Chapter 18
Re-evaluation of the Neuronal Centrosome as a Generator of Microtubules for Axons and Dendrites

Peter W. Baas and Aditi Falnikar

Abstract A typical vertebrate neuron extends a single axon and multiple dendrites, both of which are rich in highly organized arrays of microtubules that serve essential functions. In simpler cell types, microtubules are organized by their attachment to a centralized nucleating structure such as the centrosome. In axons and dendrites, however, microtubules are not attached to the centrosome or any recognizable organizing structure. Over a decade ago, we proposed that the neuronal centrosome acts as a "generator" of microtubules for the axon and dendrites. Our studies suggested that the neuronal centrosome is highly active, especially during development, nucleating and releasing microtubules into the cell body. The released microtubules are then actively transported into the axon and dendrites by molecular motor proteins. In migrating neurons, most of the microtubules are attached to the centrosome, suggesting that significant changes in the nucleation or release of microtubules from the centrosome occur as neurons cease migration and begin to form their axonal and dendritic arbors. Recent studies suggest that the centrosome eventually becomes inactive as neurons mature, and that microtubule numbers are increased by other mechanisms, such as the severing of existing microtubules. Exactly how important the centrosome is for early stages of differentiation remains unclear, and the possibility exists that the centrosome may be re-activated in more mature neurons to meet particular challenges that may arise. Here we review historical as well as contemporary data on the neuronal centrosome, with emphasis on its potential role as a generator of microtubules.

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18.1 Introduction

Neurons are arguably the cell type in nature with the greatest dependence upon sophisticated arrays of highly organized microtubules for their form and function. A typical vertebrate neuron extends a single axon and multiple dendrites, both of which are rich in microtubules. The microtubule arrays within these processes are essential for providing architectural support, for enabling axons and dendrites to take on different shapes and branching patterns, and for supporting bidirectional organelle transport (Baas and Buster 2004). Many of the most fundamental differences between axons and dendrites directly or indirectly result from distinct patterns of microtubule orientation in each type of process. In the axon, nearly all of the microtubules are oriented with their plus ends distal to the cell body, whereas in the dendrite, the microtubules have a mixed pattern of orientation (Baas and Lin 2011). In most textbooks, microtubules are said to be organized mainly by their attachment to microtubule-organizing centers such as the centrosome (Alberts et al. 2007), but amazingly, the highly organized microtubules in axons and dendrites are not attached to the centrosome or any recognizable organizing structure (Baas and Yu 1996). Instead, the microtubules are free at both ends, and take on various lengths within the axon and dendrites. The shortest microtubules are highly mobile, moving rapidly within the axon (Wang and Brown 2002) and perhaps the dendrite as well (Sharp et al. 1995). One of the questions that has driven our laboratory for many years is how microtubules become organized in the axon and dendrites if not via attachment to an organizing center. Another question is whether the centrosome (located in the cell body of the neuron) has any importance for generating or organizing the neuronal microtubule arrays, or alternatively, whether it is a vestigial structure with no function.

Over a decade ago, we embarked on a series of studies the results of which led us to propose that the neuronal centrosome acts as a "generator" of microtubules for the axon and dendrites (Ahmad and Baas 1995; Ahmad et al. 1994, 1998, 1999; Baas 1996; Yu et al. 1993). The premise was that the neuronal centrosome is highly active, especially during development, nucleating and releasing microtubules into the cell body. The released microtubules are then actively transported into the axon and dendrites by molecular motor proteins. The relevant motors transport the microtubules specifically with their plus or minus end leading, and thereby establish the distinct patterns of microtubule polarity orientation in each type of process (Baas and Ahmad 1993; Sharp et al. 1995, 1997; Yu et al. 1997). In this view, the centrosome does not contribute to the polarity orientation of microtubules in either type of process, except perhaps to create an initial bias of plus ends outward in the cell body as the microtubules transit away from the centrosome (Ahmad and Baas 1995). One of the main roles that we envisioned for the centrosome was to nucleate microtubules in a regulated fashion with the appropriate lattice structure, as de novo nucleation of microtubules would presumably result in a variety of different protofilament numbers comprising the lattice (Baas and Joshi 1992; Yu et al. 1993). Another role for the centrosome, as a

kind of centralized "generator" of product, was to impose a level of control on the amount of microtubule polymer and the numbers of microtubules available at critical stages of neuronal development (Baas 1996). We envisioned the activity of the centrosome as being pulsatile, delivering bursts of new microtubules for example just prior to dendritic differentiation or when needed to supply a rapid increase in axonal growth.

