Balanced regulation of microtubule dynamics during the cell cycle: a contemporary view

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Summary
Assembly of mitotic and meiotic spindles into an elliptical bipolar shape is an example of morphogenetic processes that involve local chromosomal regulation of microtubule dynamics for proper spatial microtubule assembly. Global microtubule dynamics during the cell cycle and local microtubule dynamics during spindle assembly are regulated by a balance between microtubule stabilizing and destabilizing factors. How a chromosome-induced phosphorylation gradient may be generated and modulate spindle microtubule assembly through balanced regulation of the activity of microtubule-associated proteins and Stathmin/Op18 is analyzed. BioEssays 1999;21:53–60. © 1999 John Wiley & Sons, Inc.

Introduction
Microtubules (MTs) represent one of the polymer systems of the eukaryotic cytoskeleton. They are essential for a wide variety of cellular functions, including mitosis, transport, cell motility, cell shape, and polarity. How the cytoskeleton organizes itself into the dynamic asymmetric arrays required for function is one of the most challenging questions in cell biology.

During interphase, MTs form a radial array emanating from the centrosome, which is the principal cellular MT nucleator. In addition to centrosomal MTs, free MTs not associated with the centrosome are observed during interphase. At the onset of mitosis, there is a dramatic rearrangement of the interphase MT array. The radial interphase MT array depolymerizes and repolymerizes into the elliptical bipolar shape of the spindle essential for cell division (as described later and outlined in Fig. 2A). How MTs can undergo such distinct three-dimensional rearrangements from interphase to mitosis is largely unknown. In this review, we will examine the molecular machinery that regulates this spectacular rearrangement of MTs during the cell cycle, and in particular, during spindle assembly.

The building blocks of MTs are tubulin dimers. Tubulin dimers polymerize into a 25-nm-diameter MT filament via the formation of 13 protofilaments and with concomitant GTP hydrolysis (Fig. 1). MTs are asymmetric, or so-called “polar” polymers. They are polar because each tubulin dimer is composed of an α- and a β-subunit (only 50% identical), and because of the uniform polar arrangement of these heterodimers in the MT lattice. This polarity is used by motor proteins to sort cargo and MTs spatially. The energy for the movement of motor proteins comes from the hydrolysis of ATP coupled with the translocation of the motor along MTs. MT polarity also results in MTs having a fast-growing and a slow-growing end, the plus- and minus-ends, respectively. The plus-end extends from the spindle poles/centrosomes toward the chromosomes, and the minus-end is often associated with the spindle pole/centrosome. 

An understanding of how the three-dimensional rearrangement of MTs is achieved during the cell cycle requires knowledge about the basic molecular polymerization characteristics of MTs. However, MTs have very complex polymerization characteristics. The “dynamic instability” model offers a satisfactory theoretical framework for the description of many of the MT polymerization phenomena observed in vitro and in vivo; it is used here to explain how MTs polymerize during the cell cycle. In the dynamic instability model, a MT polymerizes with a certain “growth rate” (v+), and depolymerizes with a certain “shrinkage rate” (v−). The transition from growth to shrinkage is called a “catastrophe” and occurs with a certain frequency (fcat). The opposite event, from shrinkage to growth, is called a “rescue” and occurs with a frequency (fres) (Fig. 1). Regulation of the value of these four parameters seems sufficient to regulate MT polymerization during the cell cycle (A more comprehensive model would include the frequency of MT nucleation, fnuc as a fifth parameter).

During the past fifteen years, work on MT turnover has been strongly dominated and guided by the dynamic instability model. However, MTs may also turn over by so-called “treadmilling”, where tubulin subunits continuously added to one end of MTs “treadmill” through the MTs and subsequently are lost by continuous depolymerization at the other end. It is important to realize...
that MT length changes occur at a rate of up to 1 µm/min by treadmilling and up to 20 µm/min by dynamic instability. Therefore “treadmilling” conveys slow plasticity to the MTs, whereas “dynamic instability”, in particular, is required for more extensive and fast rearrangements of the MT network. For example, the ob-namic instability”, in particular, is required for more extensive and ”treadmilling” conveys slow plasticity to the MTs, whereas ”dy-treadmilling and up to 20 µm/min by dynamic instability. Therefore that MT length changes occur at a rate of up to 1 µm/min by polymization of MTs require the tubulin heterodimers to have GTP bound, which is hydrolyzed rapidly upon incorporation into the MT lattice. (From ref. 82, with permission from the ASCB.)

