Maize meiotic spindles assemble around chromatin and do not require paired chromosomes

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SUMMARY

To understand how the meiotic spindle is formed and maintained in higher plants, we studied the organization of microtubule arrays in wild-type maize meiocytes and three maize meiotic mutants, *desynaptic1* (*dsy1*), *desynaptic2* (*dsy2*), and *absence of first division* (*afd*). All three meiotic mutations have abnormal chromosome pairing and produce univalents by diakinesis. Using these three mutants, we investigated how the absence of paired homologous chromosomes affects the assembly and maintenance of the meiotic spindle. Before nuclear envelope breakdown, in wild-type meiocytes, there were no bipolar microtubule arrays. Instead, these structures formed after nuclear envelope breakdown and were associated with the chromosomes. The presence of univalent chromosomes in *dsy1*, *dsy2*, and *afd* meiocytes and of unpaired sister chromatids in the *afd* meiocytes did not affect the formation of bipolar spindles. However, alignment of chromosomes on the metaphase plate and subsequent anaphase chromosome segregation were perturbed. We propose a model for spindle formation in maize meiocytes in which microtubules initially appear around the chromosomes during prometaphase and then the microtubules self-organize. However, this process does not require paired kinetochores to establish spindle bipolarity.

Key words: Maize, Meiocyte, Spindle, Desynaptic mutation

INTRODUCTION

The assembly of the spindle is a dynamic process. However, despite years of study, the basic principles that govern this process are unknown (Hyams, 1996; Vernos and Karsenti, 1995), and there is still debate over the exact mechanisms of spindle formation. There are two models that describe spindle assembly, the 'search and capture' model and the 'self-assembly' model. In the 'search and capture' model, centrosomes nucleate and organize the spindle microtubules (Kirschner and Mitchison, 1986). The minus ends of the microtubules are at the centrosomes, and the microtubules are captured and stabilized when their plus ends contact kinetochores, which are specialized protein complexes that assemble onto centromeric DNA (Kirschner and Mitchison, 1986). In this model, bipolarity and the orientation of the spindle microtubules are generated by the newly separated centrosomes even before nuclear envelope breakdown. This model is based on observations of somatic and early embryonic cells of animals (Vernos and Karsenti, 1995). Although somatic higher plant cells lack conspicious centrosomes, bipolar spindle arrays are formed before nuclear envelope breakdown, suggesting that in broad detail somatic plant cells follow a similar strategy of spindle assembly (Baskin and Cande, 1990; Palevitz, 1993).

During meiosis in some animal species, spindles form using a different pathway which relies on 'self-assembly' of the spindle. According to this model, after nuclear envelope breakdown, microtubules grow from multiple sites around condensed chromatin, and then the microtubules self-organize into a spindle in the absence of centrosomes or discrete microtubule-organizing centers (Steffen et al., 1986; Albertson and Thomson, 1993; Heald et al., 1996; Theurkauf and Hawley, 1992; Vernos and Karsenti, 1995). During female meiosis in *Drosophila* (Theurkauf and Hawley, 1992) and *Xenopus* (Vernos and Karsenti, 1995), meiotic spindle assembly seems to involve randomly oriented growth of microtubules around chromatin, followed by the self-organization of the microtubules into bipolar arrays. In these cells, which appear to lack discrete centrosomes, centrosomal material is recruited to the minus ends of the microtubules after the bipolar arrays are formed. In support of this model, Heald et al. (1996) have demonstrated that bipolar spindles assemble around DNA-coated beads incubated in *Xenopus* egg extracts. Because the spindles assembled around the beads in the absence of centrosomes and kinetochores, it was concluded that establishment of bipolarity is an intrinsic property of the newly forming microtubule arrays associated with the chromatin.

We were interested in how the meiotic spindle is formed and maintained in higher plants that lack distinct centrosome structures (Smirnova and Bajer, 1992). In particular, we wanted to determine whether paired homologous chromosomes are essential for the assembly and maintenance of the bipolar spindle during meiosis. Maize is an excellent organism for studying

meiotic spindle formation because there are mutants available that are defective in the commitment to meiosis, synapsis, and spindle formation (Golubovskaya et al., 1992; Golubovskaya, 1989; Neuffer et al., 1997; Staiger and Cande, 1990, 1991).

We chose to compare spindle formation in wild-type meiocytes and three meiotic mutants, *desynaptic1* (*dsy1*), *desynaptic2* (*dsy2*), and *absence of first division* (*afd*). All three are monogenic recessive meiotic mutations that have abnormal chromosome pairing, presumably due to defects in the synaptonemal complex (SC) rather than in kinetochore function (Golubovskaya et al., 1992; Golubovskaya, 1989; Golubovskaya and Mashnenkov, 1975, 1976). The results of ultrastructural analyses of *dsy1* and *dsy2* are similar (Golubovskaya, 1989). Pairing and synapsis are compromised; consequently, the bivalents never form or fall apart prematurely, producing univalents (Golubovskaya and Mashnenkov, 1976). By diakinesis in *dsy1* mutant plants, over 90% of the meiocytes contain 16 to 20 univalents per cell. The univalents undergo nondisjunction at meiosis I, and the sister chromatids separate at anaphase II. In *afd* homozygous plants, typical stages of prophase I, such as leptotene, zygotene, pachytene, and diplotene, are absent, and the reductional division of meiosis I is replaced by an equational division (Golubovskaya et al., 1992; Golubovskaya, 1989; Golubovskaya and Mashnenkov, 1975). Short SC fragments form early in prophase I in the *afd* mutant plants, but they soon disappear, producing 20 univalents. Then at metaphase I, the univalents align at the metaphase plate. At anaphase I, the 20 sister chromatids separate as a result of a premature division of the centromeres, and the chromatids move to opposite poles. At the end of the first meiotic division, each daughter cell contains 20 chromosomes. At anaphase and telophase of the second meiotic division, chromatids move randomly to the two poles, generating almost 100% abnormal tetrads.

