Selective Stabilization of Tau in Axons and Microtubule-associated Protein 2C in Cell Bodies and Dendrites Contributes to Polarized Localization of Cytoskeletal Proteins in Mature Neurons

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Abstract. In mature neurons, tau is abundant in axons, whereas microtubule-associated protein 2 (MAP2) and MAP2C are specifically localized in dendrites. Known mechanisms involved in the compartmentalization of these cytoskeletal proteins include the differential localization of mRNA (MAP2 mRNA in dendrites, MAP2C mRNA in cell body, and Tau mRNA in proximal axon revealed by in situ hybridization) (Garner, C.C., R.P. Tucker, and A. Matus. 1988. Nature (Lond.). 336: 674--677; Litman, P., J. Barg, L. Rindzooski, and I. Ginzburg. 1993. Neuron. 10:627-638), suppressed transit of MAP2 into axons (revealed by cDNA transfection into neurons) (Kanai, Y., and N. Hirokawa. 1995. Neuron. 14:421-432), and differential turnover of MAP2 in axons vs dendrites (Okabe, S., and N. Hirokawa. 1989. Proc. Natl. Acad. Sci. USA. 86:4127-4131). To investigate whether differential turnover of MAPs contributes to localization of other major MAPs in general, we microinjected biotinylated tau, MAP2C, or MAP2 into mature spinal cord neurons in culture (~3 wk) and then analyzed their fates by antibiotin immunocytochemistry. Initially, each was detected in axons and dendrites, although tau persisted only in axons, whereas MAP2C and MAP2 were restricted to cell bodies and dendrites. Injected MAP2C and MAP2 bound to dendritic microtubules more firmly than to microtubules in axons, while injected tau bound to axonal microtubules more firmly than to microtubules in dendrites. Thus, beyond contributions from mRNA localization and selective axonal transport, compartmentalization of each of the three major MAPs occurs through local differential turnover.

Different compartmentalization of the cytoskeletal elements in nerve cells is an important feature occurring in the highly polarized structural differentiation shown by dendrites and axons of these cells (Dotti and Banker, 1987; Cleveland, 1990; Hirokawa, 1994). Among the cytoskeletal proteins showing a polarized distribution, microtubule-associated protein 2 (MAP2) is specifically localized in dendrites (Bernhardt and Matus, 1984; Caceres et al., 1984; Cumming et al., 1994; De Camilli et al., 1984), while tau is abundant in axons (Binder et al., 1985; Kosik and Finch, 1987; Peng et al., 1986; Brion et al., 1988; Mandell and Banker, 1995). Furthermore, MAP2C, an isoform derived from the MAP2 gene by alternative splicing, is specifically localized in both axons and dendrites in juvenile neurons (Tucker et al., 1988a; Meichsner et al., 1993), whereas it is no longer present in axons in mature neurons (Tucker et al., 1988b). Recent molecular biological approaches have revealed that cDNAs of MAP2 (1828 amino acids), MAP2C (467 amino acids), and tau (432 amino acids) are composed of an NH2-terminal projection domain having different lengths, with homologous microtubule-binding domains consisting of three to four repeats of 18 amino acids being located at the COOH-terminal region (reviewed by Goedert et al., 1991). Moreover, when MAP2 and tau are expressed in nonneuronal cells, these cells grow thin processes in which microtubules (MT) form bundles (Kanai et al., 1989; Lewis et al., 1989; Knops et al., 1991). In fact, MT bundles resembling those found in dendrites and axons are formed in the thin processes induced by expression of MAP2 and tau, respectively (Knops et al., 1991; Chen et al., 1992). Our recent gene targeting study of tau protein indicated its importance in the organization and stabilization of MTs in small caliber axons and also suggested that several MAPs may play a group role in affecting their functions (Harada et al., 1994). Hence, the polarized distribution of MAPs may have an important relationship with neuronal morphogen-
bodies (Garner et al., 1988; Litman et al., 1993). These MAPs, tau and MAP2C, is lacking. No earlier studies have proposed that differential localization of mRNA results in the final localization of MAPs.

The second mechanism demonstrated is differential turnover of MAP2 in the axon vs in the dendrites. This possibility arose from the microinjection of biotin-labeled MAP2 into cultured mature spinal cord neurons and subsequent analysis of the localization of injected MAP2 in the axon and dendrites (Okabe and Hirokawa, 1989). This study indicated that injected MAP2 is conveyed to both dendrites and the axon after 1 d, but MAP2 in the axon turned over quickly and disappeared ~3-4 d after microinjection, while MAP2 is stabilized in dendrites ~3-4 d after microinjection.

The third mechanism is the suppressed transit into axons. This was shown for MAP2 by transfection of a CDNA encoding it into cultured spinal cord neurons (Kanai and Hirokawa, 1995). Although tau and MAP2C were transported into the axon, MAP2 was not.

Although all three mechanisms are apparently used to localize MAP2, the relative contribution of each for two of the known MAPs, tau and MAP2C, is lacking. No earlier efforts have examined the potential that differential turnover of these in axons and dendrites may contribute to compartmentalization. In the case of MAP2C, mRNA is confined to the cell body (Garner et al., 1988) and despite its transport of the protein into the axon (Kanai and Hirokawa, 1995), MAP2C is compartmentalized in cell bodies and dendrites in mature neurons (Tucker et al., 1988b). Strongly suggests that compartmentalization of MAP2C requires differential stabilization in the cell body and dendrites.

To test this and to extend the analysis of differential stability to tau, we have microinjected biotin-labeled tau, biotin-MAP2C, and biotin-MAP2 into the mature spinal cord neurons, and subsequent analyses of the fates of injected MAPs at light microscopic and electron microscopic levels were performed.

