Four Loci on Abnormal Chromosome 10 Contribute to Meiotic Drive in Maize

Evelyn N. Hiatt*,† and R. Kelly Dawe*,‡,1

*Department of Genetics and ‡Department of Plant Biology, University of Georgia, Athens, Georgia 30602 and †Department of Biology, Kentucky Wesleyan College, Owensboro, Kentucky 42301

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ABSTRACT

We provide a genetic analysis of the meiotic drive system on maize abnormal chromosome 10 (Ab10) that causes preferential segregation of specific chromosomal regions to the reproductive megaspore. The data indicate that at least four chromosomal regions contribute to meiotic drive, each providing distinct functions that can be differentiated from each other genetically and/or phenotypically. Previous reports established that meiotic drive requires neocentromere activity at specific tandem repeat arrays (knobs) and that two regions on Ab10 are involved in *trans*-activating neocentromeres. Here we confirm and extend data suggesting that only one of the neocentromere-activating regions is sufficient to move many knobs. We also confirm the localization of a locus/loci on Ab10, thought to be a prerequisite for meiotic drive, which promotes recombination in structural heterozygotes. In addition, we identified two new and independent functions required for meiotic drive. One was identified through the characterization of a deletion derivative of Ab10 [Df(L)] and another as a newly identified meiotic drive mutation (*suppressor of meiotic drive 3*). In the absence of either function, meiotic drive is abolished but neocentromere activity and the recombination effect typical of Ab10 are unaffected. These results demonstrate that neocentromere activity and increased recombination are not the only events required for meiotic drive.

MEIOTIC drive occurs when a chromosome or chromosomal segment is transmitted to progeny at frequencies higher than expected from Mendelian segregation. Meiotic drive has been documented in a variety of animal, plant, and fungal species (Lyttle 1993), and for each well-characterized meiotic drive system the underlying mechanism for segregation distortion differs. One example of meiotic drive is the preferential segregation phenomenon associated with Abnormal chromosome 10 (Ab10) in maize (Rhoades 1942). In heterozygotes, ~70–75% of the female gametes carry Ab10 after the chromosome undergoes meiosis with normal chromosome 10 (N10), a frequency that is significantly higher than the 50% expected under random segregation. The nonrandom segregation event associated with Ab10 involves the genetically controlled induction of centromeric-like activity at previously inactive heterochromatic regions, an enhancement of recombination throughout the genome, and the poorly understood forces that coordinate nuclear architecture. Kato (1976) found that ~2% of cultivated maize strains and 10% of teosinte strains in Mexico and Guatemala carry the Ab10 form of chromosome 10. All the genetic information necessary for meiotic drive is carried in a structural polymorphism at the end of the long arm of Ab10 (Rhoades 1952). The polymorphic portion of Ab10 can be divided into four distinct regions (Figure 1). Closest to the centromere is the *differential segment*, which contains three prominent chromomeres not found on N10. The differential segment will often pair with the end of N10, but will not recombine with it (Kikudome 1959). Next to the differential segment is the *central euchromatin*, which contains a transposed and inverted portion of N10 (Rhoades and Dempsey 1985). The inverted segment encompasses a ≥14 map unit (MU) distance, including the *W2*, *O7*, and *L13* loci. A deeply staining heterochromatic region known as a *knob* is adjacent to the proximal euchromatin. Knobs may be found at any of 21 other cytological positions (Kato 1976) and all knobs appear to contain tandem repeat arrays composed of a 180-bp repeat (Peacock *et al.* 1981) and/or a 350-bp (TR-1) repeat (Ananiev *et al.* 1998). The fourth distinct region is a short stretch of euchromatin called the *distal tip*. It has been estimated that a minimum of five independent breaks would be required to convert a normal chromosome 10 into an abnormal chromosome 10 (Rhoades and Dempsey 1985).

A variety of evidence indicates that the heterochromatic knobs have an indispensable role in Ab10-mediated meiotic drive. In at least three cases loci linked to knobs on other chromosomes have been shown to display meiotic drive when Ab10 is present, suggesting that most if not all knobs are preferentially segregated in response to *trans*-acting factors encoded by Ab10 (Longley 1945; Rhoades and Dempsey 1985). Perhaps the most striking evidence in favor of the role for knobs in meiotic drive is the fact that each knob is capable of

[1]*Corresponding author:* Department of Plant Biology, Miller Plant Sciences Bldg., University of Georgia, Athens, GA 30602.
E-mail: kelly@dogwood.botany.uga.edu

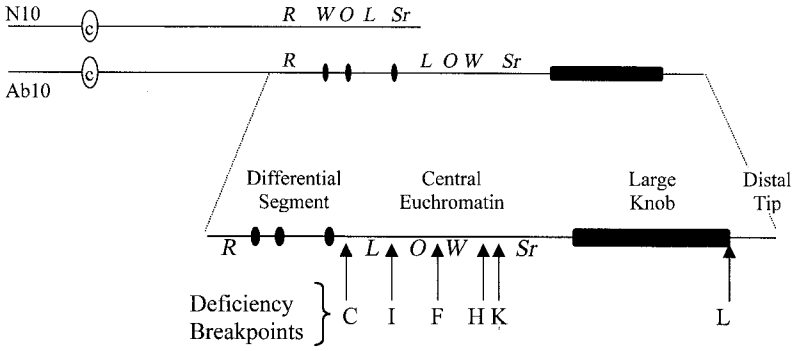

FIGURE 1.—Schematic diagram of the N10 and Ab10 chromosomes. Centromeres are indicated by "c." Mapped loci are indicated by *R, W, O, L,* and *Sr* and the breakpoints for five Ab10 deficiency chromosomes are indicated. The deficiency chromosomes identified as Df(C), Df(I), Df(H), Df(F), and Df(K) were isolated by RHOADES and DEMPSEY (1985). The origin of Df(L) is discussed in the text.

behaving as a facultative centromere, known as a neocentromere, when Ab10 is in the genome. In meiosis I and II, neocentromeres are visible as long extensions of chromosome arms terminated by knobs (RHOADES and VILKOMERSON 1942; RHOADES 1952; PEACOCK *et al.* 1981; DAWE and CANDE 1996; YU *et al.* 1997; DAWE *et al.* 1999). Both the 180-bp and TR-1 repeat arrays are driven poleward. TR-1 arrays are transported more rapidly than the 180-bp arrays and are activated by a gene or genes proximal to the Df(I) deficiency breakpoint (Figure 1; HIATT *et al.* 2002). The 180-bp arrays appear to move slower on the spindle and are *trans*-activated either exclusively by a gene(s) distal to the Df(K) breakpoint or by the combined activities of these and other factors on Ab10 (HIATT *et al.* 2002). A mutation mapping to Ab10 (*suppressor of meiotic drive 1*, or *smd1*) causes neocentromere activity and meiotic drive to be reduced to similar levels (DAWE and CANDE 1996), further suggesting that neocentromeres have a necessary function in meiotic drive.

