Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 10369–10373, October 1995
Neurobiology

Functional implications for the microtubule-associated protein tau: Localization in oligodendrocytes

PATRIZIA LOPRESTI*†, SARA SZUCHET‡, SOZOS CH. PAPASOZOMENOS§, RAYMOND P. ZINKOWSKI*, AND LESTER I. BINDER*¶

*Molecular Geriatrics Corporation, Lake Bluff, IL 60044; ‡Department of Neurology and the Brain Research Institute, University of Chicago, Chicago, IL 60637; and §Department of Pathology and Laboratory Medicine, University of Texas Medical School, Houston, TX 77030

Communicated by Philip Siekevitz, The Rockefeller University, New York, NY, July 17, 1995

ABSTRACT **We present evidence that the microtubule-associated protein tau is present in oligodendrocytes (OLGs), the central nervous system cells that make myelin. By showing that tau is distributed in a pattern similar to that of myelin basic protein, our results suggest a possible involvement of tau in some aspect of myelination. Tau protein has been identified in OLGs *in situ* and *in vitro*. In interfascicular OLGs, tau localization, revealed by monoclonal antibody Tau-5, was confined to the cell somata. However, in cultured ovine OLGs with an exuberant network of processes, tau was detected in cell somata, cellular processes, and membrane expansions at the tips of these processes. Moreover, in such cultures, tau appeared localized adjacent to or coincident with myelin basic protein in membrane expansions along and at the ends of the cellular processes. The presence of tau mRNA was documented using fluorescence *in situ* hybridization. The distribution of the tau mRNA was similar to that of the tau protein. Western blot analysis of cultured OLGs showed the presence of many tau isoforms. Together, these results demonstrate that tau is a genuine oligodendrocyte protein and pave the way for determining its functional role in these cells.**

Tau is known to be a microtubule stabilization factor in the long axonal processes of neurons (1–3). Original immunolocalization studies supported this role in that monoclonal antibodies (mAbs) to tau appeared to stain axons exclusively (2, 4, 5). Since these studies, numerous reports have demonstrated the presence of tau in the somatodendritic and astrocytic compartments in the brain (6–8), although phosphatase treatment was usually necessary to visualize nonaxonal tau (6). Tau proteins are produced by the alternate splicing of a single mRNA transcript resulting in six isoforms in the central nervous system (CNS) (9–11). Tau's primary structure shows a large number of Pro-Gly repeats predictive of a highly flexible molecule (12, 13). Moreover, the 17% of serines and threonines present in tau and their potential of undergoing phosphorylation adds to the number of possible conformational states that tau protein may attain (14). It is this multiplicity of forms that introduces elements of uncertainty and complexity in tau's immunocytochemical detection.

Tau isoforms have been found localized differentially in subcellular compartments suggesting functions other than and in addition to its role as a microtubule-associated protein (MAP). Ultrastructural studies demonstrated that tau was associated with microtubules and ribosomes (6). In cultured neuroblastoma cells, the association of tau with microtubules in the cytoplasm was equivocal, but tau was found aligned with the fibrillar regions of nucleoli within the nucleus (15). Here, we reveal another facet to tau's interactions by reporting its segregation with myelin basic protein (MBP).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

During development, interfascicular oligodendrocytes (OLGs) extend numerous processes that surround axons to elaborate the myelin sheath. OLG processes are very rich in microtubules and in actin-containing microfilaments. Similarly, cultured OLGs elongate their processes to elaborate membrane expansions into the myelin-like structures (16–19). It is at the site of myelin assembly that MBP mRNA is translated. To reach its site of translation, this mRNA is translocated long distances from the cell body (20–23), establishing yet another example of "local synthesis" similar to that occurring at the base of dendritic spines in the neuron (24). Presumably, microtubules are involved in this transport process. Prior to this study, the only other MAP described in oligodendrocytes was MAP1B (25).

Other reports have suggested that tau is present in OLGs. For example, tau was found to localize in perineuronal OLGs in gray matter (6). Autoclaving of sections from human brain has allowed staining of OLGs with Abs to the paired helical filaments (PHFs) that form in the abnormal neurons in Alzheimer disease (26). PHFs are largely composed of tau (27). This report unambiguously demonstrates the presence of tau in OLGs *in situ* and *in vitro* by immunocytochemical, fluorescence *in situ* hybridization (FISH), and immunoblotting techniques. Furthermore, by showing that tau might be associated with MBP, our results implicate tau in myelinogenesis.

METHODS

Immunohistochemistry. Rat brains were fixed by vascular perfusion with PLP [2% paraformaldehyde/75 mM lysine/10 mM sodium periodate in phosphate-buffered saline (PBS), pH 7.4]. Sections (20 μm) were cut on a vibratome and exposed to various concentrations of mAb Tau-5 (IgG1 subclass). Antibody binding was visualized following incubation in mouse ClonoPAP (Sternberger–Meyer, Jarrettsville, MD) using H_2O_2 and diaminobenzidine as the chromogen (28).

