Functional Redundancy in the Maize Meiotic Kinetochore

Hong-Guo Yu* and R. Kelly Dawe*‡

*Department of Botany and ‡Department of Genetics, University of Georgia, Athens, Georgia 30602

Abstract. Kinetochores can be thought of as having three major functions in chromosome segregation: (a) moving plateward at prometaphase; (b) participating in spindle checkpoint control; and (c) moving poleward at anaphase. Normally, kinetochores cooperate with opposed sister kinetochores (mitosis, meiosis II) or paired homologous kinetochores (meiosis I) to carry out these functions. Here we exploit three- and four-dimensional light microscopy and the maize meiotic mutant *absence of first division 1* (*afd1*) to investigate the properties of single kinetochores. As an outcome of premature sister kinetochore separation in *afd1* meiocytes, all of the chromosomes at meiosis II carry single kinetochores. Approximately 60% of the single kinetochore chromosomes align at the spindle equator during prometaphase/metaphase II, whereas acentric fragments, also generated by *afd1*, fail to align at the equator. Immunocytochemistry suggests that the plateward movement occurs in part because the single kinetochores separate into half kinetochore units. Single kinetochores stain positive for spindle checkpoint proteins during prometaphase, but lose their staining as tension is applied to the half kinetochores. At anaphase, ~6% of the kinetochores develop stable interactions with microtubules (kinetochore fibers) from both spindle poles. Our data indicate that maize meiotic kinetochores are plastic, redundant structures that can carry out each of their major functions in duplicate.

Key words: kinetochore • checkpoint • meiosis • misdivision • *afd1*

Introduction

The congression of chromosomes to the metaphase plate and subsequent poleward movement at anaphase are complex processes that occur with remarkable accuracy during cell division. An important organelle in chromosome movement is the kinetochore, a protein complex that associates with centromeric DNA (for reviews see Rieder and Salmon, 1998; Maney et al., 1999). Through the interaction with spindle microtubules, kinetochore proteins have direct roles in propelling chromosomes toward the equatorial plane at prometaphase (e.g., Schaar et al., 1997; Wood et al., 1997), and subsequently away to opposite spindle poles at anaphase (Nicklas, 1989). In addition, a handful of kinetochore proteins participate in the spindle checkpoint pathway, which ensures that chromosomes align correctly at the metaphase plate before anaphase begins (for reviews see Rudner and Murray, 1996; Skibbens and Hieter, 1998; Amon, 1999). Even a single unaligned chromosome can activate the spindle checkpoint and prohibit anaphase onset (Li and Nicklas, 1995; Rieder et al., 1995). It has been proposed that tension registered at the kinetochore is either directly or indirectly involved in the spindle checkpoint, at least in meiosis (Li and Nicklas, 1995, 1997; Nicklas, 1997; Yu et al., 1999).

Normally, chromosomes possess either two sister kinetochores (mitosis/meiosis II) or the paired kinetochores from homologous chromosomes (meiosis I). A widely held view is that kinetochore pairs are required to ensure that sister/homologous chromosomes segregate to opposite poles. The natural polarity of opposed kinetochores matches the bipolarity of the spindle, allowing the chromosomes to adopt a stable position at the spindle midzone (Rieder and Salmon, 1994, 1998; Nicklas, 1997). However, the importance of paired kinetochores in chromosome congression was questioned by Khodjakov et al. (1997), who used laser ablation to experimentally remove a kinetochore from each of 50 mammalian mitotic chromosomes. The remaining single kinetochores were sufficient to generate the congression of 38% of the chromosomes analyzed. Electron microscopy of three cells revealed that the single kinetochores were distorted and attached to microtubules from both poles. These, and similar data involving detached kinetochore fragments (Zinkowski et al., 1991; Christy et al., 1995; Wise and Brinkley, 1997), suggest that mitotic mammalian kinetochores are composed of subunits that can interact with microtubules independently (Khodjakov et al., 1997). In contrast, single kinetochore chromosomes failed to align at the spindle equator

Address correspondence to R. Kelly Dawe, Department of Botany, Miller Plant Sciences Bldg., University of Georgia, Athens, GA 30602. Tel.: 706-542-1658. Fax: 706-542-1805. E-mail: kelly@dogwood.botany.uga.edu

when the same technique was applied to African blood lily (*Haemanthus*) endosperm cells (Khodjakov et al., 1996), suggesting that single kinetochores and/or their interactions with the spindle differ among species or cell types.

Here, we extend the analysis of single kinetochores to maize meiotic cells. For a source of material, we exploit the phenotype of the maize meiotic mutant *absence of first division 1* (*afd1*)[1] (Golubovskaya and Mashnenkov, 1975), which, as a result of premature sister kinetochore separation at meiosis I, produces cells at meiosis II that contain a complete set of single kinetochore chromosomes. By analyzing these single kinetochore chromosomes in detail, we demonstrate that they can align with ~60% accuracy at metaphase II by interacting with kinetochore fibers from opposite spindle poles. During alignment, the single kinetochores appear to divide into halves that are capable of functioning independently. The connections established by half kinetochores are stable enough to dissociate/dephosphorylate two well-studied spindle checkpoint proteins. Finally, in anaphase, considerable poleward force was generated by the half kinetochores, stretching and nearly separating the kinetochores into two parts.

Materials and Methods

Plant Materials

The original stocks carrying the recessive *afd1* mutation were provided by Inna Golubovskaya (N.I. Vavilov Institute of Plant Industry Research, St. Petersburg, Russia). This strain was crossed once to the inbred line KYS, and a single resulting *Afd1*/*afd1* plant was self-crossed to generate all of the material used here. Homozygous *afd1/afd1* plants were identified cytologically in microsporocytes.

Immunolocalization

Meiocytes (Yu et al., 1999) or anthers (Yu et al., 1997) from both wild-type and mutant plants were fixed and processed as described previously (Yu et al., 1999). For the analysis of mitosis in *afd1* plants, the tips of prop roots were excised, fixed, and sectioned on a cryostat (Yu et al., 1999). The maize centromere protein (CENPC) antibodies, maize MAD2 antibodies, 3F3/2 mAb (a gift from Gary Gorbsky, University of Virginia, Charlottesville; Gorbsky and Ricketts, 1993), and mAb against α-tubulin (a gift from David Asai, Purdue University, West Lafayette, IN; Asai et al., 1982) were used as described previously (Yu et al., 1999). The CENPC and MAD2 antibodies were detected by rhodamine-conjugated goat anti–rabbit secondary antibodies, and the 3F3/2 and α-tubulin mAbs were detected by FITC-conjugated goat anti–mouse secondary antibodies (secondary antibodies were purchased from Jackson ImmunoResearch Laboratories). In double labeling studies, primary antibodies were incubated simultaneously. Chromosomal DNA was stained with diamino phenylindole (DAPI) at 0.1 μg/ml.

In Situ Hybridization

For in situ hybridization, a maize centromeric satellite tandem repeat called CentC (Ananiev et al., 1998) was PCR amplified from genomic DNA derived from the inbred line W23 (primers were 5′-GATTGGGCATGTTCGTTGTG and 5′-CACTACTTTAGGTCCAAAAC). Two clones of the ~155-bp PCR product were sequenced to verify their identity as CentC. Gel-purified PCR products were labeled with fluorescently tagged dUTP and used as probes for in situ hybridization as described previously (Yu et al., 1997), except that the denaturing temperature was reduced to 90°C. In experiments where CENPC and CentC were both labeled, immunolocalization of CENPC was performed first, followed by in situ hybridization.

[1]*Abbreviations used in this paper:* 3D, three-dimensional; *afd1*, *absence of first division 1*; CENPC, centromere protein C; CentC, maize centromeric tandem repeat.

Microscopy and Data Analysis

Except where specifically noted in the text, all data were collected using a DeltaVision SA3.1 three-dimensional (3D) light microscope workstation as described previously (Yu et al., 1997). The data were processed by constrained iterative deconvolution. For the analysis of meiosis in living cells, meiocytes were cultured in a synthetic culture medium supplemented with the vital DNA stain Syto12 (Yu et al., 1997). Cells regularly survive in this medium for >6 h. Time lapse 3D (4D) data were collected at intervals from 1 to 30 min depending on the experiment.

To estimate the frequency of single kinetochore chromosome alignment in *afd1* cells, we first determined that a rectangle with a width of 2 μm encompassed all the kinetochores in four wild-type metaphase II cells. Based on this estimate, a rectangle with a width of 2 μm was applied to the equator of the metaphase II spindles in six *afd1* cells (see Fig. 5). The placement of the rectangle in *afd1* cells was necessarily subjective, but in each case it was positioned roughly at the equator of the spindle and at right angles to the spindle axis. If a kinetochore was located within the rectangle, it was counted as aligned at the metaphase plate.

