REVIEW: CELL CYCLE

The Mitotic Spindle: A Self-Made Machine

E. Karsenti[1,2] and I. Vernos[1]

The mitotic spindle is a highly dynamic molecular machine composed of tubulin, motors, and other molecules. It assembles around the chromosomes and distributes the duplicated genome to the daughter cells during mitosis. The biochemical and physical principles that govern the assembly of this machine are still unclear. However, accumulated discoveries indicate that chromosomes play a key role. Apparently, they generate a local cytoplasmic state that supports the nucleation and growth of microtubules. Then soluble and chromosome-associated molecular motors sort them into a bipolar array. The emerging picture is that spindle assembly is governed by a combination of modular principles and that their relative contribution may vary in different cell types and in various organisms.

The mitotic spindle is an aesthetically attractive structure (Fig. 1A). It is also a molecular machine capable of distributing the genome to the daughter cells with stunning precision. The spindle is built of microtubules that are used as tracks to move chromosomes precisely during cell division. In the spindle, microtubules are arranged in two antiparallel arrays with their plus ends at the equator and their minus ends at the poles, whatever the detailed shape of the spindle (Fig. 1) (*1–3*). Although the images shown in Fig. 1 seem to have the precision and quality of a very stable, almost crystalline, structure, the spindle is, in fact, very dynamic: it is a dissipative, steady-state structure (*4*).

Here, we explain our ideas on the mechanism of spindle assembly, mostly on the basis of experiments carried out in frog eggs and egg extracts. They are presented historically to show how the concepts emerged. Recent reviews cover the evolution of ideas on spindle organization and function (*2*, *5*).

Posing the Questions

The first investigations of spindle assembly were simple observations of cell division in various living cells (*6*). In animal cells, during interphase, microtubules often originate from a central spot, called the centrosome, and radiate in an astral configuration throughout the cytoplasm. When the cell replicates its DNA, the centrosome duplicates. At the onset of mitosis (in prophase), the duplicated centrosomes move around the nucleus while nucleating two asters of microtubules. At some point during this process, the nuclear envelope disassembles, astral microtubules shorten, and at the same time, the centrosomes seem to nucleate many more microtubules that soon start to grow asymmetrically toward the chromosomes (Fig. 1C) (*7*).

There are variations on this theme (*8*). In plant cells and in the oocytes of some species, there are no centrosomes. In this case, microtubules do not arise from discrete spots but seem to be nucleated around the chromosomes before becoming organized into a spindle (Fig. 1D) (*9*, *10*). In yeast, the nuclear envelope does not disassemble, and the spindle forms inside the nucleus (Fig. 1A) (*3*, *11*). These observations and video microscopy experiments indicate that microtubule dynamics and organization are regulated during the cell cycle (*8*). In fact, the half-life of microtubules is on the order of minutes to hours in interphase and drops to a minute or less in mitosis, but it may vary for the different kinds of microtubules present in the spindle: (i) Astral microtubules originate from the poles and radiate out into the cytoplasm (Fig. 1A) (*1*, *4*), (ii) some microtubules connect the poles to specific sites on the chromosomes, the kinetochores, and finally (iii) a variable number of microtubules originate from the spindle poles and overlap in an antiparallel way at the spindle equator or interact with chromosome arms (Fig. 1A).

These observations raise several questions: (i) What are the signals that lead to a change in microtubule dynamics when cells enter mitosis? (ii) How are various microtubule populations generated around the chromosomes? (iii) How is the length of microtubules determined in the spindle, and how is the size of the spindle thus determined? (iv) How are microtubules nucleated around chromosomes, and only there, in cells lacking centrosomes? (v) How are highly dynamic microtubules organized into a precise antiparallel array around the chromosomes and oriented with their plus end at the equator? (vi) How are the poles of a spindle defined in the absence of centrosomes? And finally, (vii) how are all these processes coordinated in space and time to generate a geometric structure that maintains itself at steady state?

Two hypotheses were put forward to explain how a spindle forms. According to one, formulated by Bovery (*12*) at the beginning of the 20th century and then taken up by Mazia (*13*), centrosomes are the center of division of the cell, and the spindle forms from astral microtubules that interact with the chromosomes. More recently, the discovery of microtubule dynamic instability led to the search-and-capture model, according to which highly dynamic microtubules nucleated by the two centrosomes are captured and stabilized by kinetochores, thereby forming a bipolar spindle (*14*). According to the other hypothesis, the chromosomes are the source of spindle organization. Microtubules are nucleated by kinetochores or by whole chromosomes and then organized into a bipolar array by some kind of cross-linking activities (*15*, *16*). In this model, centrosomes may influence spindle assembly mostly by orienting the spindle in the cell but are not essential organizers of spindle bipolarity. In fact, concepts from each model may contribute to different extents to the mechanism of spindle assembly in different species.

