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Rapid turnover of microtubule-associated protein MAP2 in the axon revealed by microinjection of biotinylated MAP2 into cultured neurons

(neuronal cytoskeleton/axonal transport/spinal cord neurons)

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ABSTRACT **We studied the mechanism of compartmentation of microtubule-associated protein 2 (MAP2) in the dendrites and cell bodies by using microinjection of biotin-labeled MAP2 into mature spinal cord neurons in culture. MAP2 molecules microinjected into the nerve cell body were distributed not only throughout the cytoplasm of the cell body and dendrites, but also in the axon as far as a few millimeters from the cell body within 24 hr after injection. However, when injected cells were incubated for more than 3 days, the amount of biotin-labeled MAP2 in the axon decreased remarkably compared with that in the dendrites. This indicates that there is no sorting mechanism in the cell body for the transport of MAP2 selectively into the dendrites but that the turnover rate of MAP2 in the axons differs from that in the dendrites. To further characterize the mechanism of MAP2 compartmentation, we performed immunoelectron microscopy of injected cells and detergent extraction of microinjected cells prior to immunocytochemistry with anti-biotin. The results strongly suggest that a large part of axonal MAP2 is not associated with cytoskeleton and that this weak association of MAP2 favors selective loss of MAP2 from the axon.**

The formation of two distinct classes of neuronal processes, axons and dendrites, is a fundamental step in neuronal morphogenesis because the functional polarity of nerve cells is established through this event. The cellular mechanism that determines the distinctive shapes of dendrites and axons is not clear, but several lines of evidence indicate that the cytoskeleton is one of the important endogeneous factors that control the elaborate shape of neuronal processes (1–3). Furthermore, recent studies have shown that axons and dendrites are not identical in the composition of their cytoskeletal proteins (4–6). Since microtubule-associated protein 2 (MAP2), one of the major microtubule-associated proteins in nervous tissue, is preferentially localized in dendrites and cell bodies, it is possible that this protein may be an intrinsic factor that regulates the shape of neurites as dendrites.

Although biochemical properties and immunocytochemical distribution of MAP2 have been thoroughly investigated (7–12), the molecular mechanism of MAP2 compartmentation is not known. The technique developed for the microinjection of haptenized cytoskeletal proteins combined with hapten-mediated immunocytochemistry has enabled us to examine directly the pattern of incorporation and turnover of cytoskeletal proteins in living cells (13–15). Thus, to directly answer the problem of MAP2 compartmentation, we microinjected biotin-labeled MAP2 (biotin-MAP2) into mouse spinal cord neurons, which were fully differentiated for 2–4 weeks in culture, and examined the distribution of injected molecules by using anti-biotin antibody. Our results indicate that injected MAP2 molecules are moved into both the dendrites and axons but are not associated tightly with the axonal cytoskeleton and are eliminated rapidly from the axon.

MATERIALS AND METHODS

Preparation of Biotin-MAP2. Porcine brain microtubules (MTs) were prepared by temperature-dependent assembly and disassembly (16). Two hundred milligrams of MT proteins in 40 ml of assembly buffer [20% (vol/vol) glycerol/100 mM Pipes, pH 6.8/1 mM $MgCl_2$/1 mM EGTA] containing 1 mM GTP was heated to 37°C for 30 min, and 40 mg of *N*-hydroxysuccinimidyl biotin (17) was added. The biotin labeling was allowed to proceed for 15 min after which the reaction was quenched by adding 400 mg of sodium glutamate. The labeled MTs were further purified by two assembly–disassembly cycles. Biotin-MAP2 was separated from MTs by the method described by Hirokawa *et al.* (12). Supernatants of the second cycle were boiled at 95°C for 5 min in the presence of 0.75 M NaCl, 2 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride and were then centrifuged at 80,000 × *g* for 40 min at 4°C. Proteins in the supernatants were sedimented by adding ammonium sulfate to 50% saturation and centrifuged at 80,000 × *g* for 45 min at 4°C. The pellet was resuspended in a solution containing 20 mM Pipes, 1 mM $MgCl_2$, 1 mM EGTA, and 0.75 M NaCl at pH 6.8 and was dialyzed against the same buffer. The sample was applied to a Superose 6 preparative grade gel-filtration column (Pharmacia). Fractions containing MAP2 were further concentrated by precipitation with 50% saturated ammonium sulfate. The pellet of ammonium sulfate precipitation was dissolved in injection buffer (50 mM potassium glutamate, pH 6.8/100 mM KCl/1 mM $MgCl_2$/1 mM EGTA).

