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Minireview

Molecules and Cells

Mechanism, Function and Regulation of Microtubule-Dependent Microtubule Amplification in Mitosis

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Mitotic spindle mediates the segregation of chromosomes in the cell cycle and the proper function of the spindle is crucial to the high fidelity of chromosome segregation and to the stability of the genome. Nucleation of microtubules (MTs) from centrosomes and chromatin represents two well-characterized pathways essential for the assembly of a dynamic spindle in mitosis. Recently, we identified a third MT nucleation pathway, in which existing MTs in the spindle act as a template to promote the nucleation and polymerization of MTs, thereby efficiently amplifying MTs in the spindle. We will review here our current understanding on the molecular mechanism, the physiological function and the cell-cycle regulation of MT amplification.

INTRODUCTION

Mitotic spindle is a dynamic assembly of microtubules (MTs), biopolymers polymerized from the α/β-tubulin subunits (Gadde and Heald, 2004). MTs in the spindle grow and shrink, and search the 3-dimensional cellular space for kinetochores, the protein structure on the mitotic chromosomes where the spindle MTs attach (Kirschner and Mitchison, 1986; Maiato et al., 2004). The force generated on kinetochores by attached MTs then congresses chromosomes to the center of the cell at the metaphase plate. When all the chromosomes in a cell are aligned at the metaphase, anaphase ensues and dynamic depolymerization of attached MTs segregates chromosomes toward two daughter cells. Thus, timely assembly, dynamic turnover, and proper function of the mitotic spindle is essential for the high fidelity of chromosome segregation and for the genomic stability in the cell cycle (Gadde and Heald, 2004; Scholey et al., 2003).

Nucleation of MTs is the initiating events for the spindle assembly. It is well established that MTs can be nucleated from centrosomes and around chromatin in mitosis (Wiese and Zheng, 2006). Recently, we demonstrated that MTs can also polymerize using existing spindle MTs as templates in mammalian cells in a process we termed MT-dependent "MT amplification" (Zhu et al., 2008). All three mechanisms of nucleation of MTs require the γ-tubulin ring complex (γTuRC), a multisubunit protein complex in which the γ-tubulin subunit serves as a template to initiate the polymerization of the α/β-tubulin into a MT (Wiese and Zheng, 2006). γ-tubulin is localized not only to centrosomes, but also to MTs in the spindle (Luders et al., 2006), consistent with our observation that existing MTs promote the polymerization of new MTs through associated γ-tubulin. When MTs are repolymerized in mitotic cells after release from the arrest of nocodazole, a MT destabilizing drug, γ-tubulin also exists as foci on the chromatin and MTs nucleate and polymerize from these foci (Luders et al., 2006).

γ-tubulin is targeted to centrosomes, the spindle, and chromatin by NEDD1, an accessory subunit in the γTuRC (Haren et al., 2006; Luders et al., 2006). A central question in mitosis is how NEDD1 is recruited to these mitotic structures. We recently reported that the Polo-like kinase, Plk1, an essential mitotic kinase (Barr et al., 2004; Petronczki et al., 2008; van de Weerdt and Medema, 2006), controls the localization of NEDD1 on both the spindle and centrosomes (Zhu et al., 2008). In addition, a novel spindle-associate protein, FAM29A, recruits NEDD1 to the spindle, but not to centrosomes, and FAM29A is required for MT-dependent spindle amplification (Zhu et al., 2008). This review will focus on the molecular mechanism, the physiological function, and the cell-cycle regulation of the MT-dependent MT amplification and on the role of FAM29A, NEDD1, and Plk1 in the MT amplification.

MT-Dependent MT amplification in mammalian cells

Several earlier reports suggest that MTs can polymerize from the side of existing MTs in plant cells, in yeast, and in *Drosophila* to amplify cytoskeleton structure locally (Goshima et al., 2008; Janson et al., 2005; Murata et al., 2005). For example, plant cells do not have a defined microtubule-organizing center and interphase MTs are present at the cell cortex as a well oriented array. It has been reported that these cortical MTs recruit cytosolic γ-tubulin, which promotes the nucleation of MTs as branches on the cortical MTs (Murata et al., 2005). In yeast, γ-tubulin is associated along the length of interphase MTs and new MTs are nucleated from these non-centrosomal

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γ-tubulin complexes, which efficiently drives the formation of bipolar MT bundles in interphase (Janson et al., 2005). Recently, an Augmin complex consisting of the Dgt2-6 proteins has been shown to be required for centrosome-independent MT generation within the spindle in *Drosophila* culture cells (Goshima et al., 2008), suggesting a possibility that spindle MTs can serve as templates to form more MTs. Although the molecular mechanism in these earlier studies remains to be characterized, these reports suggest that non-centrosomal MT amplification may represent an evolutionarily conserved mechanism for MT nucleation and polymerization.

