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# Actin Immunoreactivity Localizes with Segregated Microtubules and Membranous Organelles and in the Subaxolemmal Region in the β,β′-Iminodipropionitrile Axon

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**Using the peroxidase-antiperoxidase staining technique with monoclonal and polyclonal antibodies against actin, we found that, in β,β′-iminodipropionitrile (IDPN)-treated axons, actin immunoreactivity was codistributed with segregated microtubules and membranous organelles in the central region and excluded from the peripheral axoplasm occupied by neurofilaments. Actin immunoreactivity was also present in the subaxolemmal region. Fast axonal transport is localized in the central region of the IDPN axon (Papasozomenos et al., 1982a). As both microtubules and actin are present in the central region of IDPN axons, a possible role of actin in fast axonal transport warrants further investigation.**

Intoxication of rats by intraperitoneal injection of β,β′-iminodipropionitrile (IDPN) results in reorganization of axoplasmic organelles with displacement of neurofilaments toward the periphery, and of microtubules and membranous organelles toward the center of axons (Papasozomenos, 1986; Papasozomenos et al., 1981, 1982a, b, 1985). Concomitantly with the segregation of microtubules from neurofilaments in the extraspinal portion of axons, the axonal transport of neurofilament proteins is selectively and severely impaired (Griffin et al., 1978), and accumulation of neurofilaments occurs in the intraspinal portion of motor axons (Chou and Hartmann 1964, 1965). Accumulation of neurofilaments starts at the intraspinal–extraspinal junction of motor axons and proceeds centripetally (Papasozomenos et al., 1985; Yokoyama et al., 1980). Despite the microtubule–neurofilament segregation, the transport of tubulin and actin and the rates of fast anterograde and retrograde transports remain normal (Griffin et al., 1978; Yokoyama et al., 1980). In addition, rapidly transported proteins are found in the central region of axons, occupied by microtubules and membranous organelles, and no fast-moving proteins are present in the peripheral axoplasm, occupied by neurofilaments (Papasozomenos et al., 1982a, b). Thus, the IDPN axon provides an experimental model to examine the interactions of the various cytoskeletal elements and their role in axonal transport.

While the properties of fast axonal transport are well known, the mechanism has not yet been elucidated (reviewed in Schliwa, 1984). Several microtubule-associated models of axonal transport have been proposed. In 2 theoretical publications, Weiss and Gross (1983) and Gross and Weiss (1983) suggested that axonal organelles are carried in low-viscosity microstreams generated by and around microtubules. In the "transport filament" model, Ochs (1982) hypothesized that materials are transported bound to an as yet unidentified filament that moves along the axon by microtubular side arms. More recently, this microtubule-based motility complex has been shown to include a novel force-generating ATPase (kinesin) (Gilbert et al., 1985; Lasek and Brady, 1985; Vale et al., 1985). The role of actin, if any, in fast axonal transport remains to be defined. While intraaxonal depolymerization of actin filaments inhibits fast axonal transport, intraaxonal injection of substances that in other motility systems inhibit myosin ATPase has no effect on axonal transport (Brady, 1984; Goldberg, 1982; Nemhauser and Goldberg, 1985). Because of these findings, a structural role of actin in the axonal cytoskeleton has been suggested (Goldberg, 1982).

IDPN-treated axons were examined to determine if actin is segregated to the central region of the axon where microtubules and fast transport are localized (Papasozomenos et al., 1982a, b). Using the peroxidase–antiperoxidase technique for light- and electron-microscopic immunohistochemistry, we found that 2 monoclonal and 2 polyclonal anti-actin antibodies localize in the central region of IDPN-treated axons, occupied by microtubules and membranous organelles. In addition, actin immunoreactivity was present in the subaxolemmal region. Thus, both microtubules and actin are present in the central region of IDPN axons where fast transport takes place. A portion of this study has been previously reported in abstract form (Papasozomenos et al., 1984).

## Materials and Methods

### *Anti-actin antibodies*

#### *Monoclonal*

Preparation, characterization, and specificity of monoclonal antibodies against Japanese quail pectoral muscle actin, designated 3G7 and 3F10, have been described elsewhere (Caceres et al., 1983; Payne, 1983). In addition, for the present study, the specificities of these 2 monoclonal hybridoma antibodies were tested on nitrocellulose immunoblots of homogenates of whole mouse cerebellum and the lumbar segment of spinal cord. Tissues were dissected and immediately placed in saline solution, pH 7.8, containing 2% SDS, 0.5% Tween-20, 1% β-mercaptoethanol, and 5% ethyl ether. The samples were homogenized, placed in boiling water for 5 min, and centrifuged to remove insoluble material. The samples were electrophoresed on a 13% SDS–polyacrylamide slab gel and then Western blotted onto nitrocellulose (Towbin et al., 1979). The proteins on the nitrocellulose sheets were visualized using amido black staining. Localization of the antibodies, 3G7 and 3F10, was performed using a peroxidase-labeled anti-mouse immunoglobulin (Tago, Burlingame, CA) and *O*-dianisidine substrate. Their specificity is shown in Figure 1. For immunostaining, the antibodies were used as hybridoma tissue culture supernatant that was ammonium sulfate precipitated and

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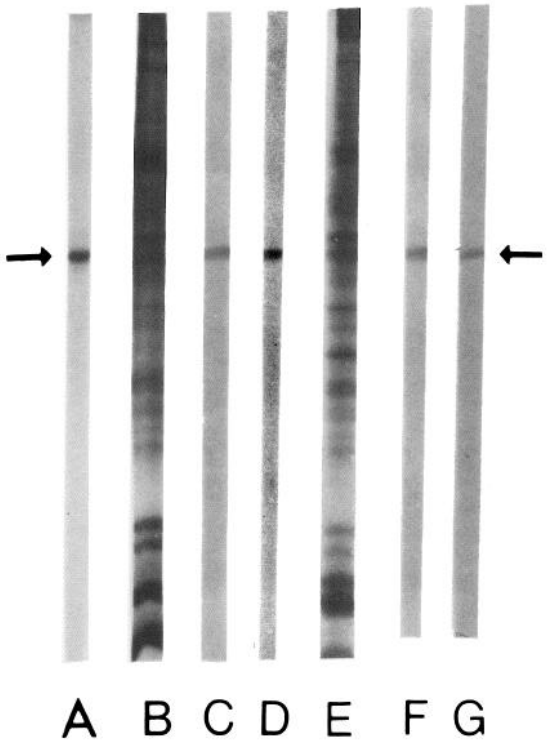


*Figure 1.* Specificity of monoclonal anti-actin antibodies 3G7 and 3F10 is demonstrated using nitrocellulose immunoblots, prepared as is described in Materials and Methods. *A,* Adult quail breast muscle actin, amido black staining. *B,* Adult mouse spinal cord, amido black staining. *C,* Western blot examining the binding of 3G7 (2 μg/ml) on the adult mouse spinal cord electrophoretic gel of *B. D,* as in *C,* but examining 3F10 (2 μg/ml). *E,* Adult mouse cerebellum, amido black staining. *F,* Western blot examining the binding of 3G7 (2 μg/ml) on adult mouse cerebellum electrophoretic gel of *E. G,* as in *F,* but examining 3F10 (2 μg/ml). Both antibodies stain only a protein with the same electrophoretic mobility (42,000 Da) as actin.

purified on a protein A–Sepharose 4B affinity column (Pharmacia, Uppsala, Sweden) at a concentration of 2–5 μg/ml or as ascites fluid, which was protein A–Sepharose 4B affinity purified at a dilution of 1:500–1:1000.

