Chromosome Size-Dependent Control of Meiotic Reciprocal Recombination in *Saccharomyces cerevisiae*: The Role of Crossover Interference

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ABSTRACT

In the yeast *Saccharomyces cerevisiae*, small chromosomes undergo meiotic reciprocal recombination (crossing over) at rates (centimorgans per kilobases) greater than those of large chromosomes, and recombination rates respond directly to changes in the total size of a chromosomal DNA molecule. This phenomenon, termed chromosome size-dependent control of meiotic reciprocal recombination, has been suggested to be important for ensuring that homologous chromosomes cross over during meiosis. The mechanism of this regulation was investigated by analyzing recombination in identical genetic intervals present on different size chromosomes. The results indicate that chromosome size-dependent control is due to different amounts of crossover interference. Large chromosomes have high levels of interference while small chromosomes have much lower levels of interference. A model for how crossover interference directly responds to chromosome size is presented. In addition, chromosome size-dependent control was shown to lower the frequency of homologous chromosomes that failed to undergo crossovers, suggesting that this control is an integral part of the mechanism for ensuring meiotic crossing over between homologous chromosomes.

DURING meiosis, homologous chromosomes pair, undergo reciprocal recombination (crossing over or chiasma formation), and then disjoin from each other. Failure to cross over can lead to chromosomal nondisjunction and aneuploidy (Baker *et al.* 1976). In the yeast *Saccharomyces cerevisiae*, every pair of chromosomes recombines at least once in virtually every nucleus and meiotic nondisjunction is infrequent (Sora *et al.* 1982; Kaback *et al.* 1989). The mechanisms that ensure that homologues cross over with each other are not understood and appear complex. Small chromosomes undergo reciprocal recombination at rates higher [expressed as centimorgans per kilobase pair (cM/kb)] than large chromosomes (Kaback *et al.* 1989; Mortimer *et al.* 1989; Riles *et al.* 1993). Furthermore, genetically marked segments of chromosome *I*, the smallest chromosome (Link and Olson 1991), undergo recombination at even higher rates when the chromosome is bisected into smaller functional chromosome fragments and at lower rates when these segments are translocated to a larger chromosome. Thus, yeast has a mechanism that regulates meiotic recombination rates by responding directly to chromosome size (Kaback *et al.* 1992). As this mechanism raises rates of reciprocal recombination on the smallest chromosomes, it probably plays a role in ensuring crossing over. The log of the physical size of each chromosome appears to be proportional to the −log of the rate of reciprocal recombination (Mortimer *et al.* 1992). This relationship suggests that chromosome size-dependent control of recombination could have a complex mechanism.

cis-acting DNA sequences also control recombination rates. Several recombination hot spots that can induce relatively high levels of both nonreciprocal (gene conversion) and reciprocal recombination in small regions of the chromosome have been characterized (Kawasaki 1979; Fogel *et al.* 1981; Coleman *et al.* 1986; Nicolas *et al.* 1989; White *et al.* 1991; Malone *et al.* 1992; Goldway *et al.* 1993). These hot spots correspond to DNA double-strand break sites that serve as initiation sites for meiotic recombination (Nicolas *et al.* 1989). Several hot spots have been found on chromosome *I*, including the well-characterized *PYK1* and *CYS3* regions (Coleman *et al.* 1986; de Massey *et al.* 1995). In fact, four out of five known hot spots are on small chromosomes, an observation consistent with an increased density of hot spots on small chromosomes. An increased hot spot density could also play a role in increasing recombination rates on small chromosomes. However, reciprocal recombination near hot spots is subject to chromosome size-dependent control, suggesting that size control is superimposable upon any control by *cis*-acting sequences (Kaback *et al.* 1992). Accordingly, size-dependent control may be responsible for the observation that most reciprocal recombination hot spots have been found on small chromosomes.

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Crossover or chiasma interference also controls recombination. Positive crossover interference is defined by the observation that double crossovers are less frequent than predicted by a random distribution of crossovers (Muller 1916). The probability of a second crossover increases with distance from the first crossover. Positive interference should be thought of as a process by which a crossover initiates a mechanism for inhibiting further reciprocal recombination over some length of the chromosome. Interference affects large amounts of DNA. A single crossover can inhibit crossovers over >100 kb of DNA in yeast (Mortimer and Fogel 1974; D. Kaback, unpublished observations) and possibly megabases in humans. It conceivably could affect an entire chromosome (King and Mortimer 1990). The molecular mechanism of interference is not known. Recent evidence suggests that the synaptonemal complex (SC) is involved. In *S. cerevisiae*, the *ZIP1* gene encodes a component of the SC (Sym *et al.* 1993; Sym and Roeder 1995). A *zip1* null mutant both fails to form SC and exhibits no chiasma interference (Sym and Roeder 1994). In addition, *Schizosaccharomyces pombe* and *Aspergillus nidulans* neither make SC nor show crossover interference (Egel-Mitani *et al.* 1982; Bahler *et al.* 1993; Munz 1994). Several mechanisms for crossover interference that involve the synaptonemal complex have been proposed but remain untested (Maguire 1977; King and Mortimer 1990). Crossover interference has been suggested to regulate reciprocal recombination so that each pair of homologues does not recombine excessively and chiasmata are evenly distributed over both single chromosomes and the entire genome. As positive interference is thought to distribute crossovers, it could be involved in chromosome size-dependent control of reciprocal recombination.

In this report, additional studies on chromosome size-dependent control of meiotic reciprocal recombination are described and the mechanism of this process is explored. Size-dependent control is shown to correlate with different amounts of crossover interference on different size chromosomes and a model is introduced for how interference could vary as a function of chromosome size. We also demonstrate that size-dependent control is indeed part of the mechanism for ensuring crossing over.

MATERIALS AND METHODS

Growth and genetic manipulation of yeast: Strains and their genotypes are listed in Table 1. Control strains for bisection experiments were isogenic and produced by reconstituting full-length chromosomes from bisected ones (Guacci and Kaback 1991; Kaback *et al.* 1992). Controls for translocations were congenic and were produced from translocation heterozygotes carrying the noted markers. All strains were maintained, grown, and sporulated on standard media as previously described (Sherman *et al.* 1986). Asci were dissected and analyzed as previously described (Sherman *et al.* 1986). Tetrad data from the small number of three viable-spored asci were consistent with data from four viable-spored asci and the two data sets combined.

Recombinant DNA manipulation and yeast transformation: Standard techniques were used for construction and bacterial amplification of all recombinant DNA plasmids (Maniatis *et al.* 1982). Recombinant DNA molecules were introduced into yeast by the method of Ito *et al.* (1983) using 0.5 m LiCl. Integrative transformation was confirmed by DNA blot hybridization (Southern 1975).