We recently demonstrated that MT-dependent MT amplification exists in mammalian cells and that FAM29A is a key mediator for this process (Zhu et al., 2008). We show that FAM29A associates with the entire mitotic spindle and that depletion of FAM29A reduces the density of spindle MTs by 60%. MT repolymerization assay in cells released from nocodazole indicates that FAM29A is not required for the initial nucleation of MTs from centrosomes and chromatin, but is essential for subsequent growth of MTs and for the assembly of the spindle. Analysis of the localization of EB1, a MT plus-end tracking protein, in FAM29A-depleted cells also indicates that FAM29A is not required for the nucleation of MTs around spindle poles, but controls the growth of MTs within the mitotic spindle. At the molecular level, FAM29A interacts with the NEDD1-γ-tubulin complex and this interaction is specific to mitosis. FAM29A targets the NEDD1-γ-tubulin complex to the mitotic spindle. The localization of NEDD1 and γ-tubulin on spindle MTs is dependent on FAM29A, but not vice versa. In the MT repolymerization assay, the initial localization of NEDD1 to centrosomes and to chromatin foci is not affected by the depletion of FAM29A. However, subsequent growth of NEDD1, and therefore the growth of MTs, in these foci, are blocked in the absence of FAM29A. We conclude that FAM29A recruits the NEDD1-γ-tubulin complex to the spindle to amplify MTs. Interestingly, FAM29A is only associated with MTs located within, but not outside, the sphere of condensed chromosomes in early prometaphase cells. Although the mechanistic basis for this remains to be determined, this differential localization indicates that the FAM29A pathway only promotes the polymerization of a subset of MTs in mitosis.

Centrosomes and chromatin are the two best-characterized centers of MT nucleation during mitosis (Gadde and Heald, 2004; Wiese and Zheng, 2006). However, the FAM29A-mediated MT polymerization is not associated with the centrosome and chromatin structures, as the FAM29A pathway acts on MTs after their initial nucleation from centrosomes and chromatin to promote the nucleation and polymerization of MTs from the side of existing MTs. Sequence analysis indicates that FAM29A shares a weak sequence homolog to Dgt6, a subunit of the Augmin complex in *Drosophila* (32% of aa identity over an 100-aa region in the N-terminal regions of the proteins) (Goshima et al., 2008; Zhu et al., 2008). Thus, the FAM29A-mediated MT amplification is an evolutionarily conserved mechanism in mitosis.

Physiological function of MT amplification in mitosis

FAM29A-dependent MT amplification is required for efficient chromosome congression and segregation in mammalian cells (Zhu et al., 2008). Depletion of FAM29A weakens the MT-kinetochore attachment due to a reduced number of kinetochore MTs, activates the spindle checkpoint and delays the mitotic progression. FAM29A-mediated MT amplification regulates spindle assembly and promotes k-fiber maturation in mitosis.

MT amplification and spindle assembly

Depletion of FAM29A reduces the MT density within the spindle and accumulates cells at prometaphase and metaphase (Zhu et al., 2008). Although bipolar spindle forms in depleted cells, metaphase cells have a higher frequency of generating multiple spindle poles in the absence of FAM29A. Furthermore, MT repolymerization assay indicates that the lack of MT amplification prevents the efficient formation of the bipolar spindle upon release from nocodazole. Thus, nucleation of MTs from centrosomes and chromatin alone is not sufficient for the formation of stable bipolar spindle and FAM29A-mediated MT polymerization is required for efficient spindle assembly and for maintaining the structure integrity of bipolarity. Depletion of FAM29A increases the amount of astral MTs outside the spindle. This phenotype presumably results from a higher concentration of free α/β-tubulin in the cytosol due to a 60%-reduction in the amount of spindle MTs in FAM29A-depleted cells. We propose that nucleation of MT polymerization from centrosomes and chromatin (Wiese and Zheng, 2006), sorting of MTs into a spindle structure (Gadde and Heald, 2004; Kline-Smith and Walczak, 2004), and FAM29A-mediated MT amplification (Zhu et al., 2008) are three essential steps of spindle assembly.

