Chromosomes Initiate Spindle Assembly upon Experimental Dissolution of the Nuclear Envelope in Grasshopper Spermatocytes

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Abstract. Chromosomes are known to enhance spindle microtubule assembly in grasshopper spermatocytes, which suggested to us that chromosomes might play an essential role in the initiation of spindle formation. Chromosomes might, for example, activate other spindle components such as centrosomes and tubulin subunits upon the breakdown of the nuclear envelope. We tested this possibility in living grasshopper spermatocytes. We ruptured the nuclear envelope during prophase, which prematurely exposed the centrosomes to chromosomes and nuclear sap. Spindle assembly was promptly initiated. In contrast, assembly of the spindle was completely inhibited if the nucleus was mechanically removed from a late prophase cell. Other experiments showed that the trigger for spindle assembly is associated with the chromosomes; other constituents of the nucleus cannot initiate spindle assembly in the absence of the chromosomes.

The initiation of spindle assembly required centrosomes as well as chromosomes. Extracting centrosomes from late prophase cells completely inhibited spindle assembly after dissolution of the nuclear envelope. We conclude that the normal formation of a bipolar spindle in grasshopper spermatocytes is regulated by chromosomes. A possible explanation is an activator, perhaps a chromosomal protein (Yeo, J.-P., F. Alderuccio, and B.-H. Toh. 1994*a*. *Nature (Lond.)*. 367: 288–291), that promotes and stabilizes the assembly of astral microtubules and thus promotes assembly of the spindle.

CHROMOSOMES in some cells dramatically affect spindle microtubule assembly or stability. Chromosomes can regulate the content of spindle microtubules in grasshopper spermatocytes (Marek, 1978; Nicklas and Gordon, 1985; Zhang and Nicklas, 1995), and chromosomes promote microtubule assembly in their vicinity in *Drosophila* spermatocytes (Church et al., 1986) and *Drosophila* oocytes (Theurkauf and Hawley, 1992). In *Xenopus* eggs injected with cellular components, astral microtubule growth is enhanced only at the centrosomes associated with the injected nuclei, not at free centrosomes (Karsenti et al., 1984). Also in *Xenopus*, demembranated sperm nuclei added to an egg extract induce the assembly of polarized microtubule arrays that are biased toward chromatin (Sawin and Mitchison, 1991).

What is the utility of such chromosomal effects on microtubules? An intriguing possibility is that chromosomes play a critical role in normal spindle formation in some cells. For instance, other participants in spindle assembly, the centrosomes and tubulin subunits, might be activated by exposure to chromosomes when the nuclear envelope breaks down. Previous work shows that the nucleus is necessary for spindle formation in some cells, e.g., in echinoderm embryos, in which centrosomes cannot organize a bipolar spindle in the absence of a nucleus (Sluder et al., 1986; references in Sawin and Mitchison, 1991). But any role for the chromosomes as opposed to other constituents of the nucleus is not established, and those other constituents could be involved (Kallajoki et al., 1992).

We set out to test decisively in grasshopper spermatocytes whether chromosomes can play a role in the normal pathway of spindle assembly. We find that mechanical disruption of the nuclear envelope in prophase leads to premature formation of an apparently normal spindle. We show that the activator of spindle formation is associated with chromosomes; other nuclear constituents alone cannot trigger spindle assembly. Our results also show that while chromosomes in grasshopper spermatocytes initiate spindle formation, centrosomes are also required, as in almost all other cells (Mazia, 1985).

Materials and Methods

Materials

Spermatocytes of the grasshopper *Chortophaga australior* (Rehn and Hebard) were cultured according to Nicklas et al. (1982) except in a different micromanipulation chamber (Kiehart, 1982). Because prophase cells in the same cluster of the preparation are naturally synchronized in the same

