

Review

The mitotic spindle and actin tails

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Abstract

To segregate their chromosomes, eukaryotic cells rely on a dynamic structure made of microtubules: the mitotic spindle. This structure can form in cells lacking centrosomes, because their chromosomes also nucleate microtubules. This second assembly pathway is observed even in some cells that naturally have centrosomes, for example when the centrosomes are ablated by laser surgery. Recent results have started to address the complementary question of whether centrosome-nucleated microtubules alone could sustain the formation of a functional mitotic spindle. We wonder in this respect whether lower eukaryotes such as yeasts are different from higher eukaryotes such as vertebrates.
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1. Introduction

The microtubules forming the mitotic spindle of a vertebrate somatic cell could be classified from their nucleation pathway (Figure 1A): microtubules nucleated by centrosomes would be called centrosomal microtubules, and microtubules nucleated off the chromosomes, chromosomal microtubules (Karsenti and Vernos, 2001; Wittmann *et al.*, 2001). There are many examples of spindles in which centrosomal microtubules do not exist like in plants and in meiotic systems (Waters and Salmon, 1997). Moreover, it has been shown that even in cells containing centrosomes, spindles could form in their absence suggesting that chromosomal microtubules can be sufficient to form a spindle (Khodjakov *et al.*, 2000). It remains unclear however, whether a spindle could form only out of centrosomal microtubules. There is growing evidence that this may not work in higher eukaryotes (Carazo-Salas *et al.*, 2001; Garrett *et al.*, 2002; Gruss *et al.*, 2002) (Figure 1B). Indeed, in the absence of Ran-GTP or the protein TPX2 that is required for the nucleation of chromosomal microtubules, there is no bipolar spindle assembly, no chromosome segregation, and cells are blocked in mitosis. This raises questions about the underlying physical principles that govern the organization of microtubules into a mitotic spindle and why centrosomal microtu-

bules alone seem to fail to self-organize into a bipolar spindle in vertebrate somatic cells.

2. Generation versus selection

The search-and-capture model describes how bipolar spindle assembly could result from the capture and selective stabilization of centrosome-nucleated microtubules by the kinetochores or simply chromatin, (for example by motors like CenPE, Xklp1 or Xkid; (Vernos *et al.*, 1995; Wood *et al.*, 1997; Antonio *et al.*, 2000)). This mechanism based on *selection* does not seem to be sufficient, at least in the examples mentioned above, where formation of a robust bipolar spindle requires continuous microtubule *generation* in the cytoplasm closely surrounding the chromosomes. What seems to happen is that microtubules are constantly being nucleated close to the chromatin surface in addition to those that are nucleated by the centrosomes (Figure 1C and 2A). This nucleation is dependant on the generation of Ran-GTP and the activity of the TPX2 protein. This continuous generation around the chromosomes compensates for a constant loss of microtubules due to dynamic instability and treadmilling (Figure 3A). In the absence of such microtubules, the microtubules nucleated by the centrosomes (Figure 1B) even if locally stabilized and captured by chromosomes do not form a stable anti-parallel array probably because the rate of capture, the residence time on chromosomes or the cross linking activity of motors at microtubule plus ends are not

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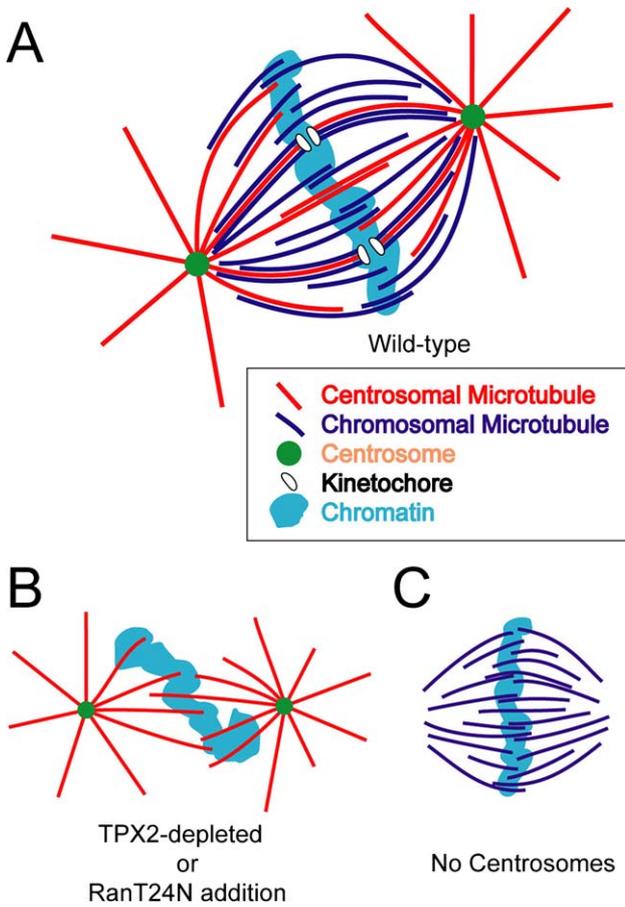