### *Polyclonal*

Two polyclonal antibodies were used: rabbit affinity-purified anti-actin antibodies to chicken gizzard smooth muscle at a concentration of 20 μg/ml (a gift from Dr. I. M. Herman, Department of Anatomy and Cellular Biology, Tufts University) and an anti-actin serum raised in rabbits also against chicken gizzard smooth muscle actin, diluted 1:500 (a gift from Dr. P. C. Letourneau, Department of Anatomy, University of Minnesota). All antibodies were kept in small aliquots at −70°C and diluted just prior to use.

### *IDPN administration*

Male Sprague-Dawley rats, weighing 200–250 gm, received a total of 2 mg/gm body weight IDPN (Eastman Kodak, Rochester, NY) divided into 4 equal doses and given every 3 d by intraperitoneal injection. IDPN was diluted 1:5 in saline and the pH adjusted to 7.4 with hydrochloric acid. Controls were given similar injections of saline.

### *Immunohistochemistry*

Control and IDPN-treated rats were sacrificed 1, 2, 3, 4, 5, and 6 weeks following the fourth injection. Under Nembutal anesthesia, the animals were perfused through the left ventricle with 100–150 ml of PBS followed by 800 ml of a mixture of 4% paraformaldehyde and 0.25% glutaraldehyde in 120 mM sodium potassium phosphate buffer (pH 7.4) at 37°C. Tissue samples were taken from the lumbar (L) segment of spinal cord, the $L_5$ ventral and dorsal roots, the sciatic and optic nerves, brain stem, and cerebellum. They were further fixed in 4% paraformaldehyde (pH 7.4) at 4°C overnight. Thirty- or 40-μm-thick sections were cut on a vibratome and processed for light- and electron-microscopic immunohistochemistry. To facilitate cross-sectioning, samples of nerve roots, optic and sciatic nerves just prior to sectioning were mounted in 7% (wt/vol) molten agar in 50 mM Tris-HCl, pH 7.6, and immediately cooled at 4°C.

For immunostaining, the peroxidase–antiperoxidase technique (Sternberger et al., 1970; Straus, 1982) was used. Free-floating sections were incubated with (1) 10% normal rabbit (Sternberger-Meyer, Jarrettsville, MD) or normal goat (Miles Laboratories, Elkhart, IN) serum, depending on the species of the secondary antibody, to reduce nonspecific binding; (2) primary antibody for 18–36 hr at 4°C; (3) rabbit anti-mouse IgG, diluted 1:40 (Sternberger-Meyer, Jarrettsville, MD), or goat anti-rabbit IgG (Cappel Laboratories, West Chester, PA), diluted 1:50, for 90 min; (4) mouse peroxidase–antiperoxidase prepared from monoclonal antibody, diluted 1:150 (Sternberger-Meyer, Jarrettsville, MD), or rabbit peroxidase–antiperoxidase (Cappel Laboratories, West Chester, PA), diluted 1:200, for 90 min; and (5) 0.075% diaminobenzidine tetrahydrochloride and 0.015% hydrogen peroxide in 50 mM Tris-HCl, 10 mM imidazole, pH 7.6, for 5 min. The sections were washed 3 times for a total of 90 min between steps (2) and (3) and between (3) and (4). Washings and dilutions of antibodies were done with 1% normal rabbit or goat serum in 50 mM Tris-HCl, 0.15 M sodium chloride, pH 7.6.

For electron-microscopic immunohistochemistry, immunostained sections were osmicated in 1% $OsO_4$ for 30 min, dehydrated in graded alcohols, passed through propylene oxide, and flat-embedded in Epon according to standard procedures. Ultrathin sections were mounted on uncoated grids and viewed without prior staining with heavy metals.

To test the staining specificity, antibodies 3G7 and 3F10 were preabsorbed with a molar excess of electrophoretically pure actin from adult quail pectoral muscle according to standard techniques. Also, 1% normal rabbit or goat serum in 50 mM Tris-HCl, 0.15 M sodium chloride, pH 7.6, was substituted for the primary antibodies.

Several sections were also embedded in paraffin, Epon, and polyethylene glycol and immunostained as has been previously described (Papasozomenos et al., 1985).

## Results

### *Specificity of anti-actin antibodies*

The specificities of the 2 monoclonal anti-actin antibodies, 3G7 and 3F10, are shown in Figure 1. It is evident that in the complex extracts from mouse cerebellum and lumbar spinal cord, both antibodies stain only a protein with the same electrophoretic mobility (42,000 Da) as adult quail breast muscle actin. Moreover, in solid-phase enzyme-linked immunoassay, both 3G7 and 3F10 were positive to actin isolated from species throughout evolution but had no cross-reactivity to a large number of examined proteins, including purified quail muscle myosin, tropomyosin, and tubulin from many different species, including bovine brain (data not shown).

The specificities of the anti-actin polyclonal antibodies have been reported by Drs. I. M. Herman and P. C. Letourneau (Herman and D'amore, 1985; Herman and Pollard, 1979; Herman et al., 1981; Letourneau, 1981, 1983; Marsh and Letourneau, 1984).

### *Localization of actin immunoreactivity in IDPN-treated axons*

#### *Monoclonal anti-actin antibodies (3G7 and 3F10)*

Because the most reliable, consistent and reproducible results were obtained with vibratome sections, while paraffin-, polyethylene glycol-, and Epon-embedded sections produced less than optimal results, observations made only on vibratome sections are reported.