Electron Microscopic Examination. Vibratome sections (20 μm thick) were fixed and processed for immunostaining using mAb Tau-5 or a control Ab and the peroxidase-antiperoxidase technique as described (29). Following immunostaining, sections were exposed to 1% OsO_4 for 30 min, dehydrated in graded alcohols, passed through propylene oxide, and flat-embedded in Epon according to standard procedures. Ultrathin sections were picked up on uncoated 200-mesh nickel

Abbreviations: OLG, oligodendrocyte; FISH, fluorescence *in situ* hybridization; mAb, monoclonal antibody; MAP, microtubule-associated protein; MBP, myelin basic protein; CNS, central nervous system.

†Present address: Department of Pediatrics, Joseph P. Kennedy Jr. Mental Retardation Research Center, University of Chicago, Chicago, IL 60637.

¶To whom reprint requests should be sent at the present address: Department of Cell and Molecular Biology, Northwestern University Medical School, 303 E. Chicago Avenue, Chicago, IL 60611.

grids and viewed with a JEOL 100CX II electron microscope operated at 60 kV without prior staining with heavy metals.

Cell Culture. OLGs from 6-month-old lamb brains were isolated (30) and kept in long-term culture by a modification of the method previously described (31).

Immunofluorescence. Indirect immunofluorescence was performed by methods standard in our laboratory (15). Cells were cultured on poly(D-lysine)-coated glass slides and fixed with 3% formaldehyde for 30 min at room temperature. Briefly, the cells were permeabilized with 100% ethanol (−20°C), rinsed in PBS, and kept in 1% bovine serum albumin for 10 min. The cells were then incubated with the corresponding Ab followed by incubation with an appropriate fluorescein- or Texas red-conjugated secondary Ab. All incubations were done for 30 min at 37°C in a humidified atmosphere, and each incubation was followed by three 5-min washes in PBS. Controls in which the primary mAbs were deleted were routinely performed. The processed glass coverslips were mounted onto glass slides with a 10:1 (vol/vol) mixture of glycerol/PBS containing 0.1% *p*-phenylenediamine (Sigma) to reduce photobleaching and then sealed with nail polish.

FISH. A human three-repeat tau cDNA (courtesy of Gloria Lee, Harvard Medical School, Boston) was amplified as described (32). Tau probe (≈10 mg) was random primed with digoxigenin-dUTP (Boehringer Mannheim). Ten nanograms of the resultant digoxigenin-labeled tau cDNA was used per slide. Hybridization solution was made fresh each time and contained 50% formamide, 5× standard saline citrate (SSC), 10× dextran sulfate, and PBS made in diethylpyrocarbonate-treated water. Hybridization was performed at 42°C overnight in a 100-μl volume. After hybridization, coverslips were washed several times to a high stringency as follows: (*i*) 20 min in 50% formamide/2× SSC at 37°C, (*ii*) 20 min in 2× SSC at 37°C, (*iii*) 20 min in 1× SSC at room temperature, (*iv*) 20 min in 1× SSC at 65°C, and (*v*) 20 min in 0.5× SSC at 65°C. Probes were detected using fluorescein isothiocyanate-conjugated affinity-purified sheep anti-digoxigenin (Boehringer Mannheim).

SDS/PAGE and Western Blotting. Cells were harvested in PBS, 5 mM EDTA, and a cocktail of protease inhibitors (1 μg/ml each of chymostatin, leupeptin, antipain, and pepstatin and 1 mM phenylmethylsulfonyl fluoride = CLAPP). After centrifugation the pellet was dissolved in 62.5 mM Tris, pH 6.8/1% SDS/CLAPP. The sample was boiled for ≈5 min, and after centrifugation the supernatant was frozen at −80°C. Protein concentrations were determined by a modification of the method of Lowry *et al.* (33). Proteins were separated by SDS/PAGE using a 5–12% linear polyacrylamide gradient gel (34) and were transferred to nitrocellulose (35). Resulting blots were probed with various primary and secondary Abs prior to signal detection using chemiluminescence.

