Current Biology, Vol. 13, 1728–1733, September 30, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.cub.2003.09.006

Long-Range Communication between Chromatin and Microtubules in *Xenopus* Egg Extracts

Rafael E. Carazo-Salas[1,*] and Eric Karsenti*
Cell Biology and Biophysics Programme
European Molecular Biology Laboratory
Meyerhofstrasse 1
D-69117 Heidelberg
Germany

Summary

The mitotic spindle of animal cells is a bipolar array of microtubules that guides chromosome segregation during cell division [1]. It has been proposed that during spindle assembly chromatin can positively influence microtubule stability at a distance from its surface throughout its neighboring cytoplasm [2]. However, such an "à distance" effect has never been visualized directly. Here, we have used centrosomal microtubules and chromatin beads to probe the regulation of microtubule behavior around chromatin in *Xenopus* egg extracts. We show that, in this system, chromatin does affect microtubule formation at a distance, inducing preferential orientation of centrosomal microtubules in its direction. Moreover, this asymmetric distribution of microtubules is translated into a directional migration of centrosomal asters toward chromatin and their steady-state repositioning within 10 μm of chromatin. To our knowledge, this is the first direct evidence of a long-range guidance effect at the subcellular level.

Results

A series of results have suggested the existence of an "à distance" effect of chromatin on microtubules [3–7]. Using egg extracts Dogterom et al. [8] made a first attempt at visualizing such effects. However, they used low-density chromatin sources and hydrophobic glass chambers that weakened the spatial concentration of potential chromatin-generated signals and confined aster assembly to the plane of the glass. As a result, the asters appeared isotropic [9], and a long-range effect could only be inferred from statistical [8] evidence. More recently, biochemical approaches have suggested that chromatin could generate a gradient of Ran-GTP having an effect on microtubule nucleation, dynamics, and organization at a distance from chromatin [2]. However, such effects have not been examined directly yet, and attempts to visualize the gradient of Ran-GTP have suggested a fairly short-range signaling effect [10].

We therefore devised a novel assay that overcomes the technical difficulties encountered in previous attempts in order to try to solve this long-standing problem (Figure 1A). We mixed artificial chromatin aggregates lacking kinetochores ("chromatin beads" [11, 12]) with purified human centrosomes [13] and M-phase arrested *Xenopus* egg extract [14] and enclosed a drop of this mixture in a sealed chamber of pretreated glass (see Supplemental Data). This produced a random distribution of centrosomal microtubule asters and chromatin throughout the chamber. Although this approach limited the number of chromatin-aster couples appropriate for observation, it removed most of the limitations encountered previously [9]. The configurations in which a centrosomal aster and a chromatin aggregate were found within a distance of a few tens of microns were recorded by time-lapse video microscopy.

Observation after 15–20 min of incubation revealed a profuse assembly of microtubules linking centrosomes to chromatin ($n > 50$ cases observed). Both the length and the density of centrosomal microtubules appeared higher in the direction of chromatin (Figure 1B). In addition, we observed that chromatin-generated microtubules (Figure 1B, arrowheads) appeared to physically merge with centrosomal microtubules to stabilize the centrosome/chromatin interaction (Figure 1B, small arrows).

During the first 10 min, however, microtubules were only nucleated by centrosomes, and no microtubule assembly was observed around chromatin, as previously reported [15]. Strikingly, centrosomal microtubules appeared to grow preferentially toward the chromatin mass ($n > 25$ cases observed in more than ten different egg extract preparations; see Figure 1C and supplemental Movie 1). Although initially nucleated isotropically, they were gradually stabilized in the direction of chromatin (Figures 1C and 1D), often without ever contacting it directly (Figure 1C, arrows). We saw such a persistent anisotropy in centrosomal microtubule organization in 100% of the cases in which a centrosome was within approximately 20 μm from chromatin, in all focal planes, and it clearly occurred in the absence of any physical interaction between the centrosome and chromatin as detected both by tubulin fluorescence and by DNA decoration with Hoechst dye (not shown). Moreover, it was not due to random variations in microtubule assembly around the center of the aster becuase it was completely absent in samples devoid of chromatin (Figure 1E) that displayed an average centrosomal microtubule length of less than 10 μm, consistent with previous reports [16]. In addition, little or no anisotropy was found in samples containing chromatin beads with low DNA content, and no anisotropy was observed in samples containing DNA beads on which chromatin had not been assembled yet (not shown).

