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REVIEW

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A role of microtubules during the formation of cell processes in neuronal and non-neuronal cells

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Abstract This review discusses the role of microtubules in the formation of processes from neuronal and non-neuronal cells. In elongating axons of the neuron, tubulin molecules are transported toward the end of pre-existing microtubules, which may be nucleated at the centrosome, via a mechanism called slow axonal flow. Two different hypotheses are presented to explain this mechanism; the transport of soluble monomers and/or oligomers versus the transport of polymerized microtubules. The majority of tubulin seems to be transported as small oligomers as shown by the data presented so far. Alternatively, an active transport of polymerized microtubules driven by microtubule-based motor proteins is postulated as being responsible for the non-uniform polarity of microtubule bundles in dendrites of the neuron. Microtubule-associated proteins (MAPs) play a crucial role in stabilizing the microtubular arrays, whereas the non-uniform polarity of microtubules may be established with the aid of microtubule-based motor proteins. The signals activating centrosomal proteins and MAPs, resulting in process formation, include phosphorylation and dephosphorylation of these proteins. Not only neuronal cells, but also renal glomerular podocytes develop prominent cell processes equipped with well-organized microtubular cytoskeletons, and intermediate and actin filaments. A novel cell culture system for podocytes, in which process formation can be induced, should provide further evidence that microtubules play a pivotal role in process formation of non-neuronal cells.

Key words Cell process · Cytoskeleton · Microtubule · Neuron · Podocyte

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Introduction to the cell processes

Each cell has its cell-type-specific morphology, and thus we can find a clear morphological diversity in the world of the cell: some cells are highly polarized, some are flat or elongated, and some are arborized bearing cell processes. One of the goals for cell biologists is to understand the mechanism by which the diversity of cell shape is achieved. Morphological diversity is evidently established and maintained by, for example, the heterogeneous cytoskeletal system, extracellular matrices, interaction with neighboring cells, and mechanical stimuli.

The cell process has been paid particular attention with respect to morphological heterogeneity. It is a very attractive system that requires intra- and extra-cellular mechanisms to withstand surface tension on the plasma membrane. The long history of histology has revealed several kinds of cells with prominent cell processes in situ, including neurons, glial cells, and renal glomerular podocytes.

Among process-bearing cells, the neuron has been, so far, the most intensely studied, because neurons possess two types of highly polarized cell processes, viz., the axons and dendrites, and because culture systems allowing selective pharmacological and cell biological experiments have long been established. Furthermore, the neuron is undoubtedly the unit of brain function, which represents one of the largest enigmas for mankind. Neurites contain microtubules (MTs), neurofilaments (the neuronal subtype of intermediate filaments), and actin filaments as the three major cytoskeletal components, whereas the content of each differs between the axon and the dendrite (Hirokawa 1982, 1993). These cytoskeletal components are crosslinked with each other by many associated proteins. The proteins associated with MTs are called microtubule-associated proteins (MAPs) and were first described and characterized in neuronal cells (reviewed in Hirokawa 1993; Lee 1993). It is well known that the axon and the dendrite contain different MAPs. Although the neuron still forms the center of interest of many researchers, our knowledge is now expanding from neurons to non-neuronal cells.

We will first discuss the important theme of the dynamic architecture of neuronal MTs in this review. Based on these discussions, we will then try to extend our knowledge to microtubular organization in non-neuronal cells, especially in the renal glomerular podocyte.

Transport of tubulin, polarity of microtubules, and microtubular organizing centers in neurons

Microtubules are polarized structures with fast-growing (plus-) and slow-growing (minus-) ends. In many cells, minus-ends of MTs have been shown to be bound with or closely located to the centrosome, an important microtubular organizing center (MTOC) that facilitates the nucleation of MTs, i.e., the formation of new polymer from subunits. In these cases, MTs grow only at the plus-end; tubulin subunits are added onto the fast-growing end of the pre-existing MTs. The polarity of MTs in vivo has been visualized in electron micrographs by dynein-arm decoration (Haimo 1982) and tubulin-hook decoration (Heidemann 1991). As the polarity of MTs differs between the two types of neuronal processes (Baas et al. 1989), we will separately discuss microtubular organization in axons and in dendrites.

MTs in axons: mechanism of the slow axonal transport of tubulin

It is now well known that MTs in axons are oriented in the "plus-end-distal" fashion (see, for example, Baas et al. 1989). The plus-end-distal MTs in the axons are assumed to nucleate at, or closely related to, the centrosome located in the cell body and then elongate from the pre-existing MTs. During the recovery phase from MT-depolymerization in cultured neurons, MTs are observed to nucleate from the centrosome, showing that the centrosome is active in these neurons (Yu et al. 1993).

The molecular unit of MTs, tubulin, must be transported along the axoplasm toward the tip of the growing ends, as axons possess no machinery for protein synthesis. Slow axonal flow is the transporting system for cytoskeletal proteins, whereas fast axonal flow conveys membrane-bound organelles. There are two major theories for the mechanism by which tubulin molecules are transported along the axon. According to one hypothesis (Okabe and Hirokawa 1993c, b), tubulin is transported as monomers and/or oligomers that can be easily washed out from permeabilized cells. In contrast, the other hypothesis claims that tubulin is transported not only as these relatively small forms, but also as polymers, and that this transport is driven by some motor molecule (Baas 1997).

By using microinjection of labeled molecules, Hirokawa's group has repeatedly shown that tubulin is transported in the soluble form (i.e., monomer, dimer, and/or oligomer) along the axon of cultured neurons (Okabe and Hirokawa 1988, 1990, 1992, 1993a, b; Funakoshi et al. 1996) and living neurons in situ (Takeda et al. 1995). Other groups have also provided evidence for the transport of monomers or oligomers by microinjection and metabolic labeling (Sabry et al. 1995; Campenot et al. 1996; Miller and Joshi 1996), and it has been shown that tubulin is added preferentially onto the plus-end of pre-existing MTs (Okabe and Hirokawa 1988; Baas and Ahmed 1992; Li and Black 1996). This theory proposes that MTs are stationary, with the polymer in the axoplasm and tubulin dimers being added onto the plus-end (i.e., the distal end) of the pre-exiting MTs during axonal growth.

Although MTs provide the structural basis for the motor proteins conveying vesicles in the axoplasm (Hirokawa 1996), MTs themselves are thought to be stationary and cannot be transported along the axon, because MTs are bridged with each other and with other cytoskeletal elements, such as neurofilaments, via MAPs and other molecules (Hirokawa 1982; Zagon et al. 1986). MAPs compete for binding onto MTs with the MT-based motor proteins, viz., kinesin and dynein (Hagiwara et al. 1994). Therefore, MAPs may generally inhibit the translocation of MTs in microtubular arrays in vivo. MT-based organelle transport is thought to be promoted only along a limited microtubular "highway" in the axoplasm (Miller et al. 1987); this route may lack or contain small numbers of MAPs to facilitate the binding of motor proteins onto MTs.

On the other hand, Baas and co-workers continue to argue that MTs can be transported as polymers (Baas 1997). This hypothesis is based on several approaches, including pharmacological, electron-microscopic, and molecular biological experiments. They propose the polymer-transporting-model for both axons and dendrites (dendritic machinery will be discussed later.)

In cultured neurons during the recovery phase of MT arrays after depolymerization with nocodazole, MTs nucleate at the centrosomes, thereafter being released (Yu et al. 1993). It has been claimed that MT-severing proteins, such as katanin, which is reported to be localized at centrosomes (McNally et al. 1996), are actively involved in this releasing process (Baas 1997). The released MTs are considered to move into both axons and dendrites in the presence of nano-molar concentration of vinblastine, an MT-depolymerizing toxin thought to inhibit both nucleation and elongation of MTs (Ahmed and Baas 1995; Sharp et al. 1995). However, Miller and Joshi (1996) have reported the assembly of MTs even in the presence of vinblastine at such concentrations. As discussed later, our data on cultured podocytes also show that some MTs elongate even in the presence of vinblastine, although their distribution pattern seems abnormal; in these experiments, cells are exposed at maximum to a ten-times higher concentration of vinblastine than that used by Baas's group (N. Kobayashi et al., in preparation). Therefore, Baas's argument based on the pharmacological data should be more carefully interpreted. Miller and Joshi (1996) have reinterpreted the data of Baas's group based on dynamic instability theory (Mitchson and Kirschner 1984; Walker et al. 1988). However, Baas (1997) has criticized such objections and insists on his own interpretation. Nevertheless, the implications of such

experiments with vinblastine to inhibit MT dynamics are still a matter of debate.

In a recent study (Yu et al. 1996), Baas's group have taken advantage of electron microscopy with high spacial resolution coupled with microinjection of biotinylated tubulin into cultured neurons. They have observed that most MTs in the newly elongated regions of growing axons can be labeled by anti-biotin markers, showing that these MTs are assembled from exogenous tubulin subunits after microinjection. At the same time, the authors have also observed non-labeled MTs that appear to contain no exogenous tubulin, in these regions. They postulate that these MTs are assembled before the microinjection of labeled tubulin and are then transported into the growing regions. However, one could also argue that such non-labeled MTs are made from tubulin synthesized after the injected tubulin subunits are spent, because all the MTs in one fixed region need not be assembled at the same time, the incorporation of tubulin subunits at the end of MTs occurring at all times all over the axoplasm.