RESULTS

***In Situ* Localization of Tau in OLGs Using mAb Tau-5.** Immunohistochemical examination of vibratome sections with mAb Tau-5 revealed intense staining of interfascicular OLGs in the corpus collosum of adult rats (Fig. 1). Similar cell-specific staining patterns were observed in all regions of CNS white matter examined. An ultrastructural analysis confirmed these results (Fig. 1 *Inset*); axons showed no Tau-5 immunoreactivity. This outcome is striking when compared with results obtained with other tau Abs such as Tau-1, 5E2, or Tau46, which only stained axonal profiles (2, 36). These observations can be interpreted as indicating that mAb Tau-5 (*i*) recognizes a site unique to OLG tau, (*ii*) detects an epitope revealed when tau assumes a distinct conformational state, or (*iii*) crossreacts with another OLG protein. The first possibility can be ruled out because the epitope for Tau-5 maps to sequence 210–241 of tau, a sequence present in all isoforms (data not shown).

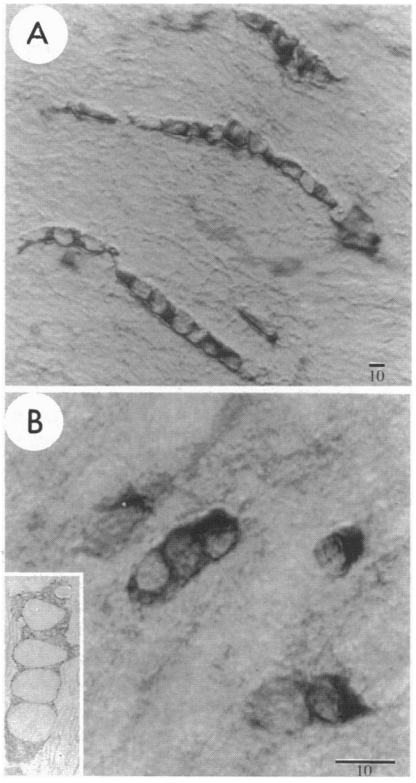

FIG. 1. Tau-5 immunostaining of rat interfascicular oligodendrocytes, *in situ*. PLP-fixed vibratome sections (10 μm) from the corpus callosum of the rat (*A*, low power; *B*, high power). (Bars = 10 μm.) Note the intense staining of the cytoplasm in the cell bodies and the absence of nuclear staining. Also note that Tau-5 does not stain axons under these fixation conditions. (*Inset*) Ultrastructural localization of Tau-5 processed from a similar section of corpus collosum.

Experiments were designed to distinguish between the second and third interpretations listed above.

Tau Protein Is Present in Cultured OLGs. It was incumbent on us to demonstrate that tau and not an immunologically crossreacting protein is present in OLGs. For these reasons, we searched for the presence of *bona fide* tau in cultured OLGs from 6-month-old ovine white matter. We chose this culture system for three reasons: (*i*) these are pure cultures of OLGs (>98%), (*ii*) the cells have been well characterized, structurally and functionally, and (*iii*) cultures can be maintained for an extended time spanning from differentiation to myelination (37). Characteristic features of long-term OLG cultures are well established. Such cultures consist of cells that have elaborated an extensive network of processes with the formation of many multilamellar structures that resemble myelin (38). The range of cell morphologies seen in these cultures fits the classical description of OLG subtypes (for review, see ref. 39).

Four-week-old cultures were examined by immunofluorescence using mAbs Tau-5 and Tau-1; these antibodies recognize distinct epitopes on the tau molecule. The staining localized intensely in the cell somata (Fig. 2) and in most OLG processes (Fig. 3 *B* and *D*). The tips of OLG processes exhibited a granular pattern of tau staining (Fig. 3*B*) similar to punctate morphological entities observed under phase or by scanning electron microscopy (40). Moreover, membranous expansions located along the processes also exhibited the same punctate staining (data not shown). In a parallel study with mixed cultures obtained from neonatal rat brains, we have also stained OLGs identified as MBP-positive cells with these mAbs (P.L. and L.I.B., unpublished data). Control experiments, in which incubations were performed without addition of the primary Abs, lacked background fluorescence (data not shown).

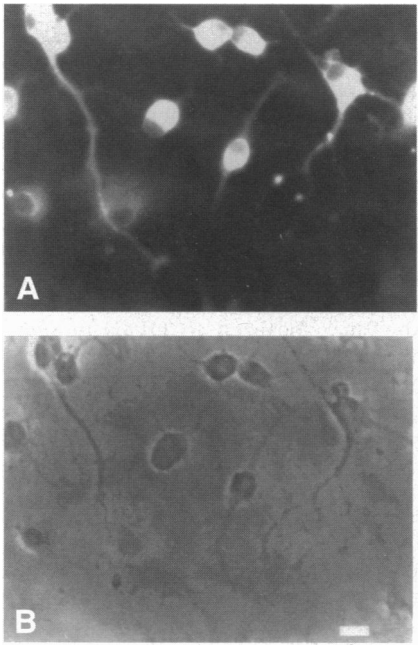

FIG. 2. Staining of OLGs with Tau-5. Four-week-old cultured OLGs as seen by Tau-5 immunofluorescence (*A*) and by phase-contrast microscopy (*B*). (Bar = 20 μm.) OLGs show strong cytoplasmic staining (*A*).