To better characterize this long-range effect, we then quantitated the degree of anisotropy of asters exposed to chromatin by drawing through each aster center an axis perpendicular to the aster/chromatin axis and comparing microtubule assembly toward and away from chromatin. We first identified the longest microtubule/

*Correspondence: Rafael.Carazo-Salas@cancer.org.uk (R.E.C.-S.); karsenti@embl-heidelberg.de (E.K.)
[1]Present address: Cell Cycle Laboratory, Cancer Research UK, London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3PX, United Kingdom.

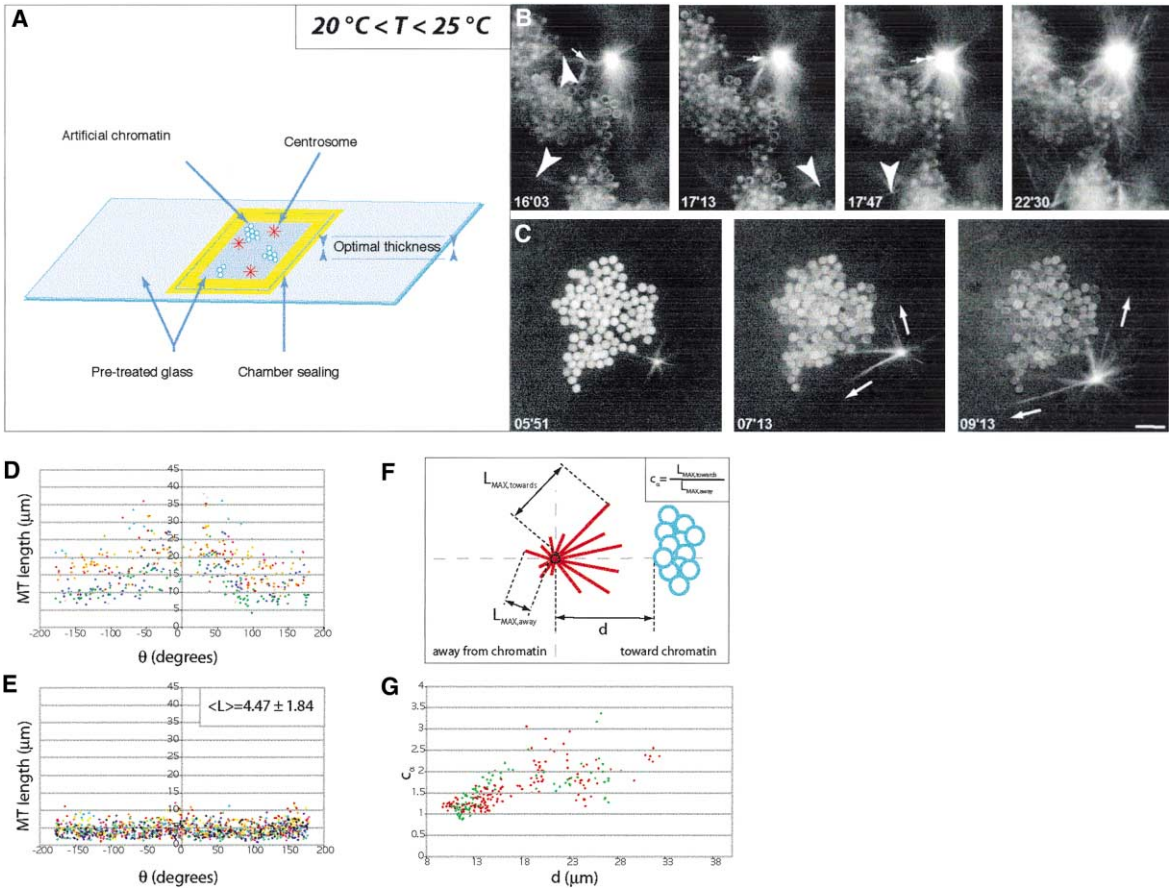

Figure 1. Anisotropic Behavior of Centrosomal Microtubules around Chromatin

(A) Experimental assay allowing rare astral-chromatin configurations to be observed.

(B) Centrosomal and chromatin microtubules (arrowheads) interact in a complex way (small arrows) to establish stable centrosome-to-chromatin contact.