IDPN produced reorganization of axoplasmic organelles with displacement of microtubules and membranous organelles towards the center and of neurofilaments towards the periphery of the extraspinal axons, as has been previously described in detail (Papasozomenos, 1986; Papasozomenos et al., 1981,

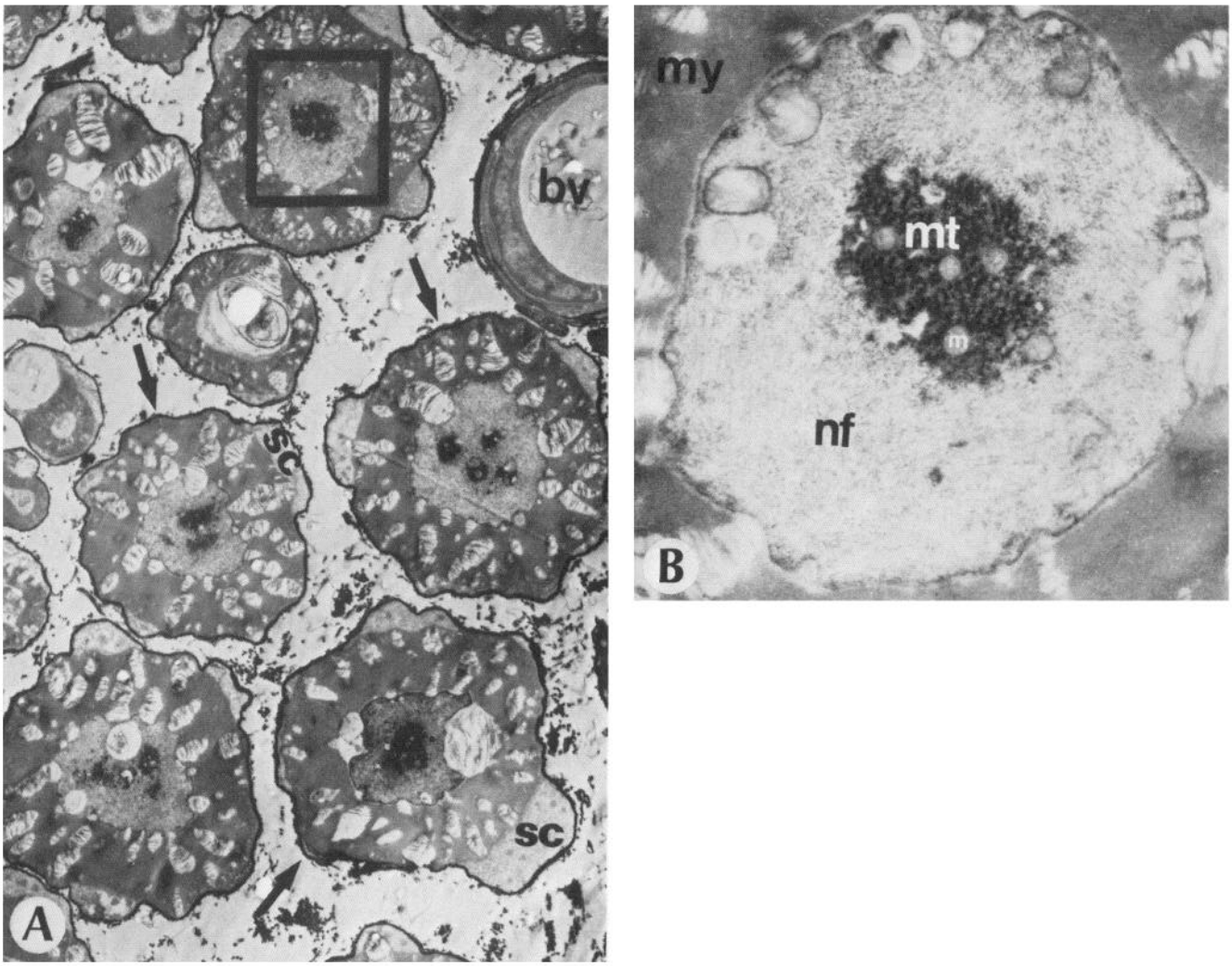


*Figure 2.* Electron micrographs of cross sections of axons in $L_5$ anterior spinal root 1 week after IDPN administration, immunostained with 3F10 (5 μg/ml). The *boxed area* in *A* is shown at higher magnification in *B*. Intense immunostaining is present in the central region of axons occupied by microtubules (*mt*), mitochondria (*m*), and other membranous organelles. Weak immunoreactivity is seen in the subaxolemmal region. No immunostaining is present in the peripheral axoplasm occupied by neurofilaments (*nf*). Schwann cells (*sc*), myelin sheaths (*my*), and walls of blood vessels (*bv*) are unstained, but at more superficial levels of sectioning Schwann cells were also stained. Intense staining of Schwann cell basal laminae (*arrows*) is nonspecific, as explained in the text and Figure 3. Identical results were obtained with 3G7. Similar results were obtained in posterior spinal roots. *A*, ×4800; *B*, ×23,250.

1982a, b; 1985). Both 3G7 and 3F10 localized in the central region of axons, which is also occupied by microtubules (Fig. 2). Most of the axons showed only a single stained area in the center, although in a few axons, 2 or more smaller separate immunoreactive areas were observed in the central region. Mitochondrial and other membranous organelles present in the central region were negative. Weak immunoreactivity was also found in the subaxolemmal region. The rest of the axoplasm, occupied by neurofilaments, remained unstained. Myelin sheaths and blood vessels did not stain. At superficial levels of sectioning, Schwann cells were intensely stained, but at deeper levels, while the axons were still strongly labeled, the Schwann cells frequently became negative (Fig. 2*A*). This finding is most probably due to deeper penetration of the immunochemicals in axons than in Schwann cells.

In peripheral nerves, intense nonspecific immunostaining of the Schwann cell basal laminae was present not only with anti-actin but with every monoclonal antibody (anti-β-tubulin, anti-MAP2, etc.) that we have examined (Fig. 2*A*). To investigate the origin of this nonspecific immunostaining, the primary and link antibodies and the peroxidase–antiperoxidase complex were alternatively omitted from the staining sequence. The nonspecific staining was observed even when the anti-actin antibodies were omitted from the staining sequence, but was abolished by omitting the link antibody (rabbit anti-mouse IgG) (Fig. 3). It is apparently due to a nonspecific binding of rabbit anti-mouse IgG (Sternberger-Meyer, Jarrettsville, MD) to a component of the rat Schwann cell basal lamina. This nonspecific binding was not present when the polyclonal anti-actin antibodies were used (see below). It was also absent from sections that had been embedded in paraffin, Epon, and polyethylene glycol prior to immunostaining.

Absorption of 3G7 and 3F10 with molar excess of actin abolished the immunostaining.