Materials and Methods

Preparation of Biotin-labeled Proteins

Porcine brain MTs were prepared by temperature-dependent assembly and disassembly (Shelanski et al., 1973). MT proteins were conjugated with N-hydroxy-succinimidyld biotin (Kristofferson et al., 1986; Okabe and Hirokawa, 1989). Briefly, we heated 500 mg of MT proteins in 100 ml of assembly buffer (25% (vol/vol) glycerol, 100 mM Pipes, 1 mM EGTA, pH 6.8) containing 1 mM ATP and 10 mg/ml leupeptin at 37°C for 5 min and then added 100 mg of N-hydroxy-succinimidyld biotin. The biotin labeling was allowed to proceed for 15 min, after which the reaction was quenched by adding 1 g of potassium glutamate. The labeled MTs were further purified by two assembly-disassembly cycles. Biotin-tau and MAP2 were separated from MTs by a method described previously (Hirokawa et al., 1986a, b). MAP2C was purified from porcine brain according to Takeuchi et al. (1992). Purified MAP2C was incubated with tubulin purified from porcine brains by phosphocellulose column chromatography in one-third vol glycerol in PEM (0.1 M Pipes, 1 mM EGTA, 1 mM MgCl2, pH 6.8) at 37°C for 30 min. The resultant MT proteins were labeled with N-hydroxy-succinimidyld biotin as described above. After the reaction was quenched by K-glutamate, the MT proteins were centrifuged at 36,000 rpm (L8-58 Ultracentrifuge; Beckman Instruments, Inc., Fullerton, CA) at 35°C for 30 min. The pellets were suspended in 1 M NaCl-PEM at 4°C for 40 min, which was then applied to a PD10 column (Pharmacia Biotech AB, Uppsala, Sweden) to remove tubulin. The flow-through was collected and centrifuged at 2°C for 10 min. After adding 2 mM DT, the supernatant was boiled for 5 min, immediately cooled on ice for 30 min, and then brought to 50% saturation in ammonium sulfate by the addition of saturated ammonium sulfate. The sample was stirred for 30 min at 4°C and centrifuged at 12,000 g for 20 min at 4°C to precipitate biotin-labeled MAP2C. The precipitate was suspended in PEM buffer containing 0.75 M NaCl and then dialyzed against PEM.

Binding of Biotin-labeled Proteins to MT

Tau, biotin-tau, MAP2, biotin-MAP2, MAP2C, biotin-MAP2C, and tubulin in PEM buffer were centrifuged (TL-100; Beckman Instruments, Inc.) at 55,000 rpm for 30 min at 2°C. The supernatants were used for the experiments. All of these MAPs were mixed with tubulin (concentrations of MAPs and tubulin were 1.5 and 2 mg/ml, respectively) and incubated at 37°C for 30 min in the presence of 1 mM GTP. Then they were again centrifuged at 55,000 rpm for 30 min at 35°C. Supernatants and pellets were separated, and the pellets were suspended in PEM of equal volume to the supernatant. The same volume of supernatants and pellets was subsequently analyzed by SDS-PAGE.

Cell Culture and Microinjection

Spinal cord neurons of mouse embryos were cultured according to the method of Ranson et al. (1977). Mature neurons (~3-wk culture) were used. PC12 cells were cultured and fused using polyethylene glycol as described previously (Okabe and Hirokawa, 1988). Microinjection was performed according to Graessmann et al. (1980). For microinjection, the stored sample was dialyzed and diluted with an injection buffer (50 mM potassium glutamate, 1 mM MgCl2, pH 6.8) to 2-2.3 mg/ml. Cells were placed on a Diaphot inverted microscope (Nikon Inc., Garden City, NY) and then pressure injected using a micromanipulator (Narishige Scientific Laboratory, Tokyo, Japan). During the injection procedure, the cells were videotaped with a time-lapse videocorder to determine the exact incubation time after injection. The volume injected per cell was estimated by the method of Graessmann et al. (1980). Under a constant flow rate of the sample solution, we recorded the movement within the pipette and determined the sample solution flow rate. Using this flow rate and the mean injection time per cell, the volume injected per cell was estimated at <10-10 ml. Because the cell body of the smallest cell used was ~30 mm in diameter and mostly hemispheric in shape, and because the height of the cell body was ~5 mm, the calculated volume of this cell body was ~2.4 × 10-9 ml. From these data, the injected volume was estimated at ~4.2% of the cell body volume.

Immunofluorescence Localization and Immunoblotting of Native Tau, MAP2, and MAP2C in Spinal Cord Neurons in Culture

Cultured neurons (~3-wk culture) were fixed with 2% paraformaldehyde and 0.1% glutaraldehyde in PEM buffer for 30 min at room temperature (RT). After washing with PBS, they were permeabilized by immersion in 100% methanol at ~20°C for 6 min. Next, they were washed with PBS and incubated with 5% skim milk in PBS containing 10% normal goat serum for 30 min at 35°C. As the first antibodies, we used 100-fold diluted affinity-purified anti-MAP2 rabbit IgG (Hirokawa et al., 1988a, b) and 50-fold diluted anti-tau mouse mAb Tau-1 (Binder et al., 1985, 1986). In some cases, affinity-purified anti-MAP2 mouse mAb (HM-2; Sigma Chemical Co., St. Louis, MO) was used as the first antibody. The cells were stained with these first antibodies for 1 h at 35°C. After thorough washing, they were stained for 1 h at 35°C with 100-fold diluted FITC-labeled anti-rabbit IgG or rhodamine-labeled anti-mouse goat IgG in 5% skim milk, and 10% non-natural.
mal goat serum in PBS for 1 h at 35°C. Control cells were incubated with normal rabbit IgG or normal mouse IgG as the first antibodies. After washing, cells were mounted and observed with a microscope (Axiophoto; Zeiss, Inc., Thornwood, NY). In the phosphate treatment study, cells were incubated with 20 U/ml calf intestine alkaline phosphatase (TOYOBO, Tokyo, Japan) in alkal phosphatase buffer (50 mM Tris, pH 8.0) at 37°C for 2 h (Papasozomenos and Binder, 1987; Kanai et al., 1989). After washing with PBS, the cells were stained as described above.

For Western blotting, MT proteins were purified from cultured spinal cord cells (~3-wk culture) by the taxol-dependent procedure described by Vallee (1982). The cells were scraped from culture dishes after washing with PBS and then homogenized in 1.5 vol of PEM. The homogenates were centrifuged at 180,000 g for 90 rain at 4°C, the supernatant was collected, and GTP and taxol were added (final 1 mM GTP, 20 mM taxol). This was followed by warming the supernatant at 37°C for 15 min to allow MT polymerization, after which they were centrifuged at 100,000 g for 40 min at 30°C. The resultant pellets (MT proteins) were separated by SDS-PAGE and transferred onto nitrocellulose sheets. The sheets were immunoblotted using anti-MAP2 mAb (HM2; Sigma Chemical Co.), which stains MAP2A, B, C, and anti-tau mAb (tau-1; Boehringer Mannheim Biochemicals, Indianapolis, IN), followed by 125I goat anti-mouse IgG (ICN Pharmaceuticals, Inc., Irvine, CA).

