

## Induction of centromeric activity in maize by *suppressor of meiotic drive 1*

R. KELLY DAWE\*<sup>†‡</sup> AND W. ZACHEUS CANDE\*

\*Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720; and <sup>†</sup>Departments of Botany and Genetics, 4613 Plant Sciences, University of Georgia, Athens, GA 30602

Communicated by Michael Freeling, University of California, Berkeley, CA, April 23, 1996 (received for review February 7, 1996)

**ABSTRACT** The Abnormal chromosome 10 (Ab10) in maize causes normally-quiescent blocks of heterochromatin called knobs to function as meiotic centromeres. Under these circumstances genetic markers associated with knobs exhibit meiotic drive, i.e., they are preferentially transmitted to progeny. Here we describe a mutation called *suppressor of meiotic drive (smd1)* that partially suppresses meiotic drive, and demonstrate that *smd1* causes a quantitative reduction in the mobility of knobs on the meiotic spindle. We conclude that *Smd1* encodes a product that is necessary for the activation of ectopic centromeres, and that meiotic drive occurs as a consequence of the resulting change in chromosome movement. As a genetic system, Ab10 offers a new and powerful approach for analyzing centromere/kinetochore function.

A variety of evidence indicates that tandemly repeated DNA sequences have a central role in the organization and function of the higher eukaryotic centromere. DNA repeats such as the mammalian  $\alpha$ -satellite, which interacts with kinetochore proteins (1), and other well-conserved sequences (2), are thought to have required functions in chromosome disjunction and segregation. Similar centromeric elements have recently been identified in higher plants (3–5), including a centric sequence from the maize B chromosome (6). A notable feature of the maize centric sequence is its strong homology (72% over a 90-bp region) to specialized heterochromatic regions called knobs that are found in distal locations on the arms of maize chromosomes (6).

Knobs have been observed at 22 loci on all 10 maize chromosomes (7) and are composed primarily of a 180-bp element distributed in tandem arrays (8, 9). This type of organization (tandem repeats of  $\approx$ 180 bp) is typical of other known centromeric elements (4, 5, 10). Perhaps the most convincing evidence of the knob/centromere homology is the fact that knobs can function as meiotic centromeres when a variant of chromosome 10, known as Abnormal 10 (Ab10), is present in the cell. In most strains of maize the knobs are inactive and lag behind the true centromeres at anaphase. However, when Ab10 is present, knob loci form “neocentromeres” that are pulled ahead of the true centromeres (8, 11). The effects can be dramatic, causing chromosome arms to be stretched from the metaphase plate all the way to the spindle (11).

Ab10 has the additional effect of causing the preferential segregation, or meiotic drive, of knobs and loci linked to knobs (12). Meiotic drive describes the outcome of any genetic system that serves to increase the transmission of a chromosome or chromosomal segment by distorting normal Mendelian segregation (13). Examples of meiotic drive have been found in nearly every organism that has been studied extensively at the genetic level, including *Drosophila* (14), maize (15), man (16), mouse (17), and *Neurospora* (18). In well-studied examples like the *Drosophila Segregation distorter* system and the *t*-haplotypes

of mouse, one homolog is preferentially transmitted because it carries a drive locus, or multiple drive loci (17), that can inactivate the sperm carrying the other homolog. The result is that a pair of genetic markers that would normally be passed to progeny in a 1:1 ratio can be found in ratios as high as 99:1 (14, 19). In maize, there is no gamete inactivation and meiotic drive is less pronounced, distorting test cross ratios anywhere between 1:1 and 3:1 depending on the genetic distance of a marker from a knob (12). The preferential segregation is not limited to markers on chromosome 10, but can be detected with any gene linked to a knob as long as Ab10 is present (at least three chromosomes other than 10 have been studied; refs. 20 and 21).

