Differential Axonal Transport of Isotubulins in the Motor Axons of the Rat Sciatic Nerve

P. Denoulet,* G. Filliatreau,‡ B. de Néchaud,* F. Gros,* and L. Di Giamberardino‡

*Biochimie Cellulaire, Collège de France, 75231 Paris Cedex 05, France; and
‡Service Hospitalier Frédéric Joliot, Département de Biologie, C.E.A., 91406 Orsay, France

Abstract. The axonal transport of the diverse isotubulins in the motor axons of the rat sciatic nerve was studied by two-dimensional polyacrylamide gel electrophoresis after intraspinal injection of [^{35}S]methionine. 3 wk after injection, the nerve segments carrying the labeled axonal proteins of the slow components a (SCa) and b (SCb) of axonal transport were homogenized in a cytoskeleton-stabilizing buffer and two distinct fractions, cytoskeletal (pellet, insoluble) and soluble (supernatant), were obtained by centrifugation. About two-thirds of the transported-labeled tubulin moved with SCa, the remainder with SCb. In both waves, tubulin was found to be associated mainly with the cytoskeletal fraction. The same isoforms of tubulin were transported with SCa and SCb; however, the level of a neuron-specific β-tubulin subcomponent, termed β', composed of two related isotubulins β'_1 and β'_2, was significantly greater in SCb than in SCa, relative to the other tubulin isoforms. In addition, certain specific isotubulins were unequally distributed between the cytoskeletal and the soluble fractions. In SCa as well as in SCb, α''-isotubulins were completely soluble in the motor axons. By contrast, α''' and β'_2-isotubulins, both posttranslationally modified isoforms, were always recovered in the cytoskeletal fraction and thus may represent isotubulins restricted to microtubule polymers. The different distribution of isotubulins suggests that a recruitment of tubulin isoforms, including specific posttranslational modifications of defined isoforms (such as, at least, phosphorylation of β' and acetylation of α'), might be involved in the assembly of distinct subsets of axonal microtubules displaying differential properties of stability, velocity and perhaps of function.

THE main function of axonal transport is to supply the axons and their nerve endings with macromolecules used in the renewal of structural components and in synaptic functions. These macromolecules are conveyed from the cell bodies to the axonal extremities in distinct waves differing by their protein composition and by their transport rate, ranging from a few millimeters per day (slow axonal transport) to a few hundred millimeters per day (fast axonal transport) (for review, see Grafstein and Forman, 1980; Lasek et al., 1984).

Slow axonal transport, in particular, is concerned with the protein renewal of the axonal cytoskeleton (Willard et al., 1974; Lasek and Hoffman, 1976; Lorenz and Willard, 1978; Black and Lasek, 1980). Two components with different flow rates have been described for slow axonal transport: the slowest, referred to as slow component a (SCa)[1], mainly consists of the protein subunits of microtubules and neurofilaments which are transported at ~1 mm/d, while the other, slow component b (SCb), carries a more heterogeneous protein population including actin and associated proteins at 2–4 mm/d (Black and Lasek, 1980; Tytell et al., 1981).

Several reports dealing with the axonal transport of microtubules have led to the proposal that distinct subclasses of microtubules, differing by their relative velocity and/or stability, could coexist within the axon; these diverse subsets of microtubules could differ by their interaction with the neurofilament network (Willard and Simon, 1983; Tashiro and Komiya, 1983; Tashiro et al., 1984) and/or by a differential resistance towards low temperature or calcium ion (Brady et al., 1984). In addition, tubulin, the major constituent of microtubules, is known to display an extensive heterogeneity especially in the nervous system where several neurospecific isotubulins have been characterized (Denoulet et al., 1982*a*,*b*; Moura Neto et al., 1983; Denoulet et al., 1986). The different isotubulins have been shown to include both primary translation products and proteins posttranslationally modified. In particular, α', β'_1, β_3, and β_4 are primary products; α'', α''', β'_2, and the acidic β are derived by posttranslational modifications (Denoulet et al., 1986, 1988; Eddé et al., 1987, 1989).

On the basis of these data, we analyzed the isotubulin compositions of microtubules carried by SCa and SCb by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE)

1. *Abbreviations used in this paper*: SCa, component a of the slow axonal transport; SCb, component b of the slow axonal transport; 2-D PAGE, two-dimensional polyacrylamide gel electrophoresis.

and we compared the isotubulin populations moving along the axons in the soluble or polymerized state.

