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Global and local control of microtubule destabilization promoted by a catastrophe kinesin MCAK/XKCM1

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Abstract

Traditionally, kinesins have been identified as proteins that use the energy of ATP to translocate along microtubules. However, in the last decade some kinesin-like proteins were found to destabilize microtubule ends. The kinesins that destabilize microtubules are known as "catastrophe kinesins". Analyses of a *Xenopus* member of the catastrophe kinesins called MCAK/XKCM1 have shown that, in fact, catastrophe kinesins are essential for controlling the distribution of microtubules by inducing their depolymerization. Therefore, unraveling the mechanisms of how microtubule destabilization promoted by these catastrophe kinesins is controlled is essential for understanding how microtubules in a cell are distributed. Here we give an overview of the studies that have focused on the global and local control of microtubule destabilization promoted by MCAK/XKCM1.

Introduction

Microtubules assemble by polymerization of heterodimeric subunits of α- and β-tubulin. Analysis of the polymerization of pure tubulin has established that microtubules contain a fast-growing end terminated by the β-tubulin subunit (called the plus end) and a slow-growing end (called the minus end) (reviewed in Desai and Mitchison, 1997; Howard and Hyman, 2003). Dynamic microtubules are found in two major states, either growing or shrinking, and they transit stochastically between these two states, a behavior known as dynamic instability (Mitchison and Kirschner, 1984; Horio and Hotani, 1986; Walker *et al*., 1988). A transition from growth to shrinkage is called a catastrophe, and a transition from shrinkage to growth is a rescue (Walker *et al*., 1988). Thus, microtubule behavior can be described by four parameters: growth rate, shrinkage rate, catastrophe frequency, and rescue frequency. In animal cells, microtubules are nucleated at centrosomes by mechanisms that probably involve γ-tubulin (reviewed in Job *et al*., 2003). Once nucleated microtubules grow out with their plus ends leading into the cytoplasm. The stability of microtubule plus ends is supposed to be regulated by the opposing activity of molecules that destabilize plus ends and molecules that stabilize plus ends. Although the stability of plus ends of microtubules growing from centrosomes changes during both the cell cycle and assembly of a mitotic spindle, little is known about how these changes are controlled.

Microtubules growing from centrosomes *in vivo* were also found to exhibit dynamic instability (Cassimeris *et al*., 1988; Hayden *et al*., 1990; Rusan *et al*., 2001). However, the analyses of parameters of dynamic instability of microtubule plus ends revealed that microtubules assembled *in vivo* undergo more frequent catastrophes than microtubules polymerized from pure tubulin *in vitro* and suggested that factors exist to induce catastrophes under physiological conditions. In the last decade, a family of kinesin-like proteins that destabilize microtubules was discovered to destabilize microtubule ends (reviewed in Hertzer *et al*., 2003; Moore and Wordeman, 2004; Wordeman, 2005). These kinesins are known as "catastrophe kinesins" due to their ability to induce catastrophes. One of the catastrophe kinesins, *Xenopus* XKCM1 is a member of the internal motor domain subfamily of the kinesin superfamily (kinesin-13 subfamily; originally known as Kin I subfamily for '*kin*esin-*i*nternal'). A study using *Xenopus* egg extracts showed that suppression of XKCM1 by immunodepletion or addition of inhibitory antibodies increased the steady state length of microtubules and blocked proper spindle formation consequently (Walczak *et al*., 1996).

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Furthermore, the inhibition of XKCM1 decreased catastrophe frequency four fold in high-speed supernatant and seven fold in low-speed supernatant from frog egg extracts, suggesting that XKCM1 is a potent catastrophe-promoting factor in *Xenopus* egg extracts (Walczak *et al*., 1996; Tournebize *et al*., 2000). MCAK, a mammalian homologue of XKCM1 and the founding member of the catastrophe kinesins, localizes to centromeres in M phase (Wordeman and Mitchison, 1995). *In vitro* studies of XKCM1 and XKIF2, another member of kinesin-13 subfamily in *Xenopus*, showed that, unlike the conventional kinesins which use the energy of ATP to translocate along microtubule lattice (Sharp *et al*., 2000), catastrophe kinesins use ATP to destabilize microtubule ends (Desai *et al*., 1999; Figure 1). In this review we discuss the regulation of microtubule destabilization promoted by XKCM1/MCAK, focusing on work using egg extracts and purified proteins in *Xenopus* systems. For a more general discussion of microtubule destabilizing kinesin molecules, we suggest the following excellent reviews (Walczak, 2000; Hertzer *et al*., 2003; Ovechkina and Wordeman, 2003; Moore and Wordeman, 2004; Wordeman, 2005).

