Acentrosomal microtubule assembly in mitosis: the where, when and how

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Keywords

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Abstract

In mitosis the cell assembles the bipolar spindle, a microtubule-based apparatus that segregates the duplicated chromosomes into the two daughter cells. Most animal cells enter mitosis with duplicated centrosomes that provide an active source of dynamic microtubules (MTs). However, it is now established that spindle assembly relies on the nucleation of acentrosomal MTs occurring around the chromosomes after nuclear envelope breakdown, and on pre-existing microtubules. Where chromosome-dependent MT nucleation occurs, when MT amplification takes place and how the two pathways function are still key questions that generate some controversies. Here, we review the literature to try to reconcile the data and present an integrated model accounting for acentrosomal microtubule assembly in the dividing cell.

Text

Introduction

When the cell enters mitosis, the relatively stable interphase microtubules (MT) disassemble and highly dynamic MTs organize and build the bipolar spindle, the molecular machine that drives chromosome segregation. Most animal cells enter mitosis with two centrosomes that actively nucleate dynamic MTs [1, 2]. In addition, acentrosomal pathways trigger MT assembly in the dividing cells [3]. These MTs are now recognized as essential and sufficient for the assembly of a functional bipolar spindle. This is particularly obvious in cells that are naturally devoid of centrosomes (like plants cells and vertebrate oocytes for example) [4], but bipolar spindles also assemble in cells experimentally manipulated to eliminate their centrosomes [5-7]. More strikingly, flies engineered to lack functional centrosomes were shown to develop till adulthood [8, 9]. Altogether these data underscore the essential role of acentrosomal MT assembly pathways for cell division.

Two main mechanisms drive acentrosomal MT assembly in the dividing cells. One of them is triggered by the chromosomes (RanGTP and CPC-dependent pathways, see boxes 1 and 2) and the other drives MT amplification through a MT-dependent MT nucleation mechanism (Augmin pathway, see box 3). Although the identification of these pathways has been instrumental to explain how the spindle forms, there are currently some controversies on important questions. Where precisely chromosome-dependent MT nucleation occurs is one of them. Although this pathway can only function after nuclear envelope breakdown, it is not clear whether this is also the case for the augmin-dependent pathway. Finally, how the two acentrosomal MT nucleation pathways work and whether they are independent or not is currently unclear.

Here we review the data that have accumulated over the years on acentrosomal MT assembly in the dividing cell. Trying to reconcile these data and some controversies, we envision that acentrosomal MT assembly occurs through a sequence of events starting with RanGTP-dependent MT nucleation in the vicinity of the chromosomes followed by MT stabilization in the kinetochore area, and MT amplification through the augmin-dependent MT nucleation pathway. Finally, we propose a mechanism for the integration of the acentrosomal MT assembly pathways in mitosis, leading to the formation of the kinetochore fibers (K-fibers) within the bipolar spindle.

Chromosome-dependent MT assembly in the dividing cell

The assembly of MTs in the proximity of the chromosomes was reported as early as in the 70s and the 80s by various groups, based on observations done upon MT regrowth after MT-depolymerizing drug washouts [10-12] and *in vitro* MT nucleation assays on chromosomes [13-16]. Altogether the data suggested that mitotic chromosomes could induce MT assembly in a region close to their kinetochores [17].

Some reports on γ-tubulin localization at or close to the kinetochores in mitosis [18, 19] fueled the idea that kinetochores could in fact directly nucleate MTs during mitosis [20]. However, this also posed a problem because the nucleation of MTs at the kinetochores would inevitably result in their "reversed" polarity with their minus-end embedded at the kinetochore, and their plus-end extending away towards the spindle poles. This orientation has never been observed in animal cells [21-23]. It has however been shown to occur transiently in budding yeast and favor the search and capture of the chromosomes [24]. The mechanism in this case

involves a mitotic MAP associated to the kinetochore and not canonical γ-tubulin dependent MT nucleation [24].