We also noted, however, that it would be difficult to envision how the centrosome could be called upon to generate and deploy bursts of new microtubules to be used far down the length of the axon, for example, in the formation of a collateral branch. On this basis, we posited that existing microtubules in the axon or the dendrites may undergo localized severing events that could transform a single long microtubule into a population of many short ones (Joshi and Baas 1993). Each short microtubule would inherit the lattice structure of the parent microtubule, and each short microtubule would theoretically have the capability of assembling into a new long microtubule. This would render the axon or dendrites, once formed, less dependent upon or perhaps entirely independent of microtubule nucleation events at the centrosome. Since positing these ideas, we have confirmed that sites of impending branch formation do, indeed, display local severing of microtubules (Yu et al. 1994), and we have identified two different microtubule-severing proteins that participate in axonal branch formation (Qiang et al. 2010; Yu et al. 2008). Of course, this begs the question of whether microtubule severing could completely obviate the need for an active centrosome, even within the cell body.

Over the past decade, most studies on the neuronal centrosome have focused on neuronal migration, a phase of development some neurons undergo prior to axonal and dendritic development. In migrating neurons, most of the microtubules are attached to the centrosome, and this is important for pulling along the centrosome (and accompanying nucleus and cell body) as the neuron journeys to its final destination (Higginbotham and Gleeson 2007). This raises the question of what happens when the neuron ceases migration and sets forth to differentiate an axon and dendritic arbor. Is there an upregulation of microtubule severing such that all microtubules nucleated at the centrosome are now released? Does that centrosome gradually lose its nucleating potency during development, or is the ability to nucleate microtubules retained and used at key moments in development? In adult neurons, is there a slow but steady flow of new microtubules from the centrosome, or does the centrosome become quiescent in terms of manufacturing new microtubules? These questions remain unanswered but there has recently been new interest in whether or not the neuronal centrosome serves as a hub for microtubule-based activity relevant to neuronal differentiation (de Anda et al. 2005; Stiess et al. 2010; Stiess and Bradke 2011). Here, we review the older literature, summarize exciting new findings, and ponder the unanswered questions.

18.2 Location, Location, or Not Location?

The idea that axonal microtubules have a centrosomal origin is actually a rather old one. 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". Even so, a common theme of these earlier studies was that the position of the centrosome in the cell body of the neuron had no consistent correlation with the point of origin of the axon, and there was certainly no direct continuity between the microtubule array of the axon and the centrosome (Lyser 1964, 1968; Sharp et al. 1982). These observations also held true in the case of cultured rat hippocampal neurons (Baas et al. 1988; Dotti and Banker 1991) as well as various other types of neurons in culture we have studied over the years, which include rat sympathetic neurons (Yu et al. 2001) and chicken dorsal root ganglion neurons (Baas and Heidemann 1986). A lack of correlation between the position of the centrosome and the location of the axon (or dendrites) is consistent with the centrosome ejecting microtubules into the cell body that may ultimately come to reside in axons and dendrites, without the microtubules dragging the centrosome with them in the direction of the relevant axon or dendrite (see Fig. 18.1). This would distinguish a neuron that has stopped migrating and started elaborating its axonal and dendritic arbors from a migrating neuron, in which the centrosome is dragged toward the leading process by its attached microtubules.

An interesting exception to the location rule was reported in the case of cultured cerebellar granule neurons, which have a somewhat unique developmental pattern in the culture dish (Zmuda and Rivas 1998). After these neurons cease migrating, they extend an initial axon, then a secondary axon, and finally multiple dendrites. The centrosome is first positioned near where the initial axon develops and then moves to where the secondary axon develops, suggesting that the position of the centrosome is related to the development of each of the two axons. Perhaps having two axons and a single centrosome demands that the centrosome is nearest the one that is undergoing the most active phase of growth, whereas in neurons with only one axon and one centrosome, the position of the centrosome is not so important. Perhaps the centrosome is dragged toward the axon into which microtubules are being most actively transported. The same molecular motors responsible for transporting microtubules into the axon would pull on the microtubules while they are still attached to the centrosome, and thereby move the centrosome toward the relevant axon.

In a more recent paper on cultured hippocampal neurons, the laboratory of Carlos Dotti revisited his earlier result on centrosome location relative to axonal differentiation (de Anda et al. 2005). They reported that the axon consistently arose

