule populations located in regions of active growth. Among the neuronal proteins that could regulate microtubule stabilization are the microtubule-associated proteins or MAPs (Matus, 1988). One of them, MAP1B, is the first that is expressed during "in situ" neuronal development. Analysis of a MAP1B mutant mouse line has revealed that it could have a role in axon formation (González-Billault et al., 2000, 2001). Such a role may explain the neurological defects found in this and other MAP1B mutants (Edelmann et al., 1996; Meixner et al., 2000; Takei et al., 2000). By using antisense oligonucleotides, it was also found that the lack of tau (Caceres and Kosik, 1990; Caceres et al., 1991) also produces some defects in neurite formation (Brugg et al., 1993; DiTella et al., 1996).

Because the different MAPs could have a similar function and it could result in the complementation of those functions, it could be of interest to know the result of simultaneous depletion of some of these MAPs. In this way a good model to analyze the morphological changes of a neuron is the primary culture of hippocampal neurons (Banker and Cowan, 1977). It has been described the morphological changes that neurons suffer during their in vitro development (Dotti et al., 1988). By using that type of analysis we have tested the role of MAP1B, MAP2 and tau proteins on hippocampal neuron development by using cells derived from a control as from a MAP1B mutant line (Chowdhury et al., 1997; González-Billault and Avila, 2000) and testing the effect of antisense oligonucleotides to prevent the expression of tau (Caceres and Kosik, 1990; Caceres et al., 1991) and MAP2 (Caceres et al., 1992) proteins in those cells.

Our results indicate that the expression of MAP1B and MAP2 is required to start the morphological transformations that occur in the transition from Stage I to the following developmental stages (Dotti et al., 1988). The role for these structural MAPs in subsequent stages of development is also indicated.

MATERIALS AND METHODS

Cell Culture and Antisense Experiments

Hippocampal neurons were cultured as described by Banker and Cowan (1977). Pregnant *Map1b* heterozygous fe-

Contract grant sponsor: Spanish CICYT; Contract grant sponsor: Comunidad de Madrid; Contract grant sponsor: Fundación Ramón Areces.

C. González-Billault and M. Engelke contributed equally to this work.

*Correspondence to: Jesús Avila, Centro de Biología Molecular Severo Ochoa, CSIC, Universidad Autónoma de Madrid, Campus Cantoblanco, 28049, Madrid, Spain. E-mail: javila@cbm.uam.es

Received 18 November 2001; Revised 26 November 2001; Accepted 27 November 2001

males were sacrificed at gestational day 18, and the embryos were removed in sterility. Spinal cords were frozen for further confirmation of the genotype. Dissociated hippocampal neurons were plated on glass-coverslips coated with 1 mg/ml poly-L-lysine and then with 20 μg/ml laminin. After 3 hr in medium containing 10% horse serum (Gibco-BRL, Gaithersburg, MD) cells were supplemented with N2 (GIBCO-BRL) as described by Bottenstein and Sato (1979). For the inhibition of tau expression the oligonucleotide RT11-14 (Caceres and Kosik, 1990) (5′-GGT TCA GCC ATG CTG CTT CAA AGC C-3′) was used and for the inhibition of MAP2 expression the oligonucleotide RM 2-1 (Caceres et al., 1992) (5′- CTG GTC AGC CAT CCT TCA GAT CTC T-3′) was used. Both oligonucleotides were phosphorothioate modified (ISOGEN). The oligonucleotides were added to the culture medium 3 hr after plating the neurons at concentration ranging from 0–20 μM. After 24 hr of culture cells were fixed and analyzed.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde and 4% saccharose for 20 min at 37°C, and then washed with PBS. Cells were then permeabilized with 0.2% Triton X-100 in PBS during 5 min at room temperature. Then coverslips were blocked with 5% bovine serum albumin in PBS during 1 hr at room temperature. Primary antibodies were incubated in 1% bovine serum albumin in PBS, overnight at 4°C in a humid chamber. The day after coverslips were washed three times with PBS, incubated with secondary antibodies for 1 hr at room temperature, washed again three times in PBS, and finally mounted using the FluorSave reagent (Calbiochem, La Jolla, CA). Anti-tyrosinated tubulin antibody (clone A1.2, Sigma Chemical Company, St. Louis, MO) was used diluted 1:200; anti-tau (clone Tau1, Boehringer-Mannheim Biochemicals, Indianapolis, IN) was used diluted 1:100, polyclonal anti-MAP2 antibody (Sanchez-Martin et al., 1998) was used diluted at 1:2,000. Phalloidin-TRITC (Sigma) was used at 5 μg/ml.