To evaluate the effect of tension on the dephosphorylation of the 3F3/2 antigen at the kinetochore, 3F3/2 staining was first normalized for kinetochore size by dividing it by the intensity of CENPC staining. This was done for all the kinetochores in two *afd1* prometaphase II cells that did not overlap with another kinetochore or with the background 3F3/2 staining. A square composed of 10 × 10 pixels (pixel size, 0.1103 μm) was used to cover the kinetochore. The gray level intensity of the CENPC and 3F3/2 staining within the square was obtained from three contiguous sections (section thickness, 0.25 μm), averaged, and subtracted from the background intensity. Kinetochore edges were identified as the position half way from the tip to the base of a one-dimensional plot profile drawn over the kinetochore. The longest axis of the kinetochore was used as the length, except when it was spherical, and the diameter was used. To analyze the relationship between staining intensity and kinetochore length, we tested linear, log linear, and power models using maximum coeefficient of determination (R^2) as our optimality criterion (using SAS statistical analysis software at the University of Georgia Research Computing Resource Facility).

Results

Sister Kinetochores Separate Prematurely during Meiosis I in afd1 Meiocytes

We have recently identified and characterized a maize homologue of CENPC, a constitutive kinetochore protein (Dawe et al., 1999). Anti-CENPC antibodies effectively label each of the 20 chromosomes of a diploid maize cell at all stages of the cell cycle. As a first step in our study, we used affinity-purified anti-CENPC antibodies to confirm the phenotypic description of *afd1* given by Golubovskaya and colleagues (Golubovskaya and Mashnenkov, 1975; Golubovskaya et al., 1992). Fig. 1 illustrates a comparison of kinetochores from wild-type (left) and sibling *afd1* (right) plants at various stages of meiosis I. A complete description of kinetochore morphology in wild-type cells can also be found in our previous report (Dawe et al., 1999). The earliest detectable prophase stage in *afd1* plants is a diplotene-like stage, which in wild-type cells is typified by partially condensed and desynapsed chromosomes. All four (homologous and sister) kinetochores are usually associated at this stage in wild-type cells (Fig. 1 A, and data not shown), such that only 10 CENPC-positive spots are usually observed. Consistent with the assertion that minimal chromosome pairing occurs in *afd1* meiocytes (Golubovskaya and Mashnenkov, 1975; Golubovskaya, 1989), 20 CENPC-positive spots were generally observed at the diplotene-like stage of mutant cells (Fig. 1 B, and data not shown).

After diplotene and prometaphase I, the sister kinetochores in wild-type cells stay conjoined but separate slightly, revealing a doublet structure (Dawe et al., 1999).

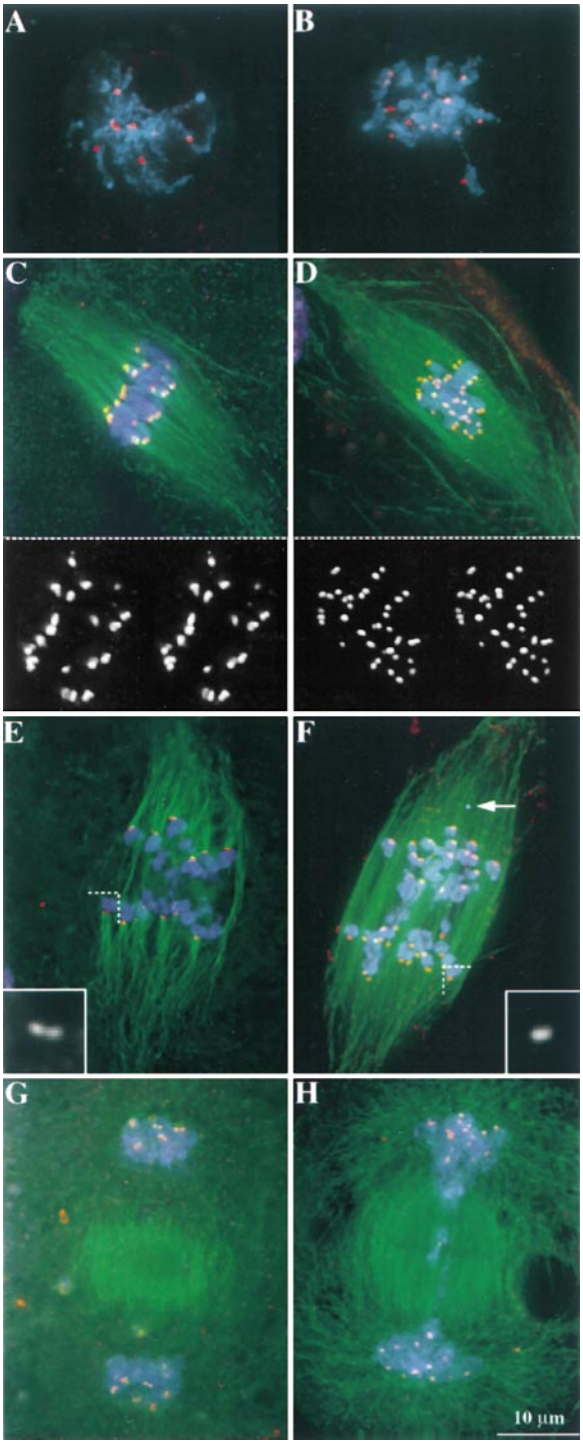

Figure 1. Sister kinetochore separation at meiosis I in the *afd1* mutant. All images are partial projections from 3D data sets. CENPC staining is shown in red, microtubules in green, and chromosomes in blue. Images on the left are from wild-type (*Afd1/afd1* or *Afd1/Afd1*) plants; images on the right are from mutant (*afd1/afd1*) plants. A and B, Diplotene. The earliest meiotic stage observed in *afd1* meiocytes is a diplotene-like stage. C and D, Metaphase I. The kinetochores often appear double in wild-type cells, whereas the kinetochores in *afd1* cells appear single. The bottom section of each frame is a stereo pair representing all of the kinetochores in these cells. E and F, Anaphase I. Sister kinetochores are conjoined in wild-type cells (inset in E, 3× magnification relative to scale bar), whereas they have disjoined and are single in *afd1* cells (inset in F). The arrow in F indicates an acentric chromosome fragment. G and H, Telophase I.

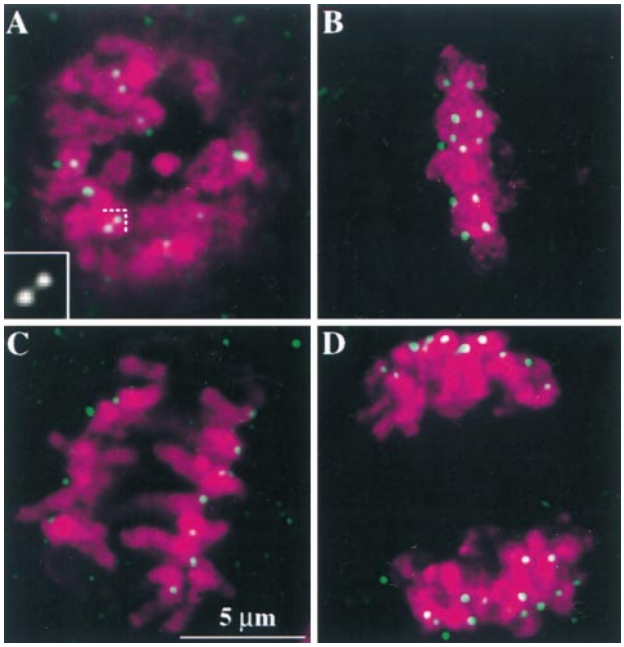

Figure 2. Mitotic chromosome segregation in *afd1* root tip cells. All images are partial projections from 3D data sets. Chromosomes are shown in magenta and CENPC staining in green. A, Prophase. Sister kinetochores can be distinguished from each other at this stage (inset, 2× magnification relative to scale bar). B, Metaphase. Sister kinetochores align at the metaphase plate. C, Anaphase. D, Telophase.

Approximately ten doublet kinetochores are the norm for each half spindle at metaphase I (Fig. 1 C), anaphase I (Fig. 1 E), and telophase I (Fig. 1 G). However, in *afd1* cells, ~20 single kinetochores were observed in each half spindle at all 3 stages (Fig. 1, D, F, and H; only the stereo pair in Fig. 1 D shows all the kinetochores). These data confirm the conclusion, made by Golubovskaya and Mashnenkov (1975), that sister kinetochores separate prematurely in *afd1* plants to generate single kinetochore chromosomes before meiosis II. Our data also support the data of Chan and Cande (1998), who demonstrated that meiosis I spindle formation is essentially unaltered by the *afd1* mutation (Fig. 1). Finally, we observed a low frequency of small chromosome fragments in *afd1* plants that lacked visible CENPC staining (Fig. 1 F, discussed below).