More direct evidence for MT-transport has been obtained from experiments on living giant axons of squid (Terasaki et al. 1995). When fluorescence-labeled and taxol-stabilized MTs are injected into the giant axons, they move anterogradely. The maximal speed of transport (0.25 mm/s) is half that for vesicle transport by kinesin, which drives the fast axonal flow (Vale et al. 1985a, b), but because their movement is saltatory, the average rate (0.03 mm/s) is approximately at the reported rate of slow axonal flow. This phenomenon implies that not only the membrane organelles, but also polymerized cytoskeletal elements are transported along the axon actively by distinct motor proteins. On the other hand, Miller and Joshi (1996) have reported that stabilized MTs remain within the cell body of cultured neurons, even 30 h after injection. As they suggest, this kind of transport could be dependent on the size of the injected objects. One can also argue that these experiments represent a model not for slow cytoskeletal transport but for fast vesicle transport. Although it is important to note that polymerized MTs can be transported in the living axonal cytoplasm, more precise methods for detecting the movement of single or thin bundles of MTs may be required to establish the occurrence of the translocation of MTs in the axoplasm in vivo.

Most data presented so far support the transport of soluble forms of the tubulin molecule by slow axonal flow, whereas only limited evidence exists for the transport of the polymer form. Although the latter is still an attractive model and should be more carefully verified, it is probably reasonable to conclude at present that the majority of tubulin is transported in the soluble form, i.e., as monomers, dimers, and/or oligomers.

Non-uniform orientation of MTs in the dendrites: do dendrites have transporting mechanisms, too?

Baas and colleagues (1989) have reported that the polarity of the MTs in dendrites changes from the uniform (plus-end-distal) to the non-uniform (both plus- and minus-end-distal) type during the maturation of cultured hippocampal neurons. By tubulin-hook decoration and reconstruction of serial ultrathin sections, they have found that the proximal part of the dendrite contains more minus-end-distal MTs than the distal part, showing a gradient in the content of minus-end-distal MTs. They also noticed that the increase in the amount of minus-end-distal MTs was dependent on the developmental stages of the cultured neurons.

In order to explain this phenomenon, the authors have presented a hypothesis that minus-end-distal MTs are actively transported by anti-parallel sliding along the pre-existing plus-end-distal MTs. As a candidate for such a motor protein, they considered a member of the kinesin superfamily, CHO1/MKLP1, which was originally found in mitotic spindles, and which acts as a motor to induce anti-parallel sliding of MTs (Nislow et al. 1992). Thick cell processes containing tight MT-bundles are reported to be induced in Sf9 cells transfected with truncated cDNAs for CHO1/MKLP1 consisting of its N-terminal half, which represents its motor activity and is necessary to bind onto MTs (Kuriyama et al. 1994). Sharp et al. (1996) have found that, like MTs in dendrites of cultured neurons, the microtubular orientation in these "dendrite-like" thick cell processes is non-uniform and that the frequency of minus-end-distal MTs in these processes increases in a time-dependent manner. Next, this group showed not only the expression of CHO1/MKLP1 protein in a neuroblastoma cell line, but also the translocation of this motor molecule into the dendrite-like processes during neurite elongation, and finally they could inhibit the establishment of non-uniform MT-orientation by treatment with anti-sense oligonucleotides for this motor protein (Yu et al. 1997). The reason that they could induce only a few cell processes by transfection with full-length cDNA of CHO1/MKLP1 into Sf9 cells (Kuriyama et al. 1994) is, however, still open for discussion. In any case, these data strongly suggest, for the first time, that motor proteins are involved in the microtubular organization of cell processes, and that the same motor protein has a dual relevance both in the mitotic spindle of proliferating cells and in the cytoskeletal reorganization of differentiated cells.

Microinjected tubulin subunits have been shown to form short MT-fragments in cell bodies probably first around the centrosomes, and thereafter, the labeled molecules move into dendrites in cultured neurons (Wang et al. 1996a). Although exogenous tubulin molecules are probably assembled at centrosomes and subsequently disassembled to supply subunits for new assembly in developing dendrites, the data from microinjection studies seem to corroborate the transport of MTs as polymers. In parallel, the addition of exogenous tubulin onto the pre-existing MTs has also been observed.

Taken together, these data lead to the hypothesis that MTs are oriented non-uniformly in dendrites, a feature that is indeed observed in cultured neurons (Baas et al. 1989). First, plus-end-distal MTs arrays might be estab-

lished as discussed for axons (see above). Second, fragments of MTs might be assembled at centrosomes and then transported into the growing dendrites by MT-based motor proteins in a minus-end-distal fashion. Third, tubulin subunits might be added onto these MTs to complete the non-uniform orientation of MTs. Transported MTs would soon be stablilized by crossbridging with other MTs and neurofilaments, e.g., via MAP2 (Hirokawa et al. 1988), in the proximal dendritic cytoplasm, resulting in the relatively higher content of minus-end-distal MTs in the proximal region of dendrites in the later stages; this would be consistent with the previous observation (Baas et al. 1989). This hypothesis has to be discussed and verified further; nevertheless, the data provides an attractive story for microtubular organization in dendrites.

As to the functional significance of such non-uniform arrangement of MTs in dendrites, it has been proposed that minus-end-distal MTs are responsible for the transport of organelles required only in dendrites, e.g., the Golgi apparatus (Baas et al. 1989). Cargos meant only for dendrites could be easily segregated, if neurons express some MT-based minus-end-directed motor proteins (i.e., motors transporting their cargos along MTs toward the minus-end) that will be never able to enter into the axoplasm where minus-ends of MTs are oriented toward the cell body. A candidate for such dendritic motors has recently been found and designated KIFC2 (Hirokawa 1996; Hanlon et al. 1997; Saito et al. 1997). KIFC2, a member of the kinesin superfamily, is reported to be a neuron-specific minus-end-directed motor protein that is expressed exclusively in dendrites. Such molecules might contribute to the transport of membrane organelles only in the dendrite. Together with the transport of MTs themselves, we now have to accept the mechanism of "dendritic flow" as an attractive theme for cell biology, in addition to axonal flow.

One has to be careful to distinguish the de novo nucleation of MTs from their elongation. Whereas the elongation of MTs occurs at the end of pre-existing MTs, MTOCs are required to nucleate MTs under physiological conditions (reviewed in Thaler and Haimo 1996). From the view-point of MT-nucleation, two opposing possibilities might explain the non-uniform polarity of MTs in dendrites: (1) MTs, which are nucleated at the centrosome, are transported as polymers in a minus-end-oriented fashion (Sharp et al. 1995); (2) within dendrites, MTOCs might exist that facilitate MT-nucleation in both directions (plus-end-distal and minus-end-distal) or exclusively toward the proximal orientation. However, if the centrosome is the only MTOC in neurons, then minus-end-distal MTs must be transported from cell bodies into dendrites. As discussed below, the first postulate, i.e. the polymer transport of minus-end-distal MTs, should represent the actual phenomenon.

Gamma-tubulin is a new member of the tubulin family and binds the minus-end of MTs (Li and Joshi 1995). Several groups have provided evidence that gamma-tubulin can nucleate the assembly of MTs by making a ring-like structure for the nucleating core of MTs (Shu and Joshi 1995; Zheng et al. 1995). Immunocytochemistry has indicated that gamma-tubulin is localized only around the centrosomes in cultured neurons (Baas and Joshi 1992). Even when overexpressed, gamma-tubulin remains in the neuronal cell body and is not distributed into the neurites (Miller and Joshi 1996). Furthermore, this molecule is thought to be necessary for MT-nucleation in fibroblasts and in neurons. In the former, formation of the mitotic spindles is totally inhibited by an anti-gamma-tubulin antibody (Joshi et al. 1992). In the latter (Ahmed et al. 1994), neurite growth has been shown to be totally abolished in one half of neurons injected with an antibody against gamma-tubulin during the recovery phase after MT-depolymerization, whereas in the other half of the injected cells, the amount of MTs is reduced, although they can elongate neurites. Thus, the centrosome seems to be the only gamma-tubulin-containing organelle and also appears to represent the only MTOC in neurons. These findings further support the hypothesis of MT-transport in dendrites.

Are there any other MTOCs that allow the de novo nucleation of MTs in the dendritic cytoplasm in both distal and proximal directions? By a biochemical approach, gamma-tubulin has also been found in the cytosolic fractions of a lymphoblastic cell line (Moudjou et al. 1996). Microinjection of biotin-labeled tubulin into PC12 cells has shown that exogenous tubulin molecules are not only incorporated into pre-existing MTs, but also nucleated from electron-dense materials whose nature is not clear (Okabe and Hirokawa 1988). Takemura and colleagues (1995) have reported that MTs are nucleated, but not related to the centrosome, in the peripheral cytoplasm of fibroblasts that are transfected with MAP2c cDNA. At present, we cannot completely exclude the presence of MTOCs other than the centrosome. Nevertheless, the inhibition of neurite growth by anti-gamma-tubulin antibody clearly shows that the majority of MTs in neurons are nucleated at centrosomes.