Construction of a genetically marked 60-kb bisection chromosome: Chromosome *I* was bisected by homologous recombination with a small linear centromere containing plasmid pLF273, as previously described (Guacci and Kaback 1991). This plasmid was constructed from plasmid pVG7 (Guacci and Kaback 1991) by inserting the 2.2-kb *Bgl*II fragment from the YAL049 region of chromosome *I* and a 1.6-kb *Sph*I telomere containing fragment from pKR56 (a gift from V. Zakian). Cleavage of this plasmid with *Bam*HI resulted in a 10-kb linear centromere-containing minichromosome with telomeres at both ends that was introduced into yeast strain VG37-8B. Stable Ura^+ transformants were screened by pulsed-field gel electrophoresis (transverse alternating field electrophoresis; TAFE; Gardiner and Patterson 1988). Putative bisections were confirmed by DNA blot hybridization using appropriate chromosome *I* probes (Southern 1975). The yeast *HIS3* and *TRP1* genes were introduced by one-step gene replacement (Rothstein 1991) on 1.7-kb *Bam*HI and 0.8-kb *Pml*I-*Stu*I fragments, respectively, at positions 8682 and 51,836 near each end of the small 60-kb bisection chromosome (Y. Su and D. Kaback, unpublished results). Isogenic control strains containing reconstituted full-length chromosomes were isolated as previously described (Guacci and Kaback 1991).

Construction of a chromosome *I*-chromosome *II* reciprocal translocation: A reciprocal translocation (Tx II) was constructed in strain YNN285 (Fasullo and Davis 1987, 1988) that placed ~550 kb from chromosome *II* on the right end of chromosome *I* and ~5 kb from chromosome *I* on the remaining ~300 kb from chromosome *II* (Figures 1 and 2). The construct was produced as previously described (Fasullo and Davis 1987, 1988; Kaback *et al.* 1992), except a 5.0-kb *Eco*RI target fragment from plasmid pYY67 (Steensma *et al.* 1989) was inserted in the YIp5-based plasmid containing the 5′ half of the *HIS3* gene. The 5.0-kb target fragment contains the *PHO11* gene and comes from the region that begins 4.5 kb from the right end of chromosome *I*. Yeast transformants were screened for the translocation by TAFE (Gardiner and Patterson 1988) and putative translocations confirmed by DNA blot hybridization using appropriate chromosome *I* probes (Southern 1975). Markers were introduced into the translocation strains by conventional genetic crosses. The yeast *ADE2* gene was inserted on a 2.3-kb *Bgl*II fragment in the *Bgl*II site at the right end of the *PHO11* gene on the chromosome *I–II* translocation by one-step gene replacement (Rothstein 1991).

Insertion of the *ARG4* gene to the left of *MAK16*: To enable a determination of the total amount of recombination on bisection II (Table 1), a marker was placed within 2 kb of its left end. A 2.0-kb *Hpa*I fragment from plasmid pKML1 containing the *S. cerevisiae ARG4* gene (supplied by Karen Lusnak; Beacham *et al.* 1984) was inserted at the Klenow DNA polymerase "filled-in" *Bam*HI site of plasmid pVG59 (Guacci and Kaback 1991). A *Cla*I-*Sph*I restriction fragment containing the insertion was integrated by one-step gene replacement (Rothstein 1991) at the appropriate site on chromosome *I* in strains JM31, JM32, and JM32R. This insertion, *iARG4*, was in the nonessential *FUN38*(*DRS2*) gene (Ripmaster *et al.* 1993; Barton and Kaback 1994) and in heterozy-

TABLE 1

Yeast strains

Strain	Genotype
DB1	*MAT***a** *CDC24 CDC19* [YLpVG47 *URA3*] *ade1::HIS3 pho11::LEU2 TRP1 leu2-3,112 his3-11,1 ura3-1* *MAT*α *cdc24-4 cdc19-1*[a] [YLpVG47 *URA3*] *ADE1 PHO11 trp1 leu2-3,112 his3-11,1 ura3-1*
DB1R	*MAT***a** *CDC24 CDC19 ade1::HIS3 pho11::LEU2 TRP leu2-3,112 his3-11,15 ura3-1* *MAT*α *cdc24-4 cdc19-1*[a] *ADE1 PHO11 trp1 leu2-3,112 his3-11,15 ura3-1*
JM31	*MAT***a** [YLpVG59 *URA3*] *iARG4 fun30:LEU2 ADE1 0 TRP1 leu2-3,112 his3-11,15 ura3-1 met10* *MAT*α [YLpVG59 *URA3*] *0 FUN30 ade1 pHIS3 trp1 leu2-3,112 his3-11,15 ura3-1 MET10*
JM31R	*MAT***a** *FUN30 ADE1 0 TRP1 leu2-3,112 his3-11,15 ura3-1 met10* *MAT*α *fun30::LEU2 ade1 pHIS3 trp1 leu2-3,112 his3-11,15 ura3-1 MET10*
JM32	*MAT***a** *CDC24* [YLpVG59 *URA3*] *0 FUN30 ADE1 pHIS3 trp1 leu2-3,112 his3-11,15 ura3 MET10* *MAT*α *cdc24-5* [YLpVG59 *URA3*] *iARG4 fun30::LEU2 ade1 0 TRP1 leu2-3,112 his3-11,15 ura3-1 met10*
JM32R	*MAT***a** *CDC24 0 FUN30 ADE1 pHIS3 trp1 leu2-3,112 his3-11,15 ura3-1 MET10* *MAT*α *cdc24-5 iARG4 fun30::LEU2 ade1 0 TRP1 leu2-3,112 his3-11,15 ura3-1 met10*
JL51	*MAT***a** *0 iTRP1* [YLpLF273*URA3*] *CDC24 FUN30 ADE1 trp1 leu2-3,112 his3 ura3 arg4* *MAT*α *iHIS3 0* [YLpLF273*URA3*] *cdc24-4 fun30::LEU2 ade1 trp1 leu2-3,112 his3 ura3 arg4*
JL52	*MAT***a** *0 iTRP1 CDC24 FUN30 ADE1 trp1 leu2-3,112 his3 ura3 arg4* *MAT*α *iHIS3 0 cdc24-4 fun30::LEU2 ade1 trp1 leu2-3,112 his3 ura3 arg4*
DH11	*MAT***a** *ade1 cdc15-1 pho11::LEU2* [*URA3 HIS3*-Tx I] *TRP1 leu2-3,112 his3-11,15 ura3-1* *MAT*α *ADE1 CDC15 PHO11* [*URA3 HIS3*-Tx I] *trp1 leu2-3,112 his3-11,15 ura3-1*
DH19	*MAT***a** *ade1 cdc15-1 pho11::LEU2 TRP1 leu2-3,112 his3-11,15 ura3-1* *MAT*α *ADE1 CDC15 PHO11 trp1 leu2-3,112 his3-11,15 ura3-1*
DH12	*MAT***a** *cdc24-4 cdc19-1*[a] *FUN30* [*URA3 HIS3*-Tx I] *TRP1 leu2-3,112 his3-11,15 ura3-1* *MAT*α *CDC24 CDC19 fun30::LEU2* [*URA3 HIS3*-Tx I] *trp1 leu2-3,112 his3-11,15 ura3-1*
DH13	*MAT***a** *cdc24-4 cdc19-1*[a] *FUN30 TRP1 leu2-3,112 his3-11,15 ura3-1* *MAT*α *CDC24 CDC19 fun30::LEU2 trp1 leu2-3,112 his3-11,15 ura3-1*
CAB21	*MAT***a** *fun30::LEU2 ADE1* [*URA3 HIS3*-Tx II] *TRP1 leu2-3,112 ura3 his3 CDC27 PET9* *MAT*α *FUN30 ade1* [*URA3 HIS3*-Tx II] *trp1 leu2-3,112 ura3 his3 cdc27-1 pet9*
CAB22	*MAT***a** *fun30::LEU2 ade1* [URA3 HIS3-Tx II] *TRP1 leu2-3,112 ura3 his3 CDC27 pet9* *MAT*α *FUN30 ADE1* [*URA3 HIS3*-Tx II] *trp1 leu2-3,112 ura3 his3 cdc27-1 PET9*
CAB25	*MAT***a** *FUN30 ADE1 trp1 leu2-3,112 his3 ura3 CDC27 pet9* *MAT*α *fun30::LEU2 ade1 TRP1 leu2-3,112 his3 ura3 cdc27-1 PET9*
CAB36	*MAT***a** *ADE1 0* [*URA3 HIS3*-Tx II] *TRP1 ade2-101 leu2-3,112 ura3 his3* *MAT*α *ade1 pho11::ADE2* [*URA3 HIS3*-Tx II] *trp1 ade2-101 leu2-3,112 ura3 his3*
CAB38	*MAT***a** *ADE1 0 TRP1 ade2-101 leu2-3,112 ura3 his3* *MAT*α *ade1 pho11::ADE2 trp1 ade2-101 leu2-3,112 ura3 his3*