To investigate the mitotic phenotype of the *afd1* mutation, we extended our studies to somatic cells from *afd1* plants. Data acquired from the cells in prop roots (aerial roots extending from the base of the stem) indicate that mitosis in mutant plants is essentially the same as was documented for normal maize mitosis (Yu et al., 1999). As shown in Fig. 2, the sister kinetochores can be distinguished from each other at the earliest stages of mitotic prophase (though they are often still connected; Fig. 2 A), and a complete separation of sister kinetochores occurs as early as prometaphase (not shown). Sister kinetochores then orient (Fig. 2 B) and segregate (Fig. 2 C) to opposite spindle poles. Acentric chromosome fragments were not observed in any of 16 anaphase/telophase cells from 2 mutant plants. Meiosis I and mitosis in the *afd1* mutant can be distinguished from each other by several criteria (compare Figs. 1 and 2). The distinct differences in the timing of kinetochore separation, chromosome condensation pat-

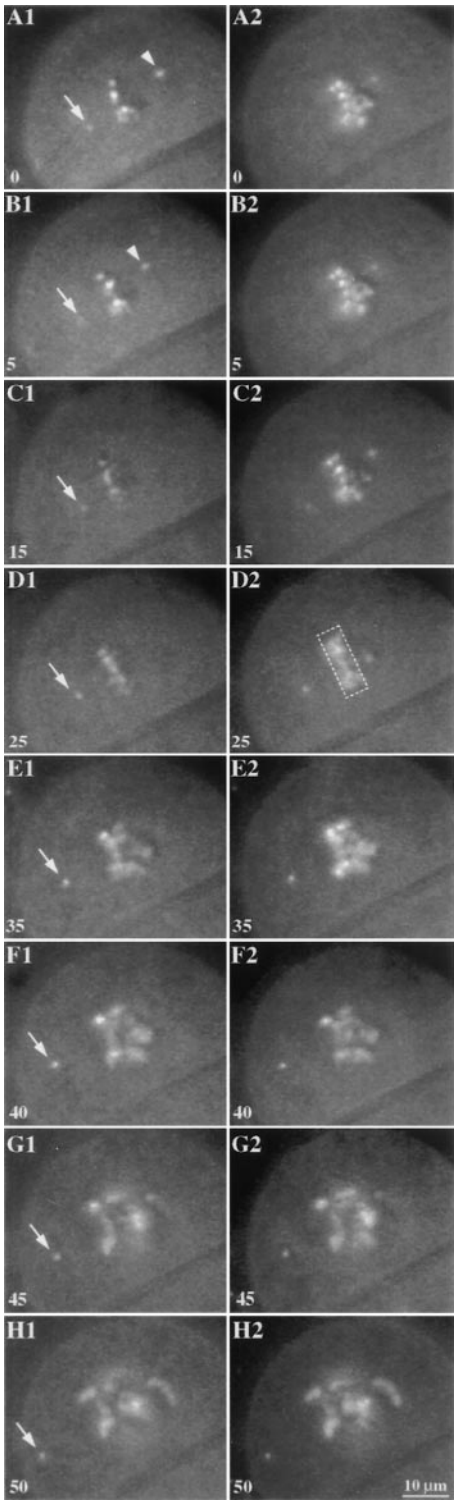

Figure 3. Meiosis II chromosome alignment and segregation in a living *afd1* cell. Images on the left show the same optical section taken at a series of time points. On the right are projections of four optical sections over a different region of the cell. Time in minutes is shown in the bottom left of each frame. Arrowheads in A1 and B1 show a single kinetochore chromosome moving toward the equatorial plate. Arrows in A1–H1 show a chromosome fragment moving away from the equator during metaphase and anaphase. The rectangle in D2 indicates the metaphase plate.

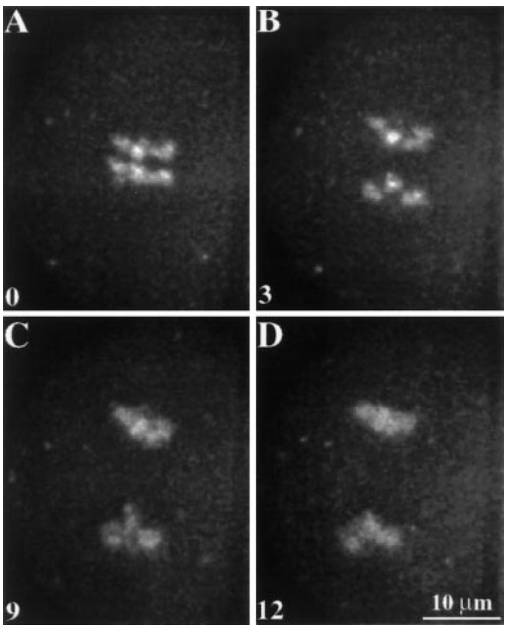

Figure 4. Anaphase II in a living wild-type cell. Shown here are partial projections from 3D data sets. Time in minutes is shown in the bottom left of each frame.

terns, and spindle morphology (mitotic spindles can be seen in Yu et al., 1999) suggest that the *afd1* mutation does not substitute meiosis I with a mitotic division.

4D Analysis of Meiosis II in the afd1 Mutant

To determine whether the single kinetochore chromosomes generated by the *afd1* mutation align at the metaphase II plate, we first employed 3D time lapse (4D) microscopy. As described previously (Yu et al., 1997), live meiocytes were extruded into a culture medium and stained with the vital DNA stain Syto12. A total of 32 cells from *afd1* plants was observed undergoing meiosis II. For 12 of the cells, data collection began before metaphase II and included all or part of prometaphase. As shown in Fig. 3, A–D, a clearly identifiable metaphase plate was formed. Once at the metaphase plate (Fig. 3 D), the single kinetochore chromosomes oscillated back and forth in a manner similar to wild-type cells (Fig. 3, A–D; Yu et al., 1997). The full prophase–metaphase II alignment process was observed in two cells, where prometaphase lasted ~1 h longer (a total of ~150 min) than expected for a wild-type meiocyte (~90 min; Yu et al., 1997).

In an additional 6 wild-type cells and 20 cells from sibling *afd1* plants, the earliest stages recorded were metaphase or early anaphase II. In each cell where the start of anaphase was documented, all of the chromosomes appeared to begin poleward movement together. The orderly chromosome segregation characteristic of a wild-type cell is shown in Fig. 4. In mutant cells, however, normal chromosome segregation was not observed. Instead, the chromosomes demonstrated erratic behavior typified by irregular rates of movement and frequent changes in direction. The rates of chromosome movement for individual chromosomes varied from <0.4 to 1.4 μm/min (compared with a consistent ~0.78 μm/min in wild-type plants,