Fig. 1. The microtubules of the spindle are usually classified based on their properties, e.g. inter-polar microtubules are the ones connecting two centrosomes, kinetochore microtubules are attached to the kinetochores, astral microtubules point outwards, etc. In A) the microtubules are classified according to their place of birth. *Centrosomal microtubules* are nucleated by the centrosomes, while *chromosomal microtubules* are nucleated around the chromosomes. Kinetochore fibers could be bundles of both types of microtubules. B) Centrosomal microtubules alone are unable to form sustained contact to the chromosomes C) Bipolar spindles can form in the absence of centrosomes, i.e. with only chromosomal microtubules.

sufficient to stabilize such an anti-parallel array. Formation of a spindle seems to require instead the constant generation of a large number of microtubules directly in the vicinity of chromosomes.

3. Microtubule and actin tails

An amusing parallel can be made between the nucleation of microtubules by chromosomes and the actin-mediated propelling system of some bacteria, vesicles and viruses (Frischknecht and Way, 2001; Ploubidou and Way, 2001; Carlier *et al.*, 2003). For example, *Listeria* moves within an infected cell by inducing the formation of a tail of actin filaments behind itself (Figure 3B). Broadly speaking, there are three requirements for an object to be moved this way. 1) The object should induce the assembly of filaments at or close to its surface. 2) As the filaments grow, they should push on the object. 3) The system of the object and filaments

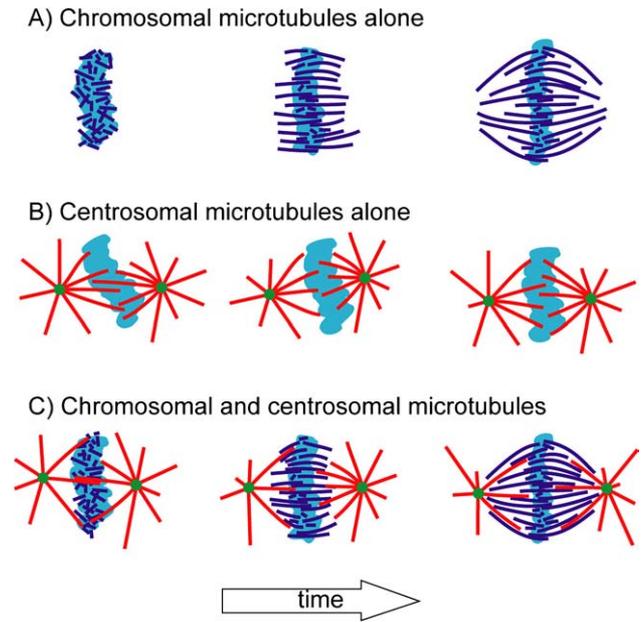


Fig. 2. Functional spindles can form in the absence of centrosomes, showing that centrosomal microtubules are not essential to this process. In the absence of TPX2, which is needed for the generation of chromosomal microtubules, the microtubules nucleated by centrosomes can only make transient contact with the chromatin. Kinetochore fibers have been omitted.

should adopt a mono-directional configuration. For the argument developed here, the molecular details of how forces are generated is secondary. We do not need to distinguish whether filaments push on the object directly (actin) or indirectly through molecular motor (microtubules).

For *Listeria*, the three requirements are met, although there are still debates about the detailed mechanism of how forces are exactly produced, with models including thermal ratchets (van Oudenaarden and Theriot, 1999), or physical pressure induced by the gel of cross linked actin filaments (Gerbal *et al.*, 2000). Requirement (3) simply means that in the moving system, the tail extends mostly on one side of the object. In this mono-directional configuration, the pushing forces of the filaments will add up to produce persistent directional motion (Wiesner *et al.*, 2003). Bacteria present an asymmetric surface, and this certainly favors the emergence of a mono-directional tail. However, experiments performed with spherical beads indicated that a radial symmetry could be broken when initially present (Theriot, 2000).