MT amplification and k-fiber formation

At prometaphase, individual MT nucleated from centrosomes grows and shrinks to search and capture individual chromosome (Kirschner and Mitchison, 1986; Maiato et al., 2004; Scholey et al., 2003). However, a single attached MT is not sufficient to generate adequate force for chromosome congression and a mature kinetochore fiber consists of 25-30 MTs (McIntosh et al., 2002). FAM29A plays a key role in the formation of the mature kinetochore fiber from a single attached MT (Zhu et al., 2008). In FAM29A-depleted cells, bipolar spindle forms and the majority of chromosomes congresses to the metaphase plate after a kinetic delay, indicating that k-fibers can form with a reduced level of FAM29A. However, FAM29A preferentially associates with k-fibers and depletion of FAM29A reduces the k-MT intensity and weakens MT-kinetochore interactions. These observations provide evidence for a direct role of FAM29A-mediated MT amplification in the maturation of k-fibers. Our study supports a model in which once a single MT captures a kinetochore and therefore is stabilized, the FAM29A pathway uses this attached MT as a template to quickly generate mature k-fibers to stabilize the kinetochore-MT interactions. The poleward flux then actively transports the minus ends of MTs in the k-fiber toward the spindle pole to stably connect the pole to the kinetochore.

Regulation of the MT amplification by the polo-like kinase

Polo-like kinase, Plk1, is an essential kinase that controls the entry into mitosis, the maturation of centrosomes, the formation of a bipolar spindle, the stable attachment of the MTs to kinetochores, the congression and segregation of chromosomes, as well as cytokinesis (Barr et al., 2004; Petronczki et al., 2008; van de Weerdt and Medema, 2006). We initially identified FAM29A as a Plk1-interacting protein in a proteomic study, raising the possibility that Plk1 may control the MT-dependent MT amplification (Zhu et al., 2008). We found that Plk1, FAM29A, and NEDD1 form three binary complexes. Through sequential Plk1-FAM29A and FAM29A-NEDD1 interactions, Plk1 targets FAM29A to the spindle and the MT-associated

FAM29A then recruits the NEDD1-γ-tubulin complex, which, in turn, initiates *de nova* nucleation and polymerization of MTs on the spindle. Plk1 also targets NEDD1 to centrosomes through a direct Plk1-NEDD1 interaction and the centrosomal recruitment of NEDD1 is independent of FAM29A. Recruitment of FAM29A to the spindle and of NEDD1 to centrosomes both requires the kinase activity of the Plk1, indicating an essential role of Plk1 in the nucleation of MTs from both the spindle and centrosomes. Interestingly, the intracellular levels of FAM29A determine the abundances of the Plk1-FAM29A and FAM29A-NEDD1 complexes, which then affects the intracellular levels of the Plk1-NEDD1 complex. Thus, the levels of FAM29A determine the partition of NEDD1 between the spindle and centrosomes, which then controls the relative contribution of the centrosome- vs. spindle-mediated nucleation of MTs in the assembly of the mitotic spindle.

Microtubule amplification beyond the cell cycle

MT amplification is not restricted to the mitotic spindle. It has been reported that MT amplification is involved in generating anti-parallel MT bundles in the cytoskeleton structure in yeast (Janson et al., 2005) and is required for the formation of branched cortical MT network in plant cells (Murata et al., 2005). In mammalian cells, the mechanism for forming MT arrays distal from centrosomes in cells such as neurons, polarized epithelial cells, and myotubes is not well understood. We speculate that the FAM29A-mediated MT amplification may function beyond the cell cycle in other physiological processes. For example, terminally differentiated neurons have axons and dendrites that are several feet away from centrosomes and local organization of MTs using existing MTs as a template could be an efficient mechanism to reinforce the cytoskeleton structure through a positive feedback loop. Thus, MT amplification ensures a local regulation at places where MTs are needed. We propose that the same FAM29A-NEDD1-γ-tubulin complex may act in neuronal cells and other specialized cell type under a different regulatory input that controls the association of FAM29A with interphase MT cytoskeleton.

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