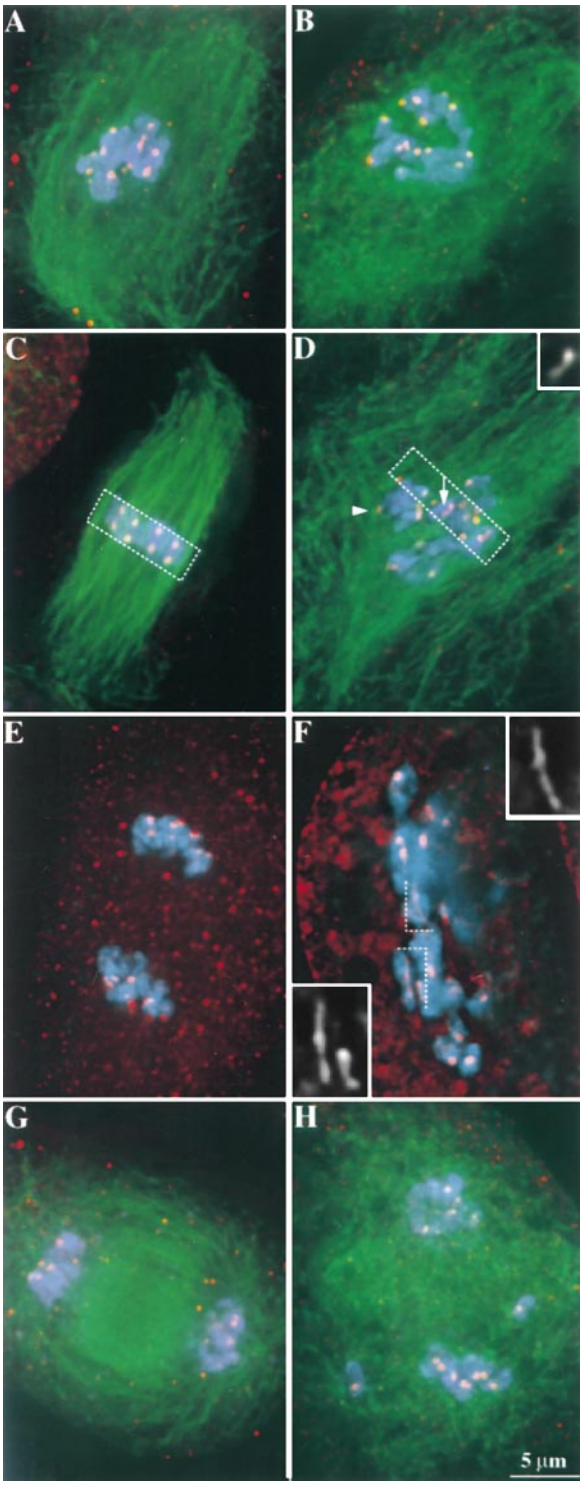

Figure 5. Kinetochore morphology in wild-type and *afd1* cells at meiosis II. CENPC staining is shown in red, microtubules in green, and chromosomes in blue. Images from wild-type plants are on the left; those from *afd1* plants are on the right. (A and B) Prometaphase II. The spindle is still amorphous at this stage. (C and D) Metaphase II. Kinetochore pairs align at the equator in wild-type cells. A majority of single kinetochores align at the equator in *afd1* cells to form a rough metaphase plate (indicated by a rectangle; see Materials and Methods for details). The arrow and inset (2× magnification relative to scale bar) in D indicates a bioriented single kinetochore that is stretched between the poles. Unaligned kinetochores appear spherical (arrowhead in D). (E and F) Anaphase II. Sister kinetochores segregate from each other in wild-type cells. Chromosome movement in *afd1* is erratic, with chromosomes located along the length of the spindle. Single kinetochores can be stretched up to five times their normal diameter (insets in F, 2× magnifications relative to scale bar). (G and H) Telophase II. Multiple nuclei are formed in *afd1* cells. There is no shift in spindle orientation as the cells proceed through the cell cycle.

Fig. 3; Yu et al., 1999). Anaphase II in mutant cells was typically three to four times longer than is characteristic for wild-type cells; the cell shown in Fig. 3 remained in anaphase for ~100 min before the distinct nuclei structures characteristic of telophase were observed (not shown, but see Fig. 5 H).

The chromosome fragments generated during meiosis I were observed in living *afd1* cells as small Syto12-stained structures. One such fragment was observed in the cell illustrated in Fig. 3 (arrows). The fragment moved slowly towards a spindle pole at 0.22 μm/min during prometaphase and remained suspended in the spindle throughout metaphase II. The same fragment moved rapidly poleward during mid-anaphase II at 0.71 μm/min (Fig. 3, E–H).

A Majority of Single Kinetochore Chromosomes, but Not Acentric Fragments, Aligns at the Spindle Midzone

By treating fixed cells with anti-CENPC antibodies, we were able to view the position and morphology of the single kinetochores during meiosis II. These data are shown in Fig. 5, with control meiocytes from wild-type plants (left) and meiocytes from sibling *afd1* plants (right). The spindle in *afd1* meiocytes was usually irregular in shape (Fig. 5 D), though a basic bipolar structure was always observed. As in living cells, a majority of the single kinetochores chromosomes appeared to align at the spindle equator in metaphase II (Fig. 5 D). We did not observe any examples of kinetochore-carrying chromosomes located at the spindle poles. Using the thickness of the metaphase II plate in wild-type cells as a standard (see Materials and Methods), we estimated from a sample of six *afd1* cells that 60 ± 16% of the single kinetochore chromosomes congressed to the spindle equator (Fig. 5 D; note rectangles in C and D). Those chromosomes that aligned at the plate frequently took on a stretched appearance (Fig. 5 D, arrow and inset), whereas those that failed to align at the plate usually appeared spherical (Fig. 5 D, arrowhead).

Among 72 prometaphase–metaphase II *afd1* cells that were analyzed in detail (from 6 plants), 32 possessed at least 1 acentric chromosome fragment, i.e., a small DAPI-stained body that lacked detectable CENPC staining. In contrast, a survey of 922 wild-type cells at the same stages revealed no visible fragments (these data were obtained by standard 2D microscopy from 3 wild-type siblings of mutant plants). The localization of acentric fragments in the spindle can be used to assess the direction of the forces prevailing on chromosome arms. Among the acentric fragments scored in *afd1* plants, 4 were located in the vicinity of the spindle midzone, whereas a majority of 28 (88%) were located in a polar region. Acentric fragments that

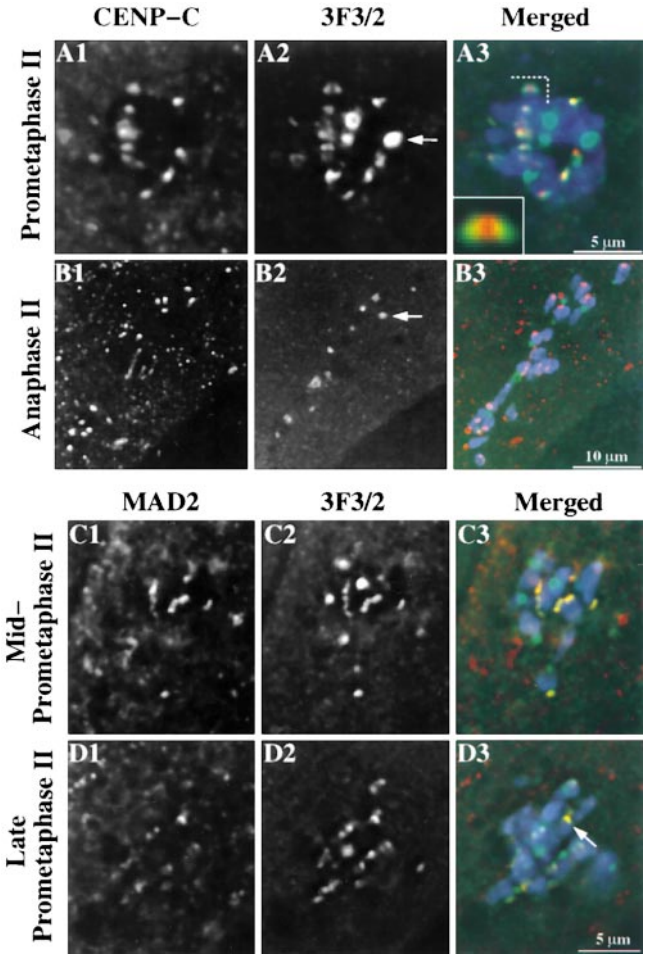

Figure 6. Spatial and temporal organization of the 3F3/2 antigen and MAD2 protein on single kinetochores in *afd1* cells. All images are partial projections from 3D data sets. 3F3/2 staining is shown in green, CENPC staining in red (A3 and B3), and chromosomes in blue. MAD2 staining (C3) is shown in red. (A1–A3) 3F3/2 staining at prometaphase II kinetochores. The 3F3/2 antibody also recognizes nonkinetochore sites (arrow). 3F3/2 staining is divided and localized to the ends of stretched single kinetochores (inset in A3, 4× magnification relative to scale bar). (B1–B3) 3F3/2 staining is not present at kinetochores during anaphase II, though background staining is apparent at nonkinetochore sites (arrow). (C1–C3) Colocalization of MAD2 and the 3F3/2 antigen on single kinetochores at mid-prometaphase II. 16 (of the 20) kinetochores were stained by MAD2 this cell. (D1–D3) Same as C1–C3, except at late prometaphase II. 12 kinetochores were stained by MAD2 in this cell. The arrow indicates a spherical kinetochore that is brightly stained with both antibodies; most of the other kinetochores are weakly stained.

were intermingled among the chromosomes at the midzone would have been difficult to detect, so it is possible that we have overestimated the proportion of fragments that migrated to a pole. At a minimum, however, the data show that while no kinetochore carrying chromosomes was found near the spindle poles, a substantial number of acentric fragments was. The simplest interpretation is that kinetochores, not motile forces associated with chromosome arms, are responsible for the alignment of single kinetochore chromosome at the spindle midzone.

