Bundling, sliding, and pulling microtubules in cells and in silico

Jonathon Howard[1] and Iva M. Tolić-Nørrelykke[1]

[1]Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Pfotenhauerstrasse 108, 01307 Dresden, Germany

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Microtubules and other proteins self-organize into complex dynamic structures such as the mitotic spindle, which separates the chromosomes during cell division. Much is known about the individual molecular players involved in assembly and positioning of the mitotic spindle, but how they act together to generate the often unexpected behavior of the whole microtubule system is not understood. Two recent papers use a combination of experimental (imaging) and theoretical (computer simulation) methods to explore the formation of bipolar linear microtubule arrays in fission yeast and the oscillatory movement of the mitotic spindle in the nematode worm. In the simulation approach, the rules for the interactions of the components (microtubules and microtubule-associated proteins) are specified and the evolution of the system is followed, with the aim of identifying the minimal set of components that can mimic the real system. The work on fission yeast concludes that bipolar microtubule structures can arise from self-organization of microtubules through nucleators, bundlers, and sliders, without a requirement for a special microtubule-organizing center. The work on the worm embryo suggests that both the positive feedback that drives oscillations and the centering force that limits their amplitude may arise from microtubule pulling forces. The systems approach exemplified by these papers should stimulate new experiments aimed at discovering the principles of cellular organization. [DOI: 10.2976/1.2740563]

CORRESPONDENCE
Joe Howard: howard@mpi-cbg.de; Iva Tolić-Nørrelykke: tolic@mpi-cbg.de

A living cell is a complex system in which a large number of different molecules combine and interact to generate complex structures and functions. One of the main subcellular systems that organize the cell interior is the microtubule cytoskeleton. Microtubules form when molecules of the protein tubulin bind to each other to form 25-nm-wide tubes. With the help of other molecules, microtubules self-organize into complex (and often very beautiful) dynamic structures such as the mitotic spindle, which separates the chromosomes during cell division. A large amount of genetic, cell biological, and biochemical work has gone into identifying molecules necessary for microtubule organization: these microtubule-associated proteins can cross-link, bundle, move, stabilize, and destabilize the microtubules. But how does one start to put all this together to show whether these molecules and their interactions are sufficient to generate the often unexpected behavior of the whole microtubule system? Two recent papers use a potentially powerful approach towards synthesizing molecular and cellular studies: by simulating individual microtubules and microtubule-associated proteins with specific interaction rules, Janson *et al.* (Janson *et al.*, 2007) and Kozlowski *et al.* (Kozlowski *et al.*, 2007) explore *in silico* the formation of bipolar microtubule arrays in fission yeast and the movement of the mitotic spindle in the nematode worm.

To perform their specific function in the cell, microtubules have to arrange into a specific geometric form. In an aster, microtubules grow from a single point, the pole, into all directions. In a linear array, microtubules are aligned with each other in a parallel or an antiparallel configuration. A mitotic spindle is a combination of both: it consists of two asters

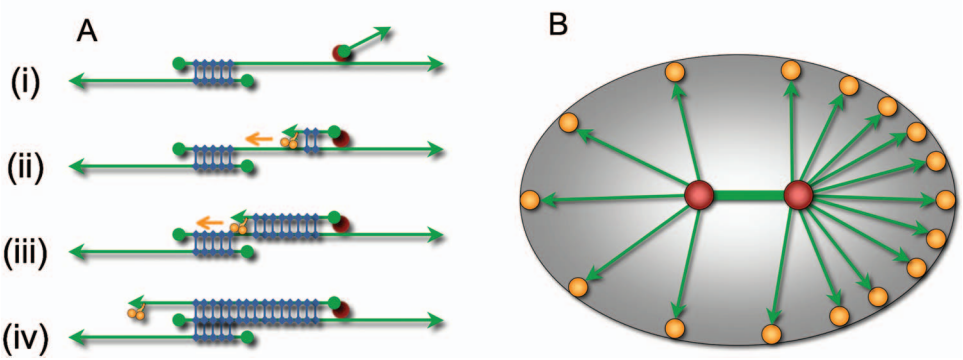

Figure 1. (A) Bundling model. From the top: (i) a nucleator (red) binds to the surface of the microtubule and nucleates a baby microtubule (short arrow); (ii) a motor (orange) binds to the plus (arrowed) end of the baby microtubule and pulls it towards the minus (round) end of the mother, (iii) the microtubule slides and grows, picking up additional bundling proteins (blue) which slow down the sliding; (iv) an antiparallel bundle is formed. (B) Force generators (orange) at the cortex bind to the plus (arrowed) ends of astral microtubules and reel them in towards the cortex. Posterior (right) movement is due to more force generators pulling towards the right. An imbalance between upwards and downwards forces on the poles is thought to lead to a rocking of the spindle (a vertical oscillation of each pole that is out of phase with the other).

