Microtubules cut and run

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There is broad agreement that cells reconfigure their microtubules through rapid bouts of assembly and disassembly, as described by the mechanism known as dynamic instability. However, many cell types have complex patterns of microtubule organization that are not entirely explicable by dynamic instability. There is growing evidence that microtubules can be moved into new patterns of organization by forces generated by molecular motor proteins. Studies on several cell types support a model called 'cut and run' in which long microtubules are stationary, but relatively short microtubules are mobile. In this model, cells mobilize their microtubules by severing them into short pieces, using enzymes such as katanin and spastin that break the lattice of the microtubule polymer. After being reorganized, the short microtubules can once again elongate and lose their mobility. Microtubule severing is also crucial for a variation of 'cut and run' in which the severed microtubules are reorganized by means of treadmilling.

Introduction

Microtubules are prominent cytoskeletal elements that undergo dramatic alterations in organization and distribution during important cellular events such as mitosis, migration and the outgrowth of processes. Dynamic instability is a potent mechanism whereby cells can try out various configurations of microtubules through rapid microtubule assembly and disassembly before selectively stabilizing the most suitable option [1,2]. However, this mechanism is not sufficient to explain the entirety of microtubule behaviors observed in cells such as the reconfiguration of microtubules that have already been stabilized by factors that bind to the surface or the ends of the microtubules. Recent studies on a variety of cell types, some of which have highly specialized microtubule arrays, provide evidence for another pathway by which microtubules change their configuration during morphogenesis. This pathway, which can be explained by a model we call 'cut and run,' involves the breakage of microtubules into short pieces that are highly susceptible to movement. The crux of this model is that long microtubules are relatively immobile, whereas short microtubules are quite mobile. Thus, in order for a cell to transform its microtubule array from one type of organization to another, the long immobile microtubules are severed into short pieces that rapidly move into a new configuration, after which the short pieces once again elongate and lose their mobility.

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Available online 29 August 2005

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Enzymes that sever microtubules

The best-characterized microtubule-severing protein is termed katanin, named after the Japanese samurai sword. Katanin consists of a P60 subunit, which is the enzyme that severs microtubules, and a non-enzymatic P80 subunit that targets katanin to the centrosome. Elegant studies using FRET suggest that six P60-katanin subunits form a hexamer that presumably wraps around the microtubule and generates the torque needed to break its lattice [3]. While katanin is indeed present at the centrosome of mitotic, interphase and terminally post-mitotic cells, it also has a widespread distribution throughout the cytoplasm of many cell types, for example appearing in all compartments of the neuron such as axons, dendrites, growth cones and cell bodies [4]. Plant orthologs for P60-katanin have been referred to by terms including AtP60 and AtKSS, but we will simply refer to them here as plant katanin. Competing with katanin for popularity in recent literature is a protein called spastin, which is another AAA enzyme with a great deal of homology to P60-katanin. Spastin was originally identified several years ago as a protein that undergoes mutations that give rise to spastic paraplegia, a debilitating neuropathy in humans. Spastin has been shown to sever microtubules when overexpressed in cells [5] and when recombinant spastin is applied to lysed cells [6].

Early studies on katanin earmarked it as a protein that severs microtubules during mitosis. Katanin activity, assessed by the degree to which microtubules were severed, was found to be higher in mitotic extracts than in interphase extracts [7]. This observation, together with the association of katanin with the centrosome, prompted the conclusion that the enzyme severs microtubules from the centrosome during M-phase to permit tubulin subunits to flux through the polymer during chromosome segregation [8]. By contrast, most microtubules in simple interphase cells are quite long and emanate from the centrosome to the periphery, with no obvious need for the microtubules to be severed. Interestingly, however, the most active work on katanin in recent years has not been on mitosis but, rather, on the role of microtubule severing in the regulation of specialized microtubule arrays in neurons and plant cells. We posit that, during mitosis, the

microtubules are cut from the centrosome but are not permitted to run very far, presumably owing to forces (or proteins such as ninein, see later) that hold the cut minus-ends within or near the centrosome. By contrast, in cells such as neurons with elaborate morphologies, the cut microtubules are re-oriented and deployed over potentially long distances.

Microtubule release from the centrosome

Microtubule release and transport from the centrosome was first observed in cellular extracts [9]. The movement, which was shown to be based on dynein, occurred by means of motor protein molecules pushing against a glass coverslip. Although the release and movement occurred from *bona fide* centrosomes, there was still concern as to whether the phenomenon reflected physiological events. In a major breakthrough, Keating and colleagues [10] observed the release and transport of microtubules from the centrosome of living epithelial cells. The microtubules moved outward towards the periphery of the cell, with the plus-ends of the microtubules leading, as would be expected of a dynein-driven process. To ensure that the movement was *bona fide* transport, the authors made photobleached marks on individual microtubules and watched them move.