Single Kinetochore Chromosome Alignment Occurs by Tension-sensitive Interactions with the Spindle

In prior studies, we described the localization of two spindle checkpoint proteins on maize mitotic and meiotic kinetochores (Yu et al., 1999). MAD2 is a widely conserved checkpoint protein that binds specifically to unaligned kinetochores (for review see Amon, 1999). The 3F3/2 antigen, also a presumed checkpoint protein, is a phosphoepitope that is sensitive to tension applied at the kinetochore (Nicklas et al., 1995; Nicklas, 1997). Antibodies to both proteins recognize an outer domain of the kinetochore in wild-type maize meiocytes (Yu et al., 1999). Similarly, single kinetochore chromosomes at prometaphase II stain brightly with both the 3F3/2 antibody and the MAD2 antibody. These data are shown in Fig. 6. The single kinetochores progressively lost 3F3/2 and MAD2 staining as the cells proceeded through prometaphase (Fig. 6, A, C, and D) and by anaphase II the staining was no longer detectable at kinetochores (Fig. 6 B). The 3F3/2 antibody also stains nonkinetochore sites (arrows in Fig. 6, A2 and B2; Yu et al., 1999), which can be distinguished from kinetochore staining by double labeling with either the CENPC or MAD2 antibodies.

We previously demonstrated in wild-type cells that MAD2 and 3F3/2 show nearly identical staining patterns both spatially and temporally (Yu et al., 1999). To test whether MAD2 and 3F3/2 are also colocalized on single kinetochores, we analyzed 20 double-labeled cells ranging from early to late prometaphase II. Each of the cells was fixed at a stage when the checkpoint proteins were detectable on some but not all of the kinetochores present (the average number of MAD2-stained kinetochores was 12.4). We found that 98.8% of the kinetochores that were MAD2-positive were also labeled with the 3F3/2 antibody (247/250 single kinetochores). These data lend strong support to the conclusion that 3F3/2 dephosphorylation and MAD2 dissociation are coincident (Yu et al., 1999), and indicate that 3F3/2 staining at kinetochores is a reliable marker for the presence of MAD2.

Double labeling for the 3F3/2 antigen and CENPC (Fig. 6 A) and for the 3F3/2 antigen and MAD2 (Fig. 6, C and D) indicated that the 3F3/2 antigen lies outside the CENPC domain but in the same domain as MAD2. Since nearly identical results were obtained in wild-type cells (Yu et al., 1999), our analysis suggests that the premature disjunction caused by *afd1* does not disrupt the basic composition and organization of the kinetochores. Interestingly, a bipolar staining pattern was clearly observed on several single kinetochores, with the 3F3/2 staining occupying opposite ends of stretched kinetochores (Fig. 6 A3). These data indicate that single kinetochores have the capacity to divide into half kinetochore units, with each end of the elongated kinetochore interacting independently with a pole.

The results of our previous work suggest that the loss of MAD2 and 3F3/2 staining during meiosis is correlated with the level of tension applied to the kinetochores (Yu et al., 1999). Because chromatin is elastic (Waters et al., 1996), we were able to use the distance between paired homologous

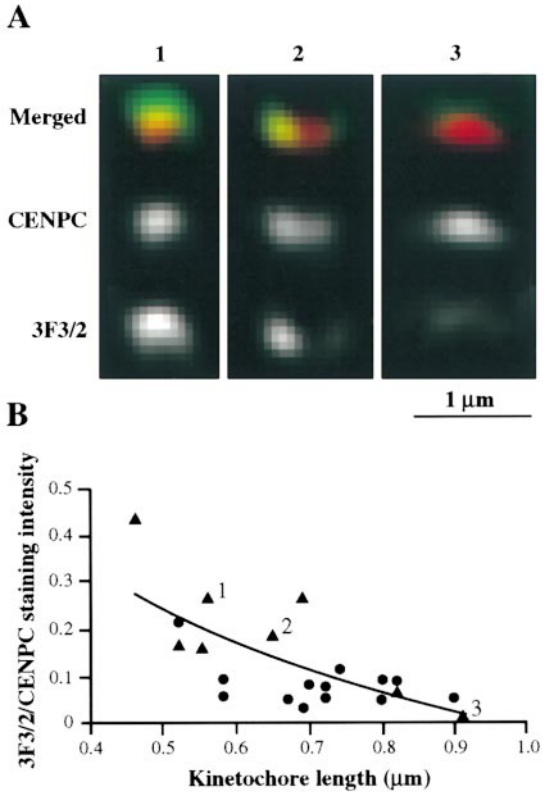

Figure 7. Normalized 3F3/2 staining intensity at single kinetochores is negatively correlated with kinetochore diameter/length. (A) Three single kinetochores from a prometaphase II cell. CENPC staining is shown in red and 3F3/2 staining in green. Images are from single optical sections. The staining intensity in each panel is unaltered from the original deconvolved image. (B) 3F3/2 staining intensity, normalized for kinetochore volume by dividing it by CENPC staining intensity, is plotted with respect to kinetochore diameter/length (see Materials and Methods for details). Data were obtained from two prometaphase II cells: (▲) kinetochores from one cell; (●) kinetochores from the second cell. Numbers next to triangles correspond to the kinetochores displayed in A. Log staining intensity is inversely proportional to log kinetochore length ($R^2 = 0.58$, $P < 0.01$).

or sister kinetochores to estimate the tension between the kinetochores and their associated kinetochore fibers (Yu et al., 1999). We used a similar assay on the single kinetochore chromosomes in *afd1* meiocytes. At mid-prometaphase II, the single kinetochores can be observed in a variety of states of alignment. Some show no evidence of a bipolar interaction with the spindle, others are stretched, indicating a bipolar interaction with the spindle, and others lie in between these two extremes. We chose two such mid-prometaphase II cells, and for each scorable kinetochore determined the 3F3/2 staining intensity (using CENPC staining to normalize for kinetochore volume) and the diameter/length of the single kinetochore. As shown in Fig. 7, there was a strong negative correlation between the two. These data indicate that the loss of 3F3/2 staining on stretched kinetochores is a result of tension applied to the single kinetochore, not an inherent limitation on the duration of the spindle checkpoint (discussed below).

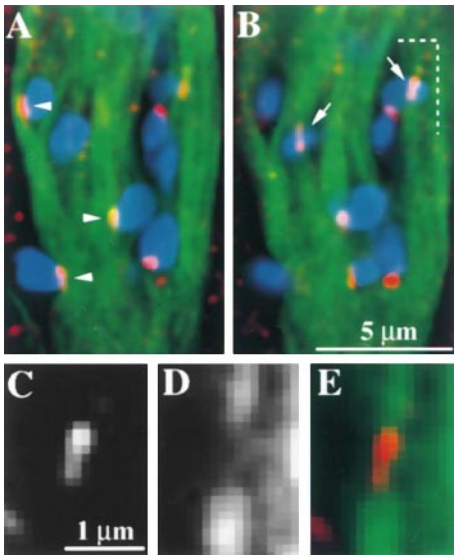

Figure 8. Interactions of single kinetochores with microtubules at anaphase II. CENPC staining is shown in red, microtubule staining in green, and chromosomes in blue. (A and B) Single optical sections taken 1 μm apart from each other. Arrowheads in A indicate kinetochores that appear to be interacting with microtubules in a tangential manner. The kinetochores indicated by arrows in B are interacting end-on with two different kinetochore fibers, one at each end of the stretched single kinetochores. (C, D, and E) 3× magnifications of the region indicated in B; C shows CENPC staining only, D microtubules only, and E the merged image.

Poleward Movement at Anaphase Causes Stretching of Single Kinetochores

Anaphase II in *afd1* meiocytes can be identified both by the absence of staining for checkpoint proteins (Fig. 6 B) and by the degree of kinetochore stretching that occurs during this stage. While kinetochore stretching during prometaphase and metaphase II was mild (Fig. 5 D and Fig. 6, A, C, and D), in anaphase II it was frequently extreme (Fig. 5 F and Fig. 6 B). An average of 24% of the single kinetochores at anaphase II were stretched into cylindrical shapes ($n = 347$ kinetochores in 18 cells), and in several cases the long axes exceeded 5 times the diameter of a normal anaphase II kinetochore (Fig. 5 F).