(C) Centrosomal microtubule assembly precedes chromatin-induced microtubule assembly. In the absence of physical contact with chromatin, centrosomal microtubules are preferentially stabilized in its direction. Among them, "flanking microtubules" grow anisotropically toward chromatin without ever contacting it (arrows). The scale bar represents 10 μm.

(D and E) Statistical significance of aster asymmetry. (D) Representative angular distributions of microtubule lengths for n = 9 different asters found in the vicinity of chromatin. (E) Angular distribution of microtubule lengths for n = 16 different asters not exposed to chromatin. Different colors represent different asters. One dot represents one microtubule tip. θ = 0 indicates the direction of the chromatin in (D) or an arbitrary angle in (E).

(F) Definition of the asymmetry coefficient, c_α.

(G) c_α as a function of the centrosome-to-chromatin distance "d" in n = 2 different asters over an approximately 5 min period of observation. Different colors represent different asters. One dot represents one time point.

microtubule bundle that was growing toward chromatin but did not contact the chromatin surface directly and measured its length. We then measured the length of the longest detectable microtubule/bundle growing away from chromatin and defined the "asymmetry coefficient" (c_α) as the ratio of the first to the second measurement (Figure 1F). Measurement of c_α for different asters, at varying distances from the chromatin surface, showed that there was a high bias of microtubules to grow toward chromatin ($c_\alpha > 2$) at distances on the order of 30 μm (n ≈ 10 cases observed). The value of the asymmetry coefficient decreased for shorter chromatin/aster distances and converged to $c_\alpha = 1$ (symmetry) for distances on the order of 10–15 μm (Figure 1G), confirming that the bias was not contact-based (short-range). As a control, at great distances (approximately 30 μm) aster microtubule densities toward and away from chromatin were comparable (not shown).

In these experiments, we often observed that asters initially positioned far from chromatin were subsequently found closer to it. Hence, we analyzed the dynamics of aster-chromatin interaction as a function of time by concentrating on the few (n = 5) centrosome/chromatin systems in which the centrosome was initially positioned far from chromatin and in which the chromatin aggregate was small enough for the system to freely move and interact inside the chamber.

Analysis of image sequences from experiments fulfilling those criteria revealed that asters underwent a period of directed movement (Figures 2C and 2D, image sequences, and see supplemental Movies 2 and 3). By computing the radial distance "d" between the aster