In physiological conditions, catastrophe frequency changes through the cell cycle. For example, in *Xenopus* egg extracts, catastrophe frequency increases five to ten fold from interphase to M phase (Belmont *et al*., 1990). Microtubule half-life is more than 5–10 min in interphase but less than a minute in mitosis (reviewed in McNally, 1996), suggesting that the modulation of catastrophe frequency is one method to control microtubule turnover during the cell cycle. Since the catastrophe kinesin XKCM1/MCAK is a major factor controlling the distribution of microtubules in cytoplasmic extracts (Belmont and Mitchison, 1996; Tournebize *et al*., 2000) and in the cell (Kline-Smith and Walczak, 2002), its microtubule destabilizing activity must be regulated either directly or indirectly during the cell cycle. In addition, the catastrophe kinesin must be regulated locally for proper mitotic spindle formation. In this review, we will give an overview of studies that have focused on the global and local control of microtubule destabilization promoted by XKCM1/MCAK. Hereafter we refer to XKCM1/MCAK as MCAK, in accordance with the growing trend towards adopting a unified name for these orthologues.

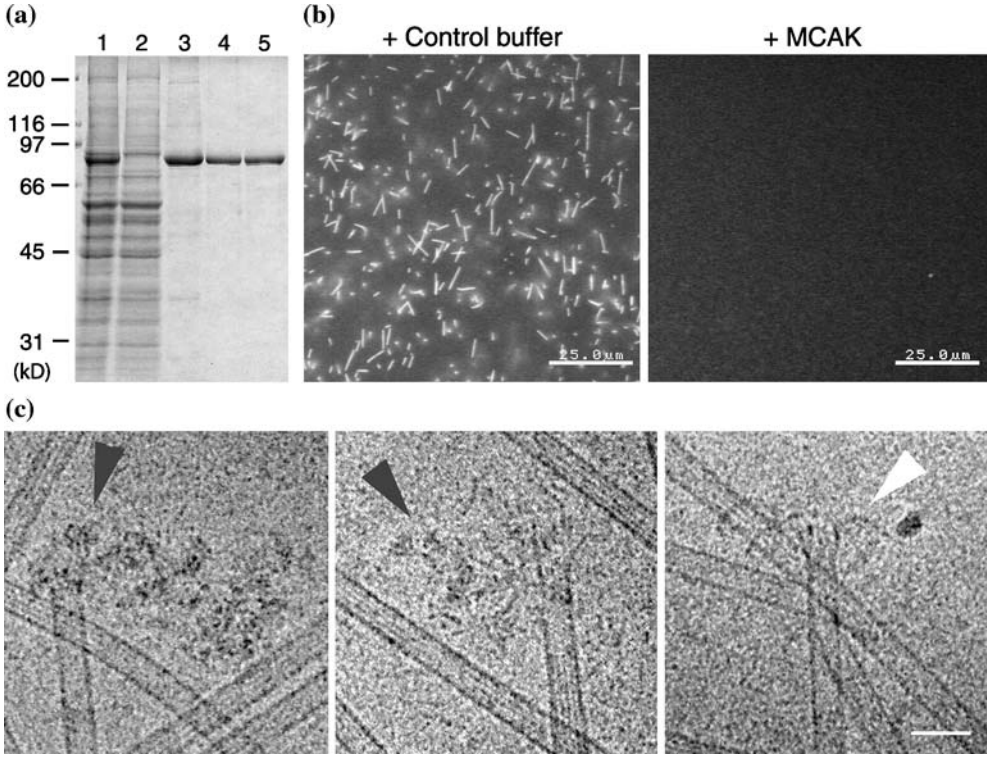

Fig. 1. Recombinant MCAK depolymerizes microtubules by destabilization of microtubule ends. (a) Purification of recombinant *Xenopus* MCAK/XKCM1. Supernatant of baculovirus-infected insect cell lysate (lane 1), HiTrap SP sepharose flowthrough (lane 2), HiTrap SP sepharose elution (lane 3), Superose 6 elution (lane 4), and Mono S elution (lane 5) were analyazed by SDS-PAGE and Coomassie Brilliant Blue staining. (b) Purified MCAK promotes disassembly of GMPCPP microtubules. Pre-polymerized GMPCPP microtubules were mixed with control buffer or 37.5 nM MCAK in the presence of 1.5 mM ATP. Panels show microscope fields after 30 min of incubation at room temperature. Bars, 25 μm. (c) MCAK induces protofilament peeling of GMPCPP microtubules. 40.8 μM MCAK was mixed with GMPCPP microtubules in the presence of 1.5 mM AMPPNP. After incubation at room temperature for 15 min, the sample was frozen in liquid ethane and analyzed using cryoelectron microscopy. Black and white arrowheads indicate large protofilaments bulbs and long peeled protofilaments, respectively. Bar, 50 nm. Adapted from Kinoshita *et al*., 2001.