Microtubule release in epithelial and migratory cells

Studies on highly polarized epithelial cells suggest that the release and transport phenomena are even more accentuated in cells with specialized morphologies. In these cells, the microtubules become organized into parallel arrays that extend from the apical to the basal membrane. Early models posited that microtubules disassemble in the region of the cell containing the centrosome, after which new microtubules are nucleated in the vicinity of the apical membrane. However, observations on the transition from the centrosomal to the noncentrosomal arrangement of microtubules suggested that microtubules actually move from one locale to the other [11]. The movement has not yet been observed directly in these cells, but it now appears that the transport of microtubules is a widely utilized mechanism across many cells types [12–14].

Microtubule release from the centrosome has been directly observed in migratory cells. As with epithelial cells and neurons (see below), microtubules were documented to move outward from the centrosome, using cytoplasmic dynein as the chief motor for their transport [15]. Not all of the microtubules were released – only the short, rapidly moving microtubules, whereas longer microtubules that extended from the centrosome to the cell periphery remained attached to the centrosome. Interestingly, a protein called ninein, which is present in the pericentriolar material, a matrix of proteins surrounding the centrosome, is thought to 'recapture' a portion of the microtubules at their minus-ends following their release [15]. Recapture is downregulated in cells such as neurons in which most or all of the microtubules are deployed to distal locales following their release [16].

Microtubule severing in neurons

Our initial interest in microtubule severing in neurons was prompted by data suggesting that microtubules are rapidly released and transported away from the centrosome almost immediately after their nucleation [17,18]. Our model maintained that all microtubules in the neuron are nucleated at the centrosome and then relocated to populate locales such as axons and dendrites (Figure 1a). Our model also addressed the question of where extra microtubules come from to supply newly forming axonal branches at sites that could be hundreds of microns away from the centrosome. We posited that perhaps the long microtubules within the axon locally fracture into short pieces to increase the number of available microtubules that can move into the branch [19]. This idea was validated by serial reconstruction of microtubules from electron micrographs [20] and live-cell imaging [21]. We also observed short microtubules being severed from looped bundles of microtubules in growth cones, followed by the transport of the short microtubules into filopodia [21]. From these studies and subsequent studies on microtubule transport down the axon [22], the conclusion emerged that the capacity of a microtubule to move is inversely proportional to its length, with only the shortest microtubules displaying rapid concerted motion [23].

