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The Role of Centromere Alignment in Meiosis I Segregation of Homologous Chromosomes in *Saccharomyces cerevisiae*

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ABSTRACT

During meiosis, homologous chromosomes pair and then segregate from each other at the first meiotic division. Homologous centromeres appear to be aligned when chromosomes are paired. The role of centromere alignment in meiotic chromosome segregation was investigated in *Saccharomyces cerevisiae* diploids that contained one intact copy of chromosome *I* and one copy bisected into two functional centromere-containing fragments. The centromere on one fragment was aligned with the centromere on the intact chromosome while the centromere on the other fragment was either aligned or misaligned. Fragments containing aligned centromeres segregated efficiently from the intact chromosome, while fragments containing misaligned centromeres segregated much less efficiently from the intact chromosome. Less efficient segregation was correlated with crossing over in the region between the misaligned centromeres. Models that suggest that these crossovers impede proper segregation by preventing either a segregation-promoting chromosome alignment on the meiotic spindle or some physical interaction between homologous centromeres are proposed.

DURING meiosis I prophase, homologous chromosomes pair and undergo crossing over to form bivalents. At later stages, the bivalents attach to the spindle and the homologues segregate from each other to reduce the number of chromosomes by half. Following pairing, homologous centromeres appear to be aligned (Scherthan *et al.* 1992). In some higher organisms, centromeres on each homologue appear to be both aligned and oriented in opposite directions. This orientation is believed to be important for spindle attachment and the subsequent segregation of the homologues (Nicklas 1997). The role of centromere alignment in meiosis I is not known. However, this alignment could be important in facilitating meiosis I segregation.

There have been several attempts to investigate the role of centromere alignment in meiosis of *Saccharomyces cerevisiae.* Koller *et al.* (1996) found that an ~15-kbp insertion adjacent to *CEN15* had a small, but noticeable, effect on that chromosome's ability to segregate from two homologous normal copies of chromosome *XV* in a trisomic strain. Their results suggested that perturbing centromere alignment might affect segregation. In other experiments, centromeres were misaligned by translocation, and meiotic segregation was examined in heterozygous diploids containing one pair of homologues with one translocated and one normal centromere. The results indicated that misaligning centromeres by 20–25 kbp had no observable effect on segregation (Surosky and Tye 1988; V. Guacci and D. Kaback, unpublished results). Unfortunately, the effect of larger misalignments could not be studied because crossovers in the region between the misaligned centromeres produced dicentric and acentric chromosomes that could not be followed during meiosis.

Chromosome bisections may be useful for examining the effect of larger centromere misalignments on meiotic chromosome segregation. *S. cerevisiae* chromosomes can be bisected into functional chromosome fragments using homologous recombination with small linear centromere-containing plasmids (Zakian *et al.* 1986; Guacci and Kaback 1991). This method places a new centromere on one of the two fragments close to the point of bisection. Bisection at the centromere produces fragments with centromeres that are both aligned with the centromere on an intact copy of that chromosome. Bisection in the middle of a chromosome arm produces fragments where one centromere is misaligned and one is aligned with the centromere on an intact copy of the chromosome. Bisections of chromosome *I* at its centromere and at two sites on the left arm have been described (Guacci and Kaback 1991; Kaback *et al.* 1999). Studies on haploids containing each of these bisections and on diploids homozygous for each of these bisections indicated that the fragments behaved as functional chromosomes and segregated with relatively high efficiency during mitosis and meiosis I and II (Guacci and Kaback 1991; Kaback *et al.* 1992, 1999). Studies on diploids that were heterozygous for another centromere bisection chromosome, where both centromeres were aligned with the centromere on the intact chromosome, indicated that both fragments efficiently segregated away from

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the intact chromosome during meiosis I (Zakian *et al.* 1986).

To investigate the role of centromere alignment in meiosis I, segregation was studied in heterozygous diploid strains of *S. cerevisiae* containing one copy of chromosome *I* bisected into two functional fragments and one intact copy of chromosome *I.* The centromere of one fragment was aligned with the centromere on the intact homologous chromosome, while the location of the centromere on the other fragment was either aligned or misaligned by 50 or 100 kbp. In these constructs, meiotic crossing over in the region between misaligned centromeres does not produce dicentric and acentric chromosomes, making it possible to follow segregation and analyze the role of centromere alignment in meiosis I segregation. The results indicated that misligned centromeres did not promote normal segregation.

MATERIALS AND METHODS

Growth and genetic analysis: Strains used in this study are listed in Table 1. Growth and sporulation media were described previously (Rose *et al.* 1990). Ura3$^-$ colonies were selected using synthetic medium containing 5-fluoro-orotic acid (5-FOA; Boeke *et al.* 1984). Tetrad analysis of chromosome *I* markers was carried out as described previously (Rose *et al.* 1990; Kaback *et al.* 1992, 1999). Partial disomy was analyzed by plating strains on 5-FOA medium to select for colonies that lost the *URA3*-marked chromosome fragment and scoring the resultant colonies for uncovered recessive markers. Statistical significance was determined by chi-square tests. Unless mentioned otherwise, *P* is the probability that results were due to chance.

Recombinant plasmids and yeast transformation: Recombinant plasmids were prepared by standard protocols (Sambrook *et al.* 1989). Plasmid YCp70 was described previously (Aguilera and Klein 1990) and is a *CEN* plasmid derived from plasmid YCp50 by substituting *LEU2* for *URA3.* Plasmids pLF237 (Barton *et al.* 1997), pLF251, and pLF278 (Su 1998) carried chromosome *I* DNA in plasmid pBS(KS)$^+$. Plasmid pCG106 (Guerra 1995) was constructed by introducing a *Not*I site between the *Nru*I and *Aat*II sites on pBR322 and introducing restriction fragments containing a *Y′* fragment (0.6-kbp *Not*I-*Eco*RI fragment), *ARS1* (0.8-kbp *Eco*RI-*Hin*dIII fragment), *ARG4* (3.0-kbp *Hin*dIII fragment), *CEN15* (4.3-kbp *Hin*dIII fragment), and another *Y′* fragment (1.6-kbp *Hin*dIII-*Not*I fragment). Yeast cells were transformed using the lithium acetate/DMSO method (Hill *et al.* 1991). Blot hybridization was carried out as described by Southern (1975) utilizing Hybond nylon membranes (Amersham Life Science, Arlington Heights, IL) and ^{32}P-labeled DNA probes produced using Multiprime (Amersham Life Science). Blots were analyzed on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Electrophoretic karyotyping: Intact chromosomal DNA was prepared as described (Rose *et al.* 1990) and separated by pulsed field gel electrophoresis (PFGE) on 1.0% (w/v) agarose in buffer containing 45 mm Tris-borate, pH 8, 1 mm EDTA using a CHEF-DR III system (Bio-Rad Laboratories, Hercules, CA). "Lambda ladder" (New England Biolabs, Beverly, MA) was used as a molecular weight standard. Electrophoresis was performed at 14° for 22 hr using 25-sec pulses at 6 V/cm at an included angle of 120°. Under these conditions, only chromosomes <500 kbp are resolved. Gels were stained for 30 min in 1.0 μg/ml (w/v) ethidium bromide. Prior to blotting, gels were irradiated with 18 mJ ultraviolet light using a Stratalinker (Stratagene, La Jolla, CA).

Translocation of the leftmost 50 kbp of chromosome *I* next to *CEN1*: 5-FOA-resistant colonies from strain JL48-1A that contains chromosome *I* bisected at YAL049 into fragments *IA*-60 and *IB*-180 were screened by PFGE and Southern blot hybridization. Homologous mitotic exchange between the *CEN1* regions of fragments *IA*-60 and *IB*-180 generated a 150-kbp translocation chromosome and a *URA3*-containing fragment that was lost. A colony (JL48-1A-F3) that contained a copy of the 150-kbp translocation and a copy of fragment *IB*-180 was identified and crossed with strain CG362-2D, which contains a copy of chromosome *I* bisected at *CEN1* into fragments *IL* and *IR* (Table 1). Spores from this cross were screened by PFGE and Southern blot hybridization to identify one containing a copy of the 150-kbp translocation chromosome (*Tx*) and a copy of a 110-kbp chromosome fragment (*IL′*). Fragment *IL′* was produced by meiotic recombination between fragments *IB*-180 and *IL* and contained the YAL049-*CEN1* interval and *URA3* (strain CG366-11C).