Other approaches had indicated that MT assembly could be triggered by the M-phase chromatin. Experiments performed in the 80s showed that injection of phage DNA into *Xenopus* eggs triggered spindle assembly suggesting that the pathway could function in the absence of kinetochores [25]. Further support for this idea was obtained by showing that DNA coated beads promoted the assembly of bipolar spindles in *Xenopus* egg extracts [26]. Moreover, experiments in grasshopper spermatocytes showed that chromatin rather than kinetochores triggered acentrosomal MT assembly [27].

How is it possible to reconcile all these data?

A major breakthrough in our understanding of the mechanism by which chromatin induces MT assembly came with the identification of the RanGTP pathway [28-30] (see box 1). This pathway is triggered by the chromatin through its associated Ran GEF, RCC1 (see box 1). Strikingly, addition of recombinant RanGTP to *Xenopus* M-phase egg extracts triggers the pathway in the absence of chromatin [28]. We now understand how RanGTP triggers MT nucleation in the M-phase cytoplasm. By releasing its direct target TPX2 from importins, RanGTP promotes the formation of a complex between TPX2 and a specific MT nucleation machinery RHAMM-NEDD1-γTURC promoting MT nucleation [31]. These mechanistic data do not suggest any need for a predefined site at which MT nucleation occurs. Moreover, the existence of a RanGTP gradient centered on the mitotic chromatin, which has been visualized in Xenopus egg extracts and in cells, strongly favors also the idea of MT nucleation occurring in the vicinity of the chromosomes rather than at predefined specific sites such as the kinetochores.

Kinetochores however do provide an excellent environment for MT stabilization (see box 2). Indeed, the chromosomal passenger complex (CPC) that localizes to the centromeres during mitosis has a major role in MT stabilization, as shown elegantly using the *Xenopus* egg extract system [32, 33]. It is therefore possible that MTs nucleated through the RanGTP dependent pathway in the proximity of the chromosomes may get preferentially stabilized in the vicinity of the kinetochores through a phosphorylation dependent mechanism involving Aurora B within the CPC complex (see box 2) [34]. In this view, the observation of γ-tubulin, or of γTuRC components, close to or at the kinetochore in MT regrowth experiments may result from the presence of very small MTs connected to the kinetochore at their plus-end and capped by the γTuRC at their minus-end [18, 20].

Based on all these data, we therefore envision that MTs are nucleated through a RanGTP-dependent pathway in the cytoplasm in the close proximity of the chromosomes [31] and become locally stabilized around the kinetochores through a CPC dependent mechanism before their capture and attachment at their plus-end [32, 33, 35, 36]. Once captured by the kinetochore, the growth of the MT results in its minus-end being pushed away towards the spindle pole [23, 37, 38]. This mechanism may favor the "search and capture" of the chromosomes [39, 40] to account for the rapid attachment of all the chromosomes observed in most animal cells, and play also an important role for K-fiber assembly [3, 41].

Augmin-dependent MT assembly – integration with the RanGTP pathway

Recently, an additional mechanism for acentrosomal MT assembly in mitosis was identified. This pathway is dependent on the octameric augmin complex also called HAUS [42-44] (see box 3). This complex recruits the γTuRC to the lattice of a

pre-existing MT, inducing the nucleation and branching of a new MT [45, 46]. This amplification mechanism drives the rapid increase of the MT mass within the spindle. The augmin pathway therefore shares with the other MT assembly pathways the essential requirement for a γTuRC-dependent MT nucleation mechanism. However, in contrast to the other pathways it also requires previously assembled MTs (see box 3).

A recent study reported the coimmunoprecipitation of augmin with TPX2 (see box 1 and above), suggesting a potential direct link between the RanGTP-dependent and the augmin-dependent MT assembly pathways [45]. Moreover, RanGTP was shown to increase the efficiency of the augmin pathway in the *Xenopus* egg extract system although the experimental setup also involved the addition of a large excess of TPX2 to the egg extract. These data suggest that the main function of RanGTP and TPX2 is to activate the augmin pathway. However, they can also be interpreted in a different way.