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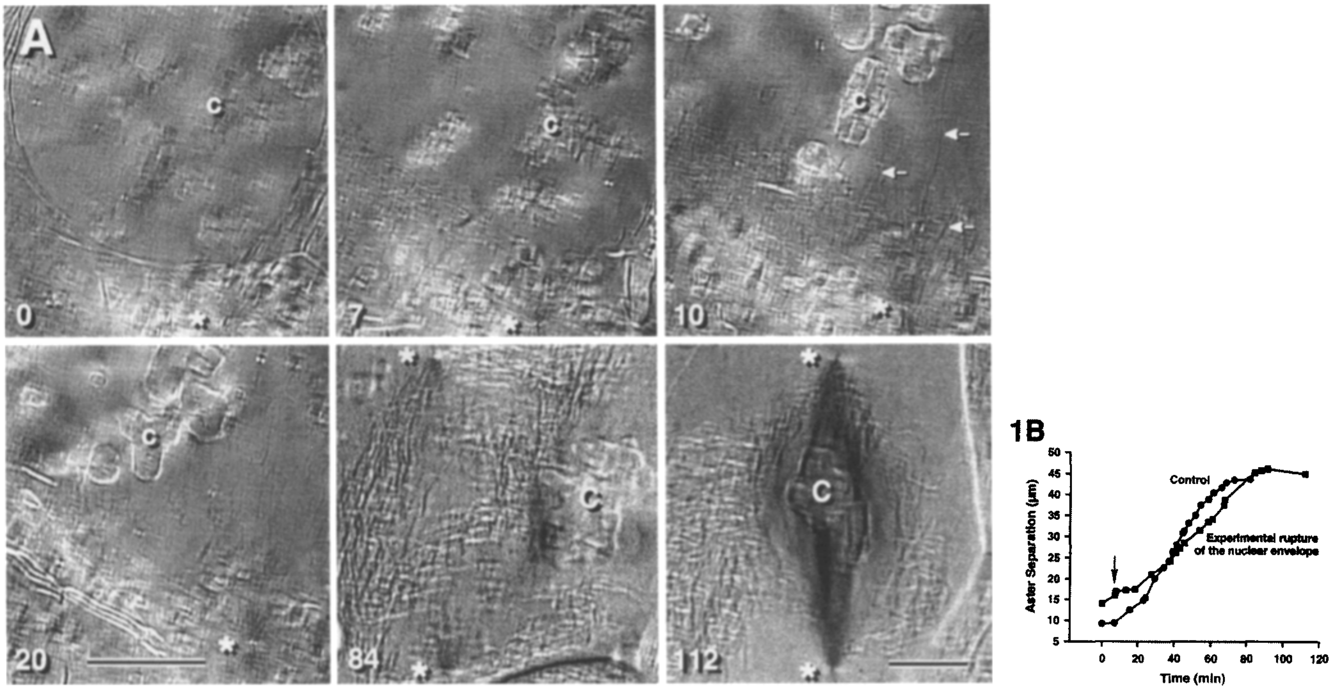

Figure 1. (*A*) The impact of nuclear contents on the initiation of spindle assembly. Time is given in min on each image. Microtubules are seen as black lines or bundles (*10 min* onward). The 0 min image shows a prophase cell with one centrosome (*) visible. Prematurely rupturing the nuclear envelope with a micromanipulation needle (*7 min*) soon results in enhanced microtubule assembly at the centrosomes and condensation of the chromosomes (*c*) (*7* and *10 min*). Spindle formation follows (*10 min* onward). Chromosomes (*C*) are clumped and entangled (*84* and *112 min*). The 0 min image is a montage of adjacent video frames taken at different focal levels so as to show both the centrosome and the nucleus. Video-enhanced polarization microscopy. (*B*) Aster separation in the experimental cell shown in *A* as compared with a control cell. *Arrow*, time of normal or experimental dissolution of the nuclear envelope. Bars, 10 µm.

cell cycle stage, they are ideal for comparison; therefore, experimental and control cells were always selected from the same cluster.

Video-enhanced Polarization Microscopy

Cells were viewed with a video-enhanced polarization microscope as described earlier (Nicklas and Ward, 1994). The condenser numerical aperture was slightly decreased to 1.2 as limited by the micromanipulation chamber used. A combination of a Nikon rectified NA 0.65/40× objective and an NA 0.65 condenser was also used on some cells to view the entire spindle. Shuttered illumination was employed to minimize the damage due to intense light. Video images were digitized, processed, and stored as previously described (Nicklas and Ward, 1994).

Micromanipulation

Prophase cells were manipulated with a fine glass needle (tip diameter less than 0.1 µm) using a piezoelectric micromanipulator (Ellis and Begg, 1981). Premature nuclear envelope rupture was achieved by repeatedly stretching and releasing the envelope with the needle until it was obviously disrupted. Removal of an intact nucleus was accomplished by mechanically dividing the cell into two unequal compartments. We first pushed the nucleus to the periphery of the cell and then indented the cell membrane around the nucleus to force the nucleus into a tiny bleb connected to the main cell by a tightly appressed membrane strand. The connecting strand was immediately severed by the micromanipulation needle to produce a non-nucleated main cell and a nucleus-containing mini-cell. Chromosomes were extracted from the cells much as described by Marek (1978) and Nicklas and Gordon (1985) except the chromosomes in prophase cells were first removed from the nucleus and then from the cell. Centrosome extraction was carried out as previously described (Zhang and Nicklas, 1995).

Results

Manipulation of Cellular Components

Grasshopper spermatocytes easily tolerate the most demanding micromanipulation. A cell without centrosomes, chromosomes and a spindle, produced by micromanipulation, can still proceed to complete a normal cytokinesis (Zhang and Nicklas, 1995). Only a small amount of cytoplasm is removed from the cell along with the extracted components.