On the basis of the involvement of neocentromeres, a model for the mechanism of meiotic drive was advanced by RHOADES (1952). In plants heterozygous for a knob, Rhoades postulated that recombination occurs between the centromere and the knob to yield heteromorphic dyads—chromosomes in which one chromatid carries a knob and the other does not. During anaphase I neocentromere activity causes the knobbed chromatids to arrive at the pole prior to the knobless chromatid. The polar orientation of knobs is presumably maintained through interkinesis and into prophase II, where the knobs again form neocentromeres. Since the meiosis II spindles are arranged end to end, the knobs can be preferentially segregated to the outermost daughters of the linear tetrad. In maize only the basal megaspore, which is expected to receive an excess of knobs following neocentromere formation, goes on to form a reproductive cell (megagametophyte). Meiotic drive does not occur in male flowers because all four of the products of meiosis form microgametophytes.

The drive mechanism as described by Rhoades requires recombination between knobs and centromeres. Close linkage or otherwise impaired recombination will result in fewer heteromorphic dyads and segregation levels closer to Mendelian expectations (RHOADES 1952; RHOADES and DEMPSEY 1966). Presumably in response to this limitation on the efficiency of meiotic drive, knobs are never found in close linkage with centromeres, but tend to cluster in the distal halves of arms (BUCKLER *et al.* 1999). In addition, Ab10 encodes a function that increases crossing over up to fivefold in regions where recombination is usually suppressed, *e.g.*, centromeric heterochromatin and structural heterozygotes (RHOADES and DEMPSEY 1966; ROBERTSON 1968; NEL 1973). Given the complexity of the events in female gametogenesis (BEDINGER and RUSSELL 1994) and the poorly understood interaction between knobs and the cytoskeleton (YU *et al.* 1997; YU 2000), it is reasonable to assume that neocentromere activity and unrestricted recombination are not the only events required for meiotic drive. For instance, it has been suggested that Ab10 may carry a locus required to maintain the polar orientation of knobs between anaphase I and prophase II (RHOADES and DEMPSEY 1990).

Several investigators have attempted to localize the functions required for meiotic drive. Traditional mapping has been hampered by the paucity of mutant alleles and by the fact that the polymorphic portion of Ab10 does not recombine with normal 10 (KIKUDOME 1959). The only mutation known to affect meiotic drive is *smd1*, which was mapped to a position somewhere distal to the differential segment (DAWE and CANDE 1996). An alternative to traditional mapping is cytogenetic mapping (HARPER and CANDE 2000), which can be readily employed on Ab10 because of the large number of terminal deficiencies that have been generated. A total of 20 deficiencies have been identified by various means (EMMERLING 1959; MILES 1970; RHOADES and DEMPSEY 1985, 1986, 1988, 1989; HIATT and DAWE 2003). Many of the chromosomes have been lost, but deficiencies covering most of the polymorphic portion are still available (Figure 1) and phenotypic analysis has been carried out on some of these (MILES 1970; RHOADES and DEMPSEY 1986, 1988, 1989; HIATT *et al.* 2002). The most notable conclusions were that deficiencies as severe as Df(I) retain neocentromere activity (HIATT *et al.* 2002), that Df(H) lacks the recombination effect (RHOADES and DEMPSEY 1989), and that Df(K) is unable to *trans*-

activate meiotic drive on chromosome 9 (Rhoades and Dempsey 1988). Overall, the data indicate that the major determinant(s) of neocentromere activity map to the proximal euchromatin (differential segment) and the major determinant(s) of the recombination effect maps to the distal half of the Ab10 structural polymorphism.

To further characterize the functions required for meiotic drive, we have taken the approach of using *Robertson's mutator* (*Mu*; Chandler and Hardeman 1992) to screen for mutants of meiotic drive (Dawe and Cande 1996; Hiatt and Dawe 2003). Although most of the mutations identified in this screen have proven to be deficiencies of the Ab10 chromosome, two mutations were identified that contain no cytologically detectable chromosome changes. One is the previously published gene, *smd1* (Dawe and Cande 1996), and the second is described here as *suppressor of meiotic drive 3* (*smd3*). Unlike *smd1*, *smd3* shows a complete loss of drive and has apparently typical levels of neocentromere activity. Through detailed analyses of *smd3*, of one of our newly identified deficiencies, Df(L), and of four other deficiencies identified by Rhoades and Dempsey (1985), we have been able to identify and map four independent functions involved in Ab10-mediated meiotic drive.

MATERIALS AND METHODS

Identification of meiotic drive mutants: An open-pollinated screen was used to identify mutants of meiotic drive (Dawe and Cande 1996; Hiatt *et al.* 2002). Plants heterozygous for Ab10 were detasseled and testcrossed by plants homozygous for N10. The cross was as follows: *r* Ab10/*R* N10 (active *Mu*) × *Rst* N10/*Rst* N10. The transposable element *Mu* was used as the primary mutagen, and alleles of the *R* gene were used as linked markers. *R* is required for pigmentation of the kernel and is linked to the Ab10 structural polymorphism by ~2 MU (Rhoades 1942). The *R* alleles used in the primary screen and subsequent tests were *R* (colored aleurone, colorless embryo); *r* (colorless); *Rst* (colorless with colored spots on the aleurone); and *Rnj* (colored aleurone cap and embryo).

In the primary screen we identified putative mutants with near-normal Mendelian segregation from among the majority of ears showing meiotic drive. Each mutant was tested for heritability over several generations. Control crosses with the Ab10 progenitor chromosome were performed to determine the level of drive in each specific environment.

Cytological analysis of the Ab10 chromosome: Strains homozygous for Ab10 and mutant derivatives [(Df(L) and *smd3*)] were grown in the greenhouse and processed for microscopy as described previously (Dawe and Cande 1996). The Ab10 chromosome was identified in pachytene preparations using the three chromomeres as cytological landmarks. All images were acquired using a (deconvolution-based) DeltaVision 3D light microscope workstation. DeltaVision modeling software was used to computationally straighten and measure the distance for each of four distinguishable regions of the Ab10 chromosome (Figure 2; Dawe *et al.* 1994; Hiatt and Dawe 2003). A *t*-test assuming unequal variances was used to compare Df(L) and *smd3* with the progenitor Ab10 over each common chromosomal region.