Western Blots

Spinal cords protein extracts were prepared by homogenization of tissue in 20 mM HEPES pH 7.4 containing 0.1 M NaCl, 10 mM NaF, 1 mM Na_3VO_4, 5 mM EDTA, 1 μM okadaic acid and proteases inhibitors, 2 mM PMSF and 10 μg/ml aprotinin, leupeptin, and pepstatin. After homogenization protein extracts was cleared by centrifuging during 10 min at 15,000 rpm in a TLX-100 centrifuge (Beckman Instruments, Palo Alto, CA). Protein extract concentration was then quantitated using the Bradford assay (Bradford, 1976). Protein samples were separated in 6% SDS-PAGE gels, transferred to nitrocellulose membranes and incubated with anti-MAP1B antibody 125 diluted 1:20 (Ulloa et al., 1994; González-Billault et al., 2000) and anti-β-galactosidase diluted 1:5,000 (Promega, Madison, WI). Additionally protein extracts from cultured neurons were done adding 2× Laemmli sample buffer (Laemmli, 1970) to Petri dishes containing neurons, and subsequently analyzed by Western blot as was described above.

RESULTS

We have described previously the effect of a drastic decrease (more than 90%) of MAP1B on neuronal development (González-Billault et al., 2000). At the cellular level, neurons lacking MAP1B display a selective inhibition of axonal formation as well as an increase in actin-based protrusive activity reflected in changes in growth cone shape and actin organization (González-Billault et al., 2001). In this study we have combined the genetic engineered model that accounts for MAP1B partial loss-of-function (González-Billault et al., 2000) and the oligonucleotide antisense technology. Antisense oligonucleotides provide a widely used tool to specifically inhibit the translation of a target protein. In the particular case of developing neurons, previous studies have shown that antisense oligonucleotides can successfully prevent the translation of tau and MAP2 proteins in cultured cerebellar macroneurons (Caceres and Kosik, 1990; Caceres et al, 1992). The effect of these oligonucleotides is MAP-specific and dose dependent (Caceres and Kosik, 1990; Caceres et al., 1992). To increase the effectiveness of the oligonucleotides we used phosphorothioate-modified oligodeoxynucleotides. This modification not only dramatically increases nuclease resistance but also fully supports RNAse H activity (Myers and Dean, 2000). Using these modified oligonucleotides we have been able to decrease their concentration to 15 μM, which is much lower than the one used in previous reports (Caceres and Kosik, 1990; Caceres et al., 1992). To analyze the suppression of tau expression we used the axonal marker tau1 (Binder et al., 1986) that recognizes a phosphorylation dependent epitope when it is dephosphorylated, and for MAP2 expression the polyclonal antibody 514 (Sanchez Martin et al., 1998) that recognizes the different isoforms of MAP2, namely MAP2A, MAP2B and MAP2C. To check the specificity of the oligonucleotide treatment we performed Western blot analysis of antisense-treated hippocampal neurons. As shown in Figure 1A, the effect of both antisense oligonucleotides was MAP specific; a working concentration of 15 μM for each oligonucleotide dramatically reduces the levels of high molecular weight MAP2 and tau. A similar effect was found in MAP1B knock-out neurons (data no shown). No effect was found when sense oligonucleotides were used (data not shown). Tubulin was chosen as a reference for relative protein concentration, and therefore the same membranes stained for MAPs expression were stripped out and incubated again, with a monoclonal antibody against β-tubulin (Fig. 1A). The genotype of the embryos used in the hippocampal cell culture was confirmed by using Western blot analyses of spinal cord protein extracts (Fig. 1B). MAP1B immunoreaction was verified in wild type protein extracts, both MAP1B and anti-β-galactosidase immunoreaction were verified in heterozygous protein extracts, whereas in homozygous extracts immunoreacted with anti-β-galactosidase.