MT assembly experiments were performed with PEM buffer (100 mM Pipes, pH 6.8/1 mM $MgCl_2$/1 mM EGTA) containing 1 mM GTP. After incubation at 37°C for 30 min, the samples were centrifuged at 100,000 × *g* at 35°C for 30 min. The supernatants and pellets were analyzed by SDS/PAGE with a 7.5% polyacrylamide running gel (18).

Cell Culture and Microinjection. Spinal cord cells were cultured according to the method of Ransom *et al.* (19) with slight modifications. Spinal cords were taken from 12- to 14-day-old mouse embryos, treated with 0.1% trypsin for 15 min at 37°C, and dissociated by repeated passage through a Pasteur pipette. The cell suspensions were plated onto polylysine-treated coverslips or 35-mm plastic dishes and maintained in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) precolostrum newborn calf serum (Mitsubishi Chemical Industries, Tokyo) and 10% (vol/vol) horse

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Abbreviations: MAP2, microtubule-associated protein 2; MT, microtubule; HRP, horseradish peroxidase.

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serum (GIBCO). Microinjection was performed according to the method of Graessmann *et al.* (20). Biotin-MAP2 was used at a concentration of 2.3 mg/ml for the microinjection. When cells were coinjected with biotin-MAP2 and horseradish peroxidase (HRP), the injected solution was adjusted to 2.3 mg of biotin-MAP2 per ml and 8.3 mg of HRP per ml. Lucifer yellow was used at a concentration of 30 mg/ml in 0.15 M LiCl (21).

PC12 cell culture and fusion were performed as described (15).

Immunofluorescence. For MAP2 staining, cells were fixed for 20 min with 2% (wt/vol) paraformaldehyde in PEM buffer. The cells were treated with cold methanol for 6 min and incubated sequentially with 5% (vol/vol) normal goat serum, polyclonal rabbit anti-MAP2 antibody (provided by Y. Ihara and K. Mori, Tokyo Metropolitan Institute of Gerontology), and rhodamine-conjugated goat anti-rabbit IgG.

When cells were microinjected with biotin-MAP2 and HRP, the samples were fixed with 2% paraformaldehyde and 0.1% glutaraldehyde in PEM buffer for 20 min, treated with 100 mM glycine in phosphate-buffered saline (PBS) for 30 min, and permeabilized with 1% Triton X-100 in PBS for 5 min. The cells were incubated sequentially with 25% normal goat serum, rabbit anti-biotin (Enzo Biochemicals), and rhodamine-conjugated goat anti-rabbit IgG. After photographs were taken, the samples were reacted with diaminobenzidine and hydrogen peroxide (22), postfixed with 1% osmium tetroxide, and rephotographed.

The procedure for the immunofluorescence of detergent-extracted cells was as follows. Injected cells were permeabilized with a solution containing 30% glycerol, 80 mM Pipes (pH 6.8), 10 mM $MgCl_2$, 1 mM EGTA, 2.5 mM GTP, and 1% Triton X-100 for 2 min and fixed with 5 mM ethylene glycol-bis(succinimidyl succinate) (23) in PEM buffer plus 1 mM GTP and 10 μM Taxol for 20 min. The cells were washed with PBS several times and quenched with 100 mM glycine/PBS for 30 min. After blocking with 5% (wt/vol) skim milk, the samples were reacted sequentially with rabbit anti-biotin antibody, fluorescein-conjugated goat anti-rabbit IgG, and fluorescein-conjugated rabbit anti-goat IgG.

All secondary antibodies were purchased from Cappel Laboratories.

Immunoelectron Microscopy. The cells injected with biotin-MAP2 were fixed with 1% glutaraldehyde in PEM buffer for 15 min, quenched with $NaBH_4$ at 1 mg/ml in PBS, and permeabilized with 1% Triton X-100. The cultures were sequentially treated with 5% skim milk, rabbit anti-biotin, goat anti-rabbit IgG conjugated with 5-nm colloidal gold (Janssen Pharmaceutica), and fluorescein-conjugated rabbit anti-goat IgG. The injected cells were marked with a needle under a fluorescence microscope. Osmification, block staining, embedding, and serial sectioning were done as described (15).