Premature Rupture of the Nuclear Envelope

We ruptured the nuclear envelope of spermatocytes in mid-diakinesis (see Figs. 1 and 5 *A*). Such cells (Fig. 1 *A*, 0 min image) can be distinguished from late prophase cells (see Fig. 3, 0 min image) in which the nuclear envelope is about to break down naturally: in diakinesis, the cytoplasm has a more granular appearance, and the chromosomes are relatively inconspicuous. If not manipulated, mid-diakinesis cells would normally remain in prophase for several hours. However, releasing the nuclear contents by mechanically breaking the envelope using a needle (Fig. 1 *A*, *7 min*) promptly initiates spindle assembly (*7 min* onward). Released prophase chromosomes are initially relatively uncondensed but quickly become as condensed as chromosomes in a normal prometaphase cell (*7*

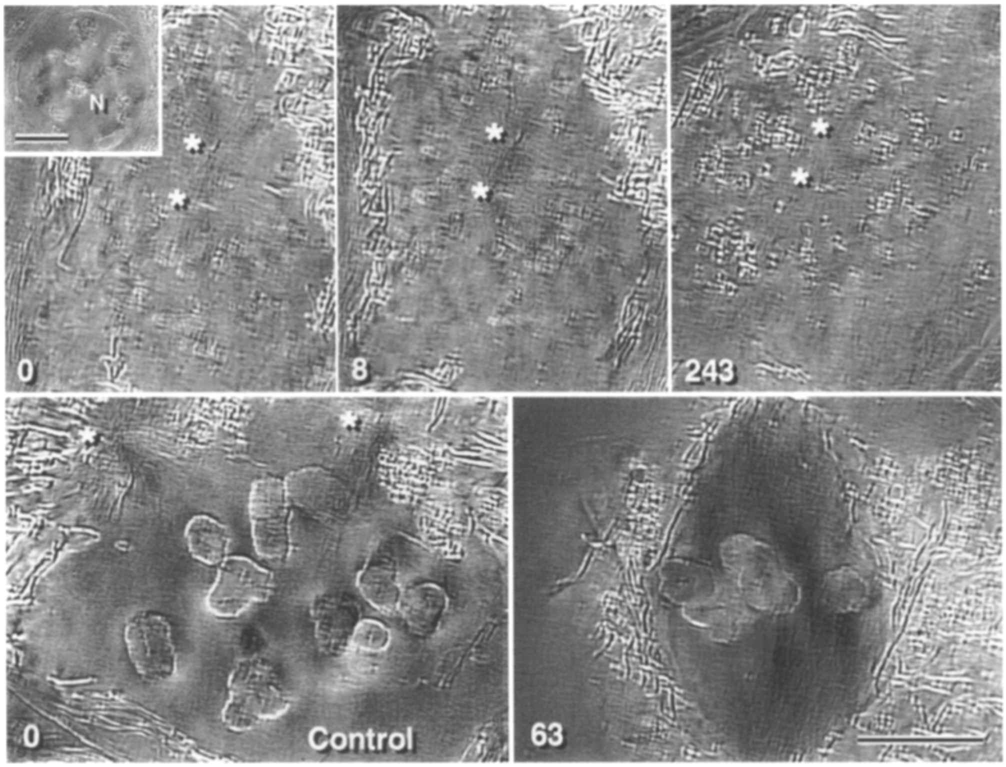

Figure 2. Centrosomes do not organize a spindle in the absence of the nucleus. After removal of the nucleus (*N*) (*0 min* image, *inset*), no spindle formed around the centrosomes (*) (*0*, *8*, and *243 min*; only one centrosome is in focus in the *243 min* image). Assembly of the spindle in an unmanipulated control cell nearby was normal (*0* and *63 min*, *Control*). Bar, 10 μm.

and *10 min*). Meanwhile, microtubules rapidly assemble at the centrosomes (*arrows*, *10 min* image). The centrosomes begin to separate soon after the envelope is broken (Fig. 1 *B*), moving at a rate (0.4 μm/min) similar to that of centrosomes associated with a normal spindle (0.5 μm/min). Chromosome congression to a metaphase plate, however, is not normal: chromosomes are tangled together in a clump and cannot be separated with a micromanipulation needle or by the spindle; independent chromosome movement is impossible. The chromosomes remain in a cluster to one side of the spindle (Fig. 1 *A*, *20 min*) even after the spindle poles are fully separated (*84 min*). The chromosome clump as a whole gradually moves into the spindle with chromosome ends (kinetochores, presumably) oriented toward opposite poles and associated with thick microtubule bundles (*112 min*). The result is a metaphase-like spindle. Control cells in the same cell cluster did not progress to nuclear envelope breakdown during over two hours of observation. Similar early stages of spindle formation were observed in three other experiments where cells were subjected to mechanical rupture of the nuclear envelope.

