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## The Neuronal Centrosome as a Generator of Microtubules for the Axon

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- I. Introduction
- II. Evidence of a Centrosomal Origin for Axonal Microtubules
  - A. Axonal Microtubules Do Not Originate within the Axon
  - B. Nucleation and Release of Microtubules from the Neuronal Centrosome
  - C. Inhibition of Microtubule Nucleation at the Centrosome Compromises Axon Growth
  - D. Centrosomal Microtubules Are Transported into the Axon
- III. Implications of a Centrosomal Origin for Axonal Microtubules
  - A. Microtubule Nucleation and Release from the Centrosome of Neurons and Nonneuronal Cells
  - B. Microtubule Translocation from the Centrosome
  - C. Regulation of Microtubule Nucleation and Release from the Neuronal Centrosome
  - D. Mechanisms for Elaborating the Axonal Microtubule Array
- IV. Concluding Remarks
- References

### I. Introduction

Neurons are remarkable cells, in terms of both their morphology and their cytoplasmic organization. The nucleus and protein synthetic machinery are housed within a small rounded cell body, but most of the cytoplasm of the neuron is extended over long distances in the form of an axon (Lasek and Brady, 1981). As a result of this compartmentation, neurons require efficient and sophisticated machinery to transport proteins manufactured within the cell body into and down the axon. Cytoplasmic organelles, membranous elements, and endocytosed materials are also actively transported within the axon, and their transport may be either in an anterograde or retrograde direction. In addition to its dependence on transport machinery, the axon also requires mechanisms that permit the generation and maintenance of a highly anisotropic morphology. These exaggerated transport and architectural needs are fulfilled by specialized cytoskeletal arrays within the axon. In particular, microtubules are prominent components of the cytoskeleton that provide structural support for the axon and also direct the transport of proteins and organelles through its cytoplasm. Microtubules are

polymers of repeating tubulin subunits, and the nucleation, assembly, and organization of these polymers are tightly regulated within living cells. Because of the exaggerated functions and fundamental importance of microtubules in the axon, there is great interest in elucidating the mechanisms by which the axonal microtubule array is generated and organized. These mechanisms are complex and involve the coordinated efforts of several types of microtubule behavior (for recent reviews see Joshi and Baas, 1993; Black, 1994).

In typical nonneuronal cells, organized microtubule arrays are generated by discrete microtubule-nucleating structures termed "centrosomes" (for review see Brinkley, 1985). *De novo* initiation of microtubules is suppressed and new microtubules arise via nucleation from these structures. The centrosome is made up of two barrel-shaped centrioles and a cloud of pericentriolar material that surrounds them. Microtubules are nucleated from the pericentriolar material and form a radial array emanating away from the centrosome. Nucleation from the centrosome regulates key features of the microtubules within the array. First, all of the microtubules assemble with their plus ends away from the centrosome, resulting in a microtubule array of uniform polarity orientation (Euteneuer and McIntosh, 1981). Second, there is a fixed number of initiation sites within the pericentriolar material and this regulates the number of microtubules within the array (Brinkley *et al.*, 1981). Third, the nature of the initiation sites constrains the lattice structure of each microtubule to 13 protofilaments (Evans *et al.*, 1985). Thus, by limiting microtubule nucleation to the centrosome, the cell tightly regulates the organization, number, and structure of its microtubules.

Microtubules in the axon are also tightly regulated with regard to these features. Axonal microtubules consist of 13 protofilaments (Tilney *et al.*, 1973), are uniformly plus-end-distal (Heidemann *et al.*, 1981, 1984; Burton and Paige, 1981; Baas *et al.*, 1988, 1991; Baas and Ahmad, 1993), and their number is coordinated with the needs of the growing axon (Yu and Baas, 1994). Given all of this, it is surprising that axonal microtubules are not attached to the centrosome or any comparable nucleating structure within the cell body of the neuron (Lyser, 1964, 1968; Sharp *et al.*, 1981). Instead, the microtubules are "free" at both ends, stopping and starting all along the length of the axon (Bray and Bunge, 1981; Tsukita and Ishikawa, 1981; Yu and Baas, 1994). This apparent paradox has led to a great deal of confusion concerning the origins of axonal microtubules and how features of their structure and organization are regulated. Over the past several years, many workers in the field have focused on "noncentrosomal" mechanisms that might account for certain features of the axonal microtubule arrays. For example, significant efforts have focused on the dynamics of microtubules within the axon itself, and in particular at its distal tip (Bamburg *et al.*, 1986; Robson and Burgoyne, 1988). However, the local dynamics of microtubules is an issue separate from their origins, and it is difficult to imagine how the same mechanisms that regulate the elongation and shortening of microtubules could also regulate their polarity orientation, number, or lattice structure.

One of the earliest ideas concerning axonal microtubules was that they do, in fact, have a centrosomal origin. As early as 1965 before “spindle tubules” and “neurotubules” were both identified as “microtubules,” Gonatas and Robbins (1965) examined the lattice structure of neurotubules in the chick embryo retina, found it to be indistinguishable from that of spindle tubules, and concluded that “neurotubules probably arise from the centrioles.” Similarly, in ultrastructural studies on rabbit embryo dorsal root ganglion neuroblasts, Tennyson (1965) concluded that neurotubules “probably originate from the centriole. . .” and “migrate into the neurite.” Despite these observations, little attention was given over almost the next three decades to the idea of a centrosomal origin for axonal microtubules, probably due to the lack of experimental evidence supporting this hypothesis.