Immunoblotting. SDS/PAGE was performed using 6% acrylamide in the running gel and 3% acrylamide in the stacking gel. After electrophoresis, gels were transferred to nitrocellulose according to the method of Towbin *et al.* (24). Blotted nitrocellulose sheets were blocked by 5% (wt/vol) bovine serum albumin and then stained with rabbit anti-biotin antibody and peroxidase-coupled secondary antibody.

RESULTS

Biotin Labeling of MAP2. To study dynamic behavior of MAP2 in nerve cells, we prepared biotin-labeled MAP2. SDS/PAGE showed that this preparation contains MAP2 as a predominant polypeptide (Fig. 1*A*, lane 2). We examined biotin-MAP2 for its ability to promote tubulin polymerization *in vitro*. MAP2 modified with biotin promoted the assembly

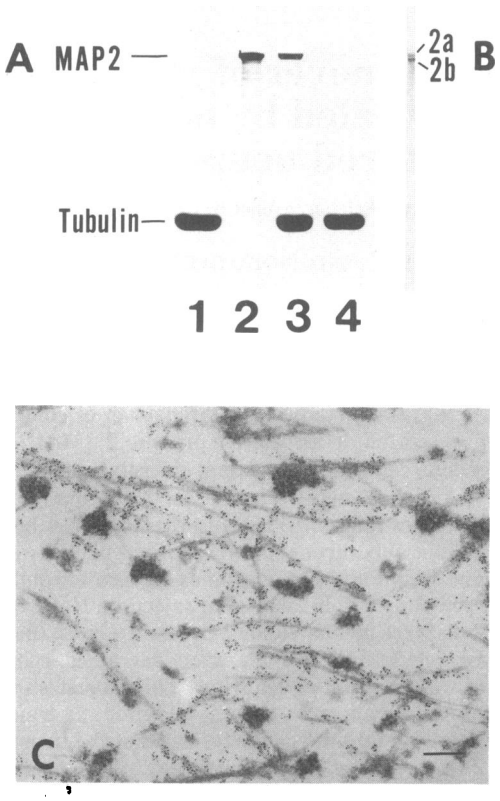

FIG. 1. Characterization of biotin-labeled MAP2. (*A*) SDS/PAGE analysis showing the activity of the biotin-MAP2 preparation in stimulating tubulin polymerization *in vitro*. Phosphocellulose column-purified tubulin (25) (lane 1) and biotin-MAP2 (lane 2) were mixed to a final concentration of 1 mg/ml and 0.13 mg/ml, respectively, heated to 37°C for 30 min, and centrifuged. The resulting pellet contained polymerized tubulin and MAP2 (lane 3), and only unpolymerized tubulin remained in the supernatant (lane 4). (*B*) Immunoblot of biotin-MAP2 reacted with an anti-biotin antibody. Anti-biotin reacted with two bands having the same electrophoretic mobility as MAP2a and MAP2b. (*C*) *In vivo* association of biotin-MAP2 with MTs revealed by the microinjection of labeled molecules into chemically fused and differentiated PC12 cells. Biotin-MAP2 associated with MTs was visualized by immunoelectron microscopy of thin sections by using a rabbit anti-biotin antibody and a goat anti-rabbit IgG conjugated with 5-nm colloidal gold. (Bar = 200 nm.)

of MTs *in vitro* at a tubulin concentration of 1 mg/ml, where there was no self-assembly (Fig. 1*A*, lane 3). Immunoblot analysis was performed to confirm that MAP2 is efficiently labeled with biotin. The biotin-MAP2 preparation was subjected to SDS/PAGE under conditions where MAP2 split into two electrophoretic bands, MAP2a and MAP2b, and then blotted onto nitrocellulose sheets. Anti-biotin reacted with only two bands having the same electrophoretic mobility as MAP2a and MAP2b (Fig. 1*B*), and there was no other distinct immunoreactivity on a nitrocellulose replica. This result indicated that this preparation contained only MAP2a and MAP2b as biotin-labeled polypeptides. To test whether labeled MAP2 is able to bind to MTs *in vivo*, we microinjected biotin-MAP2 into chemically fused PC12 cells (15, 26, 27) and performed immunoelectron microscopy with an anti-biotin antibody. Specific binding of biotin-MAP2 with MTs was revealed under electron microscopy (Fig. 1*C*), and the results indicated that a labeled fraction of our biotin-MAP2 preparation was capable of binding to MTs in living cells.