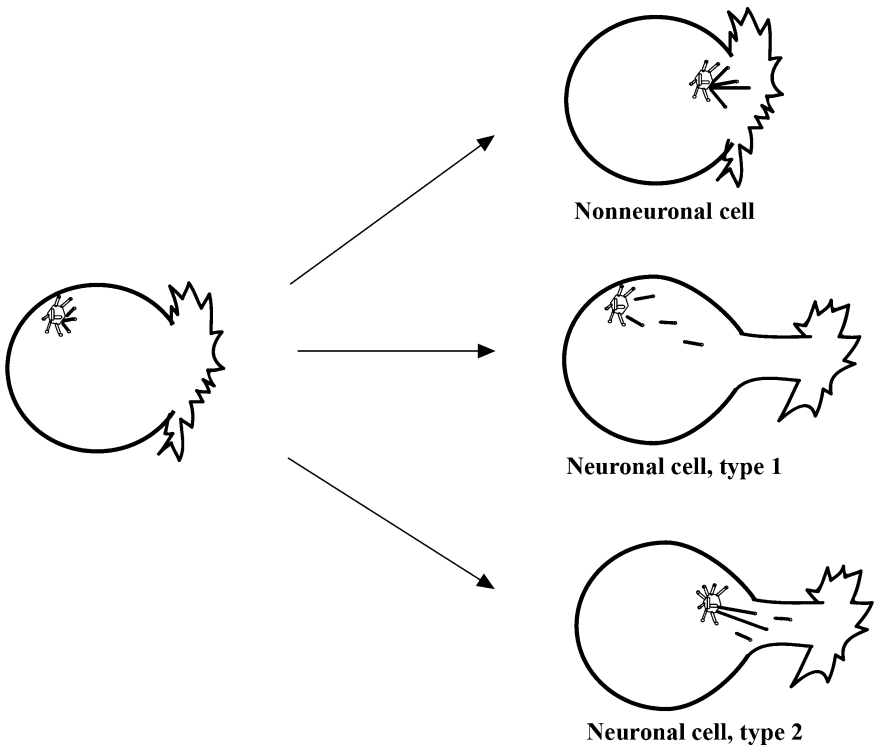

Fig. 18.1 Schematic illustration depicting how differences in microtubule behavior at the centrosome may contribute to phenotypic differences in different cell types. A pluripotent precursor cell shown on the left could give rise to either a motile non-neuronal cell or a neuron. In the case of the non-neuronal cell shown on the right at top, forces pulling on the microtubules draw the centrosome toward the leading edge of the cell as it moves. In the most typical situation of the neuron (denoted as type 1), 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. In the case of some neurons (denoted as type 2), a subset of microtubules nucleated by the centrosome remains attached to the centrosome while others are released. The same forces that transport the released microtubules into the early axon pull on the attached microtubules, drawing the centrosome toward the axon

from the first immature neurite to form after the final mitotic division of the neuroblast, and that the Golgi and endosomes (which generally accompany the centrosome) clustered in the location where the first neurite formed. These observations are surprising in light of the earlier findings on hippocampal and other types of neurons, but are consistent with the observations on cerebellar granule neurons described by Zmuda and Rivas (1998). Interestingly, they also found that ablating the centrosome precluded normal polarization of the neuron.

One of the most enduring mysteries of neuronal polarity is why most neurons have a single axon and how it is that the formation of additional axons is suppressed. It is interesting in this regard that neurons also most typically have a single centrosome. It is tempting to propose that the singularity of the axon and the singularity of the centrosome are somehow related. In the unusual case of the cerebellar granule neurons with two axons, a single centrosome changes location to serve both. However, in the recent paper by de Anda et al. (2005), they observed a small number of hippocampal neurons with two centrosomes and such neurons consistently formed two axons. On the other hand, it should be noted that cultured sympathetic neurons initially differentiate several axons after which they re-craft their morphology into a single axon and multiple dendrites (Bruckenstein and Higgins 1988); and yet, despite initially forming several axons, they only have one centrosome (Yu et al. 1993). Unlike the case with the axon, dendrites are almost always multiple in numbers, and it would be hard to fathom that the centrosome could be so mobile in the cell body as to move from dendrite to dendrite and then back to the axon to serve each neurite one at a time. Interestingly, we reported several years ago what appears to be streams of microtubules flowing from the centrosome into developing dendrites of cultured hippocampal neurons, with a location roughly centralized among the dendrites (Sharp et al. 1995; also see Fig. 18.2). No such flow of microtubules was observed between the centrosome and the axon at this stage of development. Taken together, these several findings indicate that there is no "one size fits all" scenario for the location of the neuronal centrosome. Even so, it would certainly appear that the centrosome is an important structure in the neuron, at least for the early stages of development.