In a subsequent series of experiments, immunofluorescence analyses were performed to test the consequences of antisense-induced MAP depletion on process formation in neurons derived from control and MAP1B deficient embryos. After 24 hr in culture, untreated control neurons growing on a laminin-containing substrate were at Stage

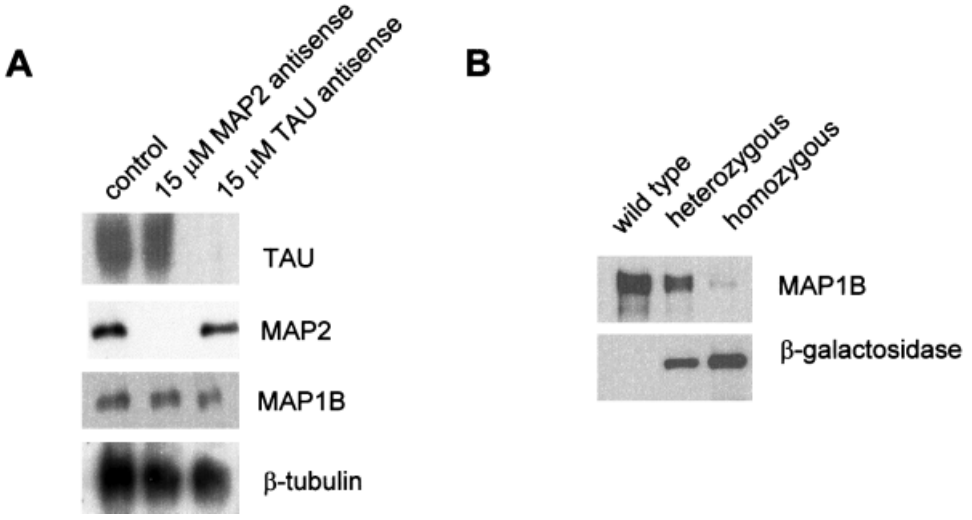

Fig. 1. Microtubule-associated proteins depletion using specific antisense oligonucleotides. **A:** Efficiency and specificity of RT11–14 and RM2-1 antisense oligonucleotides, directed against tau and MAP2 respectively. Western blots of protein extracts derived from hippocampal cell cultures in the presence of 15 μM of RT11–14 deplete tau expression without any effect on MAP2 or tubulin levels. Conversely, 15 μM of RM2-1 deplete MAP2 expression without any effect on tau or tubulin levels, after 24 hr of treatment. **B:** Genotyping of embryos used in these studies, wild type extracts react with an anti-MAP1B antibody, heterozygous extracts react with both anti-MAP1B and anti-b-galactosidase antibodies, and homozygous extracts react with an anti-b-galactosidase antibody.

III of development (Dotti et al., 1988), namely the neurons have extended a single long axon and several much shorter minor neurites. In these neurons actin was mainly present at the distal part of the neurite, including the growth cone (Fig. 2A). At the same time point many MAP1B-deficient neurons display a significant delay in axon formation, and hence in the establishment of morphological polarity. Almost 50% of the neurons at 24 hr in vitro still remain in Stage 1I (Fig. 2B) (Dotti et al., 1988).

Addition of tau antisense oligonucleotide to control and MAP1B-deficient cells result in the lack of staining with anti tau1 antibody (Fig. 2C,D). Although tau-depleted control cells showed a decrease in axonal length, most of them were capable of entering Stage III of neuritic development (Dotti et al., 1988), and hence acquire morphological polarity (Fig. 2C). By contrast, in the case of MAP1B-deficient neurons, most of the neurons remain arrested at Stage II (Dotti et al., 1988) having no neurite that could be clearly distinguishable as an axon (Fig. 2D). The results shown in Figure 2C suggests that tau-antisense treatment could affect minor process outgrowth. Thus, we have tested if it is the case by looking at different untreated or treated cells. Our results, after counting 50 minor processes of each sample indicate that the average length for a minor process from untreated cell is 0.97 μm ± 0.52 and for treated cells 0.61 μm ± 0.19. Then, a slight change, probably not very significant was observed, for tau antisense treatment.