Removal of the Nucleus in Prophase

To further test the role of the nucleus in spindle assembly, we extracted the nucleus from late prophase cells (see Fig. 5 *B*). Fig. 2 shows one of two cells in which the nucleus was removed just before the envelope was about to dissolve (note the fully condensed chromosomes in the extracted nucleus, Fig. 2, *inset*, *0 min* image). The stage of the main cell was ascertained by examining the nucleus in the mini-cell. In the mini-cell, the nuclear envelope broke down ~15 min after the operation. In the main cell, the centrosomes are visible as small asters (*0 min*), but in the absence of the nucleus, the astral microtubules failed to grow and interact to form a spindle (*0* through *243 min*). Four hours later, the centrosomes were barely visible (*243 min*). In the meantime, nearby control cells had all developed a metaphase spindle. An example is shown in the lower panels (*0* and *63 min*, *Control*).

Removal of the Chromosomes in Prophase

To determine whether the chromosomes, rather than some other parts of the nucleus, are involved in the initiation of spindle assembly, we extracted the entire complement of chromosomes prior to the breakdown of the nuclear envelope (see Fig. 5 *C*). One of three examples is shown in Fig. 3. The cell is in late prophase, as verified by the enlarged nucleus, condensed chromosomes, and well-developed asters (*0 min* image). Chromosome extraction was completed within fifteen min, leaving a cell with the nucleus containing only nuclear sap (*15 min*). The dissolution of the nuclear envelope occurred about nine min later (*24 min*), as marked by the invasion of astral microtubules (*arrows*). Despite the normal occurrence of nuclear envelope breakdown and the resulting release of the nuclear contents, the cell failed to form a spindle. Astral microtubules gradually disassembled, and asters were barely visible at the end of the observations (*44* and *94 min*). By that time, nearby control cells had entered metaphase.

Removal of the Centrosomes in Prophase

We tested whether chromosomes alone can initiate spindle assembly by eliminating the centrosomes from prophase cells (Figs. 4 and 5 *D*). The centrosomes, visible in the mini-cell produced by the operation (Fig. 4, *inset*, *0 min* image), were extracted about ten min before the dissolu-

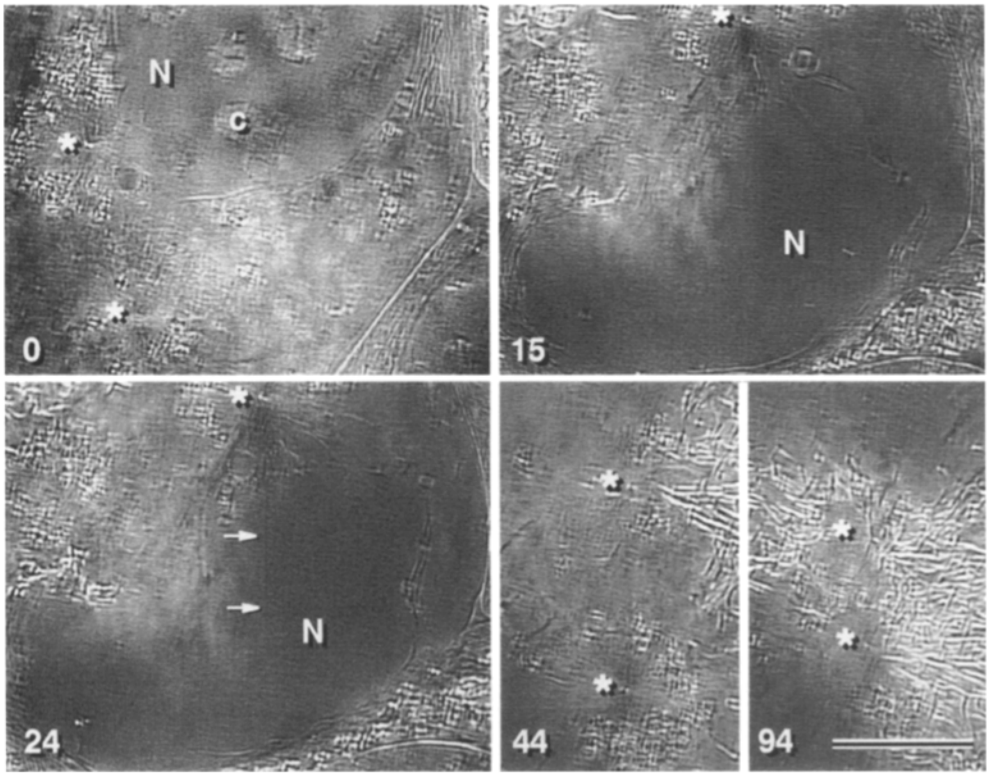

Figure 3. Nuclear activation of spindle formation does not occur in the absence of chromosomes. The 0 min image shows a late prophase cell. After chromosome extraction (*15 min*), the nuclear envelope breaks down normally (*24 min*), but centrosomes (*) fail to organize a spindle (*44* and *94 min*; only one centrosome is in focus in the *94 min* image). *N*, nucleus; *c*, chromosomes; *arrows*, microtubules. Bar, 10 μm.

tion of the nuclear envelope. Remnants of the nuclear envelope (*arrow*) are seen in the 0 min image. The entire complement of chromosomes and the nuclear sap remained in the main cell which, nevertheless, failed to form a spindle. In the absence of a spindle, the chromosomes gradually moved together (5 and 81 min), forming a cluster surrounded by the mitochondria. Three such experiments were performed with similar results.