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Role of katanin

Inhibition of katanin in cultured vertebrate neurons was first achieved by microinjection of a function-blocking antibody [4]. As a result, new microtubules nucleated from the centrosome failed to be released. In addition, microtubules became longer throughout the cell body (because they were not subjected to the severing events that normally occur) and hence failed to be efficiently transported into newly growing axons. More recently, we found that the levels of P60-katanin, the enzymatic subunit, are very high in axons that are actively growing towards their targets, but then plunge precipitously when the axon reaches its target and stops growing [24]. P60-katanin levels are also higher at the tips of growing neuronal processes at some developmental stages and are globally elevated at the developmental stage corresponding to dendritogenesis [25]. By contrast, levels of P80-katanin, the non-enzymatic, centrosome-targeting subunit, are higher in the cell body than in the processes, and are generally more uniform during development than P60-katanin levels. Based on studies so far, we believe that it is reasonable to posit that microtubule-severing proteins might play crucial roles in various aspect of neuronal morphology, such as the length, number and
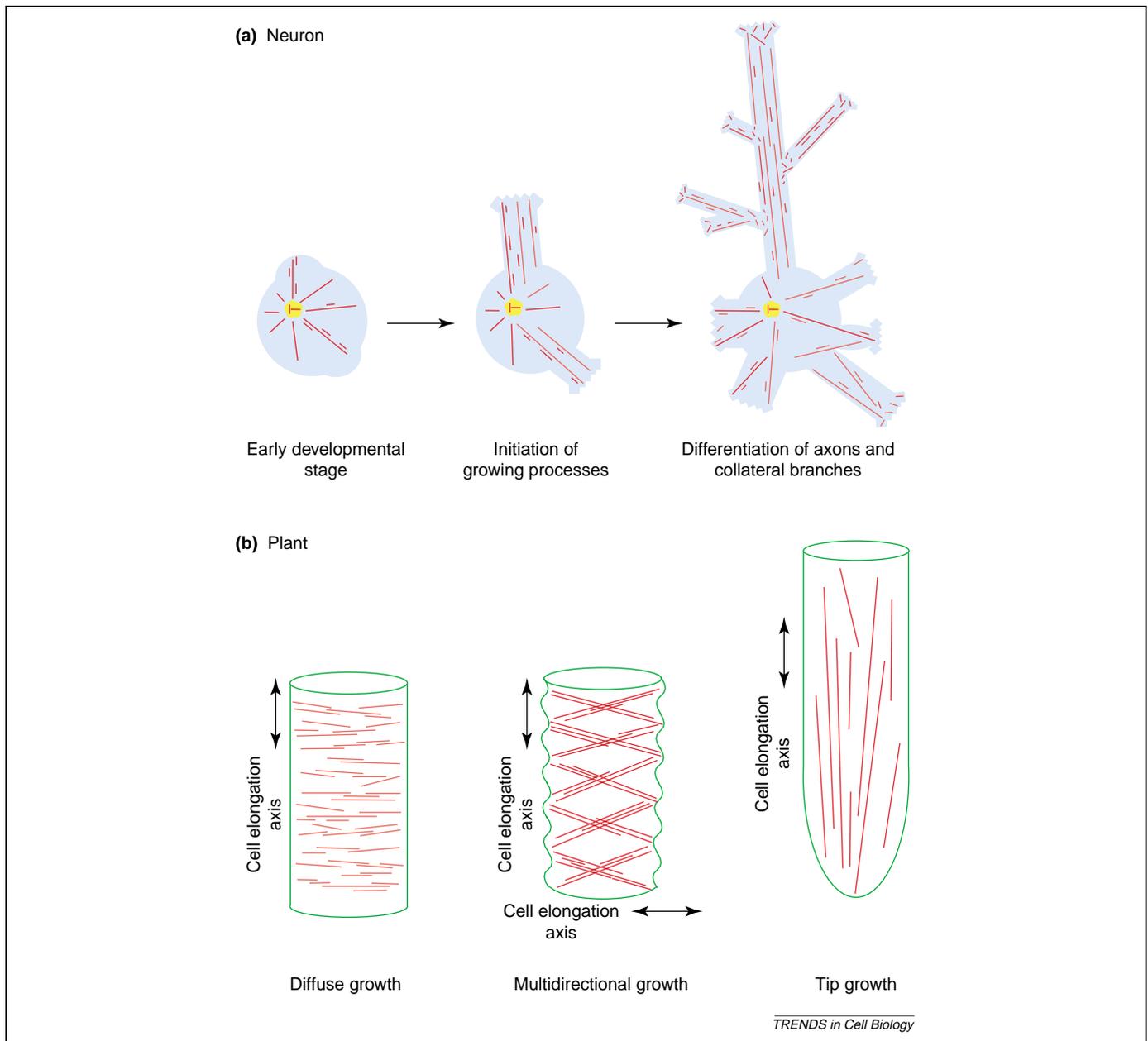
(a) Neuron

Early developmental stage

Initiation of growing processes

Differentiation of axons and collateral branches

(b) Plant

Cell elongation axis

Cell elongation axis

Cell elongation axis

Cell elongation axis

Diffuse growth

Multidirectional growth

Tip growth

TRENDS in Cell Biology

Figure 1. Microtubule reorganization and redistribution in neurons and plant cells. Panel **(a)** shows microtubules in a neuron as it develops branched axons. Microtubules are nucleated at the centrosome and then severed so they can potentially be transported into developing processes. Long microtubules are effectively immobile, whereas short microtubules can be actively transported by motor proteins. The short microtubules move along the long microtubules or along the actin cytoskeleton (not shown in the figure). Ongoing widespread but moderate severing ensures that there are always short microtubules that can move, but that the severing is not so extreme such as to lose all of the long microtubules. Additional microtubule severing occurs at sites of branch formation to produce a supply of localized short microtubules to invade the new branch. Panel **(b)** shows three different types of growth that occur for typical plant cells. Microtubules have characteristically different patterns of organization in cells undergoing each type of growth. The transition from one type of growth to another involves katanin, suggesting that the redistribution of microtubules requires that they first be severed into shorter pieces.

branching patterns of neurites, through regulation of the microtubule network.

Role of spastin

At present, it is uncertain how microtubule severing by spastin is coordinated with katanin-based microtubule severing. Spastin has become a protein of great interest because it merges the fundamental cell biology of microtubules with a neurological disorder of profound clinical importance. Interestingly, mutations in spastin might cause degeneration of longer axons not only by diminishing the supply of short microtubules required for the

generation of processes but also by the mutant spastin molecules accumulating on the microtubules and impeding their ability to participate in the transport of vesicular cargo [26]. This is evidenced by the fact that functional spastin does not appear to accumulate on the microtubules, but the mutant spastin does.