Then, we analyzed the effect of the MAP2 antisense oligonucleotide on neuronal development. The results obtained show that neurons derived from control embryos were arrested at Stage II of neuritic development. Thus, they were unable to overcome the Stage II–III transition and therefore to transform one of their minor neurite into an axon-like neurite (Dotti et al., 1988). Finally, the effect of MAP2 antisense oligonucleotides was also analyzed in MAP1B deficient neurons. In this case, the effects were even more dramatic. These neurons were not able to reach Stage II, remaining arrested at Stage I of development, which is referred as the lammelipodia stage with no neurites (Dotti et al., 1988). Most of them showed this condensation of the actin layer that precedes the formation of the minor processes and acts as a scaffold for the developing microtubules in the minor processes.

A statistical analysis of these observations was also performed. Thus, the morphology of three hundred cells (n = 300) for each condition was quantitated (Fig. 3). Cells were chosen randomly throughout the culture as long as they were not aggregated with other cells; in such a way the possible effect of direct cell–cell interactions between neurons influencing the morphology could be excluded.

In the case of MAP1B-deficient and tau-depleted cells, the neurons were found to be mainly at Stage II or III of development. The number of cells that had developed neuronal polarity was compared to the number of cells that had not reached neuronal polarity with a clear identifiable axon. This was the case for 89% of total counted neurons from MA1B deficient mice in the presence of 15 μM tau antisense. Control neurons not receiving tau antisense oligonucleotides or treated with 15 μM tau antisense oligonucleotide clearly developed morphological polarity in 84.5% and 75% of the cases, respectively (Fig. 3A). This result confirms previous findings suggest-