connected by a bundled array. The central bundle connects to the chromosomes and is responsible for their segregation, while the asters are responsible for positioning and orienting the spindle within the cell. The mitotic spindle has to be formed reproducibly at the right time and in the right place. The key question is whether the hither-to-for described molecules are sufficient to self-organize the microtubule array, or whether essential new molecules or unsuspected interactions are necessary. This is a systems biology question.

Because higher eukaryotic cells have a large number of microtubules (thousands) and often change their shape, it can be informative to study basic microtubule organization principles in a simpler cellular system. The fission yeast *Schizosaccharomyces pombe* has proven an excellent model cell because it has only about ten microtubules and a constant simple shape of a cylinder. Fission yeast microtubules form three to four antiparallel bundles that extend along the long axis of the cell. They regulate cell polarity and shape (Sawin and Nurse, 1998), center the interphase nucleus (Tran *et al.*, 2001; Tolic-Norrelykke *et al.*, 2005; Daga *et al.*, 2006) and align nascent mitotic spindles (Vogel *et al.*, 2007).

How do these linear bipolar arrays of microtubules assemble? In earlier work, Janson *et al.* (2005) showed that new microtubules can form along preexisting ones when a nucleator, the gamma-tubulin complex, binds to the surface of an existing microtubule with the help of another protein, Mto2p. In the new work (Janson *et al.*, 2007), they show that a bundler and a slider act together with the nucleator to form an antiparallel bundle of microtubules [Fig. 1(a)]. The bundler is Ase1p, a homologue of the mammalian PRC1, which associates with antiparallel overlapping microtubules in interphase bundles and in mitotic spindles. The slider is the kinesin-related motor protein Klp2p from the kinesin-14 family, which moves towards the minus (slow-growing) end of the microtubule. In their model, a baby microtubule is born at the nucleator that is attached to an older, mother microtubule. Because the bundler preferentially binds to antiparallel microtubules, babies will typically be antiparallel to the mother microtubule. The slider then slides the babies towards the minus end of the mother, which is in the central part of the cell. Similar mechanisms may operate in metazoan cells as several labs have shown that gamma-tubulin associates with the mitotic spindle where it nucleates new microtubules.

Are these components (nucleators, bundlers, and sliders) sufficient to form a linear array of antiparallel microtubules as seen in cells? How does one answer this question? The problem is that when a system, in this case the cell, consists of a large number of components (different types of molecules) that move passively by diffusion or actively by motors, modeling of the whole system can be very difficult. These difficulties have been circumvented in the Janson *et al.* paper by stochastic simulations. In this approach, the rules for the interactions of the components are specified and the evolution of the system from an initial state is followed in time. This is repeated many times with different sets of parameters and/or different initial conditions. The evolution of such "molecular automata" is not always obvious or predictable. Simulated interactions of a large number of molecules can give rise to unexpected "emergent properties" of the whole system, leading to new insights into the problem. One advantage of the approach is that the large variety of model variants, assumptions, and parameter values, can be explored in order to identify the simplest and minimal set of components that can mimic the real system, thus distinguishing between essential and nonessential elements. Such results are guidelines for further experiments as they can tell the experimenter what to measure.

Janson *et al.* have found that simulated microtubules are indeed able to self-organize into regular bipolar linear arrays.

The self-organization process appears very similar to that seen in living cells. The simulation starts with a single microtubule and includes only nucleators, bundles, and sliders. Taking either the bundler or the slider out of the simulation resulted in a somewhat less organized microtubule arrangement, suggesting that these two elements may also be necessary to explain how randomly formed microtubules become organized into a regular structure.

Bundlers and sliders, while working together in the formation of a microtubule array, act in opposite ways: bundlers try to glue two microtubules together, whereas sliders try to move them with respect to each other. How are bundling and sliding regulated and can it be predicted which one will win? Janson *et al.* propose that bundling strength depends on microtubule length, because bundlers bind along the whole length of the microtubule whereas sliding is length-independent, because sliders bind only to the plus end of the baby microtubule. Therefore, sliding velocity should decrease as the baby microtubule grows. This was observed experimentally in living cells, as well as in the simulation. The suggested interplay between bundling and sliding may be important for regulating the length of the microtubule overlap zone in the bundle.