A Source of Answers

This complicated problem could only be addressed in a system having a simple cell cycle and in which direct biochemical experiments could be performed in combination with light microscope observations. Frog eggs and then extracts made from them turned out to have these properties (*17*). Frog eggs have three interesting features (Fig. 2A).

First, the egg is laid arrested in the second metaphase of meiosis and readily enters S phase upon fertilization. From cell-cycle studies, we learned that in this system, the transition from metaphase to interphase and back again is governed by the presence or absence of a single protein, cyclin, which regulates the activity of a master cell-cycle switch, the cdc2 kinase (*18*). Second, all of the components required for the first 12 cell cycles of early development are stored in the cell during oogenesis (*17*).

Third, the giant nucleus (400 μm) of the frog oocyte, which is blocked in G_2 of meiosis, breaks open when meiosis resumes and releases stockpiled nuclear components, such as histones and all chromatin

[1]Cell Biology and Biophysics Program, EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany. [2]Institut Jacques Monod, CNRS/Universités Pierre et Marie Curie (Paris 6) and Denis Diderot (Paris 7), Place Jussieu, Paris, France. E-mail: karsenti@embl-heidelberg.de and vernos@embl-heidelberg.de

proteins, into the cytoplasm (*19*). Thus, in contrast to the cytoplasm of somatic cells, that of frog eggs is a nucleo-cytoplasmic mixture. This explains why nuclear or spindle assembly experiments can be easily carried out in frog eggs. Indeed, all the components are naturally stored in a ready-to-use state.

The Notion of Global Cytoplasmic State

Experiments carried out by Bataillon at the very beginning of the 20th century showed that somatic nuclei injected into frog eggs would adopt the cell-cycle state of the egg and make spindles during mitosis (*20*). This system was then further developed by Ziegler and Masui (*21*) and Gurdon (*22*), who also showed that somatic nuclei could be reprogrammed by the cytoplasm, opening the way to individual cloning (*23*).

Later on, the injection of any kind of DNA into frog eggs arrested in S phase was shown to result in the formation of nuclei (*24*), whereas the injection of the same DNA into eggs blocked in M phase resulted in the formation of spindles (Fig. 2B) (*25*). Because there is no transcription in the eggs, this has to be strictly governed by the periodic activation of the cdc2 kinase (*18*). This means that the cytoplasm of these eggs can only be in one of two states at this phase and that the switch between the two states is the level of cdc2 kinase activity (Fig. 2B). This also means that spindles could assemble in the absence of both centrosomes and kinetochores, because the injected plasmid DNA lacked centromere sequences and the eggs do not contain centrioles. These results lent strong support to the conjecture that chromosomes could be the main source of spindle organization in conjunction with the mitotic state of the cytoplasm.

It soon became possible to make extracts that would recapitulate faithfully in vitro most aspects of the cell cycle, microtubule dynamics, and nuclear and spindle assembly (*26–29*). It is noteworthy that spindles formed in extracts can undergo anaphase and are very similar to those assembled in the eggs, validating the use of extracts to study spindle assembly.

Clean biochemistry and video-microscopy techniques done in these extracts have revealed the respective roles of the cytoplasmic state, centrosomes, chromosomes, and kinetochores in spindle assembly (Fig. 3). In interphase extracts, microtubules self-assemble extensively and grow off centrosomes, whereas in mitotic extracts, microtubules do not self-assemble, and centrosomes nucleate only a few highly dynamic microtubules. The transition between these two states can be induced by adding purified cdc2 kinase to an interphase egg extract (*28*). This is consistent with the notion that the cytoplasmic state becomes globally inhibitory for microtubule nucleation and stability during mitosis. It was also possible to show, using a simple algebraic formula devised to predict microtubule length from the four parameters of dynamic instability, that in interphase, microtubules grow infinitely, whereas in the mitotic state, they reach a steady-state length of about 4 μm (*28*) (Fig. 3). How the cdc2 kinase induces the transition between the two states is still unknown, but this must occur through a phosphorylation-dependent change in the relative activity of factors that stabilize or destabilize the microtubules (*30*).