Process formation by bundling microtubules: a role of MAPs

In the cytoplasm, MTs consist not only of tubulin subunits, but also of several associated proteins called MAPs (reviewed in Hirokawa 1993, 1994; Kanai and Hirokawa 1993; Lee 1993; Thaler and Haimo 1996). In neurons, MAP1A, MAP1B, and MAP2 represent high molecular weight (>200 kDa) MAPs, whereas tau proteins (55–65 kDa) are the major low molecular weight MAPs. It is well known that isoforms of MAP2, viz., high molecular weight isoforms MAP2A and MAP2B, and low molecular weight isoforms MAP2c and MAP2d, are derived from a single gene by alternative splicing (Ferhat et al. 1994). Similarly, alternative splicing delivers the isoforms of tau, including six conventional small isoforms and high molecular weight isoforms specific for the peripheral nervous system (Couche et al. 1992; Goedert et al. 1992; Kanai et al. 1992). Molecular analysis of these MAPs has re-

vealed that MAP2, tau, and MAP4, which is an ubiquitous MAP in non-neuronal cells, share the conserved domain for binding with MTs, which contains 3–5 repeats of 18-amino-acid motifs called "repeats-domains" or "assembly-promoting domains" (Chapin et al. 1995). The "repeats" are located in the C-terminal region of these MAPs and there is another conserved domain with enriched proline residues in the adjacent region. These two domains are thought to be responsible for the bundling with MTs, whereas MAP1A and MAP1B are reported to possess an MT-binding domain unrelated to those of MAP2 and tau (Noble et al. 1989). The major function of MAPs is thought to be to stabilize the microtubular dynamics and to crossbridge MTs into bundles. As discussed later, some MAPs are known to connect MTs with other cytoskeletal elements.

There is a dynamic equilibrium between polymerized MTs and tubulin subunits, called dynamic instability (Mitchson and Kirschner 1984; Walker et al. 1988; Carlier 1989). According to this theory, individual MTs can switch stochastically between the slow growing phase and the fast shrinking "catastrophe" phase. Conventional MAPs can stabilize MTs by suppressing the catastrophe (Cassimeris 1993; Hirokawa 1994), whereas an MT-destabilizing protein has recently been reported to increase the rate of catastrophe, thereby facilitating cytoskeletal reorganization for mitosis (Belmont and Mitchison 1996).

Cell processes containing MT-bundles formed by MAPs

It has been clearly shown that some, but not all, MAPs induce the formation of MT-based cell processes when they are overexpressed in cells such as fibroblasts, which originally possess only actin-based cell projections (Kanai et al. 1989; Lewis et al. 1989; Baas et al. 1991; Knops et al. 1991; Chen et al. 1992; Lee and Rook 1992; Weisshaar et al. 1992; Edson et al. 1993; Takemura et al. 1992, 1995; Brandt and Lee 1994; Ferhat et al. 1996). For example, the exogenous induction of tau and MAP2c is proposed to mimic axonal elongation during neuronal development. Tau has some juvenile isoforms and MAP2c is also a type of juvenile MAP, and their expression is strongly regulated developmentally (Hirokawa 1993; Lee 1993). Excessively expressed, these MAPs facilitate the formation of cell processes filled with uniformly oriented MT-bundles like those in axons. The elevated concentration of MAPs may also influence the total amount of tubulin in MAP-overexpressing cells. Exogenous induction of tau proteins is followed by an increase in the amount of tubulin (Kanai et al. 1989), although another study has not confirmed such an increase (Barlow et al. 1994).

On the other hand, transfection of cDNAs for non-neuronal MAPs, viz., MAP4 and *Drosophila* 205 kDa MAP, has only a small effect on microtubular organization (Barlow et al. 1994). Because transfection of tau induces process formation in this experiment, one can argue that these non-neuronal MAPs have only a "weak" effect on the organization of MT-arrays, although MAP4 also contains repeat-domains and proline-rich domains, which are highly homologous to those of tau and MAP2, for binding with MTs (Chapin et al. 1995). It is reasonable that MAP4, which is ubiquitously expressed in cells without cell processes (Bulinski and Borisy 1980), can induce no process formation. The function of non-neuronal MAPs should be clarified in the future.

MT-based cell processes are induced not only by transfection of MAPs but also by overexpression of proteins in other categories. As discussed above, overexpression of CHO1/MKLP1, which is a member of the kinesin superfamily and works as an MT-based motor (Nislow et al. 1992; Kuriyama et al. 1994), induces process formation (Sharp et al. 1996). Therefore, tight bundling of MTs in the cytoplasm is able to cause the deformation of plasma membranes, resulting in process formation.

Deletion of MAPs, by applications of specific antibodies and anti-sense oligonucleotides and in knock-out mice, is another powerful strategy for investigating the function of MAPs. Anti-sense probes for tau and MAP2 have been shown to inhibit process formation in cultured neurons (Caceres and Kosik 1990; Dinsmore and Solomon 1991). MAP1B-depleted mice exhibit a severe defect in neurogenesis (Edelmann et al. 1996). Evidence strongly suggests that MAPs comprise a redundant system compensating each other's functions, i.e. the phenotype of cells in which expression of one MAP is diminished is unchanged. No obvious deleterious phenotype has been observed in cells injected with anti-MAP4 antibody, which removes MAP4 from MTs (Wang et al. 1996b); in this case, it is thought that other MAPs compensate for the function of MAP4. Mice lacking the normal tau gene are reported to have altered MT-arrays in parallel fiber axons, although these mice are born alive and are fertile (Harada et al. 1994). Because tau-lacking mice show increased levels of MAP1A, the expression of MAPs could be regulated, at least in part, to compensate their redundant function with each other. Obviously, double- and triple-knock-out mice for MAPs will provide new insights into the functional relevance of MAPs.

It is clear that not only MTs, but also neurofilaments and actin filaments are necessary for neurite formation. Some proteins are reported to crosslink these major cytoskeletal components. Neuronal MAPs, including MAP1A, MAP2, and tau, are reported to bind not only with MTs, but also with actin filaments (Correas et al. 1990; Cross et al. 1993; Pedrotti et al. 1994). MAP2c is found in actin-rich regions devoid of MTs and could influence the formation of actin gels, whereas high molecular weight isoforms of MAP2 lack such activity (Cunningham et al. 1997). MAP2d, a recently described isoform of MAP2, can stabilize actin filaments against depolymerization by cytochalasin D (Ferhat et al. 1996). MAP2 is also able to link MTs to neurofilaments in vitro and in vivo (Hirokawa et al. 1988). A subtype of spectrin, fodrin (brain spectrin), is reported to crossbridge many cytoskeletal structures in neurons (Zagon et al. 1986). Recently, plectin has been reported as a multifunctional crosslinker among cytoskeletal and other intracellular structures in

non-neuronal cells (Svitkina et al. 1996). Further studies will presumably reveal more molecules that contribute to stabilizing the complex network of the neuronal cytoskeleton.

From this viewpoint, a report should be noted showing that depolymerization of actin filaments with cytochalasin induces process formation in MAP2c-transfected cells, and that this induction is cancelled by removal of this drug (Edson et al. 1993). These cells form MT-bundles along the cell margins before addition of the actin-depolymerizing reagent (Weisshaar et al. 1992). This result strongly suggests that process formation in the MAPs-transfected cells not only depends on MT-bundles formed by excessively expressed MAPs but also on molecular and mechanical interactions with other cytoskeletal elements and plasma membranes.

Polarity of microtobules modified by motor proteins

Undoubtedly, MAPs contribute to the stabilizing and bundling of MTs, but can they affect the polarity of MTs? So far, only the occurrence of uniformly oriented MT-bundles with plus-ends pointing toward the distal portion of the processes has been detected in cells transfected with cDNAs for MAPs (Baas et al. 1991; Chen et al. 1992; Brandt and Lee 1994; Takemura et al. 1995). In dendrites, MAP2, MAP1A, and MAP1B are found, but it seems that these MAPs alone cannot establish the non-uniform MT-organization in dendrites. Adult type MAP2, a major MAP in the dendrite, can establish only the uniform orientation of MTs by transfection. MAP1B stabilizes MT-dynamics in transfected cells but does not induce MT-bundling or process formation (Noble et al. 1989; Takemura et al. 1992); therefore, it is unlikely that MAP1B is able to affect the polarity of newly formed MTs.

Can the non-uniform orientation of MTs be established by some MAPs alone or with some help from other molecules? As discussed above, we now know that the CHO1/MKLP1 motor protein is a candidate for such a molecule (Sharp et al. 1996). In MAP2c-transfected COS cells, MTs are observed to be nucleated in the cytoplasm independent of the centrosome, and subsequently, some thinner MT-bundles are further bundled into thicker ones. During these steps, MTs appear to be arranged with uniform polarity (Takemura et al. 1995), as proposed for the assembly of mitotic spindles (Hyman and Karsenti 1996). If some proteins can arrange MTs in a bi-directional fashion, then randomly nucleated MTs might be oriented and bundled non-uniformly in the cytoplasm. However, this model cannot mimic the establishment of non-uniform MT-arrays in dendrites, because minus-end-distal MTs appear later during the course of dendritic development (Baas et al. 1989).