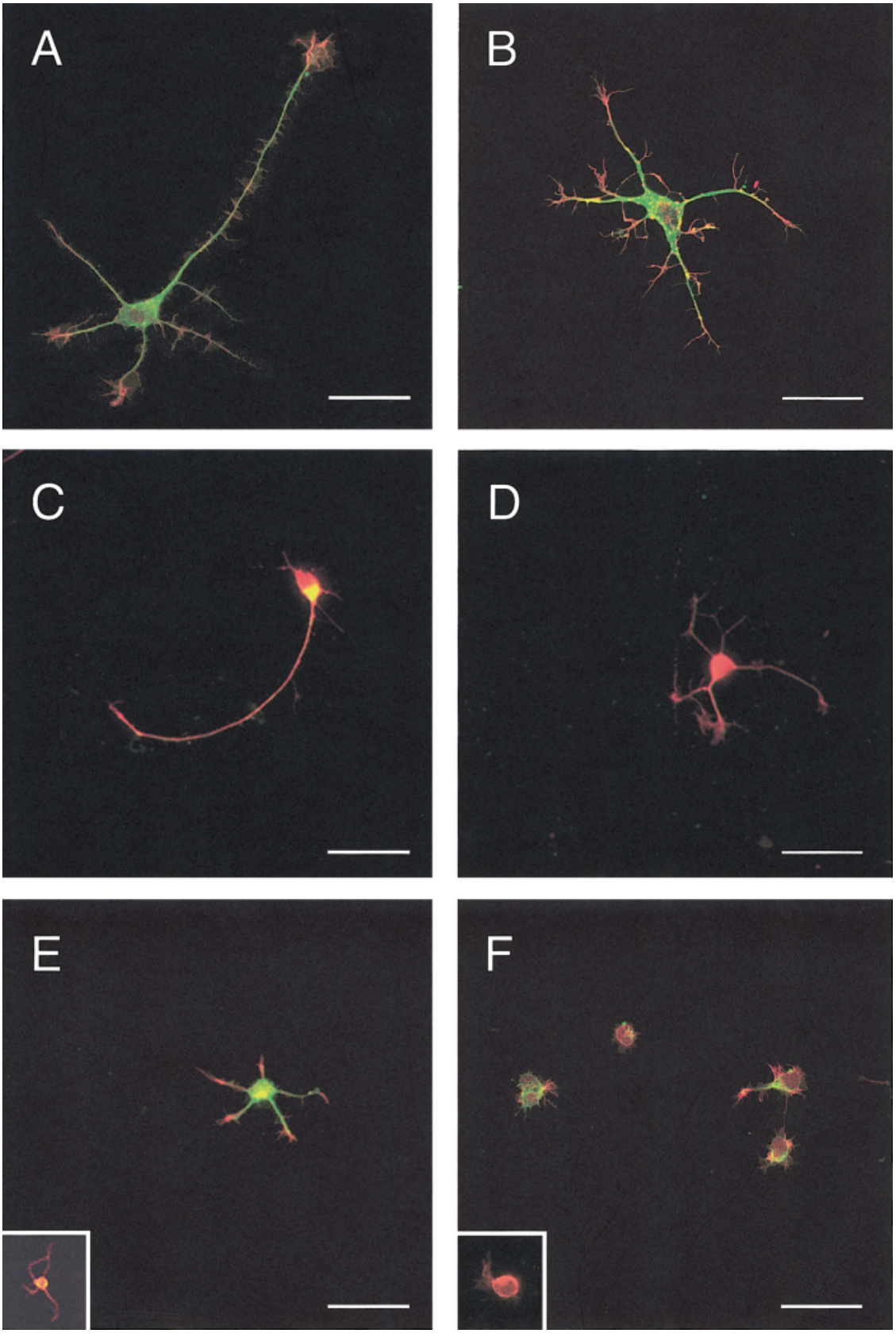

Fig. 2. Morphological analyzes of structural MAPs depleted neurons. The figure shows the appearance of hippocampal neurons from control (**A**, **C** and **E**) and MAP1B deficient (**B**, **D** and **F**) embryos, in the presence of 15 μM tau antisense (C,D) or 15 μM MAP2 antisense (E,F). Neurons are stained for α-tyrosinated tubulin (green) and Phalloidin (red) (A and B); tau (green) and β3-tubulin (red) (C,D); α-tyrosinated tubulin (green) and Phalloidin (red) (E,F) and MAP2 (green) and β-tubulin (red) (E,F insets). Cells are differentially arrested in earlier developmental stages in response to MAP1B absence and antisense effects. Scale bar = 10 μm.

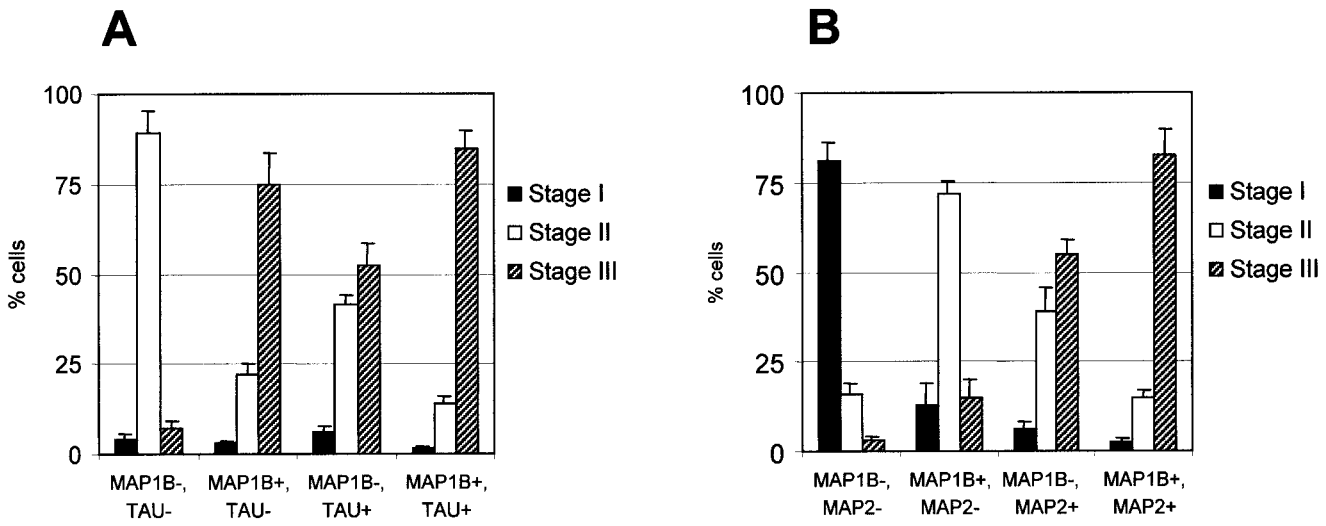

Fig. 3. Statistical analyzes of MAP1B and tau depletion or MAP1B and MAP2 depletion. Percentage of cells in Stages I–III of development in MAP1B and tau depletion (**A**) and MAP1B and MAP2 (**B**) depletion. Scale bars = mean of three independent experiments. One hundred cells were counted on each experiment.