Chromosome *I* bisections are indicated in the genotype by the presence of the integrated bisecting plasmids [YLpVG47 *URA3*] for bisection I, [YLpVG59 *URA3*] for bisection II, or [YLpLF273 *URA3*] for bisection III. R indicates an isogenic strain produced by reconstituting chromosome *I*. The two chromosome *I–II* reciprocal translocations are indicated in the genotype by the notation [*URA3 HIS3* Tx I] and [*URA3 HIS3* Tx II]. Notations *iARG4*, *iHIS3*, *iTRP1*, and *pHIS3* indicate insertions while the *0* indicates the absence of that insertion in the homologous chromosome.

[a] *CDC19* is also known as *PYK1*.

gotes did not affect spore viability or recombination between any of the other chromosome *I* markers.

Genetic calculations: PD, NPD, and TT indicate the number of parental ditype, nonparental ditype, and tetratype asci, respectively. FDS and SDS indicate the number of asci exhibiting first and second division segregation, respectively, for a given marker. Percentage recombination expressed in centimorgans was calculated as cM = 100(6NPD + TT)/2(PD + NPD + TT) or 100(SDS)/2(SDS + FDS) (Perkins 1949). FDS and SDS asci were scored with respect to *TRP1*. The probabilities of differences being due to random chance (*P*) were calculated from the results of chi-square analysis of the tetrad data.

The amount of recombination between *FUN30* and *CEN1* was calculated from the *fun30*::*LEU2-ADE1*, *fun30::LEU2-CEN1*(*TRP1*), and *CEN1*(*TRP1*)-*ADE1* data where all NPD tetrads for the *fun30::LEU2-ADE1* interval were assigned to the larger *fun30::LEU2-CEN1* interval as long as there were no crossover (SDS) tetrads in the *ADE1-CEN1* interval. This assignment is based on the reasonable assumption that double cross-

overs in the *ADE1-CEN1* interval are extremely rare. Indeed, only a single NPD tetrad out of a total of 372 asci was reported for the interval between *ADE1* and either *SPO7* or *FUN24*, markers that both map near to, but on the opposite side of, the chromosome *I* centromere (Mortimer and Schild 1985; Mortimer *et al.* 1989, 1992).

Crossover (chiasma) interference equals $1 - C$ or $1 - k$. The value C is the coefficient of coincidence and equals the observed fraction of NPD asci divided by the fraction of NPD asci expected from a random distribution of crossovers. The expected fraction of NPD asci was calculated using the equation, $NPD_{expected}/(PD + NPD + TT) = 1/2[1 - TT/(PD + NPD + TT) - (1 - 3TT/2(PD + NPD + TT))^{2/3}]$ (Papazian 1952). The value k is equivalent to the coefficient of coincidence and is calculated using the maximum likelihood method of Snow (1979; Mortimer *et al.* 1989) using the King calculation in the "Tetrads" program (courtesy of J. Kans and R. K. Mortimer; King and Mortimer 1990). The level of interference is expressed simply by the values C or k. In both cases these values decrease from 1 to 0 as interference increases. Intervals lacking NPD asci were assigned a C or k value of 0.

Analysis of recombination in the database: The Saccharomyces Genome Database (SGD; http://genome-www.stanford.edu/Saccharomyces) was searched for all gene pairs that were both physically and genetically mapped. Open reading frame (ORF) center-center distances were used for the physical distance separating each gene pair. Tetrad data for multiple crosses were summed and interference for each interval was calculated using the King calculation of the "Tetrads" program. Statistical analysis was performed using Instat (Graph Pad Software, San Diego) and JMP (SAS Institute, Inc., Cary, NC) software packages.

RESULTS

Reciprocal recombination of chromosome *I* genes contained on different size DNA molecules: Previously, reciprocal recombination of chromosome *I* genes was studied on DNA molecules that were 90–650 kb long. The highest observed rate of recombination over a >10-kb interval was ~1 cM/kb while the lowest in a >10-kb region that was not adjacent to a centromere was ~0.4 cM/kb. To determine whether rates of recombination would further increase and decrease, we constructed a functional 60-kb bisection chromosome (bisection III Figure 1) and an 800-kb translocation chromosome (translocation II; Figure 1). These chromosomes are 35% shorter and 20% longer than any of the previously examined chromosomes. Chromosomes were genetically marked and karyotypes confirmed using pulse-field gel electrophoresis (Figure 2). Rates of reciprocal recombination between each marker pair were analyzed in diploids that were homozygous for the different size chromosomes and in isogenic or congenic controls that contained normal length chromosomes (Table 2). We also show the calculated rates of recombination for previously published tetrad data for many of these same intervals to enable a thorough comparison.

The results indicated that the rate of recombination in the *iHIS3-iTRP1* interval on bisection III was 1.5 cM/kb. This interval includes most of the length of this 60-kb chromosome fragment. The rate of recombination was significantly different ($P < 0.0001$) and two times greater than the observed rate of 0.75 cM/kb for the same interval contained on the full-length control chromosome. It was 50% above the highest rates previously observed in >10-kb intervals on bisection chromosomes. A rate of 1.5 cM/kb is equal to that observed at a recombination hot spot (Kaback *et al.* 1989). Intervals on the right half of this bisection chromosome were only marginally affected by chromosome size (data not shown). In addition, the markers on the 60-kb bisection were no longer linked to the markers on the large 180-kb fragment, confirming the bisection. This linkage was restored when the chromosomes were reconstituted (data not shown).