Based on current knowledge, it seems highly likely that motor proteins are required to arrange the microtubular arrays after the assembly of MTs. The cell may utilize the same or a closely related machinery to rearrange MTs as that used during mitotic spindle formation. Here, we can summarize the possibilities regarding the elongation of MT-based cell processes from round cells. (1) MTs are nucleated by gamma-tubulin-containing complexes around the centrosome, assembled, and bundled by MAPs, the addition of tubulin subunits supporting the MT-elongation and resulting in process formation. In this case, all MTs are oriented in a plus-end-distal fashion (axonal type). (2) In addition to the MT-bundles formed as postulated in (1), minus-end-distal MTs are added later by the transport of MTs. This model results in the non-uniformly oriented MTs (dendritic type). (3) MTs are nucleated randomly in the peripheral cytoplasm of the cell body with the help of MAPs and then bundled uniformly to make thick long MT-bundles to support process formation (e.g., MAP2c-transfected cells; Takemura et al. 1995). (4) Randomly nucleated MTs are bundled in a non-uniform fashion resulting in the bi-directional polarity of MTs (only theoretical at present).

Intracellular signaling: what kind of signals initiate process formation?

The rat pheochromocytoma PC12 cell line is widely used as a model of neurite formation (Greene and Tischer 1982). This cell line shows characteristics of endocrine cells under normal culture conditions but, when it is treated with growth factors such as nerve growth factor (NGF), it begins to differentiate into a neuron-like cell with prominent cell processes. During neurite elongation, PC12 cells express juvenile MAPs, such as MAP1B (formerly also designated MAP5) and MAP3 (Brugg and Matus 1988). It is clear that increased expression of MAPs is responsible for the initiation of process formation. However, the expression of MAPs alone is not sufficient for the outgrowth of cell processes; the cell additionally requires intracellular signalling cascades that cause the modification of cytoskeletal elements.

Based on the above discussion, at least the following three elements can be considered as necessary for the initiation of process formation: (1) a sufficient amount of tubulin subunits in a state ready to be polymerized; (2) MT-nucleating activity as provided by MTOCs (centrosomes); (3) MT-assembly-promoting activity as promoted by active MAPs. Because post-translational modifications of tubulin molecules are thought to be a result of the stabilization of MTs (Khawaja et al. 1988; Webster et al. 1990), they cannot explain the initial events responsible for process formation. On the other hand, phosphorylation and dephosphorylation of centrosomal proteins and MAPs could be linked to this phenomenon.

It is postulated that both gamma-tubulin and activation of some centrosomal proteins through phosphorylation are necessary to initiate the formation of microtubular arrays of the mitotic spindle (Thaler and Haimo 1996). Activation of centrosomes may also play a role during process formation, at least in its early stages. Centrosomes in differentiated cultured neurons are still “active” enough

to supply MTs to the newly elongated neurites (Yu et al. 1993).

As discussed in the following paragraphs, protein phosphorylation inactivates several MAPs. Such inactivation of MAPs seems to be necessary to stimulate the dynamics of MTs in order to reorganize the whole MT-array. Indeed, the addition of cdc2-kinase, which can phosphorylate MAPs, is followed by disruption of MT-arrays in interphase cells (Lieuvin et al. 1994). Both transient activation of centrosomes and transient loss of microtubular stability facilitate the rapid reorganization of MT-arrays, resulting in a change of morphological phenotypes. Dephosphorylation-dependent activation of MAPs will stabilize the MTs to form rigid arrays of MTs, such as the bundling of MTs for process formation.

Phosphorylation of MAP4 during mitosis reduces its MT-stabilizing activity (Ookata et al. 1995). The activity of MAP4 appears to be regulated by phosphorylation at the serine residue and phosphorylated MAP4 cannot promote MT-assembly in vitro (Aizawa et al. 1991; Hoshi et al. 1992; Ookata et al. 1995). The cell-cycle-dependent phosphorylation followed by the loss of MT-assembly-promoting activity may be a general feature of MAPs; exogenously induced tau protein, which is specific for terminally differentiated cells in vivo, is phosphorylated in this manner (Preuss et al. 1995), whereas MAP2 is inactivated by a cell-cycle-dependent kinase, cdc2-kinase (Faruki et al. 1992), and by MAP kinase (Hoshi et al. 1992). The activity of these kinases, whose targets include cytoskeletal proteins such as MAPs, is regulated in a cell-cycle-dependent manner. They may also be controlled at the beginning of process formation. It will be interesting to determine whether the activity of MAPs is controlled by the same mechanism during process formation.

Recently, a protein kinase designated MARK has been cloned from brain (Drewes et al. 1997). MARK preferentially phosphorylates tau, MAP2, and MAP4, and phosphorylation of these MAPs by MARK at serine/threonine residues diminishes MT-stabilizing activity, resulting in the loss of MT-arrays. Because MARK is expressed at a higher level in the fetus than in the adult, it is expected that such MAP-specific kinases play roles in the reorganization of MT-arrays during early morphogenesis.

A sequence of recent data has demonstrated the relevance of protein tyrosine phosphatase during the course of process formation. First, inhibition of tyrosine phosphatases by sodium orthovanadate, which should increase the phosphorylation of proteins at their tyrosine residues, is reported to induce process formation in PC12 cells and in another neuroblastoma cell line (Rogers et al. 1994). In this case, PC12 cells are treated not with NGF, and orthovanadate partially mimics the function of NGF. Rogers et al. (1994) have also noted that the inhibition of tyrosine kinase reduces neurite formation in both vanadate- and NGF-treated PC12 cells, i.e., the initiation of neurite formation in PC12 cells requires elevated protein tyrosine phosphorylation.

Second, the inhibition of tyrosine phosphatases by orthovanadate can inhibit process formation in PC12 cells treated with growth factors (Wu and Bradshaw 1993). Third, a novel protein tyrosine phosphatase designated PTP20 is up-regulated during NGF-induced differentiation of PC12 cells; overexpression of PTP20 accelerates process formation in the presence of NGF (Aoki et al. 1996). NGF treatment is thought to cause protein tyrosine phosphorylation through receptor-type kinase Trk followed by activation of the MAP-kinase cascade (Robinson and Cobb 1997; Kaplan and Miller 1997). Studies on phosphatases clearly suggest that prolonged stimulation of this cascade works in a negative manner with respect to the differentiation of PC12 cells, and such phosphorylation-dependent signaling should be successively extinguished by phosphatases.

As is well known, the cascades consisting of tyrosine- and serine/threonine-kinases deliver the exogenous signal into the cell nucleus to modify the gene expression required for process formation. Tyrosine phosphatases may regulate the function of MAPs via inactivation of protein kinases working downstream of the cascade during process formation. Protein phosphatases counteracting protein kinases at serine/threonine residues appear to play key roles in MT-organization through the direct activation of MAPs. The function of tau seems to be regulated by dephosphorylation via protein phosphatase 2A, which may restore the MT-assembling activity of tau (Merrik et al. 1996; Sontag et al. 1996). Application of okadaic acid, which preferentially inhibits protein phosphatase 2A, suppresses neurite outgrowth (Chiou and Westhead 1992; Sasahara et al. 1996) and the stability of MTs (Shea et al. 1993) in cultured neuronal cells.

We can now assume that the cascade(s) of the various kinds of kinases will switch the exogenous signal through intracellular tyrosine phosphorylation to serine/threonine phosphorylation. Subsequently, dephosphorylation by protein phosphatases (such as PTP20) may cause inactivation of kinases acting downstream of the cascade. Finally, phosphatases (such as protein phosphatase 2A) will overcome protein kinases (such as MARK) in their competition at serine/threonine residues of MAPs, and consequently, MAPs will be activated to promote the assembly of MTs, thereby leading to process formation.