In maize, the neocentromere activity of knobs occurs in both male and female meiocytes, but meiotic drive is limited to the female. Based on this and other cytogenetic evidence Rhoades (22) proposed a model, summarized in Fig. 1, to explain meiotic drive in maize. (i) A prerequisite for meiotic drive is that a plant be heterozygous at one or more knob loci. Because most knobs are widely separated from a centromere, recombination occurs between knobs and centromeres so that the sister chromatids become heteromorphic for the presence of a knob; (ii) The spindles then interact directly with knobs to form neocentromeres, which cause the knobs and closely linked genes to lie very close to the spindle poles at telophase I; (iii) knobbed chromatids maintain their peripheral cellular location until metaphase II; and (iv) anaphase II segregates the knobbed chromosomes to the outermost cells of the linear tetrad. Finally, Rhoades argued, because only the basal cell develops into the megagametophyte, knobbed chromosomes are preferentially transmitted. Meiotic drive does not occur in the male because the tetrad is tetragonal (four-sided) and all of the products of meiosis produce gametes. The model involves a transacting drive locus (or loci) on Ab10, as well as a variable number of target loci: the knob locus within Ab10 (see below) and numerous others in the form of knobs on other chromosomes.

A large amount of genetic evidence has been amassed in support of the Rhoades model (21). However, there is no direct evidence for what is perhaps the most critical component of the model: that neocentromere formation is required for meiotic drive (19, 21). Extensive efforts to identify the cytological location of postulated neocentric-promoting loci have failed. Deletions have been recovered that lack meiotic drive, but all such deletion derivatives retain the capacity to induce neocentromeres (23–25). Here we describe a meiotic drive mutation, which upon detailed analysis proves to have a defect in neocentromere formation. This mutation opens the way to a clear understanding of meiotic drive in maize, as well as to a molecular analysis of kinetochore function in higher plants.

### MATERIALS AND METHODS

**Cytological Analysis.** Anthers were fixed for 2 hr in buffer A with 4% paraformaldehyde (26). The fixed male meiocytes

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Abbreviations: Ab10, Abnormal chromosome 10; 3D, three-dimensional. <sup>‡</sup>To whom reprint requests should be addressed at the <sup>†</sup> address.

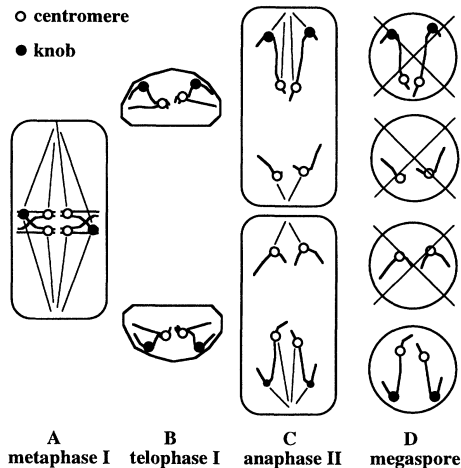


FIG. 1. The Rhoades model for meiotic drive in maize. Rhoades (22) proposed that after recombination between the centromeres and knobs (A) the knobs form neocentromeres and are pulled to the poles at telophase I (B). The knob location is maintained through metaphase II and extreme neocentric activity during anaphase II causes visible extensions of chromosome arms (C). All knobs that recombined with the centromere ultimately lie at one of the outermost megaspores, and only the basal megaspore survives to form a female gametophyte (D).

were extruded from anthers, spun down onto poly-lysine coated coverslips at 100 g, and stained with 0.1  $\mu\text{g/ml}$  of DAPI (4',6-diamidino-2-phenylindole dihydrochloride). This procedure causes a partial flattening of meiocytes (up to 50%), but increases the adherence of the cells to the coverslips. Three-dimensional (3D) data sets were produced using computer-aided wide-field 3D light microscopy (27). In Fig. 2, pachytene chromosomes were modeled interactively and computationally straightened as described (26, 29). The unique regions from six Ab10 progenitor chromosomes and eight *smd1* chromosomes (each containing two paired chromosomes) were straightened and analyzed using the PRIISM program (26, 27). Distances between six major landmarks on the unique region (between each of the three chromomeres, to the edges of the knob, and tip of the chromosome) were measured for each genotype and compared statistically. There were no significant differences when the raw distance data were compared, or when the data were first normalized to the total length of the Ab10 unique region (*t* tests at the 5% level). In Fig. 5A, the paired third chromosomes were computationally cut from a data set and volume rendered (27). In Figs. 2 and 5 the chromosomes were subjected to local contrast enhancement (28).