The rate of recombination on the 800-kb translocation II chromosome (Tx II) was significantly lower than on all smaller chromosomes including the 650-kb translocation I (Tx I) chromosome ($P = 0.005$). For the 64-kb *ADE1-PHO11* interval, the rate of recombination was 0.37 cM/kb, threefold lower than in the 90-kb bisection I chromosome, which is nine times smaller. The rate for the *fun30*::*LEU2-ADE1* interval was twofold lower than the full-length chromosome and almost fourfold lower than the 135-kb bisection II chromosome. This interval was not present in translocation I. Rates in the centromere adjacent *CEN1-ADE1* interval also were lower in translocation II compared to the smaller chromosomes ($P < 0.04$). However, no significant differences were found in this interval when the bisection and the full-length chromosomes were compared (Table 2; Kaback *et al.* 1992). Thus, it appears that this centromere adjacent region is significantly affected by size-dependent control only when the size differential is greater than threefold.

Enhanced recombination on small chromosomes lowers the fraction of homologues that fail to cross over: If all yeast chromosomes recombined at the same average rate, the smallest yeast chromosomes would fail to cross over 5% of the time (the E_0 class). The high rate of recombination normally found on chromosome *I* lowered the E_0 class to 0.2–0.4% or less (Kaback *et al.* 1989). To examine whether altering chromosome size directly affects the size of the E_0 class, the right chromosome fragments of bisections I and II and the left chromosome fragment of bisection III were genetically marked over most of their physical lengths (Figure 1). The number of E_0 asci was determined for each chromosome fragment and for the same intervals on full-length chromosomes. The results indicated that the corrected percentage of E_0 chromosomes was lower in the bisection chromosomes than for the same intervals on full-length chromosomes (Table 3). The corrected values indicated that the 135-kb bisection II chromosome failed to cross over in only 0.4% of the asci, whereas 17% of the asci on full-length chromosomes had no crossovers in these same intervals. The differences for the 90-kb and 60-

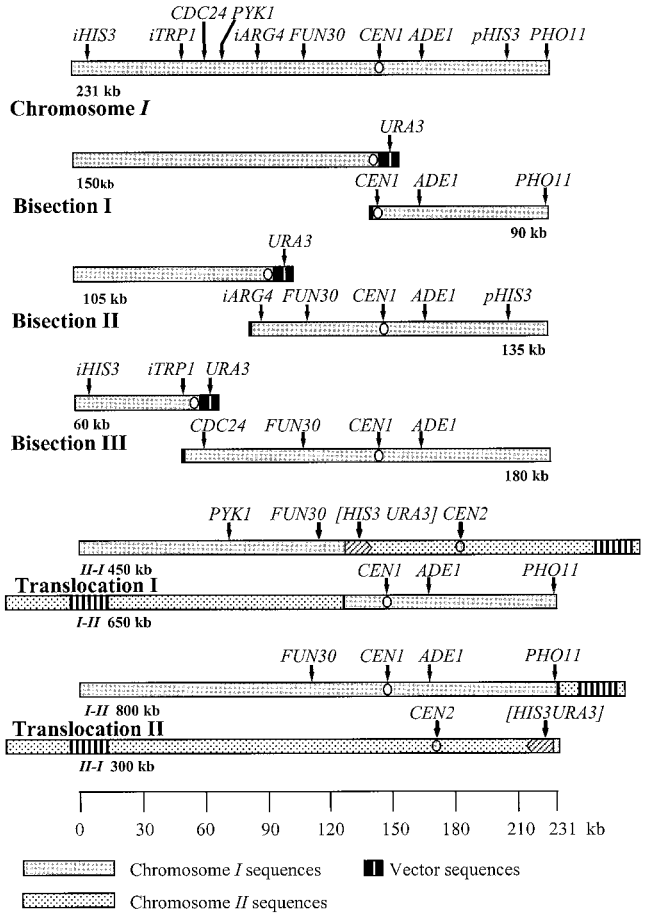


Figure 1.—Physical maps of different size chromosomal constructs showing relevant genetic markers. The approximate size in kilobases of each chromosome or functional chromosome fragment is shown. The arrow at the hatched region labeled *[HIS3 URA3]* (not drawn to scale) indicates the location of the reconstituted *HIS3* gene and the associated *URA3* selectable marker used in constructing the translocations. The hatched regions in chromosome *II* sequences indicate that the whole chromosome is not shown. *PYK1* is also known as *CDC19*.

kb bisection III chromosomes were less dramatic but significant ($P < 0.05$). These results demonstrate that the fraction of chromosomes that fail to cross over is reduced by the enhanced recombination.

The above analysis required the introduction of the *iARG4* marker near a telomere on the right half of the bisection II chromosome (Figure 1). Interestingly, the amount of recombination in the telomere adjacent *iARG4-fun30::LEU2* interval was ~2.5-fold lower than in the full-length chromosome control (Table 2). These results are consistent with the idea that telomeres inhibit meiotic reciprocal recombination. Alternatively, the lower level of recombination could be due to the removal of an initiation site for recombination as a result of bisecting the chromosome. In either case, this observed inhibition is dramatic and could mask any change in recombination rates due to chromosome size.

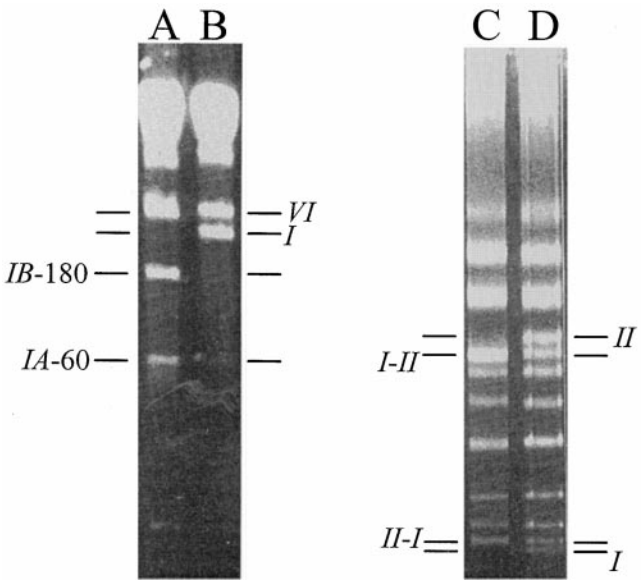


Figure 2.—Pulsed-field gel electrophoretic karyotypes of strains containing new chromosome constructs. (A) Bisection III containing 60-kb and 180-kb functional chromosome fragments. (B) Isogenic control containing reconstituted copy of chromosome *I* and the normal complement of yeast chromosomes. (C) Translocation II. (D) Congenic control containing the normal complement of yeast chromosomes. Roman numerals refer to bisection chromosomes *IA–60* and *IB–180*, reciprocal translocation chromosomes *I–II* (~800 kb) and *II–I* (~280 kb) and to normal chromosomes *I* (~231 kb), *II* (~813 kb), and *VI* (~270 kb). A and B were electrophoresed using 20-sec pulses to separate small chromosomes efficiently. C and D were electrophoresed using 60-sec pulses to display all chromosomes. Under these conditions chromosome *I–II* forms a doublet with chromosome *XIV* and chromosome *II–I* forms a doublet with chromosome *VI* as evidenced by the increased band intensities.