Cell Culture. We prepared dissociated cell cultures from mouse spinal cord and fully differentiated neurons, which were maintained for 2–4 weeks *in vitro* and were used for microinjection. Ultrastructural and electrophysiological studies have revealed that large neurons in this culture system develop two

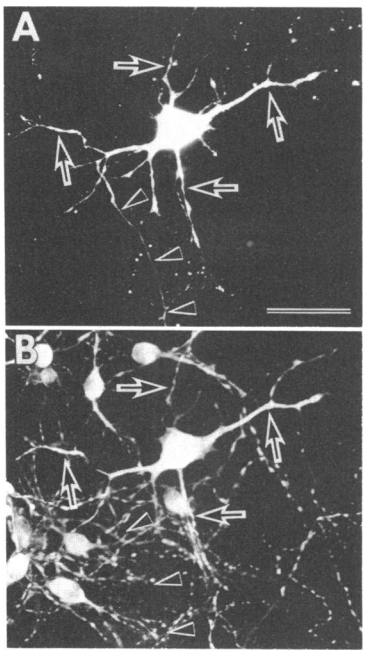

FIG. 2. Establishment of two distinct classes of neurites in mouse spinal cord neurons in culture. Spinal cord neurons were maintained for 2–4 weeks before use. (*A*) A large neuron injected with Lucifer yellow has relatively short, branching dendrites (arrows) and a long straight axon (arrowheads). (*B*) MAP2 immunoreactivity was observed only in the dendrites of the injected cell (arrows), and the axon was not stained with anti-MAP2 (arrowheads). (Bar = 50 μm.)

kinds of processes, dendrites and axons (19, 28). We examined the native pattern of MAP2 distribution in this culture system. Double-label immunocytochemistry of the cultured cells with anti-tubulin and anti-MAP2 antibodies revealed that MAP2 was not present in the axon-like thin processes that reacted with anti-tubulin (data not shown). To rule out the possibility that MAP2-positive and -negative neurites are derived from different classes of neurons, large neurons were microinjected with Lucifer yellow, fixed, and reacted with anti-MAP2 (11, 29) (Fig. 2). The intense fluorescence of Lucifer yellow revealed total arborization of neuronal processes, and all of the dendritic processes reacted with anti-MAP2. In turn, the long extending axon showed no MAP2 immunoreactivity. The results clearly indicate that MAP2 is localized to the dendrites and cell bodies of spinal cord neurons in culture, just as *in situ*, and justify the use of this culture system as a model for studying the mechanism of MAP2 compartmentation.

Distribution of Injected MAP2. Using the pressure microinjection technique, we introduced biotin-MAP2 into the cell bodies of large mature spinal cord neurons in culture. The injected biotin-MAP2 was detected by using an anti-biotin antibody and a rhodamine-labeled second antibody. Within 1 hr after injection, biotin-MAP2 molecules were distributed throughout the cytoplasm of the cell bodies and dendrites (Fig. 3*A*, arrows). In addition, they had moved into the proximal part of the axons (Fig. 3*A*, arrowheads). With longer incubation times, progressive spreading of biotin-MAP2 toward the tips of the axons was observed. Fig. 3*B* shows a cell fixed 1 day after injection. By this time, injected MAP2 had moved as far as a few millimeters from the cell body within the axon (arrowheads).

As a next step, we maintained the injected cells as long as possible and examined the distribution of injected molecules. Between 2 and 4 days after injection, we frequently observed cells whose dendrites were stained with anti-biotin, but whose axons were not. To see whether the amount of biotin-MAP2 had really decreased in the axons or if the axons themselves had degenerated due to the microinjection of MAP2, we microinjected spinal cord neurons with biotin-MAP2 plus HRP (28), stained with anti-biotin, and reacted with diaminobenzidine (22). Fig. 3 *C* and *D* show a cell fixed 3 days after injection. Total arborization of the neurites was