**Immunofluorescence Localization of Biotin-MAPs**

Cells were fixed with 0.3% glutaraldehyde in a PEM buffer for 20 min, washed with PBS, treated with 1 mg/ml NaBH₄ in PBS for 30 min, and permeabilized with 1% Triton X-100 in PBS for 30 min at RT. The cells were sequentially incubated with 5% skim milk in PBS for 30 min at RT and then again with 100-times diluted rabbit antibody (Enzo Biochem., Inc., New York, NY) in 5% skim milk in PBS for 1 h at RT. After thorough washing, they were again sequentially incubated with 5% skim milk in PBS for 30 min at RT, followed by incubation with 100-times diluted rhodamine-conjugated goat anti-rabbit IgG (Cappel Laboratories, Cochranville, PA) in 5% skim milk in PBS for 1 h at RT. For some biotin-MAP-injected cells, the cells were double stained with anti–tubulin rabbit IgG and anti–MAP2 mouse mAbs (Sato et al., 1989) as first antibodies, and rhodamine-conjugated goat anti-rabbit IgG and FITC-conjugated goat anti-mouse IgG as second antibodies. In other cases, injected cells were permeabilized with a solution containing 30% glycerol, 80 mM Pipes, pH 6.8, 10 mM MgCl₂, 1 mM EGTA, 2.5 mM GTP, and 1% Triton X-100 for 2 min, and fixed with 5 mM ethylene glycol-bis-succinimidylic sulfinate (Gorsky et al., 1987) in a PEM buffer plus 1 mM GTP and 10 mM taxol for 20 min at 37°C. The cells were washed several times with PBS and quenched with 100 mM glycine-PBS for 30 min. After blocking with 5% skim milk in PBS for 1 h, they were incubated with 100-times diluted anti–tubulin rabbit IgG in 5% skim milk in PBS for 1 h. After washing with PBS they were again incubated with 5% skim milk in PBS for 30 min at 35°C, followed by staining with 100-times diluted rhodamine-conjugated rabbit anti–goat IgG in 5% skim milk in PBS for 1 h. For PC12 cells microinjected with biotin-MAP2C, the cells were treated as described above and stained with a mixture of anti–tubulin rabbit IgG and anti–tubulin mAb (NBD-conjugated goat anti-rabbit IgG) and fluorescein goat anti-mouse IgG being used as second antibodies. Cells were examined with a Zeiss Axiophoto fluorescence microscope. The fluorescence micrographs of spinal cord neurons were taken and printed under the same conditions.

**Electron Microscopy Immunocytochemistry**

Spinal cord neurons and PC12 cells were microinjected with biotin-MAP2, -MAP2C, or -tau and permeabilized as described above. The cells were subsequently fixed with 0.3% glutaraldehyde in PEM buffer plus 1 mM GTP and 10 mM taxol for 30 min at RT and then processed for EM using anti–tubulin antibodies and colloidal gold (5 nm)–labeled secondary antibodies (Janssen Pharmaceutica, Beerse, Belgium) according to Okabe and Hirokawa (1989).

**Immunoblotting and Quantitation of MAP2 in Peripheral Nerve Axons**

MT protein was purified from mouse sciatic nerves by the taxol-dependent procedure described by Vallee (1982). Briefly, sciatic nerves from 30 mice were homogenized in 1.5 vol of PEM buffer (100 mM PMSF, 1 mg/ml leupeptin) and centrifuged at 180,000 g for 90 min at 4°C. The supernatant at this stage is referred to as cytosolic extract. Taxol was added to 20 mM and GTP to 1 mM, the solution was warmed to 37°C for 30 min, and an MT pellet was formed by centrifuging at 100,000 g for 40 min at 30°C. For the quantitation of MAP2 and tubulin, MT protein was separated by SDS-PAGE and then transferred onto nitrocellulose sheets. Purified tubulin and MAP2 from porcine brain were loaded onto the same sheets as the concentration standards. The sheets were immunoblotted using anti-MAP2 polyclonal antibodies (Hirokawa et al., 1989) followed by 125I protein A (ICN Pharmaceuticals, Inc.) or anti–a-tubulin mAb (DM1A; Sigma Chemical Co.), followed by 125I goat anti-mouse IgG (ICN Pharmaceuticals, Inc.). The quantitation of protein concentration was performed using an image analyzer (Bau 2000; Fuji Photo Film Co., Ltd., Tokyo, Japan). Immunoblotting of MAPs in sciatic nerve cytosolic extract was similarly performed. For the first antibodies, we used anti-MAP1A mAb (Shiozawa and Hirokawa, 1982), anti-MAP2 mAb (HM2; Sigma Chemical Co.) and anti-tau mAb (tau 1; Boehringer Mannheim Biochemicals).

**Axon Measurements**

To measure the length of the axon where the biotin-MAPs had been conveyed after 24 h, micrographs were printed at a magnification of 213, and the length of the fluorescent axon from the leading edge of detectable fluorescence showing higher fluorescence than the background staining of axons of un.injected neurons to the cell body was measured.

**Results**

**Biotinylated MAPs Bound to MTs In Vitro and In Vivo**

To examine whether biotinylated tau, MAP2, or MAP2C binds to tubulin, we incubated native tau, native MAP2, native MAP2C, biotin-tau, -MAP2, or -MAP2C with phosphocellulose column–purified tubulin in PEM buffer containing 1 mM GTP. After incubation at 37°C for 30 min, the samples were centrifuged at 107,000 g at 35°C for 30 min. Fig. 2A shows SDS gel electrophoresis patterns of biotinylated tau (Fig. 2, lane 1), biotinylated MAP2 (Fig. 2, lane 4), and biotinylated MAP2C (Fig. 2, lane 7); supernatants and pellets of native tau plus tubulin (Fig. 2, lane 2), biotinylated tau plus tubulin (Fig. 2, lane 3), supernatants and pellets of native MAP2 plus tubulin (Fig. 2, lane 5), and biotinylated MAP2 plus tubulin (Fig. 2, lane 6); and supernatants and pellets of native MAP2C plus tubulin (Fig. 2, lane 8) and biotinylated MAP2C plus tubulin (Fig. 2, lane 9). Note that biotin-tau, -MAP2, and -MAP2C (Fig. 2, lanes 3, 6, and 9) effectively bound to tubulin. When we microinjected biotin-tau, -MAP2, or -MAP2C into PC12 cells to determine if these proteins bound to MTs in vivo, it was found that they (Fig. 2, B, C, and D, respectively) bound to MTs that were permeabilized before fixation. These data indicate that biotin-tau, -MAP2, and -MAP2C are capable of effectively binding to tubulin both in vitro and in vivo.