Global control of MCAK-induced microtubule destabilization

Global control by a Dis1/XMAP215 family MAP

The first example of a factor that affects the microtubule destabilizing activity of MCAK came from studies of a MAP (*m*icrotubule-*a*ssociated *p*rotein) in *Xenopus* egg extracts. MAPs are known to bind to the microtubule lattice and stabilize microtubules on their own. A *Xenopus* MAP called XMAP215 was originally identified as a strong stimulator of microtubule growth in frog egg extracts (Gard and Kirschner, 1987; reviewed in Kinoshita *et al*., 2002). While *in vitro* experiments suggested that XMAP215 stimulates microtubule growth without affecting catastrophe frequency (Vasquez *et al*., 1994), immunodepletion experiments in frog egg extracts showed that XMAP215 regulates catastrophe rate as well as growth rate (Tournebize *et al*., 2000). The catastrophe frequency in XMAP215-depleted extracts was 2–7 times higher than that in mock-depleted extracts, indicating that XMAP215 promotes microtubule growth at least in part by inhibiting catastrophes (Tournebize *et al*., 2000). Interestingly, the catastrophe frequency was recovered to control levels by subsequent inhibition of MCAK activity in XMAP215-depleted extracts (Tournebize *et al*., 2000). This result indicated that XMAP215 specifically antagonizes the microtubule destabilizing activity of MCAK in *Xenopus* egg extracts.

These experiments in *Xenopus* egg extracts also suggested that both proteins could oppose each other to regulate catastrophes without any extra factors. To test this possibility, *in vitro* assays using pure tubulin were set up to examine the effects of XMAP215 on the microtubule destabilizing activities of MCAK (Kinoshita *et al*., 2001). These functional assays using purified proteins have shown that (1) XMAP215 antagonizes the activity of MCAK to shorten the length of microtubules polymerized *in vitro* (Figure 2a); (2) XMAP215 inhibits catastrophes promoted by MCAK; and (3) the combination of XMAP215 and MCAK together with pure tubulin can recapitulate the parameters of dynamic instability observed under physiological conditions (Kinoshita *et al*., 2001). Taken together with the previous results from *Xenopus* egg extracts, it was concluded that the essential features of dynamic instability in the extracts are derived from the sole action of XMAP215 and MCAK on microtubules.