**In Situ Hybridization.** Cells were prepared as described above, except that anthers were fixed in a buffer that preserves microtubules called PHEMS (30) and 0.1% Triton X-100. Under these buffer conditions neocentromeres were consistently observed. After being spun down onto coverslips, the

meiocytes were stepped through the following solutions for 5 min each: 1 $\times$  SSC (4.38 g/liter sodium citrate/8.75 g/liter NaCl), 20% deionized formamide; 2 $\times$  SSC/30% deionized formamide; 2 $\times$  SSC/50% deionized formamide. Broken pieces of coverslips were placed at four corners of a slide, and the coverslips with meiocytes were placed upside down over the broken pieces. About 70  $\mu\text{l}$  of a solution containing 2 $\times$  SSC/50% deionized formamide and 1  $\mu\text{g/ml}$  of a fluorescently labeled oligonucleotide homologous to the knob sequence was injected beneath the coverslip. The oligonucleotide (5'-AACATATGTGGGGTGAGGTGTATG-3') was labeled with the fluorescein-based dye 6-FAM (Applied Biosystems; a gift from H. W. Bass). The coverslip was sealed down with rubber cement and the slide placed on a 100°C heating block for 5 min. The oligonucleotide was allowed to anneal overnight at 28°C. The rubber cement was removed, and the coverslip stepped through the following solutions for 5 min each: 2 $\times$  SSC/20% deionized formamide/0.01% Tween 20; 1 $\times$  SSC/10% deionized formamide/0.01% Tween 20; 1 $\times$  SSC/1 $\times$  TBS (8 g/liter NaCl/0.2 g/liter KCl/3 g/liter Tris, pH 8.0); 1 $\times$  TBS; 1 $\times$  TBS/0.1  $\mu\text{g/ml}$  DAPI. The cells were then analyzed using 3D light microscopy (26).

## RESULTS

**Identification and Characterization of *smd1*.** A population of plants was generated that was heterozygous for Ab10 and the closely linked kernel pigmentation mutation *r*, which makes the kernels colorless instead of purple. The plants also carried an active transposable element family called *Robertson's Mutator* (*Mu*) which induces mutations at a high frequency (31). The *r* Ab10/*R* + plants were crossed in an isolation plot by *R-st* + /*R-st* +, and 3110 resulting ears were analyzed for the segregation of colorless and purple kernels (*R-st* produces a light spotted pattern; + indicates the cytologically normal chromosome 10). Due to the effects of meiotic drive, more than three-fourths of the kernels on most ears were colorless (a sample of 24 ears indicated a mean  $\pm$  SD of 76.5  $\pm$  3.6%). However, one ear was identified with a percentage of colorless kernels that was close to the Mendelian expectation of 50%. The mutation that caused this phenotype has been called *suppressor of meiotic drive 1* (*smd1*).

To begin a genetic analysis of *smd1*, a series of test crosses were carried out to ascertain the heritability and expressivity of the mutation, as well as to determine whether *smd1* was linked to Ab10. Plants of the constitution *r* Ab10/*R-st* +, *smd1* + were initially crossed to plants homozygous for a third *R* allele (*R-nj*, which colors only the crown of the kernel) and the ears analyzed for the preferential segregation of *r*. Among 34 resulting ears, *r* was transmitted at an average frequency of 55.4% (SD = 6.4%). None of the ears showed full meiotic drive levels of  $\approx$ 75%, providing an early indication that *smd1* is genetically linked to Ab10. Further crosses demonstrated a high degree of variability in *smd1* expression. In one experiment, plants of the constitution *r smd1* Ab10/*R* + + (*R* conditions full kernel pigmentation) were crossed to a strain

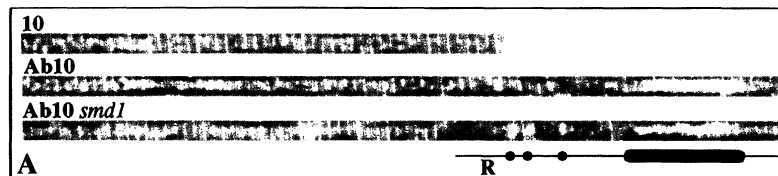


FIG. 2. Chromosome 10 comparisons. Three different chromosomes were computationally straightened from pachytene-staged meiocytes and subjected to local contrast enhancement (28). Normal chromosome 10, an Ab10, and an Ab10 from an *smd1* strain are represented. At the bottom is a schematic showing the unique features of this region of Ab10: the differential segment (see Fig. 3), a stretch of euchromatin, a large knob, and a euchromatic tip. The approximate location of the *R* locus is also shown. (Scale bar = 5  $\mu\text{m}$ .)

