Differences in the Organization of Actin in the Growth Cones Compared with the Neurites of Cultured Neurons from Chick Embryos

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ABSTRACT Sensory neurons from chick embryos were cultured on substrata that support neurite growth, and were fixed and prepared for both cytochemical localization of actin and electron microscopic observation of actin filaments in whole-mounted specimens. Samples of cells were treated with the detergent Triton X-100 before, during, or after fixation with glutaraldehyde to determine the organization of actin in simpler preparations of extracted cytoskeletons. Antibodies to actin and a fluorescent derivative of phallacidin bound strongly to the leading margins of growth cones, but in neurites the binding of these markers for actin was very weak. This was true in all cases of Triton X-100 treatment, even when cells were extracted for 4 min before fixation. In whole-mounted cytoskeletons there were bundles and networks of 6–7-nm filaments in leading edges of growth cones but very few 6–7-nm filaments were present among the microtubules and neurofilaments in the cytoskeletons of neurites. These filaments, which are prominent in growth cones, were identified as actin because they were stabilized against detergent extraction by the presence of phallacidin or the heavy meromyosin and S1 fragments of myosin. In addition, heavy meromyosin and S1 decorated these filaments as expected for binding to F-actin. Microtubules extended into growth cone margins and terminated within the network of actin filaments and bundles. Interactions between microtubule ends and these actin filaments may account for the frequently observed alignment of microtubules with filopodia at the growth cone margins.

Our knowledge of cytoplasmic filaments and their functions has been advanced by recent preparations of whole, unsectioned cells or thick sections of cells (14, 39, 53). In these studies, it has been illuminating to examine in three dimensions the interactions among filaments and their associations with other cell structures. In addition, detergents have been applied to cells with the effect of producing simplified cytoskeletons that retain many structural interactions of filaments and also allow access of antibodies and other chemical markers to the cytoskeletal fibers (3, 8, 15, 17, 18, 28, 32, 36, 41, 45, 48). Such studies have revealed new details about the roles of these filaments in cell movements and the determination of cell shape.

In this study similar methods were used to probe the distribution of actin filaments in growing nerve fibers of neurons cultured from chick embryos. This information may help clarify two prominent cell movements during nerve fiber growth: protrusion of filopodia from the nerve tip, and the movement of particulate organelles within neurites. Filamentous material with the approximate diameter of actin filaments is present in thin sections of filopodia at the leading edges of growth cones, in the cortex of neurites, and centrally within neurites, interconnecting microtubules, neurofilaments, and vesicles (9, 54). Actin has been identified in neurons and neuroblastoma cells by immunofluorescence and by visualization of heavy meromyosin (HMM) binding (11, 18, 19, 20, 22, 23, 31, 34, 44, 46). However, the disruptive effects of these various procedures on cell morphology have interfered with precise correlations of identified actin with the filamentous material seen in thin sections of embedded neurites. In addition, these reports do not agree on the distribution of actin in growing neurites and the interactions of actin filaments with microtubules.

Abbreviations used in this paper: HMM, heavy meromyosin; NBD, nitrobenzoxadiazol; PHEM buffer, 60 mM PEPES, 25 mM HEPES, 10 mM EDTA, and 2 mM MgCl₂, pH 6.9; TX-100, Triton X-100.
We have attempted to examine actin in growing neurites while preserving overall cytoskeletal organization with a combination of short detergent extractions and buffers that stabilize microtubules, neurofilaments, and actin filaments. Detergent-resistant actin filaments formed an extensive network and filament bundles in growth cone margins, but actin filaments were uncommon in cytoskeletons of neurites. This regional difference in distribution of actin filaments may be related to differences in the organization and activity of actin in growth cones vs. neurites. We also observed associations among actin filaments and microtubules in growth cones that may link mechanical forces generated at the leading edge to the structural components of neurites.

