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Tipping microtubule dynamics, one protofilament at a time

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Microtubules are polymeric tubes that switch between phases of growth and shortening, and this property is essential to drive key cellular processes. Microtubules are composed of protofilaments formed by longitudinally arranged tubulin dimers. Microtubule dynamics can be affected by structural perturbations at the plus end, such as end tapering, and targeting only a small subset of protofilaments can alter the dynamics of the whole microtubule. Microtubule lattice plasticity, including compaction along the longitudinal axis upon GTP hydrolysis and tubulin dimer loss and reinsertion along microtubule shafts can also affect microtubule dynamics or mechanics. Microtubule behaviour can be fine-tuned by post-translational modifications and tubulin isotypes, which together support the diversity of microtubule functions within and across various cell types or cell cycle and developmental stages.

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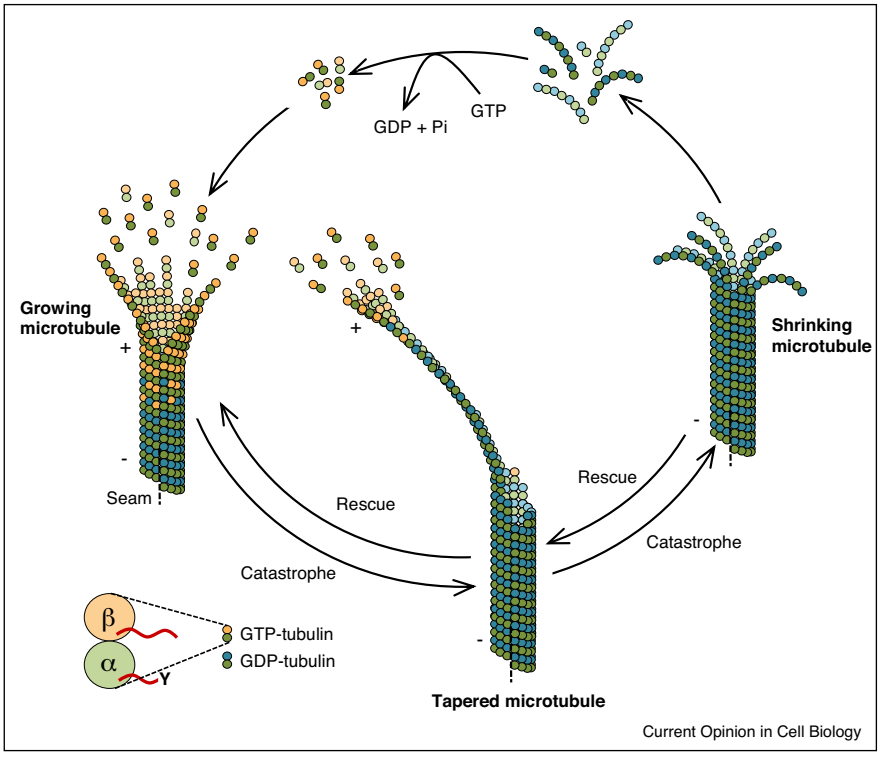
Introduction

Microtubules are built from dimers of α-tubulin and β-tubulin, which interact with each other in a head-to-tail fashion to form protofilaments, and 11–15 protofilaments, depending on the species and cell type [1–3], interact with each other laterally to form a hollow tube. The microtubule end where β-tubulin is exposed, termed the plus end, grows fast *in vitro* and serves as the major site of microtubule elongation in cells [4]. Microtubules can switch spontaneously from growth to shortening (Figure 1); furthermore, in cells they can also exhibit a paused state. Microtubules rarely pause in solutions of purified tubulin alone but can do so, for example, when exposed to a combination of microtubule growth-promoting and inhibiting factors [5]. The alternation between phases of growth and shortening, the phenomenon termed dynamic instability, is fundamental for most microtubule functions, from chromosome separation during cell division to cell reorganization during migration and differentiation. In this review, we will focus on the recent insights into how nucleotide hydrolysis and small perturbations in microtubule plus end structure bring about the transitions between different phases of dynamic instability at the microtubule plus end. We will also discuss the dynamics of microtubule shafts, which have always been seen as stable highways, but have now emerged as important sites of tubulin exchange that can affect microtubule stability and rescue from a depolymerizing state. Microtubule minus ends, which grow slowly *in vitro*, were traditionally regarded exclusively as sites of microtubule stabilization or disassembly. Recent work showed that minus ends can also display interesting and functionally important dynamics, but since this topic was reviewed recently [6], it will not be covered here. At the end, we will briefly touch upon the regulation of microtubule dynamics by tubulin isoforms and modifications, which are a major source of microtubule heterogeneity within and between different cell types and tissues.