Over the past few years, a principal goal of my laboratory has been to determine the origins of axonal microtubules. Our studies have led us back to the centrosome, which we now believe acts as a kind of “generator” of microtubules for relocation into the axon. Specifically, we have come to the conclusion that microtubules destined for the axon are nucleated at the centrosome, released from this structure, and are then actively transported into the axon. The purpose of this article is to review the observations that have led us to this conclusion, and to explore the potential implications of a centrosomal origin for axonal microtubules.

## **II. Evidence of a Centrosomal Origin for Axonal Microtubules**

### **A. Axonal Microtubules Do Not Originate within the Axon**

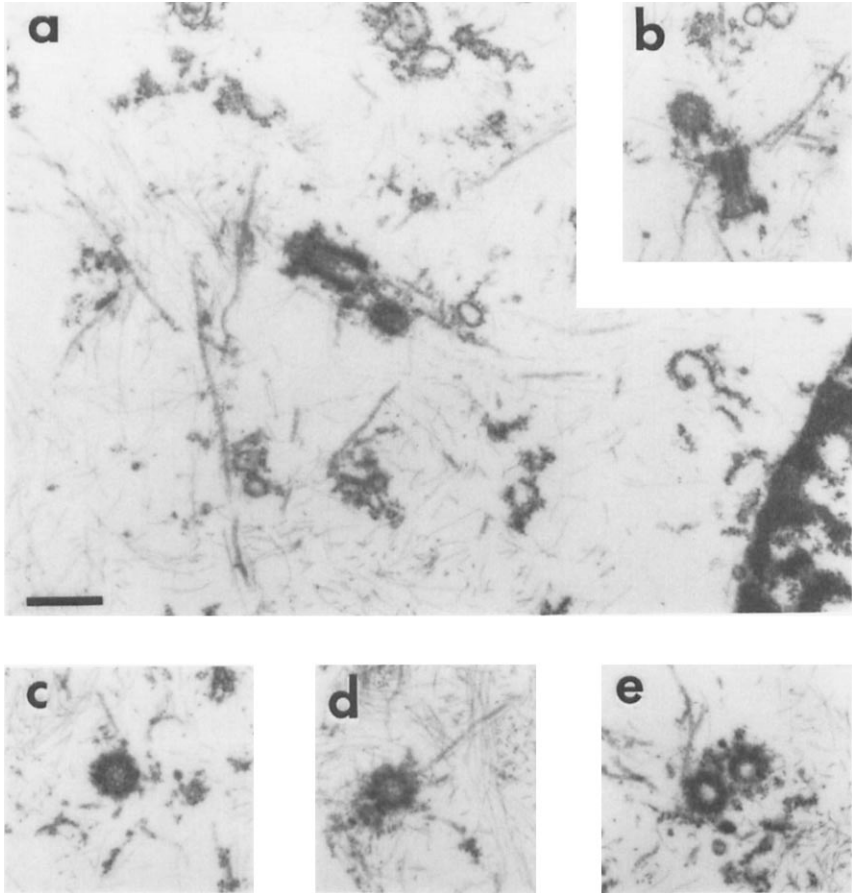
The classic method for identifying microtubule-nucleating structures within cells is to depolymerize existing microtubules with nocodazole, a potent but reversible microtubule depolymerizing agent, and then remove the drug so that microtubules can reassemble from their sites of origin. This method, first used to identify the centrosome as a microtubule-nucleating structure in nonneuronal cells (DeBrabander *et al.*, 1977, 1980), was used in my laboratory to identify potential microtubule-nucleating structures within the axons of cultured sympathetic neurons (Baas and Ahmad, 1992). After drug removal, all-new microtubule polymer arose specifically from the plus ends of the short microtubule fragments that resisted depolymerization. No microtubules arose independently of these microtubule fragments, suggesting that the plus ends of preexisting microtubules are the exclusive sites of microtubule assembly in the axon. These findings are consistent with previous work on cultured sensory neurons demonstrating that when all microtubule polymer is pharmacologically depolymerized from isolated axons, no microtubules reassemble after removal of the drug (Baas and Heidemann, 1986). Together, these studies demonstrate that no entirely new

microtubules arise within the axon itself. Thus, we concluded, by the process of elimination, that new microtubules destined for the axon must be nucleated within the cell body.

To explore the issue further, we subsequently determined the distribution of gamma-tubulin within cultured sympathetic neurons (Baas and Joshi, 1992). Gamma-tubulin is a newly discovered member of the tubulin superfamily that is present within cells at very low levels compared to the levels of alpha- or beta-tubulin. Unlike alpha and beta-tubulin, gamma-tubulin is not a component of the microtubule itself, but is localized to the sites of microtubule nucleation within a cell and has been shown to be required for microtubule nucleation in all cell types examined (Oakley and Oakley, 1989; Oakley *et al.*, 1990; Zheng *et al.*, 1991; Stearns *et al.*, 1991; Horio *et al.*, 1991; Joshi *et al.*, 1992). Using both biochemical and immunoelectron microscopic assays, we demonstrated that there is no gamma-tubulin within the axon, which is consistent with our previous conclusion that no new microtubules are nucleated within the axon. In addition, we found no gamma-tubulin anywhere in the cell body except at the centrosome, suggesting that the centrosome is the sole site for the generation of new microtubules in the neuron. On the basis of these findings, we proposed that microtubules destined for the axon are nucleated at the centrosome within the cell body of the neuron, released from this structure, and then transported into the axon.

