Posttranslational Processing of α-Tubulin during Axoplasmic Transport in CNS Axons

BEVERLY A. BROWN, RALPH A. NIXON, and CHARLES A. MAROTTA
Laboratories for Psychiatric Research and Ralph Lowell Laboratories, Mailman Research Center, McLean Hospital, Belmont, Massachusetts 02178, and Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02115. Dr. Brown's present address is New England Nuclear Corporation, Billerica, Massachusetts 01862

ABSTRACT Tubulin proteins in mouse retinal ganglion cell (RGC) neurons were analyzed to determine whether they undergo posttranslational processing during axoplasmic transport. α- and β-tubulin comprised heterogeneous proteins in the primary optic pathway (optic nerve and optic tract) when examined by two-dimensional (2D) PAGE. In addition, however, α-tubulin exhibited regional heterogeneity when consecutive 1.1-mm segments of the optic pathway were analyzed separately. In proximal segments, α-tubulin consisted of two predominant proteins separable by isoelectric point and several less abundant species. In more distal segments, these predominant proteins decreased progressively and the α-tubulin region of the gel was represented by less abundant multiple forms only; β-tubulin was the same in all segments. After intravitreal injection of [^{3}H]proline to mice, radiolabeled α- and β-tubulin heteroproteins were conveyed together at a rate of 0.1–0.2 mm/d in the slowest phase of axoplasmic transport. At 45 d postinjection, the distribution of radiolabeled heterogeneous forms of α- and β-tubulin in consecutive segments of optic pathway resembled the distribution of unlabeled proteins by 2D PAGE, indicating that regional heterogeneity of tubulin arises during axonal transport. Peptide mapping studies demonstrated that the progressive alteration of α-tubulin revealed by PAGE analysis cannot be explained by contamination of the α-tubulin region by other proteins on gels. The results are consistent with the posttranslational processing of α-tubulin during axoplasmic transport. These observations, along with the accompanying report (*J. Cell Biol.*, 1982, 94:150–158), provide additional evidence that CNS axons may be regionally specialized.

Although ubiquitous among eukaryotic cell types, microtubules are found in particularly high concentration in neurons where they occur in perikarya, axons, and dendrites (6, 38). These organelles have been implicated in a variety of cellular functions including motility, secretion, chromosome movement during mitosis, the maintenance of cell shape, and axoplasmic transport (6, 45). Microtubules are composed of the nonidentical subunit proteins α- and β-tubulin (9). Numerous lines of evidence have led to the conclusion that brain tubulin exists in heterogeneous forms. For example, by two-dimensional PAGE, both α- and β-tubulin can be separated into multiple proteins (21, 33). At least four different α-tubulins (46) and at least two β-tubulins (29) are present in porcine brain, as established by amino acid sequence analysis. The observation that heterogeneous forms of α- and β-tubulin are synthesized in response to multiple messenger RNAs (10, 13, 37, 47) is consistent with the existence of multiple genes for these proteins (12, 14). A further source of tubulin heterogeneity can be accounted for by the variety of posttranslational processes that tubulin may undergo (1, 16, 17, 19, 40, 48–50, 52, 53).

There is currently no detailed information on whether different forms of the heterogeneous α- and β-tubulin subunits are associated with specialized neuronal structures or functions. As an initial step toward examining this question, axoplasmic transport studies were combined with PAGE analysis of proteins to investigate tubulin in retinal ganglion cell (RGC) axons of the mouse. Our results indicate that multiple forms of both α- and β-tubulin are conveyed in the slowest phase of axoplasmic transport; however, several unique forms of α-tubulin are associated exclusively with proximal RGC axonal regions and are absent in distal segments of RGC axons. A portion of this work was previously reported in preliminary form (7).

MATERIALS AND METHODS

Animals

Male or female mice of the C57Bl/6J inbred strain aged 10–14 wk were used in all experimental studies. The mouse strains, initially obtained from Jackson Laboratories (Bar Harbor, ME), were bred at our facility. Mice were housed at 23°C on a 12-h light-dark cycle and maintained on Wayne Blox (Allied Mills, Chicago, IL) supplied ad libitum.

Tissue Dissections

The mice were sacrificed by cervical dislocation followed by decapitation. The primary optic pathway consisting of (*a*) optic nerve severed at the scleral surface of the eye, (*b*) optic chiasm, and (*c*) optic tract extending to the lateral geniculate nucleus, was dissected bilaterally as described in the accompanying report (43). In certain cases, the optic pathway samples were cut into consecutive 1.1-mm segments on a micrometer-calibrated slide. Tissues were frozen and stored at −70°C for up to 1 mo before analysis. Preliminary experiments, including enzyme inactivation studies using microwave irradiation, revealed no postmortem changes in protein composition during the dissection and storage procedures (43).

Tubulin Purification

Tubulin was purified from mouse brain by the assembly-disassembly procedure of Shelanski et al. (51), and was separated from copurifying proteins by phosphocellulose chromatography as described by Herzog and Weber (26). The tubulin was examined by one- and two-dimensional gels and shown to contain multiple α- and β-proteins of high purity as demonstrated in a previous report (34).

