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Microtubules and Neuronal Polarity: Lessons from Mitosis

Review

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Introduction

Neurons are the principle signaling cells of the nervous system. In order to transmit signals, neurons stop dividing early in development and direct their efforts instead toward the elaboration of elongated cellular processes. A typical vertebrate neuron extends a single axon and many dendrites. Axons are long slender processes that are specialized to transmit information, while dendrites are short tapering processes that are specialized to receive and process information. Axons and dendrites differ from one another in many aspects of structure and function, and these differences are collectively referred to as the polarity of the neuron. Several fundamental questions regarding the generation of neuronal polarity have now been identified. How is one developing process selected to become the axon, while the others are selected to become dendrites? What accounts for the unique morphological and compositional features of axons and dendrites? Why are axons long and dendrites short? How do environmental factors affect the differentiation of axons and dendrites? How do the growth cones at the tips of developing axons find their appropriate target tissues?

Several lines of evidence suggest that the cytoskeletal elements known as microtubules may be central to all of these issues. Microtubules are dynamic polymers made up of tubulin subunits. They provide architectural support for eukaryotic cells, and act as railways along which cytoplasmic constituents are actively transported. Microtubules have an intrinsic polarity. One end of the microtubule is called the plus end, while the other end is called the minus end. Although microtubule polarity was originally defined in terms of the preferential addition of tubulin subunits onto the plus end of the polymer, it is now apparent that the polarity of the microtubule is also relevant to its transport properties. Certain cytoplasmic constituents are transported preferentially toward the plus end of the microtubule, while others are transported preferentially toward the minus end. Thus, by organizing its microtubules relative to their polarity, a cell can generate an asymmetric distribution of cytoplasmic constituents. Microtubules in the axon are uniformly oriented with their plus ends distal to the cell body, while microtubules in the dendrites are of both orientations (Baas et al., 1988). As a result, different complements of cytoplasmic constituents are transported from the cell body of the neuron into each type of process (Black and Baas, 1989). Given the structural and functional roles that microtubules play within cells, it is not difficult to imagine how the distinct microtubule patterns of axons and dendrites could also profoundly affect their length and shape.

Thus, it would appear that many of the most important questions regarding neuronal polarity can best be understood by elucidating how the neuron generates its microtubule arrays. Typical mitotic cells alternate between a radial array of microtubules during interphase and a bipolar spindle of microtubules during mitosis. The microtubules are associated with their sites of nucleation within the single interphase centrosome or within the duplicated centrosomes during mitosis. Nucleation from the centrosome constrains the lattice structure of the microtubule to 13 protofilaments, and the specific association of the minus ends of the microtubules with the centrosome determines their polarity orientation (Brinkley, 1985; Evans et al., 1985). By contrast, neurons are terminally postmitotic cells that no longer form mitotic spindles. Neurons are also quite dissimilar from typical interphase cells with regard to microtubule distribution and organization. In the neuron, few microtubules are attached to the centrosome. Instead, the vast majority of the microtubules are free in the cytoplasm, where they tend to coalesce into bundles that funnel from the cell body into axons and dendrites. Despite their lack of attachment to the centrosome, the microtubules within the neuron have a consistent 13 protofilament lattice structure and are tightly regulated with regard to their polarity orientation. How is this achieved?

It generally has been assumed (if not stated) that terminally postmitotic neurons abandon the mechanisms that mitotic cells use to organize their microtubules and develop entirely novel strategies. This assumption, based on the fact that the neuronal microtubule arrays appear to be so unlike those of dividing cells, has led researchers away from the mitotic spindle as a potential model for understanding how the neuron might generate the microtubule arrays of axons and dendrites. In recent years, my laboratory has questioned the validity of this assumption. We have proposed that neurons establish their microtubule arrays using modifications of the same mechanisms used by dividing cells. If this is true, then the most important lessons regarding neuronal polarity may lie within a very unexpected place, namely the mitotic spindle.

Microtubules and the Mitotic Spindle

The mitotic spindle is the most fundamental of all microtubule arrays and the best studied. During prophase, the centrosome replicates, and each new centrosome nucleates microtubules. The minus ends of the microtubules remain in association with the centrosome from which they are nucleated, while the plus ends emanate outward. The duplicated centrosomes are driven to opposite poles of the cell (presumably as a direct result of changes in microtubule organization), and a bipolar spindle begins to take shape. During metaphase, some of the microtubules called kinetochore microtubules interact with the kinetochore regions of the chromosomes.