Axon or dendrite, which is general? A clue from the podocyte

In addition to the neuron, other cells have also been observed with prominent cell processes. In the brain, glial cells are often studied. In addition, the renal glomerular podocyte is equipped with a highly organized cytoskeleton in its cell processes. Huber and Matus (1990) have claimed that cells showing a highly polarized morphology (such as neurons, glial cells, hepatic Kupffer cells, and renal glomerular podocytes) share the same marker protein, MAP3, a neuronal and non-neuronal MAP (Huber et al. 1985). Because the molecular nature of MAP3 is as yet unclear, and because our data suggest that MAP3 is highly homologous or identical to MAP4, which is a MAP

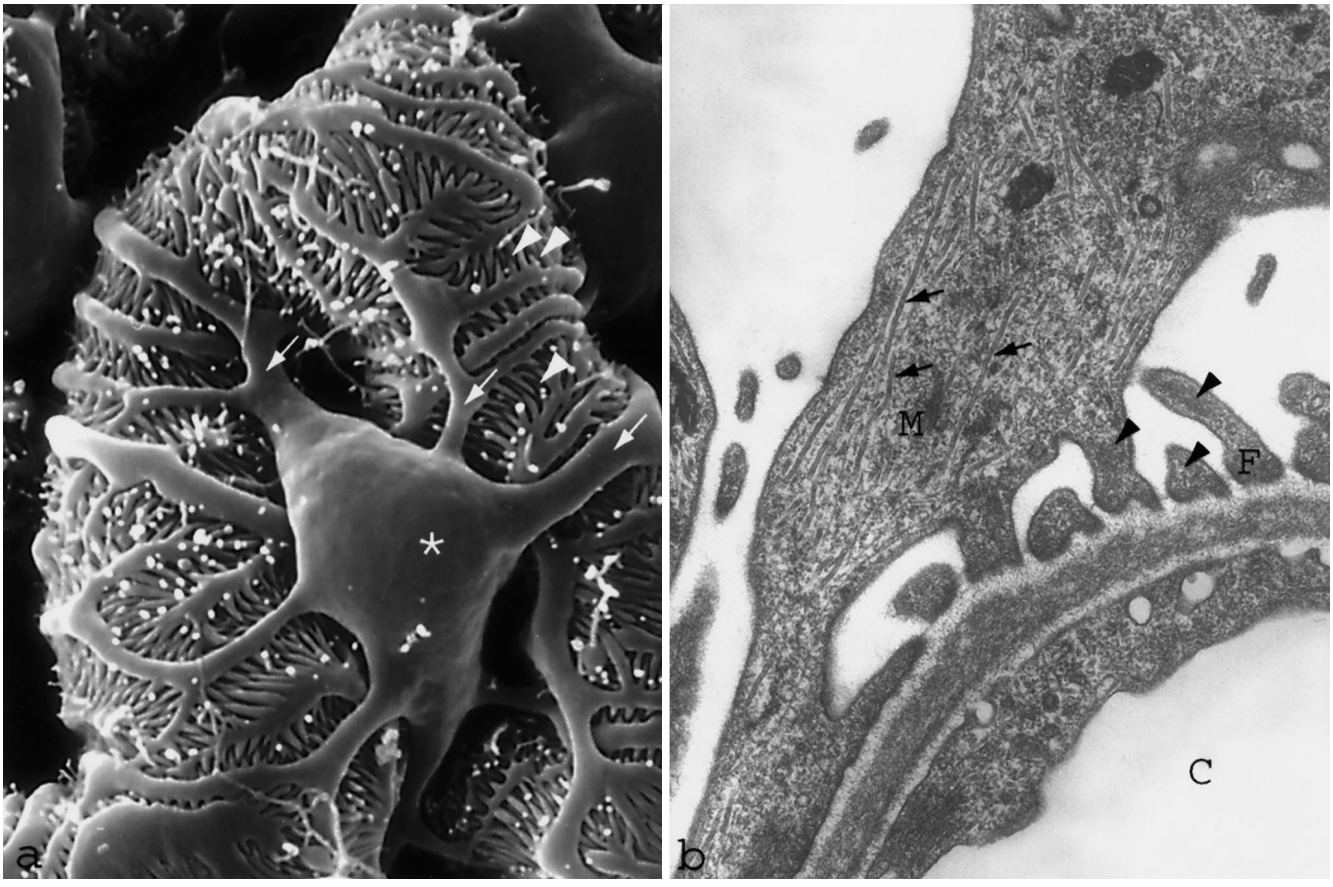

Fig. 1a, b Electron micrographs of rat renal glomerular podocytes in situ. **a** Scanning electron micrograph of podocytes viewed from Bowman's cavity. A cell body (*asterisk*) hangs in the urinary cavity by several major process (*arrows*), from which foot processes (*arrowheads*) arise; these foot processes interdigitate with those of a neighboring cell to cover the glomerular capillaries. ×5300. **b** Transmission electron micrograph showing the podocytes covering the glomerular capillary (*C*) in cross-cut view. Major processes (*M*) are equipped with microtubules (*arrows*) and intermediate filaments, whereas foot processes (*F*) are filled with tight bundles of actin filaments (*arrowheads*). The segregation of the cytoskeletal elements is clearly visible. ×33 000

ubiquitously expressed in various tissues (N. Kobayashi et al., in preparation), it is now uncertain whether MAP3 is a good marker for such a phenotype. Nevertheless, one can expect that various polarized cells share some cytoskeletal proteins in order to establish and maintain their morphology.

It is difficult to imagine that MT-bundles in cell processes of some non-neuronal cells are organized only with ubiquitous MAPs such as MAP4, which also occurs in cells without MT-based processes, e.g., HeLa cells (Bulinski and Borisy 1980), and which is not able to induce process formation by transfection in Chinese hamster ovary cells (Barlow et al. 1994). Indeed, our recent results show that podocytes express two "neuronal" MAPs, viz., tau and MAP2c (S. Sanden et al., submitted). Expression of tau and MAP2c has also been reported in oligodendrocytes (Lopresti et al. 1995; Müller et al. 1997). In addition, the expression of MAP1B is observed during process formation in cultured oligodendrocytes (Vouyiouklis and Broply 1993). Together, these data suggest that these "neuronal" MAPs are not cell-type specific but may be "phenotype-specific" proteins, which are abundant in cells with prominent cell processes.

The renal glomerular podocyte is a highly polarized cell (Fig. 1). Podocytes in situ are arborized with thick primary processes and thin foot processes and lack junctional complexes, a common feature among epithelial cells (Mundel and Kriz 1995). By electron microscopy, the well-developed cytoskeleton composed of MTs and vimentin filaments can be observed exclusively in the primary processes. Some MTs are bundled with each other and run in parallel, whereas some are located closely by the side of other cytoskeletal elements and membrane organelles. On the other hand, in the foot processes, which are much thinner and arise from the primary process, condensed bundles of actin filaments represent the core cytoskeletal structure. Some contractile proteins have been localized in these processes (Drenckhahn and Franke 1988). It is thought that the podocyte bears an actin-based contractile apparatus to counteract the capillary wall distention caused by the pressure difference between capillary and urinary spaces in renal glomeruli (Kriz et al. 1994a, b). Although the microtubular system does not seem to contribute to the production of active (energy-dependent) tension, it is still thought to play an important role in maintaining cellular morphology against mechanical