Figure 1. Microtubule dynamic instability. The growth and shortening (shrinking) phases of MT dynamic instability are persistent because thousands of tubulin heterodimers add during a single growth phase, or dissociate during a single shortening phase. The abrupt transition between growth and shortening is termed catastrophe, while the switch from shortening back to growth is termed rescue. Polymerization of MTs requires the tubulin heterodimers to have GTP bound, which is hydrolyzed rapidly upon incorporation into the MT lattice. (From ref. 82, with permission from the ASCB.)

Microtubule-associated proteins as regulators of microtubule dynamics
In interphase, MTs are stable and typically form a large radial array. In mitosis, by contrast, MTs are very dynamic and only polymerize in the area around the chromosomes (Fig. 2A). This change in MT organization requires a change in MT dynamics. The hallmark of the change in MT dynamics between interphase and mitosis is a 10-fold increase in $f_{cat}^{(5,6,12,13)}$. This change is regulated by phosphorylation by cdc2 kinase,$^{(5,6,12,14,15)}$ and probably other kinases,$^{(16,17)}$ as well as by phosphatases.$^{(6,18)}$ However, the molecular targets of these kinases and phosphatases are largely unknown and have to be identified to understand how the MT rearrangement at the interphase to mitosis transition is achieved. We will now discuss how MTs may become dynamic during mitosis.

It has been hypothesized that the increase in $f_{cat}$ at the onset of mitosis is due to the specific mitotic activation of a so-called “catastrophe factor.”$^{(19,20)}$ The definition of a bona fide catastrophe factor is a molecule that is specifically activated during mitosis and induces MT catastrophes either directly or indirectly. However, no such factor has yet been identified, as further discussed in the next section. An alternative hypothesis to the “catastrophe factor hypothesis” is that the balance between MT stabilizing and MT destabilizing factors at any time and spatial point during the cell cycle controls the dynamic state of MTs. Stabilizing factors consist of a large group of well-characterized molecules called microtubule-associated proteins (MAPs). We know from previous studies that MAPs show a phosphorylation-dependent decrease in MT affinity during mitosis (ref. 21 and references therein). Moreover, it has been shown that mitotic MAPs promote MT assembly twofold less than interphase MAPs; purified phosphorylated MAPs are also known to promote MT assembly less than the corresponding unphosphorylated MAPs (ref. 21 and references therein). Thus, because interphase MAPs are dephosphorylated, they promote MT assembly more than their mitotic counterparts. Knowledge about MT destabilizing factors and their regulation is far less advanced than that of MAPs, and encompasses Stathmin/Op18$^{(22)}$ and the motor proteins XKCM1$^{(23)}$ from Xenopus and Kar3p from Saccharomyces cerevisiae.$^{(24)}$

The increase in MT dynamics during mitosis may be explained if the destabilizing factors are constitutively active during the cell cycle. If so, they may simply overcome the residual MT stabilizing activity of mitotic MAPs and cause MTs to become dynamic.$^{(21)}$ In fact, the available data on the destabilizing factors Stathmin/Op18 and XKCM1 suggest that these are indeed constitutively active during the cell cycle.

In summary, the present experimental evidence supports a picture whereby a balance between the activity of dominant MT stabilizing factors and constitutively active MT destabilizing factors regulates the dynamic state of MTs during the cell cycle (Fig. 2B). Future work will involve testing of this hypothesis, which requires more insight into the nature, number, and cell cycle regulation of MT destabilizing factors.