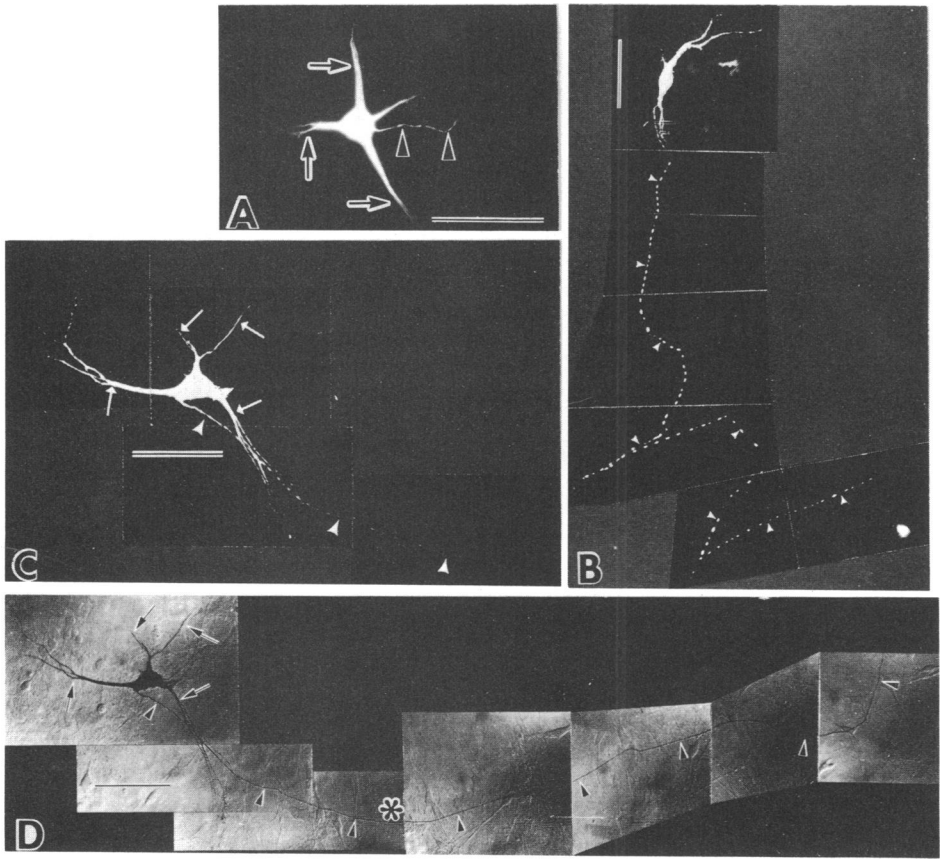

FIG. 3. Incorporation of biotin-MAP2 into the axons of spinal cord neurons and rapid turnover in them. (*A*) Anti-biotin staining of a cell fixed 1 hr after injection of biotin-MAP2. Labeled MAP2 was incorporated into both the dendrites (arrows) and the axon (arrowheads). (*B*) A spinal cord neuron fixed 1 day after injection and reacted with anti-biotin antibody shows incorporation of biotin-MAP2 into the axon (arrowheads). (*C*) Anti-biotin staining of a spinal cord neuron fixed 3 days after injection with biotin-MAP2 and HRP. Biotin-MAP2 is distributed throughout the cytoplasm of the cell body and dendrites (arrows) but is selectively lost from the axon (arrowheads). (*D*) A bright-field photomicrograph of the same cell shown in *C*. The dendrites (arrows) and axon (arrowheads) of the injected cell were visualized by HRP reaction product. The point where MAP2 staining decreased to the background level in the axon is indicated by an asterisk. (Bars = 100 μm.)

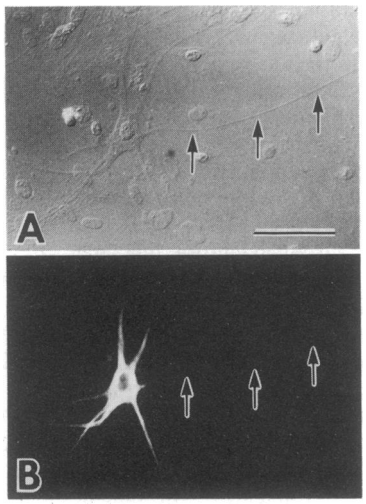

FIG. 4. Association of injected biotin-MAP-2 with the cytoskeleton studied by the extraction of soluble proteins under conditions where MTs are stable. (*A*) Differential interference contrast microscopy. (*B*) Immunofluorescence microscopy. Immunoreactivity with anti-biotin was not usually observed in the axons (arrows in *B*), which could be observed under differential interference contrast microscopy (arrows in *A*). (Bar = 50 μm.)

visualized with HRP reaction products. This cell clearly had a long extending axon (arrowheads) together with short tapering dendrites (arrows). Although all of the dendritic processes were stained with anti-biotin to their tips, the distal portion of the axon was not. This double-labeling procedure clearly indicates that the decrease of axonal staining was not due to the degeneration of axonal processes. Fig. 3*C* also showed that the axon had a uniform diameter along its length and was thicker than the distal part of most dendrites. Because all dendritic processes reacted with anti-biotin to their tips, it is not likely that the disappearance of axonal staining was due to its smaller diameter.