The amount of crossover interference changes as a function of chromosome size: The amount of crossover interference within all intervals showing NPD tetrads for at least one chromosome *I* construct was determined using the methods of Papazian (1952) and King and Mortimer (1990). The results obtained using each method were in agreement and indicated that both the C and *k* decreased significantly for a given interval as the size of the chromosome increased (Table 2). Therefore, crossover interference ([1 − *C*] or [1 − *k*]) increases with chromosome size (Figure 3). In most cases the smallest bisection chromosomes showed little interference for the measured intervals with *C* or *k* values approaching or slightly exceeding 1.0. All values >1.0 were within less than one standard error of being equal to 1.0. Therefore, there was no evidence for negative interference. In contrast to the bisections, the largest translocations usually had no NPD asci, indicating almost total interference or 100% inhibition of a second crossover within an interval. These results suggest that chromosome size-dependent control is the result of regulating the amount of crossover interference.

TABLE 2

Tetrad analysis of markers contained on different size copies of chromosome *I*

Gene pair and interval size	Chromosome and size	Strain[a]	PD (FDS)	NPD	TT (SDS)	cM	cM/kb	*C*	*k*
iHIS3-iTRP1 43 kb	Bi III 55 kb	JL51	46	21	128	65.1	1.51	0.76	0.94 ± 0.21
	WT 231 kb	JL52	104	3	146	32.4	0.75	0.15	0.29 ± 0.14
fun30::LEU2-ade1 53 kb	Bi II 135 kb	JM31/JM32	82	11	100	43.0	0.81	1.00	1.16 ± 0.23
	WT 231 kb	JM31R/JM32R/CAB25	308	5	248	24.8	0.47	0.24	0.34 ± 0.14
	TxII 800 kb	CAB21/CAB22	267	0	80	11.5	0.22	0.00	0.00 ± 0.36
ade1-pHIS3 51 kb	Bi II 135 kb	JM31/JM32	64	11	115	47.6	0.93	0.61	0.98 ± 0.23
	WT 231 kb	JM31R/JM32R	89	4	177	37.2	0.73	0.11	0.23 ± 0.10
cdc19-fun30::LEU2 44 kb	WT 231 kb	DH13	46	3	57	35.4	0.80	0.44	0.64 ± 0.29
	Tx I 450 kb	DH12	60	0	38	19.4	0.44	0.00	0.00 ± 0.39
ade1-pho11::LEU2 64 kb	Bi I 90 kb	DB1	34	17	95	67.5	1.05	0.87	1.02 ± 0.17
ade1-pho11[b]	WT 231 kb	DB1R/DH19/CAB38	113	17	321	46.9	0.73	0.38	0.42 ± 0.08
ade1-pho11::LEU2	Tx I 650 kb	DH11	63	1	107	33.0	0.52	0.05	0.13 ± 0.11
ade1-pho11::ADE2	Tx II 800 kb	CAB36	112	0	102	23.8	0.37	0.00	0.00 ± 0.09
fun30::LEU2-CEN1(trp1) 34 kb	Bi II 135 kb	JM31/JM32	96	6	98	33.5	0.99	0.62	0.82 ± 0.26
	WT 231 kb	JM31R/JM32R/CAB25	358	2	214	19.7	0.58	0.15	0.25 ± 0.15
	Tx II 800 kb	CAB21/CAB22	265	0	85	12.1	0.36	0.00	0.00 ± 0.20
CEN1(trp1)-ade1 18 kb	Bi I 90 kb	DB1	120		20	7.1	0.40		
	Bi II 135 kb	JM31/JM32	168		26	6.7	0.37		
	WT 231 kb	DB1R/CAB25/CAB38	786		133	7.2	0.40		
	Tx II 800 kb	CAB21/CAB22/CAB36	525		38	3.4	0.19		
iARG4-fun30::LEU2 18 kb	Bi II 135 kb	JM31/JM32	180	0	15	3.9	0.21		
	WT 231 kb	JM32R	122	0	26	8.8	0.49		

Marker pairs and physical size of each interval (in kilobases) are shown. Chromosome size shows each construct and the total size of the DNA molecule containing the interval. Bi I, Bi II, and Bi III refer to bisection chromosomes, WT refers to normal-length chromosomes, and Tx I and II refer to reciprocal translocations. PD, NPD, and TT refer to the number of parental ditype, nonparental ditype, and tetratype asci, respectively. For the *CEN1-ADE1* interval the number of first division segregation (FDS) and second division segregation (SDS) asci are shown in the PD and TT columns, respectively. Centimorgans (cM) were calculated as described in materials and methods. Rates of recombination (cM/kb) for each interval are shown. The values for *C* and *k* (± standard error) were calculated for all applicable intervals as described in materials and methods. Intervals with no NPD asci were assigned a *C* or *k* value of 0 ±, an error calculated on the basis of observing a single NPD ascus. However, *k* cannot be negative. Note that interference is equal to $(1-C)$ or $(1-k)$; therefore, as *C* or *k* decreases from 1 to 0, the level of interference increases.

[a] When multiple strains are listed, results shown are the sums from the analysis of each strain listed. In all cases, the individual strains showed no significant differences between them.

[b] *pho11::LEU2* or *pho11::ADE2*.

Interference changes as a function of chromosome size on real *S. cerevisiae* chromosomes: The large amount of genetic mapping data was first used to show that small chromosomes had higher rates of reciprocal recombination than large chromosomes (Kaback *et al.* 1989; Mortimer *et al.* 1989; Riles *et al.* 1993). To investigate whether this relationship correlated with changes in the level of crossover interference, we examined the SGD for all gene pairs that have been both physically and genetically mapped. We calculated and compared the *k* value of interference for each interval as a function of its physical size as described in materials and methods. Data for each chromosome were plotted in Figure 4. The calculated values were variable due to the fact that they were frequently based on very few (0–5) NPD tetrads. Nevertheless, these individual points should average out to produce an accurate description of how *k* changes as a function of interval size. A linear regression analysis indicated that *k* increases as interval size gets larger for all chromosomes.

As the fraction of NPD asci increases, it is possible to estimate *k* more accurately. There were 50 database entries containing >20 NPD tetrads where *k* had very small standard errors (<15%). When these data were examined, the *k* values fell very close to the regression line derived from all the data (Figure 4). These results suggest that the estimates produced from the entire data set for each chromosome closely approximate the actual values.

Relative levels of interference for a given size interval on each chromosome can therefore be defined by a function of the slope of the linear regression. The steeper the slope, the less interference for a given size interval on that chromosome. Therefore, the slopes for each chromosome were plotted as a function of chromosome size (Figure 5). The results indicated that the smallest chromosomes had the steepest slopes. A linear regression analysis produced a line that had a significant negative slope ($P = 0.03$, $r^2 = 0.32$), indicating that *k* for a given size interval is lower, and thus interference

TABLE 3

Homologues that fail to cross over

Interval	Chromosome	% E_0 Uncorrected	% E_0 Corrected
CEN1-ADE1-PHO11	Bisection I—90 kb	21.0	8.3
	Full length	16.5	13.0
iARG4-FUN30-ADE1-pHIS3	Bisection II—135 kb	9.9	0.4
	Full length	18.7	16.7
iHIS3-iTRP1	Bisection III—60 kb	23.6	12.8
	Full length	41.1	30.9

The percentage of asci that failed to crossover (the E_0 class) was determined from individual tetrads for the intervals shown. Uncorrected E_0 values are the percent of asci that had only PD and FDS tetrads for all marker pairs. The corrected E_0 class was calculated to account for two-strand double crossovers that give rise to PD asci and was obtained by assuming that the number of two- and four-strand double crossovers was equal (Mortimer and Fogel 1974) and subtracting the number of asci containing four-strand double crossovers (NPDs) from the total number asci with only PD and FDS for all marker pairs.

is greater on the larger chromosomes compared to the smaller ones.