Previous work has shown that augmin depletion results in a reduction of the MT mass in the spindle in various experimental systems (mammalian cells, Drosophila cells and *Xenopus* egg extracts). MT assembly is therefore not completely impaired and spindles do assemble [42, 43, 47, 48]. Indeed, Goshima et al reported that '*the Dgt complex* (i.e. augmin) *is dispensable for the initial nucleation of MTs around chromatin but is important for the subsequent amplification (…) of MTs*" [42]. Consistently, Petry et al. also stated that: "*chromatin-mediated MT nucleation (…) drives the initial MT formation and appears to be augmin independent*" [47]. Since RanGTP induces MT nucleation in a MT-free environment [28, 31], it can provide template MTs needed for the augmin complex and the subsequent MT-dependent MT amplification. It therefore appears

unlikely that the RanGTP and augmin dependent pathway constitute a unique pathway. Interestingly, it was recently shown that centrosomal MTs are also targets of the augmin-dependent amplification pathway in Drosophila embryos [49]. This suggests that the augmin-dependent amplification mechanism can work independently of the RanGTP pathway. Nonetheless, further studies will be needed to clarify the putative functional link between TPX2 and the augmin complex, since RanGTP and TPX2 dramatically increase the efficiency of the augmin pathway [44, 45] (see outstanding box).

Altogether, the data favor the idea that RanGTP and the augmin MT assembly pathways are independent pathways that contribute sequentially to the assembly of acentrosomal MTs in the M-phase cytoplasm, the augmin pathway acting downstream of the RanGTP pathway for spindle formation (see our model figure 1).

Concluding remarks

Since the early description of acentrosomal MTs in mitotic cells and the subsequent findings pointing to their essential role for building a functional bipolar spindle, some basic questions are still unresolved. Here, we have reviewed data published over the last 40 years and propose a model to account for the mechanism underlying acentrosomal MT assembly in the dividing cells (Fig. 1). RanGTP triggers the initial activation of MT nucleation and stabilization around the mitotic chromosomes (Fig. 1A). These chromosomal and centrosomal MTs are then stabilized in the proximity of the kinetochores through an Aurora-B/CPC dependent way (Fig. 1B). Concomitantly, chromosomal MTs act as template for augmin-dependent MT nucleation, promoting an efficient

mechanism for MT amplification around the chromosomes (Fig. 1C). Upon MT plus-end capture by the kinetochores, the minus-ends are pushed away towards the spindle poles (Fig. 1D) [37, 38, 50].

Acentrosomal MTs are essential for the assembly and function of the bipolar spindle [41, 51]. Since the chromosomal MTs become preferentially stabilized close to the kinetochores, they are at the right place to get efficiently captured, thereby promoting K-fiber assembly [23]. Consistently, several of the RanGTP-regulated spindle assembly factors have been found to play a role in K-fiber assembly and/or dynamics [37, 41, 52-54]. It is therefore tempting to propose that MT assembly around the chromosomes is essential for K-fiber formation (see outstanding box). The early observations of small MTs in the kinetochore area [11] could correspond to early phases in the formation of the K-fibers constituted by large MT bundles connecting the kinetochores to the spindle poles in metaphase [12]. By being nucleated [31] and stabilized [41] through specific mechanisms, the chromosomal MTs may confer to the K-fiber their characteristics in terms of dynamics and organization. This may be the reason for the essential requirement of the chromosomal MTs for accurate spindle assembly and cell division.

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Figure legend

Figure 1: Model for acentrosomal MT assembly

A. RCC1, bound to the chromosomes, induces the concentration of RanGTP around the chromatin. The RanGTP gradient (blue) promotes the dissociation of spindle assembly factors, such as TPX2, from their inhibitory binding to importins (black). This then promotes the nucleation of acentrosomal MTs around the chromosomes, in a process dependent on TPX2 and on the γTuRC.