We used wild-type meiocytes and *dsy1*, *dsy2*, and *afd* mutant plants to study how the pairing of homologous chromosomes affects the assembly and maintenance of the meiotic spindle. The two desynaptic mutants allow us to analyze the effects of univalents on meiosis I spindle formation, and *afd* allows us to analyze the effects of single sister chromatids on meiosis II spindle formation. We have developed an indirect immunofluorescence procedure utilizing confocal laser scanning microscopy to visualize microtubule arrays in maize meiocytes, while maintaining the three-dimensional structure of the cells. We found that maize meiocytes appear to follow a 'self-assembly' model for spindle assembly, in which microtubules initially appear around the chromosomes during prometaphase, followed by self-organization of the microtubules into a bipolar spindle. Although univalents behave abnormally during chromosome congression and anaphase and produce spindle defects, they do not block spindle formation, demonstrating that bivalent chromosome organization is not required for normal spindle assembly.

MATERIALS AND METHODS

Plant material

dsy1 and *dsy2* in an A344 inbred background and *afd* in a W23 background (Golubovskaya and Urbach, 1981) were obtained from Hank Bass (Florida State University) and were grown in a greenhouse at the University of California, Berkeley. After approximately 7 to 10 weeks, pre-emergent tassels were collected and scored for the mutant phenotype. *dsy1*, *dsy2*, and *afd* are monogenic recessive mutations.

Indirect immunofluorescence

The tassel of each plant was collected and wrapped in damp paper towels until dissection. Meiocytes were always used the day the tassels were harvested. The meiocytes were staged by staining the chromatin with 0.1 µg/ml of 4′,6-diamidino-2-phenylindole (DAPI, Sigma Chemical Co.) and visualizing the meiocytes using epifluorescence microscopy. Anthers (30 to 40) of the appropriate stages were placed into 2 ml of fixative solution: 8% (v/v) paraformaldehyde (PFA, Electron Microscopy Sciences) and PHEMS buffer (60 mM Pipes, 25 mM Hepes, 10 mM EGTA, 2 mM MgCl$_2$, and 0.32 M sorbitol, pH 6.8) in a small Petri dish and shaken for 2 hours at medium speed. Then the anthers were rinsed with PHEMS buffer. Using a micro-blade (Moria, micro-blade 15°, Fine Science Tools, Inc.) and forceps, the end of each anther was cut off, and the contents of the anthers were extruded from the anthers into PHEMS buffer. Aliquots (10 µl) of cell suspension were placed into 1.5 ml microcentrifuge tubes, and 10 µl of molten 3% agarose (ultra-low gelling agarose, SeaPrep 15/45, FMC Corporation, Rockland, Maine) in PHEMS buffer were added to each tube. The tubes were cooled to slightly below 15°C, allowing the agarose to solidify. The agarose block in each tube was incubated overnight at room temperature with 100 µl of 1.5% β-glucuronidase (Sigma Chemical Co., G-0751) in PHEMS buffer for 10 minutes to partially digest the cell walls. The agarose blocks were rinsed with 100 µl phosphate buffered saline (PBS; 0.14 M NaCl, 2.7 mM KCl, 10 mM Na$_2$HPO$_4$, 1.8 mM KH$_2$PO$_4$, pH 7.2). The agarose block in each tube was then incubated with 50 µl of PBS-diluted (1:1,000) mouse monoclonal antibody against sea urchin alpha-tubulin overnight at room temperature, rinsed with PBS, and then incubated overnight at room temperature with 30 µl of PBS-diluted (1:30) FITC-conjugated goat-anti-mouse antibody (Sigma Chemical Co., F-0257). After rinsing with PBS, the blocks were treated with 50 µl of 0.1 µg/ml propidium iodide for approximately 30 minutes to stain the chromosomes. The agarose blocks were rinsed with PBS then placed on glass slides. For each slide, a layer of tape was placed on each side of the slide in order to raise the coverslip. Approximately 100 µl of 100 mg/ml 1,4-diazobicyclo (2,2,2) octane (DABCO) were placed on each block (100 mg of DABCO were combined with 0.1 ml of PBS and 0.9 ml of glycerin). The slides were heated until the agarose blocks just melted completely. A coverslip was placed onto each slide, and the coverslips were sealed to the slides with fingernail polish.

Microscopy

A Zeiss Axiophot was used for epifluorescence microscopy. Laser scanning confocal microscopy was performed using a Leica TCS 4D Inverted Confocal Laser Scanning Microscope (Leica, Inc., Heidelberg, Germany), an LSM 410 Inverted Confocal Laser Scanning Microscope (Carl Zeiss, Inc., Thornwood, NY), and a Molecular Dynamics Confocal Laser Scanning Microscope (Model #1000, Molecular Dynamics, Inc., Sunnyvale, CA). Confocal images were generated and analyzed using a computer graphics workstation (Silicon Graphics, Mountain View, CA) and ImageSpace software (Molecular Dynamics, Inc.). The following Nikon objectives were used: ×40, 0.55 NA Plan; ×40, 0.95 NA PlanApo; and ×60 oil, 1.40 NA PlanApo. We also used a ×100 oil, 1.40-0.70 NA PlanApo Leica objective; a ×20, 0.50 NA Plan-Neofluar Zeiss objective; and a ×63 oil, 1.4 NA Plan-Apochromat Zeiss objective.

RESULTS

Spindle assembly in wild-type maize meiocytes

To monitor spindle assembly in normal meiocytes while

preserving the overall three-dimensional structure of the cytoskeleton, we embedded the meiocytes in agarose and processed them for indirect immunofluorescence using a monoclonal antibody that recognizes maize tubulin. With this procedure, controls lacking primary antibody showed no microtubule staining, and background staining with the secondary antibody was very low (Fig. 1A). For the stages described in the following paragraphs, 40-80 cells were examined per stage and the morphology of the spindles and microtubule arrays shown in the figures are representative of the populations of cells at that stage in development. Because of the difficulty in obtaining good material, fewer mutant meiocytes cells were examined. However, for critical stages such as prometaphase and anaphase 20-30 cells were analyzed.