Construction of chromosome fragment *IA*[*CEN4 LEU2*]-60: Strain CG325-114B, which contains a copy of chromosome *I* bisected at YAL049 into fragments *IA*-60 and *IB*-180, was transformed with *Sph*I-*Hin*dIII-digested YCp70. Ura$^-$ Leu$^+$ transformants were screened by conventional agarose gel electrophoresis and Southern blot hybridization to identify one in which *CEN1 URA3* on fragment *IA*-60 was replaced by *CEN4 LEU2.*

Chromosome *I* trisection at YAL049 and *CEN1*: Diploid strain CG365, which contains chromosome fragments *IA* [*CEN4 LEU2*]-60, *IB*-180, *IL*, and *IR*, was sporulated and Ura$^+$ Leu$^+$ spores were screened by PFGE and Southern blot hybridization to identify those that carried a copy of fragments *IA* [*CEN4 LEU2*]-60, *IL′*, and *IR.* Fragment *IL′* was produced by meiotic recombination between fragments *IB*-180 and *IL* as described above. Strains containing reconstituted copies of fragment *IB*-180 from fragments *IL′* and *IR* were isolated as described previously (Guacci and Kaback 1991).

Construction of a diploid hemizygous for the YAL049-*CEN1* interval: 5-FOA-resistant colonies of diploid strain CG410 were screened by PFGE to identify one that lost fragment *IL′*.

RESULTS

Meiotic segregation of homologous chromosomes containing aligned centromeres: The meiotic behavior of a heterozygous chromosome *I* bisection where the centromeres of both fragments were aligned with the centromere of the intact chromosome was investigated. Diploid strain CG403 contains one copy of chromosome *I* bisected at its centromere into functional fragments *IL* and *IR* and one intact copy of chromosome *I* (Figure 1A). Meiosis I segregation of both fragments from the full-length chromosome produces asci that almost always contain four viable spores. Nondisjunction of one of the fragments produces asci with two viable partially disomic spores and two inviable partially nullisomic spores. The results indicated that both fragments *IL* and *IR* segregated efficiently from the intact chromosome (94.8 and 87.9%, respectively; Figure 1B). These results are similar to those obtained using other chromosomes bisected at their centromeres (Zakian *et al.* 1986; Surosky and Tye 1988; Guerra 1995).

TABLE 1

Yeast strains

Strain	Genotype	Karyotype
JL48-1A	*MATα iTRP1 [YLpLF273 URA3] trp1 his3-11,15 leu2-3,112 arg4 ura3-1*	Chromosome *I* bisection at YAL049
JL48-1A-F3	*MATα iTRP1 [Tx] trp1 his3-11,15 leu2-3,112 arg4 ura3-1*	Chromosome *I* translocation and fragment *IB*-180
CG362-2D	*MAT***a** *[YLpVG47 URA3] trp1 his3-11,15 leu2-3,112 arg4 ura3-1 met10*	Chromosome *I* bisection at *CEN1*
CG366-11C	*MAT***a** *iTRP1 [Tx] [IL′ YLpVG47 URA3] trp1 his3-11,15 leu2-3,112 arg4 ura3-1 met10*	Chromosome *I* translocation
CG325-114B	*MATα [YLpLF273 URA3] ade1 trp1 his3-11,15 leu2-3,112 ura3-1*	Chromosome *I* bisection at YAL049
CG365	*MAT***a** *0 0 CDC24 [YLpVG47 URA3] ADE1 TRP1 his3-11,15 leu2-3,112 arg4 ura3-1* *MATα iHIS3 [YLpLF273 CEN4 LEU2] cdc24 0 ade1 trp1 his3-11,15 leu2-3,112 ARG4 ura3-1*	*Chromosome I* bisections at *CEN1* and YAL049
CG386-66C	*MATα iHIS3 [YLpLF273 CEN4 LEU2] ade1 cdc24 [IL′ YLpVG47 URA3] trp1 his3-11,15 leu2-3,112 arg4 ura3-1*	Chromosome *I* trisection
CG406-131D	*MAT***a** *iHIS3 [YLpLF273 CEN4 LEU2] cdc24 [IL′ YLpVG47 URA3] trp1 his3-11,15 leu2-3,112 arg4 ura3-1*	Chromosome *I* trisection
CG406-131D-F11	*MAT***a** *iHIS3 [YLpLF273 CEN4 LEU2] cdc24 trp1 his3-11,15 leu2-3,112 arg4 ura3-1*	Chromosome *I* bisection at YAL049[a]
CG403-3C	*MATα ade1 his3-11,15 leu2-3,112 arg4 ura3-1*	Normal
CG410	CG406-131D × CG403-3C	Heterozygous chromosome *I* trisection
CG403	*MAT***a** *0 CDC24 0 [YLpVG47 URA3] ADE1 PHO11 TRP1 his3-11,15 leu2-3,112 arg4 ura3-1* *MATα iHIS3 cdc24 iARG4 0 ade1 pho11:LEU2 trp1 his3-11,15 leu2-3,112 arg4 ura3-1*	Heterozygous chromosome *I* bisection at *CEN1*
CG391	*MAT***a** *iHIS cdc24 0 0 ade1 pho11::LEU2 trp1 his3-11,15 leu2-3,112 arg4 ura3-1* *MATα 0 CDC24 [YLpVG59 URA3] iARG4 AED1 PHO11 trp1 his3-11,15 leu2-3,112 arg4 ura3-1*	Heterozygous chromosome *I* bisection at *MAK16*
CG347	*MAT***a** *0 iTRP1 0 cdc24 FUN30 ADE1 trp1 his3-11,15 leu2-3,112 arg4 ura3-1* *MATα iHIS3 0 [YLpLF273 URA3] CDC24 fun30::LEU2 ade1 trp1 his3-11,15 leu2-3,112 arg4 ura3-1*	Heterozygous chromosome *I* bisection at YAL049
CG387	CG386-66C × CG366-11C	Chromosome *I* translocation and trisection
CG410-F3	*MAT***a** *iHIS3 [YLpLF273 CEN4 LEU2] ΔYAL049;CEN1 ADE1 trp1 his3-11,15 leu2-3,112 arg4 ura3-1* *MATα 0 0 ade1 TRP1 his3-11,15 leu2-3,112 arg4 ura3-1*	Heterozygous chromosome *I* bisection in partial hemizygote[b]
CG414	CG406-131D-F11 × CG403-3C	Heterozygous chromosome *I* bisection at YAL049

[YLpLF273 URA3], *[YLpVG47 URA3]*, and *[YLpVG59 URA3]* indicate a bisected copy of chromosome *I* at YAL049, *CEN1*, and *MAK16*, respectively. *Tx* refers to the *IA*-60;*IR* translocation chromosome. *IL′* refers to a chromosome fragment containing the YAL049-*CEN1* interval of chromosome *I*. 0 refers to the absence of an inserted sequence or plasmid. *[YLpLF273 CEN4 LEU2]* indicates a copy of chromosome *I* bisected at YAL049 that contains *CEN4 LEU2* in place of *CEN1 URA3.*

[a] Contains a reconstituted copy of fragment *IB*-180.

[b] Fragment *IL′* containing the YAL049-*CEN1* region is not present in this strain.