MATERIALS AND METHODS

Nerve Cell Culture: Dorsal root ganglia were removed from 9–11-d-old chick embryos and dissociated with trypsin (29). The resultant cell suspension was placed into a medium of 40% fresh Ham's F12, 10% newborn calf serum (Gibco Laboratories, Inc., Grand Island, NY), 50% heart conditioned medium (31), and 10 ng/ml nerve growth factor (a gift from Dr. Eric Shooter, Stanford University). 6–16 x 10⁶ cells in 1 ml of medium were plated in each 35-mm petri dish (1008, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA), containing either 75-mesh gold electron microscopic grids (Ted Pella, Inc., Tustin, CA) supporting a polyornithine-treated, carbon-coated 0.5% Pella, Inc., Tustin, CA) supporting a polyornithine-treated, carbon-coated 0.5% or 1.0% carbon film (30), or an 18-mm polyornithine-treated glass coverslip. The dishes were incubated at 37°C in a humidified 5% CO₂ incubator.

Extraction and Fixation of Cells: A buffer made by Schliwa and van Blerkom (41) to stabilize cytoskeletons was used, containing 60 mM PIPES, 100 mM NaCl, 100 mM KCl, 2.5 mM MgCl₂, 25 mM HEPES, 10 mM EGTA, and 2 mM MgCl₂, pH 6.9 (PHEM buffer). A buffer made by Schliwa and van Blerkom (41) to stabilize cytoskeletons was used, containing 60 mM PIPES, 100 mM NaCl, 100 mM KCl, 2.5 mM MgCl₂, 25 mM HEPES, 10 mM EGTA, and 2 mM MgCl₂, pH 6.9 (PHEM buffer). A culture dish was rinsed with PHEM buffer, and then the cells were either fixed and extracted simultaneously with PHEM buffer containing 0.2% Triton X-100 (TX-100) and 2% glutaraldehyde for 30 min, or extracted with PHEM buffer containing 0.2% TX-100 for 4 min, and then fixed in PHEM buffer with 2% glutaraldehyde for 30 min. The former treatment seemed to produce a limited extraction before the fixative became effective. In some cases, glutaraldehyde fixation was followed by 30-min incubation with 4% tannic acid in water for electron microscopy of cytoskeletons (2).

Phallacidin Treatment: Nitrobenzoaxadiazol (NBD)-phallacidin (Molecular Probes, Inc.) was added to PHEM buffer at 4 x 10⁻⁵ M, and neurons were extracted for 4 min with this solution containing 0.2% TX-100. Fixation with glutaraldehyde and staining with tannic acid followed.

Decoration with HMM: HMM (Sigma Chemical Co., St. Louis, MO) or the SI fragment of HMM (a gift from Dr. David Thomas, University of Minnesota) was added at 1 mg/ml to PHEM buffer containing 20% glycerol and 0.2% TX-100. Cell cultures were rinsed, extracted with this HMM solution for 4 min, rinsed twice, and fixed with 2% glutaraldehyde in PHEM buffer. Tannic acid staining followed for electron microscopic studies.

RESULTS

The cultured neurons were examined by several types of light and electron microscopy to better grasp the relationship of actin organization to overall nerve fiber morphology.

Immunofluorescence Localization of Actin

In neurons extracted with TX-100 after fixation (Figs. 1 and 2), the leading edges of growth cones were brightly fluorescent. In contrast, neurites treated this way were not noticeably brighter than the control neurites that were incubated with antibody preabsorbed with actin. This difference in staining of growth cones vs. neurites might reflect a differ-