How does Stathmin/Op18 regulate microtubule dynamics?
Since its discovery in 1983,$^{(25)}$ knowledge about the function of Stathmin/Op18 (for “relay” in Greek and Op18 for Oncoprotein of 18 kD) has been lacking, despite numerous studies.$^{(26)}$ Given its long pre-history, it was exciting when Stathmin/Op18 was discovered to interact with tubulin and destabilize MTs in vitro, apparently by increasing $f_{cat}$ 5- to 10-fold.$^{(19,22)}$ Recent
studies in vitro have, however, shown that Stathmin/Op18 does not increase $f_{cat}$; rather, it regulates MT polymerization by sequestering the pool of soluble/free tubulin.\textsuperscript{(27,28)} What are the differences, then, between these studies\textsuperscript{(22,27,28)}? The major difference between the former\textsuperscript{(22)} and the more recent studies\textsuperscript{(27,28)} is a moderate 0.7 pH units change in the buffer used. Thus, contrary to expectation, time-lapse video microscopy of MTs showed that Stathmin/Op18 simply reduced $v_p$, due to the lowered free tubulin concentration, without any effect on $f_{cat}$\textsuperscript{(28)} (despite stronger tubulin sequestration at the pH used by Curmi et al. (1997)\textsuperscript{(28)} than at the pH used by Belmont and Mitchison (1996)\textsuperscript{(22)}). Furthermore, upon immunodepletion of 95% of Stathmin/Op18 from mitotic Xenopus egg extracts, there was only a twofold reduction in the catastrophe frequency.\textsuperscript{(6)} Moreover, in Xenopus egg extracts, there is no differential phosphorylation of Stathmin/Op18 between interphase and mitosis in the absence of chromosomes,\textsuperscript{(29)} although MTs are dynamic in a mitotic Xenopus egg extract. Taken together, the present data show that Stathmin/Op18 is not the long sought-after bona fide catastrophe factor\textsuperscript{(19,22)} and most likely it simply destabilizes MTs by sequestering tubulin.\textsuperscript{(27,28)}

However, the mechanism by which Stathmin/Op18 destabilize MTs in vivo remains unclear. Stathmin/Op18 is not essential for viability\textsuperscript{(26)} or for spindle assembly,\textsuperscript{(29)} also indicating that, at most, Stathmin/Op18 modulates MT dynamics in vivo, and that it is not a major regulator of MT assembly. One hint as to the in vivo function comes from the immunoprecipitation experiments previously mentioned.\textsuperscript{(6)} These studies suggest that Stathmin/Op18 may have a minor direct effect on $f_{cat}$, which may be envisioned if the tubulin-Stathmin/Op18 complex (a “$T_2S$ complex”)\textsuperscript{(27,28)} can disturb MT polymerization through competition with free tubulin for polymerizing MT ends. However, additional studies in vivo and in Xenopus extracts are required to clarify this issue (i.e., to resolve whether or not tubulin was also depleted during these experiments\textsuperscript{(6)}). Determination of the precise in vivo function is further complicated by the very complex regulation of Stathmin/Op18 phosphorylation/activity (Fig. 3) in vivo, which is under both intra- and extracellular control.\textsuperscript{(30,32)} Phosphorylation of Stathmin/Op18 turns off the MT destabilizing activity, and it
Regulation of microtubule dynamics during spindle assembly

In the remainder of this review we will focus on how MTs assemble around chromosomes during spindle assembly. As shown in Figure 2A, the reorganization of the spatial arrangement of MTs from the interphase array to the bipolar spindle is dramatic. It is especially noteworthy that spindle MTs extend primarily from the spindle poles toward the chromosomes, and that MTs are not found elsewhere in the cytoplasm (Fig. 2A). At the molecular level, it is known that the interphase to mitotic spindle MT rearrangement involves activation of MT severing factors, (39) and a significant increase in fcat caused by a regulated change in the activity of MT stabilizing and destabilizing factors (2,5,12,36) (Fig. 2). It is unknown how the new highly dynamic centrosomal MTs elongate preferentially toward the chromosomes. Assembly of the female meiotic spindle occurs through a different pathway that involves centrosome independent assembly of MTs around chromosomes. Therefore, assembly of both the meiotic and the mitotic spindles involves selective local stabilization of MTs around the chromosomes (40–43) (Fig. 4). But how is it that MTs grow preferentially around the chromosomes during mitosis, and not elsewhere in the cytoplasm (Fig. 2A)?

Two models for spindle microtubule assembly

The model most frequently used to explain the preferential MT polymerization toward chromosomes during spindle assembly is called “search and capture.” This model proposes that dynamic MTs nucleated by centrosomes randomly search in space. When the MTs hit a kinetochore, they become captured and stabilized, and a spindle forms with time. (1,44–46) This model has its roots in the observations that MTs were preferentially found associated with, and nucleated by, centrosomes and kinetochores (for older literature, see Marek, 1978(47)). However, Marek’s classic experiments involving the removal of chromosomes from spindles, (47) as well as the observation of the site of MT re-polymerization after MT depolymerization in mitotic cells, (48) led De Brabander et al. (1981) (48) to suggest that the kinetochores perhaps were producing a diffusible factor that could locally induce MT assembly in the vicinity of kinetochores. (48) This was a new idea because it suggested that the nucleation of MTs could be enhanced by a chromosome-derived diffusible factor, and thus that MT assembly did not have to use a kinetochore or centrosome-based template. Convincing evidence for such an active non-template role of chromosomes in MT assembly came from the studies of Karsenti et al. (1984) (49) showing that oligomerized λ-phage DNA could induce spindle-like MT structures when injected into Xenopus eggs. Important was also the suggestion that chromosome areas by themselves could induce MT polymerization independently of the presence of kinetochores. (49) These experiments are the origin of the second “local stabilization” model that proposes that chromosomes actively promote spindle MT assembly (47–49). There is now ample evidence for the local stabilization model. Experiments have shown that over short distances chromosomes can induce the nucleation and assembly of MTs, as well as pro-