PAGE

Optic pathway proteins were prepared for PAGE in such a way as to minimize proteolysis and protein loss as previously described (43). The preparation of one-dimensional and two-dimensional polyacrylamide gels based upon the procedures of Laemmli (30) and of O'Farrell (44), respectively, and fluorographic and autoradiographic procedures were described in detail in previous reports (8, 33, 34, 37). LKB ampholytes (LKB Instruments, Inc., Rockville, MD) were used in isoelectric focusing gels. In all cases long slab gels were used (dimensions 140 × 260 × 1.5 mm). An Ortec densitometer was used to scan proteins of one-dimensional gels (35). Further experimental details were given in the accompanying report (43).

Peptide Mapping

Staphylococcus aureus V8 protease was used to generate peptide patterns (8, 11). Protein samples previously purified by SDS PAGE were excised from gels, homogenized, and were subjected to protease digestion during electrophoresis on 10–15% polyacrylamide gels (8).

Axonal Transport Studies

Intravitreal injections of radiolabeled amino acids were made with a glass micropipette apparatus into anesthetized mice as previously described (41). Depending on the experiment, 10–100 μCi of L-[2,3-^{3}H]proline (sp act 30–50 Ci/mmol) or L-[^{35}S]methionine (sp act 1,000 Ci/mmol), purchased from New England Nuclear (Boston, MA), was administered in a volume of 0.35 μl of phosphate-buffered saline, pH 7.4.

The rates of transport of various proteins along the retinal ganglion cell axons were determined as described in the accompanying paper (43).

RESULTS

Heterogeneity of Tubulin in the Mouse Primary Optic Pathway

Tubulin in the mouse primary optic pathway, consisting of the optic nerve (ON) and optic tract (OT), was identified initially by comparison with the purified protein. Brain tubulin from adult C57Bl/6J mice was prepared by two cycles of assembly-disassembly followed by phosphocellulose chromatography. α- and β-tubulin are of approximately equal size; however, they can be separated into several species when electrophoresed in a PAGE system containing SDS (9). In the present study, purified tubulin was separated into at least two bands after electrophoresis (Fig. 1 *A*). The more slowly migrating band was designated α-tubulin, and the more rapidly migrating band was designated β-tubulin. These two groups of proteins yield dissimilar peptides after digestion with trypsin or *S. aureus* V8 protease (8, 36, 37). When primary optic pathway was subjected to SDS PAGE, multiple protein bands in the tubulin region were observed although individual components of this region were poorly resolved (Fig. 1 *B*). ON and OT proteins were examined separately. ON protein bands comigrating with α-tubulin consisted predominantly of a broad and intensely stained band and more lightly stained bands (Fig. 1 *C*). By contrast, proteins in OT comigrating with α-tubulin were represented only by multiple species that stain relatively lightly (Fig. 1 *D*). Unlike α-tubulin protein bands, β-tubulin appeared identical in optic pathway, ON, OT, and purified tubulin.

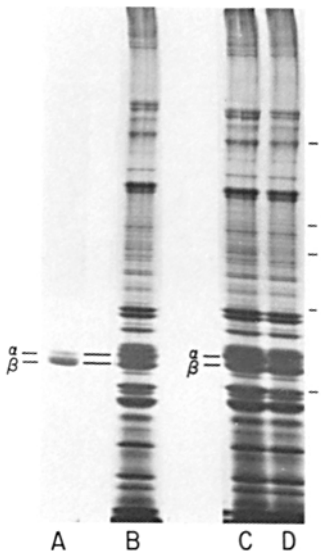

FIGURE 1 SDS PAGE of mouse primary optic pathway proteins and of tubulin. (*A*) Purified tubulin, (*B*) optic pathway proteins (optic nerve plus optic tract), (*C*) optic nerve proteins, (*D*) optic tract proteins. α- and β-tubulin bands are indicated. The standards (not shown) used to determine molecular weight values (given on figure, × 10^{-3}) were myosin, 210,000; β-galactosidase, 130,000; phosphorylase *b*, 94,000; bovine serum albumin, 68,000; ovalbumin, 43,000. All samples were electrophoresed in adjacent or nonadjacent lanes of the same 5–15% polyacrylamide gel and were visualized by Coomassie Brilliant Blue staining.

α-Tubulin Is Different on Two-dimensional Gels of ON and OT

Tubulin of the primary optic pathway migrated on two-dimensional gels to positions characteristic of α- and β-tubulin subunits from a variety of other CNS preparations (2, 10, 21, 24, 27, 33, 34, 37) including tubulin prepared by assembly-disassembly (33, 34) and by colchicine-affinity chromatography (36). As shown in Fig. 2 *A*, the optic pathway tubulin region consists of multiple proteins differing in both pI in the isoelectric focusing gel and rate of migration in the SDS gel. β-Tubulin consists of a group of proteins separable primarily by isoelectric point differences. Two-dimensional PAGE of proteins from separated ON and OT also yielded results similar to those of Fig. 1. The ON α-tubulin region consisted predominately of at least two intensely stained proteins of similar size (Fig. 2 *B*) in addition to lightly stained species, whereas OT α-tubulin displayed only a diffuse pattern of lightly stained proteins (Fig. 2 *C*). β-Tubulin appeared identical in ON and OT. As shown in Fig. 2 *D–F*, after two-dimensional PAGE, purified α- and β-tubulin overlap tubulin proteins of ON and OT.