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**Figures 1–15**

Figs. 1 and 2: Immunocytochemical localization of actin in neurites treated with TX-100 after fixation. Fig. 2 is a control neurite incubated with antibody preabsorbed with actin. Growth cone of experimental neurite (T) fluoresces brightly, while the control growth cone (t) is not stained. Neurite (N) of experimental neuron is not significantly brighter than control neurite (n). × 800. Figs. 3 and 4: Localization of actin in neurites treated simultaneously with TX-100 glutaraldehyde. Fig. 4 is a neurite of a cell incubated with antibodies preabsorbed with actin. Margins of growth cones (T) of experimental neurite are rich in actin, while the control growth cone (t) is not stained. Neurite (N) of experimental neuron is not brighter than control neurite (n). × 1,150. Figs. 5 and 6: Localization of actin in neurite treated with TX-100 before fixation. Fig. 6 is control neurite incubated with antibody preabsorbed with actin. Bright fluorescence remains at margin (arrows) of extracted growth cone (T), while control growth cone (t) does not stain. Neurite (N) of experimental neuron is very dim. Bright spot (asterisk) is artifact. × 1,250. Fig. 7: NBD-phallacidin staining of neurite treated with TX-100 after fixation. Growth cone margins (arrows) stain brighter than neurite (N). × 725. Fig. 8: NBD-phallacidin staining of neurite treated simultaneously with TX-100 and glutaraldehyde. Growth cone margins (arrow) are brighter than neurite (N). × 725. Fig. 9: NBD-phallacidin staining of neurite treated with TX-100 before fixation. Growth cone (arrow) of a preextracted neuron is stained, but neurite (N) is very dim. × 725. Fig. 10: Portion of a growth cone of a neuron extracted with TX-100 after fixation. Mitochondria (m) and microtubules (T) enter from distal portion of neurite (N) but growth cone margin contains a network of filamentous material (f). × 9,000. Fig. 11: Enlargement of area A in Fig. 10. A symmetric order of filamentous matrix is visible along concave margins (c) and in filopodia (f). Microtubules (m) run along bottom of print. × 25,000. Fig. 12: Filamentous matrix in growth cone margin of a neuron treated with TX-100 after fixation. Variable thickness of matrix segments (arrowheads) is apparent, although more uniform segments of 7 nm diam (arrows) are also present. × 69,000. Fig. 13: Portion of a neurite treated with TX-100 after fixation. Longitudinally oriented microtubules (m) are enmeshed in a filamentous matrix. 10-nm neurofilaments are not easily resolved. Uniform 7-nm thin segments (arrowheads) are apparent in cortex of neurite. × 42,000. Fig. 14: Growth cone of a neurite treated simultaneously with TX-100 and glutaraldehyde. Microtubules and mitochondria are present in dense material at base of growth cone (T). × 3,400. Fig. 15: Enlarged portion of growth cone margin outlined in Fig. 14. Network of 7-nm filaments (arrowheads) replaces the more variable matrix seen in cells extracted after fixation (Figs. 11 and 12). Bundles of 7-nm filaments (B) pass into filopodia. × 42,000.
ence in actin concentration, but might also be due to regional differences in permeability or accessibility of the antibody to actin. The relatively brighter immunofluorescence of growth cones was also present in neurons simultaneously extracted and fixed (Figs. 3 and 4) and in neurons extracted before fixation (Figs. 5 and 6). Neurites were again dim in these more extensively extracted cells, suggesting that accessibility to actin was not a reason for the low fluorescence of neurites. Rather, the data indicated that growth cones contained actin in a form that was stable to detergent extraction, while neurites lacked immunofluorescently detectable actin in a detergent stable form.

**Binding of Fluorescent Phallacidin**

Another marker for actin is phallacidin, a small peptide that has been used to localize F-actin in other cell types (1, 50, 51). Because phallacidin is much smaller than immunoglobulins, it is unlikely that accessibility to actin is a factor in regional differences in staining with phallacidin. Dorsal root ganglia neurons were extracted and fixed as described above, and then incubated with the fluorescent derivative, NBD-phallacidin. The results were identical to the distribution of antiactin (Figs. 7–9); much phallacidin binded in growth cones, even when cells were extracted before fixation, and the fluorescence of neurites was low, close to the background level of autofluorescence. Thus, binding of phallacidin also suggested that a detergent-stable form of actin was present at high levels in growth cones, but was not detectable in neurites by this method.