B. The chromosomal passenger complex CPC is located at the kinetochore. There, Aurora B phosphorylates and thus inactivates the MT-destabilizing factors MCAK and OP18. There is therefore a gradient of Aurora B dependent phosphorylation (blue) around the kinetochore acting as a hot-spot suitable for MT stabilization. MTs are therefore preferentially stabilized in the kinetochore area.

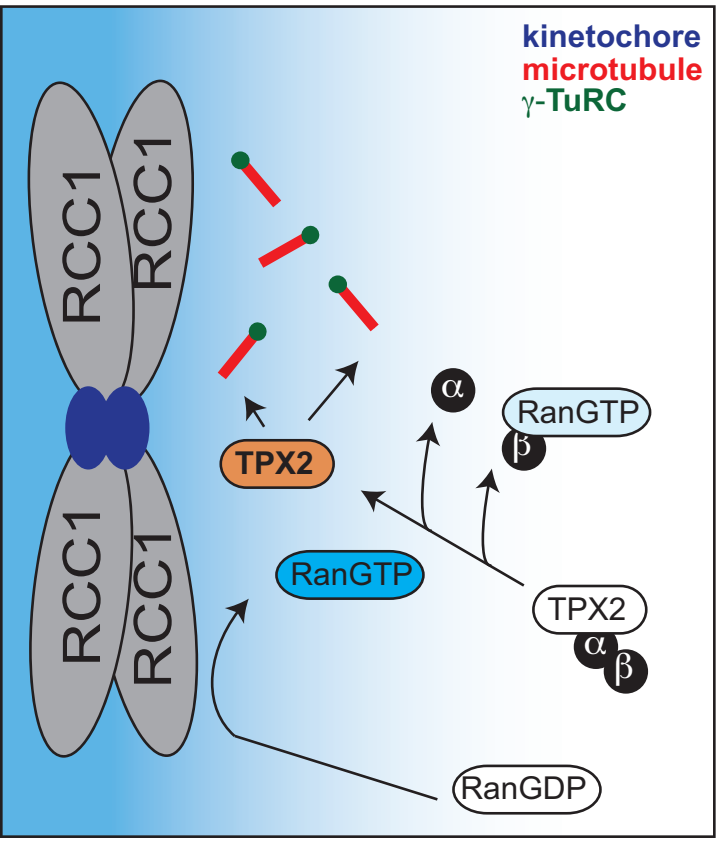
C. The augmin complex is recruited on the MT nucleated and stabilized through the RanGTP and the CPC pathways and on the centrosomal MTs. Augmin promotes MT branching an amplification, by a mechanism that seems to be potentiated by the RanGTP pathway.

D. Acentrosomal MT plus-ends are stabilized at the kinetochores through their interaction with the KMN complex. Minus-ends are pushed away towards the poles. MTs are organized in bundles and form the K-fiber.