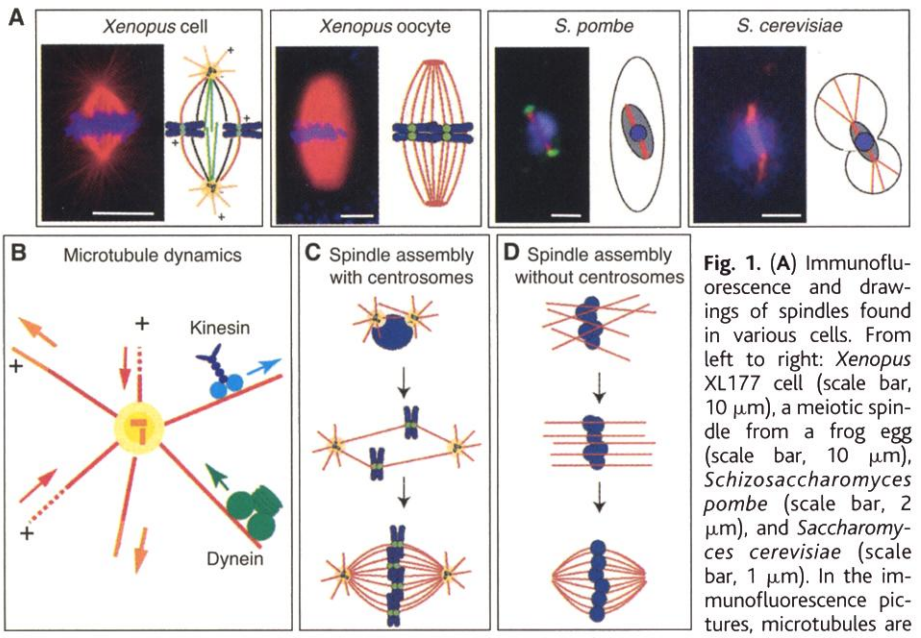

Fig. 1. (**A**) Immunofluorescence and drawings of spindles found in various cells. From left to right: *Xenopus* XL177 cell (scale bar, 10 μm), a meiotic spindle from a frog egg (scale bar, 10 μm), *Schizosaccharomyces pombe* (scale bar, 2 μm), and *Saccharomyces cerevisiae* (scale bar, 1 μm). In the immunofluorescence pictures, microtubules are red, the chromosomes are blue and the spindle pole marker sad1 (in *S. pombe*) is green. In the drawings, astral microtubules are yellow, kinetochore microtubules are red, interpolar microtubules are green, and chromosome arm microtubules are black. Note that in yeast the spindle assembles inside the nucleus, which is represented in gray in the drawings. (**B**) Microtubule dynamics, polarity, and their associated motors. Microtubules (red) at the plus end can grow (orange growing tip) or depolymerize (red dots). Minus ends are very often embedded at the centrosome (in orange). Some motors, like cytoplasmic dynein (green), move toward the minus end, whereas others, like kinesin (blue), move toward the plus end. (**C** and **D**) Pathways of spindle assembly with (C) or without (D) centrosomes. Blue, chromatin; red, microtubules; yellow, centrosomes; and green, kinetochores.