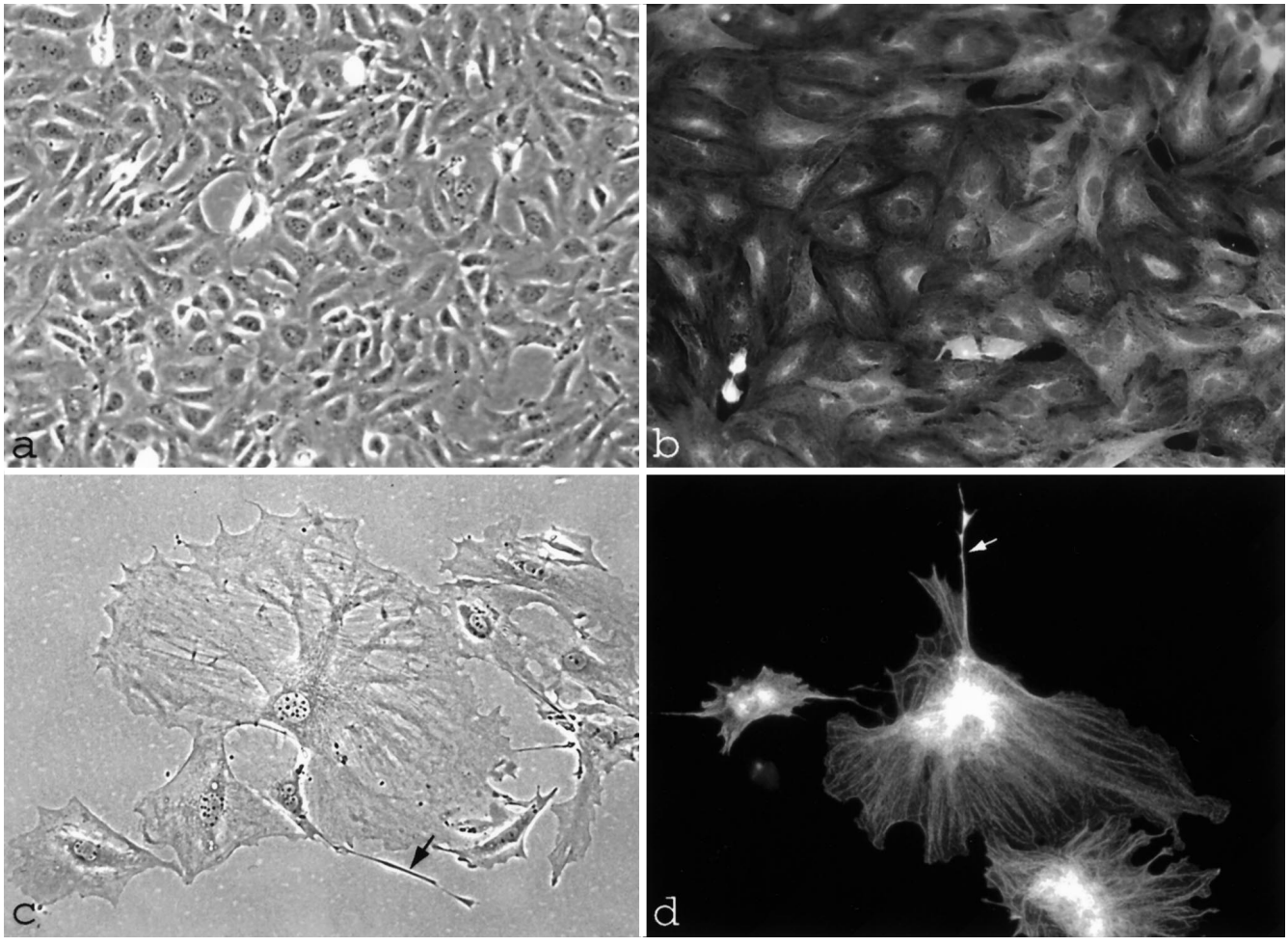

Fig. 2 Conditionally immortalized podocytes show two different phenotypes, viz., non-differentiated proliferating "cobblestone" cells, when cultured at 33°C (**a, b**) and process-bearing differentiated "arborized" cells after a temperature shift to 37°C (**c, d**). Phase-contrast microscopy (**a, c**; ×140) and anti-tubulin immunofluorescent staining (**b, d**; ×230) visualizes the morphological difference between these two phenotypes. **d** Differentiated cells are large and flat and often develop long thin cell processes filled with microtubules (*arrows*); their cytoplasm also contains well-developed microtubular arrays

stresses by means of the construction of tight filamentous networks.

Only limited data have been published on cytoskeletons in podocytes so far (Drenckhahn and Franke 1988; Parysek et al. 1984a, b; Mundel et al. 1991; Huber and Matus 1990). Recently, however, our research group has characterized a novel cytoskeletal protein, designated synaptopodin, which is expressed exclusively in neurons and podocytes and is colocalized with actin filaments (Mundel et al. 1997a). We can now propose a cytoskeletal similarity between the podocyte and the neuron.

The renal glomerular podocyte seems to share several cell biological features with the neuron. (1) Both possess long and short cell processes equipped with highly organized cytoskeletal systems. (2) Both show cytoskeletal segregation; MTs and intermediate filaments are present in primary processes of the podocyte and in neurites, whereas actin filaments are abundant in the foot processes of the former and in synaptic regions of the latter. (3) Neither has a typical intercellular adhering apparatus, such as tight and adherens junctions, and desmosomes, when they are fully differentiated. Instead, they possess cell-type-specific intercellular contacts, such as slit membranes in podocytes and synapses in neurons. (4) Both are terminally differentiated and lack mitotic activity. (5) Both show typical epithelial morphology during the course of their early development, although the neuron is derived from ectoderm, whereas the podocyte has a mesodermal origin. On the contrary, the major processes of the podocyte are homogeneous and lack such differences as observed between axons and dendrites.

In order to clarify the cell biological characteristics of glomerular podocytes, many groups have struggled for a long time to establish them in a culture system to be able to use them as a research tool. So far, it has proved difficult to culture the podocyte, and some reports have probably dealt with contaminating cell types (reviewed in Mundel and Kriz 1996). Recently, however, we have discovered a protocol to induce the differentiation of podocytes in culture (Mundel et al. 1997b) and have subsequently established a conditionally immortalized cell line that shows many characteristics of podocytes in situ (P.

Mundel et al., submitted). Using this cell line, one can easily induce the formation of cell processes following differentiation. This cell line should facilitate studies of the detailed function and cytoskeletal organization of the podocyte (Fig. 2).

Taking advantage of this cell line, we have found that process formation in cultured podocytes is dependent on normal kinetics of MTs. Vinblastine, which suppresses normal MT-kinetics, inhibits process formation in a dose-dependent and reversible manner. After depolymerization of MTs by nocodazole, recovery of MT-arrays begins in the vicinity of cell nuclei, and centrosomes seem to be the only MTOC in cultured podocytes, as in neurons. This is the first study showing the involvement of MTs in process formation of non-neuronal cells (Kobayashi et al., in preparation).

In conclusion, we now possess a powerful tool for investigating the cell biology of the podocyte, which has characteristics comparable to neurons. The results obtained from the culture system, together with data on podocytes in situ, should expand our knowledge regarding the generalized features of cells showing polarized morphology. In parallel, we hope to obtain data from other culture systems of polarized non-neuronal cells. Finally, we aim to understand cellular events during process formation as a generalized concept, where it is unnecessary to use the prefix "non".

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References

Ahmed FJ, Baas PW (1995) Microtubules released from the neuronal centrosomes are transported into the axon. J Cell Sci 108:2761–2769

Ahmed FJ, Joshi HC, Centnze VE, Baas PW (1994) Inhibition of microtubule nucleation at the neuronal centrosome compromises axon growth. Neuron 12:271–280

Aizawa H, Kamijo M, Ohba Y, Mori A, Okuhara K, Kawasaki H, Murofushi H, Suzuki K, Yasuda H (1991) Microtubule destabilization by cdc2/H1 histone kinase: phosphorylation of a "Pro-rich region" in the microtubule-binding domain of MAP4. Biochem Biophys Res Commun 179:1620–1626

Aoki N, Yamaguchi-Aoki Y, Ullrich A (1996) The novel protein-tyrosine phosphatase PTP20 is a positive regulator of PC12 cell neuronal differentiation. J Biol Chem 271:29422–29426

Baas PW (1997) Microtubules and axonal growth. Curr Opin Cell Biol 9:29–36

Baas PW, Ahmed FJ (1992) The plus end of stable microtubules are exclusive nucleating structure for microtubules in the axon. J Cell Biol 116:1231–1241

Baas PW, Joshi HC (1992) Gamma-tubulin distribution in the neuron: implications for the origins of neuritic microtubules. J Cell Biol 119:171–178

Baas PW, Black MM, Banker GA (1989) Changes in microtubule polarity orientation during the development of hippocampal neurons in culture. J Cell Biol 109:3085–3094

Baas PW, Pienkowski TP, Kosik KS (1991) Processes induced by tau expression in Sf9 cells have an axon-like microtubule organization. J Cell Biol 115:1333–1344

Barlow S, Gonzalez-Garay ML, West RR, Olmsted JB, Cabral F (1994) Stable expression of heterogeneous microtubule-associated proteins in Chinese hamster ovary cells: evidence for differing roles of MAPs in microtubule organization. J Cell Biol 126:1017–1029

Belmont LD, Mitchison TJ (1996) Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. Cell 84:623–631

Brandt R, Lee G (1994) Orientation, assembly and stability of microtubule bundles induced by a fragment of tau protein. Cell Motil Cytoskeleton 28:143–154

Brugg B, Matus A (1988) PC12 cells express juvenile microtubule-associated proteins during nerve growth factor-induced neurite outgrowth. J Cell Biol 107:643–650

Bulinski JC, Borisy GG (1980) Immunofluorescence localization of HeLa cell microtubule-associated proteins on microtubules in vitro and in vivo. J Cell Biol 87:792–801

Caceres A, Kosik KS (1990) Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. Nature 343:461–463

Campenot RB, Lund K, Senger DL (1996) Delivery of newly synthesized tubulin to rapidly growing distal axons of rat sympathetic neurons in compartmented cultures. J Cell Biol 135:701–709

Carlier MF (1989) Role of nucleotide hydrolysis in the dynamics of actin filaments and microtubules. Int Rev Cytol 115:139–170

Cassimeris L (1993) Regulation of microtubule dynamics instability. Cell Motil Cytoskeleton 26:275–281

Chapin SJ, Lue CM, Yu MT, Bulinski JC (1995) Differential expression of alternatively spliced forms of MAP4: a repertoire of structurally different microtubule-binding domains. Biochemistry 34:2289–2301

Chen J, Kanai Y, Cowan NJ, Hirokawa N (1992) Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. Nature 360:674–677

Chiou JY, Westhead EW (1992) Okadaic acid, a protein phosphatase inhibitor, inhibits nerve growth factor-directed neurite outgrowth in PC12 cells. J Neurochem 59:1963–1966

Correas I, Padilla R, Avila J (1990) The tubulin-binding sequence of brain microtubule-associated proteins, tau and MAP-2, is also involved in actin binding. Biochem J 269:61–64

Couche D, Mavilia C, Georgieff IS, Liem RK, Shelanski ML, Nunez J (1992) Primary structure of high molecular weight tau present in the peripheral nervous system. Proc Natl Acad Sci USA 89:4378–4381

Cross D, Vial C, Maccioni RB (1993) A tau-like protein interacts with stress fibers and microtubules in human and rodent cultured cell lines. J Cell Sci 105:51–60

Cunningham CC, Leclerc N, Flanagan LA, Lu M, Janmey PA, Kosik KS (1997) Microtubule-associated protein 2c reorganizes both microtubules and microfilaments into distinct cytological structures in an actin-binding protein-280-deficient melanoma cell line. J Cell Biol 136:845–857

Dinsmore JH, Solomon F (1991) Inhibition of MAP2 expression affects both morphological and cell division phenotypes of neuronal differentiation. Cell 64:817–826