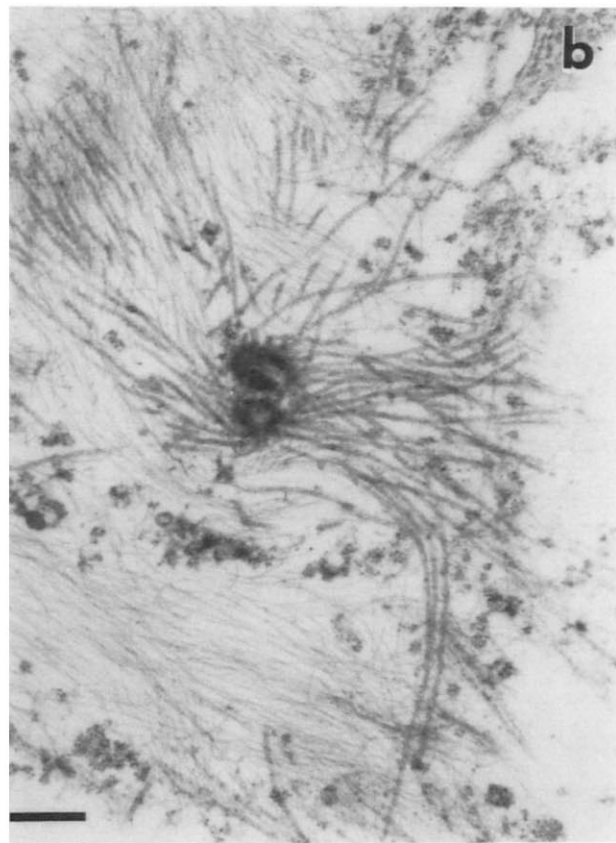
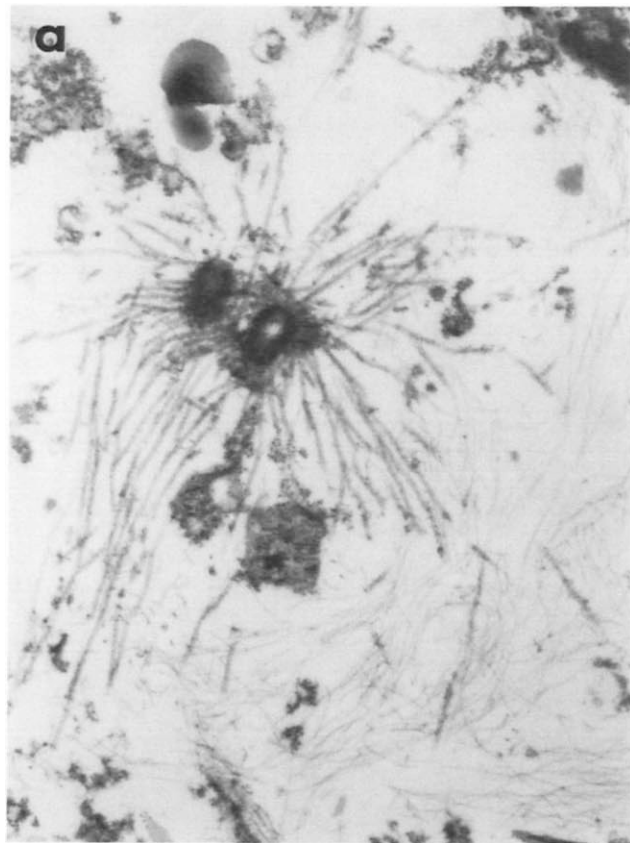
## **B. Nucleation and Release of Microtubules from the Neuronal Centrosome**

If our reasoning is correct, the neuronal centrosome must be an extremely potent microtubule-nucleating structure, capable of generating hundreds upon hundreds of microtubules for the growth and maintenance of elaborate axonal arbors. In addition, it is implicit in our proposal that the neuronal centrosome has the capacity to rapidly release the microtubules it nucleates, so that they can be exported into and down the length of the axon. Electron microscopic analyses of different kinds of neurons at different developmental stages vary with regard to the appearance of the centrosome, but most studies reveal relatively few microtubules directly attached to the centrosome. In our studies on cultured sympathetic neurons, generally fewer than 10 and often no microtubules were attached to the centrosome (Baas and Joshi, 1992; Yu *et al.*, 1993; see Fig. 1). These observations raised the possibility that axonal microtubules may not originate at the centrosome and that the neuronal centrosome may actually be relatively inactive. Alternatively, however, the nucleation and release of microtubules from the neuronal centrosome may be so rapid that there is insufficient time for substantial numbers of attached microtubules to accumulate at the centrosome before they are released. To address this issue, we tested the capacity of the neuronal centrosome to act as a microtubule-nucleating structure using the same drug-recovery regime used in our previous work on the axon (Yu *et al.*, 1993).