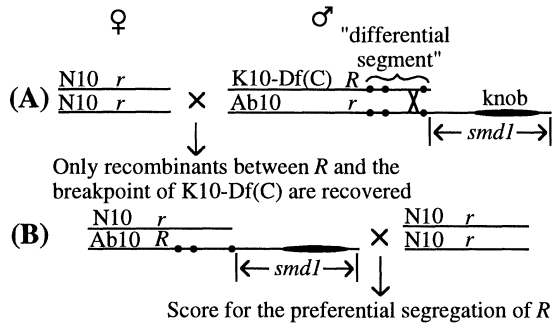


FIG. 3. Crossing scheme for mapping *smd1*. Recombination events were selected between *R* and the breakpoint of *K10-Df(C)* in cross (A), due to the fact that *K10-Df(C)* is lethal to the male gametophyte. In the cross shown in B, the recombinants were test crossed to determine whether *smd1* was present. All of the recombinants showed reduced preferential segregation of *R*, indicating that *smd1* lies distal to the differential segment.

homozygous for *R-st*, and the ears assayed for the preferential segregation of *r*. Of 12 ears, *r* was transmitted at an average frequency of 64.1%, with the values ranging between 52.5 and 72.7%. When seeds from one of the resulting ears with high meiotic drive (72.7%) were planted and crossed, in the next generation only  $50.1 \pm 8.9\%$  of the resulting progeny carried *r* (mean  $\pm$  SD,  $n = 6$  ears); when seeds from an ear showing low meiotic drive (52.5%) were planted and crossed,  $56.7 \pm 1.3\%$  of the progeny carried *r* ( $n = 4$  ears). These and other similar data suggest that the variable expressivity of *smd1* is not a heritable (epi)genetic phenomenon. The variable *smd1* expressivity could be the result of either environmental conditions or genetic background effects.

**Cytological Analysis of *smd1*.** Examples of a normal chromosome 10, an Ab10, and an Ab10 from an *smd1* strain are

illustrated in Fig. 2. To obtain these data, 3D data sets from pachytene-staged meiocytes were collected using wide-field light microscopy (27). The 10th chromosomes were located within data sets, their paths interactively traced, and the modeled paths used to linearize the chromosomes (26, 29). As shown in Fig. 2, the region unique to Ab10 contains three prominent chromomeres, an intervening unique euchromatic region, a deeply staining heterochromatic knob, and a euchromatic tip. Visual comparisons indicated that there were no gross cytological abnormalities associated with the *smd1* mutation. A statistical analysis of the straightened chromosomes also failed to reveal any significant differences (see *Materials and Methods*). Thus, the cytological data indicate that *smd1* is either a transposon-induced mutation (most likely *Mu*), a point mutation, or a small deletion that is not detectable using our cytological techniques.

**Genetic Mapping of *smd1*.** To determine if *smd1* was located within the unique region of Ab10, a variation of deletion mapping was employed. Rhoades and Dempsey (24) had previously identified a deletion derivative of Ab10 called *K10-Df(C)*, which lacks the large knob of Ab10 as well as most of the euchromatic segment that lies between the three chromomeres and the large knob. The *K10-Df(C)* deficiency is illustrated in Fig. 3. The chromomere-containing region that is retained in *K10-Df(C)* has been called the "differential segment" (24). Because *K10-Df(C)* is deficient for a large portion of Ab10, including genes known to be found on the normal chromosome 10, this derivative is inefficiently transmitted through the female and never transmitted through the male. Hence, in the sequence of two crosses shown in Fig. 3, it was possible to quickly map *smd1* relative to the differential segment.