Discussion

Our results demonstrate that in grasshopper spermatocytes, premature release of the nuclear contents can initiate spindle assembly (Figs. 1 and 5 *A*). This occurs in a cytoplasmic environment in which very little microtubule birefringence was seen at the centrosomes prior to their exposure to the nuclear components. Our results agree well with the findings in *Xenopus* eggs in which centrosomes coinjected with demembranated nuclei are activated only when a nucleus is nearby (Karsenti et al., 1984). These results, obtained with entirely different experimental approaches and different cellular systems, provide strong support for the proposition that normal spindle formation in some cells may be activated by nuclear components released upon breakdown of the nuclear envelope.

No spindle forms if all chromosomes are removed from the nucleus before the nuclear envelope breaks down (Figs. 3 and 5 *C*). Thus, the active factor involved in the nuclear activation of spindle assembly is associated with chromosomes. Other nuclear constituents may also be required, but cannot initiate spindle assembly by themselves.

How might chromosomes initiate spindle assembly? Astral microtubules are stabilized when captured by each chromosome's kinetochores (Salmon, 1975; Mitchison and Kirschner, 1985; Nicklas and Kubai, 1985) and this by itself is important in spindle organization (Kirschner and Mitch-

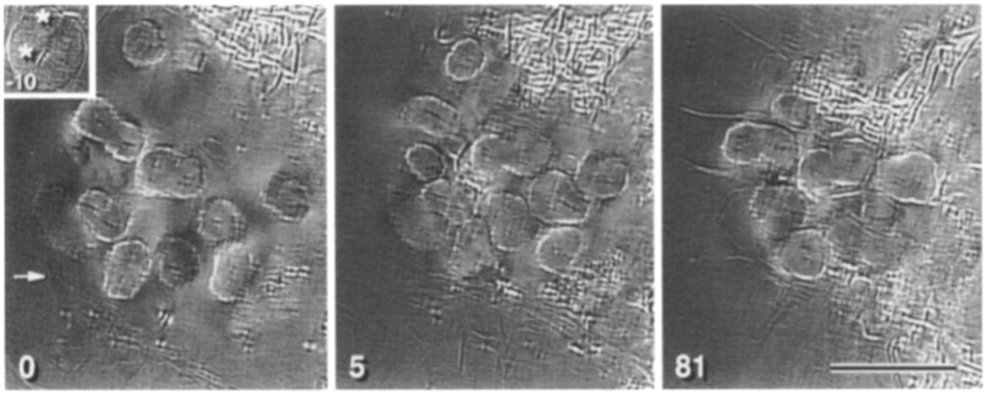

Figure 4. Nuclear activation of spindle formation does not occur in the absence of centrosomes. After removal of centrosomes (*) in late prophase (*10 min* image, *inset*), the cell fails to form a spindle (*0* through *81 min*) despite normal breakdown of the nuclear envelope (*0 min*). Arrow, remnant of nuclear envelope. Bar, 10 μm.