**Fig. 1** Electron micrographs of cultured rat sympathetic neurons in the region of the centrosome. (a) Centrosome (middle), a portion of the nucleus (lower right), and many unattached microtubules. (b) Different section through the same centrosome shown in a. A small number of microtubules can be seen attached to the centrosome in each section. (c–e) Centrosomes from three other control neurons, each with microtubules in the vicinity. (c) No attached microtubules. (d and e) One attached microtubule. Analyses of all sections through each centrosome reveal a total of fewer than 10 attached microtubules per centrosome. In all cases, centrosomes consisted of two centrioles, which were usually perpendicular to one another (a and b). In some cases, only one centriole of the pair appeared on an individual thin section (c and d). In other cases, as expected of a postmitotic cell, the two centrioles were off-perpendicular and even parallel to one another in a few rare instances (e). Adapted from Yu *et al.* (1993). Bar, 0.4  $\mu\text{m}$ .

The results of these studies were informative. Within a few minutes of drug removal, hundreds of microtubules reassembled in the region of the centrosome, and most of these microtubules were clearly attached to the centrosome (Fig. 2).



A portion of the microtubules was not attached to the centrosome, but was aligned side-by-side with the attached microtubules, suggesting that the unattached microtubules had been released from the centrosome after their nucleation. In addition, unattached microtubules were present in the cell body at decreasing levels with increasing distance from the centrosome. By 30 min the microtubule array was indistinguishable from that of control neurons, suggesting that the hundreds of microtubules nucleated from the centrosome after the first few minutes were subsequently released and translocated away from the centrosome. These results demonstrated that the neuronal centrosome is a highly potent microtubule-nucleating structure, and they provided strong indirect support for the idea that microtubules nucleated from the centrosome are released for translocation into other regions of the neuron.

### C. Inhibition of Microtubule Nucleation at the Centrosome Compromises Axon Growth

The studies described above indicated that the centrosome could in theory act as a generator of microtubules for redistribution into the axon, but they did not test whether centrosomal microtubules are essential for the initiation and growth of the axon. To address this issue, we next determined the effects on axon growth of experimentally disabling the neuronal centrosome (Ahmad *et al.*, 1994). Our strategy was to microinject into cultured sympathetic neurons the same antibody to gamma-tubulin previously shown to recognize the neuronal centrosome (Baas and Joshi, 1992) and previously shown to arrest microtubule nucleation at the centrosome when microinjected into nonneuronal cells (Joshi *et al.*, 1992). If centrosomally derived microtubules are required for the growth of the axon, we would expect inhibition of centrosome function to compromise or inhibit axon growth.

To assess the effects of centrosomal inhibition over a 2-hr time period (the time period over which the antibody is effective), it was also necessary to deplete the neuron experimentally of preexisting microtubules. These microtubules, presumably already nucleated and released from the centrosome, are assembly-competent and capable of supporting substantial levels of axon growth in the

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**Fig. 2** Electron micrographs of two different neurons treated for 6 hr with 10  $\mu\text{g/ml}$  nocodazole, rinsed free of the drug, and permitted to recover for 5 min. Microtubule reassembly from the centrosome is dramatic, with high levels of attached microtubules. Also apparent are other microtubules not directly attached to the centrosome. These microtubules are aligned with the attached microtubules as if they were once attached and then released from the centrosome. Analyses of every section through each centrosome were required to define and score attached and unattached microtubules. Adapted from Yu *et al.* (1993). Bar, 0.4  $\mu\text{m}$ .

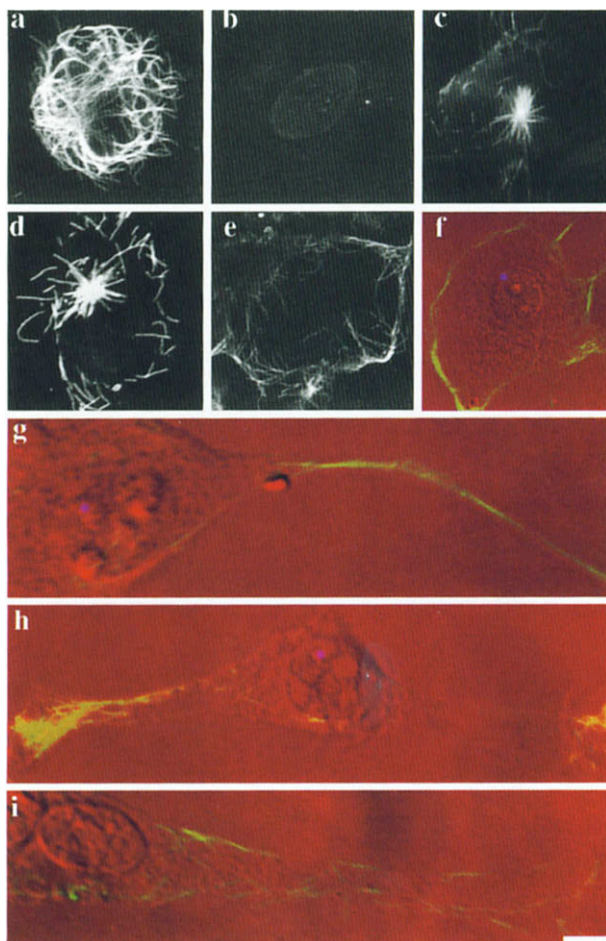
absence of further microtubule assembly (Baas and Ahmad, 1993). After depolymerizing microtubules with nocodazole, the antibody was microinjected into neurons, and then the drug was rinsed from the cultures. Reassembly of microtubules over the next 2 hr was severely diminished under these conditions, and axon growth was either compromised or completely abolished. These results indicated that a functional centrosome is important for axon growth.