*Polyclonal anti-actin antibodies*

Both polyclonal anti-actin antibodies (provided by Drs. Ira M. Herman and Paul C. Letourneau) also localized in the central

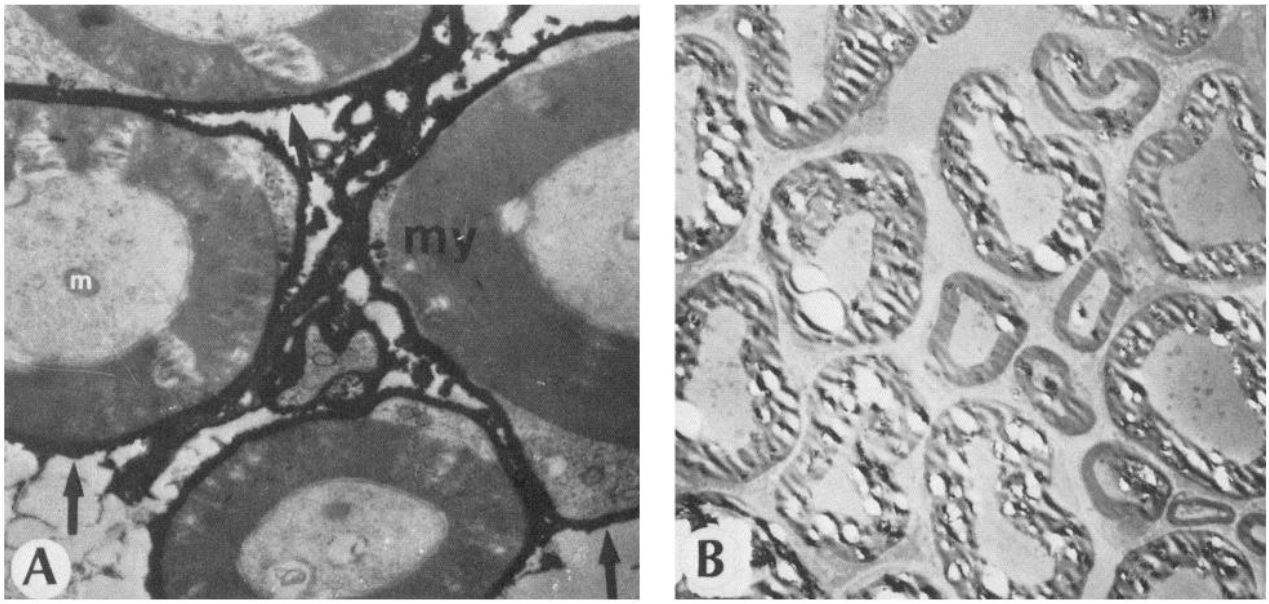


*Figure 3.* To demonstrate the nonspecific nature of the staining of the Schwann cell basal laminae (*arrows*), 1% normal rabbit serum was substituted either for 3F10 and 3G7 in *A* or rabbit anti-mouse IgG (Sternberger-Meyer, Jarrettsville, MD) in *B*. In *A*, staining of basal laminae is still present, although no staining of axons is seen. Staining of axons and basal laminae is abolished in *B*, where rabbit anti-mouse IgG was omitted from the staining sequence. Note also the lack of staining in the subaxolemmal region. Sections are adjacent to sections in Figure 2. Abbreviations are as in Figure 2. *A*, ×17,100; *B*, ×3000.

region of axons occupied by microtubules (Figs. 4, 5). In addition, especially with Dr. Herman's antibody, an annulus of intense immunoreactivity of about 100 nm in width was observed in the subaxolemmal region (Fig. 4). Neurofilaments remained unstained. Schwann cells were strongly positive at the higher levels of sectioning (Fig. 4*A*) but, as was the case with the monoclonal antibodies, also became negative at deeper levels while the axons were still intensely stained (Fig. 5).

***Localization of actin immunoreactivity in control rats using monoclonal anti-actin antibodies (3G7 and 3F10)***

With light microscopy, a uniform and rather intense staining of axons was observed in all areas examined, i.e., anterior and posterior spinal nerve roots, sciatic and optic nerves, and the white matter tracts of spinal cord, brain stem, and cerebellum (Fig. 7*A*). The only exceptions were the granular cell axons in

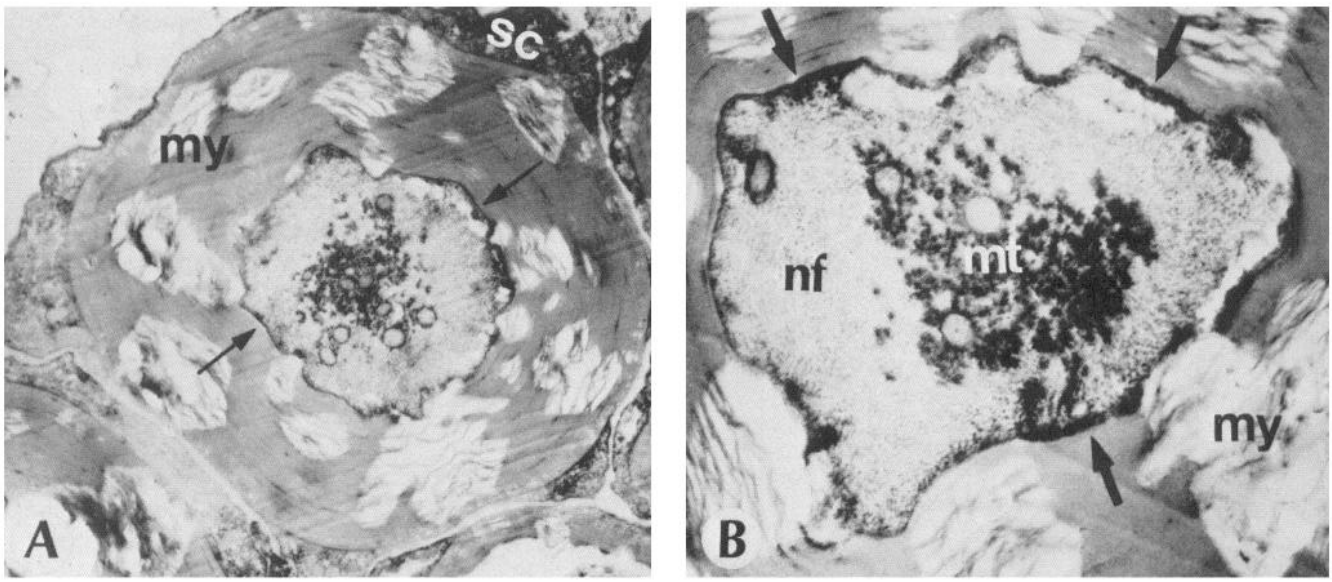


*Figure 4.* Electron micrographs of cross sections of axons in $L_5$ anterior spinal root 5 weeks after IDPN administration, immunostained with affinity-purified anti-actin antibodies to chicken gizzard smooth muscle actin at a concentration of 20 μg/ml (provided by Dr. I. M. Herman). Abbreviations are as in Figure 2. Intense staining is codistributed with microtubules and membranous organelles in the central region of axons but also in a subaxolemmal annulus (*arrows*) approximately 100 nm wide. In *A*, strong staining of Schwann cells is also present. Note the absence of nonspecific immunostaining of Schwann cell basal laminae. *A*, ×8850; *B*, ×19,950.