The results reported here for motor axons of the rat sciatic nerve indicate that the same set of isoforms of tubulin are transported with SCa and SCb. However, a specific β-tubulin subcomponent, termed β', is enriched in SCb. Furthermore, the distribution of the transported proteins between soluble and cytoskeletal fractions has shown that whether they are transported in SCa or in SCb, α''-isotubulins are completely soluble in the motor axons whereas α''' and β'_2-isotubulins are always recovered in the cytoskeletal, microtubule-containing fraction. It is hypothesized from this differential distribution of tubulin isoforms that a specific recruitment of isotubulins might be involved in the assembly of distinct subsets of axonal microtubules displaying differential properties of stability and perhaps of function.

Materials and Methods

Radiolabeling of Axonal Proteins

Female Wistar rats (200 g body weight) were injected in the right ventral horn of the L_3–L_5 lumbar cord with 150 μCi of [^{35}S]methionine (1,200 Ci/mmol; CEA, Saclay, France) (Filliatreau et al., 1988). 3 wk later, the animals were killed and the right sciatic nerves dissected in toto. The nerves were cut into 6-mm segments that were each homogenized in 150 μl of ice-cold cytoskeleton-stabilizing buffer (100 mM Pipes, pH 6.9, 5 mM EGTA, 5 mM $MgCl_2$, 20% glycerol, 5% DMSO, 0.5% Triton X-100) containing 1 mM PMSF, 1 mg/ml benzamidine, and 0.16 mg/ml bacitracin (Filliatreau et al., 1988). Total ^{35}S-radioactivity was determined on an aliquot of each crude homogenate. From these measurements, four successive 6-mm nerve segments were chosen and pooled as representative of the two nerve regions containing SCa and SCb, respectively. The pooled homogenates were then centrifuged for 20 min at 80,000 *g* in a refrigerated airfuge (Beckman Instruments, Inc., Palo Alto, CA).

2-D Polyacrylamide Gel Electrophoresis

For subsequent 2-D PAGE, pellets were resuspended in 300 μl of lysis buffer (9.5 M urea, 2% wt/vol NP-40, 5% vol/vol 2-mercaptoethanol, 5% vol/vol LKB, pH 3.5–10.0, Ampholines). Supernatants were dialyzed before lyophilization, then resuspended in 150 μl of lysis buffer. 100 μl pellet and 75 μl supernatant aliquots were subjected to 2-D PAGE according to O'Farrell (1975). In some experiments, the pH gradient in the focusing gel was modified to increase the resolution between the diverse β-tubulin isoforms. In these cases, only pH 5–7 LKB Ampholines were used instead of the classical 4:1 mixture of 5–8 and 3.5–10.0 Ampholines, respectively. The second dimension was carried out on 24-cm-high, 8% polyacrylamide SDS slab gels as previously described (Denoulet et al., 1982*a*).

After the run, gels were stained with Coomassie Blue, soaked 15 min in the fluorographic reagent Amplify (Amersham Corp., Arlington Heights, IL), and dried under vacuum. Radioactive polypeptides were detected by fluorography using X AR-5 films (Eastman Kodak Co., Rochester, NY). Exposure time varied from 2 to 4 wk.

For silver staining (Morrissey, 1981), samples were diluted 1:20 before loading.

The major slowly transported proteins, including the diverse tubulin isoforms, have been identified by several criteria such as electrophoretic coordinates or comigration, peptide mapping (Cleveland et al., 1977) and immunoblotting (Denoulet et al., 1982*a*, 1986; Eddé et al., 1983; Filliatreau et al., 1988).

Determination of Radioactivity in Two-Dimensional Gels

After 2-D PAGE, Coomassie-stained spots corresponding to the major axonal polypeptides were cut from dried gels. Each spot was placed into a scintillation vial, covered with 500 μl of 30% H_2O_2, and incubated overnight at 70°C. 400 μl of BTS (Beckman Instruments, Inc.) were added followed by 30 min incubation at 40°C. ^{35}S-Radioactivity was measured by scintillation counting with 10 ml Beckman Ready-Solv NA.

Immunodetection of Proteins

After 2-D PAGE, proteins were electrophoretically transferred from SDS slab gels into nitrocellulose sheets as described by Towbin et al. (1979) in 25 mM Tris, 192 mM glycine, pH 8.3, containing 20% methanol. Transferred proteins were rapidly detected by Ponceau Red (0.2% in 3% TCA) and their locations identified with a pencil mark. All of the following steps were carried out at room temperature under gentle shaking; the same PBST buffer (0.1% Tween 20 in PBS) was used to block and wash the nitrocellulose as well as to dilute the antibodies. After a 60-min blocking step, the primary antibody (mouse monoclonal anti-α-tubulin, anti-β-tubulin, or anti-neurofilament subunit, purchased from Amersham Corp. and diluted as specified by the manufacturer) was added to the blot in a sealed plastic bag for 1–2 h. The first antibody was then withdrawn and the blot washed twice for 10 min. The second peroxidase-labeled, anti-mouse IgG antibody (Diagnostics Pasteur, Marnes-la-Coquette, France) was added for 30–60 min and the blot washed again twice for 15 min. Peroxidase activity was revealed with diaminobenzidine (500 μg/ml) in 10 mM Tris, pH 7.6, in the presence of H_2O_2. The reaction was stopped by soaking the blot for 5 min in diluted HCl.