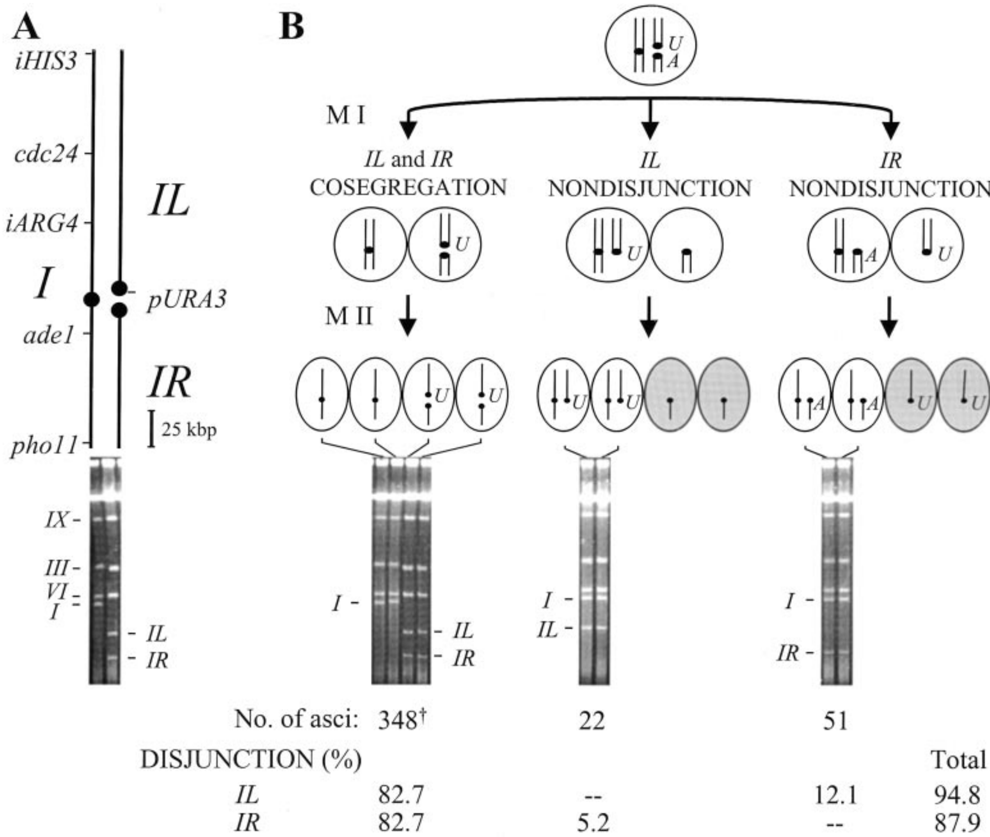

Figure 1.—Meiotic segregation of aligned centromeres in a diploid heterozygous for a chromosome *I* bisection at *CEN1.* (A) Physical maps of chromosome *I* and PFGE analysis of the haploid parents of strain CG403. *CEN1* is indicated by the solid circle, *pURA3* denotes the integrated linear bisection plasmid YLpVG47 (Guacci and Kaback 1991), *iARG4* and *iHIS3* denote insertions produced by one-step gene replacement (Kaback *et al.* 1999). PFGE used conditions that only resolved chromosomes <500 kbp. (B) Meiotic segregation of chromosome fragments *IL* and *IR* from the intact copy of chromosome *I.* Segregation was scored using spore viability. Nullisomic inviable spores are shaded. Nondisjunction of chromosome fragments *IL* and *IR* was scored using *URA3* (*U*) and *ADE1* (*A*), respectively. All aneuploidy was confirmed by PFGE. PFGE of all viable spores from a representative ascus is shown below each tetrad class. Disjunction is the percentage of asci segregating each chromosome fragment from the intact copy of chromosome *I.* Totals are the combined percentage disjunction from each tetrad class showing segregation of that fragment from the intact chromosome. Chromosome *I* segregation could not be classified in a total of 55 asci: 6 exhibited aberrant (4:0, 3:1, or 1:3) *URA3* segregation, 20 contained one viable spore with an intact copy of chromosome *I* and one viable spore with a bisected copy of chromosome *I*, 6 contained two viable euploid spores with either bisected or intact copies of chromosome *I*, and 23 contained either one or no viable spores. (†) Includes 55 asci with three viable spores.

Meiotic recombination levels were analyzed along the length of chromosome *I* and were found to be slightly greater than the published values (data not shown). Chromosome *I* fragments that contained a crossover segregated from the full-length chromosome *I* in 93–96% of the asci (Table 2A). Fragments containing no detectable crossovers segregated from the intact chromosome in 70–77% of the asci (Table 2A). Segregation of the nonrecombinant fragments was significantly less efficient than recombinant fragments ($P < 0.0001$) but was not random ($P < 0.01$). This nonrandom segregation could be due to either distributive disjunction or the segregation of chromosomes containing undetected two-strand double crossovers, possibilities that were not investigated. These results demonstrate that bisection fragments that contain centromeres that are both aligned with the centromere on the full-length chromosome segregate relatively efficiently from the full-length chromosome and that the most efficient disjunction is associated with crossing over.

Meiotic segregation of homologous chromosomes containing misaligned centromeres: To determine whether homologous centromere alignment is important in meiosis I disjunction, segregation was analyzed in a strain (CG391) that contains one copy of chromosome *I* bisected near the *MAK16* gene and one full-length copy of chromosome *I.* In this strain, the centromere of the left bisection fragment (*IA*-110) is located ∼50 kbp from the centromere on the intact copy of chromosome *I*, while the centromeres on the right fragment (*IB*-140) and the intact chromosome are aligned (Figure 2A). Since a crossover between the left arm of fragment *IB*-140 and the intact chromosome leads to a reconstituted chromatid, these analyses followed the centromeres of each fragment and the intact chromosome (Figure 2B). The results indicated that the aligned centromere of fragment *IB*-140 segregated from the centromere of the intact copy of chromosome *I* in 95.4% of the asci and underwent nondisjunction in only 4.5% of the asci. In contrast, the misaligned centromere of chromosome *IA*-110 segregated from the centromere on the intact chromosome in only 64.6% of the asci and underwent nondisjunction in 28.0% (= 6.4% + 21.6%) of the asci. Segregation of the *IA*-110 centromere could not be determined in 7.3% of the asci because they exhibited a crossover adjacent to *CEN1* on the right arm and

TABLE 2

Effect of crossing over on chromosome fragment segregation

	Strain	Chromosome fragment	Recombinant fragments[a]				Nonrecombinant fragments[b]			
			No. of asci				No. of asci			
			Disjunction	Nondisjunction	% Disjunction	P	Disjunction	Nondisjunction	% Disjunction	P
A	CG403	IL	379	15	96	<0.0001	20	6	77	<0.01
		IR	309	24	93	<0.0001	54	23	70	<0.005
B	CG391 (intercentromere crossover class)	IA-110	60	67	47	0.8	19	23	45	0.8
	(noncrossover class)	IA-110	167	18	90	<0.0001	22	9	71	<0.025
C	CG347	IA-60	27	26	51	0.9	97	91	52	0.8

Disjunction from a full-length copy of chromosome I was determined for recombinant and nonrecombinant copies of chromosome I fragments. (A) Segregation of bisection fragments containing aligned centromeres. (B) Segregation of fragment IA-110 containing a misaligned centromere. Intercentromere crossover class indicates asci that underwent an exchange in the region between the misaligned centromeres. Noncrossover class indicates asci that did not undergo exchange in the region between the misaligned centromeres. (C) Segregation of fragment IA-60 containing a misaligned centromere. P is the probability that segregation was random.

[a] Fragments exhibiting crossing over with the intact copy of chromosome I.

[b] Fragments not exhibiting crossing over with the intact copy of chromosome I.

segregated *ADE1*, the chromosome *I* centromere marker, at the second meiotic division. As there is no *a priori* reason to believe that this crossover would influence the segregation of fragment *IA*-110, exclusion of this class should not affect the conclusions. Thus, segregation of the misaligned *IA*-110 centromere from the centromere on the intact chromosome was significantly less efficient ($P < 0.0001$) than that observed for the aligned centromeres, but, nevertheless, also was not random ($P < 0.0001$).

Crossing over disrupts meiosis I segregation of chromosomes containing misaligned centromeres: To investigate why the misaligned centromere of fragment *IA*-110 segregated less efficiently than the aligned centromeres, the asci from strain CG391 were analyzed for crossovers between all chromosome *I* markers. Surprisingly, the analysis revealed that crossovers in the region between the misaligned centromeres (*iARG4-CEN1*) on the left arm of fragment *IB*-140 were correlated with random segregation of the misaligned centromere on fragment *IA*-110 with respect to the centromere on the intact chromosome (18.9% segregation *vs.* 21.6% nondisjunction; Figure 2B). Furthermore, both recombinant and nonrecombinant copies of fragment *IA*-110 segregated randomly in these asci (Table 2B). In contrast, asci with no crossovers in the *iARG4-CEN1* interval exhibited relatively efficient segregation of the fragment *IA*-110 centromere with respect to the centromere on the intact copy of chromosome *I* (45.7% segregation *vs.* 6.4% nondisjunction; Figure 2B). In this group, recombinant copies of fragment *IA*-110 segregated from the centromere on the intact chromosome in 90% of the asci, while fragments with no detectable crossovers segregated in only 71% of the asci (Table 2B). Segregation of the apparent nonrecombinant copies of fragment *IA*-110 was not random ($P < 0.025$). As above, this behavior could be due to either distributive disjunction or the segregation of chromosomes containing undetected two-strand double crossovers, possibilities that were not investigated.

