# Microtubule dynamics: **Controlling split ends**

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**The rapid switching between growth and shrinkage at microtubule ends is important for many cellular processes. Recent studies on the structure of the microtubule and on the mechanism of action of the microtubule regulators XKCM1 and OP18 have revealed how these switching events are regulated.**

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Microtubules are complex polymers assembled from heterodimers of the two GTP-binding proteins α and β tubulin. Microtubules are used universally among eukaryotes for chromosome segregation during cell division and for a plethora of more specialized functions. Two discrete properties of microtubules allow them to be used for these functions. The first of these is that microtubules act as static 'railroad tracks' on which the microtubule motor proteins — kinesin-related proteins and dyneins — transport cargo in a polarized manner through the cell. The direction of net transport by motor proteins can be changed during the cell cycle and during differentiation by altering the arrangement of microtubules in the cell.

The second key property of microtubules is that of 'dynamic instability'. This refers to the continuous switching between phases of microtubule polymerization and depolymerization, and is important for several reasons [1]. In addition to allowing cells to change the direction of net transport by motor proteins, rapid switching between growth and shrinkage also allows microtubules to probe three-dimensional space within a cell and interact with various targets. This 'search and capture' mechanism may be especially important in establishing microtubule contacts with chromosomes in the early stages of mitosis. Another role of dynamic instability is far more direct. A depolymerizing microtubule can pull a large object that is passively bound to the microtubule end, thus acting as a motor itself [2]. This aspect of microtubule dynamics has been proposed as a mechanism of chromosome movement during mitosis. The process of switching between growth and shrinkage is clearly a potential target for regulation, and recent studies have shed new light on how switching events are regulated.

Both the continuous switching between growth and shrinkage and the generation of force by a depolymerizing microtubule require a source of energy. Decades of research have pointed to GTP hydrolysis on the β tubulin subunit as the source of this energy. Microtubules assemble only in the presence of GTP-bound tubulin heterodimers but the β-tubulin-bound GTP is rapidly hydrolyzed to GDP after assembly [1]. This hydrolysis is presumed to be essential for depolymerization, because depolymerization essentially does not occur when a non-hydrolyzable GTP analog is incorporated into the β tubulin subunits of a microtubule [3]. (The GTP bound to the α subunit is non-exchangeable and never hydrolyzed.) These and other observations have led to a 'GTP cap' model in which GTP–tubulin is rapidly converted to GDP–tubulin after assembly into the microtubule. The conformation of GDP–tubulin favors depolymerization, but the GDP–tubulin within the wall of a microtubule is trapped by multiple weak lateral interactions and by a cap of GTP–tubulin subunits at the end of the microtubule.