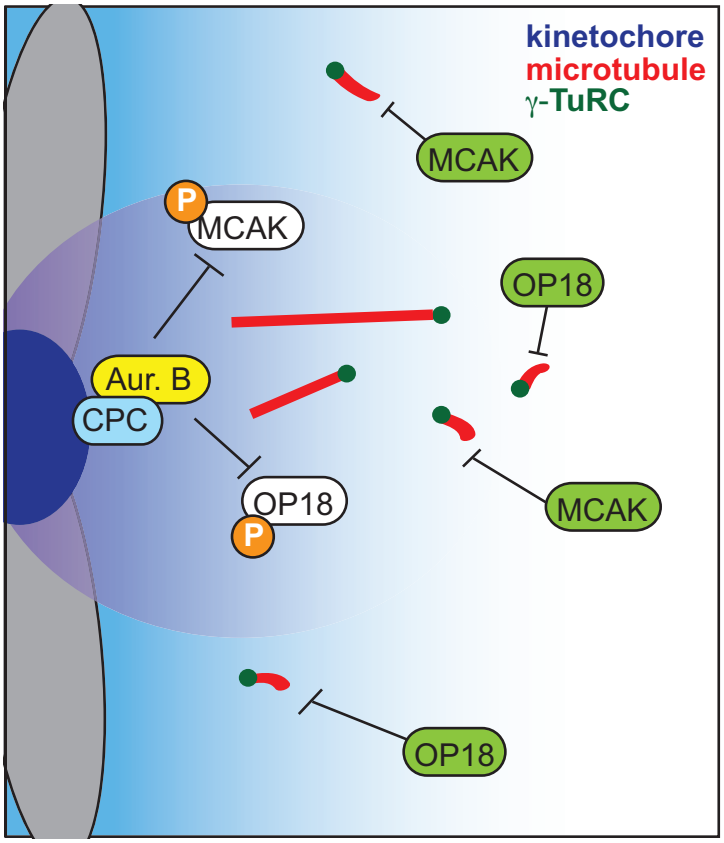
Figure

Figure 1

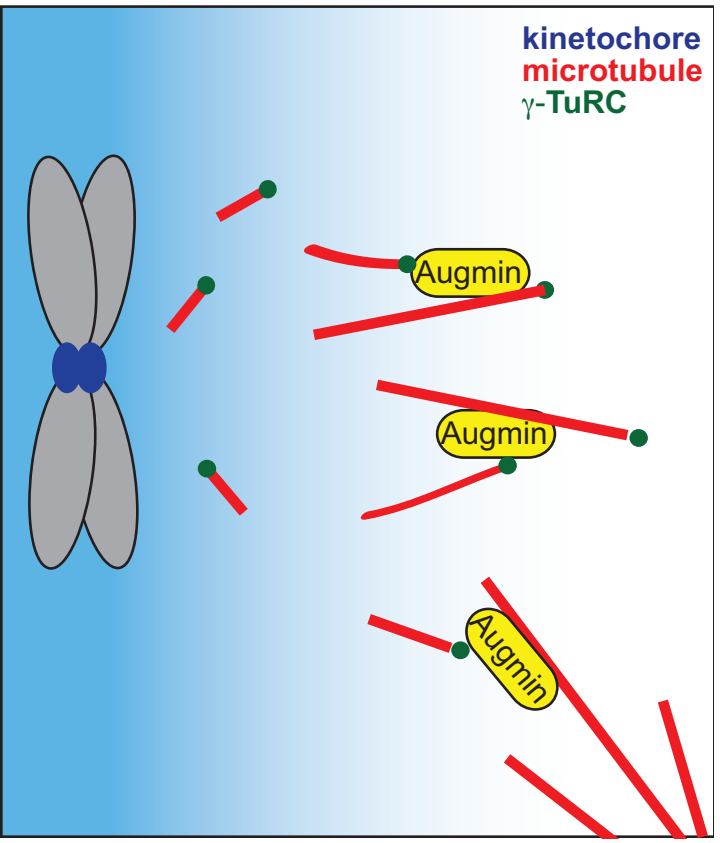
A. RanGTP-dependent MT nucleation around the chromosomes



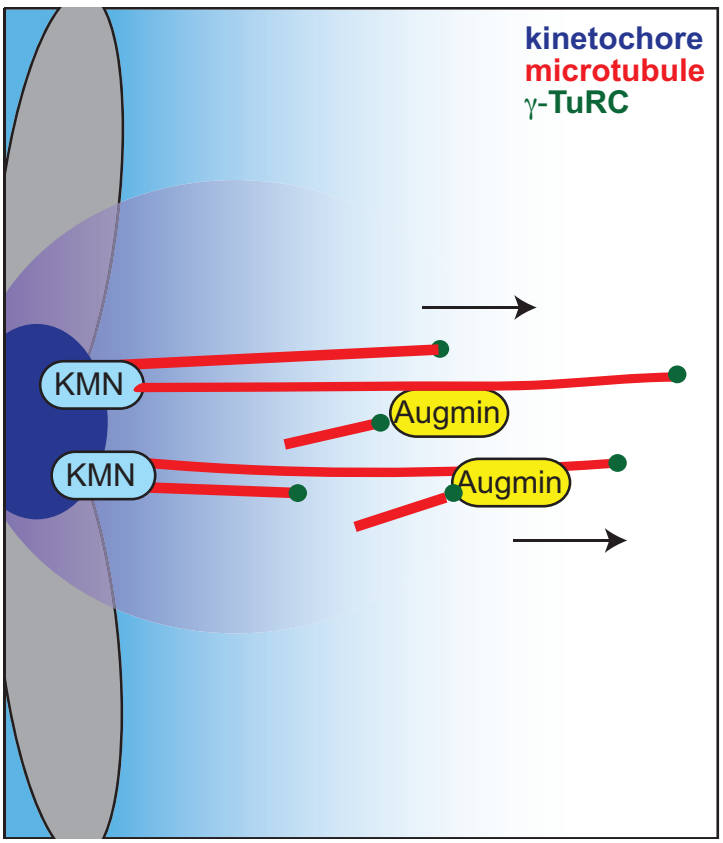
B. CPC-dependent MT stabilization around kinetochores