Results from the cross in Fig. 3A indicated a genetic distance of 7.5 map units between *R* and the *K10-Df(C)* break point (140 of 1879 kernels carried *R*). The *R*-carrying kernels were planted, and as many as possible were crossed by an *r* tester strain (Fig. 3). If *smd1* were within the differential segment, recombination between *Smd1* and the end of the *K10-Df(C)*

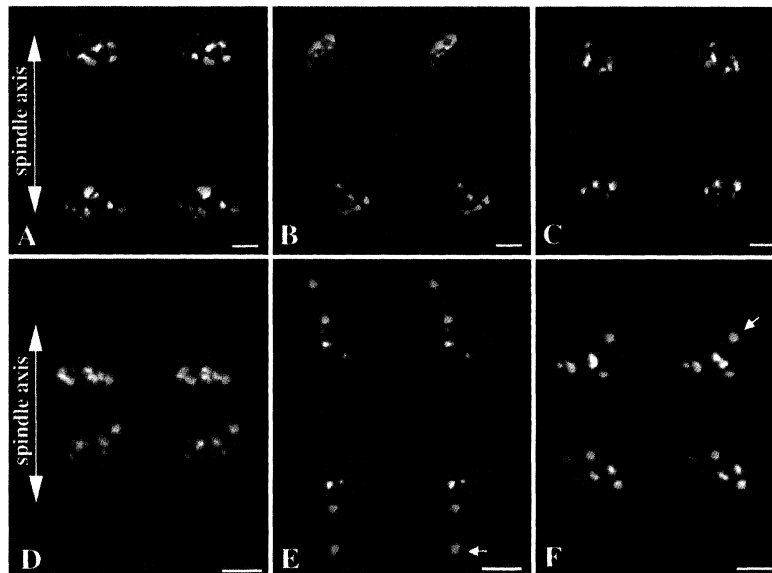


FIG. 4. Neocentric activity observed at telophase I and anaphase II. Knobs were localized by *in situ* hybridization and images were collected using 3D microscopy (26). Each panel contains a stereo pair of a single representative cell, oriented vertically so that the spindle poles are at the top and bottom. Knobs are shown in green and the chromatin in magenta. (A) Telophase I of a wild-type cell showing a lack of knob clustering. (B) Telophase I of a cell heterozygous for Ab10. Note the clustering of knobs at the spindle poles. (C) Telophase I of a cell heterozygous for *smd1* showing no knob clustering, as in B. (D) A wild-type cell in anaphase II showing that the knobs usually lag behind the bulk of the chromatin. (E) Anaphase II of a cell homozygous for Ab10. Extreme neocentric activity causes knobs to be pulled to poles, stretching the chromosome arms. (F) Anaphase II of a cell homozygous for *smd1* showing most knobs lagging but some exhibiting weak neocentric activity (arrow). (Scale bars = 5  $\mu$ m.)

breakpoint would be expected to reconstruct the progenitor Ab10 and give full meiotic drive levels. However, all of the resulting 61 ears showed a reduced level of meiotic drive consistent with the presence of *smd1* [with a mean  $\pm$  SD of  $62.8 \pm 6.0\%$ ; in control test crosses using *R* Ab10/*r* + females there were  $75.8 \pm 6.0\%$  colored kernels ( $n = 11$  ears)]. Since no ears with full meiotic drive were observed, the data suggest that *smd1* lies  $\geq 7.5$  map units distal to *R*. Whether or not *smd1* lies distal to the differential segment cannot be established using this approach, because of the possibility that there was an inhibition of crossing over near the terminus of the deficient chromosome.

**Qualitative Studies of Neocentromere Formation in *smd1* Plants.** As a first step toward understanding the cytological effects of *smd1*, meiocytes were analyzed for the presence or absence of neocentromeres. Because it was known that neocentromeres are difficult to see in meiosis I (11), and because of the possibility that reduced neocentric activity would be difficult to detect by traditional methods, a fluorescent probe for the knob sequence was used to identify neocentromeres. The probe was hybridized *in situ* to partially flattened meiocytes (see *Materials and Methods*), and the data analyzed using wide-field 3D light microscopy (26, 27). Representative cells are illustrated as stereo pairs in Fig. 4.