Is the antagonizing activity of XMAP215 against MCAK also conserved in other systems? This question has been examined by RNAi and overexpression of either the human homologue of XMAP215, TOGp/ch-TOG or MCAK, or both proteins in human tissue culture cells (Holmfeldt *et al*., 2004; Cassimeris and Morabito, 2004). Gullberg's group have shown that TOGp is required to counteract the microtubule destabilizing activity of MCAK during mitosis but not in interphase, in contrast to *Xenopus* egg extracts in which XMAP215 is required in both interphase and M phase (Holmfeldt *et al*., 2004). On the other hand, Cassimeris and Morabito have shown that TOGp and MCAK act antagonistically to regulate spindle morphology rather than microtubule length, suggesting that both proteins make smaller contributions in regulating microtubule dynamics in human tissue culture

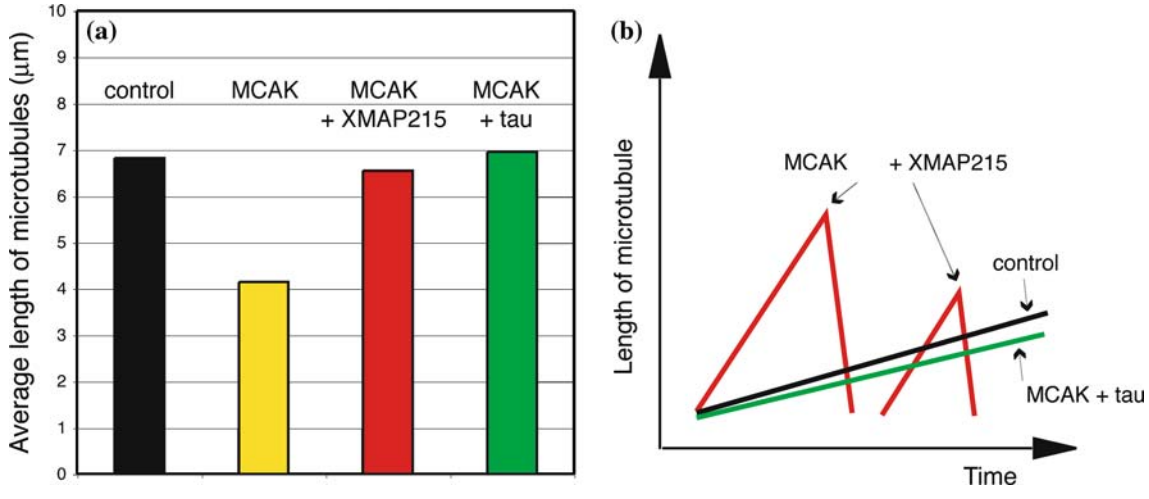

Fig. 2. Distinct roles of XMAP215 and tau in the regulation of MCAK-induced microtubule destabilization. (a) Both XMAP215 and tau antagonizes the microtubule destabilizing activity of MCAK. Plots represent the average length of microtubules fixed after assembly (for 5 min at 30 °C) from pure tubulin in the presence of the indicated proteins. Control (black), 15 μM tubulin alone; MCAK (yellow), 15 μM tubulin + 110 nM MCAK; MCAK + XMAP215 (red), 15μM tubulin + 110 nM MCAK and 200 nM XMAP215; MCAK + tau (green), 15 μM tubulin + 110 nM MCAK and 300 nM tau. (b) Comparison of the effects of XMAP215 and tau on microtubule dynamics in the presence of MCAK. Schematic plots represents life-history traces of a single microtubule observed with 25 μM tubulin alone (Control, black), tubulin + 0.2 μM MCAK and 0.8 μM XMAP215 (MCAK + XMAP215, red) or tubulin + 0.2 μM MCAK and 0.8 μM tau (MCAK + tau, green). No microtubule growth was observed in the presence of 25 μM tubulin + 0.2 μM MCAK. Adapted from Noetzel *et al*., 2005.

cells (Cassimeris and Morabito, 2004). Thus the antagonizing activity of XMAP215 against MCAK seems to be conserved to some extent even though not completely in other systems.

An outstanding question is how XMAP215 opposes MCAK mechanistically. MCAK binds to microtubule ends and probably exerts its effects there, as suggested from studies *in vitro* and *in vivo* (Walczak *et al*., 1996; Desai *et al*., 1999). In *Drosophila* and mammalian cells it appears as if kinesin-13 members surf on the ends of microtubules, like other + TIP proteins (Mennella *et al*., 2005; Moore *et al*., 2005). XMAP215 has not been shown to bind directly to microtubule ends, although there is some indirect evidence that TOGp, the mammalian homologue does (Spittle *et al*., 2000). XMAP215 could exert its effects by preventing MCAK from binding to microtubule ends. On the other hand it may work by stabilizing the lattice, or even by inhibiting the activity of the motor itself.