Spastin has now been identified in *Drosophila*, which provides more opportunities for functional analyses. Two recent studies on spastin in *Drosophila* seem to be contradictory: one study suggests that depletion of spastin causes diminution of microtubules at the synapse [27], whereas the other study suggests an increase in microtubule

levels at the synapse [28]. While the reasons for this discrepancy are uncertain, one possibility is that diminished severing of microtubules can lead to longer microtubules and an elevation in microtubule mass, but it can also lead to an impaired transport of microtubules, and hence a diminution in the delivery of microtubules to the distal axon. Thus, the opposite results in the two studies might be due to differences in the extent or timing of the deficit in microtubule severing.

Microtubule severing in plant cells

The morphology of a plant cell is determined by a rigid cell wall that contains highly organized cellulose. The orientation of the cellulose microfibers is affected by cortical microtubules that serve to guide the deposition of nascent fibers. In many plant cell types studied to date, microtubules are aligned perpendicular to the major axis of cell expansion, and this determines the orientation of cellulose microfibrils within the wall (Figure 1b). During transitions in cellular morphology, microtubules undergo major alterations in organization that are necessary for conveying cell wall materials to their appropriate destinations [29] (Figure 1b). In plant cells, which lack a traditional centrosome, katanin accumulates at the preprophase/prophase nuclear envelope, where spindle microtubules are nucleated, indicating that, regardless of whether the spindle is centriolar or acentriolar, katanin is positioned to affect spindle formation [29,31–34]. Almost three decade ago, Hardham and Gunning demonstrated that the cortical microtubule array comprises short overlapping microtubules of mean length 2–4 microns [30]. Mutations in plant katanin have recently been shown to alter transitions in microtubule organization [31–33], presumably by compromising the capacity of the cell to regulate microtubule length. In turn, there are deficits in the proper deposition of both cellulose and hemicellulose.

The validity of a motor-based 'cut and run' model (Figure 2) for plants is uncertain, given that live-cell studies have not revealed *bona fide* transport of the microtubule lattice in any plant cells studied to date [34]. Nevertheless, it seems counterintuitive that plants would never utilize motor-driven transport as a means to reconfigure microtubules after they are severed, given that plants certainly express a wide variety of molecular motors [34]. Therefore we suspect that more work on different kinds of plant cells will reveal evidence for motor-driven transport. For now, the available studies on plant cells suggest a variation of the 'cut and run' model in which the microtubules are cut by katanin but 'run' by a mechanism called treadmilling. During treadmilling, microtubules add subunits to one end while simultaneously losing subunits from the other end (Figure 3). This results in the redistribution of microtubules by an assembly/disassembly-based mechanism that gives the appearance of transport. Redistribution of microtubules by treadmilling has also been observed in fish melanophores [35]. In such cells as these, microtubule severing might also be important for uncapping minus-ends of microtubules and hence permitting treadmilling to occur.

Regulating 'cut and run' in cells

What mechanisms determine when and where microtubules will cut and run? We suspect that the motor proteins that transport microtubules are not selective for short microtubules but cannot move the longer microtubules owing to drag imposed upon them by crosslinks with other components of the cytoplasm. This raises questions such as the threshold length for microtubules to move, and what determines the threshold. In the axon, the longest microtubules observed to move are less than 10 microns in length [22], but we suspect that the threshold probably varies depending on the cell type and the degree to which the microtubules are crosslinked with other structures. In this view, it is actually the severing event that sets forth the transport because it renders the microtubule short enough to be moved (Figure 2). As for treadmilling, longer microtubules are less likely to treadmill because they would be more likely to accumulate factors that stabilize them (Figure 3); thus, it makes sense that severing of long microtubules would also be a key step in their redistribution.

An important clue to the regulation of microtubule severing comes from live-cell imaging of simple fibroblastic cells in which microtubules are observed to break upon fairly modest bending [2]. By contrast, microtubules do not tend to break when bent in purified microtubule preparations [36]. Moreover, microtubules become highly contorted in neuronal axons that undergo retraction, but they show no indication of breakage [37]. These studies suggest a model whereby katanin (or another severing protein) accesses the microtubule when it undergoes bending and causes it to break in fibroblasts. Certain positions along the length of the microtubule, termed 'lattice flaws', are thought to be more prone to breakage by severing proteins [38]. Neurons presumably contain factors that suppress microtubule severing in response to such bending. Indeed, we have shown that microtubules in the axonal compartment of the neuron are relatively more resistant to severing by katanin than microtubules in any other neuronal compartment [25]. Interestingly, actively growing axons are particularly rich in katanin and yet their microtubules also display a higher degree of resistance to breakage. We suspect that these seemingly contradictory observations reflect the need in the axon for both short mobile microtubules and longer microtubules to act as a substrate along which the short microtubules are transported [39].