The acentric rescue assay for neocentromere activity and recombination effect: The assay to quantify neocentromeric activity and recombination effect requires that the Ab10 genotypes be tested in plants that are heterozygous for an inversion on chromosome 3 (Inv3a) and homozygous for the knob within the inversion (K3L; Rhoades and Dempsey 1953; Dawe and Cande 1996). The crosses shown below generated the necessary offspring, which were analyzed cytologically at meiosis I for recombination and neocentromeric activity (where *Df** indicates the chromosomes Df(I), Df(F), Df(H), Df(K), or Df(L), and N3 indicates a normal chromosome 3).

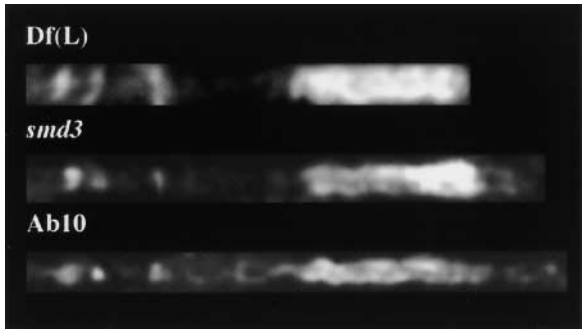

Figure 2.—Computationally straightened Ab10 and Ab10 derivatives. The progenitor Ab10 and the *smd3* chromosomes are structurally indistinguishable. The Df(L) chromosome lacks the distal euchromatic tip.

$$\text{Cross 1: } \frac{r\ smd3}{Rst\ \text{N10}}\ \frac{\text{N3}}{\text{K3L N3}} \times \frac{R\ \text{Ab10}}{Rnj\ \text{N10}}\ \frac{\text{Inv3a K3L}}{\text{Inv3a K3L}}$$

$$\text{Cross 2: } \frac{R\ Df^*}{Rst\ \text{N10}}\ \frac{\text{N3}}{\text{K3L N3}} \times \frac{R\ \text{Ab10}}{Rnj\ \text{N10}}\ \frac{\text{Inv3a K3L}}{\text{K3L N3}}$$

These crosses provide the positive control (*R* Ab10/*Rst* N10), the negative control (*Rnj* N10/*Rst* N10), and the experimental material (*r smd3* or *R Df**/*Rnj* N10) as full sibs (thereby controlling for genetic background). To differentiate between the *R* Ab10/*Rst* N10 positive control and *R* Ab10/*r smd3* (cross 1) or *R* Ab10/*r Df** (cross 2) genotypes, plants were maintained after meiocyte removal and self or testcrossed to confirm the genotype. Inv3a and K3L were scored cytologically (Figure 3). The inversion can be identified by the presence of dicentric bridges at anaphase I, and K3L can be identified by hybridizing the cells with probes for the 180-bp or the TR-1 knob repeat sequence (Yu *et al.* 1997; Hiatt *et al.* 2002). When the inversion is heterozygous and the knob is homozygous, all the acentric fragments are expected to stain positive for the knob. In practice, ~90% of the acentric fragments stain positive for the knob in homozygous K3L strains (the remaining 10% of the fragments are presumably from other breakage events in the cell). In heterozgyous K3L plants, ~60% of the fragments stain positive for the knob.

Anaphase I cells were stained with 4′,6-diamidino-2-phenylindole and assigned to one of four categories: (1) nonrecombinant (no bridges or fragments); (2) recombinant with bridge and fragment present; (3) recombinant with bridge and rescued fragment (no fragment observed, assumed to be pulled to a pole); and (4) recombinant with fragment only (rare class resulting from a specific double crossover; see Burnham 1962). For quantification of neocentromeric activity, the anaphase I cells with a bridge present were divided into two groups: fragment absent (rescued) and fragment present. To calculate the percentage of rescue, the number of cells with the fragment absent was divided by the total number of cells with bridges. For quantification of recombination, all cells in anaphase I were counted and separated into two groups:

nonrecombinant and recombinant (cells with bridges and/or fragments). The percentage of recombination was obtained from the number of recombinants divided by the total number of anaphase I cells. The proportions were compared between mutants and their corresponding positive (Ab10) and negative (N10) controls using a *Z*-statistic (Hiatt 2000).

Complementation tests: For complementation tests where both forms of Ab10 contained the large knob [*e.g.*, *smd3* and Df(L)], a marker on another chromosome was required. However, if one of the versions of Ab10 used [*e.g.*, Df(K)] did not include the large knob, then the *R* locus could be used as a marker. We measured the effects of *smd1*, *smd3*, and Df(L) on a large knob at the end of the small arm of chromosome 9 (K9S). A terminal deficiency of chromosome 9 known as *white deficiency* (*wd*) indicated the presence of K9S (Kikudome 1959). K9S is distal to *wd*, so there is no recombination between the knob and *wd*. Plants with various chromosome 10 backgrounds and heterozygous for K9S/*wd* were testcrossed to *wd*/*wd* males (*wd* homozygotes can be grown to maturity by covering the deficiency with a ring chromosome carrying *Wd*). Drive was measured in the progeny by comparing the number of *Wd*/*wd* (green seedlings) to *wd*/*wd* (white seedlings). We also tested to make sure that the *wd* deficiency was fully transmitted through the female in our stocks. When heterozygous with *wd*, N9 segregated at 52.9 ± 2.9% (n = 612 seedlings from 13 ears in an Ab10 background) and K9S segregated at 50.9 ± 5.2% (n = 2078 seedlings from 23 ears in a N10 background).

In complementation and dominant/recessive tests, pairwise comparisons between relevant groups were used to compare the segregation ratios (level of drive) of the different genotypes. Crosses were performed at different times with positive (Ab10/N10, Ab10/Ab10) and negative (N10/N10) controls present in each set of crosses. Data from the same genotype but different crossing episodes were first compared by chi-square analysis; if there were no significant differences, the data were combined. A *Z*-statistic was used for pairwise comparisons to appropriate control crosses with significance determined at the 0.01 probability level (Hiatt 2000).