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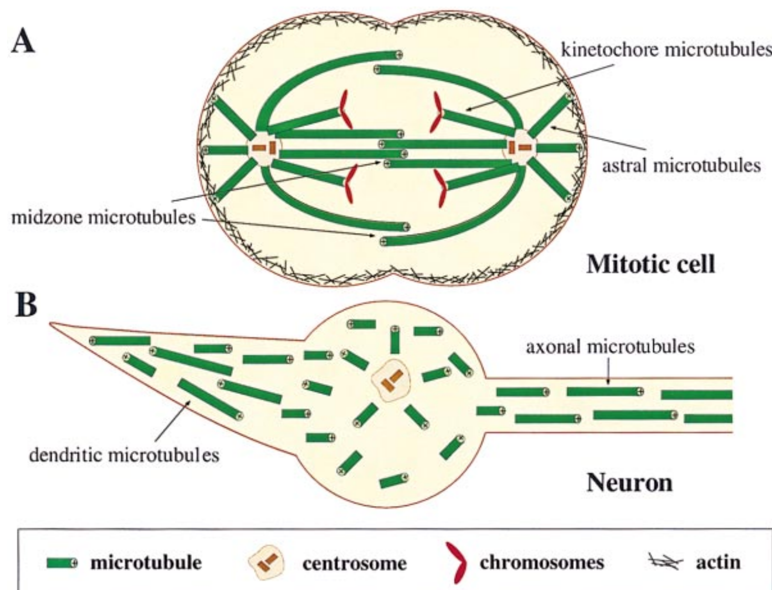

Figure 1. Schematic Illustrations of the Microtubule Arrays of the Mitotic Spindle and the Postmitotic Neuron

(A) shows the mitotic spindle (in anaphase), while (B) shows the postmitotic neuron. The mitotic spindle consists of two partially overlapping half-spindles. Each half-spindle emanates from a centrosome. Astral microtubules extend to the cell periphery and interact with the cell cortex. Kinetochore microtubules extend inward and interact with chromosomes. Nonkinetochore microtubules from each half-spindle overlap in the midzone and are referred to as midzone microtubules in this region. The minus ends of the microtubules are associated with the centrosome, while the plus ends emanate away from it. Thus, the mitotic spindle consists of regions in which microtubules are uniformly oriented (the regions of the astral microtubules) and another region in which microtubules are nonuniformly oriented (the midzone, where oppositely oriented microtubules from each pole interdigitate). The postmitotic neuron extends two distinct types of processes. The axon consists of microtubules that are uniformly oriented with their plus ends distal to the cell body. The dendrites consist of microtubules that are nonuniformly oriented. Thus, with regard to microtubule polarity orientation, the axon is similar to the astral microtubules of the spindle, while the dendrites are similar to the midzone microtubules.

Other microtubules called nonkinetochore microtubules do not associate with kinetochores but instead overlap with oppositely oriented or "antiparallel" microtubules from the other pole within the region of the spindle known as the midzone. Still other microtubules called astral microtubules extend toward the cell periphery, where they interact with the cell cortex. During early anaphase, the kinetochore microtubules depolymerize and draw the chromosomes toward each pole. During late anaphase, forces are generated between the antiparallel microtubules within the midzone and between the astral microtubules and the cell cortex. These forces drive the two half-spindles apart. Figure 1A shows a schematic illustration of a cell in early anaphase. During telophase, the nuclear membrane reforms. Shortly thereafter, the half-spindles reorganize into typical monoastral interphase microtubule arrays.

Although these stages of microtubule reorganization during mitosis have long been recognized, the mechanisms underlying them have remained mysterious for years. Even early workers noted that the relevant changes in microtubule organization probably cannot be attributed entirely to the assembly and disassembly of the microtubule polymers. It is now clear that the formation and functioning of the mitotic spindle depend on forces generated by molecular motor proteins that include cytoplasmic dynein and a variety of specialized kinesin-related proteins. For example, cytoplasmic dynein appears to be important for generating forces between microtubules and the cell cortex during both prophase and late anaphase (Carminati and Stearns, 1997; Busson et al., 1998; Inoue et al., 1998). The kinesin-related proteins in the *bim*C family appear to be important for driving apart the duplicated centrosomes during prophase (see, e.g., Blangy et al., 1995) and may have other functions as well later in mitosis (Gaglio et al., 1996; Kashina et al., 1997). The kinesin-related proteins in the CHO1/MKLP1 family appear to be important for driving apart the antiparallel microtubules in the spindle midzone during late anaphase (Sellitto and Kuriyama, 1988; Nislow et al., 1990, 1992). Several other motor proteins have also been identified within the mitotic spindles of different species. These various motors generate forces that can be either complementary or antagonistic to one another. Tight regulation of these forces accounts for many of the key changes in microtubule organization and distribution that occur during mitosis.

Quite remarkably, recent in vitro studies have shown that molecular motor proteins and other components of mitotic extracts can organize microtubules into bipolar spindles in the complete absence of centrosomes (Heald et al., 1996). The spindles form via the movement of individual microtubules by motor proteins that specify whether the plus end or the minus end of the microtubule leads during the movement. Given these results, the question arises as to whether or not centrosomes play any crucial role whatsoever within the mitotic spindles of living cells. Other recent studies suggest that they do, specifically in the nucleation of new microtubules. The pericentriolar material of the centrosome contains ring-like microtubule-nucleating structures (Moritz et al., 1995; Zheng et al., 1995). These structures consist of γ-tubulin (the nucleating protein) and a variety of other proteins that form a ring with the appropriate diameter to constrain the lattice structure of the microtubule to thirteen protofilaments. Thus, it would appear that the centrosome is important for the nucleation of microtubules, and for determining their lattice structure. However, the centrosome may not be as important as once thought for the organization of microtubules, which would appear to be more the responsibility of forces generated by molecular motors.