18.3 Why is the Neuronal Centrosome Important?

The centrosome is best known in eukaryotic cells as a microtubule-organizing center that organizes microtubules by virtue of its microtubule-nucleating properties. The centrosome consists of two barrel-shaped centrioles surrounded by amorphous pericentriolar material (Alberts et al. 2007). Among the components of the pericentriolar material are structures known as γ-TuRCs (gamma-tubulin ring complexes). Each γ-TuRC, which consists of gamma-tubulin together with several other proteins, is a template for nucleating a microtubule. Microtubules are nucleated from the γ-TuRCs in such a way that the plus ends of the microtubules grow away from the centrosome. Thus, if the microtubules remain attached to the centrosome, they form a radial array of uniform polarity orientation (Euteneuer and McIntosh 1981; Schiebel 2000; Teixido-Travesa et al. 2010). Such a radial array, typical of simple interphase cells, is able to direct organelle traffic by virtue of the tendency of different types of organelles to interact with specific motors that move toward either plus or minus ends of microtubules. This is why, for example, the Golgi apparatus tends to cluster at the centrosome; because membranous elements that comprise the Golgi are transported by cytoplasmic dynein toward

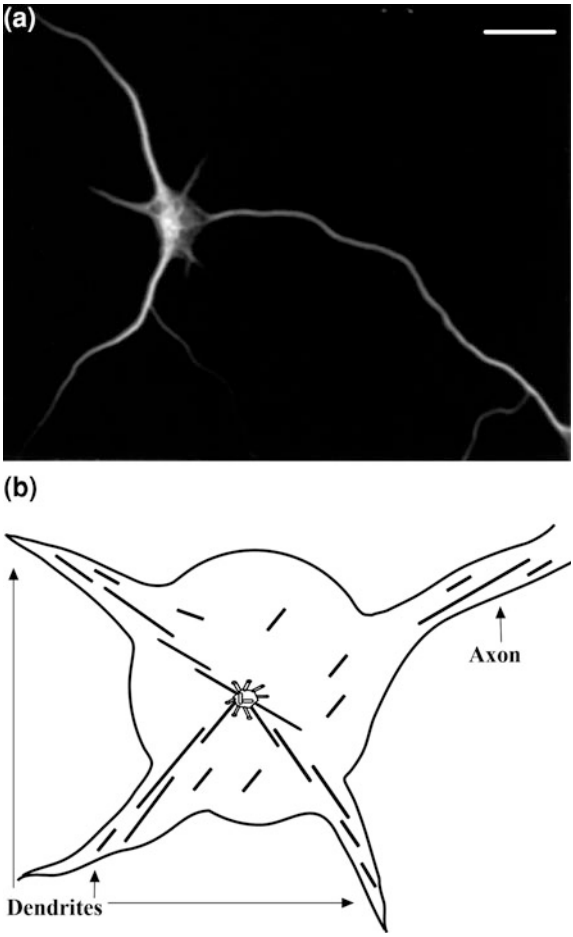

Fig. 18.2 Distribution of microtubules in cultured embryonic rat hippocampal neurons in the context of dendritic development. **a** A dendrite-bearing neuron immunostained for microtubules. The image is presented in a quantitative scale in which *white* indicates the highest intensity, *black* indicates the least, and shades of *gray* indicate intermediate levels. The cell body contains high levels of microtubules within a discrete region. This region is continuous with high levels of polymer within the developing dendrites. Adapted from Sharp et al. (1995). Bar, 20 μm. **b** Schematic illustration of a dendrite-bearing neuron, depicting a stream of microtubules emerging from the centrosome and flowing into the developing dendrites. The centrosome itself occupies a location that is roughly central in reference to developing dendrites

minus ends of microtubules (Corthesy-Theulaz et al. 1992). As a general but not universal principle, very little gamma-tubulin is located in cells anywhere except the centrosome (or other microtubule-organizing centers) and de novo nucleation of microtubules is suppressed in the cytoplasm relative to nucleation from such structures (Alberts et al. 2007). Nucleation from structural templates also serves the purpose of constraining the lattice of the microtubule to a consistent number of protofilaments (typically 13 in most vertebrate cells) (Evans et al. 1985) although there are other factors that influence protofilament number as well (Fourniol et al. 2010; Moores et al. 2004).

The centrosome is generally positioned in the center of the cell (hence the name centrosome) and this location is determined by a balance of forces that act upon the microtubules that emanate from the centrosome while remaining attached to it (Euteneuer and Schliwa 1992; Vallee and Stehman 2005). Without the attached microtubules, there is nothing for molecular motors to pull on in order to center the

centrosome and its position becomes less centralized (Burakov et al. 2003). Based on observations from other cell types, we have posited that the reason why the centrosome relocates toward a particular neurite, if it does, is that the machinery that transports microtubules strongly favors that particular neurite at that particular moment in development (Baas 1996). Thus, the microtubules that are released from the centrosome would flow into the relevant neurite but those that are not yet released would react to the motor-driven forces by pulling the centrosome toward that particular neurite. Thus, in neurons, we would speculate that the degree to which the location of the centrosome is predictive of where an axon or dendrite emerges from the cell body probably relates to how active the centrosome is in nucleating microtubules as well as the degree to which or rate at which the microtubules are released once nucleated. In other words, if the centrosome is not very active at nucleating microtubules, it would not be relocated toward any particular neurite. If the centrosome is highly active at nucleating microtubules but most or all of the microtubules are almost immediately released upon nucleation, the centrosome would not be relocated toward any particular neurite. Also, if the relevant motors do not favor any particular neurite, the centrosome would not be relocated toward any particular neurite. These points are schematically illustrated in Figs. 18.1 and 18.2b. It is difficult to imagine a scenario by which the centrosome would relocate without being active at nucleating microtubules.