Detergent Extraction of Injected Cells. Our results suggest that the turnover rate of MAP2 is not uniform in the neuronal cells. One possible explanation for this is that the mode of association of MAP2 with the cytoskeleton in axons is different from that in dendrites. To test this possibility, we extracted the cells with Triton X-100 before fixation and examined biotin-MAP2 immunoreactivity in the cytoskeletal preparation of the injected cells (30). Fig. 4 shows a cell that was permeabilized before fixation and reacted with anti-biotin antibody. The dendritic cytoskeleton was intensely stained with anti-biotin, whereas the anti-biotin staining of the axon was weak compared with that of the dendrites. This result indicates that MAP2 binds to cytoskeleton more firmly in the dendrites than in the axons.

Electron Microscopic Immunocytochemistry. To further analyze the interaction of injected MAP2 with the neuronal cytoskeleton, we performed immunoelectron microscopy of microinjected cells. Fig. 5*A* shows a distal part of the dendrite where many MTs are running parallel. Gold particles, which indicate the presence of biotin-MAP2, were localized on the fibrillar matrix between the MTs (arrows). The overall pattern of biotin-MAP2 localization in the dendrites was identical to that of native MAP2 localization documented by previous immunohistochemical studies (1, 11, 12). In turn, specific association of gold particles with the cytoskeleton was not unambiguous in the axons. Fig. 5*B* shows the axon of the same cell as in Fig. 5*A*, and gold particles were distributed randomly within the axoplasm and not restricted to the close vicinity of MTs. Taken together with the results of detergent extraction experiments, the random distribution of biotin-MAP2 in the axon suggests a weak association of injected MAP2 with the axonal MTs.

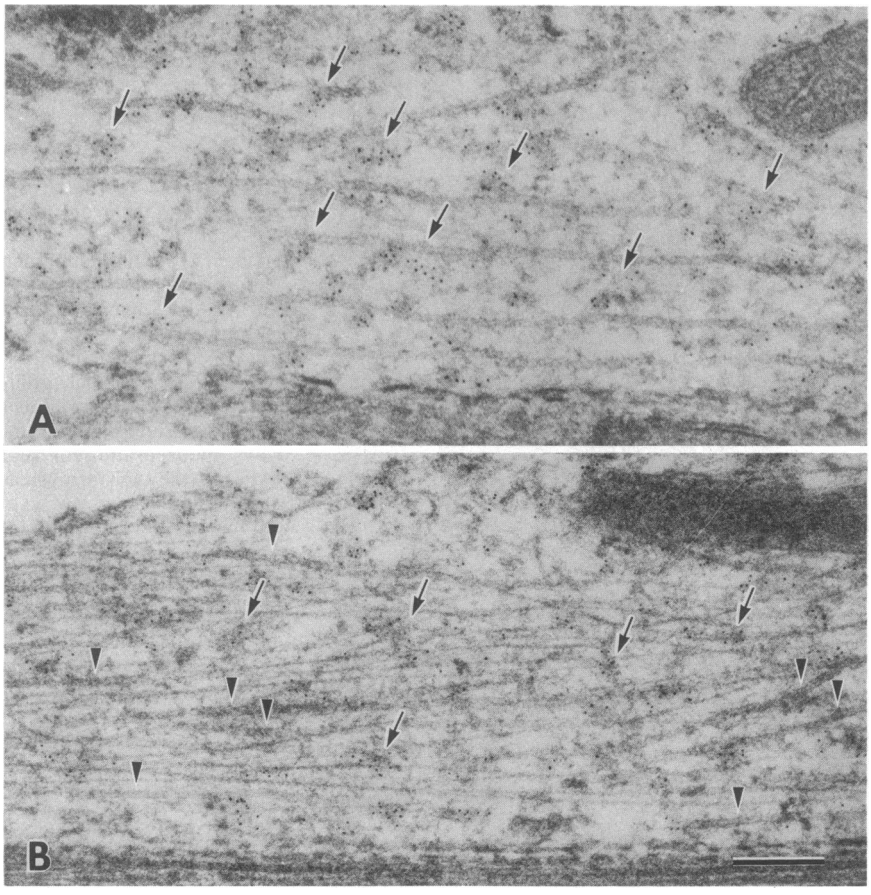

FIG. 5. Electron micrographs of a spinal cord neuron injected with biotin-MAP2 3 hr before fixation and reacted with rabbit anti-biotin and 5-nm colloidal gold–anti-rabbit IgG. (*A*) Distal part of a dendrite where many MTs are running parallel. Gold particles (arrows) are associated with fuzzy structures between the MTs. (*B*) Proximal part of the axon containing parallel running MTs (arrowheads) and neurofilaments. Gold particles not associated with MTs are indicated by arrows to illustrate the random distribution of biotin-MAP2 in the axoplasm. (Bar = 200 nm.)