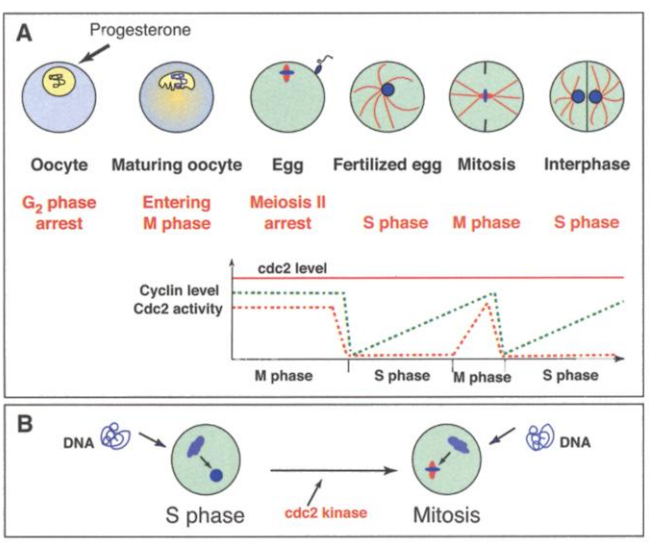

Fig. 2. (**A**) The frog egg system. The oocyte is typically 1 mm and the nucleus 400 μm in diameter. Nuclear components (yellow) are released in the cytoplasm (blue) during maturation induced by progesterone. The nucleo-cytoplasmic mixture produced (green) persists roughly until the midblastula transition. Microtubules are in red and chromatin in blue. The early cell cycle is driven by periodic accumulation and degradation of cyclin that activates the cdc2 kinase when its concentration reaches a threshold. (**B**) The frog egg cytoplasm: a two-state network. Injection of DNA into a frog egg arrested in S phase results in the formation of nuclei. The same DNA injected into an egg arrested in M phase induces spindle assembly. The cytoplasmic state transition is induced solely by the activation of the cdc2 kinase.

The Notion of Local Cytoplasmic State

In mitotic extracts, spindles assemble around artificial chromosomes in the absence of centrosomes and kinetochores (*31*) (Fig. 3). How could chromatin induce the assembly of a large number of relatively long microtubules in a cytoplasm in which even centrosomes nucleate only a few short microtubules? There are two possibilities. The surface of the chromosomes could physically nucleate microtubules, or the chromosomes could locally modify the cytoplasm to favor microtubule growth. Close observation of microtubule assembly around chromatin or chromosomes seemed to support this second possibility (*15*, *32*). Nice chromosome micromanipulation experiments in cells further strengthened this impression (*33*, *34*).

The Notion of Steady-State Gradient

How could chromosomes influence the cytoplasm to support microtubule growth? Because microtubule dynamics are regulated by phosphorylation, the first possibility is a phosphorylation gradient of regulatory factors around chromosomes (*35*). This was tested theoretically and found to be possible under certain conditions of kinetic parameters (*36*). This completely hypothetical idea got some support in the case of a known regulator of microtubule dynamics, the small molecule stathmin. Stathmin is moderately phosphorylated both in interphase and mitotic extracts, and it actively destabilizes microtubules. Chromatin beads induce a hyperphosphorylation of stathmin (as measured globally) on sites known to inactivate its destabilizing effect on microtubules (*37*) and a chromosomal kinase (Plx1) that may do this has recently been identified (*38*). A gradient of phosphorylated stathmin could therefore lead to microtubule stabilization around chromatin (Fig. 4A). However, the existence of such a gradient remains controversial (*39*).

The notion of dynamic gradients in the cell had also emerged in the field of nucleo-cytoplasmic transport in interphase. The shuttling of proteins with nuclear localization signal (NLS) through nuclear pores is governed by the state of the small Ran guanosine triphosphatase (GTPase). Ran is mostly bound to GTP in the nucleus and to GDP (guanosine diphosphate) in the cytoplasm (*40*, *41*). The steady-state ratio between the GTP- and GDP-bound states of Ran depends on the relative activity or concentration of GTPase-activating proteins (GAPs, required for Ran to hydrolyze GTP) and GTP exchange factors (GEFs), required to load Ran with GTP. The steady-state asymmetric distribution of Ran-GTP between nucleus and cytoplasm is the result of the localization of the Ran GEF (RCC1) on chromosomes and of the GAP in the cytoplasm (*42*) (Fig. 4B). Something similar seems to happen in mitosis, but in the absence of a nuclear envelope (*43*, *44*). The induction of microtubule nucleation and stabilization by chromatin requires the production of Ran-GTP. This nucleation is probably induced by a local high level of Ran-GTP around chromosomes, because this can be mimicked by the addition of a Ran mutant locked in the GTP-bound state to a mitotic extract (*45*). One downstream effector of Ran has been identified, a microtubule-associated protein, named TPX2 (Fig. 4B) (*46*). But more regulators are expect-