Complicating this issue is the observation that XMAP215 itself can destabilize the ends of microtubules (Shirasu-Hiza *et al*., 2002). To date this has only been observed for microtubules stabilized with GMPCPP, a slowly hydrolyzable analogue of GTP. There is no evidence so far *in vivo* that XMAP215 destabilizes microtubules, although Msps, a *Drosophila* orthologue of XMAP215, acts as an anti-pausing factor in interphase *Drosophila* S2 culture cells (Brittle and Ohkura, 2005). One possibility is that this destabilization is a consequence of the interaction between XMAP215 and the non-hydrolyzable microtubule lattice. However, it may also represent some aspect of the mechanism of XMAP215's action on microtubules. For instance, if XMAP215 helps tubulin dimers associate with microtubule ends, then the absence of tubulin dimers in solution may cause XMAP215 to start removing dimers from the end.

Global control by a classical MAP tau

The above functional analysis of XMAP215 and MCAK raised the following question: is the opposing activity of XMAP215 against MCAK unique to XMAP215 itself, or a general feature of all classes of known MAPs? To address this question, the effect of tau, a member of the classical' MAPs, on the microtubule destabilizing activity of MCAK was examined (Noetzel *et al*., 2005). All classical MAPs can be characterized by the conserved repeat motifs that are important for microtubule-binding (Lewis *et al*., 1988). Tau is a neuronal MAP that specifically localizes to axons, and is known to moderately increase the growth rate, strongly suppress the catastrophe rate and slow the depolymerization rate of microtubule assembly *in vitro* (Drechsel *et al*., 1992). A fixed-time point assay using pure tubulin has shown that, like XMAP215, tau also suppresses the decrease of microtubule length by MCAK, suggesting that it is also an antagonizer of MCAK (Figure 2a, Noetzel *et al*., 2005). However, a real-time assay for *in vitro* microtubule dynamics has shown that tau completely blocks catastrophes promoted by MCAK whereas XMAP215 partially inhibits catastrophes (Figure 2b, Noetzel *et al*., 2005). These analyses showed that tau also acts as an antagonizer of microtubule destabilizing activity of MCAK but likely acts *via* a different mechanism of inhibition than XMAP215. Interestingly, when MCAK was added to growing microtubules in the presence of low concentration of tau, the growth rate of microtubules decreased despite the complete absence of catastrophes (Noetzel *et al*., 2005). This suggests that, in certain conditions, MCAK can influence growth rate as well as catastrophe rate. It is still unclear mechanistically how XMAP215 and tau differentially suppress microtubule destabilization promoted by MCAK although one possible clue is that stoichiometrically less XMAP215 is bound to microtubules (1:20 = MAP: tubulin) than classical MAPs (1:4–10) (Desai and Mitchison, 1997).

Tau is highly expressed in neuronal cells while the expression of XMAP215 is abundant in proliferating cells. However, it is not known if MCAK is expressed at this stage. More likely, tau would oppose the activity of XKIF2/Kif2a, which is another member of the kinesin-13 family reported to be expressed in neurons (Noda *et al*., 1995; Morfini, 1997) and which has also been shown to be a microtubule destabilizer (Desai *et al*., 1999). It is possible that tau alters the activity of XKIF2 as well as with MCAK, because the *in vitro* activities of MCAK and XKIF2 are very similar (Desai *et al*., 1999). This suggests that the microtubule destabilizing activity of kinesin-13 family members could be regulated differently in post-mitotic differentiated cells compared to proliferating cells, according to the opposing MAP that is expressed in the particular cell type. Thus, XMAP215 is a more appropriate antagonizer of MCAK to generate dynamic microtubules for spindle assembly and function. In contrast, tau generates stable microtubules required for neuronal functions. More generally, there are multiple different kinesin-13 family members expressed at different places and different times. Regulation of different kinesin-13 members by different MAPs provides a potentially rich source of local regulation of microtubule dynamics. It is still unknown how other MAPs act on MCAK-induced microtubule destabilization although MAP4, another classical MAP, and EB1 were reported to suppress microtubule destabilization promoted by overexpression of MCAK in tissue culture cells (Holmfeldt *et al*., 2002; Moore *et al*., 2005). In *Drosophila* interphase S2 cells, EB1 is required for targeting KLP10A, a fly kinesin-13, to microtubule plus ends (Mennella *et al*., 2005). It would be an interesting question if EB1 also regulated the activity of kinesin-13 directly.