Results

The SCa and SCb Waves

3 wk after intraspinal injection of [^{35}S]methionine in the region of the sciatic motor neuron cell bodies, total radioactivity was determined in successive 6-mm nerve segments. Two peaks of radioactivity, referred to here as SCa and SCb, were observed (Fig. 1). The radioactivity associated with SCa was measured to be about twice the radioactivity associated with SCb.

Two-dimensional Analysis of Axonal Proteins Transported in the Soluble and Cytoskeletal Fractions of SCa and SCb

Four nerve segments of each peak region were pooled, homogenized in the cytoskeleton-stabilizing buffer, and centrifuged according to Materials and Methods. The insoluble proteins and the stabilized polymers were recovered in the pellet whereas the soluble and depolymerized proteins remained in the supernatant (Filliatreau et al., 1988). Four nerve fractions were thus obtained through this procedure: SCa pellet, SCa supernatant, SCb pellet, and SCb supernatant. Aliquots from each fraction were analyzed by 2-D PAGE (Fig. 2). Labeled neurofilament subunits were detected almost exclusively in the pellet fraction of SCa (Fig. 2*a*). Labeled soluble enzymes, such as nerve-specific enolase (NSE) and brain creatine phosphokinase (CPK), were only recovered in the supernatants from both waves (Fig. 2, *b* and *d*), however mostly in SCb. A detailed analysis of the transport of axonal proteins has been published in a separate paper (Filliatreau et al., 1988).

Labeled tubulin was detected in variable amounts in the four compartments. Determination of ^{35}S-radioactivity in tubulin spots indicated that about two-thirds (65% $\pm$ 3) of the transported tubulin moved with SCa, the remainder with SCb. The partition of labeled tubulin between pellet and supernatant was found to be very similar in SCa and in SCb, i.e., ~80% in the cytoskeletal fraction vs. 20% in the soluble fraction. The isoform composition of tubulin was different both between SCa and SCb with an enrichment of β' isoforms in SCb, and between pellet and supernatant for at least the α-tubulin isoforms. However, the large differences existing in the amounts of radioactivity in the tubulin subunits be-

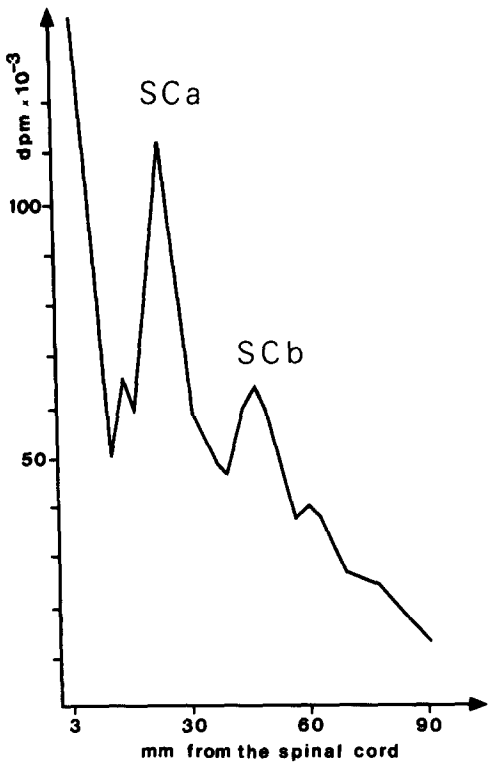

Figure 1. Identification of SCa and SCb waves of the slow axonal transport. As indicated in Materials and Methods, animals were injected with labeled amino acids in the ventral horn of the spinal cord and their sciatic nerve dissected out in toto 3 wk later. The nerve was then cut into 6-mm segments and total radioactivity determined in each segment. The amount of radioactivity is plotted against the distance from the spinal cord.

tween the four fractions did not allow a detailed qualitative comparison of the isotubulin compositions. Therefore, the four samples were reanalyzed after adjustments of the loading volumes as described in the following section.

Differential Transport of the Different Isotubulins in the Sciatic Motor Axons

After two-dimensional separation of the proteins from the four fractions, Coomassie-stained spots corresponding to the tubulin subunits (α, β', β) were excised from the gels and the amount of ^{35}S-radioactivity determined by scintillation counting. The loading volumes of the four samples were tentatively adjusted to comparable amounts of labeled tubulin and then subjected to a second 2-D PAGE. In spite of this attempt, the SCa pellet appeared overloaded (Fig. 3).