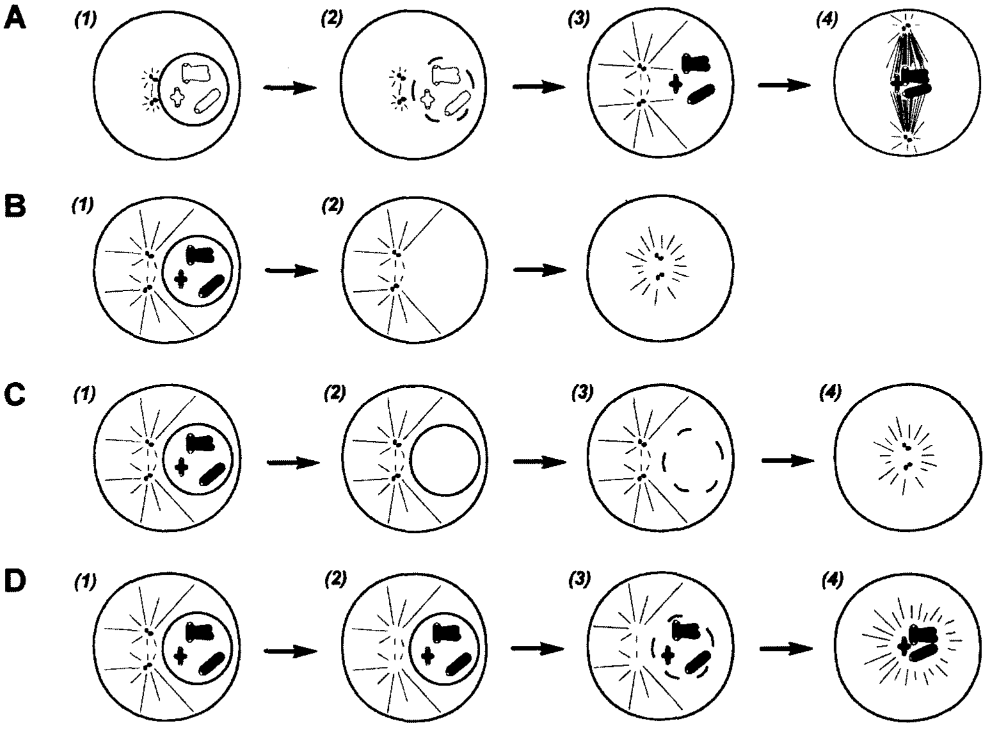

Figure 5. Summary of experimental designs and results. For simplicity, only three chromosomes are shown. (*A*) Premature rupture of the nuclear envelope in prophase triggers spindle assembly. (*1*) Prophase cell several hours before nuclear envelope breakdown; chromosomes are still relatively uncondensed (shown in outline) and asters (*black dots surrounded by short lines*) are relatively inconspicuous. (*2*) Nuclear envelope is ruptured with a micromanipulation needle. (*3*) Released chromosomes immediately become condensed (*black*) and astral microtubules (*fine lines*) rapidly assemble at the centrosomes. (*4*) Asters separate from each other; chromosomes move as a group into the spindle while more microtubules assemble at the poles. (*B*) Removal of the nucleus from the cell inhibits spindle assembly. (*1*) Late prophase cell with condensed chromosomes and prominent asters. (*2*) Nucleus is removed some min before natural breakdown of the nuclear envelope would have occurred. (*3*) In the absence of the nucleus, asters do not separate and astral microtubules gradually disassemble. No spindle forms. (*C*) Extracting chromosomes from the nucleus in late prophase inhibits spindle assembly. (*1*) Late prophase cell some minutes before nuclear envelope breakdown. (*2*) Chromosomes are extracted without rupturing the nuclear envelope. (*3*) Nuclear envelope breaks down naturally. (*4*) Asters do not separate and astral microtubules disassemble. No spindle forms. (*D*) Extracting centrosomes in late prophase inhibits spindle assembly. (*1*) Late prophase cell some min before nuclear envelope breakdown. (*2*) Centrosomes are removed from the cell. (*3*) Nuclear envelope breaks down naturally. (*4*) Former astral microtubules disassemble and the chromosomes move together to form a cluster. No spindle forms.

ison, 1986). Stabilization of kinetochore microtubules is not the only chromosomal role in spindle assembly, however, at least in some cells. In grasshopper spermatocytes, a second spindle forms after the asters from the first spindle are moved to the cytoplasm (Zhang and Nicklas, 1995). The presence of a single chromosome on the first spindle permits the second spindle to form even though that spindle lacks chromosomes and kinetochore microtubules. Similarly, in *Xenopus* egg extracts, the effect of the sperm nucleus on spindle assembly is independent of specific kinetochore-microtubule interactions (Sawin and Mitchison, 1991). Therefore, some additional effect of chromosomes needs to be considered and the obvious one is the impact of chromosomes on microtubule assembly/stability (Karsenti, et al., 1984; Sawin and Mitchison, 1991; Zhang and Nicklas, 1995). A plausible model is a diffusible chromosomal factor that stabilizes microtubules; upon breakdown of the nuclear envelope, the centrosomal microtubules would be exposed to this factor and stabilized, leading to a rapid increase in microtubule concentration at centrosomes. A candidate for the chromosomal factor has been identified, regulator of mitotic spindle assembly-1 (RMSA-1)[1], a new chromosomal protein, which is essential for mitotic spindle assembly (Yeo et al. 1994*a*). A RMSA-1–like protein is also found on the meiotic chromosomes of crane fly spermatocytes (Yeo et al., 1994*b*) and may well be present in grasshopper spermatocytes. Other candidates are kinesin-like motor proteins associated with chromosome arms. For instance, an antibody to a novel chromosomal protein, Xklpl (*Xenopus* kinesin-like protein), causes spindle instability (Vernos et al., 1995). An impact of chromosomes on microtubule content during normal spindle formation is suggested by some earlier observations of an increase in microtubule content soon after nuclear envelope breakdown (Inoué and Sato, 1967; Roos, 1973).

Despite their indispensability in the initiation of spindle formation, chromosomes per se are not required to maintain spindle structure in grasshopper spermatocytes. A spindle, once formed, can persist even after all the chromosomes are removed from the cell. The spindle is well organized and functionally normal, though its microtubule content is reduced (Zhang and Nicklas, 1995). Why is a chromosome needed for spindle initiation but not for the maintenance of the spindle? Perhaps once the chromosomal factor binds to the spindle, the chromosome itself is no longer essential and can be removed. Intriguingly, however, if a single chromosome is left in the cytoplasm rather than removed from the cell, the spindle disassembles (Zhang and Nicklas, 1995). Why should a chromosome that does not affect spindle stability if it is removed from the cell

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[1] *Abbreviation used in this paper:* RMSA-1, regulator of mitotic spindle assembly-1.

cause the spindle to disappear if present in the cytoplasm? Perhaps when left in the cell rather than removed, the chromosome recruits to itself the active factor and causes the spindle to disassemble.