TABLE I. Development of Neuronal Polarity After 24 hr Over Poli-L-Lysine*

Genotype	% cells in Stage 2	SD	Cell number
Control	58.2	3.70	180
MAP1B deficient	61.8	5.26	142

*Four experiments were done in each case.

ing that axon elongation of neurons plated over laminin depends mainly on MAP1B function (DiTella et al., 1996). These results were confirmed analyzing the number of cells at Stage II after 24 hr of culture over poly-L-lysine. When plated over poly-L-lysine the number of cells remaining in Stage II was quite similar between control and MAP1B deficient neurons (Table I).

The statistical analysis of the effect of MAP1B-deficient and MAP2-depleted cells was performed by counting cells that had been remaining in Stage I of development and cells that had overcome this stage, because a previous report indicated that cerebellar neurons remained arrested in Stage I of development due to the effect of MAP2 antisense (Caceres et al., 1992). This made clear that only the combination of MAP1B and MAP2 depletion lead to an arrestment in Stage I (81%), whereas neurons in which MAP1B was present could reach the Stage II of development in a 72% (Fig. 3B). This difference with previous results using the same oligonucleotide could be explained in terms of the substrate where neurons where plated (Caceres et al., 1992). It has been shown that laminin can enhance axonal elongation, shortening the period that neurons required to reach Stage III of development (Lien et al., 1992). Moreover, changes in the substrate can alter the development of polarity in a MAP-sensitive way as have been indicated in previous studies (DiTella et al., 1996).

DISCUSSION

In this report we have analyzed the role of structural MAPs in the development of hippocampal neuronal polarity. It is very clear from previous findings that some of these MAPs can have some redundant roles (DiTella et al., 1996; Takei et al., 2000; González-Billault et al., 2001). We described here that MAP1B and MAP2 could have some synergistic roles that could support the transition from Stage I–II of development of polarity. MAP1B and tau or MAP1B and MAP2 could have some redundant function that could explain the transition from Stage II–III of development. The synergistic and compensational effects, in which MAP1B could compensate the loss of MAP2 or tau expression, should be only verified on neurons plated over laminin, an extracellular matrix protein that selectively enhances axonal elongation (Lien et al., 1992). It is quite clear from these and previous results that the substrate where neurons are cultured is not a trivial issue. Laminin represents a more physiological substrate as compared to poly-L-lysine. Thus, we propose that neurons require the presence of certain structural MAPs depending on the substrate where they are, to substantiate the dramatic morphological changes that account for the development of neuronal polarity. These requirements are summarized in the Figure 4. The transition from the lammelipodial stage (Stage I) to the minor processes stage (Stage II) should require the presence of either MAP2 or MAP1B, being both required to induce cell polarity. The absence of one of these proteins clearly produce different effects, suggesting that not a redundancy but a synergistic effects that are only partial are occurring, and pointing out

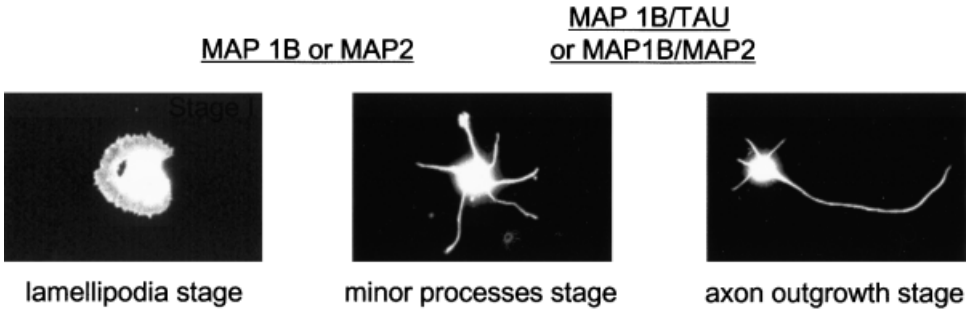

Fig. 4. Contribution of structural MAPs to the development of neuronal polarity. The model suggest the synergistic and redundant functions of structural MAPs in the development of hippocampal neuronal polarity. MAP1B or MAP2 should be required to overcome the transition from Stage I to Stage II, whereas MAP1B/MAP2 or MAP1B/tau should be necessary to complete the transition from Stage II to Stage II of development.