When the axonally transported tubulins were compared on the basis of their partition between SCa and SCb, the most striking difference observed was the higher proportion of labeled β'-isotubulins in SCb as compared to SCa, in the pellet as well as in the supernatant (Fig. 3, compare *a* and *b* with *c* and *d*). The ratios of radioactivities associated with β' vs. β-tubulin isoforms are 0.53 ± 0.05 and 0.56 ± 0.07 in SCa pellet and supernatant, and 0.97 ± 0.06 and 0.93 ± 0.07 in SCb pellet and supernatant. respectively.

When cytoskeletal and soluble isotubulins were compared (Fig. 3, *a* and *c* with *b* and *d*), two main qualitative differences were observed. First, α' and α'''-isotubulins were exclusively recovered in the cytoskeletal fraction of SCa and SCb (Fig. 3, *a* and *c*), whereas α''-isotubulin was found only in the soluble fraction (Fig. 3, *b* and *d*). The discrete segregation of the α-tubulin isoforms between pellet and supernatant is more clearly seen in Fig. 4 where tracings of typical two-dimensional fluorographs, as those shown in Fig. 3, were superimposed. Furthermore, when total nerve α-isotubulins were immunodetected (Fig. 5, *a* and *c*), the α'''-isotubulins were clearly revealed only in the insoluble fraction while the α'- and α''-isotubulins were found in both the soluble and the cytoskeletal fractions, by either silver staining (Fig. 5, *b* and *d*) or by immunoblotting (Fig. 5, *a* and *c*). In these experiments, not only the labeled, axonally carried tubulin was detected but also cold tubulin present in the whole sciatic nerve segment analyzed. Thus, although α'- and α''-isotubulins appear to be clearly segregated during their transport in motor axons, α' in the cytoskeletal fraction and α'' in the soluble fraction, these isotubulins could also exist under different states in the other sciatic nerve structures such as sensory fibers or nerve supporting cells.

Another striking difference concerned the distribution of the β'-subcomponents of the β-tubulin subunit. Whereas the β'_1-isotype was present in both cytoskeletal and soluble fractions, β'_2-isotubulin was always found in the insoluble fraction (Fig. 3, *a* and *c*; Figs. 4 and 5, *b* and *d*). The strict compartmentation of β'_2-isoform in the cytoskeletal fraction is more obvious on the gels presented in Fig. 6 in which the pH gradient in the focusing gel was slightly modified to enhance the electrophoretic separation of the various β-isotubulins (see Materials and Methods). The fluorographs confirm that the axonally transported β'_2-isotubulin is found only in the microtubule-containing fraction (Fig. 6 *b*) in SCa as well as in SCb.

The other β-isoforms of axonally transported tubulin were present in both the pellet and supernatant fractions although the acidic β-isotubulins were predominantly found in the insoluble fraction (see Figs. 3, 4, 5, and 6).

Discussion

In this study, we examined, by 2-D gel electrophoresis, the proteins conveyed by the two main components of the slow transport (SCa and SCb) along the motor axons of the rat sciatic nerve. The nerve proteins were fractionated in a cytoskeleton-stabilizing buffer. Most of the axonal microtubules stabilized in their polymerized state were recovered in the pellet while the soluble and/or solubilized tubulin remained in the supernatant (Filliatreau et al., 1988). The isoform composition of the transported tubulin, in the SCa and SCb waves on one hand and in the polymerized and soluble fractions on the other hand, was then investigated.

The Two Rate Components of Transported Microtubules

As seen in two-dimensional fluorographs, the same isoforms of tubulin are transported by SCa and SCb but the relative proportions of the diverse isotubulins, however, are widely different in the two rate components. In particular, in SCb, the isotubulin population is enriched in β'-isoforms (see Figs. 2 and 3). This increase in the relative quantity of labeled β'-isoforms at the expense of other transported β-iso-