In wild-type cells, the kinetochores appear to interact with microtubules in a tangential way early in prometaphase and then display distinct end-on interactions with kinetochores in late prometaphase through anaphase (data not shown, and Fig. 1, C and E). To analyze the interaction of single kinetochores with microtubules, we analyzed a set of 153 chromosomes in 8 anaphase II cells. Of 36 stretched single kinetochores, 27 showed primarily tangential interactions with microtubules, while 9 others showed the distinct end-on interactions characteristic of wild-type cells. As shown in Fig. 8, B–E, when end-on interactions were apparent, there were two kinetochore fibers from opposite poles interacting with the ends of the stretched kinetochore. It is likely that the microtubules that appeared to interact tangentially with stretched kinetochores (Fig. 8 A)

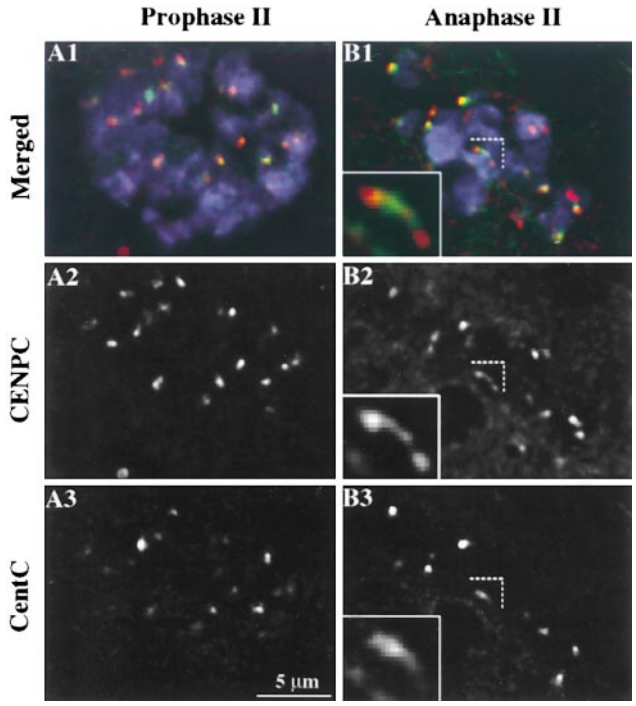

Figure 9. Separation of half kinetochore units at meiosis II in *afd1* cells. CENPC staining is shown in red, centromere (CentC) staining in green, and chromosomes in blue. Images are partial projections from 3D data sets. (A1–A3) Prophase II. Centromere staining overlaps with kinetochore staining. (B1–B3) Anaphase II. A stretched single kinetochore has nearly separated into two parts (insets, 3× magnification relative to scale bar).

also extended away from the kinetochores in both directions, but other possibilities cannot be excluded.

The observation that a single kinetochore can be stretched between two spindle poles suggests that the force may occasionally divide the kinetochore into two parts. That this is indeed the case was demonstrated by a combination of CENPC immunolocalization and in situ hybridization using an ~155-bp centromeric DNA repeat called CentC (Ananiev et al., 1998). At prophase II, CentC colocalized well with the CENPC-stained kinetochores (Fig. 9 A), although CentC staining varied considerably from chromosome to chromosome (as documented previously; Ananiev et al., 1998). However, on several anaphase chromosomes (eight chromosomes in five anaphase II cells), the kinetochores had clearly separated into two units that were joined by a thin thread of kinetochore material (Fig. 9 B). In each case, the two kinetochore units were attached by centromeric DNA, ruling out the possibility that these were rare examples of normal (i.e., two-chromatid) chromosomes that may have segregated correctly in meiosis I (in fact, we have no evidence that such chromosomes are ever present at meiosis II in the *afd1* mutant). We made an effort to quantify the frequency with which centromere/kinetochores were actually broken during anaphase II, i.e., misdivided (Darlington, 1937), by counting the number of CENPC spots in 10 telophase II cells from *afd1* plants. Misdivision would be expected to increase the number of kinetochores to a value significantly greater than the expected number of 20. The average kinetochore number in the 10 *afd1* cells was 19.78 ± 1.20, which was not significantly different from the kinetochore number in 8 wild-type telophase II cells (19.80 ± 0.84).

Discussion

Here we demonstrate that meiotic kinetochores, which move to one spindle pole with high fidelity under normal circumstances, will regularly interact with two poles if they are denied a sister kinetochore. The evidence suggests that single kinetochore alignment involves the same basic processes employed during normal chromosome alignment, and that in anaphase, a significant fraction of the single kinetochores divides into half kinetochore units that interact independently with the spindle. In discussing our results, we first evaluate the *afd1* phenotype in relation to other published data, and follow with our interpretations of how the single kinetochore chromosomes align, interact with spindle checkpoint proteins, and finally segregate in anaphase.

afd1 Causes a Defect in Sister Chromatid Cohesion at Meiosis I

Sister kinetochores are normally conjoined in meiosis I and then disjoin to move to opposite spindle poles in meiosis II. However, as described by Golubovskaya and colleagues, these events are significantly altered by the *afd1* mutation (Golubovskaya and Mashnenkov, 1975; Golubovskaya and Khristolyubova, 1985; Golubovskaya, 1989; Golubovskaya et al., 1992). Plants homozygous for *afd1* appear to skip the early prophase stages of meiosis I and then to prematurely segregate the sister kinetochores to opposite poles. The single kinetochore chromosomes released during meiosis I are then carried through to meiosis II, where they form a haphazard metaphase plate and then segregate randomly at anaphase II (Golubovskaya and Mashnenkov, 1975; Golubovskaya et al., 1992; Chan and Cande, 1998). Using antibodies to the recently identified maize CENPC protein (Dawe et al., 1999), we were able to confirm this phenotype and demonstrate that the equational segregation of sister kinetochores at meiosis I is close to 100% (Fig. 1).

To explain the *afd1* phenotype, Golubovskaya and colleagues proposed that the mutation causes a substitution of meiosis I with mitosis-like division, i.e., that meiosis I is absent in *afd1* plants (Golubovskaya and Mashnenkov, 1975; Golubovskaya and Khristolyubova, 1985; Golubovskaya, 1989). However, our immunocytochemical analysis suggests that the phenotype is more complex than this. Although some prophase I stages cannot be identified (roughly leptotene to pachytene), sister kinetochore separation in *afd1* meiocytes starts after nuclear envelope breakdown (Fig. 1, A and B) as is characteristic of normal meiosis I cells (Dawe et al., 1999). This contrasts with mitosis (Fig. 2 A) or normal meiosis II (Dawe et al., 1999), where kinetochore separation is readily apparent at prophase. Further, the chromatin condensation patterns and spindle morphology of the first division (Fig. 1, B and D) more closely resemble meiosis I in wild-type cells (Fig. 1, A and C) than mitosis (Fig. 2; Yu et al., 1999). We also show here that a low level of chromosome fragmentation occurs during meiosis I in *afd1* plants and that many of these fragments lack kinetochores (Fig. 1 H).

The available data suggest that *Afd1* encodes a cohesin such as budding yeast rec8p, a meiosis-specific rad21-like protein (Klein et al., 1999). Mutations in *REC8* cause premature separation of sister chromatids and inhibit reciprocal recombination. The chromosome fragmentation caused by *afd1* may be evidence of aborted recombination events, since meiotic recombination involves the formation of double-strand breaks (Roeder, 1997; Zickler and Kleckner, 1999). *REC8*/*RAD21*-like genes are widely conserved in eukaryotes (Watanabe and Nurse, 1999), and mutants of a meiosis-specific *RAD21*-like gene called *SYN1/DIF1* have recently been described in *Arabidopsis* (Bai et al., 1999; Bhatt et al., 1999). Consistent with a homology between the two genes, there are clear similarities between the *afd1* phenotype and the *Arabidopsis syn1/dif1* phenotype (Bai et al., 1999; Bhatt et al., 1999).

Recent data from both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* suggest that cohesin is not required to maintain the integrity of the mitotic kinetochore (Tomoyuki et al., 1999; Takahashi et al., 2000). These data are consistent with our own data, showing that at least three kinetochore proteins (CENPC, MAD2, and the 3F3/2 antigen) show the stage-specific (Fig. 6) and sub-kinetochore localization (Figs. 6, A3 and C3) typical of wild-type kinetochores (Yu et al., 1999). Although we cannot rule out the possibility that *afd1* has subtle effects on the morphology or makeup of the kinetochore, the available data suggest that the sister kinetochores are separated but otherwise undisturbed as they enter meiosis II.

Single Meiotic Kinetochores Regularly Align at the Metaphase Plate

Using both living and fixed specimens, we demonstrate that ~60% of single kinetochore chromosomes align at the metaphase II plate (Figs. 3 and 5), whereas ~88% of acentric fragments move in the opposite direction towards a pole (Fig. 3). These data support the observations of Khodjakov et al. (1997), who, with data obtained from a different class of cell division (mitosis) and a distantly related species (rat kangaroo), showed that single kinetochores are capable of autoaligning at metaphase. Our data are also in agreement with earlier data from the same group showing that acentric fragments in *Haemanthus* mitotic cells move poleward during chromosome alignment (Khodjakov et al., 1996).