#### **D. Centrosomal Microtubules Are Transported into the Axon**

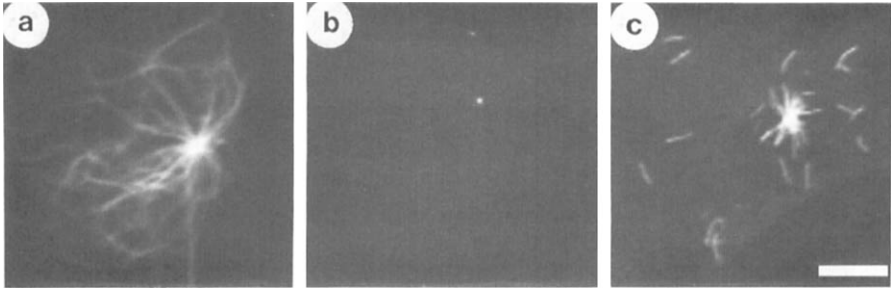
The studies described above documented that the neuronal centrosome is a potent microtubule-nucleating structure and that inhibition of its microtubule-nucleating capacity is detrimental to both microtubule assembly and axon growth. These studies strongly suggest that the microtubules nucleated at the centrosome are the same microtubules that are used to construct the axonal microtubule array. To test this more directly, we would like to be able to visualize within living cells the movement of individual microtubules from the centrosome into the axon. Achieving this goal will be a technical challenge. Even when microtubule assembly at the centrosome is synchronized using the nocodazole recovery regime, ongoing elongation of microtubules and nucleation of additional microtubules occurs so rapidly that it is impossible to follow their fate. To simplify matters, we have recently developed a kind of "pulse-chase" regime that permits the study of a small population of microtubules nucleated at the centrosome (Ahmad and Baas, 1995). While not permitting the direct observation of microtubule movements, this technique has provided strong indirect support for the transport of centrosomal microtubules into the axon.

After drug treatment to depolymerize microtubules, and a few minutes of microtubule reassembly at the centrosome, nanomolar levels of vinblastine were added to neuron cultures. Vinblastine is an anti-microtubule drug that when used at nanomolar levels suppresses further microtubule assembly while not substantially depolymerizing existing microtubules. Thus, any alterations in the microtubule array that occur after the addition of vinblastine must be the result of microtubule movements from one location in the cell to another. As expected, microtubule levels remained roughly the same after the addition of vinblastine, as did the lengths of individual microtubules (1–5  $\mu\text{m}$ ) over time. Within minutes, unattached microtubules began to appear in the cytoplasm, and by 10 min many of these had reached the periphery of the cell body. By 1 hr, few or no microtubules were attached to the centrosome and the vast majority of the microtubules were concentrated at the cell periphery. In the case of the neurons that were able to grow axons under these conditions, microtubules appeared progressively farther down the axons with increasing time. These results, shown in Fig. 3, demonstrate that microtubules derived from the centrosome are transported from cell center to cell periphery and then into and down the axon.

Also of interest were the fibroblastic nonneuronal cells in the same cultures.



**Figure 3.** Release and translocation of microtubules from the neuronal centrosome to the cell periphery and into developing axons. Freshly plated control rat sympathetic neurons showed a dense array of microtubules (a), while nocodazole-treated neurons showed few or none (b). Neurons recovered for 3 min showed a small population of microtubules emanating from the centrosome (not shown). After 3 min in vinblastine, most microtubules remained attached, but a small number of unattached microtubules were apparent (c). After 10 min, both attached and unattached microtubules were apparent, with some of the unattached microtubules appearing at the cell periphery (d). By 1 hr, microtubules were concentrated at the periphery of the cell body, and some could be seen funneling into developing axons (e and f). In f, superimposed on the immunofluorescence image of microtubules (in green) are the differential-interference-contrast image showing the cell's morphology (in orange) and the immunofluorescence image of the centrosome (in purple), which appears in a different optical section (see text). No microtubules were attached to the centrosome at 1 hr. (g-i) Three different neurons which grew axons during a 6-hr incubation in vinblastine. The neurons are shown in the same color format as used in f. In each case, a small number of microtubules remained in the cell body (none were attached to the centrosome), but most had translocated into and down the axon. (g) A cell with an axon showing a relatively even distribution of microtubules. (h) A cell with two axons, each showing a clustering of microtubules in the distal region of the axon. (i) An advantageous axon in which the ends of individual microtubules can be discerned. Adapted from Ahmad and Baas (1995). Bar, 5  $\mu$ m.



**Fig. 4** Release of microtubules from the centrosome of nonneuronal cells. Shown are nonneuronal fibroblastic cells from the same cultures (treated under the same experimental paradigm) as those containing the neurons shown in Fig. 3. (a) Control cell with a characteristic microtubule array emanating from the centrosome. (b) Cell treated with nocodazole for 6 hr, with no detectable microtubules remaining. (c) Cell treated with nocodazole for 6 hr, recovered for 3 min, and then exposed to vinblastine for 30 min. Most microtubules remained attached to the centrosome. Unattached microtubules were apparent, but numbered fewer than 20 and appeared dispersed in the cytoplasm, not concentrated at the cell periphery. Adapted from Ahmad and Baas (1995). Bar, 5  $\mu\text{m}$ .