Coupling GTP hydrolysis to structural changes in microtubules and catastrophe induction

Tubulin subunit addition during microtubule polymerization is coupled to GTP hydrolysis: the residues in both α and β-tubulin subunits complete the catalytic core for GTP hydrolysis and undergo structural changes upon hydrolysis (see [7] and references therein). Polymerizing microtubules have a stabilizing cap of GTP-bound tubulin subunits (GTP cap) at their ends, whereas the loss of this cap results in a switch from growth to depolymerisation (catastrophe). It has been proposed that GTP hydrolysis leads to a conformational strain at the E-site (GTP-binding site on β-tubulin), and that this strain is relieved upon depolymerisation, resulting in the formation of peeling protofilaments [7,8]. Recent high resolution cryo-electron microscopy (cryo-EM) studies of mammalian microtubules bound to GDP or GTP analogue GMPCPP indicated that GTP hydrolysis leads to structural rearrangements in both α-tubulin and β-tubulin and to microtubule lattice compaction along the longitudinal inter-dimer interface [7,9]. However, GTP-hydrolysis-dependent lattice compaction was not observed in microtubules grown from tubulin derived from budding or fission yeast [10•,11•]. This highlights species-specific

Figure 1



Microtubule polymerization–depolymerization cycle. Microtubules polymerize by the addition of GTP-bound tubulin dimers. Loss of the GTP-cap leads to microtubule destabilization and the switch to a shrinking state (a catastrophe) with peeling protofilaments. Microtubules have been proposed to go through a tapered intermediate state [8,12,15], although we note that alternative models with a blunt end constituting an intermediate between different phases of microtubule dynamics also exist [17]. When the stabilizing GTP cap at such a tapered end is lost beyond a certain threshold, a microtubule can switch to catastrophe; alternatively, it can regain a complete plus end structure and keep growing. Depolymerising microtubules can transit back to the polymerization phase (a rescue). A scheme of tubulin dimer at the bottom shows the flexible acidic tails of α-tubulin and β-tubulin (red lines); Y, the C-terminal tyrosine of α-tubulin.

differences in the structural plasticity of microtubule lattices. It should be noted here that we are still missing a complete picture of the structural transitions in microtubule lattice associated with GTP hydrolysis and that the data obtained with GTP analogues should be interpreted with some caution.

The exact structure of a microtubule plus end is a matter of debate. Importantly, there are indications that such ends are often not blunt: cryo-EM analysis of microtubules assembled *in vitro* has shown that a ~30–40% of the growing microtubule ends can be tapered and display curved sheet-like extensions that range from 50 to 2000 nm in length [8,12,13]. The length of these sheet-like structures at the growing ends was shown to increase with increasing growth rates in a tubulin concentration-dependent manner [8]. Furthermore, it has been proposed that catastrophe induction involves the accumulation of defects like lagging protofilaments when a microtubule keeps growing for a longer period of time and undergoes ageing [14]. Such defects could lead to destabilization of a growing microtubule, possibly resulting in a tapered tip structure with a reduced stabilizing GTP-cap that predisposes it to a catastrophe [14,15,16•]. Experimentally observed sheet-like extensions might thus correspond to ageing, catastrophe-prone tip structures (Figure 1). It should be noted, however, that correlative observations of the structure and dynamics of microtubule ends in frog egg extracts led to the suggestion that metastable intermediates between different phases of microtubule dynamics correspond to blunt ends [17]. Furthermore, modelling suggested that the dynamic evolution of microtubule tip structure could explain age-dependent microtubule catastrophes in the absence of visible changes in microtubule tip configuration [18]. It is clear that more work is needed to understand what is happening at the microtubule plus end undergoing a catastrophe.

Visualization of stabilizing cap at microtubule ends was greatly facilitated by the discovery that the binding of the proteins of the End Binding (EB) family to microtubules is very sensitive to the nucleotide state of tubulin. EBs recognize a pocket on the microtubule in close proximity