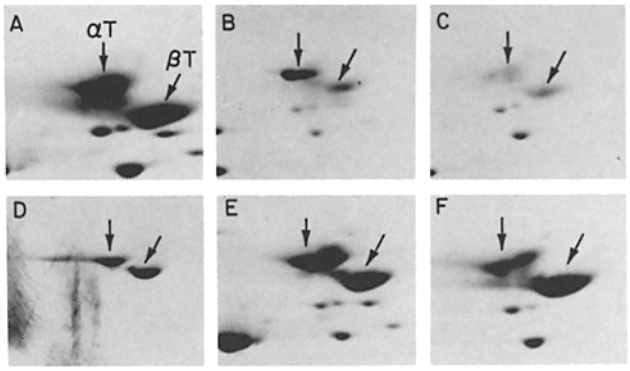

FIGURE 2 Two-dimensional stained gels of optic pathway proteins showing the tubulin region of each gel; the basic end of the isoelectric focusing gel was on the left. The α- and β-tubulin proteins are indicated for each gel. (*A*) Optic pathway proteins (optic nerve plus optic tract), (*B*) optic nerve proteins, (*C*) optic tract proteins, (*D*) purified tubulin, (*E*) mixture of purified tubulin and optic nerve proteins, (*F*) mixture of purified tubulin and optic tract proteins.

Progressive Alteration of Optic Pathway α-Tubulin

To further define apparent regional differences in tubulins, we used SDS PAGE to examine the distribution of these proteins in consecutive 1.1-mm segments of the optic pathway. After electrophoresis the proteins of each gel lane were scanned with a densitometer (see Materials and Methods). The results are shown in Fig. 3. As before, α-tubulin gel bands were identified by comparison with pure tubulin, and were observed to change progressively from segment 1 to segment 9 (arrows, Fig. 3). In proximal segments of the ON, α-tubulin was represented predominantly as darkly stained bands. In OT segments these bands become increasingly less intense. β-Tubulin appeared unchanged.

Progressive proximodistal changes in α-tubulin heterogeneity were further established by two-dimensional PAGE of each of the nine nerve segments as shown in Fig. 4. In segments 1–4, two predominant proteins separable by isoelectric point and several less intensely stained proteins were present in the α-tubulin position of the gel. In more distal segments, the two predominant proteins decreased relative to other species (segments 5 and 6, Fig. 4 *E* and *F*), and α-tubulin was represented by multiple species that stained relatively lightly (segments 7–9, Fig. 4 *G–I*). The results are consistent with the change in α-tubulin proteins when the nine segments were examined by one-dimensional SDS PAGE and densitometry (Fig. 3). It is interesting to note that in segment 9 (Fig. 4 *I*) the composition of α-tubulin heteroproteins resembles the pattern from superior colliculus (Fig. 4 *J*) and from cerebral cortex (not shown). Similar to previous results, β-tubulin exhibited no detectable variation among optic pathway segments.

Alteration of Tubulin in RGC Axons during Axoplasmic Transport

Axoplasmic transport studies were undertaken to further examine the regional heterogeneity of tubulin in the primary optic pathway. After intravitreal administration of L-[^{3}H]proline to mice, optic pathway proteins were analyzed by SDS PAGE followed by fluorography. Prominently labeled proteins corresponding to α- and β-tubulin migrated together in the slowest of five separate groups of axonally transported proteins (43) at a rate of 0.1–0.2 mm/d.

The segmental distribution of heterogeneous forms of radioactive tubulin subunits along RGC axons was examined at selected postinjection intervals by subjecting consecutive 1.1-mm segments of optic pathway to two-dimensional PAGE and autoradiography. 15 d after injection of L-[^{3}H]proline (Fig. 5), intensely labeled heterogeneous forms of α- and β-tubulin were present in proximal axonal segments. Both α- and β-tubulin had just reached the optic chiasm (Fig. 5 *E*) at 15 d postinjection. By 45 d postinjection (Fig. 6), α- and β-tubulin were present along the entire length of the primary optic pathway. Comparison of Fig. 6 with Fig. 4, which is the corresponding stained protein pattern, indicates that the segmental distribution of radioactive and stained α- and β-tubulin proteins are similar at 45 d postinjection; all forms of α-tubulin present in the stained gel (Fig. 4) were radioactively labeled (Fig. 6). Thus, at 45 d postinjection, and possibly earlier, the distribution of heterogeneous forms of α- and β-tubulin conveyed by axonal transport closely resembles the distribution of unlabeled heterogeneous forms present in all segments of the RGC axon.

Peptide Mapping of α-Tubulin from Optic Pathway Segments

Amino acid sequence analysis of brain α-tubulin has definitively established the highly conserved nature (46) of this electrophoretically dissimilar group of proteins. α-Tubulin microheterogeneity has been shown to be confined to a small portion of the sequence, and these alterations are not discernible when large peptides are generated by *S. aureus* V8 protease digestion (8, 11). This peptide mapping procedure was used in the present study to establish whether radiolabeled tubulin conveyed in the slow phase of axoplasmic transport was contaminated by polypeptides unrelated to α- and β-tubulin in some optic pathway segments. This possibility would be indicated by a changing peptide map pattern of tubulin obtained from the different ON and OT segments. Proteins in RGC axons were labeled by intravitreal injection of L-[^{35}S]methionine to mice. At 45 d postinjection, the optic pathway proteins of consecutive 1.1-mm segments were separated on a one-dimensional gel. After staining, the α- and β-tubulin region was removed from each lane, the proteins were digested with