Local control of MCAK-induced microtubule destabilization in mitosis

Local control by ICIS and Aurora B on centromeres and chromosome arms

In addition to the global regulation throughout the cytoplasm, the microtubule destabilizing activity of MCAK is also regulated locally. MCAK was originally identified as a kinesin-like protein localized on centromeres in M phase (Wordeman and Mitchison, 1995). Centromeres are essential regions on chromosomes for faithful segregation of sister chromosomes into daughter cells. When cells enter mitosis, mitotic-specific organelles called kinetochores assemble on centromeric regions of sister chromatids and capture microtubules nucleated from each spindle pole to establish bi-oriented chromosomes. Due to its localization on centromeres during mitosis, the behavior of microtubules attached on kinetochores, so-called kinetochore microtubules, can be regulated by MCAK. How is MCAK activity regulated on centromeres? A clue to the regulation of MCAK activity on centromeres came from the study of a microtubule associated protein, ICIS (Ohi *et al*., 2003). ICIS associates with MCAK and localizes to inner centromeres in an MCAK-dependent manner in *Xenopus* egg extracts. It stimulates the microtubule destabilizing activity of MCAK *in vitro* and its inhibition in *Xenopus* extracts blocks normal spindle assembly and causes huge microtubule asters, with long microtubules forming around condensed chromosomes, which are often seen in MCAK-depleted extracts (Ohi *et al*., 2003). Therefore ICIS may function as a stimulator of MCAK activity on the inner centromere and this appears to be required for proper mitotic spindle assembly. Interestingly, ICIS associates not only with MCAK but also with INCENP and Aurora B, components of chromosome passenger complexes that are thought to be important for chromosome bi-orientation (Tanaka *et al*., 2002). Recent studies have shown that MCAK is phosphorylated by Aurora B *in vitro* and *in vivo* and that Aurora B phosphorylation of MCAK inhibits its microtubule destabilizing activity *in vitro*. (Andrews *et al*., 2004; Lan *et al*., 2004; Ohi *et al*., 2004) Aurora B phosphorylation of MCAK seems to be required for the proper localization of MCAK on inner centromeres in *Xenopus* systems (Lan *et al*., 2004; Ohi *et al*., 2004). In human tissue culture cells, the non-phosphorylatable mutants of MCAK at Aurora B target sites can still localize on centromeres but the expression of either the non-phosphorylatable or the phospho-mimic mutants increases the frequency of defects in the establishment of bi-oriented chromosomes (Andrews *et al*., 2004). These results suggest that Aurora B regulates kinetochore bi-orientation through its phosphorylation of MCAK on centromeres.

The chromosome passenger complex that includes Aurora B is not only located on mitotic centromeres but also found on non-centromeric regions along the chromosome arms. Funabiki's group has shown that Aurora B phosphorylation of MCAK is required for the stabilization of microtubules nucleated around chromatin in *Xenopus* egg extracts (Sampath *et al*., 2004). Thus proper bipolar spindle formation and stabilization appear to require regulation of MCAK activity both at centromeres and along chromosome arms (Figure 3).