**Electron Microscopy**

Neurons, cultured on formvar films over gold grids, were treated with TX-100 after, during, or before fixation. The grids were examined to determine which structural features are characteristic of each extraction regimen and to identify the ultrastructural components responsible for the great difference in binding of antiactin and phallacidin to growth cones vs. neurites.

**Fixation before Extraction**

Neurites that were extracted with TX-100 after glutaraldehyde fixation and not postfixed with osmium tetroxide lacked electron-dense membrane boundaries. The cytoplasmic spaces of growth cones and neurites were defined by the limits of a matrix or lattice of irregular filamentous segments, within which microtubules and mitochondria were the major identifiable organelles (Figs. 10–13). This matrix resembles the microtrabecular lattice described in unextracted neurons and other cells by Porter and co-workers (14, 53). The segments of the matrix were irregular in length and width, varying to 10 nm or more wide (Figs. 12, 13). However, among the segments we saw thin, smooth filaments 6–7 nm wide, the size of actin filaments.

This matrix was the only distinguishable feature in the actin-rich growth cone margin (Figs. 10–12). In filopodia, at borders of lamellipodia and along concave borders of the growth cone margin, the filaments of the matrix were densely packed and showed a parallel orientation (Fig. 11). These were areas of high concentration of actin. The matrix also filled neurites, penetrating from the cortex inward among the longitudinal microtubules (Fig. 13). The 10-nm neurofilaments were not readily distinguished from the irregular segments of the matrix. Some thin, 6–7-nm filaments were seen in the neurite cortex.

**Simultaneous Extraction and Fixation**

Dorsal root ganglia cells were simultaneously fixed and extracted by adding TX-100 and glutaraldehyde to the same buffer. Despite having an open, obviously extracted, appearance, growth cones treated this way retained their overall morphology, including filopodia (Fig. 14). Instead of an irregular matrix (Fig. 12), the growth cone margin contained a network of longer, more regular 6–7-nm filaments (Fig. 15). These filaments were recognized more clearly as filaments than the irregular lattice in cells extracted after fixation (cf. Figs. 12 and 15). These filaments were grouped into bundles that passed down the length of filopodia (Fig. 15), and a filament network was present throughout the leading edge of growth cones. These findings reproduced the cytochemical localizations of actin.

Microtubules and neurofilaments were readily identifiable in the bases of growth cones and in neurites (Fig. 16). Very few 6–7-nm filaments were present in neurites that were simultaneously fixed and extracted. There was no regular
spacings or orientation of the 6–7-nm filaments in these neurite cytoskeletons, though they often were roughly perpendicular to the longitudinally oriented microtubules and neurofilaments.

**Extraction before Fixation**

The effects on living neurites of treatment with 0.2% TX-100 in PHEM buffer were monitored by light microscopy. Within seconds of adding detergent, the leading edges of growth cones, including all filopodia, disappeared, and the neurites were reduced to a core structure that was stable for many minutes of further incubation (Figs. 20 and 21).