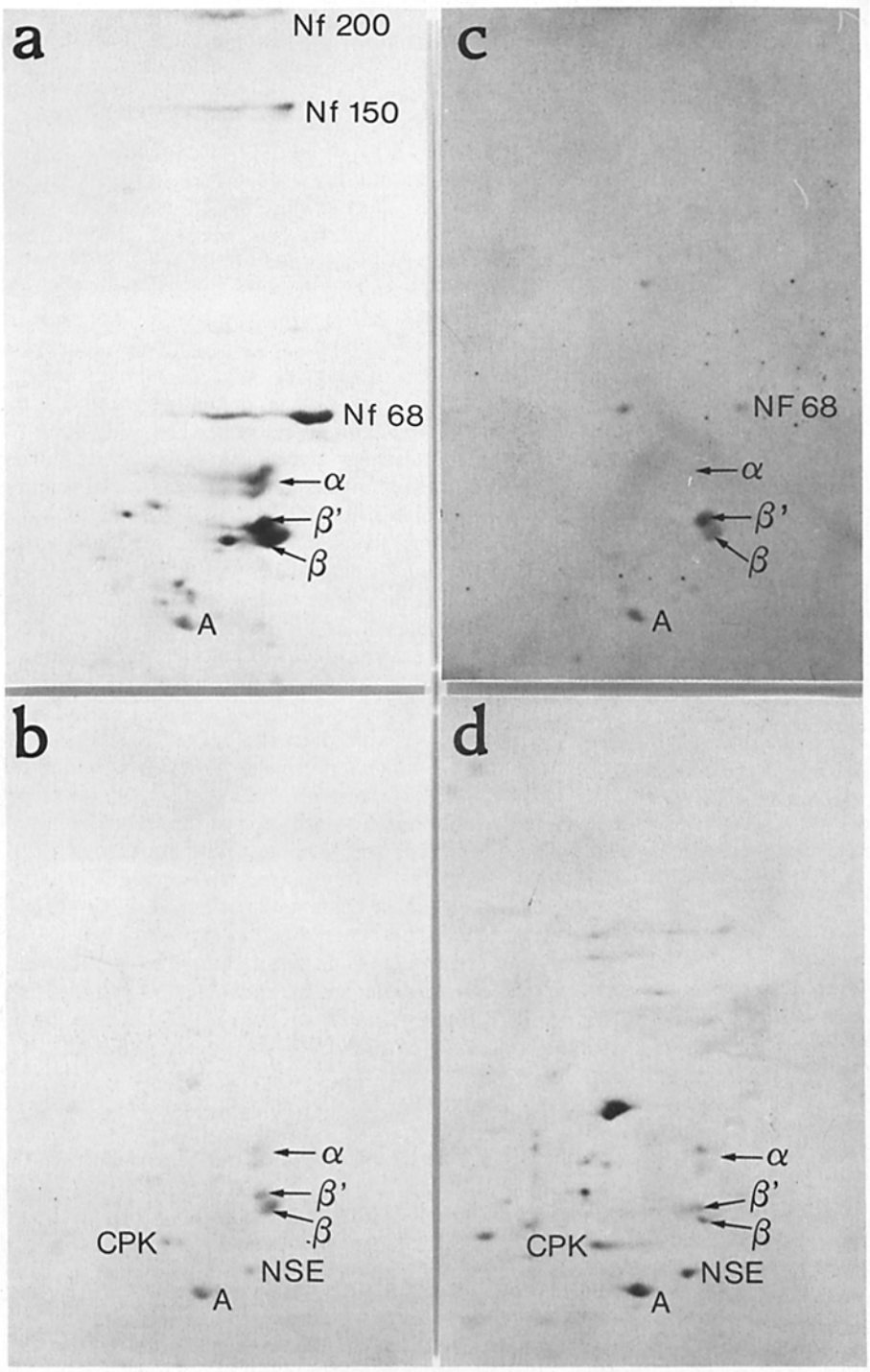

Figure 2. Two-dimensional fluorographs of axonally carried proteins recovered in the cytoskeletal (*a* and *c*) and soluble (*b* and *d*) fractions of SCa (*a* and *b*) and SCb (*c* and *d*). The labeled nerve segments corresponding to SCa and SCb, respectively, were pooled, homogenized, and centrifuged as described in Materials and Methods. Proteins from each compartment were subjected to 2-D PAGE and labeled axonally carried proteins detected by fluorography. *Nf 200*, *Nf 150*, and *Nf 68*: neurofilament subunits. α, β, and β': tubulin subunits. *A*, actin; *NSE*, nerve-specific enolase; *CPK*, brain creatin phosphokinase. Migration in the first dimension IEF gel was from left (basic) to right (acidic).

tubulins observed in SCb may reflect a differential, faster transport rate of β'-isotubulins since the protein ratio of β'- to β-isoforms was found constant over the nerve length analyzed here, as judged by Coomassie staining of two-dimensional gels. Since the insoluble fraction contains polymerized microtubules (Brady and Black, 1986; Filliatreau et al., 1988), this observation may suggest that a subset of axonal microtubules, characterized by a distinct isotubulin composition, is transported with SCb. This hypothesis is also supported by the studies of Tashiro and Komiya (1983) which concluded that two subpopulations of transported tubulin can be differentiated by their transport rate and isoform composition in both the sensory and motor axons of the sciatic nerve. This enrichment of the SCb-associated microtubules in β'-isotubulins at the expense of the other transported β-tubulin isoforms cannot be accounted for by posttranslational modifications transforming these β into β'-isotubulins during the axonal transport since it has been shown that β'_1-isotubulin is a distinct primary translation product coded by a specific 1.8-kb mRNA (Denoulet et al.,