Whether or not it is functionally important that the centrosome is located where it is, in various types of neurons at particular stages of development, remains to be seen. Certainly, if there are multiple options for where the microtubules released from the centrosome could be transported, a location near the hillock of the relevant neurite would be an advantage for directing microtubules into that neurite. Another possibility is that the location of the centrosome could be functionally important but for other reasons, such as providing a flow of Golgi-derived vesicles. Support for this idea comes from work showing in cultured hippocampal neurons a particularly robust flow of membranous elements into the immature process that develops into the axon (Bradke and Dotti 1997). Yet another possibility is that the centrosome is important for reasons related to the various proteins that gather together to form the pericentriolar material. For example, the pericentriolar material is rich in kinases (Hames et al. 2005), and hence the centrosome could act as a processing center to phosphorylate functionally important proteins. Alternatively, the pericentriolar material might act as a sink for various proteins that would otherwise, and under certain circumstances, be widely distributed in the neuron. This could apply not only to proteins such as kinases, but also to proteins directly related to microtubule nucleation. In such a scenario, it may not be essential that the centrosome nucleates microtubules, but by sequestering the proteins needed for microtubule nucleation, the centrosome ensures that microtubule nucleation does not occur in other locales, where it would be problematic. Whatever the case, it may become important, as we ponder the entirety of the data on the neuronal centrosome, to think more expansively on the potential roles that it may play in organizing the cytoplasm and directing various events relevant to the axon and dendrites.

As noted earlier, our original interest in the neuronal centrosome was as a "generator" of new microtubules for axons and dendrites. However, we should note that there are exceptions to the rule of microtubule nucleation being constrained to centrosomes, as gamma-tubulin can redistribute to new locations in certain cell types (Bugnard et al. 2005). In fact, we previously proposed that the non-uniform orientation of dendritic microtubules might result from centrosomal proteins being relocated from the centrosome into dendrites at early stages of their differentiation (Baas et al. 1989). There are also examples in the literature where de novo nucleation of microtubules has been observed (Yvon and Wadsworth 1997) but not commonly, as haphazard nucleation of new microtubules would probably make more problems for cells than solutions. In studies directed at testing for de novo nucleation of microtubules in axons, we found no evidence for it, as all new assembly was observed to occur via elongation of existing polymers (see below).

18.4 Older Data on Microtubules and the Neuronal Centrosome

Our studies positing the neuronal centrosome as a generator of microtubules for the axon were conducted in the 1990s, and utilized tools that had been previously used in other cell types. The classic method for identifying sites of microtubule nucleation in cells is to depolymerize existing microtubules with nocodazole, 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 site of microtubule nucleation in other cell types, (De Brabander et al. 1977, 1980), was used by our laboratory to identify potential sites of microtubule nucleation in the axons of cultured rat sympathetic neurons (Baas and Ahmad 1992). After drug removal, all new microtubule assembly arose from the plus ends of the stable microtubules that resisted depolymerization. No microtubules arose independently of existing microtubules, suggesting that the plus ends of pre-existing microtubules are the exclusive sites of microtubule assembly in the axon. These findings were consistent with previous work on cultured sensory neurons demonstrating that when all microtubule polymer is pharmacologically depolymerized from isolated axons, no reassembly occurred after removal of the drug (Baas and Heidemann 1986). Based on these findings, we concluded that entirely new microtubules destined for the axon must be nucleated within the cell body. To explore the issue further, we investigated the distribution of gamma-tubulin in these neurons (Baas and Joshi 1992). Using both biochemical and immunoelectron microscopic assays, we found no evidence for gamma-tubulin in the axon. In addition, we found no appreciable levels of gamma-tubulin anywhere in the cell body except at the centrosome, suggesting that the centrosome is the sole site for the nucleation of new microtubules for the entire neuron. On the basis of these findings, we proposed that

microtubules destined for the axon are nucleated at the centrosome, released, and then transported into 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 ten and often no microtubules were observed to be attached to the centrosome (Baas and Joshi 1992; Yu et al. 1993). 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 nucleate large numbers of microtubules, using the same drug-recovery regime that we used on the axon (Yu et al. 1993). 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 it (Fig. 18.3). Some of the microtubules were not attached to the centrosome, but were 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 after removing the drug, the microtubule array was indistinguishable from that of control neurons, suggesting that the hundreds of microtubules nucleated from the centrosome were subsequently released and translocated away from the centrosome.