There are also notable differences between our data and those published previously. Perhaps the most significant is the demonstration by Khodjakov et al. (1996) that *Haemanthus* single kinetochore chromosomes fail to align at the spindle midzone. An explanation for this may lie in the fact that meiotic kinetochores display morphological plasticity in the course of their normal function, first associating closely with a sister kinetochore in meiosis I and then subsequently changing their behavior to dissociate from the sister in meiosis II. The inherent capacity for remodeling may make the meiotic kinetochore especially susceptible to aberrant alignment during meiosis II. Another notable difference is that acentric chromosome fragments move plateward during prometaphase in mammalian mitotic cells (Rieder and Salmon, 1994), not poleward as in *Haemanthus* mitosis or maize meiosis. This difference is probably related to the function of centrosomes, which organize the spindle poles in animal mitotic cells, but which are absent in all higher plant cells and the meiotic cells of some animals (Smirnova and Bajer, 1992; Rieder et al., 1993). The active polymerization of microtubules outward from centrosomes is thought to be part of the force that drives chromosome fragments plateward (Rieder and Salmon, 1994). In cells that lack centrosomes, the dominant force affecting the movement of acentric fragments may be the poleward flux of tubulin monomers within the spindle (Sawin and Mitchison, 1994).

Single Kinetochores Interact Normally with Spindle Checkpoint Proteins and Demonstrate Anaphase Motility

In normal cells, sister/homologous kinetochores orient towards opposite spindle poles and generate tension with attached kinetochore fibers (Nicklas, 1997). The kinetochores move farther away from each other as increasing tension is applied during prometaphase, such that there is a rough correlation between tension and kinetochore–kinetochore distance (Waters et al., 1996, 1998). MAD2 and 3F3/2 staining decrease as kinetochore–kinetochore distance increases during maize meiosis, suggesting that the release/dephosphorylation of these proteins is tension sensitive (Yu et al., 1999). Here we demonstrate the same effect on single kinetochore chromosomes. Single kinetochores showed variable degrees of stretching during prometaphase (Fig. 7 A), and stretching was negatively correlated with 3F3/2 staining intensity (Fig. 7 B). The double staining required for these experiments revealed that many of the single kinetochores had divided into half kinetochore units. A distinct bipolar staining pattern was observed, where 3F3/2 staining was fully divided and localized at the poles of the elongated kinetochores (Fig. 6 A3).

It is known that tension at the kinetochore makes kinetochore fibers more stable and/or promotes microtubule bundling within the fiber (Ault and Nicklas, 1989; Nicklas and Ward, 1994). Therefore, it is likely that once bipolar connections are established by the single kinetochore, the resulting tension stabilizes the interaction and serves to further separate and define the half kinetochore units. For the numerous kinetochores that did not appear to adopt a bipolar interaction with the spindle (e.g., those lying outside the rectangle in Fig. 5 D), the loss of MAD2 and 3F3/2 staining may be the result of a normal "timing out" of the checkpoint. In the presence of microtubule-destabilizing drugs that activate the spindle checkpoint in budding yeast, the cell cycle is delayed for a period of ~6 h, after which the cell cycle proceeds regardless of the state of the chromosomes (Hoyt et al., 1991). Similarly, Li and Nicklas (1995) demonstrated a 5–6-h delay when a single unaligned chromosome was present during insect meiosis. In our study of the *afd1* mutant, the full prophase–metaphase II period was documented in only two cells. Prometaphase II extended for ~1 h longer than expected in these cells, suggesting that the meiosis II spindle checkpoint times out relatively quickly in maize.

Anaphase II in the *afd1* mutant is marked by two events: the loss of staining for checkpoint proteins (Fig. 6 B), and a significant increase in the stretching of many sin-

gle kinetochores (up to five times their normal diameter; Fig. 5 F). In many cases, the kinetochore stretching appeared to be caused by prometaphase-like tangential interactions with microtubules (Fig. 8 A), whereas in others, there were clearly identifiable kinetochore fibers interacting with each end of the stretched kinetochores (Fig. 8 B). The type of end-on interactions shown in Fig. 8 B occurred on 25% of the stretched kinetochores and 6% of all single kinetochores. These data support the idea that single kinetochores can divide into half kinetochore units, and indicate that each unit can undergo poleward motility.

Centromere/Kinetochore Redundancy

A large body of literature suggests that the centromeric DNA, which underlies the kinetochore and may be entwined within it (for review see Choo, 1997), has a redundant structure. The bulk of the cytological evidence for redundancy comes from experiments where centromeres are split by a process known as centromere misdivision (Darlington, 1937). One method for detecting misdivision is to observe the behavior of univalents at meiosis I (unpaired chromosomes) or single kinetochore chromosomes at meiosis II. Such chromosomes often appear to break at the centromeres, in some species, such as wheat, at frequencies close to 40% (Sears, 1952). More recently, the molecular analysis of centromeres in *Drosophila* and *Arabidopsis* has revealed highly reiterated sequence elements (Sun et al., 1997; Copenhaver et al., 1999). Maize centromeres appear to have a similar structure. Several abundant sequence repeats have been identified at maize centromeres (Kaszas and Birchler, 1996; Ananiev et al., 1998), and the centromere of the B chromosome can be reduced by misdivision to ~10% of its natural size and still retain function (Kaszas and Birchler, 1996).

Other evidence suggests that the centromere–kinetochore complex has a visibly redundant external structure. Lima-de-Faria (1958) reviewed this literature, which in some cases provides convincing descriptive evidence for half kinetochore units on single kinetochore chromosomes (i.e., at anaphase II; see Figures 45 and 46 of Lima-de-Faria, 1958). More recently, Mole-Bajer et al. (1990) used kinetochore-specific human (calcinosis, Raynaud phenomenon, esophageal dismotility, sclerodactyly, telangiectasia [CREST]) autoantisera to show that, under enhanced immunogold detection conditions, the mitotic kinetochores in *Haemanthus* appear to be composed of two (and sometimes four) distinct units. Similarly, when mammalian mitotic kinetochores are artificially stretched, a repetitive staining pattern is observed (Zinkowski et al., 1991). We show here using functional assays and specific antibodies to a kinetochore outer domain that the maize meiotic kinetochore is divisible into two parts. We consider it unlikely that kinetochores are especially prone or otherwise limited to being divided into two parts. As discussed above, it is probably the interaction with the spindle and the stabilizing properties of kinetochore–microtubule attachment that is responsible for the twofold redundancy we have observed.

Östergren (1947) suggested that the half kinetochore units that were sometimes visible in the light microscope might independently orient towards different spindle poles and cause misdivision (see also Lima-de-Faria, 1956, for additional references and further discussion). Our data support this model for centromere misdivision. However, in maize the high frequency of single kinetochore alignment (~60%) and stretching at anaphase (~23%) does not result in a comparable level of centromere breakage. In our data set of 10 telophase II cells, we found no evidence for centromere–kinetochore misdivision. Therefore, the data suggest that while maize half kinetochore units frequently orient towards opposite spindle poles, this biorientation is usually resolved by one half of the kinetochore releasing its attachment. The frequency with which a bipolar kinetochore orientation results in centric misdivision is likely to be a species-specific parameter that depends on the size and sequence of the centromere as well as the molecular makeup of the kinetochore.

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References

Amon, A. 1999. The spindle checkpoint. *Curr. Opin. Genet. Dev.* 9:69–75.

Ananiev, E.V., R.L. Phillips, and H.W. Rines. 1998. Chromosome-specific molecular organization of maize (*Zea mays L.*) centromeric regions. *Proc. Natl. Acad. Sci. USA*. 95:13073–13078.

Asai, D.J., C.J. Brokaw, W.C. Thompson, and L. Wilson. 1982. Two different monoclonal antibodies to tubulin inhibit the bending of reactivated sea urchin spermatozoa. *Cell Motil.* 2:599–614.

Ault, J.G., and R.B. Nicklas. 1989. Tension, microtubule rearrangements, and the proper distribution of chromosomes in mitosis. *Chromosoma.* 98:33–39.

Bai, X., B.N. Peirson, F. Dong, C. Xue, and C.A. Makaroff. 1999. Isolation and characterization of *SYN1*, a *RAD21*-like gene essential for meiosis in *Arabidopsis*. *Plant Cell.* 11:417–430.

Bhatt, A.M., C. Lister, T. Page, P. Fransz, K. Findlay, G.H. Jones, H.G. Dickinson, and C. Dean. 1999. The *DIF1* gene of *Arabidopsis* is required for meiotic chromosome segregation and belongs to the *REC8/RAD21* cohesin gene family. *Plant J.* 19:463–472.

Chan, A., and W.Z. Cande. 1998. Maize meiotic spindles assemble around chromatin and do not require paired chromosomes. *J. Cell Sci.* 111:3507–3515.

Choo, K.H.A. 1997. The Centromere. Oxford University Press, New York. 304 pp.

Christy, C.S., M. Deden, and J.A. Snyder. 1995. Localization of kinetochore fragments isolated from single chromatids in mitotic CHO cells. *Protoplasma.* 186:193–200.