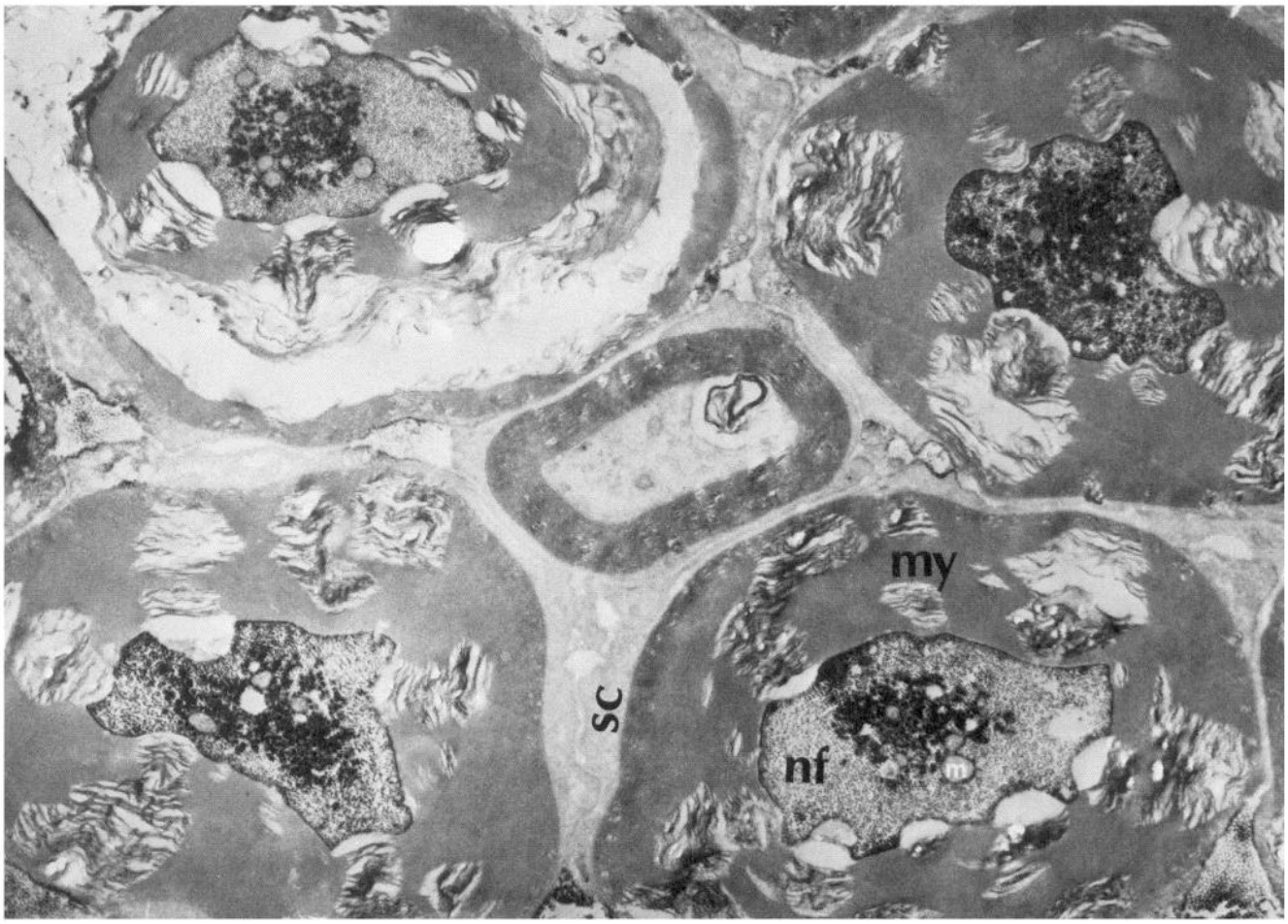


*Figure 5.* Electron micrograph of cross sections of axons in $L_5$ anterior spinal nerve root 5 weeks after IDPN administration, immunostained with anti-actin serum against chicken gizzard smooth muscle actin, diluted 1:500 (provided by Dr. P. C. Letourneau). Using this anti-actin serum, actin immunoreactivity is also codistributed with segregated microtubules and membranous organelles in the central region of 4 IDPN axons. Note also the absence of nonspecific binding to Schwann cell basal laminae. Abbreviations are the same as in Figure 2. ×11,390.

the molecular layer of cerebellum, which, as was demonstrated with the EM, remained unstained (Fig. 9*B*). With electron microscopy, in peripheral axons the immunoreactive product was concentrated on or around individual and small groups of microtubules and frequently in the subaxolemmal region (Fig. 6*A*). However, the staining intensity was weaker in untreated than in IDPN axons; this difference in staining intensity was due to segregation and thus increased the concentration of actin in the central region of IDPN axons. In white matter tracts of spinal cord and other CNS regions, strong immunostaining was concentrated on or around microtubules and membranous organelles, but focally diffuse staining and, in several axons, a subaxolemmal annulus of immunoreactivity were also present (Fig. 6*B*). Similar results were obtained with the polyclonal antibodies (data not shown).

At the light-microscopic level, cell bodies were uniformly stained, with the exceptions of Purkinje cells and ependymal cells, which showed no immunoreactivity (Fig. 9*A*), and spinal motor neurons, which frequently showed accentuation of staining at the cell periphery (Fig. 7*A*). At the EM level, immunoreactive product was present over the entire cytoplasm with accentuation around mitochondria. Staining of Nissl bodies was weaker than the rest of the neuronal cytoplasm. Mitochondrial matrix remained unstained. Both 3G7 and 3F10 stained intensely nuclei of all neurons, astrocytes, and ependymal cells. Nucleoli did not stain (Figs. 7, 9*A*). With the EM, nuclei showed diffuse but not homogeneous immunostaining. While the inner leaflet of nuclear membrane was intensely stained, the outer leaflet was negative (Fig. 7*B*). That these 2 monoclonal anti-actin antibodies stained neuronal and glial nuclei was not a surprise, since actin has been found to be the major non-histone protein of the nuclear matrix (Armbruster et al., 1983; Capco and Penman, 1983; Capco et al., 1982; Douvas et al., 1975), and nuclear actin and myosin are involved in nucleocytoplasmic transport (Schindler and Jiang, 1986).