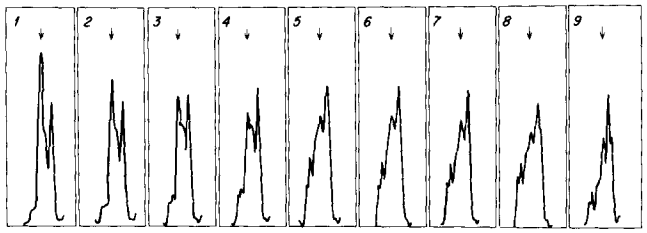

FIGURE 3 Densitometric scans of primary optic pathway proteins. The optic pathway was excised and divided into nine 1.1-mm segments. Segment 1 was adjacent to the scleral surface of the eye, segment 5 includes the optic chiasm, and segment 9 overlaid the lateral geniculate nucleus. The proteins of each segment were electrophoresed on adjacent lanes of an SDS polyacrylamide gel. After staining and destaining, the gel was scanned with an Ortec densitometer and the tracings were recorded automatically. For each gel the tracing shows the tubulin region only; the α-tubulin peak is indicated by an arrow and the β-tubulin peak is to the right. Tracings of proteins from consecutive optic pathway segments are shown with segment 1 at the left of the figure and segment 9 at the right.

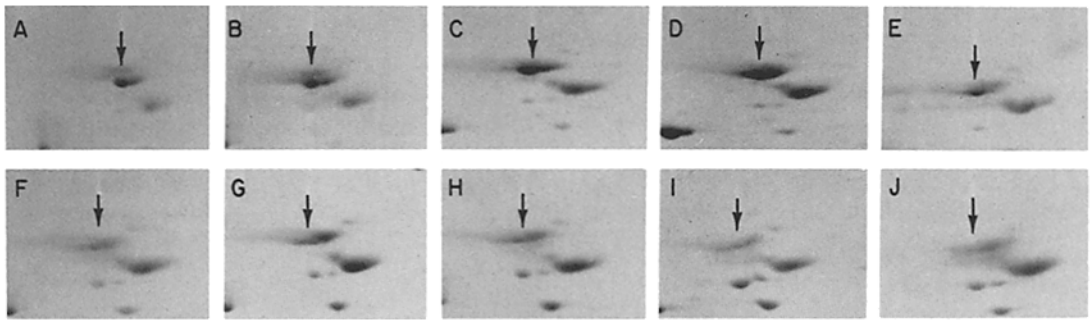

FIGURE 4 Two-dimensional stained gels of primary optic pathway proteins showing the tubulin region of each gel. The primary optic pathway was excised and divided into nine segments and the proteins of each segment were separated on two-dimensional gels. Panels *A–I* show the proteins of consecutive segments 1–9 (see legend to Fig. 3); α-tubulin is indicated by an arrow. Panel *J* shows tubulin of the superior colliculus.

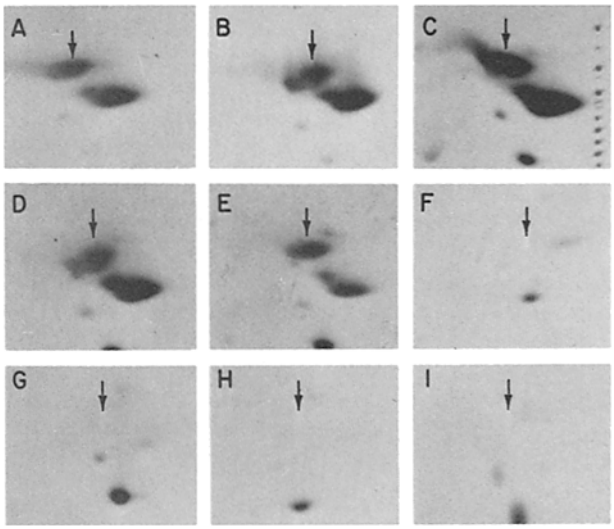

FIGURE 5 The distribution of radiolabeled tubulin in consecutive segments of the primary optic pathway at 15 d postinjection of L-[^{3}H]proline. Radioactive proteins from each segment were separated on two-dimensional gels; after electrophoresis, the gels were subjected to fluorography. Panels *A–I* show the tubulin region of each gel corresponding, consecutively, to segments 1–9 of the optic pathway. The arrow indicates the position of α-tubulin.

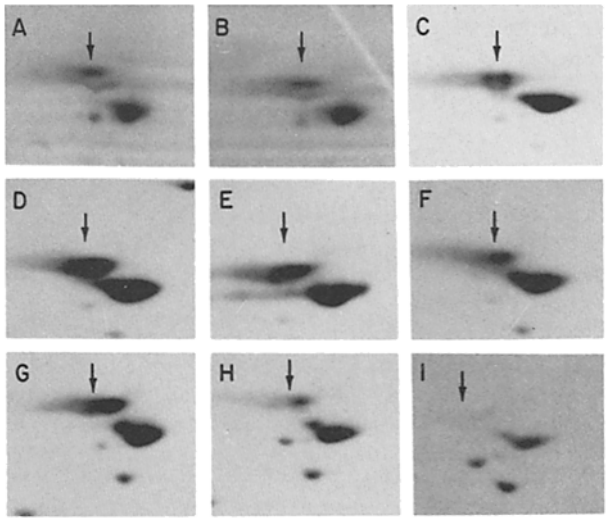

FIGURE 6 The distribution of radiolabeled tubulin in consecutive segments of the primary optic pathway at 45 d postinjection of L-[^{3}H]proline. Panels *A–I* show the tubulin region of each gel corresponding, consecutively, to segments 1–9 of the optic pathway. The arrow indicates the position of α-tubulin.