RESULTS

Isolation and characterization of Df(L): *Df (L) causes a complete loss of meiotic drive:* The Df(L) derivative of Ab10 was identified among the progeny of a cross designed to recover mutations of meiotic drive (Dawe and Cande 1996). As a marker in the mutant screen and in subsequent tests, we used the *R* gene, which is linked to the Ab10 structural polymorphism by ~2 MU (Rhoades 1942). Plants of the constitution *r* Ab10/R N10 and carrying an active family of *Robertson's mutator* transposable elements (Chandler and Hardeman 1992) were open pollinated by plants homozygous for *Rst* (an allele distinct from both *r* and *R*; see materials and methods). Since Ab10 was heterozygous in the females of this cross, all except rare mutant individuals demonstrated meiotic drive for the *r* allele. This same screen was performed in four separate seasons from 1991 to 1999 and produced a frequency of meiotic drive mutations in the range of 0.08% (Hiatt and Dawe 2003). On the original ear containing the Df(L) chromosome, 55% (93/168) of the kernels carried the *r* allele linked to Ab10. These *r*/*Rst* progeny were planted and again testcrossed, this time to plants carrying the N10 chromosome linked to *Rnj*, which can be readily distinguished from the *Rst* allele. An average of 47.4% of the progeny carried the *r* allele in this series of crosses, indicating that Df(L) not only abolished meiotic drive, but also reduced segregation of the Ab10 chromosome to levels below Mendelian expectations (Hiatt and Dawe 2003). Additional Df(L) isolates have also been found (DfL-2 and DfL-3; Hiatt and Dawe 2003), but only the initial Df(L) isolated was used in the studies described here.

Df(L) maps genetically to Ab10: To verify that the Df(L) mutation maps to Ab10, we made use of the Ab10 deficiency Df(C), which is not transmitted through the male (Rhoades and Dempsey 1985; Dawe and Cande 1996). In a testcross where *R* Df(C)/*r* Df(L) is the male, the only *R*-carrying kernels transmitted will be recombinants between *R* and the breakpoint of Df(C). If the lesion responsible for suppression of meiotic drive on Df(L) maps distal to the Df(C) breakpoint, all the progeny of the cross will fail to show meiotic drive when tested in the next generation. Consistent with this expectation, all of 36 such recombinants showed <49% colored kernels when testcrossed. The average segregation of the *R* Df(L) chromosome in these crosses was 44.3% (n = 10,957 kernels from 36 ears), which is consistent with our earlier estimates of Df(L) segregation ratios (Hiatt and Dawe 2003).

Df(L) lacks the distal tip of Ab10: To determine if any cytological abnormalities were associated with the Df(L) mutation, pachytene chromosomes from Df(L)/Df(L) homozygotes were analyzed by 3D light microscopy. The Df(L) chromosomes from five different meiocytes were identified by the three chromomeres typical of Ab10, their paths through the cells modeled (Dawe *et al.* 1994), and the chromosomes computationally straightened (Figure 2). The data were compared to similar measurements from nine Ab10 progenitor chromosomes. As is readily apparent, Df(L) lacks the distal tip of the chromosome. We also found that the large knob of Df(L) was significantly smaller than the same region on progenitor Ab10, suggesting that the breakpoint may have occurred within the distal portion of the knob [the Df(L) knob was 4.8 μm ± 0.5 while the Ab10 knob was 6.0 μm ± 1.4]. The other euchromatic regions of progenitor Ab10 and Df(L) were indistinguishable by cytological measurements (see materials and methods). These cytological data, in conjunction with the genetic analysis above, suggest that meiotic drive is suppressed by a loss of genetic information in the distal tip of Ab10.

Df(L) acts in trans to suppress meiotic drive on chromosome 9: Genetic information on Ab10 acts *in trans* to cause meiotic drive at knobs located elsewhere in the genome. To determine if the gene(s) absent in Df(L) is involved in this *trans*-activity, the effects of Df(L) on the meiotic drive of a terminal knob on the short arm of chromo-

TABLE 1

Segregation of K9S as marked by *Wd* in various Ab10 backgrounds

Genotype	% K9S segregation[a]	No. of seedlings[b]
N10/N10	56.0 ± 4.7	466 (5)
N10/Df(L)	52.2 ± 8.1	498 (5)
N10/*smd3*	56.5 ± 0.1[c]	377 (2)
Ab10/*Smd1*	57.7 ± 6.9[c]	546 (4)
Ab10/*smd3*	65.5 ± 3.3[d]	744 (4)
Df(L)/*smd3*	67.7 ± 1.6[d]	554 (4)
Ab10/N10	67.6 ± 7.7[d]	1064 (8)
Ab10/Ab10	66.8 ± 9.9	734 (9)

[a] The percentage of offspring (average of multiple families) that carried the K9S chromosome as determined by a green seedling phenotype ± standard deviation.

[b] Number of seedlings counted for each genotype. Number of families represented is indicated in parentheses.

[c] Segregation in the N10/*smd3* and Ab10/*Smd1* genotypes was not significantly different ($P < 0.01$) from that in the N10 control, but was significantly different from that in the Ab10 control.

[d] Segregation in the Ab10/*smd3*, Df(L)/*smd3*, and Ab10/N10 genotypes was not significantly different ($P < 0.01$) from that in the Ab10 control, but was significantly different from that in the N10 control.

TABLE 2

Segregation ratios for the Df(K) chromosome when paired with Ab10, N10, or *smd3*

Genotype	% segregation[a]	Kernels counted
Df(K)/Ab10	30.8 ± 2.7[b]	$n = 3264$ (10)[c]
Df(K)/N10[d]	51.6 ± 3.5[b,e]	$n = 5158$ (18)
Df(K)/*smd3*	53.5 ± 5.1[b,e]	$n = 639$ (5)

[a] Meiotic drive was also measured in Ab10/N10 positive controls. Ab10 segregation was 76.9 ± 6.7% ($n = 10{,}210$ kernels on 25 ears).

[b] Segregation for the Df(K) chromosome (average of multiple ears) as marked by R ± the standard deviation.

[c] Total number of kernels counted. In parentheses are the numbers of ears from which the data were derived.

[d] Data were combined from crosses carried out in the field in 1999 and in the greenhouse in 2000.

[e] There was no significant difference ($P < 0.01$) between segregation values in Df(K)/N10 and Df(K)/*smd3* genotypes, and both were significantly different from those in Df(K)/Ab10.

some 9 (K9S) was measured. Plants heterozygous for K9S/*wd* and heterozygous for Df(L), heterozygous for Ab10 (positive control), or homozygous for N10 were testcrossed to *wd/wd* N10/N10 plants. As shown in Table 1, the results indicate that while Ab10 induces significant levels of meiotic drive at K9S, Df(L) does not. These data indicate that a factor(s) required for Ab10 to induce meiotic drive *in trans* is missing from the Df(L) chromosome. Since Df(L) lacks a relatively large segment of the Ab10 chromosome, we will refer to the factor(s) present there as the distal tip function.