**Cultured Spinal Cord Neurons Develop Polarized Distribution of Tau, MAP2, and MAP2C**

First we confirmed whether the cultured mature spinal cord neurons (~3 wk) compartmentalize native tau mainly to axons and MAP2 and MAP2C to dendrites. Double staining with anti-tau antibodies and anti-MAP2 antibodies indicates that anti-tau antibodies (Tau-1) mainly stained axons, whereas anti-MAP2 antibodies specifically stained dendrites. In addition, these antibodies can only stain neurons. Because Tau-1 does not recognize tau proteins phosphorylated at tau-1 site, and because it was suggested that tau proteins phosphorylated at tau-1 site exist in certain

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Differential localization of mRNA

MAP2 mRNA extends into dendrites (Garner et al. 1988)

tau mRNA extends into proximal axon (Litićan et al. 1993)

MAP2c mRNA is confined to cell body (Garner et al. 1988)

Transfection of MAP2cDNAs (Kanai and Hirokawa 1995)

MAP2

MAP2c

Microinjection of biotin MAP2 (Okabe and Hirokawa 1989)

1 day

4 day

Microinjection of biotin tau

Microinjection of biotin MAP2c

Figure 1. Possible mechanisms for the compartmentalization of MAP2, MAP2C, and tau based on previous studies and subjects of the present study.

amounts in dendrites and in cell bodies in vivo (Papasozomenos and Binder, 1987), we stained the spinal cord neurons with tau-1 antibodies after treatment with alkaline phosphatase (Papasozomenos and Binder, 1987). Tau-1 stained many axons and some dendrites and cell bodies. In terms of the dendritic staining, double staining with anti-MAP2 antibodies revealed that tau-1 staining tended to be restricted to cell bodies and some proximal dendrites, while many dendrites were not stained. Thus, our data demonstrate that mature spinal cord neurons localize tau abundantly in axons, whereas cell bodies and some proximal dendrites localize tau phosphorylated at tau-1 site in small amounts. Concerning the localization of MAP2 and MAP2C, it should be realized that antibodies that specifically stain MAP2C are not available. However, because anti-MAP2 antibodies that recognize MAP2A, MAP2B, and MAP2C (HM-2; Sigma Chemical Co.) stain only cell bodies and dendrites, MAP2C is not localized immunofluorescently in the axon of spinal cord neurons at this stage. Immunoblotting of MT proteins from spinal cord-cultured

Figure 2. (A) SDS-PAGE analysis of biotinylated tau, MAP2, and MAP2C stained with Coomassie brilliant blue. (Lane 1) Biotinylated tau; (lane 2) supernatant (S) and pellet (P) of tau plus tubulin; (lane 3) supernatant (S) and pellet (P) of biotinylated tau plus tubulin; (lane 4) biotinylated MAP2; (lane 5) supernatant (S) and pellet (P) of MAP2 plus tubulin; (lane 6) supernatant (S) and pellet (P) of biotinylated MAP2 plus tubulin; (lane 7) biotinylated MAP2C; (lane 8) supernatant (S) and pellet (P) of MAP2C plus tubulin; (lane 9) supernatant (S) and pellet (P) of biotinylated MAP2C plus tubulin. Note that biotinylated tau, MAP2, and MAP2C efficiently bind to tubulin. Electron micrographs (B–D) of PC12 cells that were permeabilized, fixed, and stained with anti-biotin antibodies and colloidal gold–labeled second antibodies after microinjection of biotin-tau (B), -MAP2 (C), or -MAP2C (D). Note that injected tau, MAP2, and MAP2C efficiently bind to microtubules. B–D are the same magnification. Bar, 200 nm.
cells showed the existence of tau, MAP2, and MAP2C (data not shown). These data, in conjunction with immunofluorescence microscopy results, confirm the evidence that mature spinal cord neurons localize MAP2C in cell bodies and in dendrites.

**Microinjection of Biotin-labeled MAPs into Mature Spinal Cord Neurons in Culture and Analysis of Their Fates and MT Binding**

Equal amounts of biotinylated tau, MAP2, and MAP2C were separately microinjected into primary-cultured mature spinal cord neurons, with the microinjected cells then being fixed after specific time periods and processed for immunofluorescent microscopy using anti-biotin first antibodies and rhodamine-labeled second antibodies. 24 h after the injection, tau, MAP2, and MAP2C were found in both axons and dendrites in 100% of the cells examined (Tau, 95 cells; MAP2, 92 cells; MAP2C, 107 cells). Tau, MAP2, and MAP2C in the axon moved progressively, traveling 2.30 ± 0.85 mm (n = 22), 1.58 ± 0.49 mm (n = 30), and 1.7 ± 0.42 mm (n = 10), respectively, away from cell bodies after 24 h. Since biotin-MAP2 was detected with anti-biotin antibodies in axons that normally are not stained with anti-MAP2 antibodies, the question arises whether anti-biotin staining really reflects the localization of labeled MAP2. To clarify this, we double stained the microinjected neurons with anti-biotin antibodies and anti-MAP2 antibodies. MAP2 was detected in the axons of a microinjected neuron.