If the Rhoades model for meiotic drive is correct (Fig. 1), an important role of neocentromeres is to bring the knobs to the poleward side of the newly formed nuclei after chromosome segregation in telophase I (Fig. 1B). Since meiotic drive is nearly complete when Ab10 is present in only a single copy (21), the peripheral localization should be visible in Ab10/+ heterozygotes. The data indicated that knobs are randomly placed in wild-type cells (Fig. 4A), and as predicted by Rhoades (22), distinctly peripheral in the Ab10/+ heterozygote (Fig. 4B). In the *smd1*/+ heterozygote, an apparently random knob localization similar to wild type was observed (Fig. 4C).

More direct evidence of neocentromere activity was obtained by studying anaphase II in homozygous stocks, the conditions where neocentromeres produce dramatic poleward extensions (8, 11). Because most knobs are located toward the ends of chromosome arms (7), knobs are expected to trail behind the bulk of the chromatid. As expected, when wild-type stocks were hybridized with the knob, the signal was located on the lagging chromosome arms (Fig. 4D). By contrast, when anaphase II cells were observed in Ab10 homozygotes, the signal was found at the tips of extreme poleward extensions (Fig. 4E). In homozygous *smd1* plants, an intermediate phenotype was observed where some knobs lagged behind the bulk of the chromatid and others showed weak neocentric activity (Fig. 4F, arrow). Thus, the qualitative data not only support the Rhoades model for meiotic drive (Fig. 1), but indicate that *smd1* causes a significant reduction in neocentromere activity.

**Quantitative Studies of Neocentromere Function in *smd1* Plants.** To obtain a more precise estimate of the reduction in neocentric activity conditioned by *smd1*, we used acentric fragments generated by recombination within a heterozygous paracentric inversion (Fig. 5). Acentric fragments generated this way normally lag at the spindle midzone during anaphase I unless the fragments carry knobs and the neocentric activity of Ab10 is present in the cell; in which case the fragments are pulled to a pole (15). We were interested in determining if the apparently weak neocentromere formation typical of *smd1* (Fig. 4F) could be measured as a reduced capacity to pull a knobbed acentric fragment from the spindle midzone. To maximize the efficiency of the assay, stocks were created that were heterozygous for a paracentric inversion of chromosome 3 (Inv3a), but homozygous for a large knob within the inversion loop. Fig. 5A illustrates the pairing configuration of the inverted chromosome, as well as the two-paired knobs. This genetic constitution ensures that wherever recombination oc-

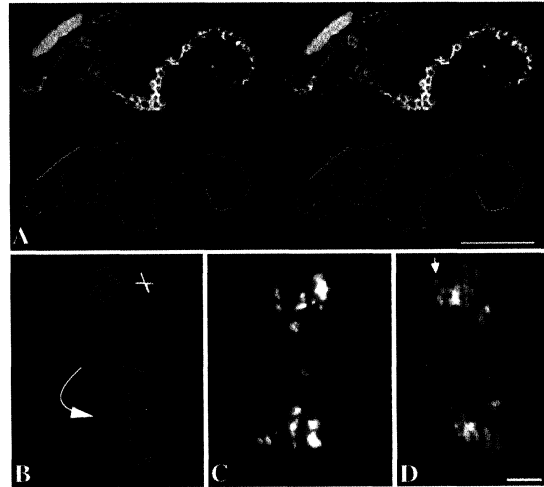


FIG. 5. The behavior of knobbed acentric fragments at anaphase I. (A) A heterozygous paracentric inversion of chromosome 3 (Inv3a) at pachytene showing a large knob on both chromosomes. The chromosome is shown as a stereo pair with the approximate paths of the homologous chromosomes shown below. (B) Schematic illustration of how acentric fragments are generated by a heterozygous paracentric inversion. Any recombination event within the inversion at pachytene (*upper*) will generate a dicentric bridge and an acentric fragment carrying the knob at anaphase I (*lower*). The nonrecombinant chromatids will also carry the knob. (C) An anaphase I cell from a wild-type plant that has been hybridized with a knob-specific probe. The knob sequence is detected within the acentric fragment, the bridge, and in the nonrecombinant chromatids. (D) An anaphase I cell from a plant homozygous for Ab10 showing the acentric fragment (arrow) being pulled to a pole (this cell was not hybridized with the knob probe). Both C and D are projections of 3D data sets. (Scale bar = 5  $\mu$ m.)

urs within the inversion, the acentric fragment contains a knob (Fig. 5B).

