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Regulation of microtubule dynamic instability

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Abstract

Proper regulation of MT (microtubule) dynamics is essential for various vital processes, including the segregation of chromosomes, directional cell migration and differentiation. MT assembly and disassembly is modulated by a complex network of intracellular factors that co-operate or antagonize each other, are highly regulated in space and time and are thus attuned to the cell cycle and differentiation processes. While we only begin to appreciate how the concerted action of MT stabilizers and destabilizers shapes different MT patterns, a clear picture of how individual factors affect the MT structure is emerging. In this paper, we review the current knowledge about proteins that modulate MT dynamic instability.

Introduction

MTs (microtubules) form a cytoplasmic network composed of hollow tubes that assemble from α/β-tubulin heterodimers. Due to the polarized nature of the tubulin dimer, the generated MT is also polarized: β-tubulin is exposed at the plus end, whereas the α-subunit is exposed at the minus end. MTs are dynamic and can rapidly switch between phases of growth and shrinkage, a process called dynamic instability [1]. MTs play an essential role in separating sister chromatids during mitosis and have multiple functions in non-dividing cells, for example in intracellular transport, positioning of intracellular organelles, cell migration and differentiation [2]. All these processes require the organization of MTs into arrays with different geometry and density, which depends on the generation of new MTs as well as on the proper regulation of their dynamic behaviour. New MT ends can be formed by two main types of mechanisms: *de novo* MT nucleation [3] and mechanical or enzymatic breakage of pre-existing MTs. The latter mechanism depends on severing proteins such as spastin and katanin, the AAA (ATPase associated with various cellular activities) family ATPases that use ATP as a source of energy necessary for their action ([4] and references therein).

Free MT ends can elongate or shorten, and even mild suppression of MT dynamics by low doses of MT-stabilizing or -destabilizing drugs has a profound effect on the organization of the mitotic apparatus, directional migration and even synaptogenesis in neuronal cells [5–7]. Cells express an arsenal of MT-modulating factors, some of which promote assembly [such as XMAP215 (*Xenopus* microtubule-associated protein 215)] or disassembly [kinesin-13s and stathmin/SCG10 (superior cervical ganglion-10 protein) proteins], while others have more specific roles only on a subset of MTs. In the present review, we summarize the current knowledge about factors modulating MT dynamic instability (see Figure 1).

MT dynamics is determined largely by four parameters: (i) the speed of MT growth, (ii) the speed of MT shrinkage, (iii) the frequency of catastrophes (transitions from growth to shrinkage phase) and (iv) the frequency of rescues (transitions from shrinkage to growth phase) [8]. In addition, MT pausing can be frequently observed in animal cells. It is unclear whether pauses represent a truly undynamic and thus stable state or whether they are phases of growth and shrinkage that occur at either a very low speed or with very high transition frequencies that cannot be resolved spatially and/or temporally with the current imaging technologies. Owing to uncertainties in pause definition, the data on pauses obtained in different studies are difficult to compare and will not be discussed here.

Purified tubulin can self-assemble under certain conditions to form MT filaments that exhibit dynamic behaviour at both ends [9]. However, in intact cells and cell extracts, MT plus ends assemble and disassemble at much higher speeds than *in vitro* at the same tubulin concentration [10]. In contrast, although MT minus ends show dynamic instability, albeit at a lower rate than the plus ends *in vitro*, they are usually capped and anchored at MT organizing centres in cells. Even in specialized cell types where minus ends are free (such as most of the lamellar MTs in migrating epithelial cells [11]), growth at the minus end has never been reported (reviewed in [12]). Capping of MT minus ends requires specialized factors such as γ-tubulin and the components of the γ-TuRC (γ-tubulin ring complex), and anchoring at centrosomal or non-centrosomal sites involves ninein and/or Nezha [13–15]. However, MT minus ends can serve as sites of depolymerization in cells; their shrinkage often being regulated by the same factors that also affect plus end dynamics.