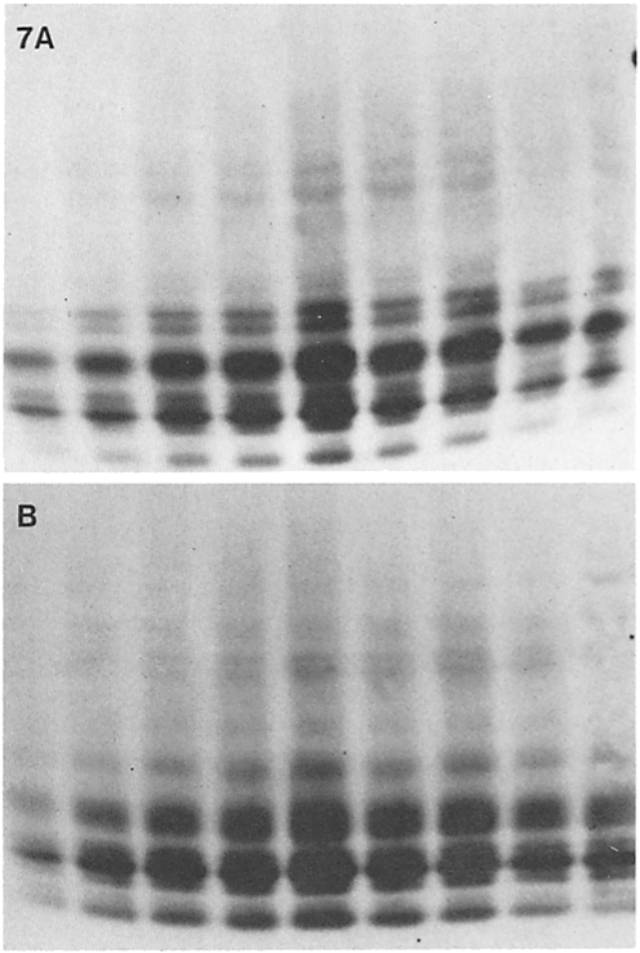

FIGURE 7 Peptide maps of α- and β-tubulin from consecutive segments of the primary optic pathway. The RGC axon was labeled with L-[^{35}S]methionine. Radioactive α- and β-tubulin was obtained from each of nine consecutive segments and subjected to digestion with *S. aureus* V8 protease; see text for details. The resulting peptides were fractionated on a 10–15% gel. After staining and destaining, the gels were fluorographed. (*A*) α-tubulin peptides, (*B*) β-tubulin peptides. In both panels optic pathway segments 1–9 are in consecutive lanes from left to right.

S. aureus V8 protease, and the resulting peptides were separated on a second SDS gel that was subjected to fluorography. α-Tubulin of each optic pathway segment yielded peptides of identical migration (Fig. 7*A*); the same result was obtained for β-tubulin (Fig. 7*B*). We conclude that the progressive altera-

tion of α-tubulin revealed by polyacrylamide gel analysis cannot be accounted for by contamination of the α-tubulin gel region by other proteins.

DISCUSSION

The present results demonstrate the synthesis and axoplasmic transport of multiple forms of α- and β-tubulin in mouse retinal ganglion cells. In addition to the microheterogeneity of the synthesized proteins, we have also observed regional changes in the composition of α-tubulin along the RGC axon. Although this regional heterogeneity arises while the proteins are being transported, differences in transport rates are not a causal factor. Rate measurements, using one-dimensional gels, indicated that the major bands of α- and β-tubulin migrated in the same phase of axoplasmic transport at 0.1–0.2 mm/d. Two-dimensional fluorographs further demonstrated that the major heterogeneous forms of both groups of proteins are transported together. Since tubulin is found in both neurons and glia and their processes, a gradient of nonneuronal cells along the optic pathway may have contributed to the regional heterogeneity of tubulin demonstrated on stained gels. This possibility, however, is inconsistent with the results of the axonal transport studies in which RGC axonal proteins were selectively labeled. The distribution of heterogeneous tubulin subunits observed on stained gels of axonal segments was similar to the distribution of multiple forms of tubulin observed by two-dimensional PAGE fluorography 45 d after administration of radioactive amino acid to the RGC. The tubulin protein alterations along the RGC axon are consistent, therefore, with the posttranslational processing of α-tubulin during axoplasmic transport. As discussed below, several possible mechanisms may account for these observations.

Recently, α-tubulin microheterogeneity was definitively established by amino acid sequence analysis (46). At least four different α-tubulins, accounted for by amino acid replacements, were found in porcine brain. An additional source of brain tubulin microheterogeneity results from several types of post-translational modification. Among these are phosphorylation (16, 21, 50, 52, 53), glycosylation (19), and the addition of amino acids (1) including the addition of tyrosine to the carboxy-terminal of α-tubulin (40, 48, 49). These modifications may cause a change in the isoelectric point of α-tubulin on two-dimensional gels. Hypothetically, the presence of modifying enzymes in specialized regions could partly explain the changing pattern of α-tubulin that occurs during axonal transport. However, posttranslational modification by covalent addition to a polypeptide appears inadequate to fully account for the relatively decreased abundance of certain of the more prominent α-tubulin proteins when optic tract is compared with optic nerve. Therefore the fate of tubulin, with respect to neuronal compartmentalization and degradation, is considered.