Drenckhahn D, Franke RP (1988) Ultrastructural organization of contractile and cytoskeletal proteins in glomerular podocytes of chicken, rat, and man. Lab Invest 59:673–682

Drewes G, Ebneth A, Preuss U, Mandelkow EM, Mandelkow E (1997) MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. Cell 89:297–308

Edelmann W, Zervas M, Costello P, Roback L, Fischer I, Hammarback JA, Cowan N, Davis P, Wainer B, Kucherlapati P (1996) Neuronal abnormalities in microtubule-associated protein 1B mutant mice. Proc Natl Acad Sci USA 93:1270–1275

Edson K, Weisshaar B, Matus A (1993) Actin depolymerization induces process formation on MAP2-transfected non-neuronal cells. Development 117:689–700

Faruki S, Dorée M, Karsenti E (1992) cdc2 Kinase-induced destabilization of MAP2-coated microtubules in *Xenopus* egg extracts. J Cell Sci 101:69–78

Ferhat L, Bernard A, Ribas de Pouplana L, Ben-Ari Y, Khrestchatisky M (1994) Structure, regional and developmental expression of MAP2d, a MAP2 splice variant encoding four microtubule-binding domains. Neurochem Int 25:327–338

Ferhat L, Represa A, Bernard A, Ben-Ari Y, Khrestchatisky M (1996) MAP2d promotes bundling and stabilization of both microtubules and microfilaments. J Cell Sci 109:1095–1103

Funakoshi T, Takeda S, Hirokawa N (1996) Active transport of photoactivated tubulin molecules in growing axons revealed by a new electron microscopic analysis. J Cell Biol 133:1347–1353

Goedert M, Spiliantini MG, Crowther RA (1992) Cloning of a big tau microtubule-associated protein characteristic of the peripheral nervous system. Proc Natl Acad Sci 89:1983–1987

Greene LA, Tischer AS (1982) PC12 pheochromocytoma cultures in neurobiological research. Adv Cell Neurobiol 3:373–415

Hagiwara H, Yorifuji H, Sato-Yoshitake R, Hirokawa N (1994) Competition between motor molecules (kinesin and cytoplasmic dynein) and fibrous microtubule-associated proteins in binding to microtubules. J Biol Chem 269:3581–3589

Haimo LT (1982) Dynein decoration of microtubules: determination of polarity. Methods Cell Biol 24:189–205

Hanlon DW, Yang Z, Goldstein LSB (1997) Characterization of KIFC2, a neuronal kinesin superfamily member in mouse. Neuron 18:439–451

Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T, Sato-Yoshitake R, Takei Y, Noda T, Hirokawa N (1994) Altered microtubule organization in small-calibre axons of mice lacking tau protein. Nature 369:488–491

Heidemann SR (1991) Microtubule polarity determination based on formation of protofilament hooks. Methods Enzymol 196:469–477

Hirokawa N (1982) Cross-linker system between neurofilaments, microtubules, and membrane organelles in frog axons revealed by the quick-freeze, deep-etching method. J Cell Biol 94:129–142

Hirokawa N (1993) The neuronal cytoskeleton: its role in neuronal morphogenesis and organelle transport. In: Hirokawa N (ed) Neuronal cytoskeleton. Morphogenesis, transport and synaptic transmission. Japan Scientific Societies and CRC, Tokyo, Japan, pp 3–32

Hirokawa N (1994) Microtubule organization and dynamics dependent on microtubule-associated proteins. Curr Opin Cell Biol 6:74–81

Hirokawa N (1996) Organelle transport along microtubules: the role of KIFs. Trends Cell Biol 6:135–141

Hirokawa N, Hisanaga S, Shiomura Y (1988) MAP2 is a component of crossbridges between microtubules and neurofilaments in the neuronal cytoskeleton in vivo and in vitro. Quick-freeze, deep-etch immunoelectron microscopy and reconstitution studies. J Neurosci 8:2769–2779

Hoshi M, Ohta K, Gotoh Y, Mori A, Murofushi H, Sakai H, Nishida E (1992) Mitogen-activated-protein-kinase-catalyzed phosphorylation of microtubule-associated proteins, microtubule-associated protein 2 and microtubule-associated protein 4, induces an alteration in their function. Eur J Biochem 203:43–52

Huber G, Matus A (1990) Microtubule-associated protein 3 (MAP3) expression in non-neuronal tissues. J Cell Sci 95:237–246

Huber G, Alaimo-Beuret D, Matus A (1985) MAP3: characterization of a novel microtubule-associated protein. J Cell Biol 100:496–507

Hyman AA, Karsenti E (1996) Morphogenic properties of microtubules and mitotic spindle assembly. Cell 84:401–410

Joshi HC, Palacios MJ, McNamara L, Cleveland DW (1992) Gamma-tubulin is a centrosomal protein required for cell cycle-dependent microtubule nucleation. Nature 356:80–83

Kanai Y, Hirokawa N (1993) Microtubule bundling by tau proteins in vivo. In: Hirokawa N (ed) Neuronal cytoskeleton. Morphogenesis, transport and synaptic transmission. Japan Scientific Societies and CRC, Tokyo, Japan, pp 207–219

Kanai Y, Takemura R, Mori H, Ihara Y, Yanagisawa M, Masaki T, Hirokawa N (1989) Expression of multiple tau isoforms and microtubule bundle formation in fibroblasts transfected with a single tau cDNA. J Cell Biol 109:1173–1184

Kanai Y, Chen J, Hirokawa N (1992) Microtubule bundling by tau proteins in vivo: analysis of functional domains. EMBO J 11:3953–3961

Kaplan DR, Miller FD (1997) Signal transduction by the neurotrophic receptors. Curr Opin Cell Biol 9:213–221

Khawaja S, Gundersen GG, Bulinski JC (1988) Enhanced stability of microtubules enriched in detyrosinated tubulin is not a direct function of detyrosination level. J Cell Biol 106:141–b150

Knops J, Kosik KS, Lee G, Pardee JD, Cohen-Gould L, McConlogue L (1991) Overexpression of tau in a nonneuronal cell induces long cellular processes. J Cell Biol 14:725–733

Kriz W, Hackenthal E, Nobiling R, Sakai T, Elger M, Hähnel (1994a) A role for podocyte to counteract capillary wall distention. Kidney Int 45:369–376

Kriz W, Mundel P, Elger M (1994b) The contractile apparatus of podocytes is arranged to counteract GBM expansion. Contrib Nephrol 107:1–9

Kuriyama R, Dragas-Granoic S, Maekawa T, Vassilev A, Khodjakov A, Kobayashi H (1994) Heterogeneity and microtubule interaction of the CHO1 antigen, a mitosis-specific kinesin-like protein: analysis of subdomains expressed in insect sf9 cells. J Cell Sci 107:3485–3499

Lee G (1993) Non-motor microtubule-associated proteins. Curr Opin Cell Biol 5:88–94

Lee G, Rook SL (1992) Expression of tau protein in non-neuronal cells: microtubule binding and stabilization. J Cell Sci 101:227–237

Lewis SA, Ivnov IE, Lee GH, Cowan NJ (1989) Organization of microtubules in dendrites and axons is determined by a short hydrophobic zipper in microtubule-associated proteins MAP2 and tau. Nature 342:498–505

Li QQ, Joshi HC (1995) Gamma-tubulin is a minus end-specific microtubule binding protein. J Cell Biol 131:207–214

Li Y, Black MM (1996) Microtubule assembly and turnover in growing axons. J Neurosci 16:531–544

Lieuvin A, Labbé JC, Dorée M, Job D (1994) Intrinsic microtubule stability in interphase cells. J Cell Biol 124:985–996

Lopresti P, Szuchet S, Papasodomenos SC, Zinkowski RP, Binder LI (1995) Functional implications for the microtubule-associated protein tau: localization in oligodendrocytes. Proc Natl Acad Sci USA 92:10369–10373

McNally FJ, Okawa K, Iwamatsu A, Vale RD (1996) Katanin, the microtubule-severing ATPase, is concentrated at centrosomes. J Cell Sci 109:561–567

Merrik SE, Demoise DC, Lee VMY (1996) Site-specific dephosphorylation of tau protein at Ser^{202}/Thr^{205} in response to microtubule depolymerization in cultured human neurons involves proteins phosphatase 2A. J Biol Chem 271:5589–5594

Miller KE, Joshi HC (1996) Tubulin transport in neurons. J Cell Biol 133:1355–1366

Miller RH, Lasek RJ, Katz MJ (1987) Prefered microtubules for vesicle transport in lobster axons Science 235:220–222

Mitchison TJ, Kirschner MW (1984) Dynamic instability of microtubule growth. Nature 312:237–242

Moudjou M, Bordes N, Paintrand M, Bornenes (1996) Gamma-tubulin in mammalian cells: the centrosomal and the cytosolic forms. J Cell Sci 109:875–887

Müller R, Heirich M, Heck S, Blohm D, Richter-Landsberg C (1997) Expression of microtubule-associated proteins MAP2 and tau in cultured brain oligodendrocytes. Cell Tissue Res 288:239–249

Mundel P, Kriz W (1995) Structure and function of podocytes: an update. Anat Embryol 192:385–397