In cross and longitudinal sections of dendrites immunoreactivity was present on microtubules but also diffusely in the cytomatrix (Fig. 8). Dendritic spines showed intense diffuse staining (Fig. 9*B*). Postsynaptic densities were also strongly positive. Axonal terminals were always negative (Figs. 8, 9*B*). Oligodendrocytes and myelin sheaths did not stain. Blood vessels also remained unstained.

## Discussion

Using monoclonal and polyclonal anti-actin antibodies and light- and electron-microscopic immunohistochemistry, we have demonstrated that actin immunoreactivity is codistributed with segregated microtubules and membranous organelles in the central region of IDPN axons. In addition, an annulus of subaxolemmal actin immunoreactivity is also present.

Monoclonal antibodies are exquisitely specific probes that recognize discrete binding domains on proteins. If the specific

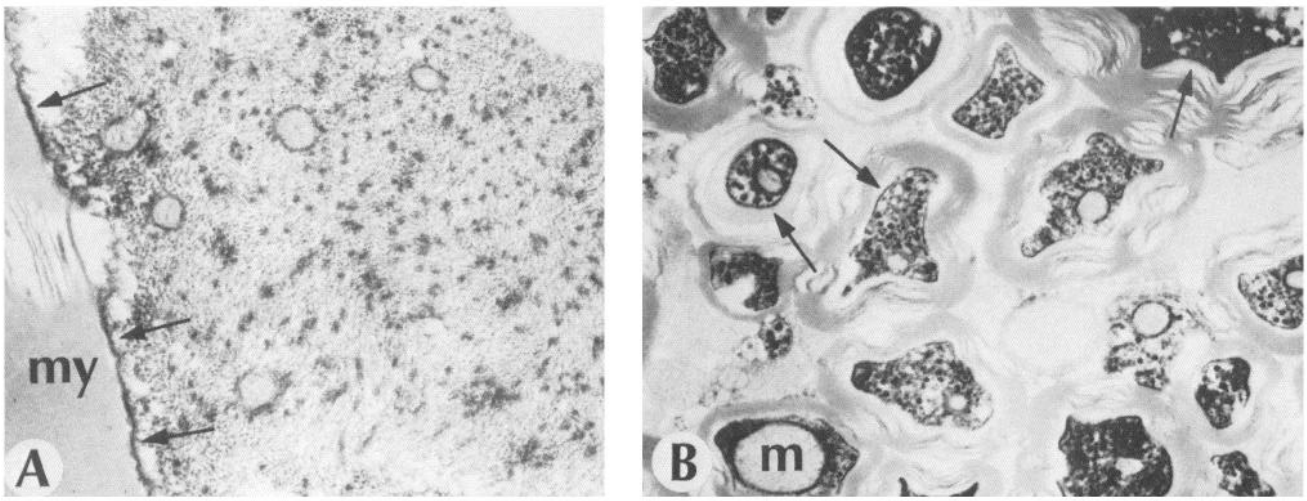


*Figure 6.* Electron micrographs of cross section of axons in $L_5$ anterior root (*A*) and white matter tracts of spinal cord (*B*) from untreated rats, immunostained with 3F10, diluted 1:500, and 3G7, diluted 1:600, respectively. Rather strong staining concentrated around individual or groups of microtubules and in the subaxolemmal region (*arrows*) is present in *A*. Note the intense immunoreactivity in axons of white matter tracts; it is frequently concentrated around microtubules and membranous organelles, but focally diffuse staining is also present. A thin annulus of subaxolemmal staining is seen in several axons (*arrows*). Abbreviations are the same as in Figure 2. *A*, ×21,014; *B*, ×19,700.

epitope is present on a polypeptide other than the immunogen, false-positive immunoreactions are possible. Indeed, unexpected cross-reactivities with monoclonal antibodies have been reported (Nigg et al., 1982; Pruss et al., 1981). In view of the finding that the molecule of α-tubulin contains regions that are homologous to sequences of actin (Ponstingl et al., 1981), the possibility that our monoclonal antibodies to actin cross-react with microtubular tubulin should have to be ruled out. In fact, the lack of reactivity with mouse and bovine brain tubulin on nitrocellulose immunoblots and with tubulin from many different species in solid-phase enzyme-linked immunoassays, as well as the codistribution of 2 well-characterized polyclonal anti-actin antibodies with microtubules, leaves almost no doubt that the observed localization indeed represents only actin immunoreactivity. In addition, similar results have recently been presented by other investigators (Nagele et al., 1985). The finding that the polyclonal antibodies frequently produced a more intense subaxolemmal staining than the monoclonal is perhaps