Tau mRNA Is Found in OLG Somata, in Their Processes, and in the Tips of These Processes. Although tau is a MAP, the localization pattern described above did not indicate binding along microtubules as would be expected. The expression of tau in neurons is targeted by localization of one form of tau mRNA to the microtubules (41, 42), where the expressed protein binds. Therefore, we sought to determine whether tau mRNA is targeted to structures other than microtubules in OLGs using FISH (see *Methods*). As illustrated (Fig. 4), tau mRNA is detected in OLG somata, in the cellular extensions, and in their tips. Interestingly, the discontinuous, punctate staining observed with the mAbs (see Figs. 3 and 6) is also seen for the mRNA in some of the cellular processes (Fig. 4 *B* and *D*). To assess the extent of nonspecific binding, we probed neonatal rat OLGs in mixed cultures. Only MBP-staining cells exhibited a FISH signal; other cells in these mixed cultures contained no detectable fluorescence signal (P.L. and L.I.B., unpublished data). These results indicate that OLGs contain tau protein and abundant tau mRNA. Moreover, tau mRNA, like MBP mRNA (22, 43), is not restricted to the cell body, suggesting that a message targeting event may be instrumental in establishing the site of tau synthesis in the processes.

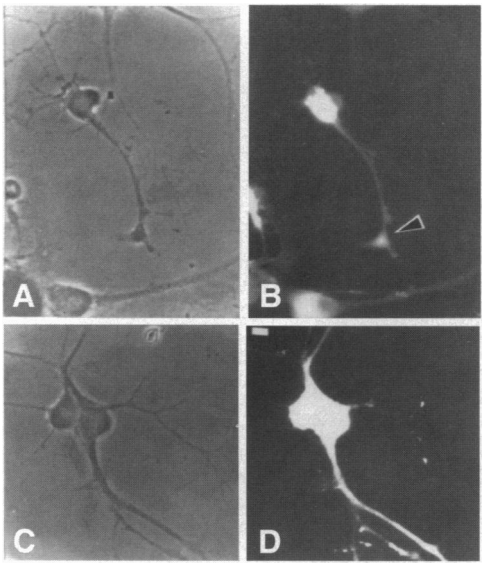

FIG. 3. Staining of OLGs with Tau-1. Two profiles of cultured oligodendrocytes are shown in phase contrast (*A* and *C*) and corresponding immunofluorescence (*B* and *D*). The cell body (*B* and *D*) and terminal membrane expansions (*B*, arrowhead) are strongly tau-positive. (Bar = 20 μm.)

OLGs Express Several Tau Isoforms. In the brain cortex, tau exists as six isoforms. It was therefore of interest to determine whether these isoforms were all present in OLGs. Extracts from lamb brain cortex and cultured OLGs isolated from aged-matched brains were resolved on SDS/PAGE and blotted with mAb Tau-1 (Fig. 5, lanes 1 and 2), Tau-5 (Fig. 5, lane 3), and Tau46 (Fig. 5, lane 4) according to standard protocols (see *Methods*). It is instructive to compare the resulting patterns; some notable differences are apparent. First, OLGs seem to have one or two additional isoforms of slightly higher molecular weight than the slowest migrating cortical tau species (Fig. 5; compare lane 1 with lanes 2–4). Second, while the relative amount is about the same for each of the isoforms in the cortex, there is variation among OLG isoforms, with a clear preponderance of some species (Fig. 5, lanes 2–4).

Tau and MBP Exhibit Similar Distribution Patterns in OLG Processes and in the Membranous Sheets at the Tips of the Processes. Analysis of the fluorescent images of tau and MBP in doubly stained OLGs revealed that the two fluorochromes localized in patterns of close apposition. Although various OLG morphologies were observed, common staining characteristics were evident (Fig. 6). In all cases, the intense staining of the cell somata with either fluorochrome precluded analysis; however, this was not the case for the cellular processes and the membranes emanating from them. The salient features of MBP and tau distributions in OLG processes and membrane expansions observed were as follows: (*i*) staining was discontinuous, often segmental (Fig. 6 *A* and *B*, arrows), (*ii*) tips of processes were clearly stained (Fig. 6 *A*, *B*, *D*, *E*, *G*, *H*, arrowheads), and (*iii*) immunoreactivity within membrane expansions appeared also to occur in patches (Fig. 6 *D*, *E*, *G*, *H*, short arrows). In all cases, controls lacking primary antibody exhibited no fluorescence signal. Together, these findings indicate that tau protein and/or its message exist in close proximity to, if not superimposed with, MBP.