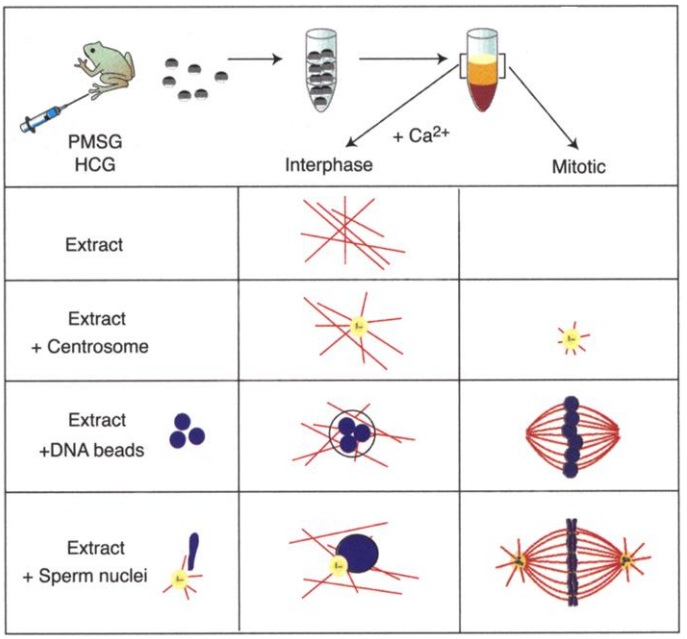

Fig. 3. Summary of microtubule behavior in interphase and mitotic extracts. Calcium is used to inactivate a cytostatic factor present in mitotic extracts and to send the extract into interphase. Addition of spindle components to egg extracts allows one to discern the relative contributions of the cytoplasmic state, centrosomes, and chromatin to spindle assembly. Yellow, centrosomes; blue, chromatin; and red, microtubules.

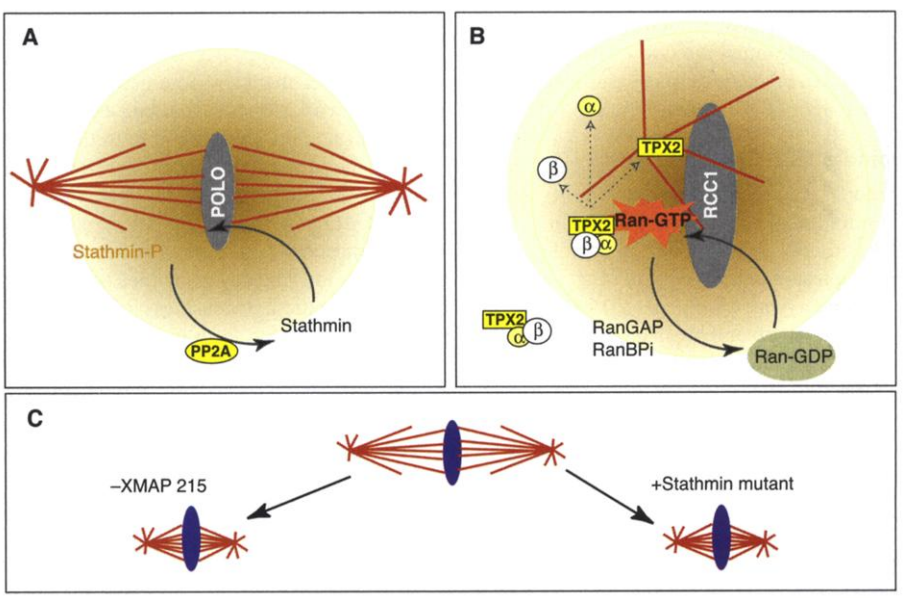

Fig. 4. (A) A hypothetical phosphorylation gradient of the microtubule-destabilizing factor stathmin. A chromosomal kinase (polo) would locally phosphorylate stathmin that is kept in a dephosphorylated state by a type 2A phosphatase in the cytoplasm. The relative kinetics of phosphorylation, dephosphorylation, and diffusion of stathmin would result in a gradient of stathmin phosphorylation. Because stathmin destabilizes microtubules when not phosphorylated, this could result in local microtubule stabilization around chromosomes. However, see text for reservations about this simple model. **(B)** A hypothetical Ran-GTP gradient around chromosomes. Ran is kept in a GDP form in the cytoplasm by a soluble Ran GTPase-activating enzyme (RanGAP) and loaded with GTP by the RCC1 GEF factor concentrated on chromosomes, resulting in a steady-state Ran-GTP gradient. The presence of Ran-GTP around chromosomes dissociates a complex made of importins (α, β) and TPX2. When released from the complex, TPX2 nucleates microtubules. The Ran gradient has other effects, such as stabilizing microtubules and activating motors. **(C)** Regulating spindle size. When microtubules are destabilized by removing a stabilizing factor like XMAP 215 or by adding a destabilizing factor like a mutant of stathmin that cannot be dephosphorylated, shorter spindles are formed.

ed to be found (*47*, *48*). Thus, in addition to the overall two-state model of the cytoplasm (interphase and mitosis), there seems to be a spatial regulation of the local state of the cytoplasm defined by localized enzymes or regulators that promote microtubule nucleation and stability around chromosomes.