In these studies, nondisjunction or recombination caused a lethal nullisomy that could not be assayed directly. Accordingly, these experiments were repeated on a chromosome *I* bisection that had a functional fragment with no essential genes where all four spores from most asci were viable and karyotypes could be directly analyzed. Diploid strain CG347 contains a full-length and a bisected copy of chromosome *I* composed of fragment *IA*-60, which contains no genes essential for vegetative growth, and fragment *IB*-180 (Figure 3A; Y. Su, J. Lamb and D. B. Kaback, unpublished results). In strains homozygous for this bisection, homologous copies of fragment *IA*-60 efficiently recombine and segregate from each other (Kaback *et al.* 1999). In strain CG347, the centromere of fragment *IA*-60 is located ~100 kbp from the centromere on the full-length chromosome, whereas the centromeres of fragment *IB*-180

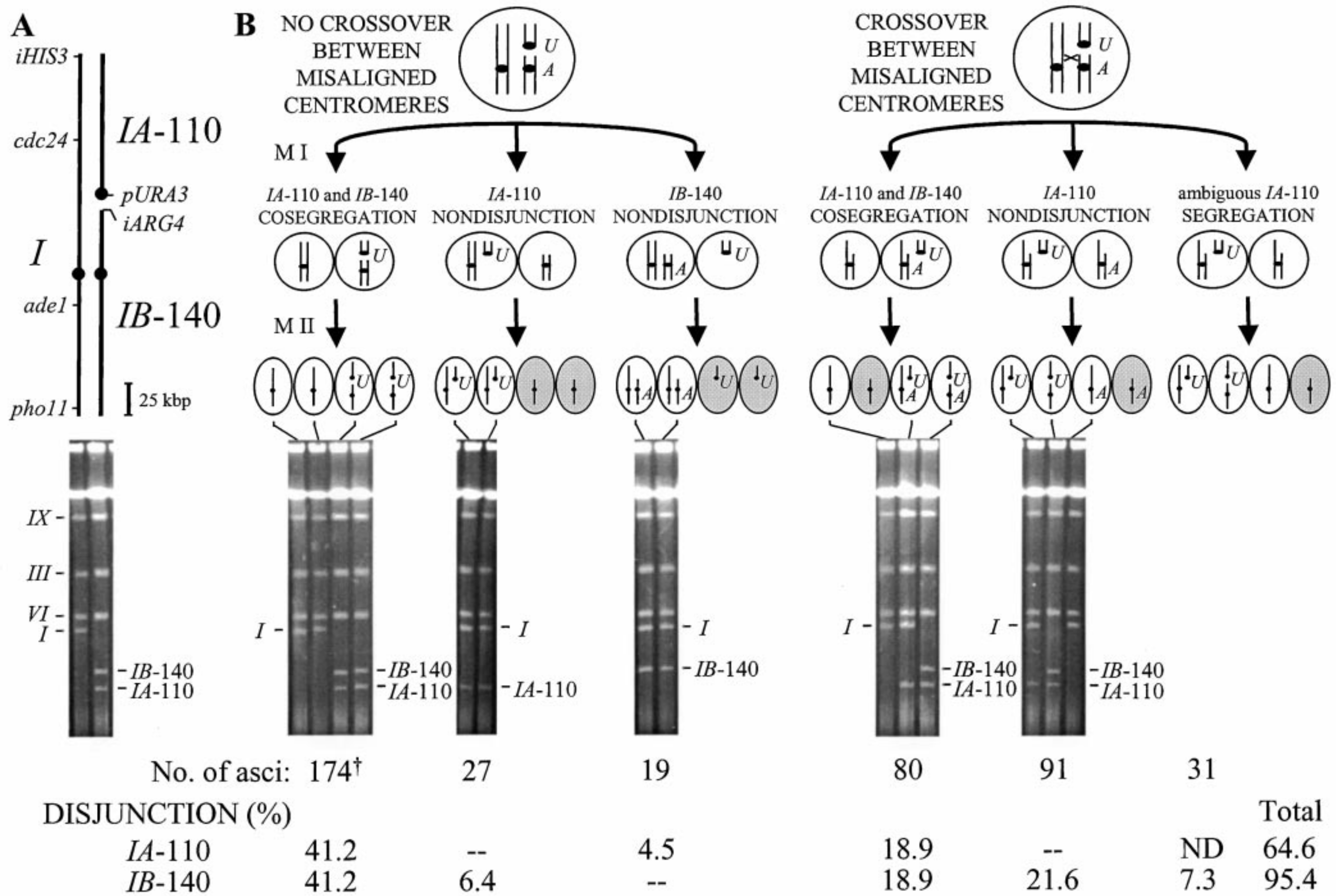

Figure 2.—Meiotic segregation of misaligned centromeres in a diploid heterozygous for a chromosome *I* bisection at *MAK16.* (A) Physical maps of chromosome *I* and PFGE analysis of haploid parents of strain CG391. The *pURA3* denotes the integrated linear bisection plasmid YLpVG59 (Guacci and Kaback 1991). Other symbols are explained in the legend to Figure 1. (B) Meiotic segregation of centromeres on chromosome fragments *IA*-110 and *IB*-140 from the centromere on the intact copy of chromosome *I.* Segregation was determined using spore viability and the centromere-linked *URA3* (*U*) marker. Inviable spores due to nullisomy are shaded. Crossing over between the misaligned centromeres on the left arm of chromosome *IB*-140 was indicated by second-division segregation (SDS) of *iARG4* with respect to *URA3.* First-division segregation (FDS) of *ADE1* (*A*) was used for scoring nondisjunction of the fragment *IB*-140 centromere in asci that did not show a crossover between misaligned centromeres and for scoring disjunction of the fragment *IB*-140 centromere from the centromere on the intact chromosome in asci that showed a crossover between the misaligned centromeres. Segregation of the chromosome *IA*-110 centromere with respect to the centromere on the intact chromosome was ambiguous and could not be determined (ND) when *ADE1* showed SDS and there was a crossover between the misaligned centromeres. Four-strand double crossovers (NPD for *iARG4*-*URA3*) were detected between the misaligned centromeres in 6 asci. PFGE indicated that 2 fell into the chromosome fragment cosegregation class, while 4 showed nondisjunction of fragment *IA*-110. These asci were included in the totals showing recombination between misaligned centromeres. Disjunction is the percentage of asci segregating each chromosome fragment centromere from the centromere of the intact copy of chromosome *I.* Chromosome *I* segregation could not be classified in 52 asci due to spore inviability not caused by nondisjunction and 6 asci due to aberrant, 4:0, 3:1, or 1:3 *URA3* segregation. (†) Includes 15 asci with three viable spores.

and the full-length chromosome are aligned. Analysis of chromosome *I* segregation was carried out as described in Figure 3B. On the basis of spore viability, which was comparable to isogenic diploids containing two intact copies of chromosome *I* (data not shown), the aligned fragment *IB*-180 centromere segregated from the centromere on the intact copy of chromosome *I* in virtually all nuclei. In contrast, the misaligned *IA*-60 centromere appeared to segregate randomly with respect to the centromere on the intact chromosome *I* (54.0% segregation *vs.* 46.0% nondisjunction; Figure 3B). Segregation of the fragment *IA*-60 centromere could not be analyzed in 25 asci (6.1% of the total analyzed) where *ADE1* segregated at the second meiotic division (not shown). As above, omission of this class should not affect the conclusions.

Meiotic reciprocal recombination was monitored over the intervals shown in Figure 3A. The frequency of crossing over was approximately equal to that found for two intact chromosomes (data not shown). Both recombinant and nonrecombinant copies of fragment *IA*-60 segregated randomly with respect to the centro-