It is known that katanin-induced microtubule severing becomes more active in interphase extracts that are depleted of a fibrous microtubule-associated protein (MAP) that is the frog homolog of MAP4 [40]. Various kinases that can potentially phosphorylate MAP4 also enhance microtubule severing in the extracts. Taken together, these results suggest a model whereby fibrous MAPs protect the lattice of the microtubule from being severed by katanin [41]. Phosphorylation of the MAPs results in their release from the microtubule and thus could enable katanin to gain access. Not all MAPs have this protective ability [42], however, indicating that specific MAPs regulate microtubule severing. We have experimentally determined that tau offers strong
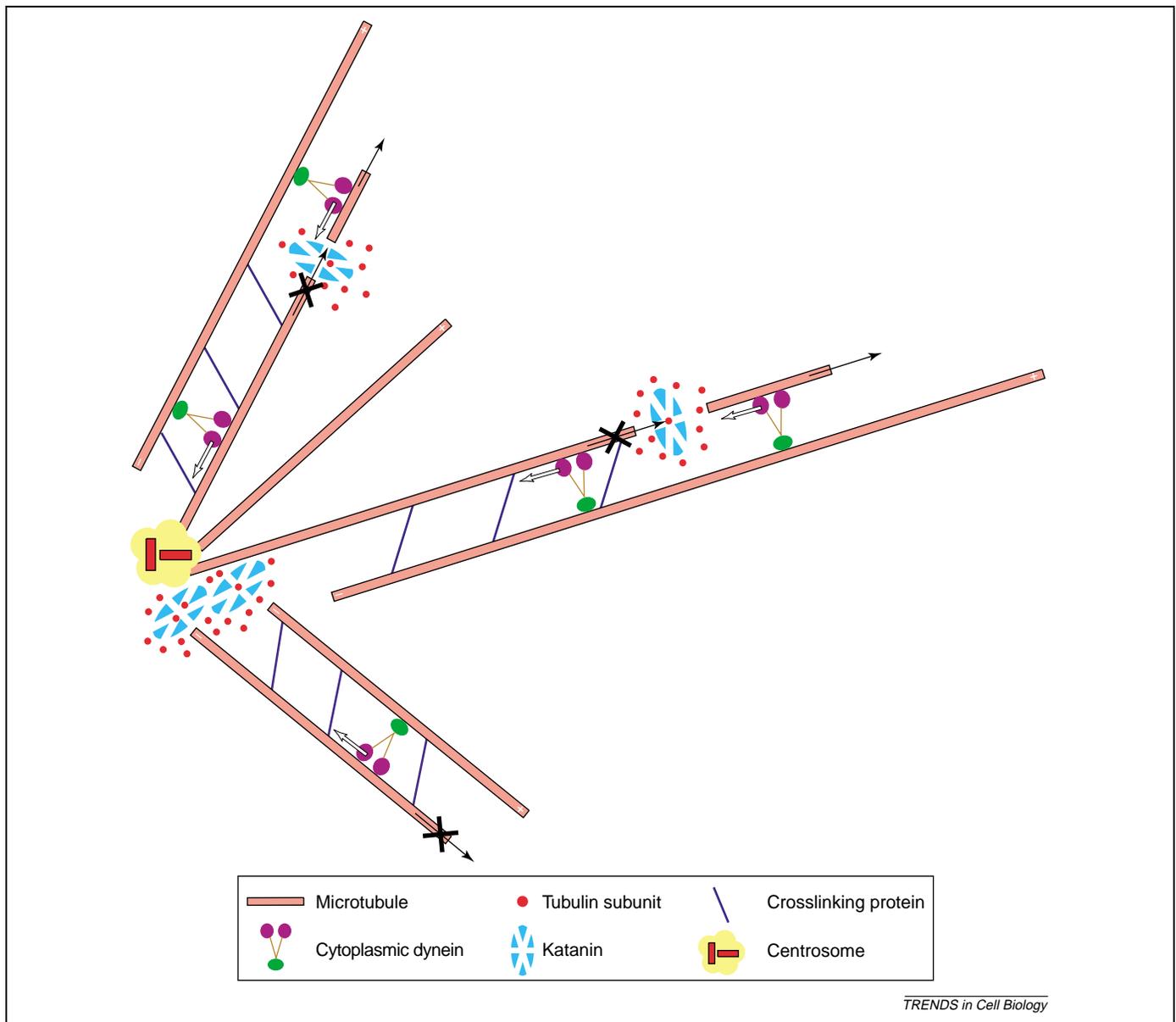
Figure 2. The 'cut and run' model for microtubule reconfiguration. Microtubules are nucleated from the centrosome, after which severing proteins such as katanin can break the microtubule free of the centrosome and can also sever the microtubule elsewhere along its length. Microtubules are crosslinked to neighboring microtubules as well as other cytoplasmic structures; this produces drag on the long microtubules that prevents them from moving in response to motor proteins such as cytoplasmic dynein. However, short microtubules (generated by severing) are less encumbered by such drag and hence are able to move in a rapid and concerted fashion in response to the motor-driven forces. As for the long microtubules, the same motor-driven forces presumably generate tensile forces that assist in their alignment and organization.