These cells showed a typical centrosomal microtubule array before drug treatment. After recovery from nocodazole, the cells showed an array of short microtubules at the centrosome similar to that observed in neurons. Following the addition of vinblastine, unattached microtubules were observed at all time points. However, most microtubules remained attached. The unattached microtubules did not exceed 10–20 in number and did not concentrate at the cell periphery even after 1 hr. These results, shown in Fig. 4, indicated that microtubules are also released from the centrosomes of nonneuronal cells, but less actively than neurons.

### III. Implications of a Centrosomal Origin for Axonal Microtubules

#### A. Microtubule Nucleation and Release from the Centrosome of Neurons and Nonneuronal Cells

Prior to our work, a small number of studies had already documented evidence for microtubule release from the centrosome of nonneuronal cells (Kitanishi-Yumura and Fukui, 1987; McBeath and Fujiwara, 1990; Belmont *et al.*, 1990). The most compelling of these studies utilized live-cell techniques to directly visualize the release and movement of microtubules from centrosomes in cytoplasmic extracts (Belmont *et al.*, 1990). Our observations on the fibroblastic cells that contaminate sympathetic neuron cultures also demonstrate release of microtubules from the centrosome. However, direct comparison with the neurons in these cultures indicates that microtubule release and translocation from the cen-

trosome are far more active in neurons than in nonneuronal cells. Within 30 min of their nucleation from the centrosome, most microtubules were released in the case of the neurons, while most remained attached in the case of the nonneuronal cells. By 10 min, many of the microtubules had reached the periphery of the neuronal cell body, and by 60 min this was true of virtually all of the microtubules. By contrast, microtubule translocation was more sluggish in the nonneuronal cells. Another difference between the microtubules in neurons and nonneuronal cells, not directly studied in our work, relates to their stability, and this difference probably contributes to the fate of the microtubules once they are released. In nonneuronal cells, it is thought that the release of a microtubule is followed by its rapid depolymerization from both ends (Kitanishi-Yumura and Fukui, 1987). We suspect that our use of vinblastine as a kinetic stabilizer permitted us to visualize released microtubules that would otherwise have depolymerized in the nonneuronal cells. In contrast, neurons normally contain high levels of microtubule-stabilizing factors, such as tau, that may prevent centrosomal microtubules from depolymerizing after their release. On the basis of these observations, we conclude that neurons differ from nonneuronal cells with regard to the efficiency of microtubule release and translocation from the centrosome as well as the fate of the microtubules following their release.

These differences are consistent with the very different microtubule arrays generated and maintained by neurons versus nonneuronal cells. Typical nonneuronal cells are mitotic and motile, while neurons are terminally postmitotic and consist of a stationary cell body from which processes grow. In nonneuronal cells, the centrosome nucleates and organizes the microtubules of the mitotic spindle as well as the microtubules that extend to the leading edge of the interphase cell to guide its motility. In neurons, microtubules are utilized for a very different purpose, the growth of the axon. It seems reasonable that the more active release of microtubules from the neuronal centrosome may be related to the unique functional and morphological demands inherent in these differences. It is interesting in this regard that neurons differentiate from pluripotent precursor cells, such as neural crest cells, that are highly motile and mitotic. We suspect that major alterations in the behavior of centrosomal microtubules occur during neuronal commitment and differentiation. Studies are underway in our laboratory to investigate these changes.

## **B. Microtubule Translocation from the Centrosome**

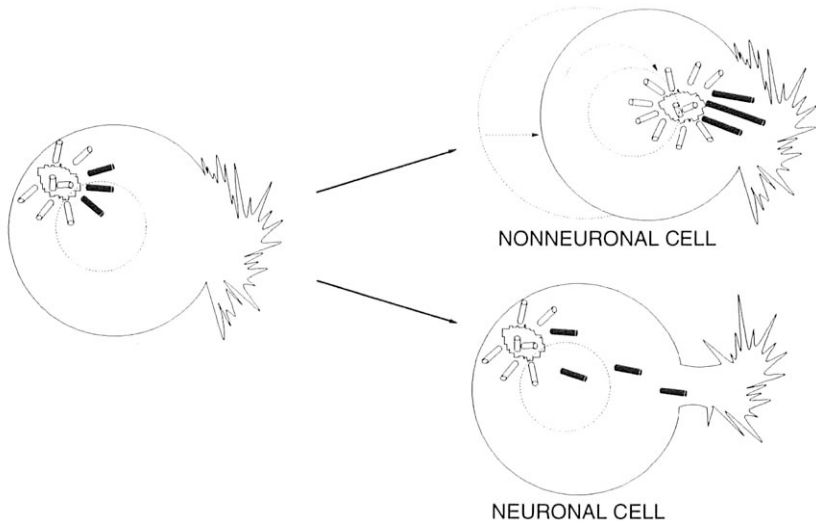
Worth additional consideration are the forces at play which act on the microtubules to transport them through the cytoplasm. In order for microtubules released from the centrosome to move away from it, there must be an active transport mechanism. In fact, such a mechanism would be consistent with a growing body of evidence concerning the capacity of molecular motors to create microtubule movements within living cells. In particular, and relevant to the

centrosome, it has now been established that motors such as kinesin and dynein are essential for the formation of the mitotic spindle as well as for microtubule movements that occur during mitosis (for review see Fuller and Wilson, 1992). Perhaps, in a postmitotic cell such as a neuron, similar mechanisms account for the movement of microtubules away from the centrosome after they are released. Our data and that of Belmont *et al.*, (1990), demonstrating that microtubules released from the centrosome are carried away from it, indicate that such an active transport mechanism exists, even in nonneuronal cells. In the neuron, these or similar mechanisms would also be necessary within the axon to continue the orderly transport of microtubules down its length. In other studies on axons elaborated in the presence of vinblastine, we have established that such transport exists and that it is the nature of the transport machinery that establishes the uniformly plus-end-distal orientation of microtubules in the axon (Baas and Ahmad, 1993).