Microtubules in the Postmitotic Neuron

Neurons develop from mitotic cells of ectodermal origin. After several divisions, these cells begin to express neuron-specific proteins, during which time they can extend and retract primitive cytoplasmic processes even prior to their terminal mitotic division (Haendel et al., 1996). This suggests that the interphase cytoplasm of the neuroblast gradually becomes more neuron-like, after which the neuroblast permanently exits the cell cycle. At this point, the neuron will never again organize its microtubules into a bipolar spindle (unless, of course, it becomes cancerous). Unlike a typical interphase cell, the terminally postmitotic neuron does not display a radial array of microtubules emanating from a centralized centrosome. Instead, the centrosome has an apparently random location in the cell body of the neuron, with few microtubules actually attached to it at most times during development (Yu et al., 1993). Microtubules are abundant throughout the cell body, but do not show any detectable pattern of organization, except in the hillock regions of developing processes into which the microtubules appear to "funnel" (Yu and Baas, 1994). Studies on cultured hippocampal neurons have shown that the initial immature processes extended by the neuron contain microtubules exclusively with a plus end–distal orientation, and that this pattern is preserved in the single process that develops into the axon (Baas et al., 1989). After the axon has differentiated, the remaining immature processes begin to develop into dendrites. As this occurs, the developing dendrites acquire a nonuniform pattern of microtubule polarity orientation that results from the gradual addition of minus end–distal microtubules (Baas et al., 1989). In addition, there is a marked increase in the total microtubule mass within axons and dendrites, an increased range of microtubule lengths, and increasing levels of particularly stable microtubule polymer (Baas et al., 1991, 1994; Yu and Baas, 1994). Figure 1B shows a schematic illustration of the microtubule arrays of a postmitotic neuron.

Given the unique features of the neuronal microtubule arrays, it is not surprising that researchers would hypothesize that these arrays are established by mechanisms that are distinct from those that organize the mitotic spindle or even a typical interphase microtubule array. Many authors have assumed that the centrosome becomes a relatively inactive structure in the postmitotic neuron and that new microtubules arise via local nucleation within axons and dendrites. This assumption is based in part on the observation that neurons express high levels of fibrous microtubule-associated proteins (MAPs) such as tau and MAP2 that can nucleate microtubule assembly in the test tube. However, the idea that widely distributed proteins such as the MAPs could nucleate microtubules in a living cell seems unattractive in that such microtubules would not be expected to have a consistent lattice structure or a specific polarity orientation. Experimental analyses have also failed to show any support for the local nucleation of microtubules within neuronal processes (Baas and Heidemann, 1986; Baas and Ahmad, 1992). Another idea, that MAPs directly determine the polarity patterns of microtubules in axons and dendrites, is also not supported by the available experimental evidence (Chen et al., 1992; Le Clerc et al., 1993; Brandt and Lee, 1994). At present, it would appear that the fibrous MAPs probably stiffen the microtubule polymers against bending (Felgner et al., 1997), determine the spacing between individual microtubules within a bundle (Black, 1987; Chen et al., 1992), and possibly contribute to the stability properties of the microtubules (see, e.g., Baas et al., 1994). However, there is no evidence suggesting that the expression of these MAPs permits the neuron to circumvent the kinds of mechanisms that other cells utilize to generate their microtubule arrays.

As suggested above, forces are generated in mitotic cells that affect the distribution and organization of microtubules by physically moving the microtubule polymers relative to other structures. Interestingly, the idea that similar forces transport microtubules down the axon was proposed over 2 decades ago (reviewed by Baas and Brown, 1997). Since its proposal, however, this idea has met numerous challenges from authors who believe that neuronal microtubules are entirely stationary structures (reviewed by Hirokawa et al., 1997). If true, this latter theory would demand a complete cessation during neuronal development of the kinds of microtubule movements that are so essential for mitosis. Such a paralysis in microtubule movements is counterintuitive because the neuron (with its elongated processes) is arguably the cell type that has the greatest need to transport microtubules. Axons and dendrites do not synthesize tubulin subunits locally, and therefore tubulin must be actively transported in some form. Transporting tubulin in the form of microtubules makes sense for several reasons (see Baas and Brown, 1997), not the least of which is the fact that the mitotic precursor cells that give rise to neurons already have the machinery to do so. Moreover, indirect analyses indicate that microtubules are indeed transported into axons (Yu et al., 1996; Slaughter et al., 1997; Ahmad et al., 1998) and dendrites (Sharp et al., 1995), and we have now directly observed microtubule transport within various regions of living neurons (E. W. Dent, G. Szebenyi, J. L. Callaway, P. W. B., and K. Kalil, unpublished data). Thus, the available evidence indicates that, in fact, postmitotic neurons do not abandon the kinds of motor-driven transport events that are so crucial to organizing microtubules within the mitotic spindle.