protection against severing by either katanin or spastin (L. Qiang and P. Baas, unpublished), suggesting that the enrichment of tau in the axon might explain why its microtubules are more resistant to breakage.

Another level of regulation for katanin might lie within its non-enzymatic P80 subunit. Biochemical studies suggest that P80-katanin has two different domains that can influence the severing properties of P60-katanin, one negatively and one positively, although the net effect is to enhance severing [43]. Katanin is typically viewed as a heterodimer, but we have recently shown that the two subunits are not present within cells at equimolar levels [25]. In fact, the ratio of the two subunits varies markedly in different tissues and at different stages of development, suggesting that the activity of the P60 subunit might be influenced by the levels of the P80 subunit. Thus far,

experimental evidence suggests that P80 augments the severing of microtubules by P60, but it is conceivable that different domains of the P80 molecule might be masked under different circumstances, permitting the available P80 to either suppress or augment microtubule severing. It is also worthwhile bearing in mind that katanin is thought to have to hexamerize to sever microtubules (and the same might be true of spastin), and hence there might be regulatory factors that either promote or deter the process of hexamerization.

Concluding remarks

We envision the regulation of microtubule severing as a multi-tiered process that involves coordination between the levels of the various severing proteins and their potential partners and antagonists, the distribution of
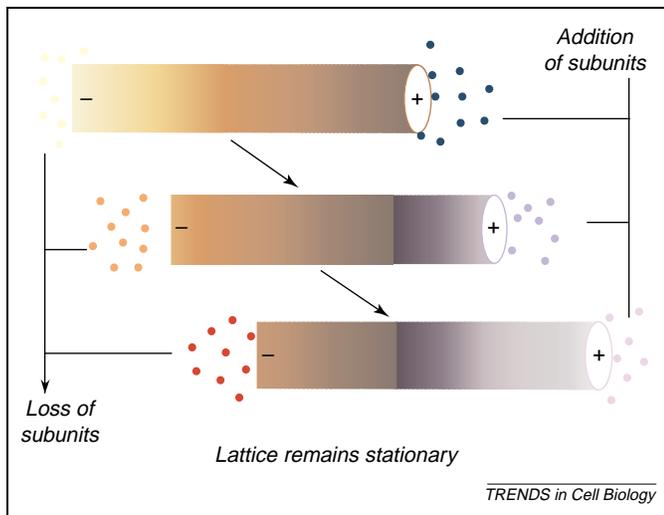
Figure 3. Treadmilling mimics microtubule transport. The phenomenon of treadmilling has been shown to result in redistribution of microtubules in certain cell types. The redistribution is similar to what can occur with motor-driven transport of microtubules, for example causing a change in microtubule distribution from the cell center to the cell periphery. However, treadmilling does not involve actual movement of the microtubule lattice. Instead, tubulin subunits are added to the plus-end at a similar degree to which they are lost from the minus-end, resulting in the appearance and effect of movement. Note in the colored schematic that regions of each color remain stationary, but the net effect is that the microtubule now appears in a new location.

these proteins and factors and signaling events that determine whether or not the severing proteins can access the microtubule lattice. For example, we suspect that signaling cascades relevant to axonal branching cause certain MAPs to be locally phosphorylated, lose their association with the microtubules and hence allow severing proteins to focally fragment microtubules at the site of the impending branch [41]. If this reasoning is correct, pathologies that result in widespread microtubule deterioration in axons might result at least in part from wholesale dissociation of MAPs from microtubules, leading to dysregulation of microtubule severing [41].