C. Augmin-dependent MT amplification on Chromosomal and centrosomal MTs



D. K-fiber formation



Boxes

Box 1: The RanGTP pathway

In the dividing cell, the association of the Ran-GEF RCC1 with the chromatin promotes a peak of GTP-bound Ran (RanGTP) close to the chromosomes generating a gradient [28-30, 55-58]. By associating with importin beta with high affinity, RanGTP promotes locally the dissociation of a number of NLS (nuclear localization signal) containing proteins called spindle assembly factors (SAFs) from importins alpha and/or beta [59, 60]. The released SAFs promote MT nucleation, stabilization and organization in the vicinity of the chromosomes. There are currently around 12 proteins identified as direct or indirect targets of RanGTP in the dividing cell [3]. One essential RanGTP target is the nuclear protein TPX2 that plays multiple roles during spindle assembly [31, 61-63]. Once released from Importins alpha/beta by RanGTP, TPX2 interacts with the kinase Aurora A locking it in an active conformation [64-66]. In this way the RanGTP gradient may be translated into an Aurora A phosphorylation gradient. We recently showed that this specific activation is part of the mechanism by which RanGTP promotes MT nucleation. Indeed, RanGTP release TPX2 that interacts with a specific MT nucleation complex including the γTuRC, NEDD1 and RHAMM and promotes the essential phosphorylation of NEDD1 on ser405 by Aurora A, altogether driving MT nucleation [31, 61, 67]. Other RanGTP targets were proposed to play a role in MT nucleation [68] and stabilization around the chromatin [3, 53, 69-72]. The RanGTP gradient is therefore translated into MT nucleation and stabilization gradients centered on the mitotic chromatin favoring the “search and capture” mechanism, the formation of the K-fibers and spindle assembly [69].

Box 2: The CPC pathway

Another mechanism was shown to promote MT assembly around the chromosomes. This pathway relies on the kinetochore-associated chromosomal passenger complex (CPC). The CPC is composed of INCENP, survivin, borealin and the Aurora-B kinase [35]. The role of the CPC in MT stabilization and spindle assembly has been demonstrated using the *Xenopus* egg extract system [32, 33, 73]. INCENP is an activator of the Aurora B kinase that phosphorylates and inhibits various MT destabilizing factors such as MCAK and OP18 [74]. The resulting Aurora-B-dependent phosphorylation gradient is postulated to favor MT assembly in the kinetochore area. However, the CPC has complex functions during mitosis as it also participates in error correction through Aurora-B dependent phosphorylation of components of the KMN network [35]. In this context the phosphorylation of NDc80 or of Ska complex components by Aurora B destabilizes kinetochore – MT attachments [75].

The CPC-dependent and RanGTP dependent mechanisms appear to act independently to favor MT assembly. In fact it is very likely that the two systems cooperate for preferential MT assembly in the kinetochore area [36, 73].