Significant evidence exists that, in addition to soluble cytoplasmic tubulin, brain and other tissues contain variable proportions of particulate tubulin as measured by colchicine-binding studies (4, 15, 18, 31, 58), and polypeptides that comigrate with tubulin in various gel electrophoresis systems have been shown to be major components of the neuronal membranes (17, 27, 28, 55–57). Membrane-associated tubulin can be purified from brain after detergent extraction (4, 5), and can be distinguished from cytoplasmic tubulin by the presence of carboxy-terminal tyrosine on the α-chains of the latter (40). There is also evidence for the higher abundance of α-tubulin in membranes of synaptic vesicles (59) and synaptosomes (25) and for a unique synaptoplasmic form of α-tubulin (36). An interesting observation on tubulin compartmentalization was made on neurites derived from cultured superior cervical ganglion cells (17). The neuritic plasma membrane contains fucosylated tubulin, at least a portion of which may be associated with the exterior neuronal surface.

Other studies have focused on the insertion of newly synthesized tubulin into membranes. Microheterogeneous forms of α- and β-tubulin have been synthesized in vitro by multiple messenger RNAs (10, 13, 37, 47). When separated on two-dimensional gels, the distribution of α- and β-tubulin subunits synthesized in vitro by brain polysomes or mRNA (10, 13, 37) resembles the pattern of radiolabeled tubulin synthesized by the retinal ganglion cell and found in the axon. Brain polysomes can be separated into free and membrane-bound fractions (34). When the fractions were translated separately in a reticulocyte cell-free protein synthesizing system, multiple forms of α- and β-tubulin were synthesized by free polysomes, whereas an α-tubulinlike protein was the major product of synthesis by bound polysomes (23). The protein was shown to copurify with unlabeled brain tubulin after two cycles of assembly and disassembly. In a separate series of experiments it was shown that tubulin synthesized by rough microsomes was incorporated, in part, into the microsomal membrane (54), and that newly synthesized α-tubulin was preferentially tightly associated with membranes. In the present study, the proportions of certain forms of α-tubulin and/or their electrophoretic migration on two-dimensional gels were observed to change during transport within the RGC axon. It is possible, therefore, that regional heterogeneity of α-tubulin proteins in ON and OT arises by the early dissociation of some forms of tubulin from the transport mechanism and subsequent compartmentalization of these proteins into membranes at proximal sites in RGC axons. Thus certain forms would not be visible on gels of distal axonal segments.

The absence of certain forms of α-tubulin in the OT, relative to ON, may also be accounted for by the action of proteolytic enzymes along RGC axons. It has been shown that a number of proteinases are present in RGC axons (41, 42). In preliminary experiments we have observed that α-tubulin is among the proteins most sensitive to proteolysis by a leupeptin-sensitive calcium-activated neutral proteinase when excised optic pathways are incubated in vitro under certain conditions. Proteolytic modification of proteins during axonal transport has been previously shown to occur in mammalian (22) and molluscan (3) neurons. It is also possible that α-tubulin undergoes more than one posttranslational event in the RGC axon. If, during transport, modification of α-tubulin occurred resulting in a shift in isoelectric point, and if this modification made the α-tubulin more susceptible to proteolytic degradation, these processes could work in concert to give rise to the relatively more diffuse spots seen on two-dimensional gels of optic tract segments.

In the accompanying study (43), we demonstrated the modification of a neurofilament protein during axoplasmic transport in RGC axons. It was shown that neurofilament proteins of 140,000 and 145,000 mol wt were prominent in proximal segments of the optic pathway, whereas, in distal segments, the two proteins occurred in addition to a more abundant 143,000 mol wt neurofilament protein. These results provided evidence for regional specialization of RGC axons with respect to enzymatic machinery as well as cytoskeletal elements. The present report has focused on structural differentiation. The data demonstrate the nonuniform distribution of heterogeneous α-tubulin forms along the length of CNS axons.

The progressive alteration of α-tubulin in the RGC axon produced a pattern of protein in the most distal segments that closely resembles tubulin in homogenates of mouse cortical proteins. The α-tubulin changes may be due to posttranslational modification, selective compartmentalization, regional specialization with respect to proteolytic enzymes, or due to a combination of these factors. Irrespective of the precise mechanism, the observed modification of α-tubulin may represent a general process for the maturation of this structural protein within neurons.

The authors are grateful to Kimberly Bishop and Donna Staton for expert technical assistance and to Leta Sinclair for assisting in the preparation of the manuscript.

Portions of this work were supported by National Institutes of Health (NIH) grants NS 15494 and RR 05404 to R. A. Nixon, and AG NS 02126 to C. A. Marotta. R. A. Nixon was an Ethel Du Pont Warren Fellow of Harvard Medical School and presently is a Fellow of the Medical Foundation, Inc. C. A. Marotta is the recipient of a MacArthur Foundation Award and of a Research Career Development Award from NIH (AG 00084). B. A. Brown was a National Institute for Mental Health Psychobiology Trainee, MH-14278 (Harvard Medical School).

Received for publication 23 November 1981, and in revised form 26 February 1982.