Identification and characterization of *smd3*: *smd3 causes a complete loss of meiotic drive:* The meiotic drive mutation *smd3* was recovered in the same screen used to identify Df(L). Similar to Df(L), the original ear from the *smd3* mutant showed an *r* segregation ratio of 42.9% ($n = 308$ kernels). The heritability of the *smd3* was then verified over three generations by crossing it alternately to either *Rnj* or *Rst* to track the segregation of *r*. When the data for all three years were averaged, the segregation of *smd3* was 45% ($n = 13{,}222$ kernels from 44 ears), a value that was significantly less than the Mendelian expectation of 50%. We never observed a crossover between *r* and the *smd3* phenotype in these experiments, suggesting that *smd3* is located in the distal portion of Ab10.

The Ab10 chromosome in smd3 strains is cytologically indistinguishable from progenitor Ab10: Seven Ab10 chromosomes from *smd3/smd3* plants were computationally straightened (one is shown in Figure 2) and compared to nine straightened progenitor Ab10 chromosomes. At the resolution afforded by the light microscope, we detected no significant length differences between the Ab10 chromosomes in progenitor and in *smd3* mutant strains. We cannot rule out the possibility that a small interstitial or terminal deletion may be present.

smd3 acts in trans and complements Df(L): To determine whether *smd3* identifies a function different from that/those absent on the Df(L) chromosome, we again made use of K9S and its closely linked *wd* marker. Control crosses demonstrated that *smd3* lacks the ability to *trans*-activate meiotic drive at K9S and that *smd3* is recessive to progenitor Ab10; *i.e.*, the segregation of K9S in the *smd3*/Ab10 and Ab10/N10 or Ab10/Ab10 backgrounds was indistinguishable (Table 1). When *smd3* was made heterozygous with Df(L), we also observed meiotic drive on chromosome 9S that was indistinguishable from that observed in the Ab10/N10 and Ab10/Ab10 controls. We did not test directly whether Df(L) is recessive to Ab10, although the facts that Df(L) is a large deletion and that it fully complements *smd3* strongly suggest that it is. Taken together, these data indicate that the two mutations complement each other and that the lesion responsible for the *smd3* phenotype maps proximal to the Df(L) breakpoint.

smd3 maps distal to the Df(K) breakpoint on Ab10: To further refine the map position of *smd3* we carried out complementation tests with Df(K), a derivative that lacks the large knob with a breakpoint just proximal to the *Sr2* locus (Figure 1). When paired with N10, Df(K) segregated in a roughly Mendelian fashion; *i.e.*, it was segregated to ~50% of the progeny (Table 2). However, when Df(K) was paired with Ab10, meiotic drive of Ab10 caused Df(K) segregation to be significantly <50% (Table 2). We reasoned that if *smd3* maps distal to the Df(K) breakpoint, the results of a Df(K)/*smd3* testcross would

be similar to the results of a Df(K)/N10 testcross; *i.e.*, they would provide no evidence for meiotic drive. In contrast, if Df(K) complemented *smd3*, the results would be similar to a Df(K)/Ab10 testcross (since the SMD3 product acts *in trans*). Consistent with the former expectation, Df(K) segregation was indistinguishable when paired with either N10 or *smd3* (Table 2). We conclude that *smd3* does not complement Df(K) and therefore maps to a region distal to the Df(K) breakpoint. These data, combined with the K9S segregation data (Table 1), indicate that *smd3* lies within the region bounded by the Df(K) and Df(L) breakpoints.

Smd1 is a dominant mutation and so cannot be used in complementation tests with smd3: The *smd1* mutation was described previously as a meiotic drive defect with a corresponding decrease in neocentromeric activity (Dawe and Cande 1996). Although the phenotypes of *smd1* and *smd3* differ with respect to neocentromeric activity, it remains possible that *smd3* is an allele of *smd1*. We initiated the complementation test by first determining whether *smd1* is recessive to Ab10 with respect to its *trans*-effect on K9S. Surprisingly, we found that the *smd1*/Ab10 heterozygote gave results that were indistinguishable from the N10/N10 negative control and significantly different from the Ab10/N10 positive controls (Table 1). These data suggest that *smd1* is a dominant mutation and as a result cannot be used in complementation tests. In recognition of this new observation, in further discussions we will refer to *smd1* as *Smd1*, to indicate that the mutation shows evidence of dominance.

Use of Inv3a to localize regions encoding the recombination effect and neocentromere activity: Df(L), *smd3*, and four other Ab10 deficiencies [(Df(I), Df(F), Df(H), and Df(K)] were analyzed for neocentromere activity and the recombination effect using a previously described assay involving Inv3a (Dawe and Cande 1996). Inv3a is a well-characterized paracentric inversion that covers >50 MU of the long arm of chromosome 3 (Rhoades and Dempsey 1953). Within the region bounded by the inversion is a large knob known as K3L, which is composed of both 180-bp repeats and TR-1 repeats (Figure 3). When recombination occurs within the inverted portion of an Inv3a/N3 heterozygote, a dicentric chromosome and acentric fragment are formed at meiosis I (Figure 3). Ab10 causes a significant increase in recombination levels in and around Inv3a (Rhoades and Dempsey 1953) such that the recombination effect can be measured by scoring for the number of dicentric bridges and/or acentric fragments. The same strains can be used to measure neocentromere activity. In normal maize strains, the acentric fragment is generally lost in the cytoplasm, whether or not it contains a knob. However, when the acentric fragment carries a knob and Ab10 is present, many of the fragments interact with the spindle and move poleward (Rhoades and Dempsey 1966; Dawe and Cande 1996). In homozygous Ab10 strains, nearly 100% of the fragments are "rescued" and pulled poleward, while in Ab10 heterozygotes the number is closer to 50% (Dawe and Cande 1996). By using this "bridge and fragment assay" in cells carrying Ab10, we were able to quantify the effects of each mutation on recombination and neocentromere activity.

The bridge and fragment assay requires that Inv3a be heterozygous and that K3L be homozygous to ensure that the fragment contains a knob. So that we could best interpret the results, we made crosses where positive control (Ab10/N10), negative control (N10/N10), and experimental material (*smd3*/N10 or Df*/N10) were segregating on individual ears carrying Inv3a and K3L (see materials and methods). The different chromosome 10 constitutions were identified using linked R alleles (Figure 3). The negative controls provide estimates of normal recombination levels within Inv3a and of the frequency of fragments that were hidden from view or may have migrated to the pole by chance. The positive controls provided estimates of the recombination effect and acentric rescue expected from the progenitor Ab10 chromosome. This strategy effectively controls for genetic background, a factor that was not considered in previous studies of the Ab10 deficiencies (Emmerling 1959; Miles 1970).