The Centrosome as a Generator of Microtubules for Axons and Dendrites

If the neuronal microtubule arrays are established by mechanisms similar to those that organize the mitotic spindle, we would expect the microtubules to be nucleated by γ-tubulin ring structures within the centrosome. To explore the nucleation sites for neuronal microtubules, we initially used immunological techniques to localize γ-tubulin within developing neurons (Baas and Joshi, 1992). We found no detectable γ-tubulin within axons or dendrites. The neuron contains a soluble pool of γ-tubulin and a pool that is associated with the centrosome. On the basis of these observations and the presumption that γ-tubulin is required for the nucleation of microtubules in all cell types, we proposed that the centrosome is the exclusive site of microtubule nucleation within the neuron. That is, microtubules destined

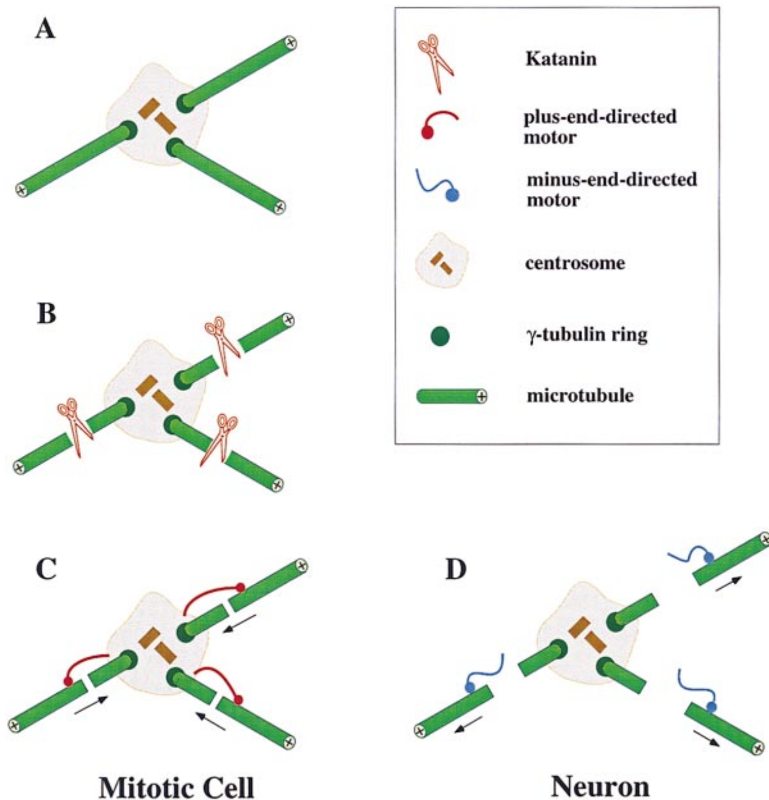

Figure 2. Schematic Illustration Showing How a Modification in the Use of Molecular Motors Could Transform the Centrosome of a Mitotic Cell into a "Generator" of Microtubules for Transport into Neuronal Processes

Microtubules are nucleated within the pericentriolar material by a ring-like structure containing γ-tubulin (A). Nucleation from this structure constrains the lattice of the microtubule to 13 protofilaments. The location of katanin, outside of the pericentriolar material, permits it to sever the microtubule while leaving the γ-tubulin-containing ring structure within the pericentriolar material (B). In the case of the mitotic cell, a plus end–directed motor protein is tethered to the centrosome, holding the severed microtubule in close proximity to it (C). In the case of the neuron, a motor protein does not tether the severed microtubules to the centrosome, thus permitting a minus end–directed motor to transport the released microtubules away from it (D).

for axons or dendrites are nucleated at the centrosome, released, and then actively transported into these processes. To test this idea, we performed drug recovery experiments in which neurons were treated with and then rinsed free of microtubule-depolymerizing drugs (Yu et al., 1993). Within the first few minutes of recovery, enormous numbers of microtubules were nucleated from the centrosome, demonstrating that the neuronal centrosome is a potent microtubule-nucleating structure. With increasing time, the numbers of microtubules attached to the centrosome were diminished, suggesting that the microtubules were rapidly released after their nucleation. As a further test of this interpretation, we injected a function-blocking γ-tubulin antibody into the neurons and found that it severely compromised the formation of new microtubules (Ahmad et al., 1994). Notably, process outgrowth was also severely compromised. These results strongly suggest that a functional centrosome is required for the nucleation of the microtubules that will eventually occupy axons and dendrites. More recently, we found that a function-blocking antibody to katanin, a potent microtubule-severing protein, inhibits the release of microtubules from the centrosome and also severely compromises process outgrowth (F. J. Ahmad, W. Yu, F. J. McNally, and P. W. B., unpublished data). On the basis of all of these results, I would conclude that the centrosome acts as a "generator" of microtubules for the neuron, rapidly nucleating them and releasing them so that they can then be conveyed into developing axons and dendrites.

