Microtubule dynamics: **Treadmilling comes around again**

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Although it is generally believed that microtubules have minus ends bound to the centrosome and free plus ends that exhibit dynamic instability, recent observations show that the minus ends can be free and that modulation of dynamic instability at both ends can result in treadmilling and flux in interphase cells.

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A principal component of the cell's cytoskeleton consists of microtubules, polarized polymers of α and β tubulin heterodimers that grow and shorten by the association and dissociation of tubulin at their ends. The fast-growing end of the microtubule is referred to as the plus end, and the slow-growing end as the minus end. A tubulin heterodimer can be added to a microtubule when both α and β tubulin have bound GTP and, after association with the polymer, the GTP bound to β tubulin (GTP–tubulin) is hydrolyzed irreversibly. A microtubule therefore consists primarily of GDP–tubulin (reviewed in [1]). This hydrolysis of GTP frees microtubule assembly from the constraints of a simple equilibrium, allowing unusual assembly behaviors such as 'dynamic instability' and 'treadmilling'.

For microtubules made from pure tubulin, dynamic instability — defined as the coexistence of microtubules in growing and shrinking populations that interconvert infrequently and stochastically — is the fundamental mechanism of assembly and disassembly (reviewed in [1]). The difference between the two states is probably due to the presence of a cap of GTP–tubulin at growing microtubule ends; this cap stabilizes the ends for growth and gives them their characteristic straight conformation. When GTP hydrolysis in the polymer catches up with assembly, GDP–tubulin is exposed on microtubule ends, resulting in a destabilizing, curved conformation that promotes rapid shortening.

The parameters of dynamic instability have been defined as the rates of growth and shortening, and the frequencies of transition from growth to shortening ('catastrophe') and from shortening to growth ('rescue'). By measuring individual plus and minus ends directly *in vitro*, it has been shown that microtubules polymerized from pure tubulin grow at 1–2 μm min^{-1} and shorten at 20–30 μm min^{-1}, switching phases stochastically less than once per minute on average, at physiological tubulin concentration ranges [2]. These parameters of dynamic instability are affected by the binding of microtubule-associated proteins (MAPs), which, for classically studied neuronal MAPs, generally stabilize microtubules by suppressing catastrophe, promoting rescue, enhancing growth rates and reducing shortening rates. MAPs are thought to regulate the dynamics and end structure of microtubules *in vivo* (reviewed in [3]).

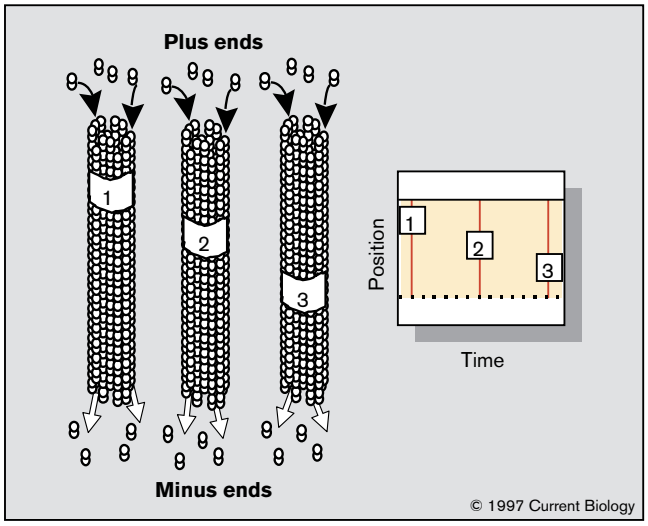
A second consequence of assembly-driven GTP hydrolysis is that, at certain tubulin concentrations, microtubules assembled from pure tubulin can undergo head-to-tail polymerization, or treadmilling [2]. Head-to-tail polymerization, as proposed originally by Wegner [4] for actin filament assembly, is defined as the steady-state unidirectional flux of subunits through the polymer as a result of continuous net assembly at one end of the polymer and continuous net disassembly at the other end. Differences between the two microtubule ends in both the critical tubulin concentration for assembly and the concentration dependency of catastrophe and rescue frequencies of dynamic instability allow pure tubulin treadmilling in a minus-to-plus direction at about 4 μm per hour [2]. Before this discovery, Margolis and Wilson [5] had used bulk steady-state assembly assays to show that self-nucleated microtubules, assembled from brain tubulin and MAPs, can treadmill *in vitro* at about 1 μm per hour, and for several years treadmilling was believed to be the fundamental mechanism of microtubule assembly. Several years later, treadmilling of individual MAP-containing microtubules at rates similar to those for pure tubulin, but in a plus-to-minus direction, was visualized by Hotani and Horio [6] using darkfield microscopy. They suggested that the binding of MAPs could suppress the phase transitions of dynamic instability and bias the kinetic properties of the two ends to allow for treadmilling (Fig. 1) [6].