On a cautionary note, the wealth of rapidly emerging data on microtubule-severing proteins and the consequences of their expression, mutation and experimental manipulation are not all readily interpretable, at least in a simple fashion, by the cut and run model. For example, mutations in *Caenorhabditis elegans* of the katanin-related protein MEI-1 have pronounced phenotypes that are not obviously due to deficits in microtubule mobility [44]. We suspect that microtubule severing has multiple consequences in addition to rendering the microtubules more mobile. For example, the severing of microtubules increases the number of microtubules and the number of free ends of microtubules. Plus-ends of microtubules are known to interact with a variety of molecules relevant to the dynamic properties of the microtubules as well as their interactions with cortical structures, actin filaments and molecular motors [45]. Some of these factors might also be important for determining whether a cut microtubule will depolymerize, treadmill or remain stable enough to be transported by a molecular motor. Of course, microtubule severing can also lead to significant depolymerization and loss of microtubule mass if the effects are particularly widespread or the microtubules are particularly labile.

Thus, as the merits of the 'cut and run' model are assessed, it is important to keep in mind that microtubule severing is likely to be crucial to a panoply of other cellular pathways and events in addition to the potential movement of the severed microtubules.

References
1 Kirschner, M. and Mitchison, T. (1986) Beyond self-assembly: from microtubules to morphogenesis. *Cell* 45, 329–342
2 Waterman-Storer, C.M. *et al*. (1997) Actomyosin-based retrograde flow of microtubules in the lamella of migrating epithelial cells influences microtubule dynamic instability and turnover and is associated with microtubule breakage and treadmilling. *J. Cell Biol.* 139, 417–434
3 Hartman, J.J. *et al*. (1999) Microtubule disassembly by ATP-dependent oligomerization of the AAA enzyme katanin. *Science* 286, 782–785
4 Ahmad, F.J. *et al*. (1999) An essential role for katanin in severing microtubules in the neuron. *J. Cell Biol.* 145, 305–315
5 Errico, A. *et al*. (2002) Spastin, a protein mutated in autosomal dominant hereditary paraplegia, is involved in microtubule dynamics. *Hum. Mol. Genet.* 11, 153–163
6 Evans, K.J. *et al*. (2005) Linking axonal degeneration to microtubule remodeling by Spastin-mediated microtubule severing. *J. Cell Biol.* 168, 599–606
7 Vale, R.D. (1991) Severing of stable microtubules by a mitotically activated protein in *Xenopus* egg extracts. *Cell* 64, 827–839
8 McNally, F.J. *et al*. (1998) Katanin is responsible for the M-phase microtubule-severing activity in *Xenopus* eggs. *Mol. Biol. Cell* 9, 1847–1861
9 Belmont, L.D. *et al*. (1990) Real-time visualization of cell cycle-dependent changes in microtubule dynamics in cytoplasmic extracts. *Cell* 62, 579–589
10 Keating, T.J. *et al*. (1997) Microtubule release from the centrosome. *Proc. Natl. Acad. Sci. U. S. A.* 94, 5078–5083
11 Mogensen, M.M. (1999) Microtubule release and capture in epithelial cells. *Biol. Cell* 91, 331–341
12 Rusan, N.M. *et al*. (2005) Centrosome fragments and microtubules are transported asymmetrically away from the division plane in anaphase. *J. Cell Biol.* 168, 21–28
13 Marrari, Y. *et al*. (2003) Analysis of microtubule movement on isolated *Xenopus* egg cortices provides evidence that the cortical rotation involves dynein as well as kinesin related proteins and is regulated by local microtubule polymerization. *Dev. Biol.* 257, 55–70
14 Adames, N.R. and Cooper, J.A. (2000) Microtubule interactions with the cell cortex causing nuclear movements in *Saccharomyces cerevisiae*. *J. Cell Biol.* 149, 863–874
15 Abal, M. *et al*. (2002) Microtubule release from the centrosome in migrating cells. *J. Cell Biol.* 159, 731–737
16 Baird, D.H. *et al*. (2004) Distribution of the microtubule-related protein ninein in developing neurons. *Neuropharmacology* 47, 677–683
17 Yu, W. *et al*. (1993) Microtubule nucleation and release from the neuronal centrosome. *J. Cell Biol.* 122, 349–359
18 Ahmad, F.J. and Baas, P.W. (1995) Microtubules released from the neuronal centrosome are transported into the axon. *J. Cell Sci.* 108, 2761–2769
19 Joshi, H.C. and Baas, P.W. (1993) A new perspective on microtubules and axon growth. *J. Cell Biol.* 121, 1191–1196
20 Yu, W. *et al*. (1994) Microtubule fragmentation and partitioning in the axon during collateral branch formation. *J. Neurosci.* 14, 5872–5884
21 Dent, E.W. *et al*. (1999) Reorganization and movement of microtubules in growth cones and developing interstitial branches. *J. Neurosci.* 19, 8894–8904
22 Wang, L. and Brown, A. (2002) Rapid movement of microtubules in axons. *Curr. Biol.* 12, 1496–1501
23 Baas, P.W. and Buster, D.W. (2004) Slow axonal transport and the genesis of neuronal morphology. *J. Neurobiol.* 58, 3–17