Because the relationship between the slope and chromosome size may not be linear, the data were replotted as *k vs.* ln chromosome size and analyzed by linear regression (not shown). The transformation produced a negative slope with a higher probability of significance ($P = 0.003$) and a closer fit to linearity in the ln scale ($r^2 = 0.45$). We also examined each chromosome using intervals that were <105 kb so that only like-size intervals on small and large chromosomes could be directly compared. These analyses revealed the same relationship as the total database except potential errors were larger due to much smaller sample sizes for each chromosome (data not shown).

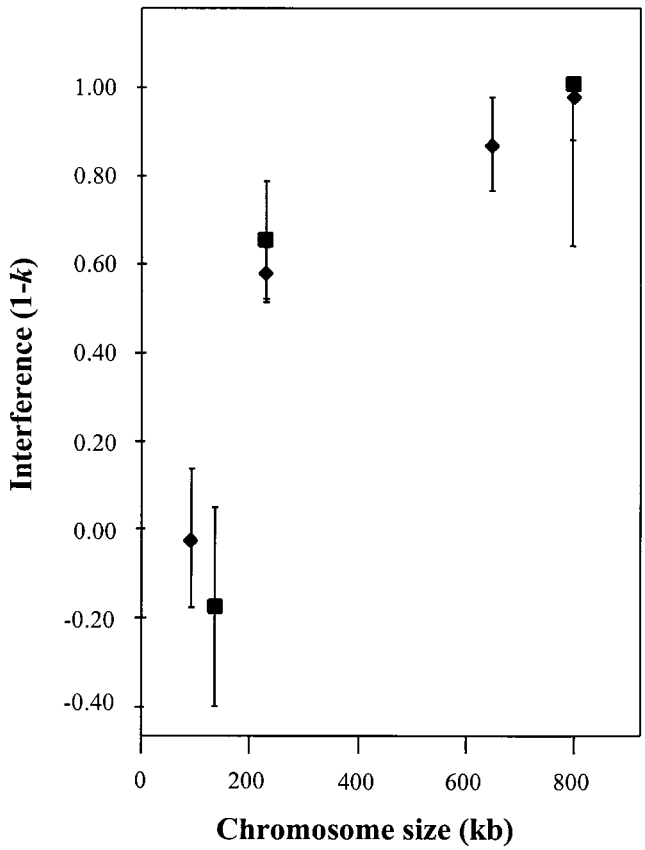


Figure 3.—Crossover interference ($1 - k$) increases with increasing chromosome size. Values calculated from Table 2 for the 53-kb *fun30::LEU2-ADE1* (■) and the 64-kb *ADE1-PHO11* (◆) intervals contained on different size copies of chromosome *I* were plotted as a function of chromosome size. Bars denote the standard error.

In summary, the database analyses are consistent with the experiments presented here showing that intervals that are the same physical size exhibit more crossover interference on large chromosomes than on small chromosomes. These observations support our suggestion that chromosome size-dependent control is the result of regulating the amount of crossover interference. A model for how interference regulates overall recombination rates as a function of chromosome size is presented below.

DISCUSSION

We have previously demonstrated that chromosome size has a direct effect on the level of meiotic reciprocal recombination. However, with one exception, the differences between the altered chromosomes and the full-length control were less than twofold and the greatest rate of recombination was ~1 cM/kb over a large region, while the smallest was ~0.4 cM/kb. This low rate was still greater than the average for the larger yeast chromosomes. The studies reported here show that further increasing the size of chromosome *I* further reduced rates of recombination to those found on the larger *S. cerevisiae* chromosomes (0.19–0.37 cM/kb). In contrast, decreasing the size of a chromosome to 60 kb, produced a reciprocal recombination rate over a 43-kb interval that was equal to that found in a recombination hot spot. A three- to fourfold difference in the rate of