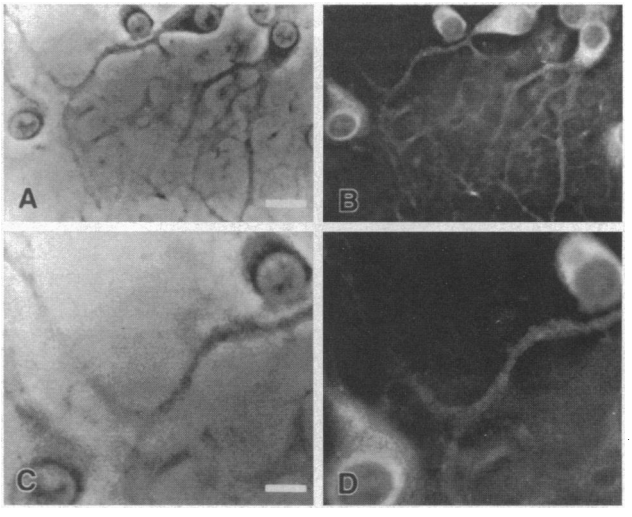

FIG. 4. *In situ* hybridization. FISH of tau mRNA in 3-week cultured OLGs (*B* and *D*). Phase-contrast micrographs of the same fields (*A* and *C*) are also shown. (Bar in *A* = 50 μm; bar in *C* = 25 μm.) Note presence of mRNA in the processes.

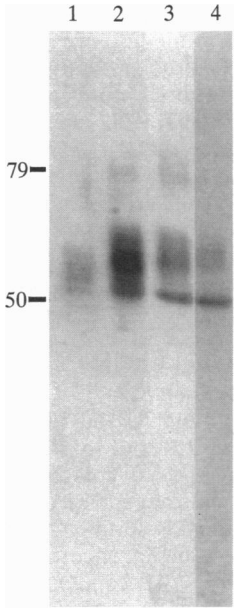

FIG. 5. Immunoblot analysis of tau in cultured OLGs using three mAbs to distinct epitopes. Many isoforms of tau can be visualized in a Tau-1 blot of lamb cortex (lane 1), whereas a different pattern of isoforms is visualized in cultured OLGs whether the blot is probed with Tau-1 (lane 2), Tau-5 (lane 3), or Tau46 (lane 4). Molecular weights are shown as $M_r \times 10^{-3}$.

DISCUSSION

This study conclusively shows that tau is present in OLGs. However, tau distribution is different in cultured OLGs when compared to those resident in the rat brain. *In situ*, only cell bodies had immunoreactivity; no antibody signal was detected in the cellular processes. In contrast, cultured OLGs displayed antibody localization in their cellular processes as well as in the membranes emanating from them. Interestingly, the staining pattern in the processes was discontinuous, appearing segmental and patch-like. The finding that, *in situ*, tau was only detected in the cell bodies is not surprising given the intricate architecture of the mature CNS and the fact that the tissues examined were obtained from mature animals. Indeed, our observation is in line with previous reports describing staining with Abs against myelin-associated proteins that demonstrated differences between developing and mature tissues (44).

Morphologically and possibly functionally, OLGs are heterogeneous (39). This heterogeneity is manifested in cell size, shape, number, and nature of the cellular processes as well as association with other cell types. The heterogeneity is evident *in situ* and *in vitro*. Yet, *in vitro*, tau distribution was similar in all OLGs in that it was concentrated in the somata, at the tips of processes, at points of processes bifurcation, and in membranous expansions.

Only mAb Tau-5 localized to OLGs *in situ*, whereas a number of other tau mAbs stained cultured OLGs. The reason for these disparate results can probably be explained by differential fixation of the flexible tau molecule (see *Results*). Fixation may also be the reason why the detection of OLG tau has been equivocal. Indeed, this report definitively demonstrates the presence of tau in these cells. Although others have presented data also suggesting that tau immunoreactivity was present in OLGs (26), biochemical and molecular biological corroboration of the immunostaining was not documented.

Tau protein comprises six isoforms produced by alternate splicing of a single mRNA species (13). Using immunoblot analysis, we observed only two or three major tau bands in cultured OLGs. To ascertain whether these bands represent an OLG-specific isoform pattern or a differentially phosphorylated single isoform would require isolation and characterization of the OLG tau transcripts. Moreover, whether different tau isoforms are expressed during OLG process extension and maturation has yet to be determined.