The breakpoints of the five deficiencies used in the bridge and fragment assay are shown in Figure 1. The most severe deficiency assayed was Df(I), which lacks most of the central euchromatin as well as the large knob and distal tip. Df(F), Df(H), and Df(K) are each deficient for less of the chromosome, and as described above, Df(L) lacks only the distal tip. The *smd3* mutation was also analyzed in the bridge and fragment assay with a cross that differed slightly from the one shown in Figure 3 (see materials and methods). The results for each mutation, along with corresponding negative and positive controls, are shown in Tables 3 and 4. Our control data were consistent with previous reports (Rhoades and Dempsey 1966; Dawe and Cande, 1996) showing that Ab10 causes significant increases in recombination over the Inv3a interval and roughly threefold increases in the rescue of acentric fragments when compared to N10 controls [the sole exception was in the Df(I) data set, where the Ab10 and N10 recombination data were similar].

Each of the four previously published deficiencies [Df(I), (F), (H), and (K)] appeared to have full neocentromere activity but to lack the recombination effect characteristic of Ab10. These data are consistent with previous observations suggesting that the recombination effect maps distal to the Df(H) breakpoint (Rhoades and Dempsey 1989) but proximal to a breakpoint within the large knob (Miles 1970) and with data from Hiatt *et al.* (2002) suggesting that a TR-1-activating neocentromere-inducing factor(s) is located proximal to the Df(I)

breakpoint. Our data also provide strong support for the suggestion made previously (Hiatt *et al.* 2002) that the TR-1-activating factor is sufficient to mobilize TR-1-containing knobs to a pole. Unexpected, however, were the observations that neither Df(L) nor *smd3* had any significant effect on recombination or acentric rescue (*i.e.*, they were indistinguishable from the Ab10 positive control), despite the fact that both completely abolished meiotic drive. As discussed below, these data, along with our mapping results, provide evidence for additional genetic complexity in the Ab10 meiotic drive system.

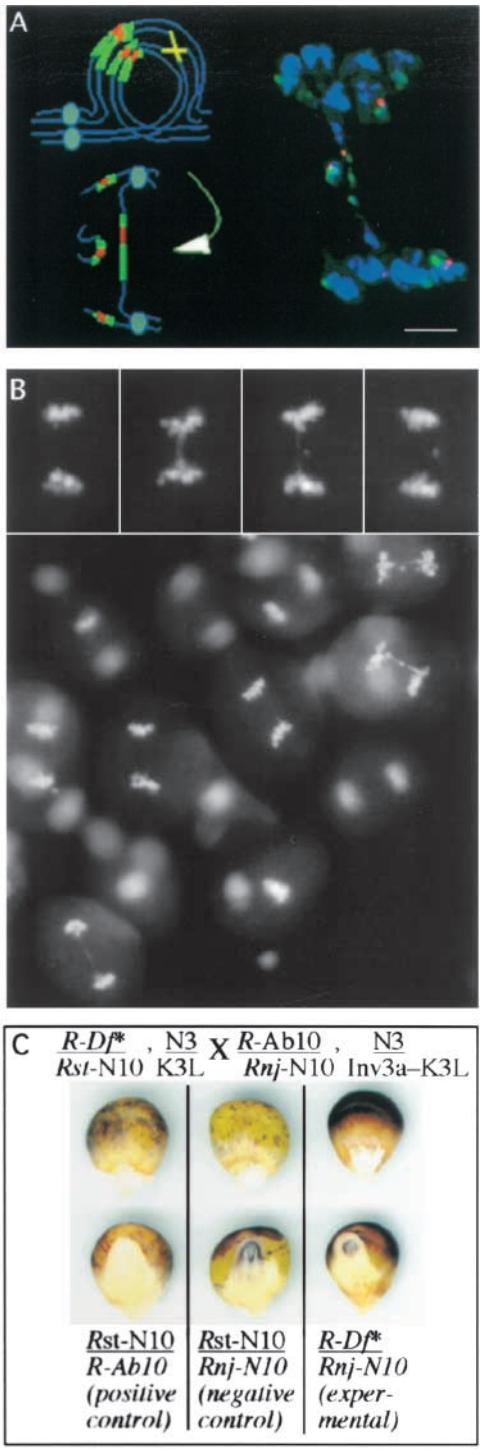

Figure 3.—The bridge and fragment assay. (A) Recombination within the inversion loop formed in an Inv3a/N3 heterozygote that is homozygous for K3L. (Left) The cytological consequences at anaphase I. (Right) An actual anaphase I figure, showing the bridge and fragment. *In situ* hybridization with the 180-bp knob repeat (green) and the TR-1 knob repeat (red) demonstrates that both are present in K3L. Bar, 5 μm. (B) Anaphase figures scored in the bridge and fragment assay. (Top) Four classes of anaphase I figures. (Left to right) No recombination; recombinant showing bridge but no fragment (fragment rescued); recombinant showing bridge and fragment (fragment not rescued); recombinant showing fragment but no bridge due to complex recombination event (fragment not rescued). (Bottom) A full field of anaphase I cells. (C) One of the crosses used in the assay and how genotypes were chosen on the basis of kernel phenotype (see materials and methods). The front and back of each of three kernels are shown.

DISCUSSION

The preferential segregation mediated by Ab10 in maize is one of the clearest examples of meiotic drive caused by modifications of chromosome segregation at meiosis (Novitski 1967). That any system has evolved to "beat Mendel's rules" via direct effects on the behavior of chromosomes is indeed remarkable, since chromosome segregation is one of the most highly regulated processes in the cell, replete with redundancies and backup mechanisms of many forms (Nicklas 1997). One might anticipate that a process evading the checks and balances on chromosome segregation would be complex and multigenic, and our data provide several lines of new evidence that this is the case. In addition to more precisely mapping two previously known phenotypes associated with Ab10, neocentromere activity and the recombination effect, we provide evidence for two additional factors. Each of the four functions can be differentiated from each other by either map position or phenotype. Interestingly, as shown in Figure 4, the loci responsible for drive appear to lie in novel portions of the Ab10 chromosome, *i.e.*, outside of the central euchromatic domain that shows strong homology to N10. Further, much of the information required for meiotic drive—the recombination effect, the product of *smd3*, and the distal tip function—maps distal to the Df(K) breakpoint, a region that is known to contain few genes essential for plant viability (Hiatt and Dawe 2003). In the following sections we discuss the evidence for each of the proposed meiotic drive functions as well as their potential roles in manipulating the movement