Microtubule dynamics *in vivo*

In living cells, the polarity of microtubules lends organization to the cell. In undifferentiated cells in culture, microtubule minus ends are believed to be anchored at the centrosome, while their plus ends radiate out to the cell periphery in the interphase array or towards the chromosomes in the mitotic spindle. High-resolution microscopy has shown that the plus ends of individual microtubules in the flat peripheral region of interphase cells undergo random growth and shortening excursions at velocities slightly higher than, but within the range typical for, dynamic instability *in vitro* [3].

Dynamic instability of individual microtubules also occurs during mitosis [7] but, in contrast to interphase cells,

Figure 1



Microtubule treadmilling behavior *in vitro* for MAP-containing microtubules, as observed by Hotani and Horio [6]. A steady net gain of tubulin subunits at the plus end and a net loss of subunits at the minus end (arrows indicate net dimer addition or removal) produce a continuous flux of marked subunits through the polymer. The white numbered blocks on the microtubules represent marked subunits in the lattice at three consecutive points in time, chronologically labeled 1, 2 and 3. The graph shows how the position of the marked block of subunits and the plus (solid line) and minus (dashed line) ends of the microtubule (red lines) changes relative to a reference point over time. The slopes of the solid and dashed lines represent velocities of growth and shortening (see Figs 2 and 3).

mitotic microtubules have also been shown to exhibit treadmilling behavior. Tubulin subunits in kinetochore fibers in tissue cells and in polarized astral microtubule arrays in mitotic *Xenopus* extracts undergo slow, steady-state treadmilling towards the minus ends at the pole or center at about 1 μm min^{-1}. As mitotic treadmilling occurs only on microtubules organized in a focused array and depends on energy input by ATP, it is mechanistically different from the biased dynamic instability that produces treadmilling of individual microtubules with two free ends as observed by Hotani and Horio [6]. To differentiate these two processes, the steady-state poleward movement of the microtubule lattice has been been termed microtubule 'flux' (reviewed in [8]).

The discovery of treadmilling during interphase

Recent improvements in the sensitivity of cameras for fluorescence imaging and the introduction of methods to reduce fluorophore-induced photodamage in living cells [9] have allowed for more detailed views of larger areas of the microtubule cytoskeleton over longer times. This has led to several interesting new observations on microtubule dynamics in interphase cells, including the recent discovery by Rodionov and Borisy [10] of the treadmilling behavior of individual microtubules in interphase. These researchers examined melanophore cell fragments microinjected with x-rhodamine–tubulin to determine how microtubules are organized in the cytoplasm in the absence of a centrosome.

In the cell fragments, microtubules were seen to organize themselves into a radial array [11], which frequently released microtubules from its center [10]. Following release, the microtubules appeared to move, while maintaining constant length, towards the cell periphery. When the lattice of individual microtubules was marked by laser photobleaching as they were released from the organizing center, the marks remained stationary with respect to the cell boundaries while the plus and minus ends maintained continuous growth and shortening, respectively, without transitions. When the plus end reached the cell edge, the minus end shortened until the microtubule depolymerized completely (Fig. 2a). These observations showed that individual microtubules with free plus and minus ends can exhibit steady treadmilling in the cytoplasm of living cells.

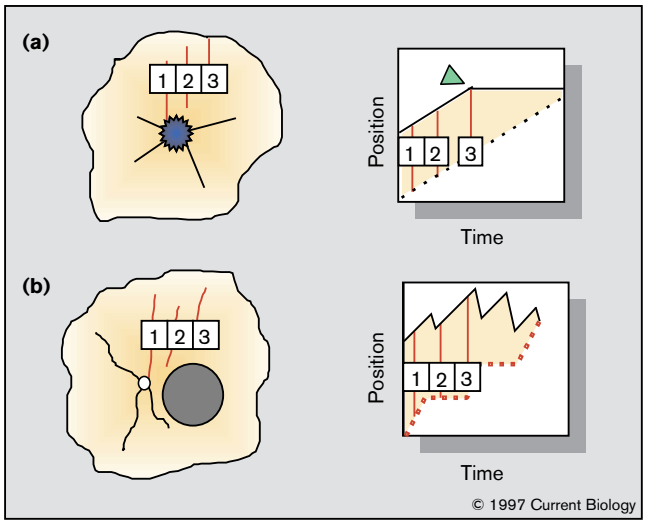
Rodionov and Borisy [10] postulated that, because the frequent release of microtubules from the organizing center in cell fragments always led to complete depolymerization by treadmilling, the free tubulin concentration in the cell fragments was higher than that in intact cells, where minus ends are bound more stably to the centrosome. The regulation of the time that minus ends reside at the centrosome could thus be a means for shifting the mechanism of microtubule turnover, in cells, from dynamic instability to treadmilling. However, the rate of treadmilling observed in the cell fragments is much higher than rates measured *in vitro* [2,5,6], being more comparable to the rates of growth and shortening measured for dynamic instability both *in vitro* [2] and *in vivo* [3]. It is likely, therefore, that the treadmilling behavior observed in melanophore fragments is caused by MAPs or factors that regulate dynamic instability in an end-specific fashion, such that the rates of assembly and disassembly are tightly controlled, catastrophe is suppressed at the plus end, and rescue is suppressed at the minus end. We call this behavior 'biased dynamic instability'.