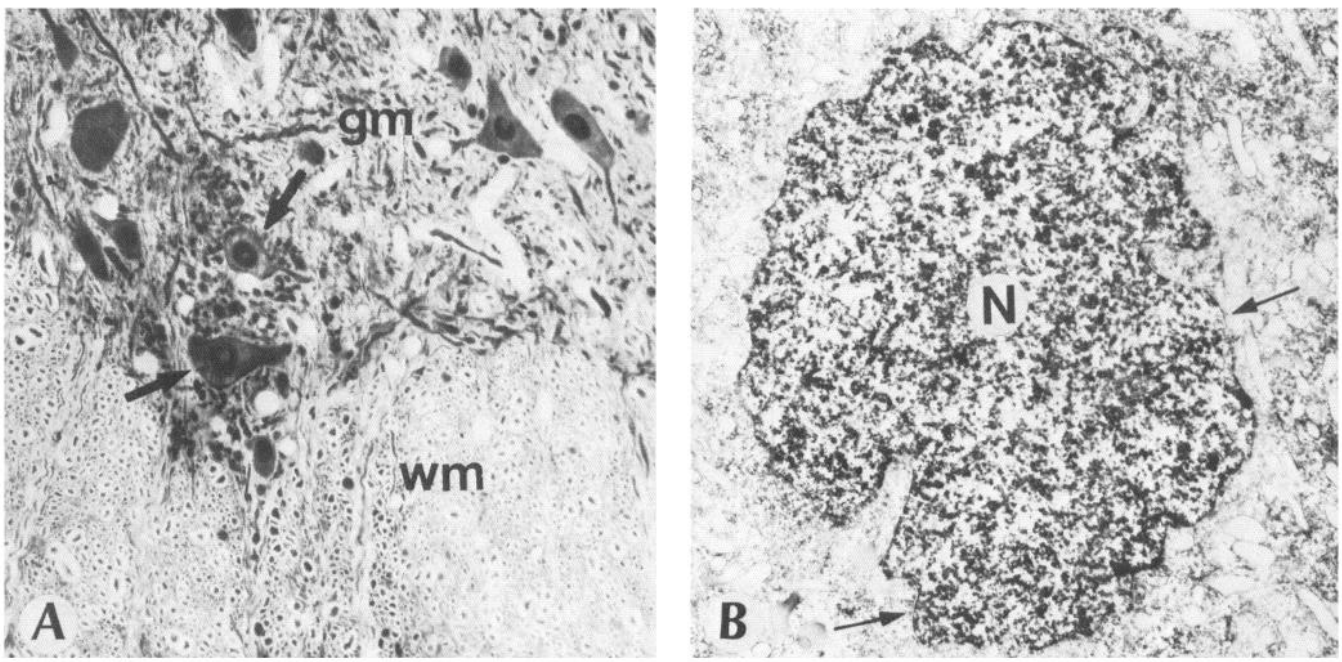


*Figure 7.* Light (*A*) and electron (*B*) micrographs of a cross section of the lumbar segment of spinal cord of a control rat immunostained with 3F10 (2 μg/ml) and 3G7, diluted 1:600, respectively. *A*, Axons and dendrites are strongly stained. In motor neurons, actin immunoreactivity is frequently accentuated at the cell periphery (*arrows*). Nuclei are intensely stained and are frequently surrounded by a cytoplasmic halo of relatively weak immunoreactivity. Nucleoli are not stained. *B*, Diffuse but not homogenous staining of the nucleus (*N*) of a motor neuron is demonstrated. Note that the outer nuclear membrane is unstained (*arrows*). *gm*, gray matter; *wm*, white matter. *A*, ×125; *B*, ×6480.

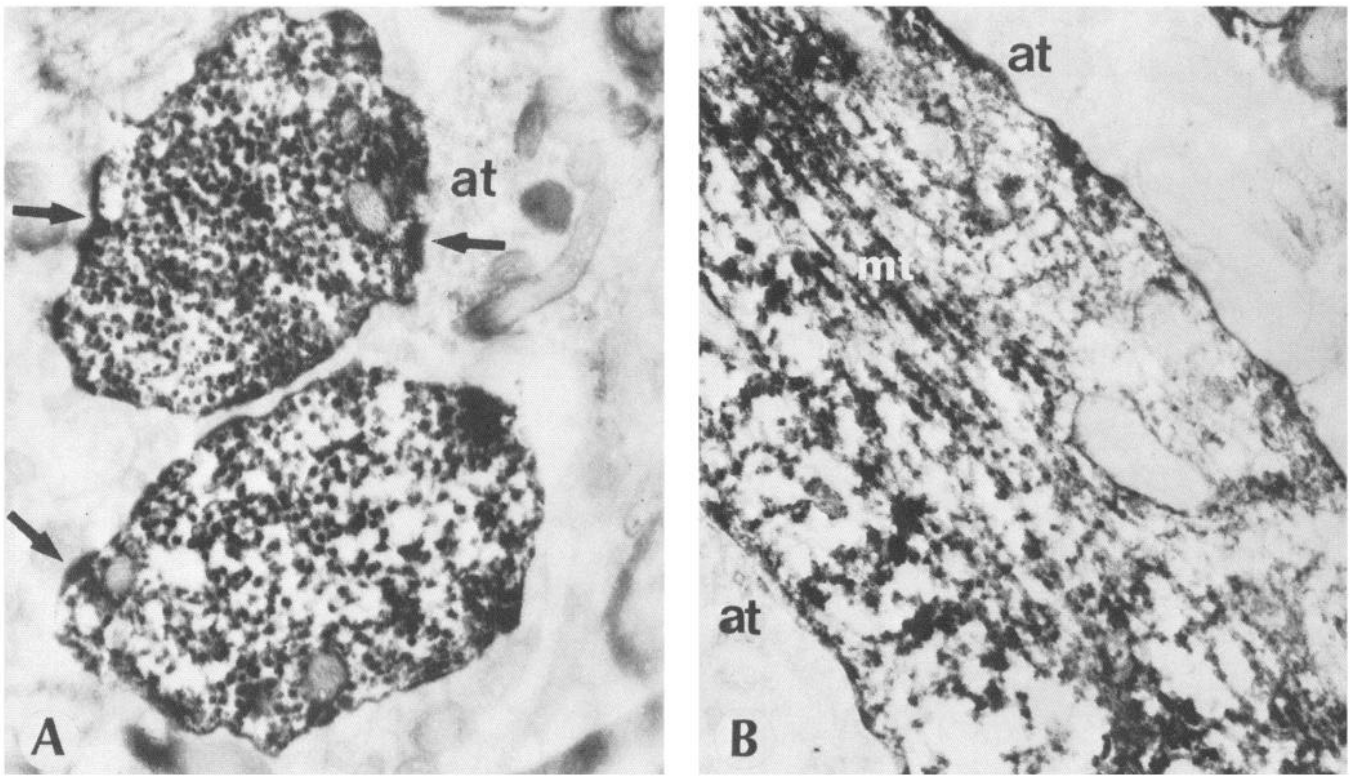


*Figure 8.* Electron micrograph of cross (*A*) and longitudinal (*B*) sections of dendrites of spinal motor neurons from a control (*A*) and IDPN-treated (*B*) animal 5 weeks after IDPN administration, immunostained with 3F10 (2 μg/ml; *A*) and 3G7 (*B*), diluted 1:500. In both sections intense immunoreactivity is present on or around microtubules but also in patches throughout the dendrites. In *B*, a bundle of microtubules (*mt*) located in the center of the dendrite is present. Postsynaptic densities are intensely stained (*arrows*). All axonal terminals (*at*) are unstained. *A*, ×20,920; *B*, ×28,020.

due to the fact that more antigenic determinants are recognized by the polyclonal compared to the monoclonal antibodies.