to differential roles for each one of these proteins in normal conditions. Looking at the cellular localization of MAP1B and MAP2 it is quite clear that these synergistic effects should be found just at the initial stages of development. Recently, Teng et al. (2001) have found that in a double knockout for MAP1B and MAP2 a certain cell polarity can be found. We think that the possible discrepancy between theirs and ours results could be based in that our MAP1B mutant shows a stronger phenotype than that of Hirokawa's group (Takei et al., 1997; González-Billault et al., 2000). Afterwards, during the transition from the minor processes stage (Stage II) to axonal stage (Stage III), MAP1B and tau or MAP1B and MAP2 proteins should be required. The fact that MAP2 and tau could both have to MAP1B as a counterpart for their appropriate functions is attractive if we consider the nature of these structural MAPs. MAP2 and tau share a tubulin binding domain (Sato-Yoshitake et al., 1989; Takemura et al., 1992) that is different from the one found in MAP1B protein (Noble et al., 1989; Zauner et al., 1992). It is well known that both tau and MAP2 are more efficient than MAP1B in their promoting and stabilizing effects over microtubules (Takemura et al., 1992). It has been demonstrated that MAP1B could have some role in the control of the dynamic properties of microtubules in transfected cells (Goold et al., 1999) and primary hippocampal neurons (González-Billault et al., 2001). In this respect it is interesting to point out that this control of the dynamic properties of microtubules is assumed to be related with a posttranslational modification of MAP1B, namely the MAP1B mode 1 of phosphorylation (Ulloa et al., 1993, 1994). This phosphorylation mode is spatially and temporally consistent with the function above described. Thus, MAP1B mode 1 of phosphorylation should be responsible for the balance of dynamic and stable microtubules within cells (Goold et al., 1999; González-Billault et al., 2001). In such a way MAP1B could act in a coordinate fashion with MAP2 and tau to control the microtubule polymerization and maintenance of the dynamic properties of the microtubular polymer.

Consistent with this speculation is the fact that two different studies conducted to inactivate the tau gene have resulted in an increase of MAP1A expression (Harada et al., 1994; Dawson et al., 2001), a protein that could play the role of MAP1B in adults, where the MAP1B expression is downregulated.

ACKNOWLEDGMENTS

We would like to acknowledge the technical assistance of M.A. Bordallo and R. Cuadros.

REFERENCES

Banker G, Cowan N. 1977. Rat hippocampal neurons in dispersed cell culture. Brain Res 126:397–425.

Binder LI, Frankfurter A, Rebhun LI. 1986. Differential localization of MAP-2 and tau in mammalian neurons in situ. Ann NY Acad Sci 466:145–166.

Bottenstein JE, Sato GH. 1979 Growth of a rat neuroblastoma cell line in serum-free supplemented medium. Proc Natl Acad Sci USA 76:514–517.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Brugg B, Reddy D, Matus A. 1993. Attenuation of microtubule-associated protein 1B expression by antisense oligodeoxynucleotides inhibits initiation of neurite outgrowth. Neuroscience 52:489–496.

Caceres A, Kosik K. 1990. Inhibition of neurite polarity by antisense oligonucleotides in primary cerebellar neurons. Nature 343:461–463.

Caceres A, Potrebic S, Kosik KS. 1991. The effect of tau antisense oligonucleotides on neurite formation of cultured cerebellar macroneurons. J Neurosci 11:1515–1523.

Caceres A, Mautino J, Kosik K. 1992. Suppression of MAP-2 in cultured cerebellar macroneurons inhibits minor neurite formation. Neuron 9:607–618.

Chowdhury K, Bonaldo P, Torres M, Stoykova A, Gruss P. 1997. Evidence for the stochastic integration of gene trap vectors into the mouse germline. Nucleic Acids Res 25:1531–1536.

Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP. 2001. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. J Cell Sci 114:1179–1187.

DiTella MC, Feiguin N, Carri N, Kosik KS, Caceres A. 1996. MAP1B/tau functional redundancy during laminin-enhanced axonal growth. J Cell Sci 109:467–477.

Dotti CG, Sullivan CA, Banker GA. 1988. The establishment of polarity by hippocampal neurons in culture. J Neurosci 8:1454–1468.

Edelmann W, Zervas M, Costello P, Roback L, Fischer I, Hammarback JA, Cowan N, Davies P, Wainer B, Kucherlapati R. 1996. Neuronal abnormalities in microtubule-associated protein 1B mutant mice. Proc Natl Acad Sci USA 93:1270–1275.