We next tested whether microtubules derived from the centrosome are essential for the initiation and growth of the axon. Our strategy was to microinject into cultured sympathetic neurons a function-blocking antibody to gamma-tubulin previously shown to arrest microtubule nucleation at the centrosome when microinjected into other cell types (Ahmad et al. 1994). We reasoned that if centrosomally derived microtubules are required for the growth of the axon, we would expect inhibition of centrosome function to compromise or inhibit axonal growth. These experiments were tricky, however, because the cell body of the neuron is packed with microtubules that had presumably (according to our hypothesis) already been nucleated and released from the centrosome. Therefore, it was also necessary to deplete the neuron experimentally of pre-existing microtubules. After depolymerizing existing microtubules with nocodazole, the antibody was microinjected into neurons, and then the drug was rinsed from the cultures. Reassembly of microtubules over the next two hours was severely diminished under these conditions, and axonal growth was either compromised or completely abolished. These results, using an admittedly complicated experimental regime, suggested that microtubules generated from the centrosome are important for axonal growth.

Finally, we set forth to test if the microtubules nucleated at the centrosome are the same microtubules that ultimately arrive in the axon. To test this, we modified our pharmacological experiments into a kind of “pulse-chase” regime that permitted us

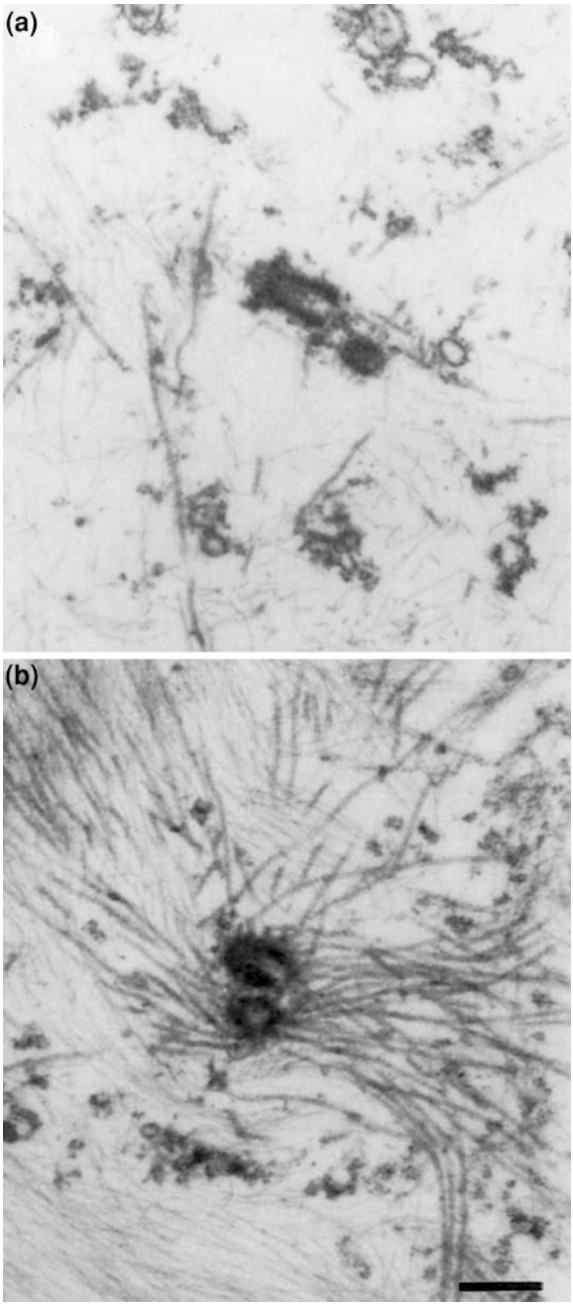

Fig. 18.3 Electron micrographs of cultured rat sympathetic neurons in the region of the centrosome. **a** A neuron showing centrosome consisting of two centrioles and multiple unattached microtubules. **b** A neuron treated for 6 h with 10 μg/ml nocodazole, rinsed free of the drug, and permitted to recover 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 μm

to follow the progress of a small population of microtubules nucleated at the centrosome (Ahmad and Baas 1995; also see Fig. 18.4). After drug treatment to depolymerize microtubules, and a few minutes of microtubule reassembly at the