There is general consensus that the cytoskeleton is involved in OLG process outgrowth and myelin assembly (18, 19). Involvement of actin and tubulin during myelin formation has been suggested in that both proteins have been shown to copurify with myelin from rat brain (45), and purified MBP

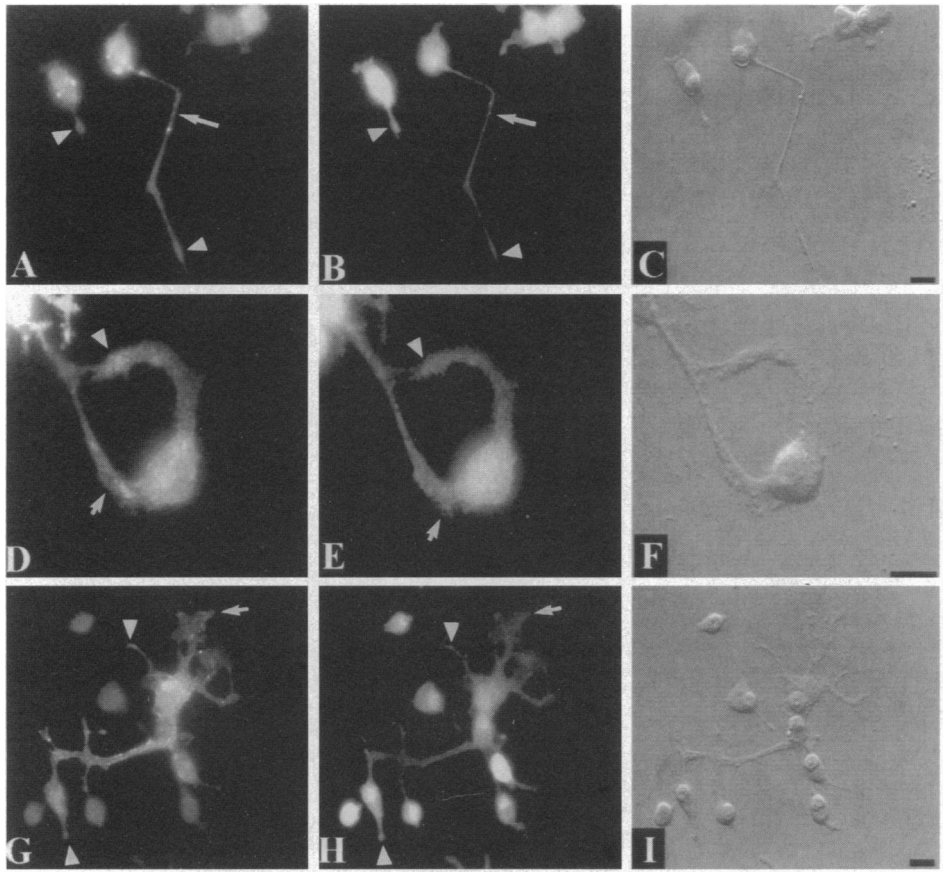

FIG. 6. Three fields of cultured OLGs (*A–C, D–F,* and *G–I*) doubly stained for tau and MBP. The cells are stained or observed as follows: immunofluorescence using anti-MBP (*A, D,* and *G*) and Tau-5 (*B, E,* and *H*); differential interference contrast optics (*C, F,* and *I*). Noteworthy is the resemblance of the distribution patterns of both proteins. Salient features are (*i*) segmental staining (arrows in *A* and *B*), (*ii*) concentration of fluorescence at the tips of the processes (arrowheads in *A, B, D, E, G,* and *H*) and (*iii*) patchy immunoreactivity in membrane expansions (short arrows in *D, E, G,* and *H*). (Bars = 10 μm.)

forms complexes with these proteins (45–47). A colocalization between MBP and the microtubule protein tubulin has also been reported (48). In a comparative study of the protein composition of highly purified fractions of OLG plasmalemma and myelin by two-dimensional gel electrophoresis, Szuchet *et al.* (49) showed that both membranes had actin and α- and β-tubulins. However, there were significant differences in their contents: OLG plasmalemma was enriched in actin relative to the tubulins; the opposite was the case for myelin. Analysis of purified CNS myelin further indicated that its cytoskeletal matrix includes MBPs and 2′,3′-cyclic nucleotide 3′-phosphohydrolases I and II (50, 51). Whereas these findings do not suggest a function for cytoskeletal elements in myelination, they underscore the likely importance of the codistribution of tau and MBP observed in our studies.

Tau and MBP were observed to localize in cell somata, in the OLG processes, and in membrane expansions at the ends of these processes. In fact, their distribution patterns suggested close proximity and/or overlap. The finding that tau is present at the tips of OLG processes is quite intriguing in the context of OLG extensions and myelination. The tips of growing OLG processes, like neuronal growth cones, are known to be actin rich (52). Previous studies indicate that tau binds and gels actin *in vitro* (53). Taken together, these data suggest the possibility that OLG tau may have a function similar to that recently proposed for neuronal tau in growth cones of cerebellar neurons (54); these authors suggest that tau may maintain the structural stability of this neuritic domain by interacting with cytoskeletal elements such as actin.