MT dynamics-modulating factors can largely be divided into MT-stabilizing and -destabilizing factors. MTs are stabilized in several ways: (i) by preventing catastrophe (this ensures MT growth persistency or prolonged interaction with target sites), (ii) by rescuing a depolymerizing MT and

Key words: dynamic instability, kinesin-13, microtubule-associated protein, microtubule dynamics, microtubule assembly and disassembly regulation, tau protein.

Abbreviations used: CLIP, cytoplasmic linker protein; EB, end-binding protein; MCAK, mitotic centromere-associated kinesin; MAP, microtubule-associated protein; MT, microtubule; SCG10, superior cervical ganglion-10 protein; +TIP, MT plus-end tracking protein; XKCM1, Xenopus kinesin catastrophe modulator-1; XMAP215, *Xenopus* microtubule-associated protein 215.

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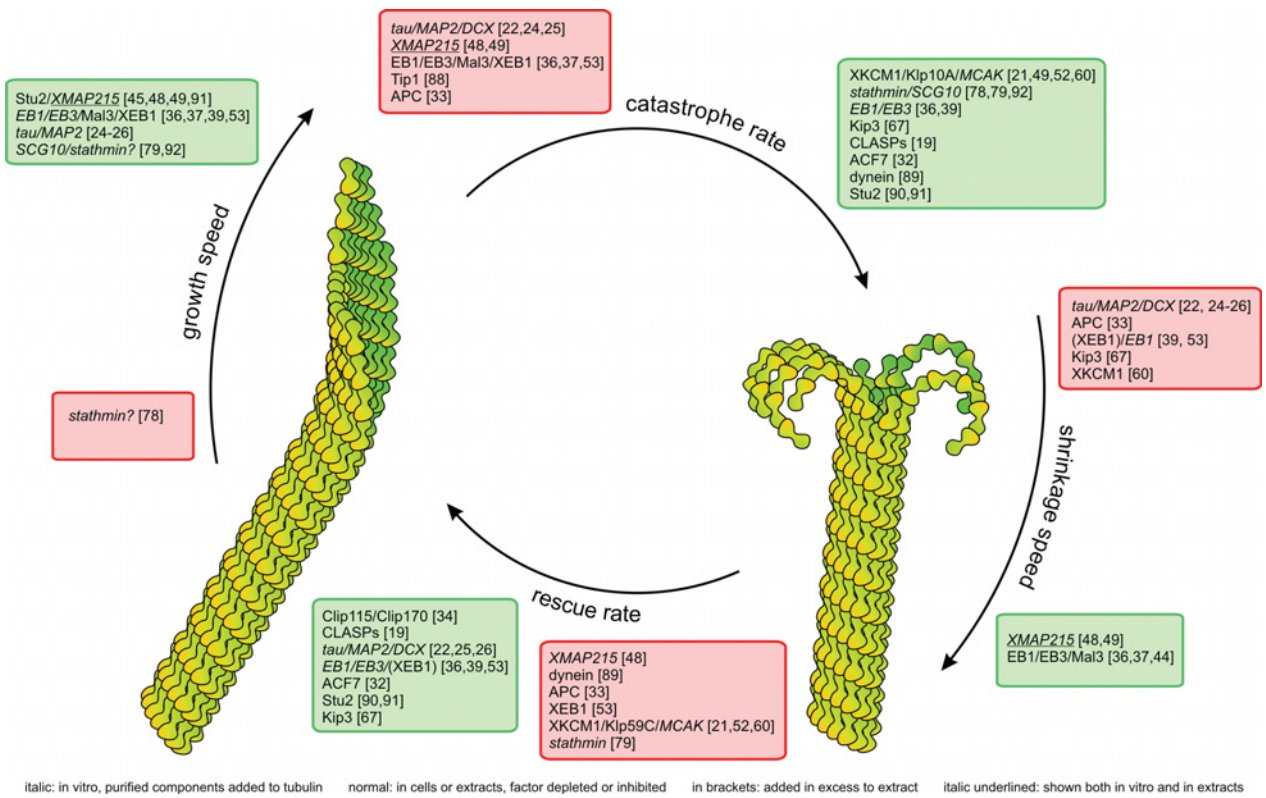
Figure 1 | Factors that modulate parameters of MT dynamic instability