Regulating Spindle Size

In egg extracts, spindles assembled around added sperm nuclei have a precise length of 25 ± 0.2 μm (*44*). In the same extracts, the steady-state length of microtubule populations nucleated by centrosomes is on the order of 4 μm (*44*, *49*). In egg extracts containing a GTP-locked form of Ran (RanQ69L), the average steady-state length of centrosome-nucleated microtubules is about 12 μm, which compares with 12.5 μm in a half-spindle (*44*). This strongly suggests that the steady-state length of the spindle is largely determined by the global microtubule dynamics imposed by the active cdc2 kinase and by a local modulation of these dynamics by Ran-GTP and phosphorylation gradients around chromosomes. How this works is not clear yet, but we know that the balance between the activity of microtubule-stabilizing and destabilizing factors (*50*) determines spindle length (Fig. 4). In addition, the balance of forces generated by molecular motors involved in organizing microtubules into a bipolar spindle must also be coordinated with microtubule dynamics to maintain the steady-state length of the spindle (*5*).

Spindle Self-Organization

The initial observations made on the kinetics of microtubule reorganization around DNA injected into frog eggs during spindle assembly suggested that some kind of cross-linking component was involved (*15*). Some years later, the involvement of motors was established in various organisms, using a genetic approach (*51*). In the past years, a large body of work carried out in frog egg extracts has led to the formulation of a model that tentatively explains how the collective action of soluble and chromosome-bound motors leads to spindle self-organization in the absence of centrosomes (*52*) (Fig. 5).

The microtubules that are randomly nucleated around chromatin are first cross-linked and aligned by the homo-tetrameric kinesin Eg5, generating an antiparallel bundle of microtubules (*53*, *54*) (Fig. 5). It is noteworthy that the generation of ran-GTP by chromatin seems to coordinate microtubule nucleation and dynamics with the activity of eg5 (*55*). At the same time, microtubules are captured by the chromatin-associated plus-end motor Xklp1, which moves microtubule minus ends away from chromatin (*56*, *57*) (Fig. 5). Finally, dynein, which was known to form asters in egg extracts (*58*), focuses microtubule minus ends into the poles (*59*).

Obviously, this model is based on experiments carried out in extracts and may account in very general terms for the mechanism of spindle assembly in systems lacking centrosomes. However, in systems where centrosomes are involved, similar principles are likely to hold. This is extensively discussed in references (*59*–*61*) but further experiments are needed to determine the generalizability of these ideas.

An Organizational Field Around Chromosomes

In this review, we have mostly summarized the principles that have emerged from a large body of work done in the frog egg system. These principles are more akin to those governing the formation of dissipative structures, than to those determining the formation of a crystal. Local inhomogeneities, gradients, and transport-dependent self-organization processes must be key principles behind spindle organization. Robustness of spindle assembly must come from guidance of the stochastic behavior of microtubules by a field. A field is defined as an area within which a force exerts an influence at every point. The question is thus, what kind of force? Gradients of regulators of microtubule dynamics and motor activities around chromosomes appear to be just that.

To what extent this view applies to all spindles is still unclear. Although there seem to be large differences between yeast and frog egg spindles (to take strikingly different examples), nothing proves that the principles are radically different. Large differences in final shapes may come from moderate variations in the parameters associated with microtubule dynamics or motors, but not necessarily from differences in fundamental principles. Also, the respective importance of self-organization, search and capture, and centrosome-dependent nucleation of microtubules obviously varies from one species to the other. Computerized models taking into account known parameters related to microtubule dynamics, microtubule numbers, types of motors involved, and their associated physical properties will certainly be required to understand fully how various spindles form (*62*). But, in any event, if the role of chromosomes as initiators of spindle organization turns out to be general, this would be a striking example of the assembly of a machine that is induced by its one purpose.

Note added in proof. Some issues that could not be addressed in this review because of space limitations are nicely discussed in (*63*).