During diakinesis, before nuclear envelope breakdown, wild-type inbreds W23 and A344 meiocytes contained networks of cytoplasmic microtubules, and there were no noticeable bipolar microtubule arrays (Fig. 1B,C,D). One difference between the two inbreds was that, in A344 meiocytes at diakinesis, approximately 90% of the A344 cells showed a bright perinuclear staining (Fig. 1B). In contrast, only about 5% to 10% of the W23 diakinesis cells had a similar microtubule distribution pattern. This perinuclear staining may be caused by an increased number of microtubules nucleated on the nuclear envelope (Staiger and Cande, 1990). During prophase I, microtubules in intercellular connections run between the meiocytes in each anther locule (Fig. 1D). To confirm our visual observations about microtubule organization at diakinesis, we quantified the distribution of fluorescence around the nuclei in 5 meiocytes. For each optical section taken through meiocytes at diakinesis and for look-through projections composed of the entire stack of optical sections taken through the nuclei of the meiocytes, the area around each nucleus was divided into twelve regions, and the mean pixel intensity of each region was determined. The mean pixel intensity reflects the number of microtubules within the region. We found that there were no peaks in mean pixel intensity value around the nucleus, consistent with our observations that bipolar microtubule arrays were absent in the cytoplasm before nuclear envelope breakdown.

At the end of diakinesis, the nuclear envelope breaks down, and the cells enter prometaphase. During prometaphase, the spindle forms and the chromosomes move to the metaphase I plate. Microtubule arrays first accumulated in the vicinity of the chromosomes just prior to chromosome congression. We did not observe any obvious localized microtubule organizing centers during prometaphase. The microtubules appeared to emanate from multiple sites in the cells and to be associated with the chromatin (Fig. 1E,F). Microtubules even appeared to emanate directly from the chromosome surface (Fig. 1F); however, large bundles of microtubules associated with the chromatin were not observed. Thus it is not possible to tell whether these microtubule-chromatin interactions are directly with the chromatin or are mediated by a kinetochore.

During metaphase I, meiocyte spindles extended from cell margin to cell margin. Some spindles had broad poles (Fig. 1G,H,I), and others had more focused poles (Fig. 1J). In some cells, the ends of the spindle flared out towards the cell membranes (Fig. 1H,I) or ran parallel to the cell cortex, with microtubules oppressed to the cell membrane (Fig. 1G). Within some spindles, microtubule bundles appeared to converge (Fig. 1I), perhaps due to lateral interactions between spindle microtubules.

During anaphase I, the spindle extended across the cell from cell margin to cell margin (Fig. 1K). Interzonal microtubules are a prominent component of the anaphase spindle. The ends of the spindle often appeared to run parallel to the cell cortex, sometimes curving dramatically along the cell membrane (Fig. 1L). Strands of microtubules often came off the sides of the spindle and extended into the cytoplasm at this stage (Fig. 1L). Finally, as the cells entered telophase, phragmoplasts formed in the spindle midzone.

desynaptic1 and *desynaptic2* maize meiocytes

The microtubule arrays in *dsy1* and *dsy2* mutant meiocytes were comparable to those seen in wild-type maize meiocytes at diakinesis (Fig. 2A). As in wild-type maize meiocytes, there were no noticeable bipolar microtubule arrays before nuclear envelope breakdown (Fig. 2A). During prometaphase I in *dsy1* and *dsy2* meiocytes, microtubules emanated from several sites in the cells and were associated with the chromosomes, and there were no obvious, localized microtubule organizing centers (Fig. 2B). As with wild-type maize meiocytes, microtubules appear to emanate directly from the chromosome surface during prometaphase (Fig. 2B).

Although *dsy1* and *dsy2* meiocytes contained univalents, they still formed bipolar spindles that were similar to the spindles seen at comparable stages in the wild-type meiocytes. At metaphase I, these spindles extended from cell margin to cell margin as in the wild-type meiocytes (Fig. 2C,D). Some chromosomes were aligned at the metaphase plate during metaphase I; more aligned chromosomes were seen in *dsy2* than in *dsy1* cells. However, many chromosomes were scattered throughout the spindle (Fig. 2C). We observed a variety of spindle abnormalities at metaphase I. Sometimes tufts of microtubules emanated away from improperly aligned chromosomes into the cytoplasm (Fig. 2C, arrow). Chromosomes were occasionally found outside of the main spindle with microtubules extending from one pole to the chromosomes and microtubules extending away from the chromosomes in the opposite direction (Fig. 2D). There were no obvious microtubule organizing centers associated with these distal microtubules.

During anaphase I in *dsy1* and *dsy2* meiocytes, the chromosomes were scattered throughout the spindle and did not segregate properly; however, the spindles still looked similar to spindles in wild-type meiocytes (Fig. 2E,F,G). At later stages, many of the *dsy1* mutant meiocytes showed partial phragmoplast formation, and there were scattered chromosomes and micronuclei (Fig. 2H). Some of the *dsy1* mutant meiocytes were capable of progressing through meiosis II. These cells also contained apparently normal spindles; however, the chromosomes did not segregate properly (Fig. 2I).

In summary, the *dsy1* and *dsy2* mutant meiocytes were capable of forming spindles, and some of the chromosomes were able to align at the metaphase I plate. However, chromosomes did not segregate properly during anaphase I. This shows that paired chromosomes are required for the proper alignment of chromosomes at the metaphase plate and for proper chromosome segregation, but they are not required for spindle formation.

Fig. 1. Single optical sections from confocal laser scanning light microscopy of wild-type maize meiocytes. The chromosomes, stained with propidium iodide, are shown in red, and the microtubules, stained with a monoclonal antibody against tubulin, are shown in green or yellow, except for A. Bars: 10 μm (F, 20 μm). (A) A single optical section of W23 meiocyte at metaphase I incubated with secondary antibody but no primary antibody. The chromosomes (white) but no microtubules are visible. Also, background staining with the secondary antibody was very low. (B) A344 meiocyte at diakinesis. The meiocyte contains a network of cytoplasmic microtubules and no noticeable bipolar microtubule arrays. The perinuclear microtubule staining was brighter than microtubule staining in the rest of the cell. (C) W23 meiocyte at diakinesis containing a network of microtubules. Before nuclear envelope breakdown, there are no noticeable bipolar microtubule arrays. (D) W23 meiocytes at diakinesis. Microtubules can be seen running between the meiocytes (arrows). (E) W23 meiocyte at early prometaphase. There