Microtubules have been postulated to be key elements in the translocation of MBP mRNA and other myelin components along cellular processes toward the sites of myelin assembly (19, 21–23, 43). As we have noted, tau mRNA and tau protein are also found at these sites. Although we have no data indicating the function of tau protein in these areas, it is likely to be important since the cell mandates its presence by specifying the colocalization of its mRNA. Since some overlap was observed in the tau and MBP signals in OLG processes, it is possible that tau interacts directly with MBP. Hence, tau might serve as a crosslinking protein between MBP, its mRNA, and the microtubule. Thus, tau would use its microtubule binding domain to "find" a microtubule for subsequent transport of the polysome complex to the sites of MBP–membrane interaction. The reason that tau is not seen localized along microtubules in this scenario is that it would only take one or a few tau molecules to accomplish such a transitory crosslinking at discrete sites on a microtubule prior to polysome complex transport. Interactions all along the length of the microtubule, as is the case observed with traditional MAP–microtubule localization, would not be expected. Definitive proof of such hypotheses requires further experimentation, the results of which would undoubtedly lead to a better understanding of tau function, mRNA targeting, and the interactions between specialized cell membranes and the cytoskeleton.

We thank Dr. Gloria Lee of the Center for Neurologic Diseases (Harvard Medical School) for providing the human tau cDNA clone used in the FISH experiments and Dr. Virginia Lee (Department of Pathology, University of Pennsylvania, Philadelphia) for her generous gift of the Tau46 mAb. We are grateful to Ms. Michelle Losekamp and Mr. Paul E. Polak for their assistance with cell isolations. This work was supported by National Institutes of Health Grants RO1 AG06969 to L.I.B., PO1 NS24575 to S.S., and P50 AG08664 to S.C.P.