As in other eukaryotic cells, actin is perhaps the most abundant neuronal protein and exists in a dynamic equilibrium between monomeric and filamentous forms (reviewed in Fifkova, 1985). It is through the versatility of the state of actin, which is regulated by actin-associated proteins, that some of the changes in viscoelastic properties of axoplasm are brought about. Although in extruded axoplasm of squid giant axon about half of the amount of actin is in monomeric form (Morris and Lasek,

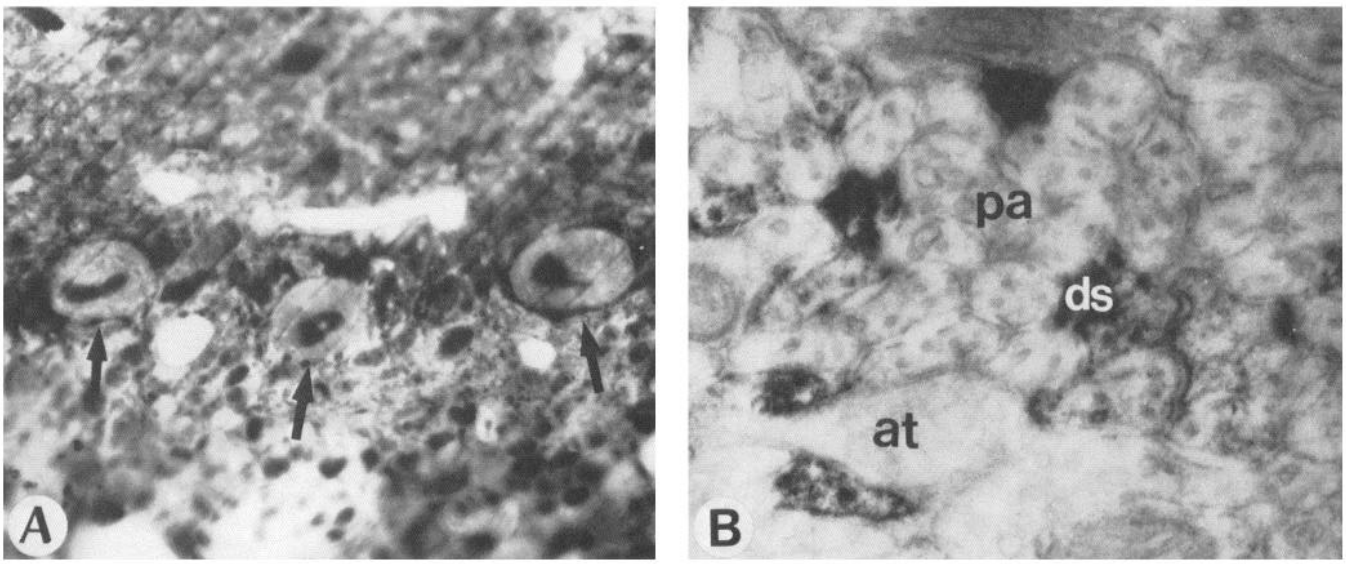


*Figure 9.* Light (*A*) and electron (*B*) micrographs of horizontal sections of a control rat, immunostained with 3F10 (2.5 μg/ml; *A*) and 3G7 (5 μg/ml; *B*). In *A*, nuclei of Purkinje cells are intensely stained, but no cytoplasmic immunoreactivity is present (*arrows*). In *B*, dendritic spines (*ds*) show intense diffuse staining but parallel axons (*pa*) and axonal terminals (*at*) are negative. *A*, ×470; *B*, ×67,500.

1984), it has been demonstrated that cellular activity determines the distribution and organization of actin (Beyers and Fujiwara, 1982; Gabbiani et al., 1983; Herman et al., 1981; Lazarides, 1976; Pesacreta et al., 1982; Wong et al., 1983). In a neuron, a transaxonal actin network and a subaxolemmal network of actin filaments are expected to be present. However, by using immunofluorescence, decoration of actin filaments with the S1 fragment of myosin, and other techniques, actin has been found in some studies to be distributed over the entire cross section of neurites (Jockusch et al., 1979; Spooner and Holladay, 1981; Yamada et al., 1971), while other investigators have found a microfilament actin-containing network predominantly located under the axolemma (Chang and Goldman, 1973; Hirokawa, 1982; Isenberg and Small, 1978; Letourneau, 1983; Metuzals and Tasaki, 1978). In addition, it has been found that the radioactivity carried by the slow component b of axonal transport, of which actin is a major component, is 2.5 times higher in the subaxolemmal region than in any other comparable region of axoplasm (Heriot et al., 1985).

Other investigators have described actin-containing filaments emerging from microtubules in neurites of neuroblastoma cells (Kuczmarski and Rosenbaum, 1979; Ross et al., 1975), attaching to axonal microtubules in the rat caudate nucleus and substantia nigra (LeBeux and Willemot, 1975), and making end-to-side contacts with microtubules in various detergent-extracted cells (Schliwa and van Blerkom, 1981). In contrast to these studies, while a subaxolemmal network of short actin filaments has been found, this, so far putative, microtubule-associated transaxonal actin network has not been visualized as a discrete filamentous system in freeze-fracture studies of axoplasm (Hirokawa, 1982; Schnapp and Reese, 1982). In addition to the above morphological studies, there is ample biochemical evidence for interaction of actin filaments with microtubules, mediated by microtubule-associated proteins, such as MAP2 (Kotani et al., 1985; Nishida et al., 1981; Pollard et al., 1984; Sattilaro and Dentler, 1982). Because the procedures that were used in our study exert a detrimental effect on the integrity of actin filaments (Lehrer, 1981; Maupin-Szamier and Pollard, 1978; Ris, 1985), no actin immunoreactivity in a filamentous form could be observed, but, due to the aggregation of microtubules in the center of IDPN axons, the codistribution of microtubules and actin has been demonstrated. This actin immunoreactivity is perhaps a component of the perimicrotubular linking system. However, it does appear to be independent of the subaxolemmal actin filaments, which, following IDPN administration, are not displaced and do not play a role in anterograde fast axonal transport (Papasozomenos et al., 1982a).

The localization of actin immunoreactivity at 2 discrete regions, i.e., in a subaxolemmal annulus and with the segregated microtubules and membranous organelles in the central region of IDPN axons, is intriguing and may help elucidate the still elusive role(s) of actin in the axon. We have previously shown (Papasozomenos et al., 1982a) that rapidly migrating proteins are exclusively found in the central region of IDPN axons, occupied by microtubules. Other investigators have demonstrated that DNase I or gelsolin, 2 proteins that depolymerize or sever actin filaments, respectively, inhibit fast axonal transport (Brady, 1984; Goldberg, 1982; Nemhauser and Goldberg, 1985). On the other hand, recent studies have suggested that actin filaments are not a component of transport filaments in the squid giant axon (Schnapp et al., 1984) and may not play a role in the generation of force necessary for transport (Vale et al., 1985). However, actin filaments appear to be essential for *in situ* fast axonal transport to occur. Perhaps, as a component of the "structured continuum" of Green (1968), the transaxonal actin network facilitates and supports transport of organelles along microtubules.

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