The differences between neurons and nonneuronal cells are also manifest in the position of the centrosome, which is tightly regulated in nonneuronal cells but variable in the cell body of the neuron. Pertinent to this issue are studies on motile nonneuronal cells in which the position of the centrosome relocates to face the direction of cell movement (Kupfer *et al.*, 1982; Rogers *et al.*, 1992). Interestingly, the repositioning of the centrosome requires microtubules that extend to the cell periphery (Euteneuer and Schliwa, 1992), and, at least in one cell type, 3T3 fibroblasts, it has been documented that a subclass of stable microtubules attached to the centrosome are "captured" by the leading edge of the cell, after which the centrosome moves in that direction (Gunderson and Bulinski, 1988). Based on these observations, it seems likely that there is some kind of pulling force on the microtubules and that this force results in the movement of the attached centrosome. In the case of neurons, our data show that release of microtubules from the centrosome is far more active than in motile nonneuronal cells. One possibility is that there is nevertheless a "pull" on the microtubules, but that this force does not cause the centrosome to change location but instead carries the microtubules away from the centrosome toward the leading edge of the cell. As a result, the cell body remains stationary, the centrosome does not change location, and the freed microtubules are transported toward the leading edge of the cell. Microtubule-rich axons are then formed between the stationary cell body and the motile leading edge, which becomes a growth cone. These ideas, shown schematically in Fig. 5, will be tested in our studies on the transformation of motile precursor cells into bona fide neurons.

### **C. Regulation of Microtubule Nucleation and Release from the Neuronal Centrosome**

The centrosome has long been recognized as a hub at which the cell can regulate the organization of its cytoplasm. As noted above, there are undoubtedly pro-



**Fig. 5** Schematic showing how differences in microtubule behavior at the centrosome may contribute to phenotypic differences between neuronal and nonneuronal cells. The cell on the left represents a pleuripotent precursor cell that could give rise to either a motile nonneuronal cell or to a neuron. In the case of the nonneuronal cell, a portion of the microtubules nucleated by the centrosome are captured by the leading edge of the cell. The motility of the leading edge pulls on the microtubules, and the attached centrosome reacts by relocating in the direction of cell movement. The entire cell moves. In the case of the neuron, the microtubules are released, and the centrosome is not relocated. Nevertheless, the microtubules are translocated toward the leading edge, which coalesces into a growth cone. The cell body remains stationary and the microtubules translocate into the space between the cell body and the growth cone, which develops into the axon.

found alterations that occur with regard to the centrosome when a precursor cell commits to a neuronal fate. There are several critical stages of development that follow the initial commitment of the neuroblast, and there are changes in the organization of the cytoplasm as well as the organization, composition, and properties of the microtubule array that accompany each of these stages (see for example Baas *et al.*, 1989; Yu and Baas, 1994; Sharp *et al.*, 1995). It is compelling to contemplate that alterations in the activity of the centrosome may be a critical feature in regulating these cytoplasmic and cytoskeletal changes. For example, when the neuron first endeavors to grow an axon, there might be intense activity of the centrosome, after which activity slows during the steady growth of the axon. A second burst of activity may occur during the development of dendrites, which also apparently require centrosomal microtubules (Baas and Joshi, 1992; D. J. Sharp and P. W. Baas, unpublished observations). Activity may slow once again when axons and dendrites have stopped growing, but may increase, for example, during regeneration in response to injury. In this manner,

the centrosome may act as a kind of "time clock," with alterations in its activity corresponding to important milestones in neuronal development.