TABLE 3

Quantification of neocentromeric activity

Plant genotype	Deficiency background[a] (%) Df(I)	Df(F)	Df(H)	Df(K)	Df(L)	*smd3*[a]
N10 (negative control)	09.6[b] ± 8.4	14.1 ± 2.1	15.7 ± 5.6	15.6 ± 4.1	14.7 ± 2.8	14.8 ± 9.2
	n = 166 (4)[c]	n = 184 (2)	n = 108 (3)	n = 122 (2)	n = 258 (3)	n = 108 (5)
Deficiency or *smd3*	40.8 ± 16.4	59.6 ± 23.6	53.0 ± 8.1	41.1 ± 18.9	44.7 ± 5.4	47.5 ± 23.3
	n = 279 (3)	n = 171 (2)	n = 249 (3)	n = 56 (3)	n = 152 (2)	n = 120 (4)
Ab10 (positive control)	45.8 ± 12.7	56.6 ± 14.7	50.9 ± 17.5	45.6 ± 6.4	44.1 ± 6.8	53.4 ± 14.8
	n = 280 (3)	n = 106 (2)	n = 228 (4)	n = 92 (2)	n = 204 (2)	n = 191 (3)

[a] There were no significant differences ($P < 0.01$) between Df(I), Df(F), Df(H), Df(K), Df(L), or *smd3* when compared to their corresponding positive controls.

[b] Percentage of anaphase I cells (average of multiple plants) with bridges in which the fragment was absent ± the standard deviation.

[c] The number of anaphase I cells observed. In parentheses is the number of individual plants used to harvest meiocytes.

and placement of chromosomes during female gametogenesis.

A major determinant of neocentromere activity maps proximal to the Df(I) breakpoint: There is little doubt that neocentromeres are required for meiotic drive in maize (Rhoades 1952; Dawe and Cande 1996), but the genetic basis for this activity remains poorly understood. Recent data suggest that genetic redundancy is built into the system by the presence of two forms of the knob satellite repeats, the 180-bp and TR-1 repeats. A previous analysis of two deficiencies [Df(I) and Df(K)] suggests that the TR-1 repeat is mobilized by information proximal to the Df(I) breakpoint and that the 180-bp repeat is mobilized by information distal to the Df(K) breakpoint, perhaps in concert with the factors required to mobilize the TR-1 repeat (Hiatt *et al.* 2002). Here we used an assay different from the one described in Hiatt *et al.* (2002) to delimit the regions of Ab10 responsible for neocentromere activity. The bridge and fragment assay measures the effects of Ab10 and its derivatives on the motility of a single knob on chromosome 3L that contains both TR-1 and 180-bp repeats (Figure 3). We show that the progressive loss of portions distal to the Df(I) breakpoint have no discernible effects on the motility of the knob (Table 3), indicating that the factor encoded in the Df(I)-proximal region is sufficient to move a knob poleward. These results support our previous data and indicate that the 180-bp-specific factor distal to the Df(K) breakpoint is not required for neocentromere activity of mixed knobs such as the K3L knob used here. We cannot yet determine whether the information proximal to the Df(I) breakpoint mobilizes

TABLE 4

Quantification of Ab10 recombination effect

Plant genotype	Deficiency background (%) Df(I)[a]	Df(F)	Df(H)	Df(K)	Df(L)[b]	*smd3*[b]
N10 (negative control)	44.9[c] ± 17.7	35.7 ± 7.0	31.2 ± 4.0	36.8 ± 13.7	37.5 ± 8.3	43.7 ± 23.7
	n = 392 (4)[d]	n = 586 (2)	n = 407 (3)	n = 391 (2)	n = 730 (3)	n = 270 (5)
Deficiency or *smd3*	34.3 ± 7.2	26.5 ± 2.0	28.3 ± 2.1	25.4 ± 6.3	44.7 ± 9.9	66.4[e] ± 2.3
	n = 869 (3)	n = 698 (2)	n = 963 (3)	n = 236 (3)	n = 374 (2)	n = 206 (4)
Ab10 (positive control)	45.8 ± 3.0	48.0 ± 4.9	46.3 ± 5.6	53.1 ± 0.8	43.8 ± 4.4	62.3[e] ± 7.5
	n = 662 (3)	n = 244 (2)	n = 600 (4)	n = 192 (2)	n = 513 (2)	n = 332 (3)

[a] The positive and negative controls for Df(I) are not significantly different ($P < 0.05$).

[b] There was no significant difference ($P < 0.05$) between Df(L) or *smd3* when compared to their corresponding Ab10 positive controls and both were significantly different from their corresponding N10 controls. All other deficiencies [(Df(I), (F), (H), and (K)] were significantly different from their corresponding Ab10 controls.

[c] Percentage of anaphase I cells (average of multiple plants) showing a bridge and/or fragment ± the standard deviation.

[d] The number of anaphase I cells observed. In parentheses is the number of individual plants used to harvest meiocytes.

[e] Data from each Ab10 derivative were compared only to positive and negative control values from full siblings (*i.e.*, to data in the same column). The apparent difference in recombination effect between the *smd3* line and the deficiency lines may be the result of genetic background and/or differences in how the data were scored. All data in the *smd3* column were collected by E.N.H. while the remaining data were collected by R.K.D.

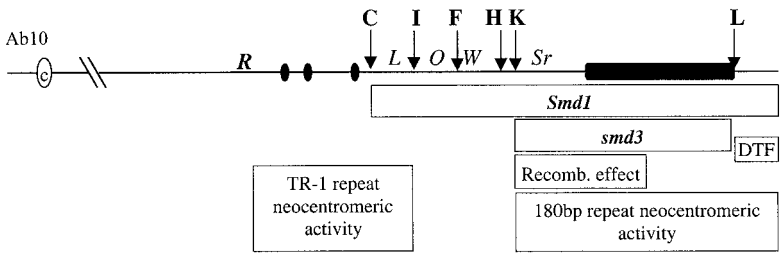

FIGURE 4.—Schematic diagram of Ab10 showing the regions associated with known meiotic drive functions. Arrows above the Ab10 diagram indicate deficiency breakpoints. The *Smd1* locus is distal to the Df(C) breakpoint (DAWE and CANDE 1996). Complementation tests localize *smd3* to a region between the Df(K) and Df(L) breakpoints. The gene(s) necessary for neocentromere activity of TR-1 repeat arrays is located proximal to the Df(I) breakpoint (HIATT *et al.* 2002). There is evidence for a second neocentromeric activity gene(s), distal to the Df(K) breakpoint, that is involved in activating neocentromeres at 180-bp repeat arrays (HIATT *et al.* 2002). In the absence of the chromatin distal to the Df(L) breakpoint, meiotic drive is abolished; this factor (or factors) is referred to as the distal tip function and indicated here as DTF. The recombination-enhancing locus (Recomb. effect) has been mapped to a region distal to the Df(K) breakpoint (Table 4) and proximal to a point somewhere near the center of the knob (MILES 1970).

only the TR-1-containing portion of the knob or also binds to and helps to mobilize the 180-bp-containing portion of the knob.