Mundel P, Kriz W (1996) Cell culture of podocytes. Exp Nephrol 4:263–266

Mundel P, Gilbert P, Kriz W (1991) Podocytes in glomerulus of rat kidney express a characteristic 44 kD protein. J Histochem Cytochem 39:1047–1056

Mundel P, Heid HW, Mundel TM, Krüger M, Reiser J, Kriz W (1997a) Synaptopodin, an actin-associated protein in telencephalic dendrites and in renal podocytes. J Cell Biol 139:193–204

Mundel, Reiser J, Kriz W (1997b) Induction of differentiation in cultured rat and human podocytes. J Am Soc Nephrol 8:697–705

Nislow C, Lombillo VA, Kuriyama R, McIntosh JR (1992) A plus-end-directed motor enzyme that moves antiparallel microtubules in vitro localizes to the interzone of mitotic spindles. Nature 359:543–547

Noble M, Lewis SA, Cowan NJ (1989) The microtubule binding domain of microtubule-associated protein MAP1B contains a repeated sequence motif unrelated to that of MAP2 and tau. J Cell Biol 109:3367–3376

Okabe S, Hirokawa N (1988) Microtubule dynamics in nerve cells: analysis using microinjection of biotinylated tubulin into PC12 cells. J Cell Biol 107:651–664

Okabe S, Hirokawa N (1990) Turnover of fluorescently labelled tubulin and actin in the axon. Nature 343:479–482

Okabe S, Hirokawa N (1992) Differential behavior of photoactivated microtubules in growing axons of mouse and frog neurons. J Cell Biol 117:105–120

Okabe S, Hirokawa N (1993a) Do photobleached fluorescent microtubules move?: re-evaluation of fluorescence laser photobleaching both in vitro and in growing *Xenopus* axons. J Cell Biol 120:1177–1186

Okabe S, Hirokawa N (1993b) Dynamics of the neuronal cytoskeleton. In: Hirokawa N (ed) Neuronal cytoskeleton. Morphogenesis, transport and synaptic transmission. Japan Scientific Societies and CRC, Tokyo, Japan, pp 161–169

Ookata K, Hisanaga S, Bulinski JC, Murofushi H, Aizawa H, Itoh TJ, Hotani H, Okumura E, Tachibana K, Kishimoto T (1995) Cyclin B interaction with microtubule-associated protein 4 (MAP4) targets p34cdc2 kinase to microtubules and is a potential regulator of M-phase microtubule dynamics. J Cell Biol 128:849–862

Parysek LM, Asnes CF, Olmsted JB (1984a) MAP4: occurrence in mouse tissues. J Cell Biol 99:1309–1315

Parysek LM, Wolosewick JJ, Olmsted JB (1984b) MAP4: a microtubule-associated protein specific for a subset of tissue microtubules. J Cell Biol 99:2287–2296

Pedrotti B, Colombo R, Islam K (1994) Microtubule associated protein MAP1A is an actin-binding and crosslinking protein. Cell Motility Cytoskeleton 29:110–116

Preuss U, Doring F, Illenberger S, Mandelkow EM (1995) Cell cycle-dependent phosphorylation and microtubule binding of tau protein stably transfected into Chinese hamster ovary cells. Mol Biol Cell 6:1397–1410

Robinson MJ, Cobb MH (1997) Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 9:180–186

Rogers MV, Buensuceso C, Montague F, Mahadevan L (1994) Vanadate stimulates differentiation and neurite outgrowth in rat pheochromocytoma PC12 cells and neurite extension in human neuroblastoma SH-SY5Y cells. Neuroscience 60:479–494

Sabry J, O'Connor TP, Kirschner MW (1995) Axonal transport of tubulin in Ti1 pioneer neurons in situ. Neuron 14:1247–1256

Saito N, Okada Y, Noda Y, Kinoshite Y, Kondo S, Hirokawa N (1997) KIFC2 is a novel neuron-specific C-terminal type kinesin superfamily motor for dendritic transport of multivesicular Body-like organelles. Neuron 18:425–438

Sasahara Y, Kobayashi T, Onodera H, Onoda M, Ohnishi M, Kato S, Kusuda K, Shima H, Nagao M, Abe H, Yanagawa Y, Hiraga A, Tamura S (1996) Okadaic acid suppresses neural differentiation-dependent expression of the neurofilament-L gene in P19 embryonal carcinoma cells by post-transcriptional modification. J Biol Chem 271:25950–25957

Sharp DJ, Yu W, Baas PW (1995) Transport of dendritic microtubules establishes their nonuniform polarity orientation. J Cell Biol 130:93–103

Sharp DJ, Kuriyama R, Baas PW (1996) Expression of a kinesin-related motor protein induces Sf9 cells to form dendrite-like processes with nonuniform microtubule polarity orientation. J Neurosci 16:4370–4375

Shea TB, Paskevich PA, Beermann ML (1993) The protein phosphatase inhibitor okadaic acid increases axonal neurofilaments and bneurite caliber, and decreases axonal microtubules in NB1a/d1 cells. J Neurosci Res 35:507–521

Shu HB, Joshi HC (1995) Gamma-tubulin can both nucleate microtubule assembly and self-assemble into novel structures in mammalian cells. J Cell Biol 130:1137–1147

Sontag E, Nunbhakdi-Craig V, Lee G, Bloom GS, Mumby MC (1996) Regulation of the phosphorylation state and microtubule binding activity of tau by protein phosphatase 2A. Neuron 17:1201–1207

Svitkina T, Verkovsky AB, Borisy GG (1996) Plectin sidearms mediate interaction of intermediate filaments with microtubules and other components of the cytoskeleton. J Cell Biol 135:991–1007

Takeda S, Funakoshi T, Hirokawa N (1995) Tubulin dynamics in neuronal axons of living zebrafish embryos. Neuron 14:1257–1264

Takemura R, Okabe S, Umeyama T, Kanai Y, Cowan NJ, Hirokawa N (1992) Increased microtubule stability and alpha tubulin acetylation in cells transfected with microtubule-associated proteins MAP1B, MAP2c or tau. J Cell Sci 103:953–964

Takemura R, Okabe S, Umeyama T, Hirokawa N (1995) Polarity orientation and assembly process of microtubule bundles in nocodazole-treated, MAP2c-transfected COS cells. Mol Biol Cell 6:981–996

Terasaki M, Schmidek A, Galbraith JA, Gallant PE, Reese TS (1995) Transport of cytoskeletal elements in the squid giant axon. Proc Natl Acad Sci USA 92:11500–11503

Thaler CD, Haimo LT (1996) Microtubules and microtubule motors: mechanisms of regulation. Int Rev Cytol 164:269–327

Vale RD, Reese TS, Sheetz MP (1985a) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell 42:39–50

Vale RD, Schnapp BJ, Reese TS, Sheetz MP (1985b) Organelle, bead, and microtubule translocations promoted by soluble factors from the squid giant axon. Cell 40:559–569

Vouyiouklis DA, Broply PJ (1993) Microtubule-associated protein MAP1B expression precedes the morphological differentiation of oligodendrocytes. J Neurosci Res 35:257–267

Walker RA, O'Brien ET, Pryer NK, Sobeiro MF, Voter WA, Erickson HP, Salmon ED (1988) Dynamic instability of individual microtubules analysed by video light microscopy: rate constants and transition frequencies. J Cell Biol 107:1437–1448

Wang J, Yu W, Baas PW, Black MM (1996a) Microtubule assembly in growing dendrites. J Neurosci 16:6065–6078

Wang XM, Peloquin JG, Zhai Y, Bulinski JC, Borisy GG (1996b) Removal of MAP4 from microtubules in vivo produces no observable phenotype at the cellular level. J Cell Biol 132:345–357

Webster DR, Wehland J, Weber K, Borisy GG (1990) Detyrosination of alpha-tubulin does not stabilize microtubules in vivo. J Cell Biol 111:113–122

Weisshaar B, Doll T, Matus A (1992) Reorganization of the microtubular cytoskeleton by embryonic microtubule-associated protein 2c (MAP2c). Development 116:1151–1161

Wu YY, Bradshaw RA (1993) Effect of nerve growth factor and fibroblast growth factor on PC12 cells: inhibition by orthovanadate. J Cell Biol 121:409–422

Yu W, Centonze VE, Ahmed FJ, Baas PW (1993) Microtubule nucleation and release from the neuronal centrosome. J Cell Biol 122:349–359

Yu W, Schwei MJ, Baas PW (1996) Microtubule transport and assembly during axon growth. J Cell Biol 133:151–157

Yu W, Sharp DJ, Kuriyama R, Mallik P, Baas PW (1997) Inhibition of a mitotic motor compromises the formation of dendrite-like processes from neuroblastoma cells. J Cell Biol 136:659–668

Zagon IS, Higbee R, Riederer BM, Goodman SR (1986) Spectrin subtypes in mammalian brain: an immunoelectron microscopic study. J Neurosci 6:2977–2986

Zheng YX, Wong ML, Alberts B, Mitchison T (1995) Nucleation of microtubule assembly by a gamma-tubulin-containing ring complex. Nature 378:578–583