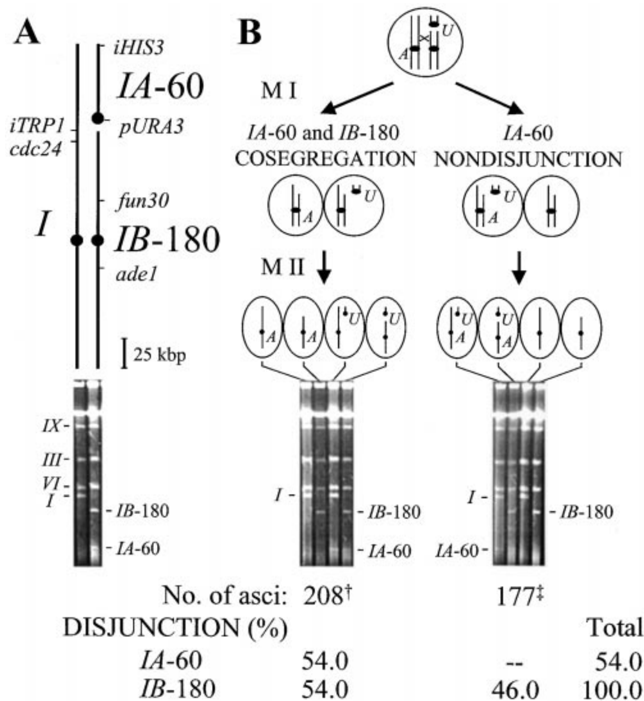

Figure 3.—Meiotic segregation of misaligned centromeres in a diploid heterozygous for a chromosome *I* bisection at YAL049. (A) Physical maps of chromosome *I* and PFGE of haploid parents of strain CG347. The *pURA3* denotes the integrated linear bisection plasmid YLpLF273, *iTRP1* denotes an insertion, and *fun30* denotes *fun30::LEU2*, both produced by one-step gene replacement (Kaback *et al.* 1992, 1999). See the legend to Figure 1 for other symbols. (B) Meiotic segregation of centromeres on chromosome fragments *IA*-60 and *IB*-180 from the centromere on the intact copy of chromosome *I.* Segregation of fragment *IA*-60 centromere was determined using the *pURA3* (*U*) marker. Segregation of the fragment *IB*-180 centromere was determined using *ADE1* (*A*) and spore viability. Crossing over between the misaligned centromeres on the left arm of chromosome *IB*-180 was scored using segregation of *fun30*, *cdc24*, or *iTRP1* with respect to *URA3.* Segregation could not be classified in 75 asci due to either SDS of *ADE1* with respect to *URA3* (25 asci), aberrant *URA3* segregation (29 asci), or spore inviability (2:2 and 1:3 viable:inviable; 21 asci). (†) Includes 64 asci with three viable spores. (‡) Includes 47 asci with three viable spores.

mere on the full-length chromosome. The left arm of fragment *IB*-180 underwent an observable exchange with the intact homologue in 88% of the asci. Correcting for the assumption that two-strand double crossovers produce asci categorized in the nonrecombinant class, the left arm of fragment *IB*-180 probably underwent crossing over in >90% of the nuclei. Accordingly, random segregation of the fragment *IA*-60 centromere must also be coincident with the occurrence of crossovers in the region separating the misaligned centromeres in most asci. The fact that the segregation class for fragment *IA*-60 was slightly greater than the nondisjunction class may be the result of the small percentage of asci that did not contain a crossover on the left arm of fragment *IB*-180. Thus, these results are identical to those obtained with the larger fragment *IA*-110. Therefore, misaligned centromeres segregate randomly when there is a crossover in the region separating them.

Centromere realignment restores meiotic segregation of homologous chromosomes: Fragment *IA*-60 is located near the end of chromosome *I* and crossovers that occur near telomeres do not promote meiosis I disjunction (Ross *et al.* 1996). To rule out the possibility that the fragment's proximity to the telomere and not centromere misalignment was responsible for its random segregation, the fragment *IA*-60 centromere was realigned with the centromere on its homologue. If telomere proximity was responsible for random segregation, the realigned fragment should still segregate randomly. Alternatively, if centromere misalignment caused random segregation, the realigned centromeres should now segregate more efficiently. Realignment was accomplished by translocating the sequences contained on fragment *IA*-60 to the region adjacent to the normal chromosome *I* centromere (Figure 4) and constructing heterozygous bisection strain CG387 (Figure 5A). In this strain, the bisected chromosome fragments consist of a modified version of fragment *IA*-60 (fragment *IA*-[*CEN4 LEU2*]-60) and fragment *IR*, while the translocation (*Tx*) is equivalent to the intact chromosome in the other experiments. The rest of the left arm of chromosome *I* is contained on a separate functional chromosome fragment *IL'* present in two copies. Analysis of segregation in this strain (Figure 5B) indicated that fragment *IA*[*CEN4 LEU2*]-60 mostly segregated from the translocation chromosome (67.5% disjunction *vs.* 32.5% nondisjunction, $P = 0.0001$). Most of the nondisjunction could be attributed to the failure of this fragment to undergo crossing over in approximately half of the asci. The recombinant copies of fragment *IA*[*CEN4 LEU2*]-60 segregated from the translocation chromosome in 89% of the asci (Table 3A). Accordingly, fragment *IA*[*CEN4 LEU2*]-60 is capable of relatively efficient segregation and there appear to be no major problems with the ability of chiasmata to promote its disjunction from the intact chromosome. In this strain the modified fragment *IA*-60 behaved like other recombinant bisection fragments containing aligned centromeres. Thus, random segregation of the fragment *IA*-60 in strain CG347 was due to its misalignment.

Eliminating crossovers in the region between misaligned centromeres restores segregation: Random segregation of chromosome fragments with misaligned centromeres was coincident with crossovers in the region between the misaligned and aligned centromeres. To determine the effect of eliminating crossovers in this region, the left arm of bisection fragment *IB*-180 was deleted, and meiotic segregation of the chromosome *I* fragments was studied. Strain CG410-F3 contains fragment *IA*[*CEN4 LEU2*]-60, fragment *IR*, and the full-length chromosome *I* (Figure 6A). Accordingly, it is hemizygous for the region between YAL049 and *CEN1*

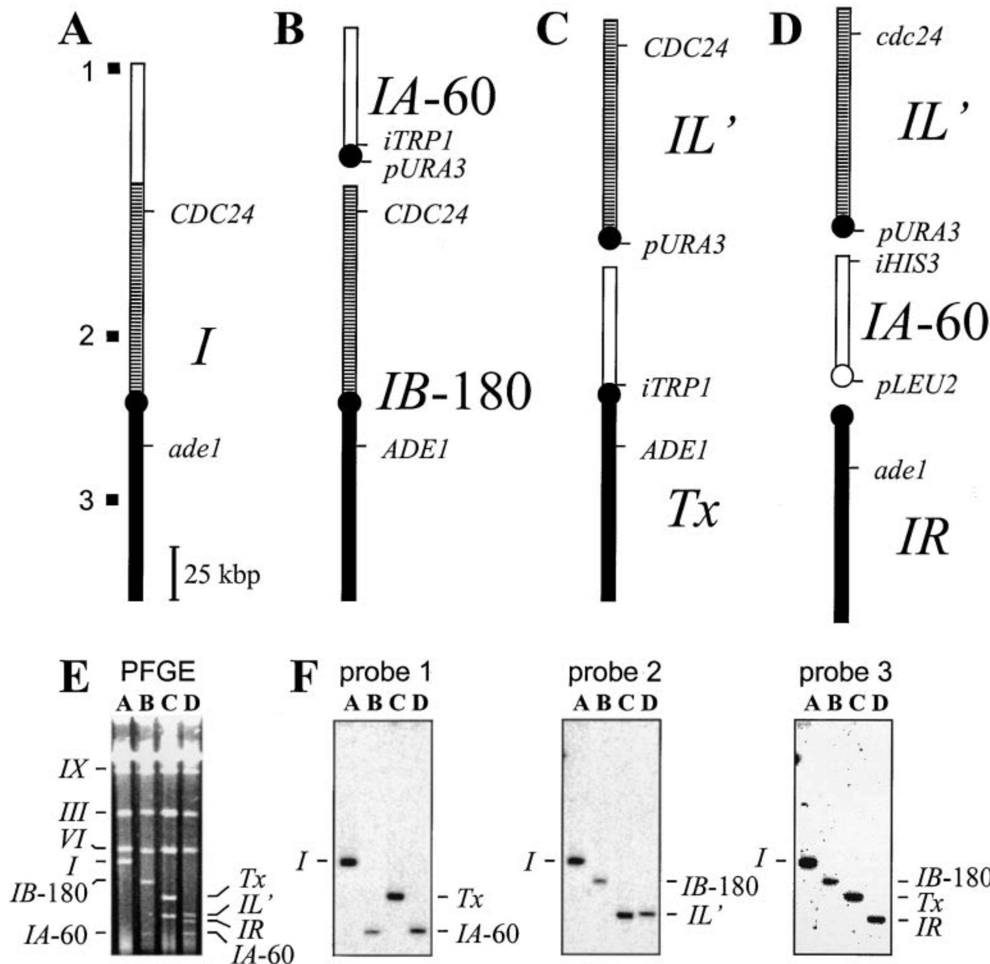

Figure 4.—Physical maps of chromosome *I* rearrangements. (A) Wild-type strain CG403-3C. (B) Bisection at YAL049 in strain JL48-1A. (C) Translocation of the leftmost 60 kbp to *CEN1* in strain CG366-11C. (D) Trisection at YAL049 and *CEN1* in strain CG386-66C. *CEN1* is indicated by the solid circle. *CEN4* is indicated by the open circle. Rearranged regions are differentially shaded. Other symbols are explained in the legend to Figure 1. Solid boxes (1–3) in A indicate approximate location of hybridization probes. Probe 1 is a 1.0-kbp *Xba*I fragment from plasmid pLF251, probe 2 is a 1.2-kbp *Hin*dIII fragment from plasmid pLF278, and probe 3 is a 3.2-kbp *Bam*HI-*Hin*dIII fragment from plasmid pLF237. (E) PFGE karyotypes of strains shown in A–D (lanes A–D). (F) Blot hybridization of strains shown in A–D using probes 1–3. Autoradiograms demonstrate that probes hybridize to the appropriate size chromosomes and chromosomal fragments depicted in A–D (lanes A–D).