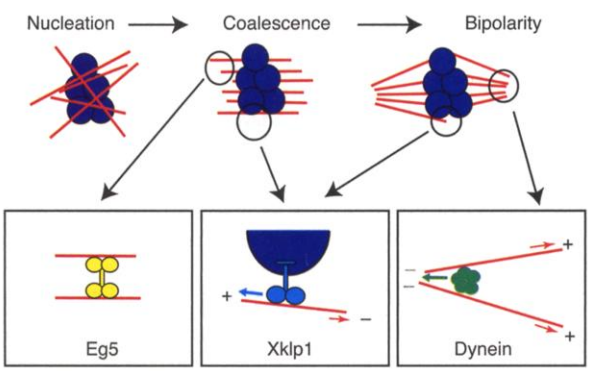

Fig. 5. Role of motors in spindle self-organization. Randomly nucleated microtubules become aligned by a cross-linking motor (eg5). The chromosome-associated plus end–directed motor Xklp1 captures microtubules on the chromatin and pushes their minus ends away, leading to their sorting into two half-spindles on each side of the chromosome mass. Finally, a multimeric form of the minus-end motor dynein focuses spindle poles. This is an intuitive working model based on many experiments carried out in egg extracts. But it probably reflects, to some extent, the principles involved in spindle assembly and stability. Red arrows indicate the direction of movement of a microtubule; arrows in the same color as a motor indicate the direction of movement of the motor.

References and Notes

1. D. A. Compton, *Annu. Rev. Biochem.* **69**, 95 (2000).
2. T. J. Mitchison, E. D. Salmon, *Nature Cell Biol.* **3**, E17 (2001).
3. M. Winey, E. T. O'Toole, *Nature Cell Biol.* **3**, E23 (2001).
4. T. Wittmann, A. Hyman, A. Desai, *Nature Cell Biol.* **3**, E28 (2001).
5. D. J. Sharp, G. C. Rogers, J. M. Scholey, *Nature* **407**, 41 (2000).
6. E. B. Wilson, *The Cell in Development and Heredity* (Macmillan, New York, ed. 3, 1925).
7. Y. Zhai, P. J. Kronebusch, P. M. Simon, G. G. Borisy, *J. Cell Biol.* **135**, 201 (1996).
8. J. C. Waters, E. D. Salmon, *Curr. Opin. Cell Biol.* **9**, 37 (1997).
9. J. W. Raff, *Curr. Biol.* **11**, R159 (2001).
10. J. De Mey, A. M. Lambert, A. S. Bajer, M. Moeremans, M. De Brabander, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1898 (1982).
11. I. M. Hagan, *J. Cell Sci.* **111**, 1603 (1998).
12. T. Boveri, *Jena Z. Med. Naturwiss.* **28**, 1 (1901).
13. D. Mazia, *Int. Rev. Cytol.* **100**, 49 (1987).
14. M. Kirschner, T. Mitchison, *Cell* **45**, 329 (1986).
15. E. Karsenti, J. Newport, R. Hubble, M. Kirschner, *J. Cell Biol.* **98**, 1730 (1984).
16. W. Steffen, H. Fuge, P. Dietz, M. Bastmeyer, G. Müller, *J. Cell Biol.* **102**, 1679 (1986).
17. M. W. Kirschner, J. Newport, J. C. Gerhart, *Trends Genet.* **1**, 41 (1985).
18. A. W. Murray, M. W. Kirschner, *Science* **246**, 614 (1989).
19. M. J. Lohka, Y. Masui, *Exp. Cell Res.* **148**, 481 (1983).
20. E. Bataillon, *Ann. Sci. Nat. Zool.* **9**, 249 (1912).
21. D. Ziegler, Y. Masui, *Dev. Biol.* **35**, 283 (1973).
22. J. B. Gurdon, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 545 (1967).
23. J. B. Gurdon, *J. Cell Sci. Suppl.* **4**, 287 (1986).
24. D. J. Forbes, M. W. Kirschner, J. W. Newport, *Cell* **34**, 13 (1983).