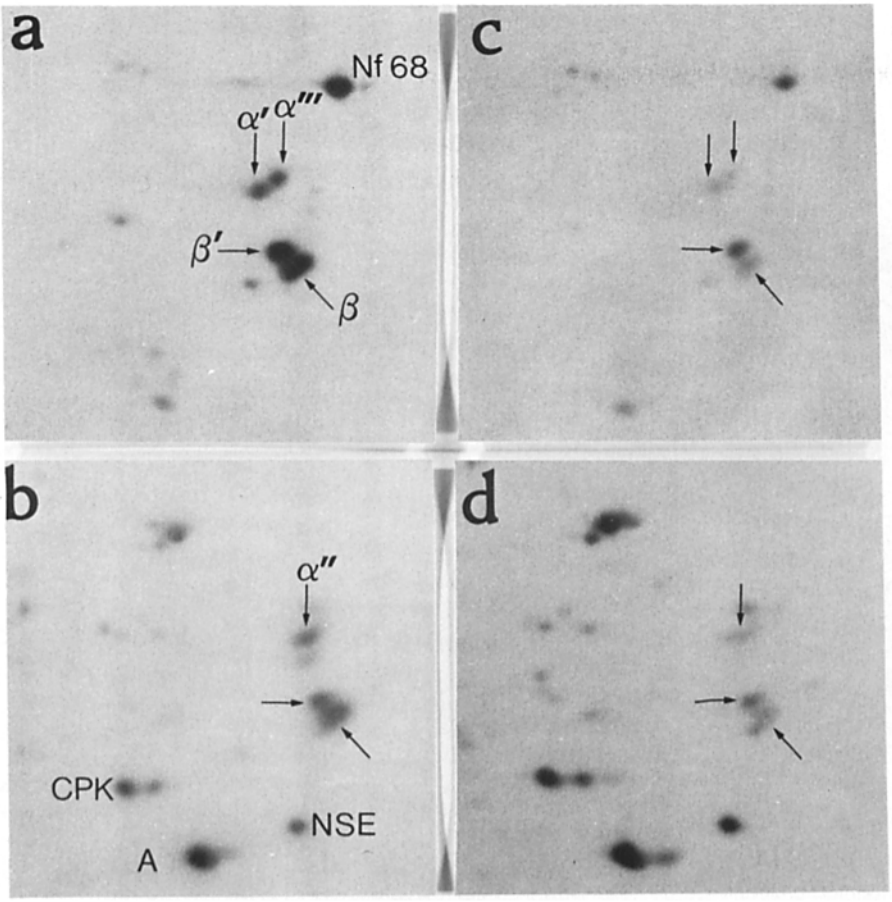

Figure 3. Specific transport of isotubulins in the cytoskeletal (*a* and *c*) and soluble (*b* and *d*) fractions of SCa (*a* and *b*) and SCb (*c* and *d*). Labeled nerve proteins were processed and analyzed as in Fig. 2. After Coomassie staining, the tubulin spots were excised from the two-dimensional gels and their radioactivity content determined. From these results, the four samples were subjected to a second 2-D PAGE to analyze comparable amounts of labeled tubulin. Only the tubulin regions of the fluorographs are shown. α′, α″, α‴ (*vertical arrows*): α-isotubulins. β′ (*horizontal arrows*) and β (*diagonal arrows*): β-tubulin subcomponents. *Nf 68*, 68 k neurofilament subunit; *A*, actin; *NSE*, nerve-specific enolase; *CPK*, brain creatine phosphokinase.

1986) and that β'_2-isotubulin derives from the β'_1-isotype by phosphorylation (Eddé et al., 1987). For these reasons, we favor the idea that the SCb-associated microtubules comprise a distinct subset of axonal microtubules, specifically enriched in β′-isotubulins and moving at a distinct rate in the motor axons.

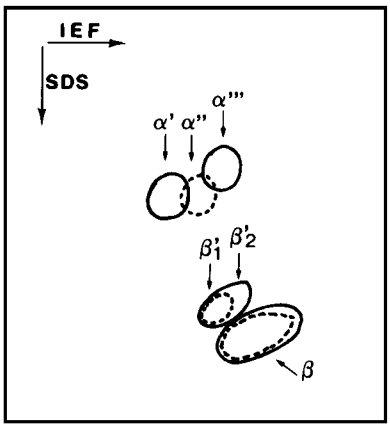

Figure 4. Tracings of tubulin spots from the fluorographs shown in Fig. 3 were superimposed to visualize the partitioning of the isotubulins between the cytoskeletal (*solid lines*) and the soluble (*dotted lines*) fractions of sciatic nerve during their transport in the motor axons.