We conclude that, in grasshopper spermatocytes, chromosomes are an essential stimulus to spindle assembly, which is initiated when the other participants in spindle formation are exposed to the chromosomes after rupture of the nuclear envelope. This arrangement automatically ties the timing of spindle formation to the regulation of nuclear envelope breakdown. Envelope breakdown is known to be regulated by cell cycle mechanisms that control cdc2 kinase and the phosphorylation of nuclear lamins (Peter et al., 1990). Spindle formation will occur at the right time without the necessity for a separate control mechanism.

Results of earlier studies led to the suggestion that biological differences between mitotic and meiotic cells are responsible for the different roles of chromosomes and centrosomes in spindle assembly (Rieder et al., 1993). In mitotic cells of echinoderm embryos (Sluder and Rieder, 1985) and newt lung (Rieder and Alexander, 1990), chromosomes cannot organize a spindle in the absence of microtubule nucleation centers, the centrosomes. In contrast, in *Drosophila* oocyte meiosis, chromosomes apparently can induce spindle assembly by themselves (Theurkauf and Hawley, 1992). This may also be true in meiotic crane fly spermatocytes (Dietz, 1966; Steffen et al., 1986), but see Rieder et al. (1993). The grasshopper spermatocytes we study certainly require centrosomes as well as chromosomes to form a spindle (Figs. 4 and 5 *D*). Centrosomes are as necessary in this meiotic system as in the somatic mitosis of echinoderm embryos and newt lung. The converse generalization, that centrosomes alone are not sufficient, is also true in both meiosis and mitosis in certain materials: our results from nuclear extraction in meiotic prophase cells (Figs. 2 and 5 *B*) are comparable to those obtained using mitotic cells (Sluder et al., 1986; references in Sawin and Mitchison, 1991). Clearly, further investigations are needed to truly understand the role of chromosomes in spindle assembly; these studies should be conducted using several different species involving both mitosis and meiosis.

The indispensable role of chromosomes in the initiation of spindle assembly in some cells makes one wonder about the many exceptions in which centrosome separation and centrosomal microtubule assembly occur before nuclear envelope breakdown (Rattner and Berns, 1976; Aubin et al., 1980; Rieder and Hard, 1990). Although in these cells chromosomes may or may not affect spindle microtubule assembly, the nucleus is certainly required in the establishment of spindle bipolarity (Sluder et al., 1986). Perhaps in these cells the chromosome as a whole plays no part, but the kinetochores remain important in establishing spindle bipolarity by selectively stabilizing polar microtubules (Kirschner and Mitchison, 1986).

The most certain exceptions to a chromosomal role in spindle initiation are cells which have extra-nuclear spindles and a nuclear envelope that never breaks down (e.g., hypermastigote flagellates and dinoflagellates; see Raikov, 1978). The converse problem occurs in cells such as yeast, in which the spindle forms within the nucleus. The spindle microtubule-nucleating centers apparently are constantly exposed to chromosomes. If so, what triggers their activation when spindle formation is required? Obviously, much remains to be learned of the strategies by which diverse cells make spindles of the proper form and at the right time.

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References

Aubin, J. E., M. Osborn, and K. Weber. 1980. Variations in the distribution and migration of centriole duplexes in mitotic PtK2 cells studied by immunofluorescence microscopy. *J. Cell Sci.* 43:177–194.

Church, K., R. B. Nicklas, and H.-P. Lin. 1986. Micromanipulated bivalents can trigger mini-spindle formation in *Drosophila melanogaster* spermatocyte cytoplasm. *J. Cell Biol.* 103:2765–2773.

Dietz, R. 1966. The dispensability of the centrioles in the spermatocyte divisions of *Pales ferruginea* (Nematocera). *Heredity (Suppl.).* 19:161–166.

Ellis, G. W., and D. A. Begg. 1981. Chromosome micromanipulation studies. *In* Mitosis/Cytokinesis. A. M. Zimmermann and A. Forer, editors. Academic Press, NY. 155–179.

Inoué, S., and H. Sato. 1967. Cell motility by labile association of molecules. *J. Gen. Physiol.* 50:259–292.

Kallajoki, M., K. Weber, and M. Osborn. 1992. Ability to organize microtubules in taxol-treated mitotic PtK$_2$ cells goes with the SPN antigen and not with the centrosome. *J. Cell Sci.* 102:91–102.

Karsenti, E., J. Newport, R. Hubble, and M. Kirschner. 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.

Kiehart, D. P. 1982. Microinjection of echinoderm eggs: apparatus and procedures. *Methods Cell Biol.* 25:13–31.