MT stability can be described by four parameters: speed of growth and shrinkage and frequency of transitions (catastrophe and rescue rate). Different MT-modulating factors affect distinct parameters either positively, hence increasing the frequency or speed (green boxes), or negatively, suppressing transitions or reducing the speed (red boxes). Factors that were shown to affect certain parameters directly on purified tubulin *in vitro* are shown in italic. Where activity shown was based on phenotypes resulting after depletion or inhibition of factors in complex extracts or cells, factors are shown in normal font. If the activity could be demonstrated in extracts as well as *in vitro*, factors are shown in italic and underlined. If the effect was only apparent when a factor was added in excess to extracts, it is shown in round brackets. Supporting data can be found in the references indicated (square brackets).



thereby decreasing shortening phases, or (iii) by decreasing shrinkage speeds. Likewise, MTs are destabilized by inducing catastrophes, preventing rescues or increasing shrinkage speeds. MT growth rate also has an effect on MT stability: increase of MT polymerization efficiency can increase the amount of MT polymer, and growth-promoting factors are often associated with MT stability. However, more rapid MT growth can also increase the frequency of catastrophes because MTs reach the cell border more rapidly. We only start to understand the mechanisms underlying these dynamic parameter changes. Assembly and disassembly rates can be modulated by catalytic activity that stabilizes a transition state of tubulin heterodimer addition/release, a strategy XMAP215 seems to apply [16]. Other proteins modify the conformation of tubulin heterodimers and introduce kinks that favour MT depolymerization and thus induce catastrophes, mechanisms that kinesin-13s and stathmin appear to employ [17,18]. Finally, changing the concentration of available tubulin heterodimers has an impact on growth speed and persistence. Thus MT destabilization due to a loss of MT-stabilizing factors can increase the free tubulin concentration and raise MT polymerization rate [19]. On the other hand, proteins that sequester tubulin heterodimers (such as stathmin and kinesin-13s) can reduce available subunits and limit polymerization efficiency [20,21].

Regulating MT assembly

Structural MAPs (MT-associated proteins) such as tau protein, MAP2, MAP4 and DCX (doublecortin) decorate the MT lattice and stabilize it [22,23]. They strongly suppress catastrophes, but also promote growth and reduce shrinkage speeds [23–26]. Tau protein can antagonize the MT-destabilizing activity of XKCM1 (Xenopus kinesin catastrophe modulator-1) [27] and can also protect MTs against katanin-dependent severing [28]. The phosphorylation status of MAPs such as tau protein is crucial for their function, because phosphorylation causes their dissociation from the lattice, making the MT accessible to severing activity or MT shrinkage [29].

Table 1 | **MT stabilizers of the ch-TOG/XMAP215 family**

Organism	Name	Description
Mammals	Ch-TOG/CKAP5	Colonic and hepatic tumour overexpressed/cytoskeleton-associated protein 5
Frog	XMAP215	*Xenopus* MAP of 215 kDa
Fruitfly	Msps	Mini-spindles
Nematode worm	ZYG-9	Zygote defective 9
Plant (*Arabidopsis*)	MOR1	Microtubule organization 1
Budding yeast	Stu2p	Suppressor of tubulin 2
Fission yeast	Dis1p/Alp14p	Distorted trichomes 1/altered growth polarity protein 14
Slime mould	Ddcp224	*Dictyostelium discoideum* centrosomal protein of 224 kDa