Stathmin/Op18

becomes highly phosphorylated during mitosis. (28,31,33–35) Although more work is required, current in vitro data suggest that Stathmin/Op18 primarily sequesters tubulin without significantly regulating fcat.

If Stathmin/Op18 primarily sequesters tubulin, how could this regulate MT dynamics in vivo? A rough calculation shows that at an in vivo concentration of 6 µM (22,29) and a maximal stoichiometry of about 1:2 (the T2 S complex) (27,28) Stathmin/Op18 could sequester almost 12 µM tubulin in vivo. Considering that about two-thirds of the total 20 µM tubulin is polymerized into MTs in vivo, (36) a factor like Stathmin/Op18 could completely regulate the amount of free tubulin available for polymerization. This may be important, as the tubulin concentration very strongly affects MT nucleation and MT dynamics in vitro. (37) For example, regulation of the binding between tubulin and Stathmin/Op18 may serve to fine-tune the amount of free tubulin during the cell cycle and at any particular spatial point. Since Stathmin/Op18 could serve to buffer the amount of free tubulin available for MT polymerization and more work should be aimed at analyzing how the free tubulin concentration influences MT dynamics in vivo. (13,38)
mote their polymerization off centrosomes over longer distances (29,42,47,49–58) (Fig. 4). It is important to realize that these two models are not mutually exclusive. Kinetochores are essential for both the congression and segregation of chromosomes, (2,59) and the efficiency of “search and capture” may be greatly enhanced through “local MT stabilization” by the chromosomes. Thus, the biggest difference between the local stabilization and the search and capture models lies in the extent of active chromosomal participation in MT formation and stabilization.

Interestingly, it appears that some systems favor one mechanism over the other, and that both mechanisms are probably involved during spindle assembly to a variable extent. Thus, in some systems, centrosomes and kinetochores may be kinetically dominant over chromosomes and drive spindle assembly, whereas chromosomes are necessary and sufficient in other systems. For example, in Xenopus eggs in the absence of centrosomes and kinetochores, (50,51) and in systems devoid of centrosomes, such as plants (56) and mouse embryos, (61) the major driving force regulating spindle assembly is “local stabilization.” In other systems, chromosome areas appear to play no role at all and spindle assembly is completely dependent on centrosomes and kinetochores. (46,58,62)

Regardless of the extent of chromosomal contribution to spindle assembly, nothing is known about how chromosomes locally regulate the biochemical environment in favor of MT assembly. In the remainder of this review, we will focus on this effect of the local stabilization model.

**How do chromosomes promote microtubule assembly?**

It has been proposed that an enzymatic activity localized to mitotic chromosomes could modify the cytoplasm locally and create an area of higher MT stability around chromosomes. (42,49,57) It was proposed that the local effect was established by a type 1 phosphatase bound onto chromosomes and was counteracted by the action of a diffusible kinase phosphorylating and inactivating a diffusible MT stabilizing molecule. (57) Since then, our understanding of MT dynamics regulation has increased. As outlined in Figure 2B, we now have knowledge about molecules that both stabilize and destabilize MTs as