Next, we examined the affinity of injected tau, MAP2, and MAP2C for microtubules in axons and dendrites. To do this, 4 h after the injection, the cells were permeabilized with 1% Triton X-100 in MT-stabilized buffer, and then fixed and processed for anti-biotin staining. This method enabled us to extract soluble proteins and observe only tau, MAP2, and MAP2C that bind to the cytoskeleton. Interestingly, although tau was found in both axons and dendrites (Fig. 3 A), MAP2 tended to be confined to dendrites and cell bodies (Fig. 3 B). MAP2C also tended to be localized in dendrites and cell bodies (Fig. 3 C). This pattern of localization of MAP2 and MAP2C did not change at 24 h after injection. However, in the case of tau, the staining in dendrites and cell bodies became weak, while persisting in the axons (Fig. 4). As shown, all three are intensely bound to the neuronal cytoskeleton. At the EM level, we found that the gold particles were scattered in the cytoplasm of axons and dendrites in nonpermeabilized cells (Fig. 5 A).
Figure 4. (A) Immunofluorescence localization of biotin-tau in a spinal cord neuron that was permeabilized and fixed 24 h after injecting biotin-tau. Labeled tau is detected clearly in the axon (arrow) for a long distance, whereas staining in dendrites is much weaker (arrowhead) and in some dendrites staining is not detected (B, thin arrows). (B) The DIC image of the same field as A. Bar, 100 mm.

However, in permeabilized cells, tau, MAP2, and MAP2C were mainly localized in MT domains, and gold particles were especially observed between or on MTs in these permeabilized cells (Figs. 5, B–C and 6 A), while in the axon of cells injected with MAP2 only a few gold particles were found in MT domains (Fig. 6 B). This pattern of localization of gold particles resembles that of the immunocytochemical localization of MAPs detected with gold-labeled second antibodies in permeabilized neurons in vivo (Shiomura and Hirokawa, 1987a, b; and Hirokawa et al., 1988b).

3 d after injecting the cells fixed without permeabilization, the localization of injected tau, MAP2, and MAP2C in the axons was notably different. Although MAP2 tended to vanish in axons or was detectable only in their proximal region (i.e., 19% of the cells examined in axons, cell bodies, and dendrites, but 81% in the cell bodies and dendrites) (Fig. 7 B), the presence of tau persisted for a considerable length in axons, namely, 53% in axons, cell bodies, and dendrites, and 47% in axons (Fig. 7 A). In contrast, MAP2C was localized in cell bodies and dendrites at 50%, and in axons, cell bodies, and dendrites at 50% (Fig. 7 C).

4 d after the injection, however, tau had almost disappeared from cell bodies and dendrites, though it persisted in axons to a remarkable length: 94% in axons, and 6% in axons, cell bodies, and dendrites (Fig. 8 A). In fact, at this stage we still found MAP2 at 80% in cell bodies and dendrites, and at 20% in axons, cell bodies, and dendrites (Fig. 8 B), although its staining tended to be weaker than at day 3 of postinjection. At this stage the injected MAP2C was localized in the axons, cell bodies, and dendrites at 33%, while it was found at 67% in cell bodies and dendrites (Fig. 8 C). The localization of biotin-tau, -MAP2, and -MAP2C in nonpermeabilized cells at several time points is summarized in Fig. 9.

Existence of MAP2 in Peripheral Nerve Detected by Immunoblotting In Vivo

Whether or not MAP2 exists in axons in vivo remains a controversy. Although several immunofluorescence studies did not detect MAP2 in axons (Bernhardt and Matus, 1984; Cumming et al., 1984; Caceres et al., 1984; De Camilli et al., 1984), Vallee (1982) reported its existence in white matter by a biochemical study. Other immunohistochemical studies also indicated that MAP2 is localized in the axons of peripheral nerves, although only in limited quantities (Papassozomenos et al., 1985; Hirokawa et al., 1985). Thus, to determine if MAP2 really exists in axons, we used immunoblotting of homogenates of peripheral nerves by anti-MAP2 antibodies. Anti-MAP2 antibodies clearly stained a band of ~280 kD, thus supporting the possibility that a small amount of MAP2 is actually present in these nerves in vivo (Fig. 10). For comparison, the same materials were immunoblotted using anti-MAP1A antibodies, anti-tau antibodies, and anti-tubulin antibodies.

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Figure 5. Electron micrographs of neurons fixed and then permeabilized (A) or permeabilized and then fixed (B and C) 4 h after micro-injection of biotin-tau. A shows a varicosity in an axon. Gold particles are scattered in the cytoplasm. B and C show axons in a permeabilized cell, where gold particles tend to be localized either between or on the microtubules, though they are not localized in the neurofilament domains (*). Bar, 100 nm.

(Fig. 10). As another experiment to quantify the amount of MAP2 in sciatic nerves relative to tubulin, we immunoblotted MT pellets, purified MAP2 and tubulin using anti-MAP2 and anti-tubulin antibodies, and then quantitated the amount to have a 1:200 weight ratio of MAP2 to tubulin.

Discussion

Cultured Spinal Cord Neurons Develop Compartamentalization of Tau, MAP2, and MAP2C

Cultured spinal cord neurons (~3 wk) revealed a characteristic staining pattern with the tau-1 antibody and anti-MAP2 antibodies similar to that for nerve cells in vivo and other mature neurons in vitro; namely, tau-1 stains the axon, while anti-MAP2 stains dendrites. In terms of tau localization, Papasozomenos and Binder (1987) suggested that tau protein phosphorylated at the tau-1 site also exists in some dendrites. Other studies, however, showed compartmentalized localization of tau in the axon with anti-tau polyclonal antibodies (Kosik and Finch, 1987; Brion et al., 1988), and recently it was shown that although differently phosphorylated tau exists in dendrites in developing neurons, it tends to be compartmentalized in the axon and cell body in mature neurons (>2 wk in culture) (Mandel and Banker, 1995). In fact, our immunofluorescence data of spinal cord neurons treated with alkaline-phosphatase also indicated that in our system tau is mainly compartmentalized in the axon, although tau proteins phosphorylated at tau-1 site are also localized in small amounts in some proximal dendrites and cell bodies.

Concerning the localization of MAP2C in juvenile neurons, it has been proven by two approaches to be localized in axons: (i) immunofluorescence microscopy using one antibody which stains both MAP2A/2B and MAP2C, and another antibody which stains only MAP2A/2B (Tucker et al., 1988a), and (ii) transfection of tagged MAP2C cDNA into cultured neurons (Meichsner et al., 1993). On the other hand, Tucker et al. (1988b) showed that MAP2C
was not localized immunofluorescently in the axon of mature neurons, and we too did not detect it in the axon of cultured spinal cord neurons. However, because our immunoblotting study indicated the existence of MAP2C in cultured spinal cord cells, and because only cell bodies and dendrites of neurons were stained with antibodies that recognize MAP2A/2B and MAP2C, it is reasonable to conclude that cultured mature spinal cord neurons compartmentalize MAP2C in cell bodies and dendrites. Thus, judging from the characteristic staining pattern we obtained using anti-tau and anti-MAP2 antibodies, we consider our cultured spinal cord neurons as a model system of mature neurons.