are no bipolar microtubule arrays showing focused poles or obvious microtubule organizing centers. The microtubules appear to emanate from multiple sites around the chromosomes. (F) W23 early prometaphase meiocytes. There are no bipolar microtubule arrays showing focused poles or obvious microtubule organizing centers. Microtubules appear to emanate from multiple sites around the chromosomes and to come directly from chromosomes (arrow). Inset shows a close-up of a chromosome. (G) W23 meiocyte at metaphase I. The spindle has broad poles and extends from cell margin to cell margin. The ends of the spindle run parallel to the cell cortex, with microtubules running along the plasma membrane (arrow). (H) W23 meiocyte at metaphase I. The poles of the spindle appear to flare out at the cortex and interact with the cell membrane (arrow). (I) Close-up of the left pole and metaphase plate of the meiocyte in H, showing converging microtubules (arrows). (J) W23 meiocyte at metaphase I. The spindle has more focused poles than at prometaphase. (K) Meiocyte at anaphase I. The spindle extends from cell margin to cell margin. (L) Meiocyte at telophase I. The phragmoplast is beginning to form. The ends of the spindle run parallel to the cell cortex, curving dramatically along the cell membrane. Strands of microtubules come off the sides of the spindle.
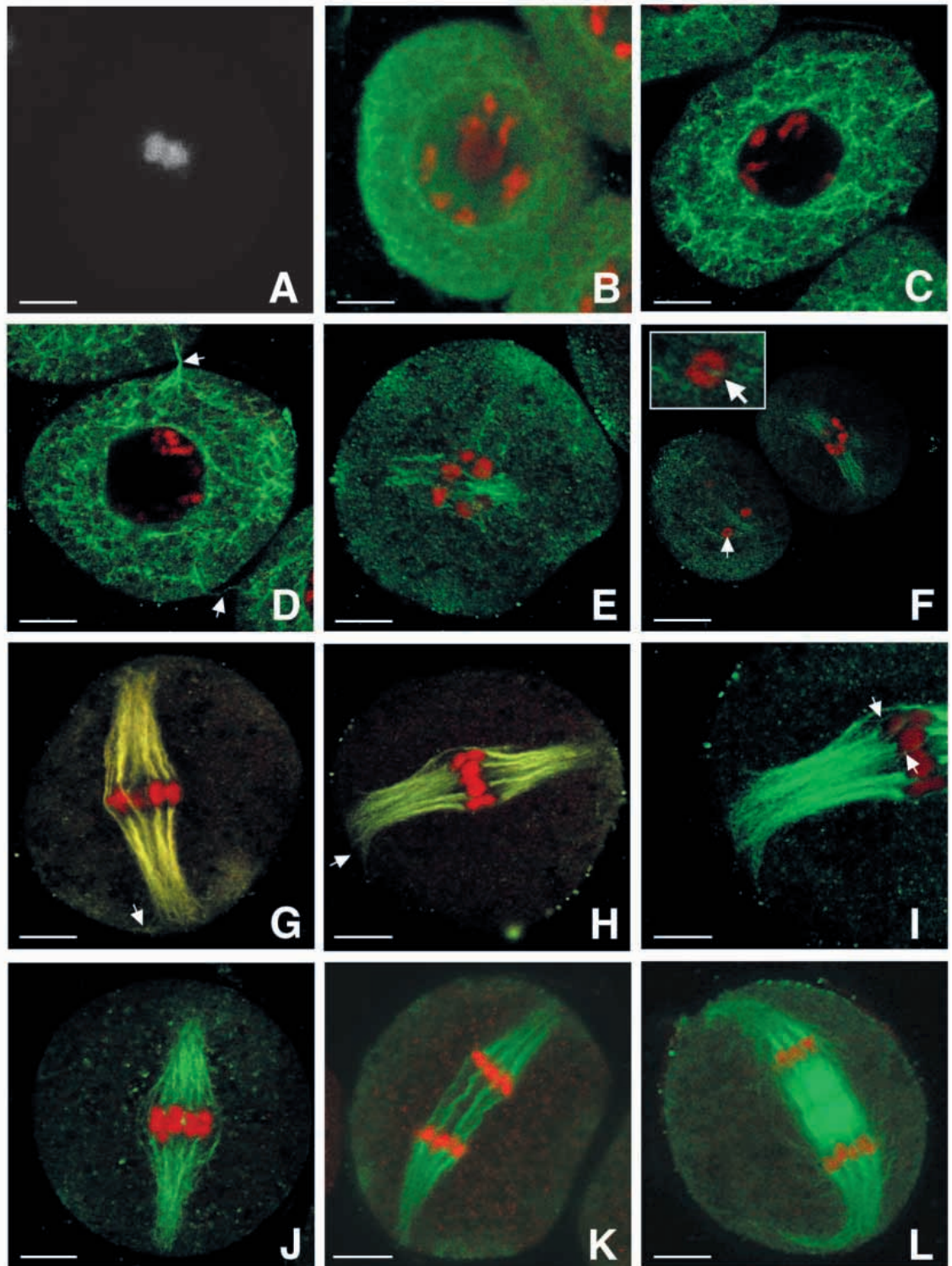
Fig. 2. Single optical sections of *dsy1* and *dsy2* meiocytes. The chromosomes, stained with propidium iodide, are shown in red, and the microtubules, stained with a monoclonal antibody against tubulin, are shown in green or yellow. Bars, 10 μm. (A) *dsy1* meiocytes at diakinesis. Univalents and the nucleolus are apparent inside the nucleus. The microtubule array in this cell is comparable to that in a wild-type maize meiocyte at diakinesis. There were no noticeable bipolar microtubule arrays before nuclear envelope breakdown. (B) *dsy1* meiocyte at prometaphase. Microtubules emanate from several sites around the chromosomes, and there are no localized microtubule organizing centers. Arrow shows microtubules coming from a chromosome (see inset). (C) *dsy1* meiocyte at metaphase I or early anaphase I. The spindle is similar to wild-type meiocyte spindles. Some of the chromosomes are aligned at the metaphase plate. However, there are also many chromosomes that are scattered along the spindle. Arrow shows a tuft