25. E. Karsenti, J. Newport, M. Kirschner, *J. Cell Biol.* **99**, 47s (1984).
26. M. Lohka, J. Maller, *J. Cell Biol.* **101**, 518 (1985).
27. J. Newport, *Cell* **48**, 205 (1987).
28. F. Verde, J. C. Labbé, M. Dorée, E. Karsenti, *Nature* **343**, 233 (1990).
29. R. Heald *et al.*, in *Cell Biology: A Laboratory Handbook*, J. Celis, Ed. (Academic Press, London, ed. 2, 1998), vol. 2, pp. 326–335.
30. S. S. Andersen, *Trends Cell Biol.* **10**, 261 (2000).
31. R. Heald *et al.*, *Nature* **382**, 420 (1996).
32. M. Dogterom, M. A. Felix, C. C. Guet, S. Leibler, *J. Cell Biol.* **133**, 125 (1996).
33. R. B. Nicklas, G. W. Gordon, *J. Cell Biol.* **100**, 1 (1985).
34. C. Waters, E. D. Salmon, *BioEssays* **17**, 911 (1995).
35. E. Karsenti, F. Verde, M. A. Félix, *Adv. Prot. Phosph.* **6**, 453 (1991).
36. M. Dogterom, thesis, Université Paris XI (1994).
37. S. S. Andersen *et al.*, *Nature* **389**, 640 (1997).
38. P. P. Budde, A. Kumagai, W. G. Dunphy, R. Heald, *J. Cell Biol.* **153**, 149 (2001).
39. T. Küntziger, O. Gavet, V. Manceau, A. Sobel, M. Bornens, *Mol. Biol. Cell* **12**, 437 (2001).
40. I. W. Mattaj, L. Englmeier, *Annu. Rev. Biochem.* **67**, 265 (1998).
41. D. Gorlich, U. Kutay, *Annu. Rev. Cell. Dev. Biol.* **15**, 607 (1999).
42. M. Hetzer, D. Bilbao-Cortes, T. C. Walther, O. J. Gruss, I. W. Mattaj, *Mol. Cell* **5**, 1013 (2000).
43. R. E. Carazo-Salas *et al.*, *Nature* **400**, 178 (1999).
44. R. E. Carazo-Salas, O. J. Gruss, I. W. Mattaj, E. Karsenti, *Nature Cell Biol.* **3**, 228 (2001).
45. J. A. Kahana, D. W. Cleveland, *J. Cell Biol.* **146**, 1205 (1999).
46. O. J. Gruss *et al.*, *Cell* **104**, 83 (2001).
47. M. V. Nachury *et al.*, *Cell* **104**, 95 (2001).
48. M. Dasso, *Cell* **104**, 321 (2001).
49. F. Verde, M. Dogterom, E. Stelzer, E. Karsenti, S. Leibler, *J. Cell Biol.* **118**, 1097 (1992).
50. R. Tournebize *et al.*, *Nature Cell Biol.* **2**, 13 (2000).
51. G. S. Bloom, S. A. Endow, *Motor Proteins 1: Kinesin* (*Protein Profile Series*, vol. 2. Academic Press, London, 1994), vol. 2, issue 1.
52. C. E. Walczak, I. Vernos, T. J. Mitchison, E. Karsenti, R. Heald, *Curr. Biol.* **8**, 903 (1998).
53. K. E. Sawin, K. LeGuellec, M. Philippe, T. J. Mitchison, *Nature* **359**, 540 (1992).
54. D. J. Sharp *et al.*, *J. Cell Biol.* **144**, 125 (1999).
55. A. Wilde *et al.*, *Nature Cell Biol.* **3**, 221 (2001).
56. I. Vernos, E. Karsenti, *Trends Cell Biol.* **5**, 297 (1995).
57. I. Vernos *et al.*, *Cell* **81**, 117 (1995).
58. F. Verde, J. M. Berrez, C. Antony, E. Karsenti, *J. Cell Biol.* **112**, 1177 (1991).
59. R. Heald, R. Tournebize, A. Habermann, E. Karsenti, A. Hyman, *J. Cell Biol.* **138**, 615 (1997).
60. E. H. Hinchcliffe, F. J. Miller, M. Cham, A. Khodjakov, G. Sluder, *Science* **291**, 1547 (2001).
61. A. Khodjakov, R. W. Cole, B. R. Oakley, C. L. Rieder, *Curr. Biol.* **10**, 59 (2000).
62. T. Surrey, F. Nedelec, S. Leibler, E. Karsenti, *Science* **292**, 1167 (2001).
63. C. L. Rieder, S. Faruki, A. Khodjakov, *Trends Cell Biol.* **41**, 413 (2001).

64. We would like to acknowledge all the people who shared our excitement about the spindle and frog eggs over the past 10 to 15 years, starting with the Kirschner lab back in the 1980s in San Francisco, and the offspring (direct or indirect), as well as all the Heidelberg connection involved in this work. We also thank I. Hagan for the picture of the *S. pombe* spindle and T. Hyman for the *S. cerevisiae* spindle shown in Fig. 1A. We tried to cite primary work as much as possible, but owing to space limitations, we must apologize to those who could not be included.