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**Figure 4.** Hypothetical model for local chromosomal regulation of Stathmin/Op18 activity and microtubule assembly. (29,51,64) Chromosome(s) (gray ellipse) somehow inhibit a diffusible type 2A phosphatase (PP2A) (small blue ellipses, kinetochores). This inhibition results in a gradient (indicated by the increased thickness of the PP2A-labeled arrow) of PP2A activity around chromosomes, if the enzyme reactivating PP2A also is freely diffusible. The gradient in PP2A activity then leads to a gradient in Stathmin/Op18 (18) phosphorylation/activity (red circle) if the kinase that phosphorylates Stathmin/Op18, cdc2, is freely diffusible. Note, it is also possible that local PP2A inactivation leads to local PKA activation which subsequently hyperphosphorylates Stathmin/Op18 (not shown). The local inactivation of Stathmin/Op18 results in release of tubulin (not shown) from phosphorylated Stathmin/Op18 (18-P), which may then contribute to the preferential MT nucleation and growth toward chromosomes in systems devoid of centrosomes, (50,51,54) and preferential growth of centrosome nucleated MTs toward chromosomes in centrosome-containing systems. (43,51,62) Whether chromosomes or centrosomes become the nucleation site for MTs may be simply a kinetic issue, as centrosomes are kinetically dominant over chromosomes for MT nucleation. (58,51,54,81) Because Stathmin/Op18 is not hyperphosphorylated in the absence of chromosomes, it is suggested that, in the absence of chromosomes, PP2A dephosphorylates Stathmin/Op18 (Op18-P) faster (thick arrow) than the corresponding phosphorylation rate by cdc2 kinase (thin arrow). Although the mechanism of Stathmin/Op18 hyperphosphorylation by chromosomes is unknown, it involves the enzymatic activities of at least PP2A, cdc2 (29) and perhaps PKA (74). Curved arrow illustrates the likelihood that MAPs and motors are locally regulated in ways comparable to that of Stathmin/Op18. However, before MAPs and motors can exert their function, MTs need to be nucleated. Stathmin/Op18 may play an important role in regulating this initial phase of MT polymerization. (29,43)
well as their regulatory enzymes. We can therefore approach the proposed model with greater molecular detail.

Is there a morphogenetic gradient of microtubule stabilizing and destabilizing factors during spindle assembly?

Let us define the sum of MT polymerization destabilizing (i.e., Stathmin/Op18 and XKCM1) and stabilizing (i.e., MAPs) activities as the “potential to polymerize MTs.” If one then imagines that chromosomes create an area with a positive potential for MT polymerization around them, this could contribute to spindle assembly. Obviously, there are no strict boundaries in the cytoplasm; such an area would consist of a region or gradient going from a positive potential close to the chromosomes to a negative potential at some distance from them. It seems likely that such local regulation of MT dynamics around chromosomes involves the same components and regulators required for global regulation of MT dynamics during the cell cycle. Thus, proper chromosome-induced local regulation of the activity of MAPs, Stathmin/Op18, and XKCM1 would be important for proper spindle assembly.

Before we analyze in greater detail the evidence for local MT dynamics regulation and a possible molecular gradient during spindle assembly, it is worth noting that gradients are common in biological, chemical, and physical systems. Specific examples of molecular gradients in biology come from neurobiology, cell motility, chemotaxis, and the morphogenesis of fruit flies. It would not be completely unexpected if a gradient in the MT polymerization potential were involved in forming the spindle.

Is there a gradient of Stathmin/Op18 and MAP activity during spindle assembly?

The first specific molecular example of how chromosomes regulate MT assembly and may generate a gradient in the potential to polymerize MTs comes from a recent paper on Stathmin/Op18. This paper reported that when increasing amounts of mitotic chromosomes were added to mitotic Xenopus egg extract, Stathmin/Op18 became increasingly phosphorylated. This was interesting because it had previously been described that during cellular mitosis Stathmin/Op18 becomes hyperphosphorylated. In the Xenopus egg extracts system, however, hyperphosphorylation required the presence of mitotic chromosomes. These experiments led to the speculation that it is the presence of mitotic chromosomes that induces Stathmin/Op18 hyperphosphorylation, and not simply the mitotic state of the cytoplasm. Interestingly, the concentration of chromosomes required to induce hyperphosphorylation of Stathmin/Op18 corresponds to a situation in which each chromosome is surrounded by a uniform cube of extract with a volume of 20–60 µm³, roughly the length of the mitotic spindle in the Xenopus system. Moreover, the observed dose-response effect of the chromosomes suggested that Stathmin/Op18 is only locally phosphorylated around chromosomes. These experiments indicate that there may be a spatial gradient in Stathmin/Op18 phosphorylation, and thus activity, around chromosomes.