Table 2 | **MT-destabilizing kinesins**

Organism	Kinesin-13	Kinesin-8	Kinesin-14
Mammals	Kif2A, Kif2B, Kif2C/MCAK	Kif18A	
Frog	XKif2, XKCM1		
Fruitfly	Klp59C, Klp59D, Klp10A	Klp67A	Ncd
Budding yeast	–	Kip3p	Kar3p
Fission yeast	–	Klp5/Klp6	Klp2

A particular class of MAPs is the +TIPs (MT plus-end tracking proteins). This is a group of evolutionarily unrelated proteins that share the ability to specifically bind to growing MT plus ends [30]. Association of +TIPs with the MT end can greatly influence MT dynamics. Many +TIPs have been implicated in stabilizing MTs by connecting them to the cell cortex (reviewed in [31]). For instance, CLASPs (cytoplasmic linker protein-associated proteins) and ACF7 (ATP-dependent chromatin assembly and remodelling factor 7) act as MT rescue and stabilizing factors at the cell cortex [19,32], whereas APC (adenomatous polyposis coli) reduces catastrophes at a subset of MTs in cellular protrusions [33]. Both of these strategies stabilize specifically those MTs that reach certain cortical domains. CLIPs (cytoplasmic linker proteins) act as cytosolic rescue factors [19,34]; the mechanism underlying their activity is enigmatic, since they preferentially associate with growing rather than depolymerizing MTs. An intriguing possibility is that the action of CLIPs and other rescue factors could be related to GTP-tubulin remnants, which are small MT lattice regions with incomplete GTP hydrolysis that were recently implicated in rescue events [35].

In addition, EB (end-binding) 1, EB3 and the fission yeast homologue Mal3 allow persistent MT growth in cells by preventing catastrophes [36,37]. EB proteins directly and autonomously interact with the tips of growing MTs [38] and it is likely that their binding coincides with the stabilization of the growing tubulin sheets [39], the lattice seam [40] or possibly even an overall effect on MT lattice structure [41]. This may not only explain how EBs prevent catastrophes, but also why EBs can increase MT growth speeds under certain conditions *in vitro* [36,39]. It should be noted that other *in vitro* studies found no effect of the EBs on MT growth rate [42,43], in line with *in vivo* observations [36,44].

The most potent protein shown to increase MT polymerization speeds is XMAP215, a widely expressed and highly conserved protein found in all eukaryotic cells (see Table 1). XMAP215 can boost the assembly by a factor of 10 *in vitro* [45]; in agreement with this observation, ZYG-9 (zygote defective 9) is the major MT growth-promoting factor in worms [46]. Stu2 as well as XMAP215 wraps round a tubulin heterodimer and facilitate its incorporation into a growing plus end [16,47]. This activity is purely catalytic and does not involve ATP hydrolysis; therefore XMAP215 can also promote the reverse reaction under conditions that favour depolymerization. Thus XMAP215 increases both growth and shrinkage rates and prevents phase transitions *in vitro* and in cell extracts [48,49]. Consistently, the *Arabidopsis* MOR1 (microtubule organization 1) promotes rapid MT growth and shrinkage [50].

Although XMAP215 is able to bind MT plus ends directly *in vitro*, *in vivo* conditions differ due to the presence of other MAPs that could regulate and/or compete with the association of XMAP215 with the MT tip. Binding to EBs, the core proteins of the +TIP complex, is often essential for +TIPs to accumulate at the MT plus end and for plus-end tracking [38,42,51,52]. An interaction between XMAP215 family members and EBs has been reported to occur in yeast and *Xenopus* egg extracts [53,54]. This interaction appears to be regulated in a cell cycle-dependent manner [53] and probably involves intermediary factors that remain to be identified. These and other observations suggest that *in vivo*, XMAP215 and its homologues might be regulated by their association with different targeting factors such as +TIPs or TACC (transforming acidic coiled-coil) proteins [55].