DISCUSSION

The molecular mechanism of the establishment of MAP2 compartmentation is one of the central questions in the field of neuronal cytoskeleton. One possible explanation for this issue is that the access of MAP2 into the axons is limited and MAP2 molecules newly synthesized in the cell body can not move into the axons. However, we have shown here that injected MAP2 can move into the axons as far as a few millimeters from the cell body within 1 day after microinjection. Furthermore, several reports have documented that in native neurons MAP2 is not totally excluded from the axons, but a small amount of MAP2 exists in the axonal processes (9, 10, 31). This evidence collectively indicates that the access of MAP2 into the axons is not so limited under physiological conditions.

Another possible mechanism for the MAP2 compartmentation is that MAP2 can move freely into the axons, but the turnover rate of MAP2 in the axons is faster than it is in the dendrites. We maintained the neurons injected with MAP2 for several days before fixation and directly showed that MAP2 molecules in the axons were selectively eliminated. Interestingly, several reports have documented that MAP2 is present in both the axons and dendrites of cultured neurons at the early stages of development and is selectively lost from the axon during subsequent maturation (32–34). The selective loss of MAP2 began after 3 days in culture, and no axonal staining with MAP2 remained by the end of the first week in culture. The similar time course of MAP2 disappearance was observed in our experiments, and it is concluded from these two experiments that the identical processes may occur in all nerve cells. Thus, it is likely that the native localization of MAP2 may be established through the same sequence observed in our injection experiments.

We have shown that injected MAP2, which moved into the axons, was not tightly associated with the axonal cytoskeleton. Because biochemical and immunological properties of tubulin molecules in the axons are different from those in cell bodies and dendrites, it is possible that axonal MTs themselves cannot interact with MAP2 molecules (35, 36). Alternatively, other types of cytoskeletal proteins such as τ may block the MAP2-binding sites on MTs in the axons. It has been documented that MAP2 and τ competitively bind to MTs *in vitro* (37), and it is possible that τ occupies the MAP2-binding sites of MTs in the axons. Furthermore, posttranslational modification of MAP2 [for example, phosphorylation (38)] may occur specifically in the axons and inhibit the association of MAP2 to the MTs.

Although the relation between the rapid turnover of MAP2 and the weak association of MAP2 with the axonal cytoskeleton is not yet clear, it is logical to hypothesize that these two molecular events are closely linked. Hollenbeck and Bray (39) have investigated the nature of phase-dense varicosities in the axons of cultured neurons and showed that these organelles, called "neuronal parcels," contain cytoskeletal components and move along the neurites bidirectionally. Similar organelles may play a role in eliminating axonal MAP2 that is not incorporated into the cytoskeleton. It is also possible that free MAP2 molecules are selectively degenerated by proteases and lost from the axons.

A major goal of this study is to understand the native turnover of MAP2 and its relation to compartmentation. Our results suggest that the somatodendritic localization of MAP2 can be explained simply by the rapid turnover of axonal MAP2 without assuming the sorting mechanism in the cell body. Namely, MAP2 molecules are synthesized in the cell body and dendrites and are mainly associated with the somatodendritic cytoskeleton. A small fraction of MAP2 in the cell body is moved into the axons, but axonal MAP2 can not interact with the cytoskeleton and turnover rapidly. As a result, the somatodendritic localization of MAP2 is established in the native neurons.

We have proposed an additional mechanism for the compartmentation of the cytoskeletal proteins in neuronal cells. The establishment of functionally distinct regions within a single cell is a general prerequisite for the specialized functions of eukaryotic cells. Whether other highly differentiated cells compartmentalize the macromolecules by their preferential association with cytoskeletal polymers is an interesting and intriguing subject for future studies.