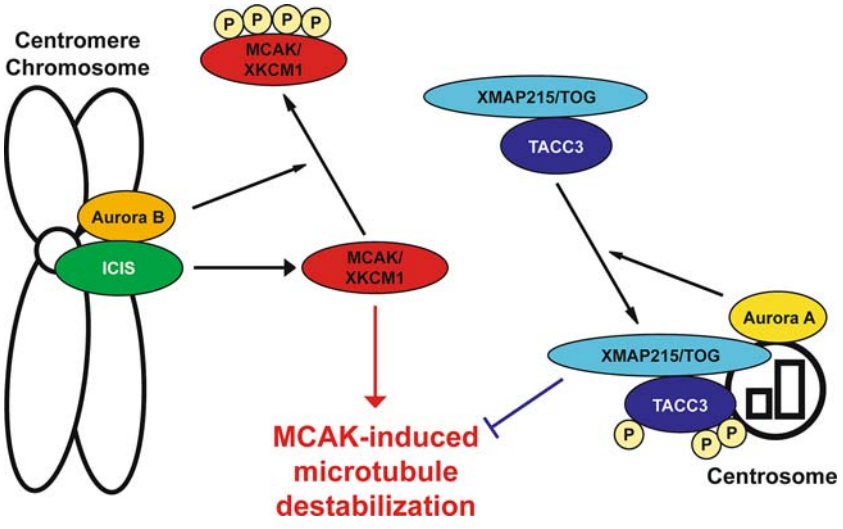

Fig. 3. A model for local control of microtubule assembly on centromeres/chromosomes and centrosomes in mitosis. A centromere protein ICIS (green) stimulates microtubule destablizing activity of MCAK (red) while Aurora B (orange) on chromosomes phosphorylates MCAK to negatively regulates its microtubule destabilizing activity. On the other hand, Aurora A (yellow) on mitotic centrosomes phosphorylates TACC3 (dark blue) to recruit a complex of TACC3 and XMAP215 (light blue) to centrosomes. The TACC3–XMAP215 complex targeted to centrosomes promotes microtubule assembly by increasing affinity of XMAP215 to microtubules and suppression of microtubule destabilizing activity of MCAK.

Local control by TACC3–XMAP215 complex on centrosomes

Although much attention has focused on the role of MCAK at centromeres, MCAK also localizes to centrosomes and spindle poles in a number of systems (Walczak *et al*., 1996; Oegema *et al*., 2001; Andrews *et al*., 2004; Gaetz and Kapoor, 2004). Therefore MCAK could also regulate microtubule growth from centrosomes. In *Xenopus*, MCAK is localized on centrosomes as well as centromeres in M phase (Walczak *et al*., 1996). Recent studies on TACC3/Maskin, a protein that interacts with XMAP215, have shown that the TACC3–XMAP215 complex is required for the inhibition of MCAK's microtubule destabilizing activity at centrosomes (Kinoshita *et al*., 2005; Peset *et al*., 2005). TACC3 is a member of the evolutionarily conserved protein family called TACC (*t*ransforming *a*cidic *c*oiled-*c*oil) (reviewed in Raff, 2002; Still *et al*., 2004). Mammalian TACC proteins contain a conserved coiled-coil domain at their C-terminus and were originally identified as gene products that have an ability to transform cells (Still *et al*., 1999a, b). D-TACC, a *Drosophila* TACC homologue, interacts with Msps, a fly XMAP215 homologue, and localizes to spindle poles and centrosomes (Cullen and Ohkura, 2001; Lee *et al*., 2001). *Xenopus* TACC3/Maskin is a component of a large ribonucleoprotein complex and is involved in sperm nuclei-induced spindle and Ran-induced microtubule aster assembly (Blower *et al*., 2005; O'Brien *et al*., 2005). Functional analyses *in vitro* have shown that the reconstituted TACC3–XMAP215 protein complex more effectively suppresses MCAK-induced microtubule depolymerization than XMAP215 alone even though TACC3 itself cannot oppose MCAK activity. This suggests that TACC3 is a regulatory subunit of the complex that facilitates XMAP215's ability to oppose MCAK activity (Kinoshita *et al*., 2005). In *Xenopus* egg extracts, TACC3 is required for microtubule growth from centrosomes specifically in M phase. By subsequent inhibition of MCAK activity in TACC3-depleted extracts, microtubules could re-grow from mitotic centrosomes, suggesting that TACC3 is required to antagonize the microtubule destabilizing activity of MCAK around mitotic centrosomes in *Xenopus* egg extracts (Kinoshita *et al*., 2005; Peset *et al*., 2005). Taken together with the results of the *in vitro* analysis of the complex, these data suggest that microtubule destabilization by MCAK is suppressed by the activity of the TACC3–XMAP215 complex specifically on mitotic centrosomes (Figure 3). Aurora A phosphorylation of TACC3 is required for targeting of the TACC3–XMAP215 complex to centrosomes and spindles (Figure 3, Kinoshita *et al*., 2005; Peset *et al*., 2005). The local inhibition of MCAK on centrosomes appears to be essential in order to provide a sufficient source of microtubules for proper spindle assembly in early mitosis.