Differential Axonal Transport of Isotubulins

We observed here that certain isoforms of tubulin are differentially partitioned in the pellet (α′, α‴, and β'_2) or in the supernatant (α″), while some others are present in both (β'_1, common and acidic β). These qualitative differences in isotubulin composition between pellet and supernatant are similar in SCa and SCb, except for the acidic β-tubulin isoforms which appear to be less abundant in SCb (see Figs. 2, 3, and 4).

Except for α′-isotypes, which are primary translation products in all tissues (Denoulet et al., 1986, 1988), all the transported tubulin isoforms that are strictly partitioned are known to be predominantly (α″) or strictly (α‴, β'_2) neurospecific and are known to derive from posttranslational modifications (Denoulet et al., 1986, 1988; Eddé et al., 1987, 1989). Among them, one isoform only (α″) is found strictly in the soluble fraction. The chemical nature of the posttranslational modification carried by α″, however, cannot be directly related to the specific solubility of this isotubulin in motor axons since, by contrast, nonlabeled α″-isotubulin was detected in the nerve-insoluble fraction (see Fig. 5, *c* and *d*), indicating that this isoform can be found associated with the insoluble structure in the unlabeled part of the sciatic nerve.

The observation that all the transported α″-isotubulins are found soluble in the motor axons could be explained either by their inability to assemble into microtubules under this form, or because they assemble only into labile structures that are depolymerized upon homogenization. An interesting possibility would be that the α″-isotubulin could serve as

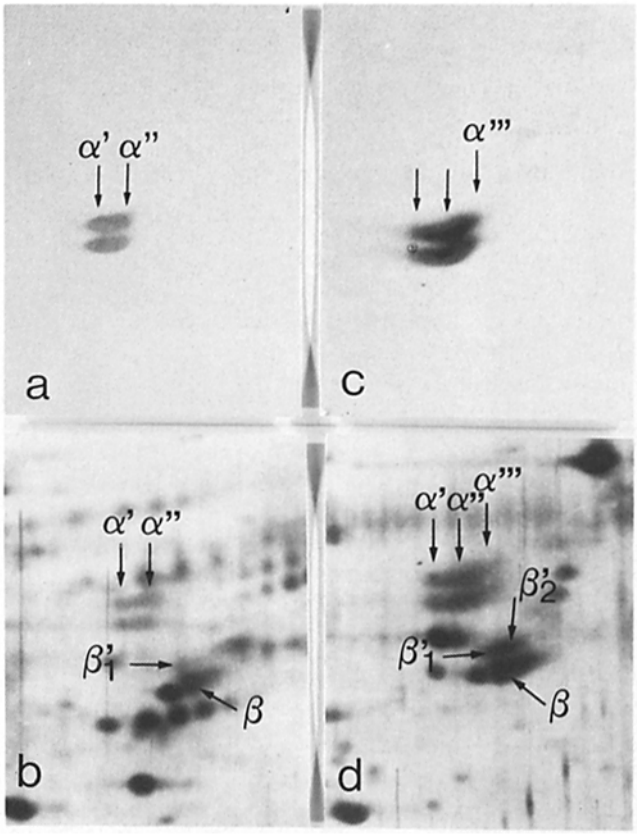

Figure 5. Immunodetection of total α-tubulin in the cytoskeletal and soluble fractions of sciatic nerve. Sciatic nerve segments were fractionated as described and proteins from the soluble (*a* and *b*) and the cytoskeletal (*c* and *d*) compartments separated by 2-D PAGE (only the tubulin regions of the two-dimensional gels are shown). Proteins were silver stained to serve as control (*b* and *d*). Proteins were also transferred onto nitrocellulose and α-tubulin polypeptides specifically detected with a monoclonal anti-α-tubulin antibody (Amersham Corp.) diluted at 1:4,000. Similar results were obtained with any segment from the nerve, except for the α'''-isotubulins that appeared to be enriched in distal segments at the expense of α'-isotubulins.

soluble precursor to the insoluble α'''-isotubulin, the posttranslational modification being in this case coupled to its polymerization (see below).

By contrast, labeled and unlabeled α'''-isotubulins are found strictly insoluble and therefore might be considered as isoforms being always stably polymerized. These isoforms arise from, at least, a posttranslational acetylation (Eddé et al., 1987, 1989) of a more basic α-isotubulin which could be α''. The two-dimensional electrophoretic coordinates of the α'''-isoforms observed here correspond to those of the posttranslationally processed α-tubulin described by Brown et al. (1982) in the axonally transported proteins in central nervous system axons. Recent preliminary experiments reinforce the idea that α''' is indeed an axonal, posttranslationally modified tubulin. When [^{35}S]methionine is injected into the spinal ventral horn to pulse-label the motor neuron proteins, labeled α''-isoforms appeared rapidly in the spinal soluble fraction while only faint traces of α'''-isoforms can be detected (Filliatreau, G., and P. Denoulet, unpublished results). By contrast, the α'''-isotubulins are found heavily labeled in the pellet fraction of the nerve proximal segments. In addition, we observed that the intensity of the α'''-isotubulin spot in two-dimensional gels increased in the more distal nerve segments, as compared to the proximal ones. All of these observations strongly suggest that an axonal α-tubulin acetylase, acting on microtubules, could produce α'''-isotubulins which would accumulate into microtubules as transport proceeds. The presence of these acetylated α'''-isotubulins might be involved in the stabilization of a subset of axonal microtubules (see Cambray-Deakin and Burgoyne, 1987; Schulze et al., 1987).