How does this compare with the situation in the mitotic spindle? After their nucleation by the centrosome, spindle microtubules are presumably released as well. Such release would be necessary for the flux of tubulin subunits that is known to occur as chromosomes move to each spindle pole. Katanin has been proposed to regulate microtubule release during mitosis, but this has not yet been proven. Mitotic cells differ from neurons in that the minus ends of the microtubules remain in close proximity to the centrosome even after they are presumably released. An interesting possibility is that there is a motor protein tethered to the centrosome that tries to move toward the plus ends of the microtubules, thereby pulling on them and keeping them near the centrosome (Sawin et al., 1992). One could imagine that another motor protein (perhaps cytoplasmic dynein; see Gaglio et al., 1996) might generate antagonistic forces that would otherwise convey the microtubules away from the centrosome. If all of this is true, then transformation of a mitotic centrosome into a "microtubule generator" might be as simple as downregulating or modifying the motor protein that draws released microtubules toward the centrosome. In the absence of this motor activity, the microtubules would be actively conveyed away from the centrosome toward the cell periphery, precisely as occurs within the postmitotic neuron. These ideas are shown schematically in Figure 2.

Cytoplasmic Dynein in the Mitotic Spindle and in Postmitotic Neurons

We recently sought to determine the motor protein that actively transports microtubules from the centrosome with their plus ends leading (Ahmad et al., 1998). This motor protein would presumably be utilized to convey microtubules with a plus end–distal orientation into the immature processes, the axon, and the dendrites of

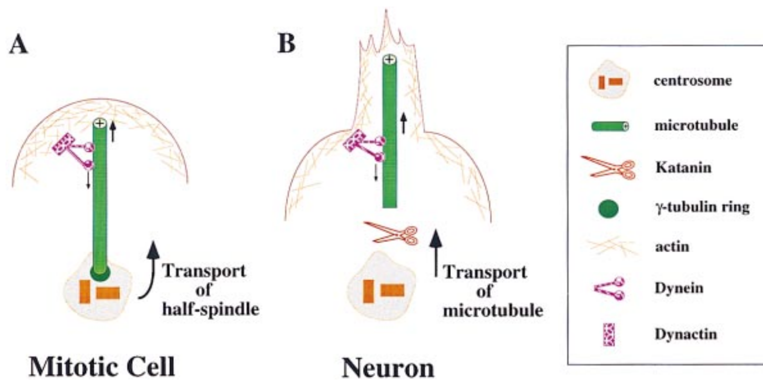

Figure 3. Schematic Illustration Showing that Similar Mechanisms Can Account for Some of the Forces that Transport the Half-Spindles during Late Anaphase and Those that Transport Microtubules with Their Plus Ends Leading into Developing Neuronal Processes

In the mitotic cell, microtubules remain associated with the centrosome, while in the neuron, microtubules are able to move away from the centrosome after their release. In both cases, cytoplasmic dynein generates forces against the actin cytomatrix that cause the microtubule to move outward. In the case of the mitotic cell, the entire half-spindle is transported (A). In the case of the neuron, the released microtubules are transported while the centrosome is left behind (B).

the developing neuron. We reasoned that cytoplasmic dynein may be a good candidate for this motor because it has the appropriate properties to transport microtubules with this orientation, assuming that the "cargo domain" is tethered to a structure with greater resistance to movement than the microtubule. To test whether cytoplasmic dynein might be the relevant motor, we performed experimental analyses on cultured neurons into which we microinjected high levels of recombinant dynamitin protein. Dynamitin is one component of dynactin, a complex of proteins required for all known functions of cytoplasmic dynein. Excess levels of dynamitin cause the dynactin complex to dissociate, thereby inhibiting the functions of cytoplasmic dynein (Echeverri et al., 1996). In our studies, microinjection of the dynamitin protein prohibited the outward transport of microtubules from the centrosome into developing processes. These results indicate that cytoplasmic dynein is required for the outward progression of microtubules from the centrosome, and they suggest that it is a likely candidate for the motor that also conveys microtubules with a plus end–distal orientation down the lengths of developing axons and dendrites.

The precise roles that cytoplasmic dynein plays in mitosis are not entirely clear. Heald and collaborators (1996) have shown that bipolar spindles that form in vitro utilize cytoplasmic dynein to transport short microtubules as cargo toward the minus ends of longer microtubules. However, such transport is not necessary for spindle formation, as the main deficit that results from removing cytoplasmic dynein is that the minus ends of the microtubules fail to focus tightly at each pole. This suggests that cytoplasmic dynein is important for "zippering" together the microtubules by translocating toward their minus ends. Moreover, as noted above, most of the forces relevant to microtubule transport generated by cytoplasmic dynein would probably tend to transport microtubules outward with their plus ends leading. Such forces would be generated via the interaction of the cargo domain of the motor with a nonmicrotubule structure and the motor domain with the microtubule itself. The best argument that such forces exist derives from evidence suggesting that the astral microtubules interact with the cell cortex in such a way as to help drive apart the duplicated centrosomes in prophase and to help drive apart the half-spindles in late anaphase (Carminati and Stearns, 1997; Busson et al., 1998; Inoue et al., 1998). Because the microtubules remain tethered to the centrosome, these forces drive the microtubule/centrosome complex toward the cell periphery. But if the microtubules were not tethered, the centrosome would remain stationary and the microtubules would be driven outward, which is precisely what happens in the neuron. This observation suggests that cytoplasmic dynein probably generates forces against similar structures in neurons and mitotic cells. Given that the cell cortex is an actin-rich region, we suspect that the structures against which these forces are generated are probably components of the actin-based cytomatrix. These ideas are shown schematically in Figure 3.