on the left arm and is expected to exhibit 2:2 segregation for viability. The centromere of fragment *IR* was aligned with the centromere of the full-length copy of chromosome *I*, while the centromere of fragment *IA*[*CEN4 LEU2*]-60 was misaligned. Meiosis I disjunction of the fragment *IA*[*CEN4 LEU2*]-60 from the intact chromosome causes it to segregate into the two inviable spores, whereas nondisjunction causes it to cosegregate with the intact chromosome into the two viable spores (Figure 7). Tetrad analysis indicated that fragment *IA*[*CEN4 LEU2*]-60 segregated from the full-length copy of chromosome *I* in a total of 74.9% of the asci. Recombinant copies disjoined 88% of the time, while nonrecombinant copies disjoined 62% of the time (Table 3B). The observed segregation was almost assuredly not due to chance ($P < 0.0001$). Therefore, the absence of homology and crossing over in the region separating aligned and misaligned centromeres enables the fragment with the misaligned centromere to behave identically to chromosome fragments containing aligned centromeres.

As a control, strain CG414 was analyzed (Figure 6B). This strain was isogenic to strain CG410-F3 except it was not a hemizygote and contained a reconstituted copy of fragment *IB*-180 in place of fragment *IR*. For all purposes, it was identical to the heterozygous bisection strain CG347, except for the presence of fragment *IA*-[*CEN4 LEU2*]-60 in place of *IA*-60. The results indicate that chromosomes in strain CG414 behaved identically to those in strain CG347. Most asci contained four viable spores, crossing over on the left arm of fragment *IB*-180 occurred in >90% of the asci, and the amount of recombination on fragment *IA*[*CEN4 LEU2*]-60 was very similar to that found at fragment *IA*-60 (data not shown). In contrast to strain CG410-F3, fragment *IA*-[*CEN4 LEU2*]-60 segregated randomly with respect to the centromere on the full-length chromosome *I* (52% disjunction *vs.* 48% nondisjunction; Table 3C). These results confirm that misaligned centromeres segregate randomly when there is a crossover in the region separating them. They also demonstrate the equivalence of fragments *IA*[*CEN4 LEU2*]-60 and *IA*-60.

Recombinant chromosome fragments containing misaligned centromeres do not behave as univalents: Random segregation of a misaligned centromere may be the result of the chromosome fragments behaving as if they were univalents. Univalent chromosomes will segregate from either an unpaired nonhomologous chromosome or a *CEN* plasmid by distributive disjunction (Daw-

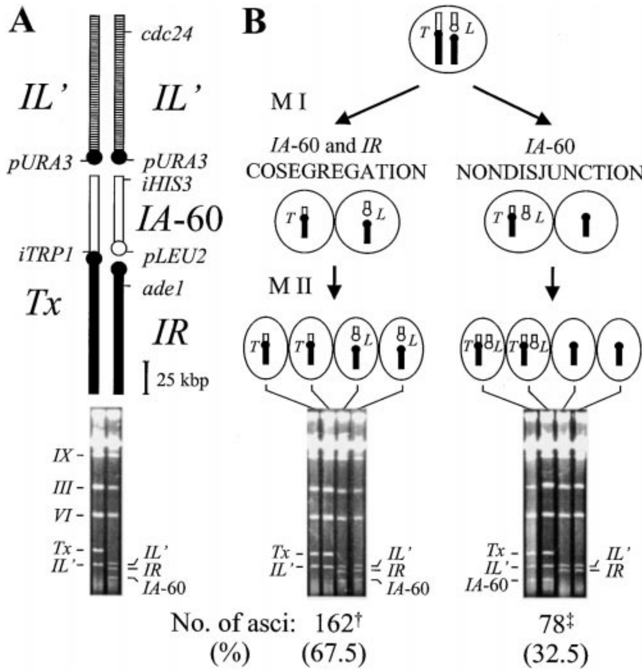

Figure 5.—Meiotic segregation of realigned centromeres in a diploid heterozygous for the chromosome *I* translocation. (A) Physical maps of chromosome *I* and PFGE analysis of haploid parents of strain CG387. Symbols are explained in the legends to Figures 1 and 4. (B) Meiotic segregation of chromosome fragment *IA*-60 from the translocation chromosome (*Tx*). Segregation was determined using the centromere markers *LEU2* (*L*) and *iTRP1* (*T*) and spore viability. PFGE from a representative ascus is shown below each tetrad class. Segregation could not be analyzed in 235 asci (not shown) because they contained two or fewer viable spores, probably due to nondisjunction of either chromosome *IR* or *IL*′ (105 asci), did not exhibit 2:2 segregation for *LEU2* or *iTRP1* (74 asci), or exhibited SDS of *LEU2* with respect to *iTRP1* (56 asci), due to precocious separation of sister chromatids or ectopic recombination. (†) Includes 37 asci with three viable spores. (‡) Includes 33 asci with three viable spores.

son *et al.* 1986; Mann and Davis 1986; Kaback 1989; Guacci and Kaback 1991). To determine whether fragment *IA*-60 behaved as a univalent and underwent distributive disjunction, *CEN* plasmid pCG106 was introduced into the chromosome *I* bisection heterozygote, CG347. The plasmid had no detectable effect on spore viability or meiotic recombination on chromosome *I* (data not shown). Tetrad analysis showed that when there was a crossover on fragment *IA*-60, the fragment did not exhibit distributive disjunction but segregated randomly with respect to both the *CEN* plasmid (Table 4) and the centromere on the intact chromosome (data not shown). These results indicate that recombinant fragments with a misaligned centromere did not behave as univalents. In contrast, nonrecombinant copies of fragment *IA*-60 segregated from the plasmid in 88% of the analyzable tetrads (Table 4; $P < 0.0001$), but randomly with respect to the centromere on the intact copy of chromosome *I* (data not shown). Therefore,

TABLE 3

Effect of crossing over and centromere alignment on the segregation of fragment *IA*[*CEN4 LEU2*]-60

	Strain	Intact homologue	Recombinant fragments[a]: No. of asci, Disjunction	Recombinant fragments[a]: No. of asci, Nondisjunction	Recombinant fragments[a]: % Disjunction	Recombinant fragments[a]: P	Nonrecombinant fragments[b]: No. of asci, Disjunction	Nonrecombinant fragments[b]: No. of asci, Nondisjunction	Nonrecombinant fragments[b]: % Disjunction	Nonrecombinant fragments[b]: P
A	CG387	Tx	72	9	89	<0.0001	55	31	64	<0.01
B	CG410-F3	I	80	11	88	<0.0001	48	29	62	<0.05
C	CG414	I	10	10	50	1.0	41	37	52	0.9

Disjunction from an intact homologue was determined for recombinant and nonrecombinant copies of fragment IA[CEN4 LEU2]-60. (A) Segregation of fragment IA[CEN4 LEU2]-60 containing an aligned centromere. (B) Segregation of fragment IA[CEN4 LEU2]-60 containing a misaligned centromere where the interval between the misaligned centromeres was hemizygous, thus preventing crossing over in this region. (C) Segregation of fragment IA[CEN4 LEU2]-60 containing a misaligned centromere where crossing over almost always occurred in the region between the misaligned centromeres. P is the probability that segregation was random.

[a] Fragments exhibiting crossing over with the intact homologue.

[b] Fragments not exhibiting crossing over with the intact homologue.