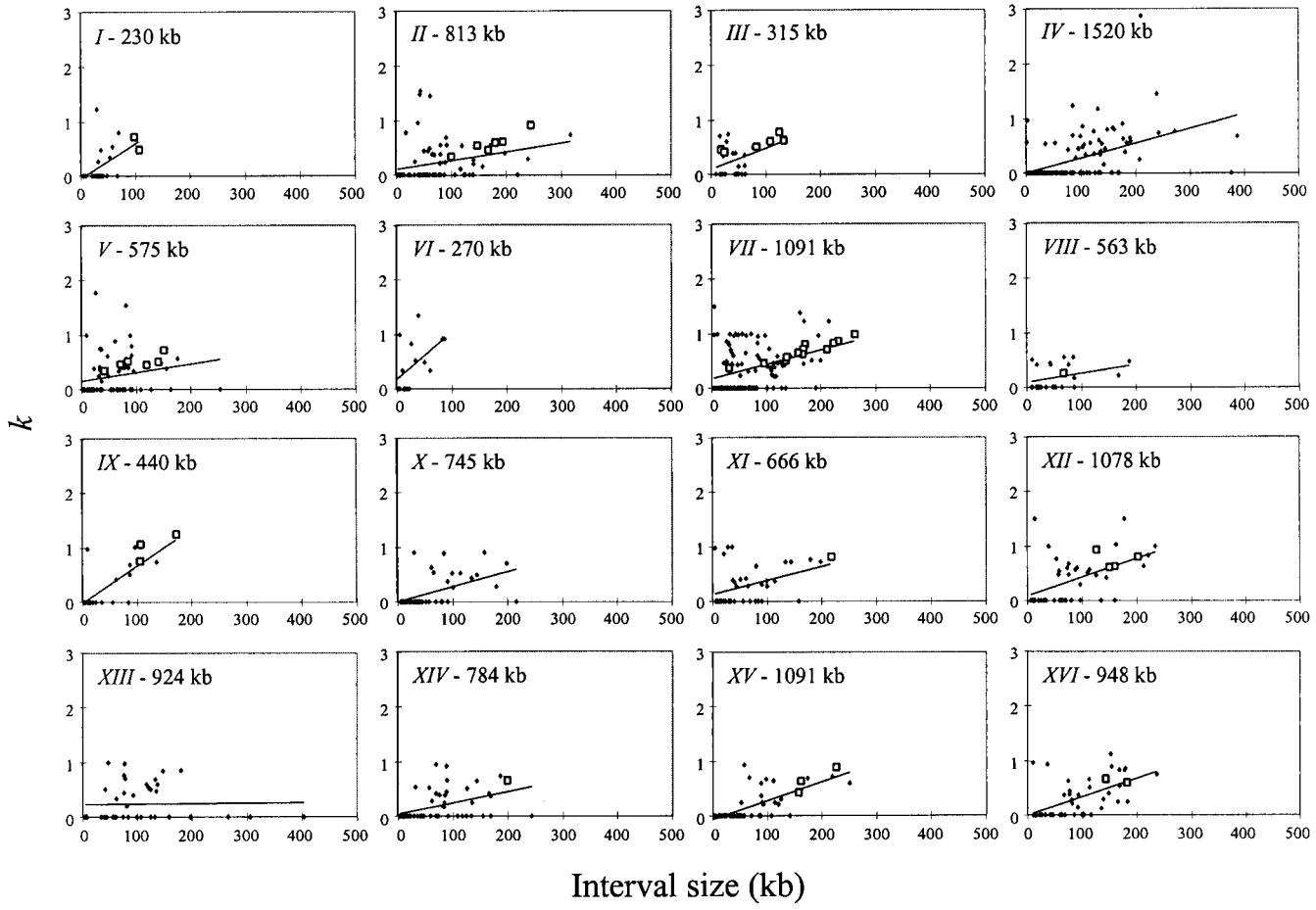


Figure 4.—Crossover interference on each *S. cerevisiae* chromosome. The value *k* (a measure of crossover interference) was calculated for each gene pair in the SGD that was both genetically and physically mapped and plotted *vs.* the physical size of each interval (◆). Intervals with >20 reported NPD asci where interference could be determined accurately (□). Physical sizes (in kilobases) are shown for each chromosome (*I–XVI*).

recombination in the same intervals was readily seen between the bisection chromosomes and the largest translocations. These comparisons were carried out between strains that did not share isogenic backgrounds. Nevertheless, with few exceptions, recombination rates in each of the full-length control chromosomes were approximately equal, making these comparisons valid.

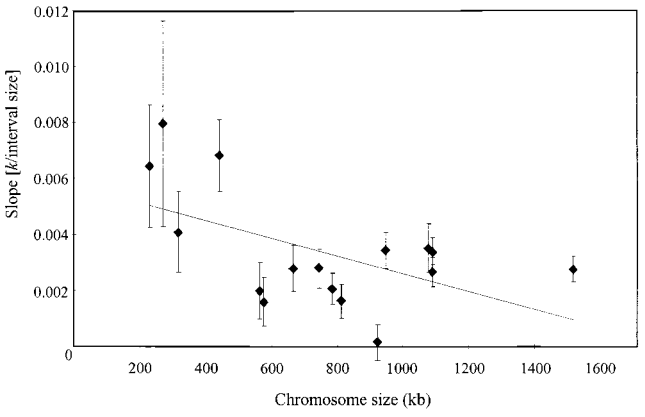


Figure 5.—Crossover interference is greater on larger chromosomes. The slopes for each chromosome (*k*/interval size) were plotted as a function of chromosome size. Smaller slopes indicate greater levels of interference. Bars indicate the standard error.

The amount of positive crossover interference was found to increase with chromosome size. Accordingly, we propose that small chromosomes have higher rates of reciprocal recombination because there is less crossover interference and large chromosomes have lower rates of recombination because there is more interference. Analysis of the database of genes that are both physically and genetically mapped supports these experimental results. The slopes derived from the measure of interference, *k vs.* interval size, were smaller for the larger chromosomes than for the smaller chromosomes (Figures 4 and 5). If we derive slopes for the chromosome *I* bisection data by extrapolating a plot of *k vs.* interval size to zero, the slopes are greater than for the intact copy of chromosome *I* (0.16–0.25 *vs.* 0.065; data not shown). Because *k* is an inverse function of interference, these results indicate that the levels of interference appear to increase with increasing chromosome size.

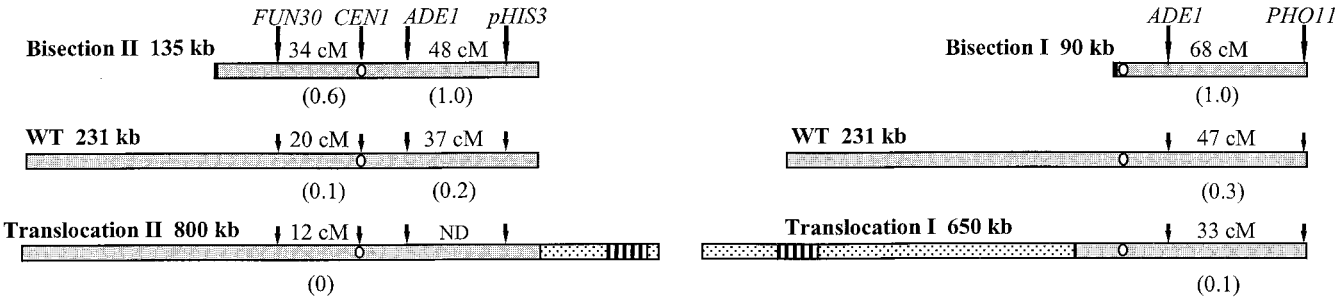


Figure 6.—Chromosome size-dependent control of reciprocal recombination passes through the centromere. Physical maps of relevant markers, chromosome lengths (in kilobases), reciprocal recombination distances (in centimorgans), and the measure of crossover interference, *k* (number in parentheses) are shown. Data are from Table 2. Averages were used for the full-length chromosomes. Lower *k* values indicate increased crossover interference. Symbols are described in Figure 1. ND, not determined.

A high degree of scatter was observed in the interference values calculated from the data base. Most of this variability is due to the small number of observed NPD asci for many of the data entries. Combining all the data for a given chromosome and plotting these data as a function of the physical size of each interval should average out this variability. Note that the slope with the largest potential error was for chromosome *VI*, which contained the fewest data points. Where interference could be accurately measured because there were 20 or more NPD asci scored, the values all fell very close to the regression line defined by the entire data set for each chromosome (Figure 5). For chromosomes *II*, *V*, and *VII*, the number of points containing >20 NPD asci was sufficiently large to define almost the same slope produced from the entire data set. Similarly, when intervals containing >500 asci analyzed were examined, the values also were very close to the regression line defined by the entire data set (data not shown). These points were mostly made up of the same points containing >20 NPD asci. Thus, the regression lines appear to closely approximate the actual values.

While we have not yet derived a suitable equation that defines the precise relationship of the *k*/interval size slopes to chromosome size, regression analysis of these points produced lines with significant negative slopes, indicative of increased interference in the larger chromosomes. Therefore, the combined results of many years of genetic mapping support our suggestion that the amount of interference in a given size interval varies as a function of chromosome size. The functions shown here may be useful in predicting the amount of crossover interference in a given size interval for each chromosome.

Large chromosomes had some very large genetically mapped intervals (>200 kb and 70 cM). In almost all of these intervals, interference was still easily observable ($k < 1.0$). These data indicate that in *S. cerevisiae* interference affects >200 kb of DNA on the large chromosomes.