As viewed by electron microscopy, the growth cone margins closely resembled neurites that were simultaneously fixed and extracted. This retention of filopodia resulted from stabilization of 7-nm filaments in bundles (F) at the edge of a neurite treated with TX-100 and I mg/ml HMM before fixation. Decorated filaments may be retained where filopodia occur at the edge of a neurite, but decorated filaments are absent from adjacent central regions of neurites, x 40,000. Fig. 32: Thickened, decorated filaments of 7 nm diam are noted by arrowheads. Some thicker filaments and linear segments (S) may contain aggregates of the HMM and S1 fragments before fixation. Increased thickness and coiled appearance of single filaments (arrowheads) are obvious, (cf. Fig. 28), but polarity of S1 decoration is not easily resolved. Thick segments (S) are bundles of decorated filaments that pass into filopodia. x 70,000. Fig. 29: Neurite of a cell treated with TX-100 and phallacidin before fixation. Microtubules (m) and neurofilaments (n) are visible, but the presence of phallacidin has not produced an increase in actin filaments in the neurite. x 70,000. Fig. 30: Network of decorated actin filaments in leading edge of a growth cone treated with TX-100 and phallacidin before fixation. Microtubules (m) and neurofilaments (n) are present, but decorated filaments are not seen in central areas of neurites. x 40,000. Fig. 32: Thickened, decorated filaments in bundles (f) at the edge of a neurite treated with TX-100 and 1 mg/ml HMM before fixation. Decorated filaments may be retained where filopodia occur at the edge of a neurite, but decorated filaments are absent from adjacent central regions of the neurite, as in Fig. 31. x 40,000. Fig. 33: Association of a microtubule (m) with microfilament bundle (b) in section from the bottom of a growth cone. Alignment of the microtubule and smooth-membraned saccules (S) with the filopodium (f) is obvious, but interaction of microfilaments with the microtubule is not clear. x 41,000.
Fig. 35 shows a growth cone extracted in the presence of phallacidin. Several microtubules projected into the leading margin, and each microtubule end may be associated with several actin filaments. Complex associations among actin filaments could be traced across the gaps between the microtubule ends and actin filament bundles in the leading margin (Figs. 35-38). We cannot resolve how the actin filaments interacted with these microtubules. Some filaments crossed over the microtubules and may have collapsed onto them during extraction, while other filaments did seem to terminate on individual microtubules.

Fig. 39 shows the margin of a growth cone extracted before fixation in the presence of the S1 fragment of myosin. Actin filaments can be recognized by S1 decoration, and several microtubules terminated near the leading margin. Actin filaments were seen associated with the microtubules, but the polarity of S1 binding to these actin filaments could not be determined (Figs. 40-42). However, some of the actin filaments that interacted with the microtubule ends also could be seen associated with actin filament bundles projecting inward from filopodia (Fig. 39). As in the phallacidin-treated preparations, some filaments crossed over microtubules, but others may terminate on microtubules. The bulbous ends of microtubules in Figs. 40-42 were a common feature of microtubules in whole-mounted growth cones. They occurred in growth cones extracted without S1 (Figs. 17 and 18), and in growth cones fixed and extracted simultaneously. Bulbous ends were not always present, and they may be more difficult to see if present in unextracted specimens or in thin sections.

**DISCUSSION**

There was a major difference in the presence of actin filaments between growth cones and neurites in detergent-treated cytoskeletons of chick sensory neurons. This difference was established cytochemically with the light microscope and also by electron microscopic demonstrations of actin filaments. Before we relate these data to previous studies of cytoskeletal fibers, we will discuss (a) what detergent treatment does to cells and (b) how actin may be affected by these procedures.

Detergents are used to make cells permeable to agents that otherwise do not enter cells, and to separate or remove cell contents for morphological and biochemical study. There are several important cautions about extraction procedures.

**Extraction before Fixation**

The destruction of membrane barriers by TX-100 results in rapid loss of 80% of cell proteins from fibroblasts, but the remaining 20% is stable to further removal (8). However, the cytoskeleton may be altered by loss of accessory molecules, by binding of extraneous molecules, or by distortions during the extraction period. For example, microtubules remain straight in cytoskeletons of neurites, as they are in thin sections, but neurofilaments are more disordered in cytoskeletons than they are in thin sections. The effects of phallacidin and myosin fragments show that the buffer can alter preservation of cytoskeletal fibers. Because of these cautions, we have examined actin distribution with three extraction methods, thin sections of unextracted cells, and photography of the extraction of living neurites.

