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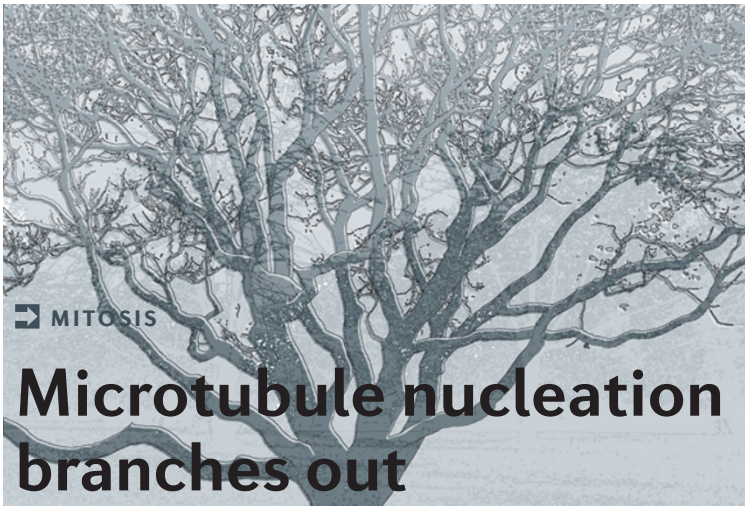

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Microtubule nucleation branches out

> Branching nucleation is an ideal mechanism for generating the parallel microtubule structure of the mitotic spindle.

Generating the mitotic spindle requires precise spatial and temporal regulation of microtubule nucleation. Vale and colleagues provide the first direct visualization of branching nucleation of microtubules (nucleation from the side of pre-existing microtubules) in animal cells.

The classic model of spindle formation involves microtubule nucleation from centrosomes and in the vicinity of chromatin, with the latter process requiring the small GTPase RAN, the coiled-coil protein TPX2 and the microtubule nucleator γTuRC (a large protein complex containing γ-tubulin). However, previous studies have shown that microtubules can form throughout the body of the spindle in the absence of centrosomes, and augmin — which recruits γTuRC to microtubules — has been implicated in this process. Augmin-dependent microtubule nucleation could involve branching nucleation, but until now this has been observed only in fission yeast and plant cells.

Cell-free extracts from *Xenopus laevis* oocytes arrested at meiosis II (which are acentrosomal) were supplemented with GFP–EB1 (to label the growing plus end of microtubules) and fluorescently tagged tubulin and then visualized by total internal reflection (TIRF) microscopy. To improve visualization, vanadate was added, which inhibits dynein-mediated microtubule sliding and prevents daughter microtubules pulling away from the mother microtubule. For the first time in a metazoan system, the authors observed the formation of new microtubules at the side of existing microtubules.

The addition of RAN•GTP by itself, and to a greater extent RAN•GTP in combination with TPX2, markedly enhanced branching microtubule nucleation, resulting in the accelerated formation of the total number of new microtubules in the extract. This resulted in large fan-shaped microtubule structures in the presence of vanadate, but branching nucleation was also observed in the absence of this compound. The authors also showed that *X. laevis* egg extract together with RAN•GTP and TPX2 could induce branching nucleation from an exogenously added pig brain microtubule tethered to the coverslip surface. This confirms that the nucleation of daughter microtubules can occur from a single parent microtubule.

Most of the daughter microtubules emerged from the mother microtubule at a shallow branch angle, such that they grew parallel to and with the same polarity as the mother microtubule. Branching nucleation is an ideal mechanism for generating the parallel microtubule structure of the mitotic spindle.

As expected, very few microtubules formed in the *X. laevis in vitro* assay in the absence of γ-tubulin. By contrast, depletion of augmin or TPX2 did not prevent microtubule nucleation and growth but completely abolished the formation of branched microtubule structures. The increased number and rate of formation of microtubules that occurs after the addition of RAN•GTP was prevented in the absence of augmin or TPX2, which indicates that branching nucleation might be the main effector mechanism of RAN-mediated spindle formation.

Kirsty Minton

ORIGINAL RESEARCH PAPER Petry, S. *et al.* Branching microtubule nucleation in *Xenopus* egg extracts mediated by Augmin and TPX2. *Cell* **152**, 768–777 (2013)
FURTHER READING Kollman, J. M. *et al.* Microtubule nucleation by γ-tubulin complexes. *Nature Rev. Mol. Cell Biol.* **12**, 709–721 (2011)