1. Weingarten, M. D., Lockwood, A. H., Hwo, S. Y. & Kirschner, M. W. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1858–1862.
2. Binder, L. I., Frankfurther, A. & Rebhun, L. I. (1985) *J. Cell Biol.* **101,** 1371–1378.
3. Drubin, D. G. & Kirschner, M. W. (1986) *J. Cell Biol.* **103,** 2739–2746.
4. Kowall, N. W. & Kosik, K. S. (1987) *Ann. Neurol.* **22,** 639–643.
5. Brion, J. P., Guilleminot, J., Couchie, D., Flament-Durand, J. & Nunez, J. (1988) *Neuroscience* **25,** 139–146.
6. Papasozomenos, S. C. & Binder, L. I. (1987) *Cell Motil. Cytoskeleton* **8,** 210–226.
7. Migheli, A., Butler, M., Brown, K. & Shelanski, M. L. (1988) *J. Neurosci.* **8,** 1846–1851.
8. Riederer, B. M. & Innocenti, G. M. (1991) *Eur. J. Neurosci.* **3,** 1134–1145.
9. Lee, G., Cowan, N. & Kirschner, M. W. (1988) *Science* **239,** 285–288.
10. Himmler, A. (1989) *Mol. Cell. Biol.* **9,** 1389–1396.
11. Himmler, A., Drechsel, D., Kirschner, M. W. & Martin, D. W. (1989) *Mol. Cell. Biol.* **9,** 1381–1388.
12. Lichtenberg, B., Mandelkow, E.-M., Hagestedt, T. & Mandelkow, E. (1988) *Nature (London)* **334,** 359–362.
13. Goedert, M., Crowther, R. A. & Garner, C. C. (1991) *Trends Neurosci.* **14,** 193–199.
14. Steiner, B., Mandelkow, E.-M., Biernat, J., Gustke, N., Meyer, H. E., Schmidt, B., Mieskes, G., Soling, H. D., Drechsel, D., Kirschner, M. W., Goedert, M. & Mandelkow, E. (1990) *EMBO J.* **9,** 3539–3544.
15. Loomis, P. A., Howard, T. H., Castleberry, R. P. & Binder, L. I. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8422–8426.
16. Szuchet, S., Polak, P. E. & Yim, S. H. (1986) *Dev. Neurosci.* **8,** 208–221.
17. Wilson, R. & Brophy, P. J. (1989) *J. Neurosci. Res.* **22,** 439–448.
18. Pfeiffer, S. E., Warrington, A. E. & Bansal, R. (1993) *Trends Cell Biol.* **3,** 191–197.
19. Brophy, P. J., Boccaccio, G. L. & Colman, D. R. (1993) *Trends Neurosci.* **16,** 515–521.
20. Benjamins, J. A., Iwata, R. & Hazlett, J. (1978) *J. Neurochem.* **31,** 1077–1085.
21. Colman, D. R., Kreibich, G., Frey, A. B. & Sabatini, D. D. (1982) *J. Cell Biol.* **95,** 598–608.
22. Trapp, B. D., Moench, T., Pulley, M., Barbosa, E., Tennekoon, G. & Griffin, J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7773–7777.
23. Barbarese, E. (1991) *J. Neurosci. Res.* **29,** 271–281.
24. Steward, O. & Banker, G. A. (1992) *Trends Neurosci.* **15,** 180–186.
25. Vouyiouklis, D. A. & Brophy, P. J. (1993) *J. Neurosci. Res.* **35,** 257–267.
26. Shin, R.-W., Iwaki, T., Kitamoto, T., Sato, Y. & Tateishi, J. (1991) *Lab. Invest.* **64,** 693–702.
27. Lee, V. M.-Y., Balin, B. J., Otvos, L., Jr., & Trojanowski, J. Q. (1991) *Science* **251,** 675–678.
28. Papasozomenos, S. C., Binder, L. I., Bender, P. K. & Payne, M. R. (1985) *J. Cell Biol.* **100,** 74–85.
29. Sternberger, L. A., Hardy, P. Y., Cuculis, J. J. & Meyer, H. G. (1970) *J. Histochem. Cytochem.* **18,** 315–333.
30. Szuchet, S., Arnason, B. G. W. & Polak, P. (1980) *J. Neurosci. Methods* **3,** 7–19.
31. Szuchet, S., Stefansson, K., Wollman, R. L., Dawson, G. & Arnason, B. G. W. (1980) *Brain Res.* **200,** 151–164.
32. Wang, Y., Loomis, P. A., Zinkowski, R. P. & Binder, L. I. (1993) *J. Cell Biol.* **121,** 257–267.
33. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
34. Laemmli, U. (1970) *Nature (London)* **227,** 680–685.
35. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
36. Kosik, K. S., Orecchio, L. D., Binder, L. I., Trojanowski, J. Q., Lee, V. M. & Lee, G. (1988) *Neuron* **1,** 817–825.
37. Yim, S. H., Szuchet, S. & Polak, P. E. (1986) *J. Biol. Chem.* **261,** 11808–11815.
38. Szuchet, S. (1987) in *Development and Regeneration*, eds. Althaus, A. & Seifert, W. (Springer, Berlin), pp. 755–777.
39. Szuchet, S. (1995) in *Neuroglial Cells*, eds. Ketlenmann, H. & Ransom, B. R. (Oxford Univ. Press, New York), in press.
40. Szuchet, S. & Dumas, M. (1983) in *Multiple Sclerosis*, ed. Antel, J. P. (Saunders, New York), pp. 729–755.
41. Sadot, E., Marx, R., Barg, J., Behar, L. & Ginzburg, I. (1994) *J. Mol. Biol.* **241,** 325–331.
42. Behar, L., Marx, R., Sadot, E., Barg, J. & Ginzburg, I. (1995) *Int. J. Dev. Neurosci.* **13,** 113–127.
43. Colman, D., Bernier, L., Staugaitis, S., Salzer, J. & Trapp, B. (1988) in *Neural Development and Regeneration*, eds. Gorio, A., Perez-Polo, J. R., de Vellis, J. & Haber, B. (Springer, Heidelberg), Vol. H22, pp. 301–309.
44. Sternberger, N. H. (1984) in *Oligodendroglia*, ed. Norton, W. T. (Plenum, New York), pp. 125–173.
45. Gozes, I. & Richter-Landsberg, C. (1978) *FEBS Lett.* **95,** 169–172.
46. Modesti, N. M. & Barra, H. S. (1986) *Biochem. Biophys. Res. Commun.* **136,** 482–489.
47. Dobrowolski, Z., Osińska, H., Mossakowska, M. & Barylko, B. (1986) *Eur. J. Cell Biol.* **42,** 17–26.
48. Dryer, C. A. & Benjamins, J. A. (1989) *J. Neurosci. Res.* **24,** 201–211.
49. Szuchet, S., Polak, P. E., Yim, S. H. & Arvanitis, D. (1988) *Glia* **1,** 141–150.
50. Pereyra, P. M., Horvath, E. & Braun, P. E. (1988) *Neurochem. Res.* **13,** 583–595.
51. Gillespie, C. S., Wilson, R., Davidson, A. & Brophy, P. J. (1989) *Biochem. J.* **260,** 689–696.
52. Kachar, B., Behar, T. & Dubois-Dalcq, M. (1986) *Cell Tissue Res.* **244,** 27–38.
53. Seldon, S. C. & Pollard, T. D. (1983) *J. Biol. Chem.* **258,** 7064–7071.
54. DiTella, M., Feiguin, F., Morfini, G. & Caceres, A. (1994) *Cell Motil. Cytoskeleton* **29,** 117–130.