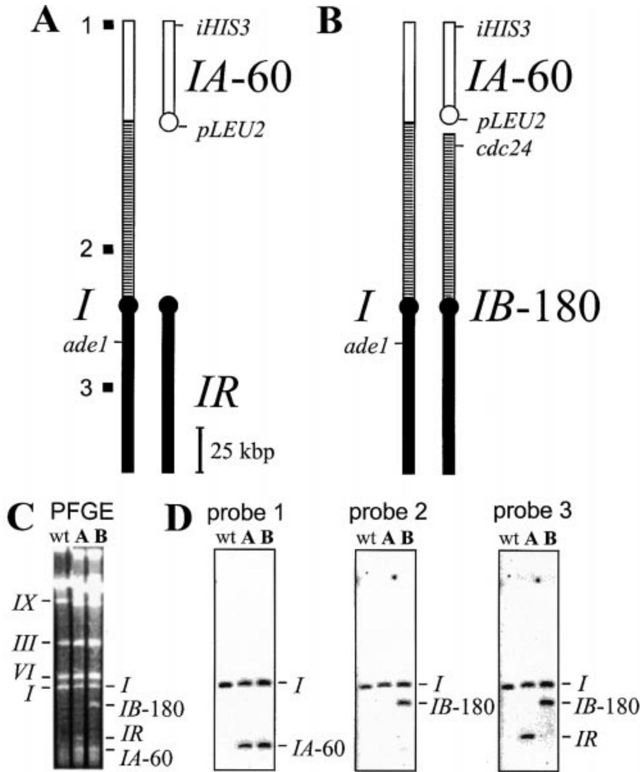

Figure 6.—Deletion of homologous sequences between the misaligned centromeres. Physical maps of chromosome *I.* (A) Deletion strain CG410-F3. Solid boxes (1–3) indicate approximate location of hybridization probes (see legend to Figure 4 for description). Other symbols are explained in the legend to Figure 1. (B) Control, strain CG414. (C) PFGE karyotypes of normal euploid control (wt, strain CG403-3C) and strains CG410-F3 (lane A) and CG414 (lane B). (D) Blot hybridization of wild type and strains shown in A and B using probes 1–3. Autoradiograms demonstrate that probes hybridize to the appropriate size chromosomes and chromosomal fragments.

the nonrecombinant fragments appeared to behave as univalents, suggesting they segregate randomly due to a failure to properly pair with their homologues.

DISCUSSION

The role of centromere alignment in meiotic chromosome segregation in *S. cerevisiae* was investigated using diploids that contained one intact copy of chromosome *I* and one copy bisected into two functional centromere-containing fragments. The centromere on one fragment was aligned with the centromere on the intact chromosome, while the centromere on the other fragment was either aligned or misaligned by 50 or 100 kbp. Aligned centromeres segregated from each other efficiently, while misaligned centromeres segregated from each other much less efficiently. In fact, the centromere that was misaligned by ~100 kbp segregated randomly. Random segregation of all misaligned centromeres was correlated with crossovers between the other chromosome fragment and the intact chromosome in the region separating the centromeres. When there were no crossovers in the region between the misaligned centromeres or when the DNA in this region was deleted on one homologue to prevent recombination, segregation appeared to be as efficient as that observed for aligned centromeres. Therefore, these crossovers are preventing disjunction of a normal chromosome from its homologous fragment.

Segregation of chromosome fragments was scored by analysis of viability in both the heterozygous *CEN1* and *MAK16* bisections. Tetrad classes were assigned on the basis of assuming normal meiosis I and II segregation of marker pairs on chromosomes and demonstrating disomy in the viable spores when there was nondisjunction. While there are no reasons to believe these assumptions are incorrect, the experiments using fragment *IA*-60 did not use spore viability as a marker and the results clearly demonstrated that the fragment segregated randomly when its centromere was misaligned and segregated correctly when its centromere was realigned. Therefore, the fragment *IA*-60 results are in complete agreement with those obtained using the other bisections.

In the experiments where the *CEN1*-linked *ADE1* gene was used to follow first-division segregation, 6–7% of the asci were excluded because there was a crossover between *ADE1* and *CEN1.* This fraction of asci is close to that expected for a ~5-cM genetic interval. There is no *a priori* reason to believe that exclusion of this class affected any of the results presented. Furthermore, recombination on the same arm distal to *ADE1* did not appear to affect segregation of the fragment containing the misaligned centromere. Nevertheless, we cannot completely eliminate the possibility that the excluded asci might quantitatively affect the results. Even if the excluded class fell entirely into the disjunction class for the misaligned centromere-containing fragment, the results would still show that misaligned centromeres segregate more poorly than aligned centromeres when there was a crossover in the region separating them.

When centromeres were aligned or there was no crossover in the region between misaligned centromeres, fragment disjunction appeared to depend on recombination with the intact chromosome. Most if not all of the nondisjunction was associated with the failure to cross over. The number of chromosomes that failed to cross over was consistent with the smaller size of the bisection chromosomes. In contrast, when centromeres were misaligned and a crossover occurred in the region between the misaligned centromeres, recombination between the intact chromosome and the misaligned centromere-containing fragment did not appear to affect its disjunction from the intact chromosome. The nonrecombinant fragments with misaligned centromeres segregated randomly but underwent distributive disjunction with a *CEN* plasmid, suggesting that these fragments might not be paired with their homologues.

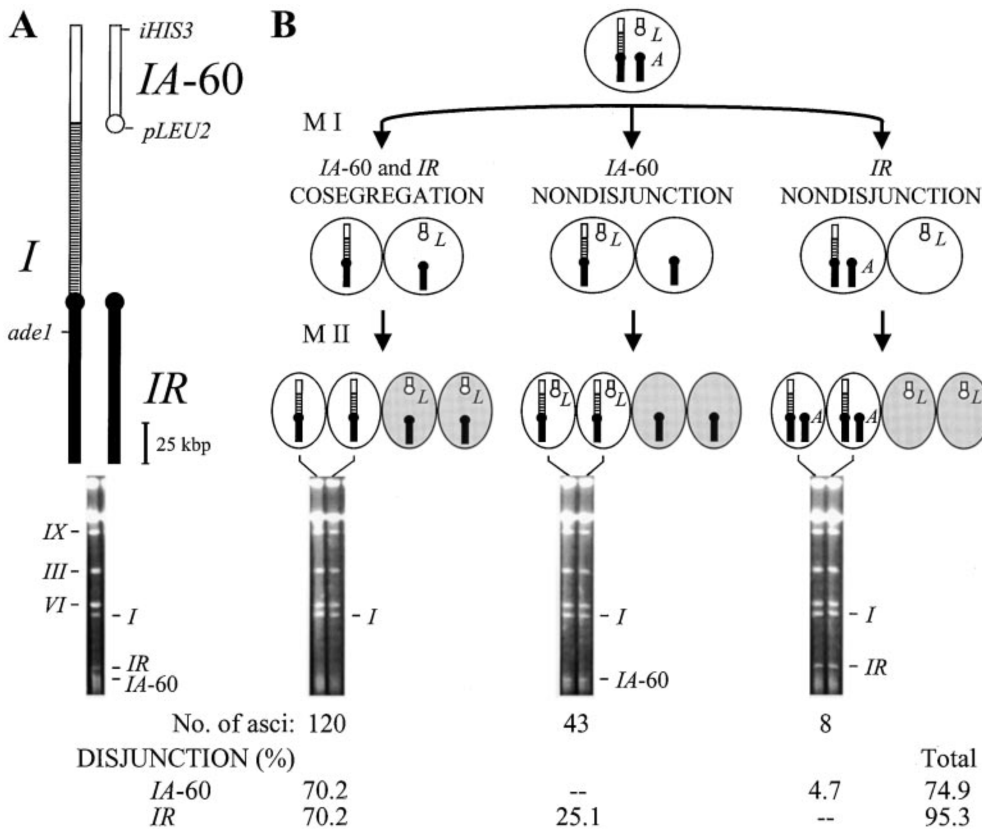

Figure 7.—Meiotic segregation of misaligned centromeres in a diploid partially hemizygous for chromosome *I*. (A) Physical map of chromosome *I* and PFGE analysis of diploid strain CG410-F3. All symbols are described in the legends to Figures 1 and 4. (B) Meiotic segregation of chromosome fragments *IA*-60 and *IR* from an intact copy of chromosome *I* was determined from segregation of their respective centromere markers *LEU2* (*L*) and *ADE1* (*A*). Inviable spores due to partial nullisomy are shaded. PFGE of all viable spores from a representative ascus is shown below each tetrad class. Only asci showing FDS of the *TRP1* gene with respect to spore viability were used. Approximately 25% of all the asci showed SDS of *ADE1* with respect to *TRP1*. Segregation of chromosome *IR* was determined in these asci by PFGE analysis where necessary. Disjunction is the percentage of asci segregating each chromosome fragment from the intact copy of chromosome *I*. A total of 47 asci could not be analyzed due to aberrant segregation of *LEU2* (18 asci), *TRP1* (5 asci), or spore inviability (24 asci with one or no viable spores).