Interestingly, a separate line of reasoning also implicates the actin cytomatrix. Prior to our experimental studies, the Pfister laboratory determined that almost all of the cytoplasmic dynein that is anterogradely transported down the axon moves in the same phase of axonal transport as the actin cytomatrix (Dillman et al., 1996). This result was initially surprising because neuroscientists had previously thought of cytoplasmic dynein exclusively as a retrograde vesicle transporter. On the basis of these new findings, it was proposed that cytoplasmic dynein transports microtubules anterogradely down the axon by generating forces against the actin cytomatrix. The cytomatrix also moves anterogradely down the axon (via another motor, presumably myosin) and therefore provides excellent resistance to backward movement. The fact that the microtubules move somewhat slower than the actin cytomatrix probably relates to an intermittent association of the microtubules with the transport machinery. Indeed, live-cell observations indicate that microtubules can move much faster than the average rates of slow axonal transport, but that they undergo intermittent stops and starts in their movement (E. W. Dent, G. Szebenyi, J. L. Callaway, P. W. B., and K. Kalil, unpublished data). I find a model involving the actin cytomatrix attractive not only because it correlates with mitotic mechanisms, but also because it is consistent with a large body of evidence suggesting a functional link between the actin and microtubule systems of the neuron (reviewed by Gavin, 1997).

CHO1/MKLP1 in the Mitotic Spindle and Postmitotic Neurons

Movement of microtubules by cytoplasmic dynein against the actin cytomatrix can explain the entry and transport

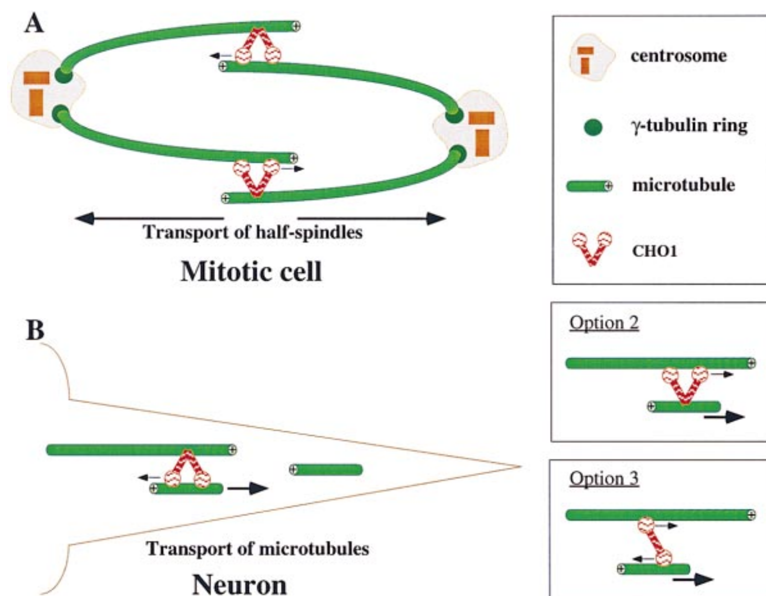

Figure 4. Schematic Illustration Showing that Similar Mechanisms Can Account for Some of the Forces that Transport the Half-Spindles during Late Anaphase and Those that Transport Microtubules with Their Minus Ends Leading into Developing Dendrites

In the mitotic cell, microtubules remain associated with the centrosome, while in the neuron, microtubules are able to move away from the centrosome after their release. In the case of the mitotic cell, CHO1/MKLP1 drives apart the oppositely oriented microtubules in the midzone by transporting the plus ends of microtubules toward the minus ends of other microtubules (A). In the case of the neuron, CHO1/MKLP1 transports minus end–distal microtubules into developing dendrites by generating forces against plus end–distal microtubules (B). Three options are shown for the means by which CHO1/MKLP1 (which is thought to form a dimer) transports minus end–distal microtubules into the dendrite. The minus end–distal microtubule is assumed to be shorter than the plus end–distal microtubule. One possibility is that the motor domains of the dimer interact with the minus end–distal microtubule, while the cargo domains interact with the plus end–distal microtubule. Because the longer microtubule has greater resistance to movement, the shorter microtubule moves with its minus end leading toward the plus end of the longer microtubule (B). The second option is that the motor domains interact with the longer plus end–distal microtubule, while the cargo domains interact with the shorter minus end–distal microtubule. In this option (which does not readily explain the polarity orientation of the minus end–distal microtubule), the minus end–distal microtubule is carried as cargo along the plus end–distal microtubule. The third option is that the two motor domains interact with the oppositely oriented microtubules, generating forces that drive the minus end of the short microtubule toward the plus end of the longer microtubule.