of microtubules coming from misaligned chromosomes. (D) *dsy2* meiocyte at metaphase I. The cell contains an apparently normal spindle, and most of the chromosomes are aligned at the metaphase plate. One chromosome lies outside of the spindle. Microtubules can be seen extending from one pole to the chromosome and extending away from the chromosome into the cytoplasm (arrow). (E) *dsy1* meiocytes at anaphase I. The chromosomes are not segregating properly and are scattered along the spindle. (F) *dsy2* meiocyte at early anaphase I. Although the spindle appears normal, chromosomes are scattered on the spindle and are not segregating properly. (G) *dsy1* meiocyte at late anaphase I or early telophase I. The spindle is similar to wild-type meiocyte spindles (compare with Fig. 1L). (H) *dsy1* meiocyte at telophase I. There is partial phragmoplast formation. Some of the chromosomes are decondensed. There are scattered chromosomes and micronuclei. (I) *dsy1* meiocyte at anaphase II. These cells contained apparently normal spindles. However, the chromosomes did not segregate properly and lie scattered along the spindles.
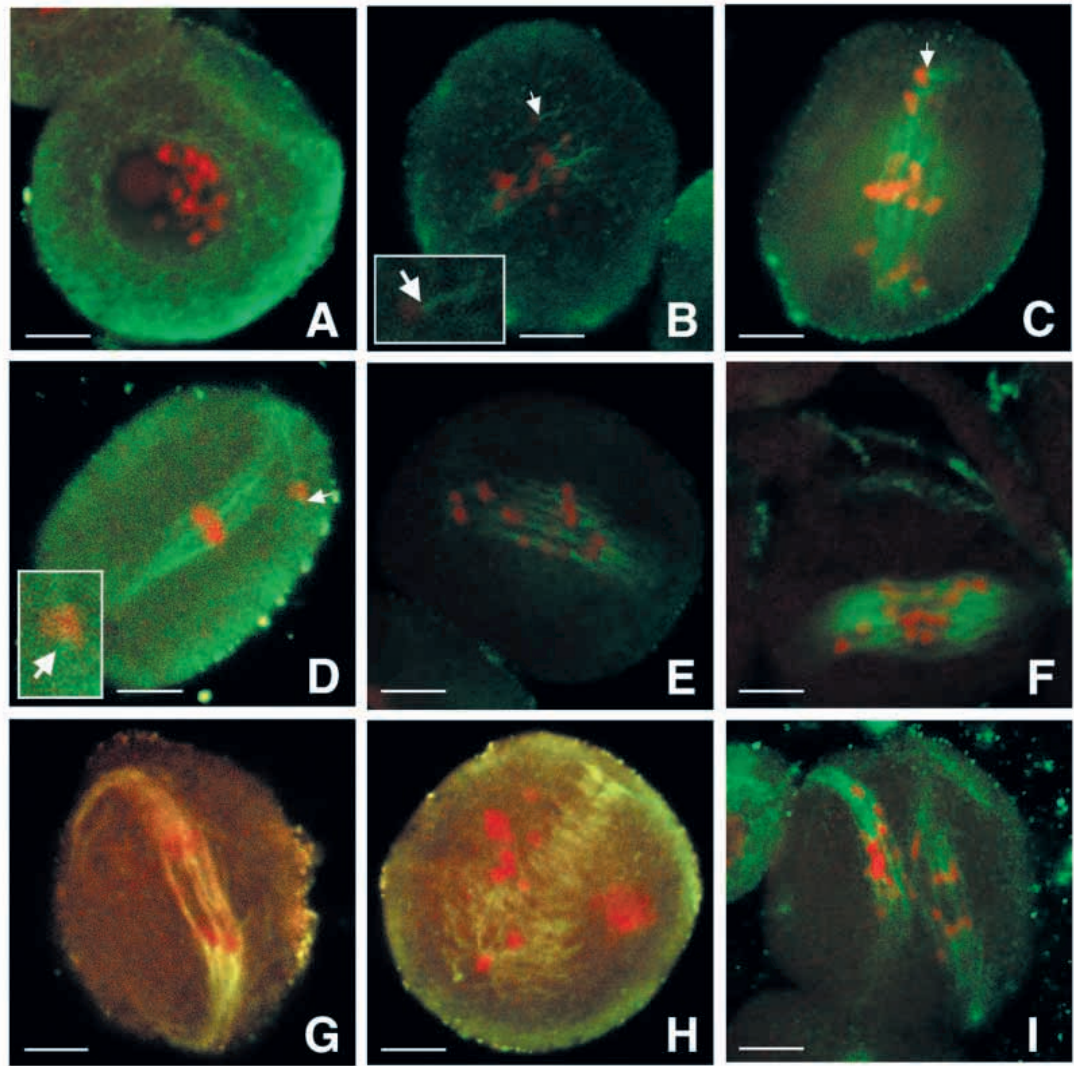
Absence of first division

At diakinesis, *afd* meiocytes have 20 univalents and sometimes more than one nucleolus (Fig. 3A) per cell. Normally, when ribosomal RNA synthesis restarts in the telophase preceding meiosis, small nucleoli reappear at the dispersed nucleolar organizer regions. These small nucleoli quickly grow and fuse to form the single large nucleolus that is also seen in many interphase cells (Anastassova-Kristeva, 1977). In *afd* meiocytes, this step is abnormal. However, the cytoplasmic microtubule arrays in *afd* meiocytes were similar to those seen in wild-type maize meiocytes at diakinesis (Fig. 3A). Furthermore, as with wild-type, *dsy1*, and *dsy2* meiocytes, during prometaphase, microtubules were associated with the

chromosomes, and there were no obvious localized microtubule organizing centers (Fig. 3B).

At metaphase I, *afd* had normal spindles, and all 20 univalents were aligned at the metaphase plate (Fig. 3C). During anaphase I, the chromosomes did not segregate properly; instead, they scattered along the spindle, although the spindle still appeared normal (Fig. 3D). *afd* mutant meiocytes at telophase I were similar to wild-type meiocytes at telophase I. However, chromosomes were sometimes caught inside and stretched across the phragmoplast (Fig. 3E).