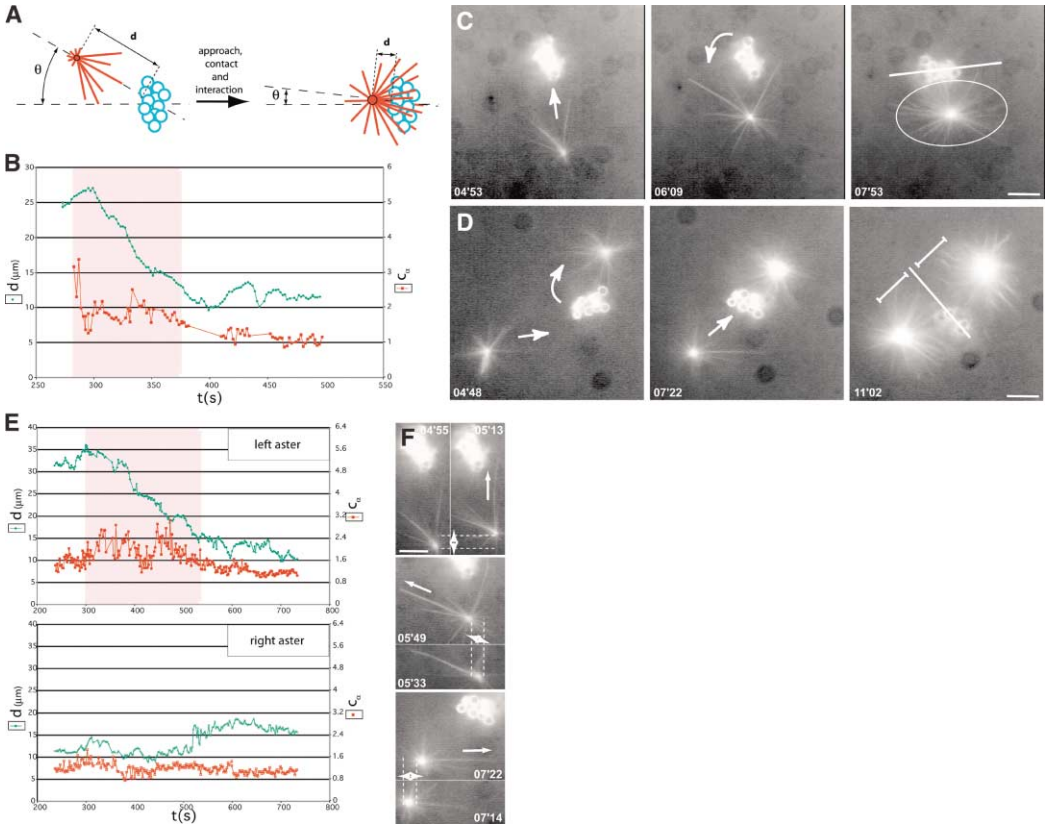

Figure 2. Directed Movement of Microtubule Asters toward Chromatin

(A) Definitions of the radial ("d") and angular ("θ") separation between asters and chromatin. (B and C) Image sequence and plot of c_α and d as a function of time for the one aster + chromatin case. (D and E) Image sequence and plot of c_α and d as a function of time for the two asters + chromatin case. In (C) and (D), solid lines indicate a long axis of chromatin, straight arrows indicate the direction of aster movement, curved arrows indicate the direction of chromatin rotation, an oval indicates aster resymmetrization, and blunt lines indicate equal aster-chromatin distances. In (B) and (E), colored (pink) regions indicate periods of high anisotropy. In (E), upper and lower quantitations correspond to the left and right asters of (D), respectively. (F) Aster displacements during the approach phase (double arrows) are parallel to the direction of the longest microtubule bundles (single arrows). Scale bars represent 10 μm.

center and the surface of chromatin (see Figure 2A for the definition) we observed that, during that period, the distance between the centrosome and the chromatin decreased from 20–30 μm to 10–15 μm (see Figures 2B and 2E). Aster movement was not random and consisted of successive, nonrectilinear, "forward" steps that followed the outgrowth of microtubule bundles toward chromatin (see Movies 2 and 3). Detailed examination of the time course of the "asymmetry coefficient" c_α for those sequences revealed that this coefficient also changed temporally and that the periods of high aster anisotropy ($c_\alpha >> 1$) correlated with the periods of approach toward chromatin (Figures 2B and 2E, top graph, colored regions). Conversely, little change in the "asymmetry coefficient" was observed when the aster did not undergo a phase of approach (see, for instance, Figure 2E, bottom graph). Close inspection of the image sequences revealed that the irregular movement of the asters was related to fluctuations in the anisotropy of microtubule elongation and that the direction of aster movement corresponded to the direction of microtubule/bundle polymerization (Figure 2F), suggesting a close correlation between the anisotropy of an aster and its movement toward chromatin.

Once in the vicinity of chromatin, the relative positions of asters and chromatin tended toward an apparent steady state. The radial distance separating them converged as a function of time to approximately 10–15 μm, and only small fluctuations around that position were observed (Figures 2B and 2E). This correlated with convergence of the asymmetry coefficient to approximately 1. Correspondingly, the angular deflection θ (Figure 2A) of the aster from its "final" position with respect to the beads converged to a pseudo-stability (Figure 3A). In cases in which one aster interacted with a chromatin aggregate (Figure 3A), this involved the chromatin mass undergoing a radical rotation ("pivoting") induced by the aster-chromatin contact (Figures 2C, middle panel, and 2D, left panel, and see supplemental Movies 2 and 3). This rotation occurred only once throughout each experiment. The "final" angle (denoted by $\theta = 0$ in Figure 3A) was approximately perpendicular to the axis joining the aster and the chromatin beads (see Movies 2 and 3). However, when two asters interacted with chromatin, no steady-state value of the angular deflection was reached (Figure 3B, top graph). Although the overall distance between chromatin and centrosomes showed again little variation (not shown) and the "pseudo-spindle" that