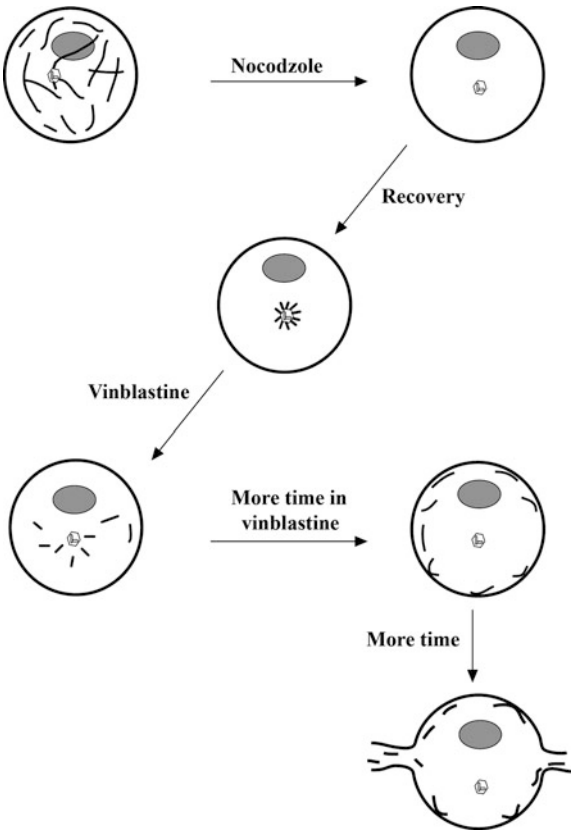

Fig. 18.4 Schematic illustration of pharmacological strategy for revealing the progression of microtubules outward from the neuronal centrosome. Nocodazole was the first drug used to depolymerize pre-existing microtubules. Next, after a brief recovery period, vinblastine was used as an anti-microtubule drug to suppress further assembly of microtubules. Microtubules redistributed over time. Adapted from Ahmad and Baas (1995)

centrosome, low levels of a second anti-microtubule drug (vinblastine) were added to the cultures to suppress further microtubule assembly while not substantially depolymerizing existing microtubules. Thus, we reasoned that any alterations in the microtubule array that occur after the addition of the second drug must be the result of microtubule movements from one location in the cell to another. Consistent with this expectation, microtubule levels remained roughly the same after the addition of vinblastine, as did the lengths of individual microtubules 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 h, few or no microtubules were attached to the centrosome and most 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 further down the axons with increasing time (see Fig. 18.4). These results suggested that microtubules derived from the centrosome are transported outward from the centrosome toward cell periphery and then into and down the length of the axon.

Due to the geometry of the neuron, the density of the microtubule array, and the pool of free tubulin in neurons, we have not been able to directly visualize

microtubules in living neurons moving from the centrosome into the axon. The issue arises as to whether the centrosome is actually needed under normal circumstances, or whether our pharmacologic regimes stress the system to a point where an otherwise unnecessary centrosome becomes necessary. In support of our interpretation, the active release of microtubules from the centrosome has been directly visualized in cellular extracts (Belmont et al. 1990) as well as living epithelial cells in a regime that involved no drug treatments (Keating et al. 1997). Moreover, in the case of the neuron, we have also shown that inhibition of katanin, a microtubule-severing protein, prohibits microtubule release from the centrosome, which in turn precludes the appearance of free microtubules in our pharmacologic regime (Ahmad et al. 1999).

18.5 Newer Data on Microtubules and the Neuronal Centrosome

Over a decade after our spate of papers on the neuronal centrosome, the laboratory of Frank Bradke has recently challenged the idea of the neuronal centrosome acting as a generator of microtubules for axons and dendrites (Stiess and Bradke 2010; Stiess et al. 2010). They favor the alternative view that the centrosome is dismantled during neuronal development such that its microtubule-nucleating duties are spread to new locations in the neuron, such as within the axon and dendrite themselves. This scenario would be similar to what has been shown for muscle cells, in which gamma-tubulin and its associated microtubule-nucleating properties are redistributed to the nuclear membrane and other sites within the cytoplasm (Bugnard et al. 2005). In fact, consistent with our original speculation for how a non-uniform microtubule polarity pattern might arise in dendrites (Baas et al. Baas et al. 1988, 1989), at least one pericentriolar protein has been shown to be present in dendrites but not axons (Ferreira et al. 1993). It is also provocative with regard to the centrosome dismantling hypothesis for neurons that Leask and colleagues reported a steady diminution in gamma-tubulin from the centrosome as dorsal root ganglion neurons mature, which would be consistent with a gradual redistribution of their pericentriolar proteins (Leask et al. 1997). Bradke's group found a similar diminution of gamma-tubulin levels as well as another key protein component of the γ-TuRCs, consistent with the idea that the capacity of the neuronal centrosome to act as a generator of microtubules wanes as the neuron matures. In addition, they found with hippocampal neurons that the nocodazole recovery regime resulted in a burst of microtubules from the centrosome early in development (Dotti and Banker 1991), but this was not the case later in development. In cultures that were several days old, neurons bearing dendrites showed no specific recovery of microtubules from the centrosome after nocodazole treatment and removal. Instead, the microtubules reassembled from sites throughout the cell body (Stiess et al. 2010).