After the first meiotic division, each daughter cell contained individual sister chromatids. At metaphase II, apparently normal bipolar spindles could form around the individual
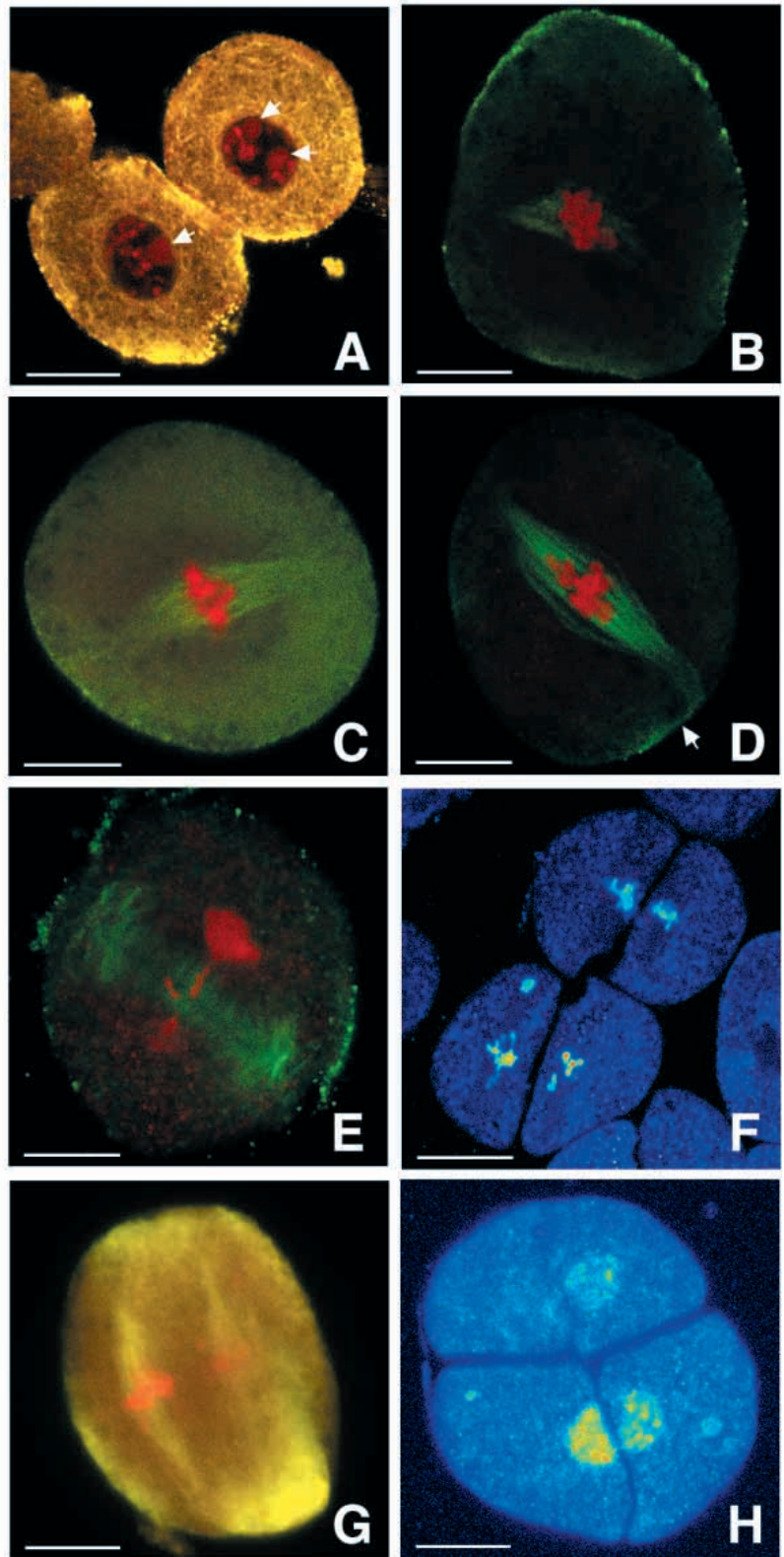
Fig. 3. Single optical sections of *afd* meiocytes. The chromosomes, stained with propidium iodide, are shown in red, and the microtubules, stained with a monoclonal antibody against tubulin, are shown in green or yellow, except for F and H. Bars, 15 µm (A and F, 20 µm). (A) Meiocytes at diakinesis. These cells contain networks of microtubules, and there are no obvious bipolar microtubule arrays before nuclear envelope breakdown. The lower cell has one nucleolus, and the upper cell has two (see arrows). (B) Meiocyte at prometaphase I. The microtubules are associated with the chromosomes, and there are no obvious localized microtubule organizing centers. (C) Meiocyte at metaphase I. The spindle appears normal, and the chromosomes are aligned at the metaphase plate. (D) Meiocyte at anaphase I. The spindle appears normal. As with the wild-type meiocytes, the ends of the spindle run parallel to the cell cortex, with microtubules along the cell membrane (arrow). However, the chromosomes are not segregating properly. (E) Meiocyte at telophase I. Some chromosomes are caught within and stretched across the phragmoplast. (F) Meiocytes at metaphase II and early anaphase II. The propidium iodide staining of chromosomes is shown in pseudocolor. In the upper dyad, the chromosomes are aligned at the metaphase plate. In the lower dyad, most of the chromosomes are aligned at the metaphase plate. These chromosomes are probably individual sister chromatids at this stage, but they are still capable of aligning at the metaphase plates. (G) Meiocyte at metaphase II (a look-through projection of 32 optical sections taken through the cells). There are a few scattered chromosomes. However, many of the chromosomes are aligned at the metaphase plates, and the spindles look normal. (H) Meiocyte at telophase II. The propidium iodide staining is shown in pseudocolor. These cells contain micronuclei and scattered chromosomes. The upper left cell looks similar to the upper right cell; however, the chromosomes in the upper left cell are out of the plane of focus in this optical section.

unpaired sister chromatids, and many of the unpaired sister chromatids were capable of aligning in the spindle midzone (Fig. 3F,G). The final tetrads that are produced are abnormal because during meiosis II, the single chromatids move randomly to the two poles (Fig. 3H).

In summary, it is not necessary to have paired sister chromatids for proper spindle formation and maintenance of normal spindle bipolarity at metaphase II. The behavior of chromosomes at metaphase II demonstrated the importance of paired sister chromatids for metaphase plate formation.