of plus end–distal microtubules down axons and dendrites and is sufficient to explain the uniformly plus end–distal orientation of axonal microtubules. However, there must be at least one additional motor activity that transports microtubules with their minus ends leading specifically into dendrites but not axons. In vitro studies have shown that the mitotic motor CHO1/MKLP1 is able to transport microtubules with their minus ends leading toward the plus ends of other microtubules (Nislow et al., 1992). Thus, it would have the appropriate properties to intercalate minus end–distal microtubules among plus end–distal microtubules in developing dendrites. As noted above, CHO1/MKLP1 is present in the midzonal region of the mitotic spindle, where it is thought to help drive the two half-spindles apart during late anaphase. Recent studies from my laboratory have shown that CHO1/MKLP1 is expressed in neurons well past their terminal mitotic division and is most highly expressed during dendritic development (Sharp et al., 1997; Ferhat et al., 1998a). In addition, we have shown that CHO1/MKLP1 is detectable only in the somatodendritic compartment of the neuron, and that inhibition of its expression with antisense oligonucleotides obliterates dendritic differentiation (Yu et al., 1997; Sharp et al., 1997). Finally, expression of a fragment of the CHO1/MKLP1 molecule in nonneuronal Sf9 cells induces these normally rounded cells to extend elongate processes with a dendrite-like morphology and a nonuniform pattern of microtubule polarity orientation (Sharp et al., 1996). Collectively, these observations strongly suggest that the same motor protein, CHO1/MKLP1, is utilized for analogous functions in the mitotic spindle and within the postmitotic neuron, namely to transport oppositely oriented microtubules relative to one another (see Figure 4).

The obvious question related to the neuron is how CHO1/MKLP1 specifically targets minus end–distal microtubules to developing dendrites (and not the axon). At present, the answer to this question is not known. However, it may be relevant that expression of the full-length CHO1/MKLP1 molecule does not induce Sf9 cells to extend dendrite-like processes (Sharp et al., 1996), even though neurons apparently express the full-length molecule rather than a truncated variant (see Sharp et al., 1997; Ferhat et al., 1998a). One possibility is that the C-terminal region contains sites that can regulate whether or not the molecule is active within the postmitotic neuron. Perhaps there is a factor specific to the somatodendritic domain of the neuron that interacts with these sites to activate the full-length CHO1/MKLP1 molecule, thus permitting it to transport minus end–distal microtubules. Additional studies will be needed to explore this possibility and to determine how the transport of minus end–distal microtubules by CHO1/MKLP1 is restricted specifically to dendrites. We find it compelling to contemplate that the targeting of CHO1/MKLP1 might somehow relate to MAP2, another cytoskeletal protein that interacts with microtubules in the somatodendritic domain of the neuron (reviewed by Matus, 1994).

Eg5 in the Mitotic Spindle and Postmitotic Neurons

In the mitotic spindle, changes in microtubule organization are regulated by complementary and antagonistic forces generated by a variety of different motor proteins. Might other mitotic motor proteins (in addition to cytoplasmic dynein and CHO1/MKLP1) play analogous roles in organizing the microtubule arrays of the postmitotic neuron? In recent studies, we have begun to investigate

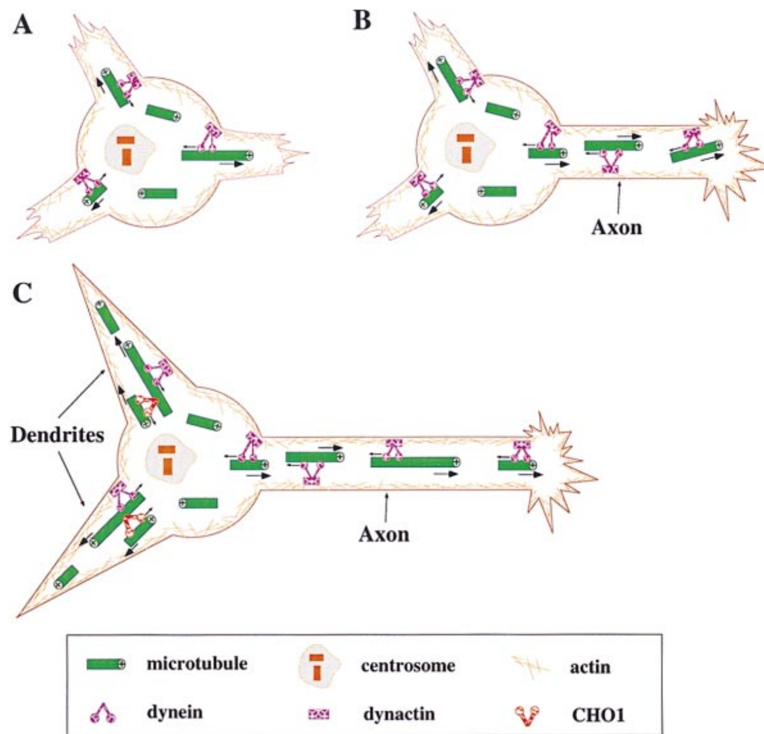

Figure 5. Schematic Illustration Summarizing a Model for the Establishment of the Microtubule Arrays of the Neuron