The behavior of knobbed acentric fragments was first assayed on cells that carried two copies of the normal chromosome 10. As expected, in a majority of cells, the acentric fragments were found lying at the midzone. When hybridized with the knob-specific probe, the knob sequence could be seen within the acentric fragment, in the dicentric chromosome bridge, and in nonrecombinant chromatids (compare Fig. 5B and C). In addition, 14% of the cells contained a bridge without a visible fragment. The fragments are assumed to have migrated to a pole in these cases, because every recombination event that generates a bridge also liberates a fragment (32). A background frequency of 14% (bridges with no fragments) is higher than what was reported in a previous study with the same inversion (33), but lower than what has been observed with other inversions (e.g., ref. 32).

In contrast with the results from wild-type cells, in cells that were homozygous for the Ab10, 98% of the acentric fragments were pulled from the midzone. Only in rare cases could the acentric fragment actually be observed among the segregating chromosomes (Fig. 5D); in the remaining cells it was inferred to have done so. When the Ab10 was reduced from two copies in the homozygote (Ab10/Ab10) to one copy in the heterozygote (Ab10/+), the number of fragments pulled to a pole fell from 98 to 65%. This result is consistent with the observation that neocentromeres are more severe when Ab10 is homozygous than when it is heterozygous (11).

The Ab10 carrying *smd1* caused significantly fewer fragments to be pulled to a pole than the corresponding Ab10 progenitor chromosome. In the homozygous *smd1* plants, 50%

Table 1. Location of knobbed acentric fragments at anaphase I in plants with different chromosome 10 constitutions

Genotype	Families/ plants*	Cells with fragment at midzone	Cells with fragment at pole <sup>†</sup>	% fragments pulled to pole actual (adjusted) <sup>‡</sup>
+/+ (wild type)	1/2	89	14	14
Ab10/Ab10	1/4	1	67	99 (85)
<i>smd1/smd1</i>	1/3	53	53	50 (36)
Ab10/+	1/3	61	120	66 (52)
<i>smd1</i> /+	2/6	146	66	30 (16)

Only cells with a dicentric bridge were counted. When two bridges were observed (four-strand double recombinants), each bridge and fragment were counted as a single event.

\*Indicates the number of different families (ears) from which seeds were drawn for planting and the number of different plants that were used to acquire the data.

<sup>†</sup>Inferred from the absence of the fragment from the midzone.

<sup>‡</sup>Indicates percent pulled to pole after subtracting the control value of 14%.

of the fragments were pulled to a pole, and in the heterozygote, only 30% were pulled to a pole. In Table 1, the data are interpreted by first subtracting the background value of 14% from the experimental values. The adjusted values suggest that *smd1* reduces the rescue of acentric fragments by 58% (from 85 to 36%) in the homozygous condition and by 69% (from 52 to 16%) in the heterozygous condition. These results should be considered as estimates, due to the difficulty in controlling for genetic background and our inability to identify fragments that may have only partially migrated to a pole. Nevertheless, the data clearly indicate that the *smd1* mutation reduces the poleward movement of knobbed acentric fragments.

## DISCUSSION

Using transposon mutagenesis, we have isolated a mutation of meiotic drive in maize called *smd1*. The combined data indicate that the wild-type copy of *smd1* (*Smd1*) provides a gene product that converts quiescent heterochromatic knobs into active meiotic centromeres. We make the following observations: (i) *smd1* causes a partial reduction in meiotic drive and demonstrates variable expressivity; (ii) the *smd1* gene is located within the unique region of Ab10, at a genetic distance of at least  $\approx 7.5$  map units distal to the *R* gene; (iii) *smd1* disrupts the asymmetrical knob localization that is observed at telophase I in Ab10 stocks (Fig. 4 B and C); (iv) *smd1* is not a cis-acting mutation, but acts in trans to affect the behavior of all knobs in a cell (Fig. 4 C and F); and finally (v) *smd1* conditions a partial defect in neocentromere motility as assayed both qualitatively (Fig. 4F) and quantitatively using acentric fragments (Table 1). The partial defect conditioned by *smd1* may indicate that *smd1* is a leaky mutation, or that *smd1* acts to enhance the function of other neocentromere components yet to be identified. Another possibility is that there are additional *Smd* loci on Ab10 that can partially compensate for the absence of *smd1*, in a manner similar to the multiple *Tcd*<sup>1</sup> genes that are found in the *tailless* haplotype meiotic drive system in mouse (17).