**Biotin-labeled MAPs Progressively Moved in the Axon at a Slow Transport Rate**

After biotinylation, tau, MAP2, and MAP2C effectively bound to MTs both in vitro and in vivo. In addition, double staining with anti-biotin antibody and anti-MAP2 antibody showed that labeled MAP2 is conveyed in the axon. Although there may be some technical limitation in determining the rate of axonal transport of cytoskeletal proteins by the microinjection method, our data (tau, MAP2, and MAP2C moved $2.30 \pm 0.85$ mm, $1.58 \pm 0.49$ mm, and $1.7 \pm 0.42$ mm, respectively, in 24 h) indicate that the rates for the biotin-labeled proteins are in the range of slow ax-

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onal transport (Grafstein and Forman, 1980; Tytell et al., 1984). In this regard, Nixon et al. (1990) recently showed that MAP1A is transported in the axon of mouse retinal ganglion cells at a rate of 1.0–1.2 mm/d. Because the labeled MAPs reached >1 mm in the axon from the cell body, and judging from the rates for the transport of labeled MAPs, the movement of these MAPs could not be explained by simple diffusion (Lasek, 1988; Sabry et al., 1995). Taken together, these results suggest that biotin-tau, -MAP2, and -MAP2C behave normally in injected

Figure 7. Immunofluorescence localization of biotinylated tau (A), biotinylated MAP2 (B), and biotinylated MAP2C (C) in spinal cord neurons fixed 3 d after microinjection. (A) Tau exists in the axon and dendrites (arrows). Although tau is detected clearly for long distance in the axon, dendritic staining is weak (arrows). Bar, 100 mm. (B) MAP2 persists in the cell body and dendrites (long arrows), but is localized only in the proximal region of the axon (short arrows). (C) MAP2C also persists in the cell body and dendrites, although in a much shorter proximal segment in the axon compared to that of tau. Bar, 100 mm.
nerve cells and are transported down the axon. However, in contrast, our recent transfection studies of cDNAs of tagged MAPs (tau, MAP2, MAP2C) into cultured mature spinal cord neurons revealed that, although tau and MAP2C are detected in the axons over a long distance from the cell body, MAP2 is only localized at the short proximal segment of axons (Kanai and Hirokawa, 1995). Obviously then, our transfection study suggests the suppression of the transit of MAP2 into axons, which is seemingly in contradiction with the results we previously obtained after microinjection of biotin-MAP2 (Okabe and Hirokawa, 1989) as well as the present results. Nevertheless, because previous studies have revealed the existence of MAP2 in axons in vivo immunocytochemically (Papasozomenos et al., 1985; Hirokawa et al., 1985) and in white matter of brains biochemically (Vallee, 1982), and because our present Western blotting data for peripheral nerves convincingly proved that MAP2 exists in them, albeit in limited amounts, we suggest that a small amount of MAP2 is probably transported down the axon in native neurons but is unstable so as to become undetectable more distally in the axon.

Differential Affinity of Tau, MAP2, and MAP2C with MTs in the Axon vs Dendrites

Our results also indicate that microinjected tau, MAP2, and MAP2C have two different forms in the neuronal cyto-
Figure 9. Relative population of neurons which localize biotin-tau, MAP2, and MAP2C in particular regions of neurons at various days after microinjection. The neurons are fixed and then permeabilized at certain time periods after microinjection of labeled proteins. Percentages refer to percentage of cells with the indicated fluorescence distribution and are based on a total area of fluorescence above background. (Dendrite, Cell body & Axon) The injected proteins are detected in dendrites, cell bodies, and axons. The injected proteins are localized along axons for a much longer distance than dendrites. (Axon) The injected proteins are only detected in axons. Neurons localize injected proteins in axons at a longer distance than dendrites. (Dendrite & Cell body) The injected proteins are detected in dendrites and cell bodies. Neurons localizing injected proteins in axons at the same or a shorter distance from the cell body in comparison with dendrites are also included.

toplasm, MT-nonbound and MT-bound forms. Because phosphorylation is known to critically control the binding of MAPs to MTs, the balance between phosphatases and kinases controls which form occurs. The kinase candidates localized in neurons that phosphorylate these MAPs are proline-directed kinases such as MAP kinase, glycogen synthase kinase 3, or members of the cyclin-dependent kinase family, and other kinases such as protein kinase A, protein kinase C, and Ca²⁺ calmodulin kinase II. By comparing the localization of injected MAPs in dendrites of permeabilized cells to that in axons, we found that biotin-tau binds to MTs in the axon and dendrites, whereas MAP2 and MAP2C tend to more firmly bind to MTs in dendrites and cell bodies in comparison with MTs in the axon 4 h after microinjection. However, 24 h after microinjection, the affinity of biotin-tau to MTs in dendrites tends to become much weaker than in the axon. Although we purified biotin-labeled MAPs by MT binding so that they would undergo this activity before the injection, there was a tendency for biotin-MAP2 and -MAP2C to have weak affinity for MTs in the axon 4 h after microinjection. In the case of tau, although it took more time, biotin-tau in dendrites inclined to lose its affinity for MTs 24 h after microinjection. Such phenomena may be caused by (a) the difference between MTs in the axon vs those in dendrites, (b) the differential phosphorylation of MAPs in the axon vs that in dendrites, or (c) the differential sorting of distinct isoforms of MAPs, especially tau, in the axon vs that in dendrites. Concerning (a), the expression of differ-

Figure 10. Immunoblot analysis of mouse sciatic nerves by anti-MAPs and anti-tubulin antibodies. The cytosolic extract from mouse sciatic nerves was separated by SDS-PAGE (7.5% running gel), transferred onto a nitrocellulose sheet, and immunobotted using anti-MAP1A mAb (lane 1), anti-MAP2 mAb (lane 2), anti-tau mAb (lane 3), anti-alpha tubulin mAb (lane 4), and normal serum (lane 5). Molecular size standards (left).