Copenhaver, G.P., K. Nickel, T. Kuromori, M.-I. Benito, S. Kaul, X. Lin, M. Bevan, G. Murphy, B. Harris, L.D. Parnell, et al. 1999. Genetic definition and sequence analysis of *Arabidopsis* centromeres. *Science.* 286:2468–2474.

Darlington, C.D. 1937. Misdivision and the genetics of the centromere. *J. Genet.* 37:342–364.

Dawe, R.K., L.M. Reed, H.-G. Yu, M.G. Muszynski, and E.N. Hiatt. 1999. A maize homolog of mammalian CENPC is a constitutive component of the inner kinetochore. *Plant Cell.* 11:1227–1238.

Golubovskaya, I.N. 1989. Meiosis in maize: *mei* genes and conception of genetic control of meiosis. *Adv. Genet.* 26:149–192.

Golubovskaya, I.N., and A.S. Mashnenkov. 1975. Genetic control of meiosis. I. Meiotic mutation in corn (*Zea mays L.*) *afd*, causing the elimination of the first meiotic division. *Soviet Genetics.* 11:810–816.

Golubovskaya, I.N., and N.B. Khristolyubova. 1985. Maize meiosis and meigenes. *In* Plant Genetics. M. Freeling, editor. Alan R. Liss, Inc., New York. 723–738.

Golubovskaya, I.N., N.A. Avalkina, and W.F. Sheridan. 1992. Effects of several meiotic mutations on female meiosis in maize. *Dev. Genet.* 13:411–424.

Gorbsky, G.J., and W.A. Ricketts. 1993. Differential expression of a phosphoepitope at the kinetochores of moving chromosomes. *J. Cell Biol.* 122:1311–1321.

Hoyt, M.A., L. Totis, and B.T. Roberts. 1991. *S. cerevisiae* genes required for cell cycle arrest in response to the loss of microtubule function. *Cell.* 66:507–517.

Kaszas, E., and J.A. Birchler. 1996. Misdivision analysis of centromere structure in maize. *EMBO (Eur. Mol. Biol. Organ.) J.* 15:5246–5255.

Khodjakov, A., R.W. Cole, A.S. Bajer, and C.L. Rieder. 1996. The force for poleward chromosome motion in *Haemanthus* cells acts along the length of the chromosome during metaphase but only at the kinetochore during anaphase. *J. Cell Biol.* 132:1093–1104.

Khodjakov, A., R.W. Cole, B.F. McEwen, K.F. Buttle, and C.L. Rieder. 1997. Chromosome fragments possessing only one kinetochore can congress to the spindle equator. *J. Cell Biol.* 136:229–240.
Klein, F., P. Mahr, M. Galova, S.B.C. Buonomo, C. Michaelis, K. Nairz, and K. Nasmyth. 1999. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell.* 98:91–103.
Li, X., and R.B. Nicklas. 1995. Mitotic forces control a cell-cycle checkpoint. *Nature.* 373:630–632.
Li, X.T., and R.B. Nicklas. 1997. Tension-sensitive kinetochore phosphorylation and the chromosome distribution checkpoint in praying mantid spermatocytes. *J. Cell Sci.* 110:537–545.
Lima-de-Faria, A. 1956. The role of the kinetochore in chromosome organization. *Hereditas.* 42:85–160.
Lima-de-Faria, A. 1958. Recent advances in the study of the kinetochore. *Int. Rev. Cytol.* 7:123–157.
Maney, T., L.M. Ginkel, A.W. Hunter, and L. Wordeman. 1999. The kinetochore of higher eucaryotes: a molecular view. *Int. Rev. Cytol.* 194:67–131.
Mole-Bajer, J., A.S. Bajer, R.P. Zinkowski, R.D. Balczon, and B.R. Brinkley. 1990. Autoantibodies from a patient with scleroderma CREST recognized kinetochores from the higher plant *Haemanthus*. *Proc. Natl. Acad. Sci. USA.* 87:3359–3603.
Nicklas, R.B. 1989. The motor for poleward chromosome movement in anaphase is in or near the kinetochore. *J. Cell Biol.* 109:2245–2255.
Nicklas, R.B. 1997. How cells get the right chromosomes. *Science.* 275:632–637.
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., S. Ward, and G.J. Gorbsky. 1995. Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J. Cell Biol.* 130:929–939.
Östergren, G. 1947. Proximal heterochromatin, structure of the centromere and the mechanism of its misdivision. *Botaniska Notiser.* 2:176–177.
Rieder, C.L., and E.D. Salmon. 1994. Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. *J. Cell Biol.* 124:223–233.
Rieder, C.L., and E.D. Salmon. 1998. The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* 8:310–318.
Rieder, C.L., J. Ault, U. Eichenlaub-Ritter, and G. Sluder. 1993. Morphogenesis of the mitotic and meiotic spindle: conclusions from one system are not necessarily applicable to the other. *In* Chromosome Segregation and Aneuploidy. B.K. Vig, editor. Springer-Verlag, Berlin. 183–197.
Rieder, C.L., R.W. Cole, A. Khodjakov, and G. Sluder. 1995. The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J. Cell Biol.* 130:941–948.
Roeder, G.S. 1997. Meiotic chromosomes: it takes two to tango. *Genes Dev.* 11: 2600–2621.
Rudner, A.D., and A.W. Murray. 1996. The spindle assembly checkpoint. *Curr. Opin. Cell Biol.* 8:773–780.
Sawin, K.E., and T.J. Mitchison. 1994. Microtubule flux in mitosis is independent of chromosomes, centrosomes, and antiparallel microtubules. *Mol. Biol. Cell.* 5:217–226.
Schaar, B.T., G.K.T. Chan, P. Maddox, E.D. Salmon, and T.J. Yen. 1997. CENP-E function at kinetochores is essential for chromosome alignment. *J. Cell Biol.* 139:1373–1382.
Sears, E.R. 1952. Misdivision of univalents in common wheat. *Chromosoma.* 4:535–550.
Skibbens, R.V., and P. Hieter. 1998. Kinetochores and the checkpoint mechanism that monitors for defects in the chromosome segregation machinery. *Annu. Rev. Genet.* 32:307–337.
Smirnova, E.A., and A.S. Bajer. 1992. Spindle poles in higher plants. *Cell Motil. Cytoskeleton.* 23:1–7.
Sun, X., J. Wahlstrom, and G. Karpen. 1997. Molecular structure of a functional *Drosophila* centromere. *Cell.* 91:1007–1019.
Takahashi, K., E.S. Chen, and M. Yanagida. 2000. Requirement of Mis6 centromere connector for localizing a CENP-A-like proteins in fission yeast. *Science.* 288:2215–2219.
Tomoyuki, T., M.P. Cosma, K. Wirth, and K. Nasmyth. 1999. Identification of cohesin association sites at centromeres and along chromosome arms. *Cell.* 98:847–858.
Watanabe, Y., and P. Nurse. 1999. Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature.* 400:461–464.
Waters, J.C., R.V. Skibbens, and E.D. Salmon. 1996. Oscillating mitotic newt lung cell kinetochores are, on average, under tension and rarely push. *J. Cell Sci.* 109:2823–2831.
Waters, J.C., R.-H. Chen, A.W. Murray, and E.D. Salmon. 1998. Localization of Mad2 to kinetochores depends on microtubule attachment, not tension. *J. Cell Biol.* 141:1181–1191.
Wise, D.A., and B.R. Brinkley. 1997. Mitosis in cells with unreplicated genomes (MUGs): spindle assembly and behavior of centromere fragments. *Cell Motil. Cytoskeleton*. 36:291–302.
Wood, K.W., R. Sakowicz, L.S.B. Goldstein, and D.W. Cleveland. 1997. CENP-E is a plus end-directed kinetochore motor required for metaphase chromosome alignment. *Cell.* 91:357–366.
Yu, H.-G., E.N. Hiatt, A. Chan, M. Sweeney, and R.K. Dawe. 1997. Neocentromere-mediated chromosome movement in maize. *J. Cell Biol.* 139:831–840.
Yu, H.-G., M.G. Muszynski, and R.K. Dawe. 1999. The maize homologue of the cell cycle checkpoint protein MAD2 reveals kinetochore substructure and contrasting mitotic and meiotic localization patterns. *J. Cell Biol.* 145:425–435.
Zickler, D., and N. Kleckner. 1999. Meiotic chromosomes: integrating structure and function. *Annu. Rev. Genet.* 33:603–754.
Zinkowski, R.P., J. Meyne, and B.R. Brinkley. 1991. The centromere–kinetochore complex: a repeat subunit model. *J. Cell Biol.* 113:1091–1110.