The observation that *smd1* conditions a partial reduction in both meiotic drive and neocentromere formation indicates that meiotic drive is reduced because neocentromere activity is impaired. This result provides strong support for the model originally proposed by Rhoades, which suggests that neocentromeres are required for meiotic drive (Fig. 1). We have also documented the unusual nuclear organization at telophase I predicted by Rhoades (ref. 22, Fig. 1), in which the knobs are pulled very close to the spindle poles of the telophase I cells (Fig. 4B). Other *in situ* hybridization data (not shown) indicate that the peripheral localization is stably maintained into prophase of the second meiotic division. Indeed, Rhoades proposed that the asymmetrical nuclear organization must be so stable that it is maintained throughout meiosis II spindle

formation, during which the neocentromeres are again pulled to the outermost megaspores (Fig. 1). Our study does not address this issue directly, because the cytological data were derived from male meiocytes where the second meiotic division occurs perpendicular to the first division (34). However, it is likely that there are genes in the distal region of Ab10 that direct or stabilize this specialized organization, because terminal deletions of Ab10 lack meiotic drive but retain neocentromere formation (23–25).

Because the 10th chromosomes are indistinguishable in Ab10 and *smd1* stocks (Fig. 2), and meiotic drive is impaired in *smd1* stocks, it is unlikely that the gross structure of Ab10 itself causes meiotic drive (as sometimes suggested, see ref. 14). On the contrary, the available data now indicate that Ab10 encodes a genic meiotic drive system in the same broad category as the *Drosophila Segregation distorter* (*SD*), *Drosophila Sex-Ratio* (*SR*), and mouse *t*-haplotype systems (reviewed in ref. 14). In each of these systems, a drive locus is linked to an insensitive form of the target locus and additional loci that enhance the effectiveness of meiotic drive. Chromosome rearrangements have occurred that cause tighter linkage among the drive elements forming what are referred to as chromosomal "haplotypes." This is similar to the Ab10 system, which does not recombine with the normal 10th chromosome due to extensive nonhomology and a large inversion (24). However, unlike *SD*, *SR*, and *t*, the drive allele(s) in the Ab10 system does not destroy the chromosomes with the target loci (knobs) but promotes their preferential segregation.

The Ab10 provides a unique opportunity for studying the meiotic kinetochore, the integrated DNA/protein complex that forms an active centromere (35). At present, very little is known about meiotic kinetochores, and mitotic kinetochores of higher eukaryotes are only accessible by immunocytochemical approaches. Because neocentromeres are induced only in the presence of Ab10, mutations can be obtained that specifically interfere with meiotic drive and neocentromere function without affecting the viability of the plant. Presumably, neocentromeres interact with proteins that are similar to the proteins of the true kinetochores. With further transposon mutagenesis and the possibility of cloning relevant genes by transposon tagging (31) we anticipate that maize Ab10 will make it possible to study both the genetics and cell biology of the meiotic kinetochore.

We are indebted to J. W. Sedat and D. A. Agard for allowing us the use of the 3D light microscope workstation and for providing invaluable support and guidance throughout this study. We are grateful to A. F. Dernburg for developing the generalized 3D *in situ* hybridization procedures and to H. W. Bass for both developing the direct-labeled oligo technique for maize meiocytes and designing the knob-specific oligonucleotide. We also thank J. E. Fowler and M. C. Brickman for helpful comments and E. Dempsey for providing the K10-Df(C) deficiency, the Ab10 progenitor chromosome, and the knobbed in-

version (Inv3a). This work was supported by a National Science Foundation Postdoctoral Fellowship to R.K.D. and a grant from the National Institutes of Health (GM48547) to W.Z.C.

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