The β'_2-isotubulin is the second isoform of tubulin always found in the cytoskeletal fraction of the sciatic nerve. As with α'''-isoform, β'_2-isotubulin might be specifically associated with stabilized microtubules (see Figs. 5 *d* and 6 *b*). It has been shown that β'_2-isoform is produced by phosphorylation (Gard and Kirschner, 1985) of the primary translation product β'_1 (Eddé et al., 1987). This β'_1-isotype, specific of the nervous system (Denoulet et al., 1982, 1986; Eddé et al., 1983), has been shown to be expressed very early during the commitment of neuronal cells in culture (Eddé et al., 1983), while the β'_2-isoform accumulates during the subsequent differentiation in neurons supporting neurite outgrowth (Eddé et al., 1981). It has also been reported (Gard and Kirschner, 1985) that tubulin phosphorylation is closely related to the amount of microtubule polymer, since tubulin assembly stimulates the phosphorylation of β-tubulin while the microtubule depolymerization rapidly decreases it. Thus, the β'_2-isotubulin, recovered only in the cytoskeletal fractions of nerve homogenates, is thought to be associated with stably polymerized microtubules. If true, the observation that β'_1-isotubulin is found in both pellet and supernatant fractions would in turn suggest that β'_1-containing microtubules

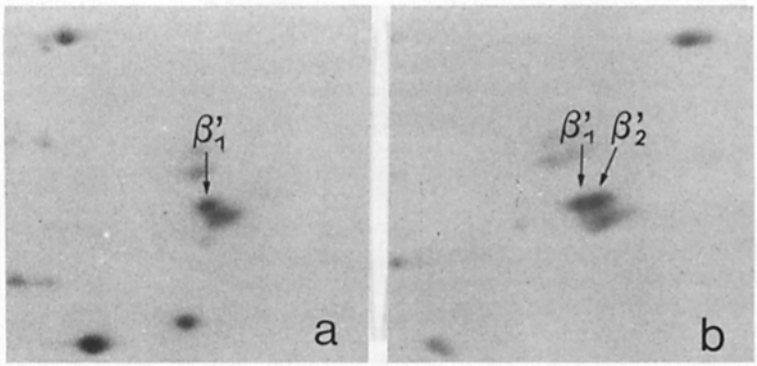

Figure 6. Specific occurrence of β'_2 in the nerve cytoskeletal fraction. Labeled protein aliquots from soluble SCa (*a*) and cytoskeletal SCb (*b*) fractions were subjected to modified isoelectric focusing gel electrophoresis (pH 5–7 LKB Ampholines only, as described in Materials and Methods) to enhance the separation of the diverse β-tubulin isoforms. The two-dimensional gels were stained then fluorographed. The same type of results were obtained with either SCa or SCb.

are in dynamic equilibrium into axons, whereas those that contain the β'_2 phosphorylated isoform are stably polymerized.

Furthermore, a parallel can be drawn between the two insoluble isotubulins, α''' and β'_2, which are both neurospecific isotubulins derived from axonal posttranslational modifications. Since the axon is unable to support any protein synthesis, posttranslational modifications provide a rapid means to modulate the structural and biochemical properties of many axonal proteins far from the nerve cell bodies. In the case of tubulin, the two distinct posttranslational modifications producing α''' and β'_2-isotubulins could, for example, allow the axon to produce microtubules characterized by an increased stability or an alternatively modulated property. They would also confer to the neuronal microtubules the ability to bind various axonal elements, like neurofilaments and specific tau polypeptides (Tytell et al., 1984), or proteins and vesicles in the process of axonal transport (see Miller et al., 1987).

In conclusion, our results show that several microtubule subpopulations, differing in their velocity and/or by relative stability in the axons, can be distinguished on the basis of their isotubulin composition. The observation that different isotubulins are found in different kinetic and physical compartments of the axon allows a functional approach to understand the role of the various components of the tubulin polymorphism. In this respect, studies of axonal transport offer an excellent in vivo model to further understand and analyze functional specificities of the different isotubulins.

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