Recombinant fragments with misaligned centromeres segregated randomly but did not undergo distributive disjunction with a plasmid, suggesting that these fragments were paired with their homologues. These results demonstrate that pairing and recombination *per se* are not sufficient to guarantee correct disjunction.

The results demonstrate that misaligned homologous centromeres will segregate randomly when there is a crossover in the region between the centromeres. Therefore, such a crossover appears to prevent normal segregation. How segregation is prevented is not known but two mechanisms may explain the behavior. It is

TABLE 4

Distributive disjunction of fragment *IA*-60 from a *CEN* plasmid

Recombinant fragments[a]			Nonrecombinant fragments[b]			
No. of asci			No. of asci			
Disjunction	Nondisjunction	% Disjunction	Disjunction	Nondisjunction	% Disjunction	*P*
13	9	59	78	11	88	0.005

Strain CG347 was transformed with plasmid pCG106. 484 asci gave rise to 333 tetrads containing four viable spores. The plasmid-borne *ARG4* marker segregated 4+:0− in 113 asci, 3+:1− in 24 asci, 2+:2− in 141 asci, 1+:3− in 14 asci, and 0+:4− in 41 asci. Segregation of fragment *IA*-60 was analyzed only in the 2+:2− asci for *ARG4*. *P* is the probability that the difference in segregation behavior between the recombinant and nonrecombinant fragments was due to chance.

[a] Fragments exhibiting crossing over with the intact copy of chromosome *I*.

[b] Fragments not exhibiting crossing over with the intact copy of chromosome *I*.

possible that misaligned centromeres segregate randomly because they may be equally capable of stably attaching to either pole of the meiotic spindle (Figure 8A). Stable attachment of kinetochores to microtubules is believed to require the tension generated by pulling against the chiasmata that hold homologues together (Nicklas 1997). A crossover in the region between the misaligned centromeres might prevent normal segregation of the fragment containing the misaligned centromere because its attachment to either side of the spindle generates approximately the same amount of tension. This tension must be generated by pulling on the chiasma between the other fragment and the intact chromosome, since there is no opposing force generated by the chiasma between the fragment containing the misaligned centromere and the intact chromosome. In contrast, when there is no crossover in the region between the misaligned centromeres (or when centromeres on bisected chromosomes are both aligned with the centromere on the intact chromosome), the most efficient way to generate tension between each kinetochore and chiasmata holding the homologues together causes the two fragments to cosegregate from the intact chromosome. Other spindle attachment arrays would generate less or unequal tension, and microtubule attachments to the spindle would not be stable enough to promote segregation.

This model is consistent with the behavior of paired multivalent translocation chromosomes in higher organisms (Rickards 1983). Reciprocal translocation heterozygotes produce balanced or unbalanced meiotic products depending on the orientation of the quadrivalent chromosome complex formed by chiasmata between the two translocation and two normal chromosomes. This orientation appears to be dependent on the relative location and orientation of chiasmata and the kinetochores and their presumed ability to generate microtubule tension from both poles of the spindle (Rickards 1964; Nicklas 1997). Similarly, Robertsonian translocation heterozygotes resemble yeast centromere bisection heterozygotes and heterozygotes containing misaligned centromeres where there are no crossovers in the region separating the centromeres. The Robertsonian translocation chromosome segregates from the two acrocentric chromosomes following the formation of a trivalent where both acrocentric kinetochores are oriented to the same pole, while the kinetochore of the translocation is oriented toward the opposite pole (Bauer *et al.* 1961; Smith 1965; Marks 1978; Hays *et al.* 1982).

If the ability to generate a stable bipolar orientation on the spindle is the only factor that dictates homologue segregation, then the physical act of aligning centromeres would play little or no role in promoting proper chromosome segregation. Indeed, misaligned centromeres were capable of segregating efficiently as long as their presumed ability to maintain only a single orienta-

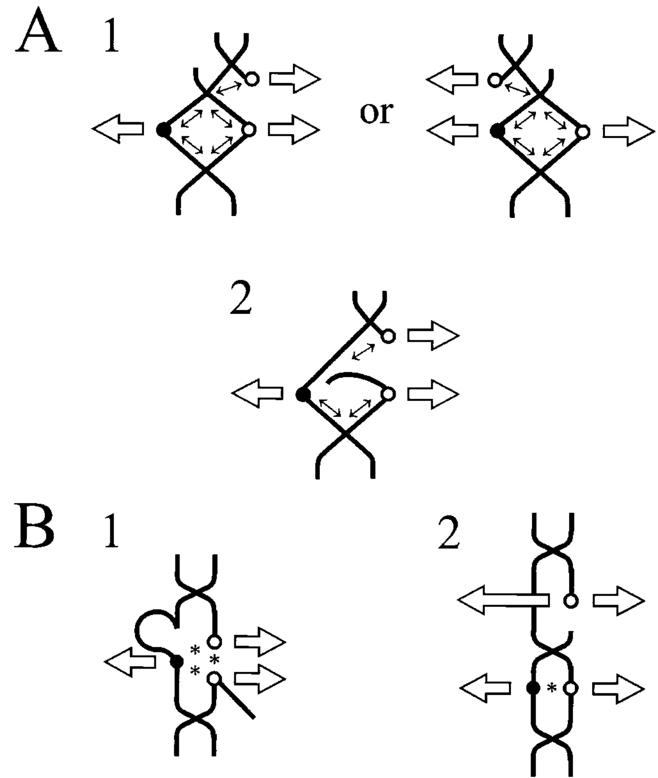

Figure 8.—Mechanisms for segregating chromosome fragments containing misaligned centromeres. (A) Tension model: (1) Crossovers between misaligned centromeres produce random segregation of the misaligned centromere-containing fragment. Attachment of the misaligned centromere to either spindle pole produces approximately equal tension because the tension is generated from the chiasmata between the intact chromosome and the other chromosome fragment (↔). (2) The absence of crossovers between the misaligned centromeres produces proper disjunction of both fragments from the intact chromosome because tension (↔) can be generated only when the centromeres of the two fragments attach to the same pole of the spindle while the centromere on the intact chromosome attaches to the opposite pole. Attachment of the misaligned centromere to the same pole as the intact chromosome would not generate sufficient tension to stabilize the microtubules because there would be no nearby opposing force. (B) The centromere interaction model: (1) The absence of crossovers between the misaligned centromeres produces proper disjunction of both fragments from the intact chromosome because centromeres on both fragments can interact either with each other or with the centromere on the intact chromosome (*). These interactions enable both fragments either to cosegregate as a single functional chromosome or to interact with and segregate from the centromere on the intact chromosome. (2) Crossovers between the misaligned centromeres produce random segregation of the misaligned centromere-containing fragment because its centromere is physically occluded from interacting either with the centromere on the other fragment or on the intact chromosome. Solid circles denote the centromere on the intact chromosome, and open circles denote centromeres on the chromosome fragments. Large open arrows indicate kinetochore movement toward the spindle poles. Chiasmata are denoted by where chromosome arms cross. For simplicity, only one chromatid is shown for each chromosome.

tion was not perturbed by crossovers in the region separating them. Nevertheless, in the absence of these crossovers, it is still possible that misaligned centromeres were segregating somewhat less efficiently than two aligned centromeres. However, any effects would have been below our detection limits.

Another possibility is that homologous centromeres physically interact with each other, and this interaction is required for segregation (Figure 8B). For example, such an interaction could be involved in organizing kinetochores so they face in opposite directions (Counce and Meyer 1973; Goldstein 1981). Alternatively, the centromeres on the two bisection fragments could interact with each other and form a complex that behaves as a single kinetochore that promotes cosegregation of the two fragments (Moses *et al.* 1979; Elder and Pathak 1980; Pathak and Lin 1981; Mahadevaiah *et al.* 1990; Davisson and Akeson 1993). In either case, a crossover could physically hinder any interaction involving misaligned centromeres. Irrespective of mechanism, chromosome fragments with misaligned centromeres segregate from their homologues much less efficiently than fragments with aligned centromeres. The inability of chromosomes with misaligned centromeres to segregate properly may serve as a control that prevents ectopically recombined chromosomes from segregating. This control may favor the production of balanced viable gametes and increase reproductive fitness.

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