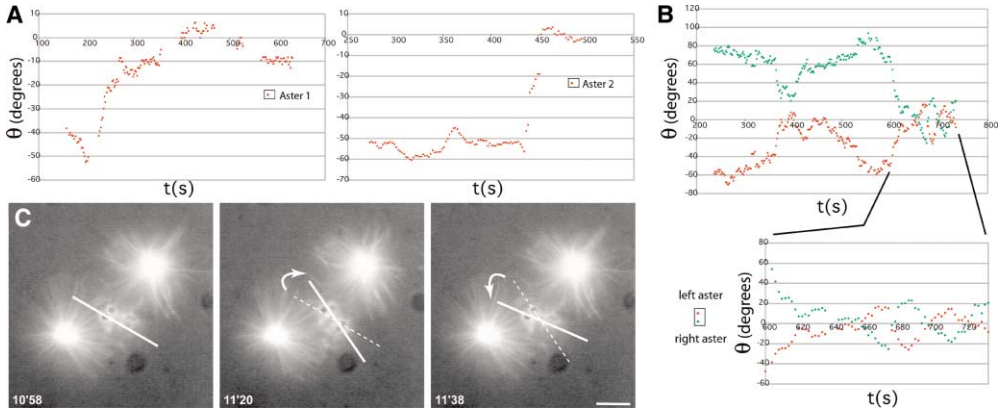

Figure 3. Pseudo-Steady-State Organization of Astral-Chromatin Systems

(A) Angular separation θ as a function of time for the one aster + chromatin case. Graphs represent different asters.

(B) θ as a function time for the two asters + chromatin case of Figure 2D.

(C) Back-and-forth, out-of-equilibrium movement of chromatin between the two asters. Solid lines: long axis of chromatin; dotted lines: previous axis position; curved arrows: direction of chromatin movement. The scale bar represents 10 μm.

formed remained globally stable, chromatin moved vigorously back and forth between the two centrosomes (Figures 3B, bottom graph enlargement, and 3C; see also Movie 4). Moreover, centrosomal microtubules were incapable of establishing a firm interaction with chromatin (not shown), suggesting that no proper spindle formed [16].

Discussion

It has long been proposed that, during spindle formation, chromatin could strongly stimulate microtubule assembly [3, 4, 7, 11, 17], even at a distance [2]. However, such an "à distance" effect had only received indirect experimental support [8, 16, 18]. Here, we provide the first direct evidence in favor of a long-range "chromatin effect." The current results conclusively demonstrate that the interplay between chromatin and microtubule arrays is not limited to short-range interaction (i.e, strict physical contact). Although these observations have been made in egg extracts, which recapitulate conditions at the interface between meiosis and mitosis of amphibian eggs [14], our results suggest that this type of long-range interaction could also occur in somatic mammalian cells during cell division and that the initial steps of spindle formation could rely on long-range cues supplied by the chromatin (Figure 4). Detailed in vivo work [6] will be required to determine the relative contributions of this and other possible mechanisms of interaction between chromatin and microtubules (including search-and-capture [19]) during spindle assembly in different cellular types.

The present experimental system did not allow us to establish a precise spatial range for this "à distance" effect, but our observations lead us to estimate that microtubules can "communicate" with chromatin at distances of a few microns to tens of microns in the apparent absence of a physical contact. They also reveal that, at short range from chromatin, the chromatin and microtubule systems interact in a complex fashion that gives rise to a host of collective movements in egg extracts. Therefore, our results are consistent with the existence of signaling gradients that have various radii of action around chromatin and which could act as determinants of steady-state centrosomal position and microtubule length and, hence, of spindle size, shape, and organization. Such gradients could be the outcome of activities known to be present around chromatin in the M phase cytoplasm. These include microtubule-associated motor activities such as cytoplasmic dynein (which is thought to be involved in aster migration in other systems [20, 21]), chromatin-mediated phosphorylation events [2], and especially, the small GTPase Ran and its effectors [22].

It will be interesting in the future to determine the exact range of the "à distance" effect and the precise nature of all the molecules implicated in the phenomena described here as well as to carry out a more detailed analysis of the effect of chromatin on microtubule dynamics parameters and on the microtubule reorganization around centrosomes. This will require developing high-throughput versions of the current approach.