The recombination-enhancer locus and its role in meiotic drive: Our analysis of five deficiencies by the bridge and fragment assay provides strong evidence that the recombination-enhancer locus originally identified by RHOADES and DEMPSEY (1966) maps to a region between the breakpoints of Df(K) and Df(L). Since Ab10-encoded recombination enhancement affects many regions of the maize genome (RHOADES and DEMPSEY 1966; ROBERTSON 1968; NEL 1973), it is likely to be a *trans*-acting factor encoded in the gene-containing euchromatic portion of this interval. This interpretation is supported by the work of MILES (1970), who showed that a deficiency retaining less than half of the large knob (called KV) appeared to retain the recombination enhancement typical of Ab10 (Figure 4).

Since the effects of Ab10 on recombination are well documented and it is known that recombination between centromeres and knobs is required for meiotic drive (RHOADES and DEMPSEY 1966), a logical assumption is that recombination enhancement is required for maximum meiotic drive. The positive effect on recombination does appear to enhance meiotic drive on the short arm of chromosome 9. KIKUDOME (1959) demonstrated a severe reduction in recombination on this chromosome when the plant was heterozygous for the large terminal knob K9S. On the standard maize genetic map, the markers *wx1* and *wd1* are separated by 49 MU, with *wd1* at the end of the chromosome and *wx1* only 5 MU from the centromere. In the presence of a large K9S knob, the *wx1-wd1* distance was measured by Kikudome at only 12.7 MU in the absence of Ab10, but 30.3 MU in the presence of Ab10. The effects were less pronounced when a small knob was heterozygous, with the increase being from 26.9 to 31.5 MU. The data suggest that Ab10 can partially reverse a severe reduction in recombination caused by knob heterozygosity. Therefore, in the case of the large knob on chromosome 9, the frequency of heteromorphic dyads (and, by inference, the effectiveness of meiotic drive) is increased significantly by the recombination effect encoded by Ab10 (Table 4). Whether or not a suppression of recombination on chromosome 10 is also relieved by the recombination-enhancing locus cannot be determined, since all the derivatives that lack the recombination effect also lack the large knob. Nevertheless, the available data suggest that the recombination-enhancer locus evolved as a component of the meiotic drive system to relieve a suppression in recombination caused by the large size of the knobs that serve as sites of neocentromere activity.

Two additional functions map to different intervals: one proximal and one distal to the large knob: Two newly identified mutations of Ab10, Df(L) and *smd3*, both have stable losses of meiotic drive but apparently unaffected neocentromeric activity and recombination effect. The similarity in phenotype between *smd3* and Df(L) prompted us to consider whether they represented defects in the same gene. We were surprised to find that the two mutations complement each other, with the Df(L) defect lying distal to the large knob and *smd3* mapping in the proximal Df(K)–Df(L) interval. Although *smd3* maps to the same interval as the recombination-enhancer locus, *smd3* causes no measurable reduction in the recombination effect, suggesting that it identifies a new function in this region.

The *smd3* mutation is the second cytologically undetectable *suppressor of meiotic drive* to be characterized. The previously described *Smd1* mutation has a variable reduction in meiotic drive that correlates with a reduction in neocentromeric activity (DAWE and CANDE 1996). Data presented here indicate that *Smd1* is dominant. The *smd3* mutation, on the other hand, is recessive and has a stable loss of meiotic drive with no reduction in neocentromeric activity or the recombination effect. The different phenotypes of *Smd1* and *smd3* suggest that they are different genes with different functions. However, because *Smd1* is dominant we have been unable to demonstrate that the two mutations are nonallelic.

What might *smd3* and the distal tip function(s) be carrying out? One possibility is that one or both func-

tions promote neocentromere activity. Supporting this view is the fact that the 180-bp-specific neocentromere factor (Hiatt *et al.* 2002) maps distal to Df(K), where both *smd3* and Df(L) are located. However, neocentromere activity is a genetically redundant process involving at least two different classes of repeats. The impairment of 180-bp-activating gene(s) would be unlikely to cause the complete losses of meiotic drive observed in Df(L) and *smd3* strains, especially since the TR-1 activating region has been shown to be sufficient to mobilize mixed knobs (containing both repeats) by two independent measures (Hiatt *et al.* 2002; Table 3). Nevertheless, we cannot rule out the possibility that *smd3* and/or the distal tip function(s) affect the motility of 180-bp repeats, since the K3L knob contains both 180-bp and TR-1 repeats (Figure 3A).

Another likely function is the maintenance of knob orientation between meiosis I and II that helps to ensure that knobbed chromatids are directed toward the basal megaspore. In their last article on Ab10, Rhoades and Dempsey (1990) postulated such a function and carried out an experiment to test its existence. They made use of the fact that in Ab10/+/+ trisomics not all of the resulting meiosis II cells receive the Ab10 chromosome. By measuring the preferential segregation of a marker linked to K9S, the authors produced data supporting the view that the Ab10 chromosome must be present in the second division daughter cell to provide a factor(s) that allows for the completion of meiotic drive. This factor is not likely to be neocentromere activity, because the protein responsible for neocentromere activity is not cell limited (neocentromeres form in a meiosis II daughter cell that has lost Ab10 by segregation in meiosis; Hiatt 2000).

Likely candidates for proteins that maintain knob orientation are those that mediate the interaction of knobs with the nuclear envelope. The available data on chromatin-nuclear envelope interactions in plants suggest that a unique set of proteins are involved (Gindullis *et al.* 1999). However, at least a subset of the factors known to mediate interactions between chromatin and the nuclear envelope in animals, such as heterochromatin protein 1 (HP1), are conserved in flowering plants (Gaudin *et al.* 2001). Interestingly, in animals, HP1 interacts with the nuclear envelope via a soluble form of tubulin (Kourmouli *et al.* 2001), suggesting a potential mechanism for the interaction of the knobs with the nuclear envelope as well as the microtubules that are thought to guide knobs to the basal megaspore (Hiatt and Dawe 2003). The products encoded by *smd3* or the distal tip function(s) may be involved in facilitating or in maintaining these interactions.

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