Figure 11. Schematic drawing of the fates of microinjected tau, MAP2C, and MAP2 based on the present and previous studies (Okabe and Hirokawa, 1989). These results clearly indicated differential turnover and selective stabilization of tau, MAP2C, and MAP2.
ent β tubulin isotypes in different regions of nerve cells might be related. Joshi and Cleveland (1989) revealed that isotype V is partially excluded from neurites of PC12 cells differentiated by NGF. However, until now, no evidence has been available to suggest that the β tubulin isotype is different between MTs in axons and dendrites. This is also the case for (c), as there is no evidence to indicate that certain isoforms of tau are preferentially localized in the axon. Therefore, the distinct MT-binding activity of injected MAPs in the axon vs. that in the cell body and dendrites could be mainly controlled by the phosphorylation of MAPs. Because microinjected MAPs were derived from whole brains, they contain several differentially phosphorylated forms (Matsuo et al., 1994). Therefore, the time difference for the injected MAPs to acquire the ability of MT binding related to their selective location in certain compartments of neurons may be caused by the degree of the presence of several distinctly phosphorylated forms in the injected MAPs. The MT-binding activity of microinjected MAP2 and MAP2C in spinal cord neurons basically revealed a similar tendency, and consequently, the critical sites for phosphorylation of MAP2 and MAP2C, which regulate microtubule binding, could reside in common MAP2 structures, namely in the short NH2-terminal region, and most probably in the MT-binding, poline-rich, and repeat regions at the COOH-terminus. Concerning the control of MT-binding activity of MAPs, phosphorylation of MAP2 and tau with kinases such as protein kinase A, Ca2+-calmodulin kinase II, MAP kinase (reviewed by Wiche et al., 1991; Drewes et al., 1992; Goedert et al., 1992b), glucose synthase kinase 3 (Mandelkow and Mandelkow, 1995), CDK5 (Bauman et al., 1993; Kobayashi et al., 1993; Paudel et al., 1993), CDC2 kinase (Ledesma et al., 1992), and proline-directed kinase (Vulliet et al., 1992) induced dissociation of MAPs from MTs, whereas in another case, phosphorylation in vivo by unknown kinases induced microtubule binding (Brugg and Matus, 1991). There is absolutely no argument that the overall process of the regulation of MT binding of MAPs by kinases is very complex indeed, and that extensive research will be required for its eventual clarification.

Multiple Sorting Mechanisms of Tau and MAP2 in Neurons

Concerning the other possible mechanisms for the compartmentalization of MAPs, our previous study using the transfection of cDNAs of tau, MAP2, MAP2C, and their various deletion mutants into primary-cultured spinal cord neurons, the same system as the one used in the present study, indicated that transit of MAP2 into the axon was inhibited, while tau and MAP2C were transported for a long distance in the axon (Kanai and Hirokawa, 1995). This study clearly showed that the suppression of MAP2 transit into the axon plays an important role, whereas it also suggests that mere suppression of the transit of MAPs is not enough to determine their selective localization as MAP2C is restricted to localization in dendrites and cell bodies despite its transit into the axon for a long distance. On the other hand, the differential localization of mRNA may work for MAP2 and tau as another mechanism for the compartmentalization of MAPs (Garner et al., 1988;Litman et al., 1993). However, in the case of MAP2C, a previous study by Garner et al. (1988) showed that MAP2C mRNA is confined to the cell body in a different manner from MAP2 mRNA, which is extended into dendrites, whereas MAP2C is compartmentalized in the cell body and dendrites in mature spinal cord neurons similarly to MAP2. Obviously, localization of MAP2C mRNA in itself cannot explain this final localization of MAP2C. This also highlights the differential turnover of MAP2C as an important mechanism for its final compartmentalization.

Based on the present study and previous studies, it is reasonable to conclude that multiple mechanisms exist that cause the selective localization of tau in the axon and MAP2 and MAP2C in dendrites in mature neurons in vivo. Some of these mechanisms are as follows: (a) the extended localization of mRNA of MAP2 in dendrites (Garner et al., 1988) and mRNA of tau in the initial segment of the axon (Litman et al., 1993), (b) the differential turnover and selective stabilization of tau, MAP2, and MAP2C (Okabe and Hirokawa, 1989, and present study), and (c) the decreased transit of MAP2 into axons (Kanai and Hirokawa, 1995). Mechanisms (a–c) probably work in concert and lead to the final characteristic compartmentalization of tau, MAP2, and MAP2C in mature neurons.

We would like to thank Dr. S. Okabe for his help at the early stage of this work. Sincere gratitude is extended to Drs. L.I. Binder and Y. Ihara for their kind gifts of tau-1 and anti-MAP2 antibodies. We also thank Ms. Y. Kawasaki, H. Sato, and Mrs. H. Fukuda for their secretarial assistance.

This research was supported by a grant-in-aid for special project research by the Ministry of Education, Science, and Culture of Japan to N. Hirokawa.

Received for publication 5 December 1994 and in revised form 2 November 1995.

References

Bauman, K., E.-M. Mandelkow, J. Biernat, H. Piwinka-Worms, and E. Mandelkow. 1993. Abnormal Alzheimer’s-like phosphorylation of tau protein by cyclin-dependent cdk2 and cdK5. FEBS Lett. 336: 417–424.

Bernhardt, R., and A. Matus. 1984. Light and electron microscopic studies of the distribution of microtubule-associated protein 2 in rat brain: a difference between dendritic and axonal cytoskeleton. J. Comp. Neurol. 226:203–211.

Binder, L.I., A. Frankfurter, and L.M. Rehbun. 1985. The distribution of tau in the mammalian central nervous system. J. Cell Biol. 101:1371–1378.

Brion, J.P., J. Guilleminot, J. Couchie, J. Flamant-Durand, and J. Nunez. 1988. Both adult and juvenile tau microtubule-associated proteins are axon specific in the developing and adult rat cerebellum. Neuroscience. 25:139–146.

Brugg, B., and A. Matus. 1991. Phosphorylation determines the binding of microtubule-associated protein 2 (MAP2) to microtubules in living cells. J. Cell Biol. 114:728–734.

Caceres, A., L.I. Banker, M.R. Payne, P. Bender, L. Rehbun, and O. Steward. 1984. Differential subcellular localization of tubulin and the microtubule-associated protein MAP2 in brain tissue as revealed by immunocytochemistry with monoclonal hybridoma antibodies. J. Neurosci. 4:394–410.