24 Karabay, A. *et al.* (2004) Axonal growth is sensitive to the levels of katanin, a protein that severs microtubules. *J. Neurosci.* 24, 5778–5788

25 Yu, W. *et al.* (2005) Regulation of microtubule severing by katanin subunits during neuronal development. *J. Neurosci.* 25, 5573–5583

26 McDermott, C.J. *et al.* (2003) Hereditary spastic paraparesis: disrupted intracellular transport associated with spastin mutation. *Ann. Neurol.* 54, 748–759

27 Sherwood, N.T. *et al.* (2004) *Drosophila* spastin regulates synaptic microtubule networks and is required for normal motor function. *PLoS Biol.* 2, e429

28 Trotta, N. *et al.* (2004) The hereditary spastic paraplegia gene, spastin, regulates microtubule stability to modulate synaptic structure and function. *Curr. Biol.* 14, 1135–1147

29 Smith, L.G. (2003) Cytoskeletal control of plant cell shape: getting the fine points. *Curr. Opin. Plant Biol.* 6, 63–73

30 Hardham, A.R. and Gunning, B.E.S. (1977) The length and disposition of cortical microtubules in plant cells fixed in glutaraldehyde-osmium tetroxide. *Planta* 134, 201–203

31 Bouquin, T. *et al.* (2003) The *Arabidopsis* lue1 mutant defines a katanin p60 ortholog involved in hormonal control of microtubule orientation during cell growth. *J. Cell Sci.* 116, 791–801

32 Burk, D.H. and Ye, Z.H. (2002) Alteration of oriented deposition of cellulose microfibrils by mutation of a katanin-like microtubule-severing protein. *Plant Cell* 14, 2145–2160

33 Webb, M. *et al.* (2002) Cell specification in the *Arabidopsis* root epidermis requires the activity of ECTOPIC ROOT HAIR 3– a katanin-p60 protein. *Development* 129, 123–131

34 Dixit, R. and Cyr, R. (2004) The cortical microtubule array: from dynamics to organization. *Plant Cell* 16, 2546–2552

35 Rodionov, V.I. and Borisy, G.G. (1997) Microtubule treadmilling *in vivo*. *Science* 275, 215–218

36 Janson, M.E. *et al.* (2003) Dynamic instability of microtubules is regulated by force. *J. Cell Biol.* 161, 1029–1034

37 He, Y. *et al.* (2002) Microtubule reconfiguration during axonal retraction induced by nitric oxide. *J. Neurosci.* 22, 5982–5991

38 Odde, D.J. *et al.* (1999) Microtubule bending and breaking in living fibroblast cells. *J. Cell Sci.* 112, 3283–3288

39 Hasaka, T.P. *et al.* (2004) Role of actin filaments in the axonal transport of microtubules. *J. Neurosci.* 24, 11291–11301

40 McNally, K.P. *et al.* (2002) Katanin-mediated microtubule severing can be regulated by multiple mechanisms. *Cell Motil. Cytoskeleton* 53, 337–349

41 Baas, P.W. and Qiang, L. (2005) Neuronal microtubules: when the MAP is the roadblock. *Trends Cell Biol.* 15, 183–187

42 Wicker-Planquart, C. *et al.* (2004) Interactions of tobacco microtubule-associated protein MAP65-1b with microtubules. *Plant J.* 39, 126–134

43 McNally, K.P. *et al.* (2000) Two domains of p80 katanin regulate microtubule severing and spindle pole targeting by p60 katanin. *J. Cell Sci.* 113, 1623–1633

44 Pang, K.M. *et al.* (2004) The minibrain kinase homolog, mbk-2, is required for spindle positioning and asymmetric cell division in early *C. elegans* embryos. *Dev. Biol.* 265, 127–139

45 Kornack, D.R. and Giger, R.J. (2005) Probing microtubule +TIPs: regulation of axon branching. *Curr. Opin. Neurobiol.* 15, 58–66