A fascinating outcome of the current results is the succession of causal relationships between the various dynamic processes observed. The presence of chromatin induces asymmetric microtubule growth toward chromatin; this then leads to a directed movement and repositioning of the asters next to the chromatin, where they reach a stable position because all their microtubules lay inside the region of preferential stability. Thus, by "reconstituting" the interaction between centrosomal microtubules and chromatin in this simple assay, we have created a subcellular "dynamical system" having intrinsic behavioral properties. This behavior seems to emerge from local ("contact") as well as long-range ("à distance") interactions between chromatin and the microtubule system. Both of these types of interaction are signatures of the self-organization behavior of larger systems ranging from multi-cellular assemblies to flying bird flocks or the formation of ant nests [23]. It is remarkable that we find such features at the sub-cellular scale. We suggest that this kind of systemic behavior could

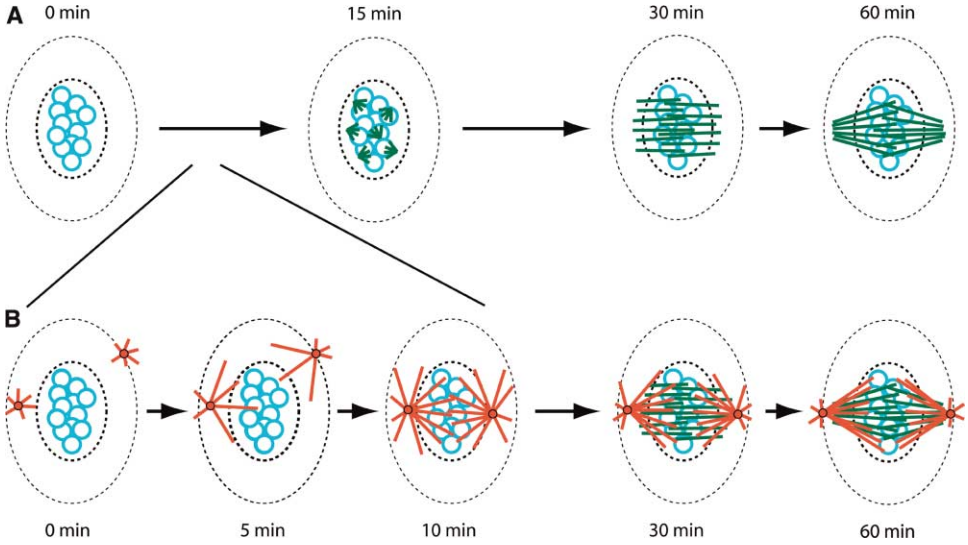

Figure 4. Proposed Model for the Radii of Communication between Centrosomes and Chromatin

Chromatin generates a morphogenetic field for microtubules during acentrosomal (A) and centrosome-assisted (B) bipolar spindle assembly by stimulating microtubule nucleation in its close proximity [11, 15, 16] (inner circle, strong dashes) and microtubule stabilization in a larger region of the cytoplasm (outer circle, weak dashes). When centrosomes are present, this long-range interaction allows centrosomal asters to sense at a distance the position of chromatin and to quickly approach it and later allows them to participate in the formation and organization of a robust spindle structure upon contacting chromatin-generated microtubules. Red: centrosomal microtubules; green: chromatin-induced microtubules.

be essential for the emergence of the mitotic spindle in general and it may largely contribute to the self-organization of other sub-cellular organelles.

Acknowledgments

We thank E. Piddini, R. Tournebize, T. Hyman, D. Chrétien, R. Heald, P. Becker, E. Steltzer, S. Leibler, F. Nédélec, T. Surrey, the Electronics and Mechanical Workshops of the European Molecular Biology Laboratory, and V. Juárez for assistance and discussions; and E. Piddini, I. Mattaj, M. Caudron, and T. Toda for critical reading of the manuscript. This work was funded by a European Molecular Biology Laboratory predoctoral fellowship, and partly by European Molecular Biology Organization and Human Frontier Science Program long-term fellowships, to R.E.C.-S.

Received: May 19, 2003
Revised: July 7, 2003
Accepted: July 28, 2003
Published: September 30, 2003