Let's now translate this to chromatin in a mitotic cell. It nucleates microtubules locally, fulfilling requirement (1). Its surface is covered by plus-end directed motors such as Xklp1 or Xkid, which push microtubules away and it thus could fulfil requirement (2). Supporting this is the observation of treadmilling in the spindle: new tubulin subunits are added close to the chromosomes to the plus end of microtubules and are lost at their minus ends, resulting in a flux of some 2 $\mu\text{m}/\text{minute}$ (Waterman-Storer *et al.*, 1998). Requirement (3) is certainly not fulfilled however, as a different symmetry is observed: the microtubules organize around chromatin preferentially in two opposite sets, on two sides of the sister-

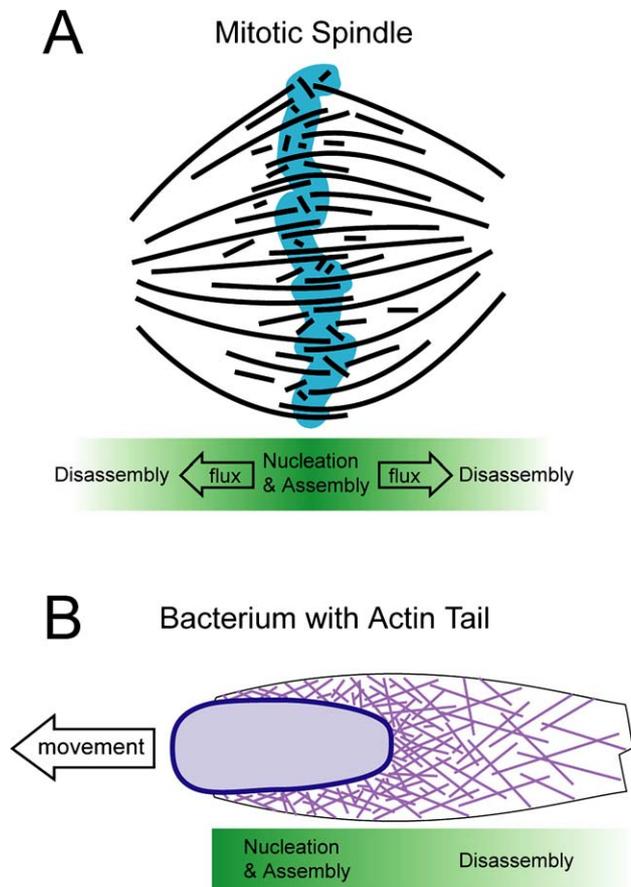


Fig. 3. The mitotic spindle is a steady state bipolar structure in which constant growth and nucleation of microtubules near the chromosomes balances disassembly at the poles. This is similar to the situation in the actin tail of moving bacteria inside a cell, where actin assembly occurs near the bacterial wall, and disassembly in the rest of the tail. The two situations are distinct by their symmetry: spindles are bipolar whereas actin tails are monopolar.

chromatids (Figure 3). Because the preferred symmetry is bi-directional and not mono-directional, the chromosomes are not propelled by microtubules, but are instead “squeezed” by them. Xkid depletion produced quite normal bipolar microtubule structures in which however chromosomes were all scattered (Vernos *et al.*, 1995; Antonio *et al.*, 2000; Funabiki and Murray, 2000). This shows on the one hand that chromokinesins such as Xkid are required for producing the forces between microtubules and chromatin (requirement 2), and on the other hand that bipolarity in this system is largely independent of the precise chromosomes position.

4. A Self-centering process

The model previously suggested (Heald *et al.*, 1996) is consistent with these observations. Indeed, if the chromatin generates microtubules in all directions and if cross-linking motors aligns them, a bi-directional configuration can emerge naturally. In more details, multimeric complexes involving motors like Eg5, dynein or ncd cross-link and by their active motion, “comb” the microtubules around the

chromatin. The combined action of these motors would primarily orient the microtubules parallel to a common axis, but in no preferred orientation. That is to say, starting from a uniform microtubule distribution, the motors would produce a “wide bundle” in which roughly 50% of the microtubules should be oriented in each of the two opposing directions. Consequently, bipolarity can simply arise from aligning the microtubules nucleated in all directions around the chromatin. This simple model explains why bipolar structures are more frequent than structures with one or three poles: because a wide bundle of aligned microtubules exactly has two directions in it.