It is unknown exactly how chromosomes regulate Stathmin/Op18 phosphorylation, and several models are possible. One model put forward by Karsenti’s and Hyman’s groups is described here (Fig. 4). The model is based on the fact that addition of okadaic acid, a chemical inhibitor of phosphatase 2A (PP2A), to mitotic Xenopus egg extract leads to a phosphorylation pattern identical to that observed in the presence of mitotic chromosomes. Thus, it was suggested that chromosomes somehow inhibit diffusible PP2A in a dose-dependent manner, and that a gradient in PP2A activity around chromosomes is established. This gradient in PP2A activity then leads to a gradient in Stathmin/Op18 phosphorylation if its kinase, most likely cdc2, is freely diffusible and globally active. The gradient in Stathmin/Op18 phosphorylation leads to a gradient in its binding activity with tubulin subunits, and ultimately to a gradient in the free tubulin concentration (highest close to the chromosomes; the latter has also been proposed by Zhai et al., ). This local increase in tubulin concentration would contribute to the preferential nucleation and growth of MTs around chromosomes (Fig. 4). Indeed, it has been shown that spindle size can be affected by the amount of free tubulin, an issue that, however, needs additional investigation (see refs. 13 and 47 for discussion and references).

At this point, it is important to note that because Stathmin/Op18 is not essential for viability or for spindle assembly, it should not be considered the principal regulator of MT polymerization around chromosomes. However, the increased MT nucleation observed around chromosomes in the absence of Stathmin/Op18 combined with the results from in vivo overexpression experiments show that Stathmin/Op18 is involved in regulating MT dynamics locally around mitotic chromosomes (Fig. 4).

One challenge to the proposed mechanism of chromosomal regulation of Stathmin/Op18 phosphorylation (Fig. 4) comes from recent reports by Gullberg’s group showing that the phosphorylation of two PKA sites (Ser16 and Ser63) are key to turning off the tubulin binding activity of Stathmin/Op18, and not phosphorylation of the cdc2 (Ser38) or MAPK (Ser25) sites (Fig. 3). The model presented in Figure 4 is not incompatible with these observations, however. Local inactivation of PP2A could cause local activation of PKA, which is known to phosphorylate Ser16 and Ser63, that would then inactivate Stathmin/Op18. Moreover, preceding phosphorylation of Ser25 and in particular Ser38 is a prerequisite for subsequent phosphorylation on Ser16 and Ser63. This suggests that phosphorylation of the cdc2 site may be the master switch for activity regulation during the cell cycle. Interestingly, a positive feedback loop between the extent of Ser16 phosphorylation and MT assembly was reported. This implies that formation of spindle MTs may be an autocatalytic process that involves local chromosomal regulation of a number of different molecules, including Stathmin/Op18. It remains an open question precisely how Stathmin/Op18 hyperphosphorylation is achieved in the presence of chromosomes during mitosis.

It will be exciting to learn how chromatin regulates the phosphorylation of Stathmin/Op18. That mechanism may be general
and could be applicable to local regulation of MAPs and motor activities (Fig. 4). For example, XMAP230, XMAP310, and E-MAP-115 are localized to spindle MTs, despite their phosphorylation-dependent reduced affinity for MTs during mitosis. Thus, it seems likely that chromosomes may reduce the phosphorylation level of these MAPs locally, as previously discussed.

A point of debate is the presence of a molecular MT polymerization gradient around chromosomes in vivo. Modelling work by Leibler and colleagues supports the possibility of a gradient. Their work shows that a molecular gradient the size of a spindle can be generated by immobilizing a phosphatase on a spherical bead, while a kinase and the respective substrate freely diffuse (this model also works with a reversed configuration of the regulators).

It may be possible to use chimeric fluorescent proteins with a phosphorylation-sensitive emission spectrum (similar to that described by Post et al., 1995) to demonstrate a gradient experimentally. Alternatively, it may be possible to show a gradient using antibodies that recognize phosphoepitopes on nondiffusible spindle-associated molecules, like MAPs. New more creative approaches may also be needed. It will be a considerable challenge in the future to test experimentally whether a gradient in the potential to polymerize MTs exists around chromosomes during spindle assembly.

Conclusions
The current understanding of MT dynamics regulation in vivo suggests that the dynamic state of MTs during the cell cycle is controlled by a balance between MT stabilizing and destabilizing factors rather than by a mitotically activated catastrophe factor (Fig. 2). Interestingly, the activity balance between these same factors also regulates local MT dynamics during spindle assembly. Most exciting is that we now start to have a molecular understanding of how the mitotic chromosomes locally regulate MT-stabilizing and MT-destabilizing factors in favor of local MT assembly (Fig. 4).

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References