References

1. Wittmann, T., Hyman, A., and Desai, A. (2001). The spindle: a dynamic assembly of microtubules and motors. Nat. Cell Biol. *3*, E28–E34.
2. Karsenti, E., and Vernos, I. (2001). The mitotic spindle: a self-made machine. Science *294*, 543–547.
3. Karsenti, E., Newport, J., Hubble, R., and Kirschner, M. (1984). Interconversion of metaphase and interphase microtubule arrays, as studied by the injection of centrosomes and nuclei into Xenopus eggs. J. Cell Biol. *98*, 1730–1745.
4. Karsenti, E., Newport, J., and Kirschner, M. (1984). Respective roles of centrosomes and chromatin in the conversion of microtubule arrays from interphase to metaphase. J. Cell Biol. *99*, 47s–54s.
5. Mitchison, T., Evans, L., Schulze, E., and Kirschner, M. (1986). Sites of microtubule assembly and disassembly in the mitotic spindle. Cell *45*, 515–527.
6. Piehl, M., and Cassimeris, L. (2003). Organization and dynamics of growing microtubule plus ends during early mitosis. Mol. Biol. Cell *14*, 916–925.
7. Nicklas, R.B., and Gordon, G.W. (1985). The total length of spindle microtubules depends on the number of chromosomes present. J. Cell Biol. *100*, 1–7.
8. Dogterom, M., Felix, M.A., Guet, C.C., and Leibler, S. (1996). Influence of M-phase chromatin on the anisotropy of microtubule asters. J. Cell Biol. *133*, 125–140.
9. Carazo-Salas, R.E. (2001). Roles of Chromatin in Mitotic Spindle Morphogenesis. PhD thesis, Université Paris 7 and European Molecular Biology Laboratory, Paris, France and Heidelberg, Germany.
10. Kalab, P., Weis, K., and Heald, R. (2002). Visualization of a Ran-GTP gradient in interphase and mitotic Xenopus egg extracts. Science *295*, 2452–2456.
11. Heald, R., Tournebize, R., Blank, T., Sandaltzopoulos, R., Becker, P., Hyman, A., and Karsenti, E. (1996). Self-organization of microtubules into bipolar spindles around artificial chromosomes in Xenopus egg extracts. Nature *382*, 420–425.
12. Carazo-Salas, R.E., Guarguaglini, G., Gruss, O.J., Segref, A., Karsenti, E., and Mattaj, I.W. (1999). Generation of GTP-bound Ran by RCC1 is required for chromatin-induced mitotic spindle formation. Nature *400*, 178–181.
13. Bornens, M., Paintrand, M., Berges, J., Marty, M.C., and Karsenti, E. (1987). Structural and chemical characterization of isolated centrosomes. Cell Motil. Cytoskeleton *8*, 238–249.
14. Murray, A.W. (1991). Cell cycle extracts. Methods Cell Biol. *36*, 581–605.
15. Heald, R., Tournebize, R., Habermann, A., Karsenti, E., and Hyman, A. (1997). Spindle assembly in Xenopus egg extracts: respective roles of centrosomes and microtubule self-organization. J. Cell Biol. *138*, 615–628.
16. Carazo-Salas, R.E., Gruss, O.J., Mattaj, I.W., and Karsenti, E. (2001). Ran-GTP coordinates regulation of microtubule nucleation and dynamics during mitotic-spindle assembly. Nat. Cell Biol. *3*, 228–234.
17. Steffen, W., Fuge, H., Dietz, R., Bastmeyer, M., and Muller, G. (1986). Aster-free spindle poles in insect spermatocytes: evidence for chromosome-induced spindle formation? J. Cell Biol. *102*, 1679–1687.
18. Wilde, A., Lizarraga, S.B., Zhang, L., Wiese, C., Gliksman, N.R.,

Walczak, C.E., and Zheng, Y. (2001). Ran stimulates spindle assembly by altering microtubule dynamics and the balance of motor activities. Nat. Cell Biol. *3*, 221–227.

19. Kirschner, M., and Mitchison, T. (1986). Beyond self-assembly: from microtubules to morphogenesis. Cell *45*, 329–342.
20. Holzbaur, E.L., and Vallee, R.B. (1994). DYNEINS: molecular structure and cellular function. Annu. Rev. Cell Biol. *10*, 339–372.
21. Vallee, R.B., and Sheetz, M.P. (1996). Targeting of motor proteins. Science *271*, 1539–1544.
22. Hetzer, M., Gruss, O.J., and Mattaj, I.W. (2002). The Ran GTPase as a marker of chromosome position in spindle formation and nuclear envelope assembly. Nat. Cell Biol. *4*, E177–E184.
23. Camazine, S., Deneubourg, J.-L., Franks, N.R., Sneyd, J., Theraulaz, G., and Bonabeau, E. (2001). *Self-Organization in Biological Systems*. (Princeton, NJ, and Oxford, UK: Princeton University Press).