Chen, J.-Y., Y. Kanai, N.J. Cowan, and N. Hirokawa. 1992. Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. Nature (Lond.). 360:674–677.

Cleveland, D.W. 1980. Microtubule MAPing. Cell. 60:701–702.

Cumming, R., R.D. Burgoyne, and N.A. Lytton. 1984. Immunofluorescence distribution of alpha tubulin, beta tubulin and microtubule-associated protein 2 during in vitro maturation of cerebellar granule cell neurons. Neuroscience. 12:775–782.

De Camilli, P., P.E. Miller, F. Navone, W.E. Theurkauf, and R.B. Vallee. 1984. Distribution of microtubule-associated protein 2 in the nervous system of the rat studied by immunofluorescence. Neuroscience. 11:819–846.

Dotti, C.G., and G.A. Banker. 1987. Experimentally induced alteration in the polarity of developing neurons. Nature (Lond.). 330:254–256.

Drewes, G., B. Lichtenberg-Kragg, F. Doring, E.-M. Mandelkow, J. Bienart, M. Doree, and E. Mandelkow. 1992. Mitogen activated protein (MAP) kinase transforms tau protein into an Alzheimer-like state. EMBO (Eur. Mol. Biol.

The Journal of Cell Biology, Volume 132, 1996 678
Grässmann, A., M. Graessmann, and C. Mueller. 1980. Microinjection of early
Garner, C.C., R.P. Tucker, and A. Matus. 1988. Selective localization of mes-
Goedert, M., E.S. Cohen, R. Jakes, and P. Cohen. 1992. p42 MAP kinase phos-
Goedert, M., R.A. Crowther, and C.C. Garner. 1991. Molecular characteriza-
Hirokawa, N., G.B. Bloom, and R.B. Vallee. 1985. Cytoskeletal architecture
Harada, A., K. Oguchi, S. Okabe, J. Kuno, S. Terada, T. Ohshima, R. Sato-
Ledesma, M.D., L. Correas, J. Avila, and J. Diaz-Nido. 1992. Implication of
Lasek, R.J. 1988. Studying the intrinsic determinants of neuronal form and
Kristofferson, D., T. Mitchison, and M. Kirschner. 1986. Direct observation of
crossbridges between microtubules and neurofibrils in vivo and in vitro.
Hirokawa et al. 1985. Cytoskeletal architecture and immunocytochemical localiza-
tonization of tau mRNA in differentiating neuronal cell culture: implications for
neuronal polarity. 
Mandel, J.W., and G.A. Banker. 1995. The microtubule cytoskeleton and the
development of neuronal polarity. 
Mandelkow, E., and E-M. Mandelkow. 1995. Microtubules and microtubule-
associated proteins. 
Matsuo, S., R.-W. Shin, M.L. Billingsley, A.V. de Voorde, M. O’Connor, J.Q.
Trotjanovalski, and V.M.-Y. Lee. 1994. Biopsy-derived adult human brain tau
is phosphorylated at many of the same sites as Alzheimer’s disease paired
helical filament tau. 
Meichner, M., T. Doll, B. Weishaar, and A. Matus. 1993. The low molecular
weight form of microtubule-associated protein 2 is transported into both ax-
ons and dendrites. 
Nixon, R.A., I. Fischer, and S.E. Lewis. 1990. Synthesis, axonal transport, and
turnover of the high molecular weight microtubule-associated protein MAP1A
in mouse retinal ganglion cells: tubulin and MAP1A display distinct trans-
port kinetics. 
Okabe, S., and N. Hirokawa. 1988. Microtubule dynamics in nerve cells: analy-
sis using microinjection of biotinylated tubulin into PC12 cells. 
Okabe, S., and N. Hirokawa. 1989. Rapid turnover of microtubule-associated
protein 2 in the axon. Proc. Natl. Acad. Sci. USA. 86:4127–4131.
Papassozomenos, S.C., and L.I. Binder. 1987. Phosphorylation determines two
different species of tau in the central nervous system. 
Perig, L.I., I. Binder, and M.M. Black. 1986. Biochemical and immunological
analyses of cytoskeletal domains of neurons. 
Ranson, B.R., E. Neale, M. Henkart, P.N. Bullock, and P.G. Nelson. 1977.
Mouse spinal cord in cell culture. 
Sabry, J., T.P. O’Connor, and M.W. Kirschner. 1995. Axonal transport of tubu-
lin in T11 pioneer neurons in situ. 
Sato, R., Y. Shiromura, H. Miyasaka, and N. Hirokawa. 1989. Molecular struc-
ture and localization of microtubule-associated protein 1B and its phosphor-
ylation-dependent expression in the developing neurons. 
Shelanski, M.L., F. Gaskin, and C.R. Cantor. 1973. Microtubule assembly in the
absence of added nucleotide. 
Shiomura, Y., and N. Hirokawa. 1987a. The molecular structure of microtubule-
associated protein 1A (MAP1A) in vivo and in vitro. An immunoelectron
microscopy and quick-freeze deep-etch study. 
Shiomura, Y., and N. Hirokawa. 1987b. Colocalization of microtubule-associ-
ted protein 1A and microtubule-associated protein 2 on the neuronal mi-
crotubules in situ revealed with double-label immunoelectron microscopy. 
Takeuchi, M., S. Hisanaga, T. Umezawa, and N. Hirokawa. 1992. The 72KD mi-
crotubule-associated protein from porcine brain. 
Tucker, R.P., L.I. Binder, and A.I. Matus. 1988a. Neuronal microtubule-associ-
ted proteins in the embryonic avian spinal cord. 
Tucker, R.P., L.I. Binder, C. Viereck, B.A. Hemmings, and A.I. Matus. 1988b.
The sequential appearance of low- and high-molecular weight forms of
MAP2 in the developing cerebellum. 
Tytell, M., S.T. Brady, and R.T. Lasek. 1984. Axonal transport of a subclass of
tau proteins: evidence for the regional differentiation of microtubules in neurons. 
Vallee, R.B. 1982. A taxol-dependent procedure for the isolation of microtu-
bules and microtubules-associated proteins (MAPs). 
Vulliet, R., S.M. Halloran, R.K. Baum, A.I. Smith, and G. Lee. 1992. Proline-
directed phosphorylation of human tau protein. 
Wiche, G., C. Oberkannins, and A. Hin blister. 1991. Molecular structure and
function of microtubule-associated proteins. 

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