**Fixation before or during Extraction**

It is not surprising that fixation with glutaraldehyde should reduce cell extraction. The extraction of a structure in the presence of glutaraldehyde depends on the chemistry of fixation, time, and temperature and concentration of fixative, and on the protein composition, concentration, and size of the structure. Simultaneous exposure to TX-100 and glutaraldehyde must involve competition between protein cross-linking and dispersal of components following the removal of membrane barriers.

The most pronounced difference in cytoskeletal morphology of neurons fixed before vs. during extraction was that the irregular cytoplasmic lattice seen when fixation preceded extraction was absent from neurites and growth cones that were simultaneously fixed and extracted (compare Figs. 10-13 with 14-16). The nature of the lattice is unknown. The lattice material may have been naturally or artifically deposited on the network of actin filaments in growth cones (Figs. 11, 12, and 13), but there was no actin network that could serve as scaffold for the lattice that is seen in neurites (Figs. 13 and 16). A similar absence of a lattice was found in detergent-extracted fibroblasts by Heuser and Kirschner (17).

**Extraction of Actin**

It is unclear whether all actin is preserved by these procedures. In nonmuscle cells, actin exists in several states (G-actin, oligomers of G-actin, and actin filaments), and each form may be fixed and extracted differently (5, 6, 47). G-actin is not cross-linked by the same concentration of glutaraldehyde that forms very high molecular weight species from F-actin (27). Thus, G-actin or small polymers may have been lost from our neurons, even when fixation preceded extraction. When extraction preceded fixation, some F-actin was lost from the growth cone margins (compare Figs. 14 and 17), but this F-actin and filopodia were preserved by including HMM, S1, or phallacidin in the extraction buffer. Because these agents can also promote actin polymerization (12, 50, 51), F-actin might have been polymerized in growth cones, but not in neurites, during extraction. However, this poly-
merization would have to occur as G-actin and other soluble components disperse rapidly in the extraction buffer. Whether a form of F-actin is stable during extraction may depend on filament length, the presence of accessory proteins, and filament associations with vesicles, microtubules, or the plasma membrane. For example, short actin filaments attached to the plasma membrane may be dispersed as TX-100 extracts membrane components.

Thus, the actin detected here was not necessarily all cellular actin, and we limit our conclusions to stating that growth cones contained a filamentous actin that could be stabilized by actin-binding agents and that was present in zones of cell surface motility. This form of actin was uncommon in neurites, except at points of lateral filopodia.

Relationship to Previous Studies of Actin in Growing Neurites

Actin has previously been reported in growth cones and neurites by immunofluorescence (19, 20, 23, 31, 34, 46), and some studies reported that actin is especially concentrated in growth cones (19, 31, 34, 46). We believe that the effects of fixatives and permeabilization on the retention of actin are responsible for many of the differences among reports of actin localization by immunofluorescence. For example, formaldehyde is inferior to glutaraldehyde in preserving growth cone morphology for immunofluorescent localization of actin (31). In addition, Spooner and Holladay (46) have shown that high dilutions of antibody are critical to demonstrating the relative abundance of actin in growth cone margins.

Electron Microscopy of Growth Cones: A network or lattice of microfilaments in growth cone margins has often been described, but the presence of microfilament bundles in filopodia and similar areas has not always been demonstrated (9, 11, 23, 26, 30, 37, 54). This discrepancy in reporting filament bundles is probably due to cautious interpretations of filamentous structures that were altered during preparation (17, 27, 35, 37, 45). Although morphological studies of the binding of S1 or HMM identify the majority of filaments in the network and bundles as actin, the relationship between the network and the bundles is not clear. Actin filaments splay out at the bases of bundles and merge with the overlapping filaments of the network.

Actin in Cultured Neurites: Yamada et al. (54) suggested that the lattice of microfilaments extends from the growth cone into the neurite and interconnects microtubules, neurofilaments, and vesicles in a continuous network. However, these projections from microtubules and neurofilaments might contain a number of proteins (10, 13, 33, 38, 40) other than actin. And, as we found, HMM-decorated filaments in the neurite-like processes of neuroblastoma cells are predominantly at the cell periphery and in lateral filopodia, with few present centrally in neurites (11, 18).