Although no factors are known to modulate dynamic instability in this particular way, there are several proteins that produce microtubule end-specific regulation. XMAP from *Xenopus* eggs specifically enhances growth and shortening rates at plus, but not minus ends [12], while ncd — a minus-end directed, kinesin-related protein — has been shown to selectively depolymerize the minus ends of taxol-stabilized microtubules [13]. XKCM-1, a microtubule-catastrophe-promoting protein with predicted plus-end microtubule motor capabilities, has also been identified in *Xenopus*, and it is likely that its microtubule regulatory activity is restricted to plus ends [14].

Treadmilling behavior of an individual microtubule *in vivo* with two free ends may also occur for a short period of time in a less specifically regulated way, simply by the random chance that normal dynamic instability becomes coordinated between the two microtubule ends, resulting in net growth at one end and net shortening at the other. There are two recent examples of this. Keating *et al.* [15] examined microtubule dynamics at the centrosome in PtK cells and found that the minus end of a microtubule released from the centrosome could shorten rapidly, with intermittent pauses, towards its randomly growing and shortening plus end. If plus-end dynamic instability resulted in net growth, the microtubule could be ejected from the centrosomal region by treadmilling until it was eventually consumed by minus-end shortening (Fig. 2b). We have observed a similar phenomenon in the lamella of migrating newt lung epithelial cells (C.M.W-S. and E.D.S., unpublished observations), where we have seen many examples of microtubule breakage associated with local microtubule buckling. Following breakage, a newly formed free minus end is either stabilized or undergoes intermittent minus-end shortening that fails to 'catch up' with its dynamic plus end, which exhibits net growth.

In addition to these examples of individual microtubule behavior, there are also recent examples in interphase cells of coordinated net assembly/disassembly at the two ends of microtubules in a large array, similar to flux in mitotic microtubule arrays. We have recently documented retrograde microtubule flux in the parallel array of microtubules that grow perpendicular to the leading edge of a migrating newt lung epithelial cell (C.M.W-S. and E.D.S., unpublished observations). We used photoactivation methods to show that all perpendicular microtubules in the lamella move coordinately towards the cell center at the same rate as surface ruffles (~0.5 μm min^{-1}) in an actin-dependent and myosin-dependent manner. As the microtubules move rearward, their plus ends exhibit growth and shortening typical of dynamic instability but, over time, net growth is maintained such that the distance between the plus ends and the cell edge is kept relatively constant (Fig. 3a). Conservation of polymer mass requires that the net growth of microtubule plus ends in the lamella must be offset by continuous net shortening somewhere in the cell. We have found that more than 75% of microtubules in these cells are not attached to the centrosome (C.M.W-S. and E.D.S., unpublished observations), so we do not think that net polymer loss occurs by disassembly of centrosome-bound minus ends, as is thought to be the case in mitotic poleward microtubule flux. Thus, although apparently different in their underlying mechanisms, actomyosin-based retrograde flux of microtubules and mitotic poleward flux of microtubules both result in the spatially and temporally coordinated movement of the lattices of many microtubules in a continuous, steady-state process. In contrast to this, Rodionov and Borisy [11] have