Though it might be argued that the conclusions could have been reached using simpler direct arguments, the simulations provide a dramatic visual confirmation of the suspected process and give the viewer an appreciation of the stochastic aspects of the behavior. The important general conclusion from this work is that a special microtubule-organizing center is not required for this type of linear bipolar microtubule organization.

This work opens up a number of interesting questions. How does the motor Klp2 track plus ends of microtubules? What increases the affinity of the bundler Ase1 to antiparallel microtubules? Does Ase1 dimerize and does this create its preference for microtubule orientation? Does Ase1 binding depend on Klp2 or vice versa? How does Ase1 find the overlap regions: does it diffuse along the microtubule, or is it transported by a motor? Finally, what is the role of cell shape in the formation of a bipolar microtubule array?

Kozlowski *et al.* (Kozlowski *et al.*, 2007) take a similar combined experiment and simulation approach to understanding mitotic spindle movements in the one-cell nematode embryo. During mitosis, the spindle moves into the posterior half of the cell; because the cleavage furrow bisects the spindle, the posterior daughter is smaller than the anterior daughter. This asymmetric cell division is common during embryogenesis and neurogenesis where the two unequal daughters have different developmental fates.

Spindle movements are thought to be driven by force generators that are bound to the cortex of the cell (the inner surface of the plasma membrane that surrounds the cell). The force generators are attached to and pull on the ends of "astral" microtubules that emanate from each of the two poles of the mitotic spindle [Fig. 1(b)]. As the microtubules shorten the spindle is reeled in towards the cortex. Laser cutting experiments showed that the central spindle is indeed under tension as expected from this model (Grill *et al.*, 2001) and suggested that the posterior displacement is due to there being more force generators on the posterior side than the anterior side (Grill *et al.*, 2003). Interestingly, concomitant with the posterior spindle displacement, the spindle oscillates transversally about a point between the two asters, producing a rocking motion. The amplitude of the rocking gradually increases and then decreases during mitosis. Pecreaux *et al.* (Pecreaux *et al.*, 2006) proposed that the oscillations are driven by load-dependent detachment of the force generators: such a tendency for force generators to detach as the load increases gives rise to positive feedback. The processivity of the force generators (they do not detach right away after the load has changed) introduces a delay, which, together with the positive feedback, causes the oscillations.

Kozlowski *et al.* take quite a different approach to the question of spindle positioning. Rather than formulating the problem as a differential equation and solving for the mean position of the spindle, they make a detailed simulation of the mitotic spindle in which they include hundreds of individual microtubules per aster that interact with the cortex according to prescribed rules. They were able to find plausible rules that give rise to oscillations, and they could also account for the posterior displacement, and even for the full three-dimensional motion of the spindle imaged end on.

Kozlowski *et al.* propose an alternative mechanism underlying oscillations. They suggest that positive feedback arises from the geometry of the embryo: as the spindle moves towards the cortex on one side, more microtubule ends reach the cortex on that side than on the opposite side, leading to more attachments to force generators and to even higher pulling forces. This is different from the load-dependent detachment mechanism (Grill *et al.*, 2005), though the Kozlowski *et al.* model still includes load-dependent detachment and it is not clear whether the new model would work without it. This will be important to explore in future simulations. A potential weakness of the Kozlowski *et al.* model is that their force generators, unlike real motors, do not slow down as they become loaded. The slowing of the force generators by load is expected to damp out the positive feedback mechanism (Howard, 2006). An economical feature of the model is that the pulling forces also lead to centering of the spindle as a consequence of microtubule bending. One of the strengths of the simulation approach is that physical manipulations like laser cutting can also be performed in silico. In this way it may be possible to distinguish the different models underlying posititve feedback and spindle centering.

A general problem with modeling approaches—both via stochastic simulations in the case of the present papers or by numeral solutions of "mean-field" solutions as in earlier work—is that as the data improve, the parameters in the

models inevitably need to be changed. It is therefore a strength of the present work that the simulations are available on the server of one of the authors, Francois Nédélec (http://www.cytosim.org/). Thus the interested readers can go there and play with the parameters themselves.

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