This finding that few actin filaments are present in growing neurites can be compared with that for mature, non-growing axons. Biochemical and morphological evidence suggests that the majority of subunits of microtubules and neurofilaments are polymerized into stable structures in axons (4, 21, 24, 25, 36). Although actin, too, is a major component of axons (4, 24, 52), the distribution and organization of actin is controversial. Ellisman and Porter (14) propose that a microtrabecular lattice exists throughout the axon and contains actin filaments. However, in two studies of rapidly frozen axons (16, 42), actin filaments were identified only in the subplasmalemmal cortex, while in the regions containing neurofilaments and microtubules there is a granular and filamentous matrix that may have been preserved as microtrabeculae by Ellisman and Porter. Thus, readily identifiable actin filaments may be concentrated in cortical regions in both embryonic neurites and mature axons, but the relationship of these actin filaments to all axonal actin is unclear. Short filaments or polymers of actin may be associated closely with microtubules and neurofilaments. These may be difficult to demonstrate either morphologically or with low concentrations of antibodies (46). Thus, one should not assume that there are no actin filaments in the neuritic core, but that there may be a few that are short or difficult to see.

The actin organization in growth cones resembles that of the neurite periphery, though protrusion is suppressed along neurites. Yet, when a neurite is cut, active growth cones form at the cut ends (7, 43, 49). Transection may remove inhibiting factors or concentrate supportive factors that permit polymerization of long actin filaments and sustained protrusive activity. An obvious similarity between a growth cone and a freshly cut neurite is the presence of many ends of microtubules and neurofilaments. Perhaps complex interactions among the monomeric and polymerized forms of all three types of cytoskeletal fibers regulate the expression of protrusive activity.

Microtubules in Growth Cones and Interactions with Actin Filaments

Previous reports of electron microscopic work have stated that microtubules are present centrally or at the base of the growth cone, but they have rarely reported that microtubules enter growth cone margins or approach filopodia (9, 18, 23, 54). Two studies demonstrate immunofluorescence for tubulin in the margins of growth cones (44, 46), though electron microscopy was not done. The use of whole-mounted cells has permitted what might be stained with immunofluorescence to be compared with ultrastructure. This is especially clear in detergent-extracted neurons, where microtubules are easily traced into the extracted peripheral margins of growth cones (Fig. 17). Our findings agree with immunofluorescence studies indicating that microtubules penetrate far forward into growth cone margins. Because microtubules may depolymerize during fixation and extraction, the location of microtubule ends in a growth cone may be influenced by the extraction of tubulin.

Cytoskeletons were examined for associations that mediate the alignment of actin bundles and nearby microtubules, but the resolution was poor. Osmium tetroxide, which disrupts actin, was not used, but distortions may have also been introduced by extraction, fixation, dehydration, and critical-point drying (17, 17, 35, 37, 45). Rapid freezing and drying might reduce distortions (37), but new problems arise, because growth cones are exquisitely sensitive to temperature change or mechanical displacement and shrink within seconds while being mounted on the freezing block. Our observations do suggest that actin filaments may terminate on microtubules at a point of contact (Figs. 40-42), or that actin filaments might contact microtubules elsewhere along the filaments' length. The organization of myosin and actin filaments in a sarcomere illustrates that cytoskeletal fibers can interact laterally without end-on contact.

In summary, a detergent-stable, filamentous actin is concentrated in the growth cone margin, but is infrequent in
neurites. This actin is probably involved in motility of the growth cone margin, but use of this form of actin is highly regulated and suppressed in neurites. The small amount of this form of actin in neurites indicates that axonal transport may use another source of mechanical energy. Besides involvement in cell surface motility, this form of actin may interact with microtubules in the growth cone margin, and participate in the transmission of mechanical forces that determine the positions of structural components in the growth cone.

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