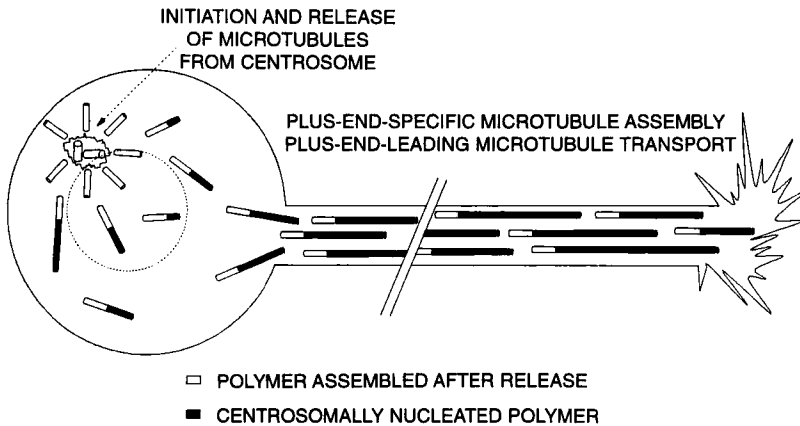
If these speculations are correct, developing neurons must have mechanisms that modulate microtubule behaviors at the centrosome during different stages of development. The capacity of the centrosome to act as a generator of microtubules may be regulated at the level of microtubule nucleation, release, transport, or at some as yet undiscovered checkpoint. Regarding microtubule nucleation, the activity of the centrosome has been shown in nonneuronal cells to depend on the phosphorylation state of certain proteins in the pericentriolar material (Centonze and Borisy, 1990), and the same might be true in neurons. Another possibility is that rates of microtubule nucleation are regulated by the need for new microtubules required to accommodate different rates of axonal and dendritic growth. If this is correct, it is likely that such a mechanism would be related to the manner by which cells monitor and autoregulate levels of assembled and unassembled tubulin (for review see Cleveland, 1988). When a microtubule leaves the cell body, we would expect the monomer/polymer equilibrium to shift toward assembly, in turn lowering the concentration of free tubulin in the cell body. As a result, the cell will synthesize more free tubulin, once again driving assembly of more polymer. If nucleation of microtubules from the centrosome is strongly favored over elongation (as our data suggest; see Yu *et al.*, 1993), this will result in an increased production of microtubules at the centrosome. With regard to microtubule release, proteins with microtubule-severing activity may be involved. For example, centrin, a protein present at the neuronal centrosome (Yu *et al.*, 1993), has been shown to have a calcium-modulated microtubule-severing activity at the basal bodies in other cell types (Sanders and Salisbury, 1989). With regard to microtubule transport, a motor that moves microtubules with plus ends leading would be required. An interesting motor protein is the yeast Kar3, which has the correct directionality and also destabilizes microtubules at their minus ends (Endow *et al.*, 1994). One possibility is that a similar protein both releases and translocates microtubules from the neuronal centrosome. Collectively, these considerations indicate that elucidating the molecular mechanisms that regulate centrosomal microtubule behaviors in the neuron will be a daunting but important task.

#### **D. Mechanisms for Elaborating the Axonal Microtubule Array**

A centrosomal origin for axonal microtubules has profound implications for the cascade of events by which the microtubule array of the axon is elaborated and organized. For example, and as noted above, if microtubules destined for the axon arise within the cell body, there must be an active transport mechanism to convey these microtubules into and down the length of the axon. In addition, there must be mechanisms for the elongation of at least some of these micro-

tubules once they arrive within the axon. Centrosomal microtubules are relatively short, a few micrometers in length, whereas microtubules in the axon can achieve lengths well over 100  $\mu\text{m}$  (Bray and Bunge, 1981; Tsukita and Ishikawa, 1981). We have argued that during their transit down the axon, many microtubules shorten or completely depolymerize to provide the subunits needed for the elongation of others (see Baas and Ahmad, 1993; Yu *et al.*, 1993; Joshi and Baas, 1993). This model, shown schematically in Fig. 6, is attractive in that it accommodates a centrosomal origin for the microtubules as well as observations from several different laboratories on microtubule transport and local assembly in the axon.

A centrosomal origin for each and every microtubule in the axon is a satisfying idea for many reasons, but it also presents a dilemma. Many axons achieve great lengths, and require rapid increases in microtubule numbers at distances far from the cell body; for example, during the formation of collateral branches. It is difficult to imagine how the great numbers of new microtubules required for collateral branch formation could derive directly from the centrosome and translocate at necessary rates over such great distances. It seems more likely that local mechanisms exist within the axon itself to account for the rapid and local increases in microtubule number required for the formation of collateral branches.



**Fig. 6** Schematic representation of our model for the elaboration of the axonal microtubule array. Microtubules destined for the axon are initiated at the centrosome and then released for translocation. Released microtubules are transported through the cytoplasm with their plus ends leading, and many of these are transported into the axon. In the schematic, the white portions of the microtubules represent the part assembled from the centrosome, while the black portions represent the part assembled after release from the centrosome. Plus ends of microtubules are directed away from the centrosome and toward the distal tip of the axon. The space between the slanted lines through the axon represents hundreds of micrometers of axon growth. During transit, the microtubules elongate specifically from their plus ends. Adapted from Joshi and Baas (1993).

In studies on axon branch formation in cultured hippocampal neurons, we have demonstrated that existing microtubules in the axon can locally fragment (Yu *et al.*, 1994). This fragmentation transforms one microtubule into many, all of which are assembly-competent and capable of rapidly elongating and all of which inherit the centrosomally derived characteristics of their predecessor microtubules. Thus, fragmentation of microtubules within the axon provides a mechanism to increase the number of "centrosomal" microtubules without having to nucleate new microtubules at the centrosome. As noted above, microtubule-severing proteins have been identified in other cell types (Sanders and Salisbury, 1989; Vale, 1991; Febvre-Chavalier and Febvre, 1992; Shiina *et al.*, 1992; McNally and Vale, 1993), and these or other proteins with similar activities may be responsible for the local fragmentation of microtubules in the axon.

#### IV. Concluding Remarks

In this article, I have reviewed several recent studies from my laboratory that point to a centrosomal origin for axonal microtubules. In addition, I have discussed the potential implications of this idea on the elaboration of the axonal microtubule array and on the establishment of the neuronal phenotype. Future efforts will be aimed at directly observing microtubule behaviors at the neuronal centrosome using real-time imaging of living cells and at elucidating the molecules and mechanisms that regulate these behaviors.

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