After their nucleation and release from the centrosome, microtubules are transported by cytoplasmic dynein against the actin cytomatrix into developing immature processes (A). Microtubule transport becomes more active in one of the immature processes compared to the others, and this process becomes the axon (B). Here, the actin cytomatrix is shown only in the cortical regions where it is known to be enriched, but the actin cytomatrix is also present throughout the cytoplasm of the neuron. CH01/MKLP1 transports microtubules with minus ends leading against oppositely oriented microtubules into the immature processes that did not become the axon, thus contributing to their transformation into dendrites (C).

the motor protein known as Eg5 (the vertebrate member of the *bim*C family of kinesin-related proteins), which is important for generating forces (presumably between antiparallel microtubules) that separate the duplicated centrosomes during prophase (reviewed by Kashina et al., 1997). In vitro analyses suggest that another role of Eg5 might be to antagonize forces generated by cytoplasmic dynein later in mitosis (Gaglio et al., 1996). We have found that Eg5 continues to be expressed in postmitotic neurons, where it is concentrated on microtubules in the distal regions of growing axons and dendrites (Ferhat et al., 1998b). Depending on the structures with which it associates, one could envision that Eg5 might either antagonize or complement the anterograde transport of microtubules by cytoplasmic dynein. Another possibility is that Eg5 might form complexes that do not actually transport microtubules but instead move along neighboring microtubules toward their plus ends, thereby "zippering" the polymers together in functionally important regions of the cytoplasm. This latter possibility is similar to the manner by which cytoplasmic dynein is thought to focus minus ends of microtubules at the centrosomes during mitosis.

Microtubules and Neuronal Polarity: Lessons from Mitosis

It is clear that terminally postmitotic neurons do not organize their microtubules into bipolar spindles. However, the observations that I have outlined in this article suggest that the axonal and dendritic microtubule arrays may be established by mechanisms very similar to those used for the formation and functioning of the mitotic spindle. Specifically, it appears that in both cases, microtubules are nucleated by γ-tubulin ring structures within the pericentriolar material and then released, presumably by katanin. After their release, the microtubules in the mitotic cell are somehow tethered to the centrosome, possibly by a plus end–directed motor protein. In the case of the neuron, the microtubules are not tethered to the centrosome, and hence they can be actively transported into axons or dendrites by available motor proteins. Initially, neurons extend several immature processes. Available evidence suggests that cytoplasmic dynein is responsible for conveying microtubules into all of these processes with plus ends leading, thereby establishing a uniformly plus end–distal microtubule polarity pattern (see Figure 5A). In order to move the microtubules, cytoplasmic dynein probably generates forces against the actin cytomatrix. This is analogous to the manner by which cytoplasmic dynein generates forces between astral microtubules and the cell cortex during mitosis. Microtubule transport becomes more active in one of the immature processes compared to the others, and as a result this process develops into the axon (Yu and Baas, 1994; see Figure 5B). Precisely how this occurs is unknown, but it may relate to an as yet unidentified motor that generates antagonistic forces within the nonaxonal processes or complementary forces within the axon.

After the axon has differentiated, CHO1/MKLP1 begins to transport microtubules with their minus ends leading against microtubules of the opposite orientation within the immature processes that did not become the axon (see Figure 5C). These forces are analogous to those generated between microtubules of opposite orientation within the spindle midzone during late anaphase. It is not known how minus end–distal microtubules are targeted to all of the processes except the axon, but this targeting may relate to other dendrite-enriched proteins such as MAP2. As the axon continues to develop, the microtubule array within the growth cone changes to accommodate the navigation of the axon

toward appropriate environmental cues. Specifically, the microtubule array invades the region of the growth cone oriented in the direction of future growth (see, e.g., Tanaka et al., 1995). Enhancing local interactions between microtubules, the actin cytomatrix, and cytoplasmic dynein could be responsible for this selective invasion. Alternatively, the relevant forces for selective microtubule invasion might be generated by Eg5 or other as yet undiscovered motor proteins. This kind of modulation of microtubule transport might also help determine the characteristic lengths that each type of process can achieve and might influence important events such as the formation of interstitial branches (Yu et al., 1994; E. W. Dent, G. Szebenyi, J. L. Callaway, P. W. B., and K. Kalil, unpublished data) or dendritic sprouts (Ferhat et al., 1998b). In addition, it is not difficult to imagine how the pertinent molecules could be regulated by environmental cues relevant to neuronal development. For example, many "mitotic" proteins are regulated by phosphorylation, and so too are many events fundamental to neuronal differentiation.

In considering these ideas, it is important to acknowledge that the mechanisms that organize microtubules during mitosis and neuronal differentiation remain controversial, and far more information is needed on both topics in order to evaluate many of the specific ideas that I have suggested in this article. Nevertheless, there is very strong evidence to support the broad conclusion that neurons do not abandon the mechanisms that mitotic cells use to organize their microtubules. Rather than "reinventing the wheel," it appears that neurons establish their microtubule arrays using modifications of the same basic blueprint that dividing cells use to organize their microtubules during mitosis. The answers to many of the most fundamental questions regarding neuronal polarity may indeed lie within the mitotic spindle.

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