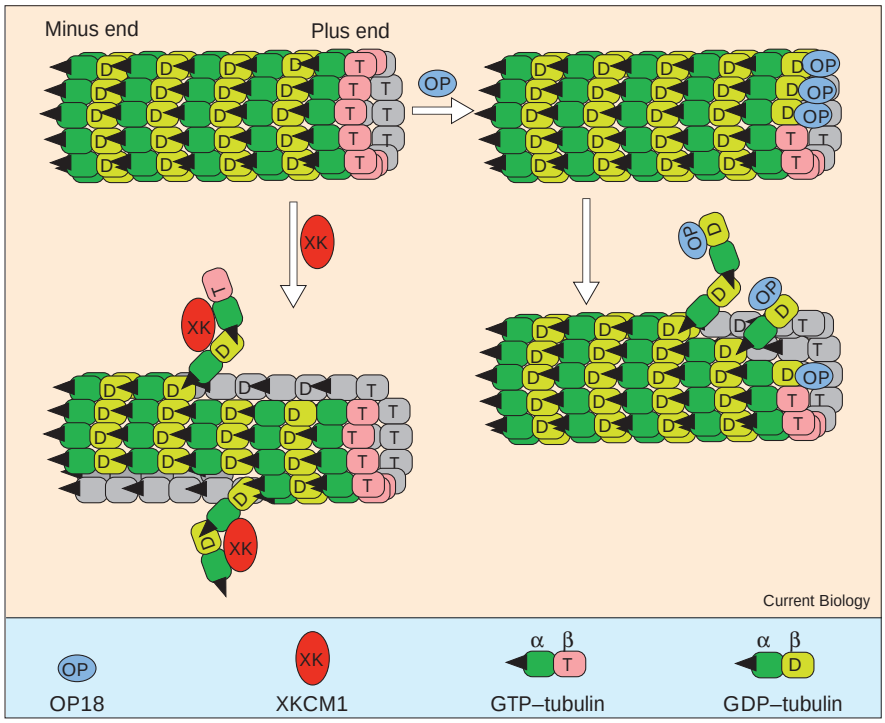
The GTP–tubulin subunits in the cap have a conformation that favors stronger lateral interactions and therefore continued polymerization. The energy of GTP hydrolysis is stored as a conformational strain within the microtubule until the GTP cap is lost by nucleotide exchange or hydrolysis. The GDP–tubulin subunits exposed at the microtubule end then lose lateral interactions and protofilaments of GDP–tubulin curl away into their favored conformation, producing 'split ends' that can be seen by cryoelectron microscopy [4]. The switch to depolymerization after loss of the GTP cap is called a catastrophe [5]. The energy released by relieving conformational strain is sufficient to power the movement of objects as large as chromosomes that are passively bound to the depolymerizing microtubule wall.

The recently determined high-resolution structure of the tubulin heterodimer within the microtubule [6] has shed new light on differences between the two ends of a microtubule. These differences are important, because the plus and minus ends of microtubules are differentially regulated in living cells. Earlier work demonstrated that, when a microtubule is cut to create a new plus end and a new minus end, the new plus end immediately undergoes a catastrophe because of the exposure of GDP–tubulin. The artificially generated minus ends, however, continue to grow [7]. This result suggested that the minus end of a microtubule might not have a GTP cap.

In the structure proposed by Nogales *et al.* [6], the β tubulin of a tubulin heterodimer is exposed at one end of the microtubule — the plus end — while α tubulin is exposed at the

**Figure 1**

Comparison of the mechanisms by which XKCM1 and OP18 induce microtubule disassembly. At upper left is a microtubule composed of GDP-bound tubulin subunits with a 'cap' of GTP-bound tubulin subunits at the plus end only. XKCM1 binds microtubule ends and induces protofilaments of microtubules to curl away, producing split ends. XKCM1 acts at both ends of the microtubule, because it does not care about the nucleotide state of the tubulin to which it binds. In contrast, OP18 appears to act by stimulating hydrolysis of GTP to GDP. At microtubule plus ends, GDP–tubulin spontaneously curls away into split ends; OP18 has little effect on minus ends, which do not have a GTP cap.




opposite, minus end. This can explain why a GTP cap forms at the plus, but not the minus, end of a microtubule. The key point is that an amino-acid side chain essential for hydrolysis of GTP on the exposed β tubulin at the plus end is found on the α tubulin of the incoming heterodimer (triangular projection in Figure 1). When a tubulin heterodimer adds to the plus end of a microtubule, contact is made between the α tubulin of the incoming heterodimer and the β tubulin exposed at the old plus end, causing hydrolysis of GTP bound to that β tubulin subunit. The GTP bound to the β tubulin subunit of the incoming heterodimer, however, remains unhydrolysed because no contact with the critical side chain of an α tubulin subunit has been made. Thus the GTP cap at the plus end consists of a single GTP-bound subunit on each protofilament. When a tubulin heterodimer adds on to the minus end, the contact is from the α tubulin subunit of the old minus end to the GTP bound to the β tubulin subunit of the incoming heterodimer, which consequently undergoes GTP hydrolysis so that no GTP cap is formed at the new minus end.