Box 3: The augmin pathway

The augmin complex also called HAUS in mammalian systems was identified in various organisms in the last few years [42-44]. It interacts with and targets the γTURC to the side of a pre-existing MT driving MT-dependent MT nucleation [46]. The augmin complex consists of 8 proteins. One of them, FAM29A, binds to the γTuRC while another one, HICE1, directly associates with the lattice of a pre-existing MT [76, 77]. The nucleation of a new MT on the lattice of a pre-existing one

results in MT branching [45, 46]. The branched MTs are then transported along the pre-existing MTs towards the spindle poles [50]. The augmin complex can in principle associate to any MT and in mitosis it acts on centrosomal and acentrosomal MTs [43, 49].

By exponentially amplifying the number of MTs in mitosis, the augmin dependent pathway constitutes a very efficient system for MT amplification [47, 49] that contributes to the robustness of the mitotic spindle. However, the contribution of the augmin-dependent MT assembly pathway to spindle assembly is probably different depending on the systems [43, 47, 78].

The augmin pathway is highly regulated by mitotic kinases such as Aurora A and PLK1, that are necessary for its activity in mitosis [76, 79].

In contrast to the RanGTP and the CPC-dependent MT assembly machineries that are nuclear in interphase and therefore can only act after nuclear envelope breakdown, the augmin complex may also play a role in MT nucleation in interphase, as this activity has been reported in plants [80-82].

Outstanding questions

- Both the γTuRC and its associated protein NEDD1 are required for the three different MT nucleation pathways described here. Interesting, each pathway involves NEDD1 phosphorylation, but through different kinases (cdk1, Nek9 and Aurora A), each targeting a single and distinct residue albeit all in the same region of the protein. How NEDD1 phosphorylation is translated into γTuRC activation and MT nucleation is however still unclear. The understanding of the activation of MT nucleation in mitosis may therefore require a better understanding of NEDD1 function and regulation [83].

- The contribution of each MT assembly pathway in the composition of the spindle differs greatly depending on the organisms, cell types, and in mitotic versus meiotic systems. Some outstanding questions are: How are these different pathways integrated to build the mitotic spindle? What is the qualitative contribution of each pathway to the function of the spindle, if any? Although we have discussed here the possibility that chromosomal MTs have an essential role in K-fiber formation, there is currently no direct evidence to support this hypothesis. Further work should explore this idea.

- The augmin-dependent pathway and its role in MT-dependent MT nucleation and amplification has only been recently described. Some important questions should still be addressed to fully understand how it works. While the function of the augmin complex in animal cells has only been described during mitosis, in plants, it was also shown to function in interphase [80, 82]. Is the augmin dependent

pathway active throughout the cell cycle in animal cells or is it restricted to mitosis like the RanGTP-dependent pathway?

- It was recently described that augmin-dependent MT nucleation sites are transported toward the spindle poles along pre-existing MTs in metaphase [50]. Does the augmin complex bind to specific predefined sites or randomly along the MT lattice? MT post-translational modifications can alter the MT binding activity of a number of MT associated proteins (MAPs) [84]. Could a related mechanism define sites for augmin-binding, and therefore where MT branching occurs within the mitotic spindle?

- The mechanism by which the augmin-dependent MT nucleation only occurs when bound to a pre-existing MT is unclear. Is there a sequential recruitment of components to the MT lattice? Does the augmin complex bind first to the MT lattice and then recruits the γTuRC, or are there preformed inactive augmin-γTuRC complexes in the cytoplasm? Work with *in vitro* reconstituted systems [44] may provide the answers to these key questions.

- A recent work suggested a putative functional link between TPX2 and the augmin complex [45]. In the light of the recent identification of a specific MT nucleation complex for the RanGTP-dependent pathway (RHAMM-NEDD1-γTuRC)[31], it will be important to determine if this complex is also used by the augmin pathway to nucleate MTs. More generally, are there different specific γTuRCs subcomplexes that are 'primed' for activation through each of the M-phase MT assembly pathways?

- Are the MTs nucleated through the distinct pathways functionally identical? There is currently only one example for the specific association of a protein to only one class of acentrosomal MTs [41]. The identification of other specific acentrosomal MT binding partners if they exist, could fuel our understanding on the function of these MTs during mitosis.