In mammalian cells, depletion of ch-TOG (colonic and hepatic tumour overexpressed) has a profound effect on mitotic spindle formation, whereas no strong interphase phenotypes have been reported so far [56]. This raises the question of whether there are additional factors responsible for the high MT growth rate in mammals.

Regulating MT disassembly

MT destabilizing proteins induce MT catastrophes, inhibit polymerization and promote disassembly. The best understood and most potent MT depolymerizers are the non-motile kinesins from the kinesin-13 family, which includes three members in mammals: Kif2A, Kif2B and Kif2C/MCAK (mitotic centromere-associated kinesin) (see Table 2).

Kinesin-13s probably have both an ATP-dependent catastrophe-promoting activity and an ATP-independent tubulin-sequestration activity [21]. MCAK binds both plus and minus ends *in vitro* [57,58] and shows the highest affinity for curved protofilaments that resemble shrinking MTs [17].

Also the crystal structure of kinesin-13 motors fits best to a curved protofilament [59,60]. Thus MCAK binding to MT ends is thought to accelerate the rate of transition to catastrophe by destabilizing the lateral interaction of protofilaments. This activity requires the consumption of ATP and it was shown that MCAK is a processive depolymerase: one MCAK dimer can remove 20 tubulin subunits before detaching [58].

In somatic vertebrate cells, MCAK has no or only minor effects on the organization of interphase MT arrays [56,61]: inhibition of MCAK in PtK2 cells led to slightly increased MT stability due to a 2-fold decrease in catastrophe frequency, a 2-fold increase in rescue frequency and, surprisingly, a mild increase in shrinkage rates [61]. Another kinesin-13, Kif2A, is strongly expressed in neuronal cells [62], and the brains of *kif2a*$^{-/-}$ mice show multiple phenotypes, including aberrantly long axonal branching and migratory defects [63]. MTs in *kif2a*$^{-/-}$ cells frequently fail to stop growing after reaching the cell edge, resulting in bent and overextended MTs; this suggests that Kif2A promotes catastrophes at the cell cortex. During mitosis, different kinesin-13 family members share the workload: whereas Kif2A and Kif2B are primarily associated with the centrosome, Kif2C/MCAK predominates at the kinetochores [64,65]; therefore these proteins are likely to affect diverse MT subpopulations differently ([5] and references therein).

Differential activities of kinesin-13 may be at least in part due to association with different partners. For example, MCAK and *Drosophila* Klp10A preferentially track growing MT plus ends [52,66] through a direct interaction with EB1 [51,52]. In contrast, Kif2A and Klp59C do not bind to EB1; and Klp59C tracks depolymerizing rather than growing MT ends [52,66].

Besides kinesin-13s, members of the kinesin-8 family (see Table 2) also promote MT depolymerization in cells. Kinesin-8s, namely Kip3 and Kif18A, disassemble MTs exclusively from the plus end in a length-dependent manner, depolymerizing long MTs more efficiently than short ones. This is explained by the fact that they are motile and exhibit a slow plus-end-directed motor activity and thus use the MT as an 'antenna' to accumulate at the plus end [67–69]; the longer the MT, the more kinesin would accumulate at its end. This characteristic would enable kinesin-8s to act as a part of the MT length-control mechanism that might be important for the alignment of chromosomes at the centre of the mitotic spindle [68,69]. Loss of kinesin-8 activity results in aberrant long spindles with hyperstable MTs in various organisms [69–72].