Problems in chromosome segregation at anaphase I were unexpected since the univalent centromere behavior at prometaphase I allowed for normal chromosome alignment on the metaphase plate.

DISCUSSION

Spindles assemble around chromosomes but do not require bivalents

Our observations of spindle formation in wild-type and desynaptic meiocytes are consistent with 'self-assembly' models for spindle assembly, in which microtubules initially grow from multiple sites around condensed chromatin and then, with the aid of motors, organize into bipolar arrays (Hyman and Karsenti, 1996). At diakinesis, there were no obvious bipolar microtubule arrays. During prometaphase, the meiocytes did not have obvious localized microtubule organizing centers. Instead, microtubules emanated from several sites around the chromosomes or directly from the chromosomes. A similar process took place in mutant meiocytes containing univalents, demonstrating that the self-assembly properties of the microtubule arrays do not require the bilateral symmetry of the paired chromosomes. This mechanism of spindle assembly is unlike that found in somatic plant cells. Despite the absence of a conspicious centrosome, in somatic plant cells, a dense accumulation of microtubules, called the prophase spindle, appears around the nucleus early in prophase before nuclear envelope breakdown (Baskin and Cande, 1990; Smirnova and Bajer, 1992; Palevitz, 1993). This multipolar prophase spindle is later transformed into a bipolar spindle around the time of nuclear envelope breakdown.

Based on our observations, we suggest a model for spindle formation in maize meiocytes based on the spindle assembly mechanisms seen in *Xenopus* and *Drosophila* oocytes (McKim and Hawley, 1995; Hyman and Karsenti, 1996). We propose that spindle formation is initiated by chromatin after nuclear envelope breakdown (Fig. 4). A bipolar spindle is formed by lateral interactions between microtubules and interactions between microtubules and chromosomes, which help to pull the microtubules together and align them into anti-parallel arrays. The kinetochores are not directly involved in setting up the bipolar microtubule arrays; however, after spindle formation, kinetochores capture microtubules to establish the functional chromosome attachment necessary for chromosome movement at anaphase. A dynamic process of spindle pole formation occurs throughout prometaphase and metaphase in maize meiocytes, leading to the elongated spindles seen at anaphase I. The spindles are short and the poles are broad and disorganized at early prometaphase, but by anaphase spindles extend all the way across the meiocytes, and the poles are tightly focused. At metaphase I and anaphase I, the ends of the spindles often appeared to run parallel to the cell surface, with microtubules oppressed against the cell membrane. These changes in spindle morphology could be due to the interaction of motor proteins between parallel microtubule arrays and/or the plasma membrane.

This model is supported by our observations of meiosis in cultured maize meiocytes (A. Chan and W. Z. Cande, unpublished). During prometaphase, there were no obvious
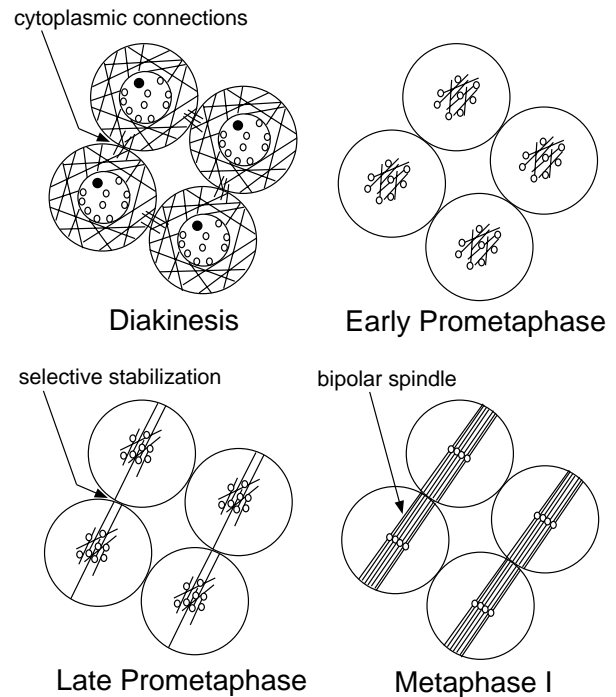
Model for Spindle Assembly in Maize Meiocytes

Fig. 4. A model for spindle formation during meiosis I in maize. A column of 4 maize meiocytes is depicted inside the anther. At diakinesis, the meiocytes are connected to each other by intercellular connections, and microtubules run through the connections. The microtubules are organized as a cytoplasmic network. After nuclear envelope breakdown at early prometaphase, the preexisting microtubules and newly forming microtubules interact with chromatin and are stablized. With the involvement of motors, such as kinesin-related proteins and dynein, during late prometaphase, the microtubule arrays are organized into antiparallel assemblies and pole material is recruited to the ends of the microtubules. Interactions of microtubules with local regions in the cortex, perhaps established by signals and proteins transported through the intracellular connections, help to establish the spindle orientation through selective stablization of microtubule ends. By metaphase I the spindle extends the width of the cell, and the spindle poles interact with the plasma membrane, becoming more focused over time.

bipolar spindles, as monitored using polarized light optics. However, the regions around the chromosomes were slightly birefringent, consistent with an association of microtubules with the chromosomes at this time. As metaphase progressed, spindle birefringence increased, and the spindles grew longer until the spindles extended the width of the cell.

Chromosomes could initiate spindle formation by several possible mechanisms. One possibility is that the chromosomes capture the plus ends of preexisting microtubules in the cytoplasm of the meiocytes, thus stabilizing the microtubules, preventing them from depolymerizing, and allowing microtubule elongation to occur (Kirschner and Mitchison, 1986; Theurkauf and Hawley, 1992). Alternatively, chromosomes may nucleate spindle microtubules (Smirnova and Bajer, 1994). Finally, chromosomes could change the local environment of the cytoplasm to favor microtubule nucleation and stabilization (Zhang and Nicklas, 1995; Heald et al., 1996;

Hyman and Karsenti, 1996). For example, in grasshopper spermatocytes, when a chromosome is placed near one pole of a newly formed spindle, the microtubule density in the half-spindle increases four times relative to the other half-spindle (Zhang and Nicklas, 1995). Displaced chromosomes in the cytoplasm of *Drosophila* spermatocytes triggered the formation of miniature spindles at sites where spindles normally do not form, and these chromosomes appeared to divide normally on the mini-spindles (Church et al., 1986).