González-Billault C, Demandt E, Wandosell F, Torres M, Bonaldo P, Stoykova A, Chowdhury K, Gruss P, Avila J, Sanchez MP. 2000. Perinatal lethality of microtubule-associated protein 1B deficient mice expressing alternative isoforms of the protein at low levels. Mol Cell Neurosci 16:408–421.

González-Billault C, Avila J. 2000. Molecular genetic approaches to microtubule-associated proteins function. Histol Histopathol 15:1177–1183.

González-Billault C, Avila J, Caceres A. 2001. Evidence for the role of microtubule-associated protein 1B in axonal formation. Mol Biol Cell 12:2087–2098.

Goold RG, Owen R, Gordon-Weeks PR. 1999 Glycogen synthase kinase 3β phosphorylation of microtubule-associated protein 1B regulates the stability of microtubules in growth cones. J Cell Sci 112:3373–3384.

Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Oshima T, Sato-Yoshitake R, Takei Y, Noda T, Hirokawa N. 1994. Altered microtubule organization in small-caliber axons of mice lacking tau protein. Nature 369:488–491.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Lien PJ, Banker G, Higgins D. 1992. Laminin selectively enhances axonal growth and accelerates the development of polarity by hippocampal neurons in culture. Dev Brain Res 69:191–197.

Matus A. 1988. Microtubule-associated proteins: their potential role in determining neuronal morphology. Annu Rev Neurosci 11:29–44.

Meixner A, Haverkamo S, Wassle H, Fuhrer S, Thalhammer J, Kropf N, Bittner R, Lassman H, Wiche G, Propst F. 2000. MAP1B is required for axon guidance and is involved in the development of the central and peripheral nervous system. J Cell Biol 151:1168–1178.

Kirschner MW, Mitchison T. 1986. Microtubule dynamics. Nature 324: 621.

Myers KJ, Dean NM. 2000. Sensible use of antisense: how to use oligonucleotides as research tools. Trends Pharmacol Sci 21:19–23.

Noble M, Lewis SA, Cowan NJ. 1989. The microtubule-binding domain of microtubule-associated protein 1B contains a repeated sequence motif unrelated to that of MAP2 and tau. J Cell Biol 109:3367–3376.

Sanchez Martin C, Diaz-Nido J, Avila J. 1998 Regulation of a site-specific phosphorylation of the microtubule-associated protein 2 during the development of cultured neurons. Neuroscience 87:861–870.

Sato-Yoshitake R, Shiomura Y, Miyasaka H, Hirokawa N. 1989. Microtubule-associated protein 1B: molecular structure, localization and phosphorylation-dependent expression in developing neurons. Neuron 3:229–223.

Takei Y, Kondo S, Harada A, Inomata S, Noda T, Hirokawa N. 1997. Delayed development of nervous system in mice homozygous for disrupted microtubule-associated protein 1B (MAP1B) gene. J Cell Biol 137:1615–1626.

Takei Y, Teng J, Harada A, Hirokawa N. 2000. Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. J Cell Biol 150:989–1000.

Takemura R, Okabe S, Uneyama T, Kanai Y, Cowan NJ, Hirokawa N. 1992. Increased microtubule stability and α-tubulin acetylation in cells transfected with microtubule-associated proteins MAP1B, MAP2 or tau. J Cell Sci 103:953–964.

Teng J, Takei Y, Harada A, Nakata T, Chen J, Hirokawa N. 2001. Synergistic effects of MAP2 and MAP1B knockout in neuronal migration, dendritic migration, dendritic outgrowth, and microtubule organization. J Cell Biol 155:65–76.

Ulloa L, Avila J, Diaz-Nido J. 1993. Heterogeneity in the phosphorylation of microtubule-associated protein MAP1B during rat brain development. J Neurochem 61:961–972.

Ulloa L, Diez-Guerra FJ, Avila J, Diaz-Nido J. 1994. Localization of differentially phosphorylated isoforms of microtubule-associated protein 1B in cultured rat hippocampal neurons. Neuroscience 61:211–223.

Zauner W, Kratz J, Stauton J, Feick P, Wiche G. 1992. Identification of two distinct microtubule-binding domains on recombinant rat MAP1B. Eur J Cell Biol 57:66–74.