Another kinesin family implicated in MT destabilization is kinesin-14s. The budding yeast Kar3, which forms a heterodimer with a non-motor polypeptide Cik1, has been shown to be a minus-end-directed motor and to slowly depolymerize taxol-stabilized MTs from the plus end [73]. It is thought that shortening of cortex-anchored MT plus ends by Kar3Cik1 is crucial for karyogamy during mating of budding yeast. Also the *Drosophila* kinesin-14 Ncd promotes MT shortening *in vitro* [73], and the deletion of Klp2 from fission yeast results in long spindles [74], suggesting an evolutionarily conserved role in negatively regulating MT stability. However, the kinesin-14 homologues HSET (human spleen, embryonic tissue and testes) and XCTK2 (*Xenopus* C-terminal kinesin 2) control spindle length, but, in contrast with Klp2, spindle length positively correlates with their presence [75]. It therefore remains to be elucidated whether MT-destabilizing activity is conserved in all kinesin-14 family members.

A completely different type of a negative regulator of MT stability is Op18/stathmin. Recent structural and thermodynamic studies on the complex formed between stathmin and tubulin provide a mechanistic model of how stathmin acts on MTs (for a recent review, see [76]). Essentially, stathmin possesses two binding sites with equal affinity for tubulin heterodimers, and occupation of both binding sites leads to the ternary tubulin–stathmin complex [20,77]. Capping of α-tubulin by the N-terminal domain of stathmin as well as the kinked structure of the ternary complex that is maintained by the C-terminal helical domain of stathmin prevents the incorporation of the sequestered tubulin subunits into protofilaments [18]. If stathmin were able to introduce such a bent conformation into tubulin subunits at the ends of MTs, this would explain the catastrophe-inducing activity of stathmin that has been reported in several studies [78–80]. Similarly to MCAK that is thought to depolymerize MTs via introducing protofilament curvature, stathmin is able to induce catastrophes at the plus as well as the minus ends of MTs [79].

During interphase, stathmin inactivation or depletion causes extensive MT polymerization and increased MT polymer content [80,81]. Long-term interference with stathmin levels revealed that stathmin acts as a positive and reversible regulator of tubulin expression, acting on the level of tubulin mRNA stability [81,82]. Further, there is a strong positive correlation between stathmin expression and cell proliferation (reviewed in [83]). The importance of the careful regulation of stathmin activity is illustrated by the observations that inhibition as well as overexpression of stathmin leads to mitotic arrest [84,85]. Mice lacking stathmin are viable, but show neurological defects in adults [86]. Neuronal cells express stathmin and three structurally related proteins [SCG10, SCLIP (SCG10-like protein) and RB3]. The regulation of MT dynamics by stathmin/SCG10-family destabilizers is thought to be crucial for neural development and plasticity (reviewed in [87]).

Concluding remarks

Regulation of MT assembly and disassembly has to be tightly controlled by multiple factors. While we begin to decipher the activities of individual factors, we are still missing the picture of how these often antagonistic activities work together to generate a certain type of MT array. For example, both XMAP215 and tau protein can antagonize the MT-destabilizing activity of XKCM1. XMAP215 does this by strongly promoting assembly and counteracting the net polymer loss, whereas tau has only minor effects on the growth speed, but is very potent in suppressing the catastrophe-promoting activity of XKCM1 [27]. Also EB

proteins probably suppress catastrophes by counteracting MT destabilizers [36]. The three-component system of tubulin, XMAP215 and XKCM1 mimics physiological MT dynamics values [10], indicating that this basic set of factors could be largely responsible for MT dynamics in cells. However, in interphase vertebrate cells, neither XMAP215 nor XKCM1/MCAK was shown to play a major role in regulating MT dynamics, suggesting that another set of factors might be performing a similar function. The development of high-throughput protein depletion technologies makes it possible to identify the molecules responsible for different aspects of MT dynamic behaviour in different systems and settings. In combination with improved imaging techniques, automated image analysis and modelling, this approach should enable us to develop a comprehensive understanding of the mechanisms governing the generation of MT arrays during cell division, polarization and differentiation.

Funding

B.v.d.V. and A.A. are supported by the Netherlands Organization for Scientific Research NWO Vici and Open programme grants. A.S. is funded by a Marie Curie Cancer Care programme grant.

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Received 13 March 2009
doi:10.1042/BST0371007