Figure 2



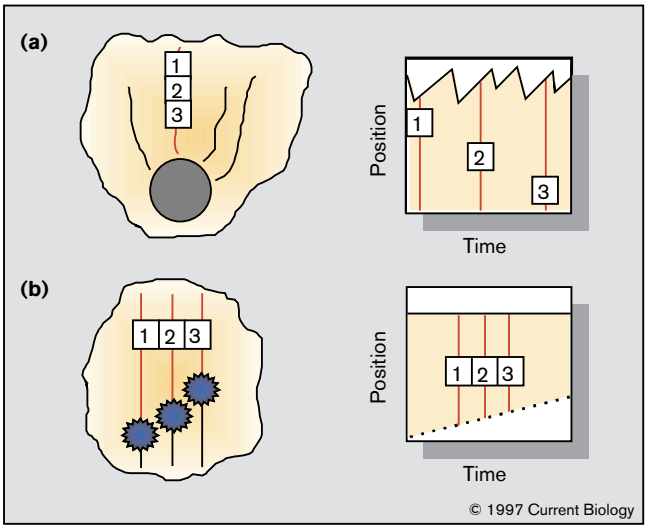
Treadmilling of microtubules with 'free' minus ends *in vivo*. The graphs are described in the Figure 1 legend. In **(a)**, the microtubule of interest is shown at three time points as it is ejected from the non-centrosomal microtubule organizing center (blue star) of a melanophore fragment [10]. The plus-end growth rate equals the minus-end shortening rate, and the microtubule exhibits no transitions. Plus-end growth ceases, but minus-end shortening continues when the microtubule reaches the cell periphery (position marked with green arrowhead on the graph). In **(b)**, a minus end is ejected from the centrosome (white circle) creating a microtubule with two free ends that treadmills towards the cell periphery. The plus end exhibits growth and shortening typical of dynamic instability; the minus end intermittently shortens and pauses, and the photobleached mark stays stationary. Note that the view of the microtubule at each consecutive timepoint is shifted to the right for clarity, but lateral shifting did not occur in the cell.

observed a non-steady-state flux activity that occurs during the self-centering of radial arrays of microtubules in melanophore fragments. They photobleached marks across microtubules emanating from the center of a radial microtubule array during the process of self-centering. This showed that, as the array moved to the fragment center, microtubules extending to the fragment periphery from either side of the organizing center had coordinated net growth and shortening (Fig. 3b).

Microtubule treadmilling: what does it do for you?

We believe that the treadmilling behavior of individual microtubules *in vivo* is the result of biased dynamic instability at the two ends of a free microtubule. That this can occur in cells is no surprise given the results of Hotani and Horio [6]. The more interesting aspect of the discovery of individual microtubule treadmilling in interphase cells, however, is that it spotlights the importance of minus-end disassembly dynamics in microtubule turnover in cells [15]. Gliksman *et al.* [16] pointed out a large discrepancy between the rates of microtubule polymer turnover measured *in vivo* and calculated values of polymer turnover

Figure 3



Flux of microtubules in interphase arrays. The graphs are described in the Figure 1 legend. In **(a)**, a photoactivated mark on a microtubule that grows perpendicular to the leading edge of a migrating epithelial cell moves toward the cell center over time. The plus end of the microtubule exhibits dynamic instability, while the mark moves steadily away from the cell edge. Because the precise location of the minus ends of microtubules are not known, they are shown to terminate near the nucleus (grey circle) and are not shown in the graph. **(b)** Three consecutive views of the same microtubule emanating from a melanophore fragment organizing center, shown from left to right within the cell fragment during the self-centering of the microtubule organizing center. The plus end and the photobleached mark stays stationary, while the minus end in the organizing center presumably shortens steadily [11].

times on the basis of measured parameters of plus-end dynamic instability in cells. There is now evidence for three ways by which free microtubule minus ends can be generated *in vivo*: first, microtubule ejection from a microtubule organizing center [10] or centrosome [14]; second, microtubule breakage (C.M.W-S. and E.D.S., unpublished observations); and third, spontaneous microtubule nucleation in cells with high endogenous tubulin concentrations [17]. Microtubule severing factors could play a role in the first two mechanisms [18]. The general consensus from these recent reports is that, *in vivo*, minus ends can either be (transiently) stabilized or intermittently shorten rapidly, but never grow. Thus, rapid microtubule turnover may be achieved by minus-end depolymerization, and may be regulated by a minus-end capping factor. As yet, there is no evidence for minus-end capping of non-centrosomal microtubules by gamma tubulin, a protein that binds to minus ends and participates in nucleation at the centrosome [1].

The discovery of treadmilling in interphase cells also resurrects old ideas about the use of molecular treadmills to generate organelle movement [5]. One can envisage how coupling to the depolymerizing minus end of a treadmilling microtubule could allow rapid plus-end-directed transport of cargo to the cell periphery. This is similar to kinesin-driven, minus-end-directed movement of cargo coupled to the depolymerizing plus end of a microtubule [19]. To extend the analogy, plus-end-directed movement by minus-end coupling could require the action of minus-end-directed, microtubule-based motors. In any case, because it now appears that the minus ends have a much more active role in microtubule dynamics *in vivo* than previously presumed, minus-end regulatory factors have new importance, and it is worth pursuing their isolation and characterization.

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