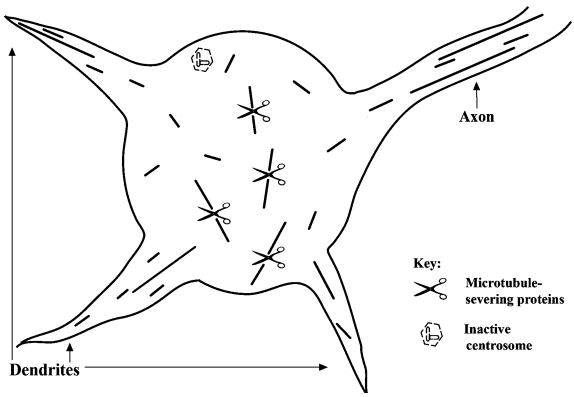

Fig. 18.5 Schematic illustration of a mature neuron showing an inactive (dismanted) centrosome and the severing of microtubules. As the neuron matures, the ability of the centrosome to nucleate microtubules diminishes. Microtubule number is increased by severing of pre-existing long microtubules into short mobile pieces, followed by the transport of short microtubules into the axon or the dendrites. These short microtubules can serve as seeds for assembly of longer microtubules

The authors posited, based on these results, that microtubule-nucleating capacity becomes de-centralized as the neuron develops such that nucleation of microtubules can occur throughout the cell body and potentially even within axons and dendrites themselves. We would agree, assuming that nucleation in this context means assembly from pre-existing microtubules, even very short fragments that are able to act as seeds for new assembly (Baas and Ahmad 1992; Baas and Black 1990; Baas and Heidemann 1986; Black et al. 1984; Brady et al. 1984; Morris and Lasek 1982). As neurons mature, it is virtually impossible to completely depolymerize the more stable microtubules, even with prolonged drug treatments, so we suspect that the reassembly of microtubules observed by Steiss and colleagues represents "nucleation" from stable microtubule fragments, but not bona fide nucleation in the de novo sense. Even so, the results reported by these authors accentuate the fact that the neuron can very ably go on "auto pilot" once a robust microtubule array has been constructed, such that a centralized factory for microtubule production can be shut down. As noted earlier (and as discussed also by Steiss and colleagues), the severing of existing microtubules in the cell body as well as in the axon and the dendrites is presumably sufficient for increasing the number of microtubules whenever and wherever needed (see Fig. 18.5).

18.6 Concluding Remarks

It may be relevant to consider that different kinds of neurons go about their business in somewhat different ways and on different timetables. For example, central and peripheral neurons may differ with regard to the importance of the

centrosome, and also neurons that bear dendrites or multiple axons may differ from those that do not bear dendrites and have the more classic single axon. Migratory neurons appear to utilize their centrosome in the most traditional fashion, as the vast majority of microtubules in the migratory neuron remain attached to the centrosome (Tsai and Gleeson 2005), while a small fraction of the microtubules are apparently released from the centrosome so that they transit down the leading process or slide their minus ends behind the centrosome (Falnikar et al. 2011). Overall, it appears that the centrosome is most important early in neuronal development, especially during neuronal migration and early axonal differentiation. After that, the preponderance of the data suggests that neurons gradually lose their dependence on the centrosome in favor of self-sustaining mechanisms for maintaining the microtubule arrays of the axon and the dendrites.

Whether or not the neuron needs a centrosome for the development of proper axons or dendrites remains a debatable point, as merely being able to form an axon or dendrite in culture may be a very different thing than being able to form the appropriate axon or dendrite within the context of a functional nervous system. In addition, it is pertinent to keep in mind that the biology community continues to be surprised by the plethora of transgenic animals that are viable in the absence of proteins believed to play important roles in cellular functions. Oftentimes, the importance of a particular protein (or in this case, an organelle) is gleaned only after cells or entire organisms are challenged in particular ways. This may be the case developmentally, and also in more mature neurons in which the centrosome appears to have become vestigial. For example, perhaps under certain circumstances, the centrosome is re-activated to enable the neuron to meet a particular challenge, such as restructuring of the dendritic arbor in response to learning or disease, or regeneration of an injured axon.

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