Spindle morphogenesis must be much more intricate than sketched above, as highly dynamic microtubules are required. Indeed, since the cytoplasmic state during metaphase disfavor microtubule assembly, a structure can be maintained only if it stabilizes microtubules, or if it continuously generates new ones, in which case it can achieve steady state. Since chromosomes generate microtubules around them, the second option might apply for the formation of mitotic spindles. That is why we can envision that under the “combing” action of motors described previously, microtubule generation around chromatin could produce a dynamic bipolar structure. Microtubule dynamics and the extent of nucleation around chromatin would determine the finite size of the spindle. The question remains open of what properties of the motors control their required collective effect on microtubules.

In this simple model, chromosomes end up automatically in the center of two equal masses of microtubules because they are the source of the microtubules. The model can be extended to include several chromosomes, if the interactions of two microtubule arrays would ultimately lead to their fusion, as was observed in *Xenopus* extracts (Mitchison, T., Salmon, E.D., 2000. Response of *Xenopus* extract mitotic spindles to mechanical perturbation, *Mol. Biol. Cell* 11 abstract 569a). This effect would involve multi-motor complexes when the two structures overlap only on their edge (Figure 4, a). At a latter stage, it could also involve the motors like Xk1p1 or Xkid present on chromosome arms (Vernos *et al.*, 1995; Antonio *et al.*, 2000; Funabiki and Murray, 2000) whose plus-end directionality makes them produce a force in the right direction. Centrosome-nucleated microtubule asters can also be added in this model; dynein oligomers (Karsenti, 1991; Heald *et al.*, 1997) which focuses the poles (Hyman and Karsenti, 1998) could attach the astral microtubules

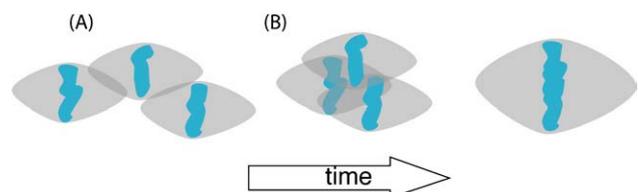


Fig. 4. Different classes of molecular motors organize the filaments generated by chromosomes into bipolar structures around them. They could also be implicated into the fusion of two separate structure. Initially (A), the motors should be able to crosslink two microtubules, while at a latter stage in the fusion (B), motors attached on chromatin can also contribute.

side-by-side with chromosomal microtubules. This model does not take into account the forces produced at kinetochores. When kinetochores are present they obviously participate in the centering process through the activity of various motors associated with them (Wood *et al.*, 1997; Yucel *et al.*, 2000; Kapoor and Compton, 2002). The generation of microtubules by chromatin nevertheless provides an independent mechanism that explains by itself why chromatin beads devoid of kinetochores are found in the center of the spindle they generate in egg extracts (Heald *et al.*, 1996). It can also explain how chromosomes find their proper position relative to the poles in mono-centrosomal spindles (Wilson *et al.*, 1997).

5. Conclusion

We have seen that spindles are bi-directional while actin tails of bacteria are mono-directional. The bi-directional symmetry is the key feature of spindle organization, as it is required for bimodal chromosome segregation. The ideas discussed here rely on observations gathered from a large collection of experiments. Recent publications (Garrett *et al.*, 2002; Gruss *et al.*, 2002) have provided a very strong qualitative support in favor of models in which chromosomes generate the microtubules necessary for their segregation. We know that spindles formed of only chromosomal microtubules are functional (Waters and Salmon, 1997). Lower eukaryotes like *S. saccharomyces* can form functional spindles in which apparently all microtubules originate from the spindle pole bodies. It will be interesting to find a vertebrate able to similarly segregate its chromosomes using only centrosomal microtubules. The principal weakness of the present article is that different levels of descriptions could not be tested together, and remained as separate unfinished elements. Fitting these pieces together into a coherent mechanistic explanation requires a quantitative analysis using a combination of studies *in vivo* and in extracts combined with theoretical modeling. This integrative step will be essential in deciphering the different spindle assembly pathways found in different species.

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