REFERENCES

1. Arce, C. A., J. A. Rodriguez, H. S. Barra, and R. Caputto. 1975. Incorporation of L-tyrosine, L-phenylalanine, and L-3,4-dihydroxyphenylalanine as single units into rat brain tubulin. *Eur. J. Biochem.* 59:145–149.
2. Berkowitz, S. A., J. Katagiri, H. K. Binder, and R. C. Williams. 1977. Separation and characterization of microtubule proteins from calf brain. *Biochemistry.* 16:5610–5617.
3. Berry, R. W., and A. W. Schwartz. 1977. Axonal transport and axonal processing of low molecular weight proteins from the abdominal ganglion of *Aplysia*. *Brain Res.* 129:75–90.
4. Bhattacharyya, B., and J. Wolff. 1975. Membrane-bound tubulin in brain and thyroid tissue. *J. Biol. Chem.* 250:7639–7649.
5. Bhattacharyya, B., and J. Wolff. 1976. Polymerization of membrane tubulin. *Nature (Lond.).* 264:576–577.
6. Bray, D., and D. Gilbert. 1981. Cytoskeleton elements in neurons. *Annu. Rev. Neurosci.* 4:505–523.
7. Brown, B. A., R. A. Nixon, and C. A. Marotta. 1981. Tubulin subunit changes during transport in mouse retinal ganglion cells. *Trans. Am. Soc. Neurochem.* 12:205.
8. Brown, B. A., R. A. Nixon, P. Strocchi, and C. A. Marotta. 1981. Characterization and comparison of neurofilament proteins from rat and mouse CNS. *J. Neurochem.* 36:143–153.
9. Bryan, J. 1974. Biochemical properties of microtubules. *Fed. Proc.* 33:152–157.
10. Bryan, R. N., G. A. Cutter, and M. Hayashi. 1978. Separate mRNAs code for tubulin subunits. *Nature (Lond.).* 272:81–83.
11. Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli. 1977. Peptide mapping by limited proteolysis in sodium dodecylsulfate and analysis by gel electrophoresis. *J. Biol. Chem.* 252:1102–1106.
12. Cleveland, D. W., S. H. Hughes, E. Stubblefield, M. W. Kirschner, and H. E. Varmus. Multiple α and β tubulin genes represent unlinked and dispersed gene families. *J. Biol. Chem.* 256:3130–3134.
13. Cleveland, D. W., M. W. Kirschner, and N. J. Cowan. 1978. Isolation of separate mRNAs for α and β-tubulin and characterization of the corresponding *in vitro* translation products. *Cell.* 15:1021–1031.
14. Cleveland, D. W., M. A. Lopata, R. J. Mac Donald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner. 1980. Number and evolutionary conservation of α and β-tubulin and cytoplasmic β and γ-actin genes using specific cloned cDNA probes. *Cell.* 20:95–105.
15. Dahl, D. R., D. Redburn, and F. E. Samson, Jr. 1970. Regional distribution of colchicine-binding (microtubular) proteins in the rat brain. *J. Neurochem.* 17:1215–1219.
16. Eipper, B. A. 1974. Rat brain tubulin and protein kinase activity. *J. Biol. Chem.* 249:1398–1406.
17. Estridge, M. 1977. Polypeptides similar to the α and β subunits of tubulin are exposed to the neuronal surface. *Nature (Lond.).* 268:60–63.
18. Feit, H., and S. Barondes. 1970. Colchicine-binding activity in particulate fractions of mouse brain. *J. Neurochem.* 17:1355–1364.
19. Feit, H., and M. L. Shelanski. 1975. Is tubulin a glycoprotein? *Biochem. Biophys. Res. Commun.* 66:920–927.
20. Feit, H., L. Slusarek, and M. L. Shelanski. 1971. Heterogeneity of tubulin subunits. *Proc. Natl. Acad. Sci. U. S. A.* 68:2028–2031.
21. Forgue, S. T., and J. L. Dahl. 1979. Rat brain tubulin: subunit heterogeneity and phosphorylation. *J. Neurochem.* 32:1015–1025.
22. Gainer, H., Y. Sarne, and M. J. Brownstein. 1977. Neurophysin biosynthesis: conversion of a putative precursor during axonal transport. *Science (Wash. D. C.)* 195:1354–1356.
23. Gilbert, J. M., P. Strocchi, B. A. Brown, and C. A. Marotta. 1981. Tubulin synthesis in rat forebrain: studies with free and membrane-bound polysomes. *J. Neurochem.* 36:839–846.
24. Gozes, I., and U. Z. Littauer. 1978. Tubulin microheterogeneity increases with rat brain maturation *Nature (Lond.).* 276:411–413.
25. Gozes, I., and U. Z. Littauer. 1979. The α subunit of tubulin is preferentially associated with presynaptic membrane. *FEBS (Fed. Eur. Biochem. Soc.). Lett.* 99:86–90.
26. Herzog, W., and K. Weber. 1977. *In vitro* assembly of pure tubulin into microtubules in the absence of microtubule-associated proteins and glycerol. *Proc. Natl. Acad. Sci. U. S. A.* 74:1860–1864.
27. Kelly, P. T., and C. W. Cotman. 1978. Synaptic proteins: characterization of tubulin and actin and identification of a distinct postsynaptic density polypeptide. *J. Cell Biol.* 79:173–183.
28. Kornguth, S. E., and E. Sunderland. 1975. Isolation and partial characterization of a tubulin-like protein from human and swine synaptosomal membranes. *Biochim. Biophys. Acta.* 393:100–114.
29. Krauhs, E., M. Little, T. Kempf, R. Hoefer-Warbinek, W. Ade, and H. Ponstingl. 1981. Complete amino acid sequence of β-tubulin from porcine brain. *Proc. Natl. Acad. Sci. U. S. A.* 78:4156–4160.
30. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
31. Lagnado, J. R., C. Lyons, and G. Wickremasinghe. 1971. The subcellular distribution of colchicine-binding protein ("microtubule protein") in rat brain. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 15:254–258.
32. Lu, R. C., and M. Elzinga. 1977. Chromatographic resolution of the subunits of calf brain tubulin. *Anal. Biochem.* 77:243–250.
33. Marotta, C. A., J. L. Harris, and J. M. Gilbert. 1978. Characterization of multiple forms of brain tubulin subunits. *J. Neurochem.* 30:1431–1440.
34. Marotta, C. A., P. Strocchi, B. A. Brown, J. A. Bonventre, and J. M. Gilbert. 1981. Gel electrophoresis methods for the characterization of neuronal fibrous proteins. Application to studies of human brain macromolecules from postmortem tissue. *In* Genetic Research Strategies in Psychobiology and Psychiatry. E. S. Gershon, S. Matthysse, X. O. Breakefield, and R. D. Ciaranello, editors. Boxwood Press, Pacific Grove, CA. 39–58.
35. Marotta, C. A., P. Strocchi, and J. M. Gilbert. 1978. Microheterogeneity of brain cytoplasmic and synaptoplasmic actins. *J. Neurochem.* 30:1441–1451.
36. Marotta, C. A., P. Strocchi, and J. M. Gilbert. 1979. Subunit structure of synaptosomal tubulin. *Brain Res.* 167:93–106.
37. Marotta, C. A., P. Strocchi, and J. M. Gilbert. 1979. Biosynthesis of heterogeneous forms of mammalian brain tubulin subunits by multiple messenger RNAs. *J. Neurochem.* 33:231–246.
38. Matus, A., R. Bernhardt, and T. Hugh-Jones. 1981. High molecular weight microtubule-associated proteins are preferentially associated with dendritic microtubules in brain. *Proc. Natl. Acad. Sci. U. S. A.* 78:3010–3014.
39. Murthy, M. R. V. 1972. Free and membrane-bound ribosomes of rat cerebral cortex. *J. Biol. Chem.* 250:4007–4021.
40. Nath, J., and M. Falvin. 1978. A structural difference between cytoplasmic and membrane-bound tubulin of brain. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 95:335–338.
41. Nixon, R. A. 1980. Protein degradation in the mouse visual system. I. Degradation of axonally transported and retinal proteins. *Brain Res.* 200:69–83.
42. Nixon, R. A. 1981. Increased axonal proteolysis in myelin-deficient mice. *Science (Wash. D. C.).* 215:999–1001.
43. Nixon, R. A., B. A. Brown, and C. A. Marotta. 1982. Posttranslational modification of a neurofilament protein during axoplasmic transport: implications for regional specialization of CNS axons. *J. Cell Biol.* 94:150–158.
44. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007–4021.
45. Olmsted, J. B., and G. G. Borisy. 1973. Microtubules. *Annu. Rev. Biochem.* 42:507–534.
46. Ponstingl, H., E. Krauhs, M. Little, and T. Kempf. 1981. Complete amino acid sequence of α-tubulin from porcine brain. *Proc. Natl. Acad. Sci. U. S. A.* 78:2757–2761.
47. Portier, M.-M., C. Jeantet, and F. Gros. 1980. Partial purification of the α-tubulin and β-tubulin messenger RNAs from rat brain. *Eur. J. Biochem.* 112:601–609.
48. Raybin, D., and M. Falvin. 1977. Enzyme which specifically adds tyrosine to the α chain of tubulin. *Biochemistry.* 16:2189–2194.
49. Raybin, D., and M. Flavin. 1977. Modification of tubulin by tyrosylation in cells and extracts and its effect on assembly in vitro. *J. Cell Biol.* 73:492–504.
50. Reddington, M., L. P. Tan, and J. R. Lagnado. 1976. The phosphorylation of brain microtubular proteins *in situ* and *in vitro*. *J. Neurochem.* 27:1229–1236.
51. Shelanski, M. L., F. Gaskin, and C. R. Cantor. 1973. Microtubule assembly in the absence of added nucleotides. *Proc. Natl. Acad. Sci. U. S. A.* 70:765–768.
52. Sloboda, R. D., S. A. Rudolph, J. L. Rosenbaum, and P. Greengard. 1975. Cyclic AMP-dependent endogenous phosphorylation of a microtubule-associated protein. *Proc. Natl. Acad. Sci. U. S. A.* 72:177–181.
53. Soifer, D. 1975. Enzymatic activity in tubulin preparations: cyclic-AMP dependent protein kinase activity of brain microtubule protein. *J. Neurochem.* 24:21–33.
54. Soifer, D., and H. Czosnek. 1980. Association of newly synthesized tubulin with brain microsomal membranes. *J. Neurochem.* 35:1128–1136.
55. Walters, B. B., and A. I. Matus. 1975. Proteins of the synaptic junction. *Biochem. Soc. Trans.* 3:109–112.
56. Walters, B. B., and A. I. Matus. 1975. Tubulin in post-synaptic junctional lattice. *Nature (Lond.).* 257:496–498.
57. Wang, Y. J., and H. R. Mahler. 1976. Topography of the synaptosomal membrane. *J. Cell Biol.* 71:639–658.
58. Wilson, L. 1970. Properties of colchicine binding protein from chick embryo brain. Interactions with vinca alkaloids and podophyllotoxin. *Biochemistry.* 9:4999–5007.
59. Zisapel, N., M. Levi, and I. Gozes. 1980. Tubulin: an integral protein of mammalian synaptic vesicle membranes. *J. Neurochem.* 34:26–32.