Kirschner, M., and T. Mitchison. 1986. Beyond self-assembly: from microtubules to morphogenesis. *Cell.* 45:329–342.

Marek, L. F. 1978. Control of spindle form and function in grasshopper spermatocytes. *Chromosoma (Berl.).* 68:367–398.

Mazia, D. 1985. The chromosome cycle and the centrosome cycle in the mitotic cycle. *Int. Rev. Cytol.* 100:49–92.

Mitchison, T. J., and M. W. Kirschner. 1985. Properties of the kinetochore in vitro. II. Microtubule capture and ATP-dependent translocation. *J. Cell Biol.* 101:766–777.

Nicklas, R. B., and G. W. Gordon. 1985. The total length of spindle microtubules depends on the number of chromosomes present. *J. Cell Biol.* 100:1–7.

Nicklas, R. B., and D. F. Kubai. 1985. Microtubules, chromosome movement, and reorientation after chromosomes are detached from the spindle by micromanipulation. *Chromosoma.* 92:313–324.

Nicklas, R. B., and S. C. Ward. 1994. Elements of error correction in mitosis: Microtubule capture, release, and tension. *J. Cell Biol.* 126:1241–1253.

Nicklas, R. B., D. F. Kubai, and T. S. Hays. 1982. Spindle microtubules and their mechanical associations after micromanipulation in anaphase. *J. Cell Biol.* 95:91–104.

Peter, M., J. Nakagawa, M. Dorée, J. C. Labbé, and E. A. Nigg. 1990. In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. *Cell.* 61:591–602.

Raikov, I. B. 1978. The Protozoan Nucleus: Morphology and Evolution. N. Bobrov and M. Verkhovtseva, editors. Springer-Verlag, NY. 72–130.

Rattner, J. B., and M. W. Berns. 1976. Distribution of microtubules during centriole separation in rat kangaroo (*Potorous*) cells. *Cytobios.* 15:37–43.

Rieder, C. L., and S. P. Alexander. 1990. Kinetochores are transported poleward along a single astral microtubule during chromosome attachment to the spindle in newt lung cells. *J. Cell Biol.* 110:81–96.

Rieder, C. L., and R. Hard. 1990. Newt lung epithelial cells: cultivation, use, and advantages for biomedical research. *Int. Rev. Cytol.* 122:153–220.

Rieder, C. L., J. G. Ault, U. Eichenlaub-Ritter, and G. Sluder. 1993. Morphogenesis of the mitotic and meiotic spindle: conclusions obtained from one system are not necessarily applicable to the other. *In* Chromosome Segregation and Aneuploidy. B. K. Vig, editor. NATO ASI Series, Vol. H72. Springer-Verlag, Berlin. 183–197.

Roos, U.-P. 1973. Light and electron microscopy of rat kangaroo cells in mitosis. I. Formation and breakdown of the mitotic apparatus. *Chromosoma (Berl.).* 40:43–82.

Salmon, E. D. 1975. Spindle microtubules: Thermodynamics of *in vivo* assembly and role in chromosome movement. *Ann. NY Acad. Sci.* 253:383–406.

Sawin, K. E., and T. J. Mitchison. 1991. Mitotic spindle assembly by two different pathways in vitro. *J. Cell Biol.* 112:925–940.

Sluder, G., and C. L. Rieder. 1985. Experimental separation of pronuclei in fertilized sea urchin eggs. Chromosomes do not organize a spindle in the absence of centrosomes. *J. Cell Biol.* 100:897–903.

Sluder, G., F. J. Miller, and C. L. Rieder. 1986. The reproduction of centrosomes: nuclear versus cytoplasmic controls. *J. Cell Biol.* 103:1873–1881.

Steffen, W., H. Fuge, R. Dietz, M. Bastmeyer, and G. Muller. 1986. Aster-free spindle poles in insect spermatocytes: evidence for chromosome-induced spindle formation. *J. Cell Biol.* 102:1679–1687.

Theurkauf, W. E., and R. S. Hawley. 1992. Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *J. Cell Biol.* 116:1167–1180.

Vernos, I., J. Raats, T. Hirano, J. Heasman, E. Karsenti, and C. Wylie. 1995. Xklp1, a chromosomal Xenopus kinesin-like protein essential for spindle organization and chromosome positioning. *Cell.* 81:117–127.

Yeo, J.-P., F. Alderuccio, and B.-H. Toh. 1994*a*. A new chromosomal protein essential for mitotic spindle assembly. *Nature (Lond.).* 367:288–291.

Yeo, J.-P., A. Forer, and B.-H. Toh. 1994*b*. A homologue of human regulator of mitotic spindle assembly protein (RMSA-1) is present in crane fly and associates with meiotic chromosomes. *J. Cell Sci.* 107:1845–1851.

Zhang, D. H., and. R. B., Nicklas. 1995. The impact of chromosomes and centrosomes on spindle assembly as observed in living cells. *J. Cell Biol.* 129: 1287–1300.