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. 1994a. Nature (Lond.). 367: 288–291), that promotes and stabilizes the assembly of astral microtubules and thus promotes assembly of the spindle.

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Materials and Methods

Materials

Spermatocytes of the grasshopper Chortophaga australis (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
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.

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.

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. 5C). 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 minutes, leaving a cell with the nucleus containing only nuclear sap (15 min). The dissolution of the nuclear envelope occurred about nine minutes 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 5D). The centrosomes, visible in the mini-cell produced by the operation (Fig. 4, inset, 0 min image), were extracted about ten minutes 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.

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.

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-
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.

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. (7) 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

RMSA-1-like protein is also found on the meiotic chromosomes of crane fly spermatocytes (Yeo et al., 1994b) 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, Xkpl (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.

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 controlcdc2 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 crank 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 both 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

Sluder, G., and C. L. Rieder. 1985. Experimental separation of pronuclei in fer-
tilized sea urchin eggs. Chromosomes do not organize a spindle in the ab-
Sluder, G., F. J. Miller, and C. L. Rieder. 1986. The reproduction of cen-
Steffen, W., H. Fuge, R. Dietz, M. Bastmeyer, and G. Muller. 1986. Aster-free
spindle poles in insect spermatocytes: evidence for chromosome-induced
Theurkauf, W. E., and R. S. Hawley. 1992. Meiotic spindle assembly in Dro-
sophila females: behavior of nonexchange chromosomes and the effects of
Xklpl, a chromosomal Xenopus kinesin-like protein essential for spindle or-
Zhang, D. H., and R. B., Nicklas. 1995. The impact of chromosomes and cen-
trosomes on spindle assembly as observed in living cells. J. Cell Biol. 129:
1287–1300.