Other possible activities of TACC3 are suggested by the original observation that TACC3 is required for regulation of translational machinery during *Xenopus* oocyte maturation (Stebbins-Boaz *et al*., 1999). It is unclear whether TACC3 has two separate functions or whether they are linked. The recent observation that a ribonucleoprotein complex including TACC3 is required for spindle assembly suggests an interesting link that could be explored in the future (Blower *et al*., 2005).

One of the puzzling aspects of this problem is that MCAK can destabilize plus and minus ends while XMAP215 only stabilizes the plus ends (Vasquez *et al*., 1994; Desai *et al*., 1999). This suggests that the mechanisms we have discussed in our review are most likely to pertain to the plus-end. It is still unclear if the XMAP215–TACC3 complex can stabilize minus ends by antagonizing MCAK-induced microtubule destabilization. Interestingly, a recent study suggests that *Drosophila* D-TACC may help Msps stabilize minus ends (Barros *et al*., 2005). However, the *in vitro* activity of Msps in microtubule assembly is still unknown, preventing any strong conclusions regarding the end-specificity of Msps and the Msps/D-TACC complex.

Recent studies have shown that another kinesin-13 family protein XKIF2/Kif2a is also located on centrosomes and spindle poles and is required for bipolar spindle assembly or for regulation of spindle length (Ganem and Compton, 2004; Gaetz and Kapoor, 2004). Surprisingly, siRNA of MCAK in Kif2a-lacking cells restores bipolar spindle assembly (Ganem and Compton, 2004). In *Xenopus* egg extracts, targeting of Kif2a but not MCAK to spindle poles requires the function of the dynein/dynactin complex, suggesting that a different mechanism targets MCAK to spindle poles (Gaetz and Kapoor, 2004). Furthermore, these results suggest that MCAK and Kif2a have different roles and undergo different regulation despite of their similar microtubule destabilizing activity measured *in vitro* and their localization on centrosomes and spindle poles. The observation that MCAK inhibition in TACC3-depleted extracts restores microtubule assembly from centrosomes suggests that the TACC3–XMAP215 complex opposes mainly MCAK-induced microtubule destabilization in *Xenopus* extracts. It is not clear how MCAK antagonizes two different activities; that of Kif2a, another kinesin-13 protein and XMAP215, a microtubule stabilizing protein. The functional relationship of these three proteins is a future question to be solved.

Concluding remarks

The microtubule destabilizing activity of MCAK is essential to produce dynamic behavior of microtubules but its activity must be rigorously controlled; hyperactivation would prevent the formation of

microtubule-based structures, leading to disastrous consequences for the cell. To secure the cell from the potential toxicity, a variety of control mechanisms seem to exist. There are indications that MCAK is regulated by additional mechanisms to those described in this review. For example, in *C. elegans*, ZYG-9, the worm orthologue of XMAP215, does not oppose to the MCAK orthologue KLP-7 suggesting that an alternative mechanism must exist to counteract MCAK activity in worm embryos (Srayko *et al.*, 2005). Also in human tissue culture cells, Ca^{2+}/calmodulin-dependent protein kinase II γ (CaMKII γ) has an overlapping function with TOGp/XMAP215 to negatively regulate the microtubule destabilizing activity of MCAK (Holmfeldt *et al.*, 2005). The extent of redundancy within the network of factors that potentially regulate MCAK activity will need to be addressed in future analyses. In addition to the spatial control of MCAK activity, we still need to know more about its temporal control in order to understand cell cycle- and developmentally-regulated reorganization of microtubule architecture inside the cell.

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