Although reviews of meiotic chromosome organization and the cytology of desynaptic mutations stress that univalents perturb spindle structure (Dawe, 1998; Koduru and Rao, 1981), we found that desynapsis had little effect on spindle formation. The bilateral kinetochore symmetry of bivalents and paired sister chromatids is not required for the establishment of a bipolar spindle at either metaphase I or II. The *dsy1* and *dsy2* mutant meiocytes were capable of forming normal-looking spindles, and many of the univalent chromosomes were aligned in the spindle midzone at metaphase I. These spindles did not fragment or become multipolar. In *afd*, all of the univalents were aligned on the metaphase plate during metaphase I, and apparently normal spindles could form around individual, unpaired sister chromatids at metaphase II. Although we saw tufts of microtubules extending into the cytoplasm from a few misaligned chromosomes at the end of metaphase I in *dsy1* and *dsy2* meiocytes (Fig. 2C), during prometaphase there were no obvious bundles of microtubules associated with the kinetochores in either mutant or normal meiocytes. These data imply that the kinetochores are not used to organize the spindle microtubules into a bipolar array during spindle formation.

The presence of intercellular connections between meiocytes could provide an explanation for why all the meiocytes in the locule of an anther progress through meiotic prophase I synchronously (Heslop-Harrison, 1966) and why all the spindles have a similiar orientation parallel to the long axis of the anther at metaphase I (Staiger and Cande, 1990). Proteins transported from cell to cell by the microtubules in the intercellular connections could play a role in spindle formation by triggering a stabilization of the bipolar microtubule arrays in a preferred orientation. For example, asymmetrically distributed cortical-associated MAPs could promote more stable microtubules in a favored cortical domain in the meiocyte.

Chromosome behavior in desynaptic meiocytes

In most models describing the mechanism of chromosome congression at prometaphase, two functional sister kinetochores and their associated kinetochore microtubules are required for the alignment of chromosomes on the metaphase plate (McIntosh and Hering, 1991; Mitchison, 1989; Rieder and Salmon, 1994; Salmon, 1989). A balance of forces, whether generated by motors associated with kinetochore microtubules, by spindle poles (polar ejection forces), or some combination of the two classes of forces is responsible for the establishment of the metaphase plate. In neither living cells (A. Chan and W. Z. Cande, unpublished) nor in fixed cells did we ever see normal metaphase plate formation in *dsy1* or *dsy2*. However, to our surprise, we found that many univalents in *dsy1* and *dsy2* spindles at metaphase I and unpaired sister chromatids in *afd* spindles at metaphase II were aligned in the spindle midzone. These results demonstrate that chromosome

alignment at the spindle midzone does not necessarily require bilaterally symmetrical kinetochores.

How do univalent chromosomes become aligned in the spindle midzone? Golubovskaya (1989) suggested that *afd* kinetochores at metaphase I have matured so that both structurally and functionally they are like the kinetochores associated with metaphase II chromosomes. It is possible a similar process could have occured with some of the aligned univalents in *dsy1* and *dsy2* meiocytes. Alternatively, kinetochores on some univalents and unpaired sister chromatids in the mutant meiocytes may have retained elements of functional bilateral symmetry that contribute to chromosome congression. At metaphase I, the kinetochores on the univalent chromosomes may be oriented perpendicular to the spindle axis so that bundles of kinetochore microtubules attached to the same kinetochore can point to opposite poles. It is also possible that the kinetochores have become fragmented or curled so that they can attach to microtubules from both poles, leading to alignment of the chromosomes on the metaphase plate. When laser microsurgery is used to cut between the two sister kinetochores on a PtK_1 prometaphase chromosome undergoing mitosis, two chromosome fragments are produced, each containing one kinetochore (Khodjakov et al., 1997). The single kinetochore on each chromosome fragment can become highly stretched or fragmented, and it can attach to microtubules from both poles, leading to alignment at the metaphase plate (Khodjakov et al., 1997). Similar kinetochore behavior could be responsible for chromosome aligment displayed by some of the univalents in these mutant meiocytes. Finally, nonkinetochore-associated motors could be involved; a prime candidate would be plus-end directed chromokinesins on the chromosome arms (McKim and Hawley, 1995; Theurkauf and Hawley, 1992; Vernos and Karsenti, 1995).

In addition to obvious chromosome alignment problems at metaphase, chromosomes did not segregate properly during anaphase I and II. Instead, they became scattered on the spindles. Golubovskaya et al. (1992) described a similiar behavior at anaphase I in plants homozygous for *dsy1-9101*, an allele of *dsy1*. The chromosome scattering seen at anaphase may be related to misalignment of the chromosomes during metaphase. Nonaligned metaphase chromosomes have to move farther on the spindle and may not reach the poles at the same time as properly aligned chromosomes, or, because of their position in the spindle, kinetochores on misaligned chromosomes may not be able to maintain their proper attachment to the spindle microtubules during anaphase.

The situation is more complex in *afd* meiocytes at anaphase I. Although the univalent chromosomes in these meiocytes are properly aligned at the metaphase I plate, they become scattered on the spindles during anaphase I. We see chromosomes at telophase I stretched across the phragmoplasts, suggesting that sister chromatids did not separate properly (Fig. 3E). This may be due to a failure to release sister chromatid cohesion at the metaphase to anaphase transition Sisters may separate late or not at all, leading to a failure or a delay of chromosome movement off the metaphase plate. That is, the centromeres on the two sisters may not split at the metaphase to anaphase transition as would be predicted if anaphase I in *afd* meiocytes was replaced by anaphase II centromere behavior. Alternatively, the *afd* chromosomes may

move at different rates during anaphase I because their kinetochores are defective.

In conclusion, the chromatin based pathway of spindle formation in maize meiocytes is similar to that observed during meiosis in *Xenopus* and *Drosophila* oocytes. Although chromosome alignment and subsequent separation at anaphase is perturbed in desynaptic mutants, spindle formation is unaffected by the absence of paired kinetochores. This suggests that kinetochores do not play a major role in spindle formation in maize meiocytes.

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