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1. Solomon, F. (1981) *J. Cell Biol.* **90,** 547–553.
2. Hirokawa, N. (1982) *J. Cell Biol.* **94,** 129–142.
3. Letourneau, P. C. & Ressler, A. H. (1984) *J. Cell Biol.* **98,** 1355–1362.
4. Matus, A., Bernhardt, R. & Hugh-Jones, T. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3010–3014.
5. De Camilli, P., Miller, P. E., Navone, F., Therkauf, W. E. & Vallee, R. B. (1984) *Neuroscience* **11,** 819–846.
6. Hirokawa, N., Glicksman, M. A. & Willard, M. B. (1984) *J. Cell Biol.* **98,** 1523–1536.
7. Sloboda, R. D., Rudolph, S. A., Rosenbaum, J. L. & Greengard, P. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 177–181.
8. Borisy, G. G., Marcum, J. M., Olmsted, J. B., Murphy, D. B. & Johnson, K. A. (1975) *Ann. N.Y. Acad. Sci.* **253,** 107–132.
9. Vallee, R. B. (1982) *J. Cell Biol.* **92,** 435–442.
10. Papasozomenos, S. C., Binder, L. I., Bender, P. K. & Payne, M. R. (1985) *J. Cell Biol.* **100,** 74–85.
11. Shiomura, Y. & Hirokawa, N. (1987) *J. Cell Biol.* **104,** 1575–1578.
12. Hirokawa, N., Hisanaga, S. & Shiomura, Y. (1988) *J. Neurosci.* **8,** 2769–2779.
13. Soltys, B. J. & Borisy, G. G. (1985) *J. Cell Biol.* **100,** 1682–1689.
14. Schulze, E. & Kirschner, M. (1986) *J. Cell Biol.* **102,** 1020–1031.
15. Okabe, S. & Hirokawa, N. (1988) *J. Cell Biol.* **107,** 651–664.
16. Shelanski, M. L., Gaskin, F. & Cantor, C. R. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 765–768.
17. Kristofferson, D., Mitchison, T. & Kirschner, M. (1986) *J. Cell Biol.* **102,** 1007–1019.
18. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
19. Ransom, B. R., Neale, E., Henkart, M., Bullock, P. N. & Nelson, P. G. (1977) *J. Neurophysiol.* **40,** 1132–1150.
20. Graessmann, A., Graessmann, M. & Mueller, C. (1980) *Methods Enzymol.* **65,** 816–825.
21. Stewart, W. W. (1978) *Cell* **104,** 9–18.
22. Graham, R. C. & Karnovsky, M. J. (1966) *J. Histochem. Cytochem.* **14,** 291–302.
23. Gorbsky, G. J., Sammak, P. J. & Borisy, G. G. (1987) *J. Cell Biol.* **104,** 9–18.
24. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
25. Weingarten, M. D., Lockwood, A. H., Hwo, S. & Kirschner, M. W. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1858–1862.
26. Greene, L. A. & Tischler, A. S. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2424–2428.
27. Hatanaka, H. (1981) *Brain Res.* **222,** 225–233.
28. Neale, E. A., MacDonald, R. L. & Nelson, P. G. (1978) *Brain Res.* **152,** 265–282.
29. Hirokawa, N. (1986) *J. Cell Biol.* **103,** 33–39.
30. Webster, R. E., Henderson, D., Osborn, M. & Weber, K. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5511–5515.
31. Hirokawa, N., Bloom, G. S. & Vallee, R. B. (1985) *J. Cell Biol.* **101,** 227–239.
32. Caceres, A., Banker, G., Steward, O., Binder, L. & Payne, M. (1984) *Dev. Brain Res.* **13,** 314–318.
33. Caceres, A., Banker, G. A. & Binder, L. (1986) *J. Neurosci.* **6,** 714–722.
34. Kosik, K. S. & Finch, E. A. (1987) *J. Neurosci.* **7,** 3142–3153.
35. Brady, S. T., Tytell, M. & Lasek, R. J. (1984) *J. Cell Biol.* **99,** 1716–1724.
36. Cambray-Deakin, M. A. & Burgoyne, R. D. (1987) *J. Cell Biol.* **104,** 1569–1574.
37. Kim, H., Jensen, C. G. & Rebhun, L. I. (1986) *Ann. N.Y. Acad. Sci.* **466,** 218–239.
38. Vallee, R. B., DiBartolomeis, M. J. & Theurkauf, W. E. (1981) *J. Cell Biol.* **90,** 568–576.
39. Hollenbeck, P. J. & Bray, D. (1987) *J. Cell Biol.* **105,** 2827– 2835.