This refined GTP cap model leads to the prediction that a protein regulating catastrophes at microtubule minus ends could act only by directly changing the conformation of tubulin protofilaments from straight to splayed or split ends. In contrast, a protein that regulates catastrophes at microtubule plus ends could act either by inducing such a conformational change or by stimulating GTP hydrolysis or exchange for GDP at the plus end. Stimulating GTP hydrolysis on tubulin subunits would have no impact on minus ends, where all the tubulin is in the GDP-bound form. Recent studies [8,9] indicate that OP18 and XKCM1, the only known catastrophe-inducing proteins, each use one of these discrete mechanisms. XKCM1 appears to induce protofilament splaying directly without affecting GTP hydrolysis, and works on both plus and minus ends [8]. OP18, in contrast, appears to induce catastrophes by stimulating GTP hydrolysis, and preferentially induces catastrophes at plus ends [9].

XKCM1 was originally identified in a screen for frog egg cDNAs encoding kinesin-related motor proteins that might be involved in mitotic spindle function. Depletion of XKCM1 from egg extracts was found to cause a marked decrease in the catastrophe frequency of microtubule ends [10]. Desai *et al.* [8] purified XKCM1 and a related protein, XKIF2, and showed that both proteins increase the catastrophe frequencies of microtubules assembled from purified frog egg tubulin. XKCM1 was found to bind specifically to both plus and minus ends of microtubules, but not along their length; it stimulated depolymerization from both ends of microtubules, even when they were assembled with a non-hydrolyzable GTP analog. Analysis of microtubule ends after incubation with XKCM1 revealed curling protofilaments or split ends. These results indicated that XKCM1 directly induces a conformational change from straight to curled protofilaments, without inducing GTP hydrolysis.

OP18 was identified as a protein that increases the catastrophe frequency of microtubules, and was found to be identical to a protein previously characterized as an abundant phosphoprotein in leukemia cells [11]. Recent work by Howell *et al.* [9] has settled a controversy over the mechanism of OP18's activity. The analysis of deletion derivatives of the protein clearly demonstrated that OP18 is bifunctional. OP18 can indirectly increase catastrophe frequencies by binding and sequestering tubulin dimers. One deletion derivative of OP18, however was found to induce catastrophes without sequestering tubulin dimers. Microtubules assembled in the presence of a non-hydrolyzable GTP analog were found to be resistant to OP18's catastrophe-inducing activity, suggesting that OP18 might act by stimulating GTP hydrolysis in the GTP cap. As would be predicted from the lack of a GTP cap at microtubule minus ends, the catastrophe-specific deletion derivative of OP18 stimulated switches to depolymerization only at the plus ends of microtubules. OP18 thus causes only split plus ends, whereas XKCM1 causes split plus and minus ends.

A recent study by Rodionov *et al.* [12] suggests that a minus-end-specific catastrophe factor may remain to be discovered. In most cell types, microtubule minus ends are difficult to visualize, either because they are embedded in a centrosome or because they are found in regions of cells where microtubules are so dense that individual ends are difficult to resolve. To provide more microtubule minus ends for analysis, cytoplasts or cell fragments were prepared that lacked centrosomes. Surprisingly, the dynamics of microtubule minus ends were found to be extremely cell-type specific. In fibroblast-like cells, microtubule minus ends exhibited rapid depolymerization matched by rapid polymerization at plus ends; the resulting 'treadmilling' caused the movement of the microtubules through the cytoplasm. In contrast, microtubule minus ends in epithelial cells were relatively static, whereas the plus ends exhibited fluctuations between growth and shrinkage [12]. These results suggest that fibroblast-like cells may contain a minus-end-specific depolymerizing protein, whereas epithelial cells have a minus-end-specific stabilizing or capping protein. This minus-end-specific depolymerizing protein must induce disassembly by a mechanism distinct from that of OP18, because there is no GTP cap at the minus end. Isolation of this minus-end-specific protein may reveal yet another mechanism for generating split ends.

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