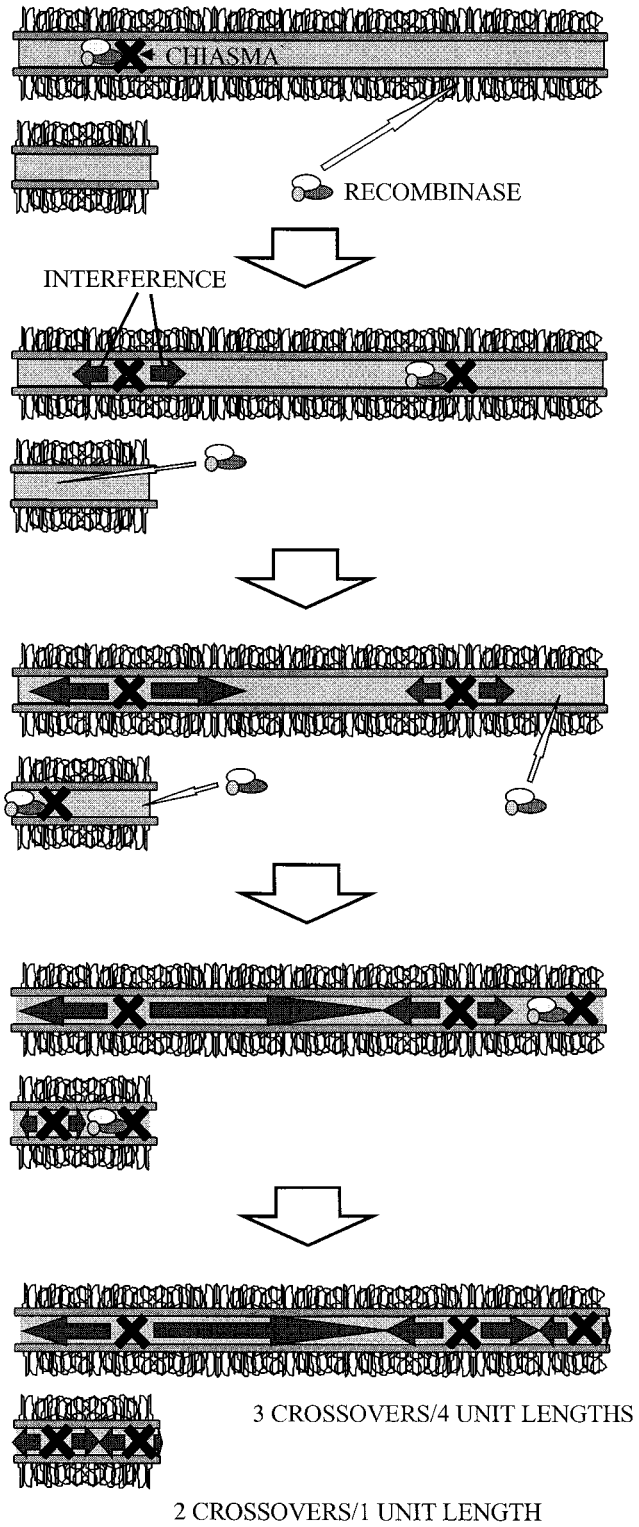
Chromosome size-dependent control appears to be a function of the size of the entire chromosome and not the size of a chromosome arm. Addition or removal of sequences from one arm of the chromosome affected recombination on the other arm (Figure 6). Because chromosome size-dependent control of recombination appears to be a function of crossover interference, it would appear that interference can pass through the centromere. This idea contrasts with the previous suggestion that interference was blocked by the centromere (Muller 1916). Rates of recombination near centromeres are lower in *S. cerevisiae* (Clarke and Carbon 1980; Lambie and Roeder 1986; Kaback *et al.* 1989). The idea that the centromere blocks interference is based on genetic mapping and does not take into consideration the fact that greater physical distances separate centromeric markers. Accordingly, interference must travel over greater physical distances near centromeres, giving the appearance of a block. This argument applies to Drosophila where centromeres are surrounded by heterochromatin that undergoes little to no meiotic reciprocal recombination.

The largest yeast chromosomes had average rates of reciprocal recombination of 0.29 cM/kb (Mortimer *et al.* 1989) and exhibited high levels of interference. The smallest yeast chromosome constructs described here had average rates of recombination of 1.0–1.5 cM/kb and exhibited little if any observable interference. If interference is indeed responsible for this size-dependent difference in rates of recombination, the ratio of these recombination rates would suggest that crossover interference might be inhibiting 75–80% of all potential crossovers on large chromosomes in yeast. The smallest bisection chromosomes showed very little interference in some of these intervals, indicating that the observed rates of reciprocal recombination may be approaching their maximum.

We also demonstrated that the fraction of chromosomes that fail to cross over is significantly reduced by the increased rate of recombination. These results show, as Muller (1916) suggested, that crossover interference is indeed involved in ensuring that chromosomes succeed in crossing over with their homologues.

The mechanism of crossover interference is unknown. Several models have been suggested and we

propose another. In most models a crossover initiates a structural change that prevents further crossing over and this change is transmitted bidirectionally down the length of the chromosome in a time-dependent manner. In the model proposed by King and Mortimer (1990), a crossover initiates polymerization of a recombination inhibitor along the length of the chromosome.



In our model, a crossover initiates a conformational chain reaction where a chromosomal component allosterically blocks recombination and causes a neighboring component to do likewise. This conformational change is analogous to arrays of falling dominoes where a crossover topples the first domino, initiating a chain reaction that prevents additional crossing over. The above two models may be kinetically similar if the rate of inhibitor polymerization parallels the rate of propagating the chain reaction. Both models are compatible with their occurrence within the framework of the synaptonemal complex (SC). In the domino model, SC components themselves undergo the conformational change. A third model proposes that zippering of the SC prevents further recombination (Maguire 1977; Egel 1978). A fourth model proposes that additional recombination is inhibited by crossover-induced release of tension (Kleckner 1997). Finally, a model has been proposed where a recombinase counts recombination intermediates, but this model does not appear to apply to yeast (Foss *et al.* 1993; Foss and Stahl 1995). The first three models all suggest that DNA close to a crossover rarely recombines again because little time is required for it to be reached by whatever causes interference. In the tension model, the level of tension is lowest near where crossovers are initiated producing the greatest inhibition.

We propose a mechanism for how interference responds directly to chromosome size (Figure 7). The model is based on the idea that interference propagates down the chromosome bidirectionally from the site of the crossover in a time-dependent manner (King and Mortimer 1990) and is most applicable to the first three mechanisms discussed above. We propose that at least one component of the crossover-forming machinery is freely diffusible, recyclable, and most important, rate limiting. For purposes of this discussion, this component will be called a recombinase. However, it may be a Holliday junction resolvase because interference

Figure 7.—Model showing how interference levels change as a function of chromosome size. A time course for a large and a small chromosome depicted in SCs is shown. The shaded arrows inside the SC denote crossover interference, which prevents further reciprocal recombination. This shaded area could represent regions where a recombination inhibitor has bound or regions that have undergone a crossover-dependent conformational change. In the event that interference is due to zippering of the SC, the shaded regions could also represent regions where SC zippering has occurred while the unshaded regions could be where the chromosomes are still present as axial elements. The recombination machinery is proposed to be both diffusible and rate limiting. Larger chromosomes are bigger targets for the recombination machinery and on average form some crossovers and initiate interference earlier than smaller chromosomes. Early crossovers are able to propagate more interference, preventing recombination in larger regions.

has been proposed to inhibit a more terminal stage of reciprocal recombination (Mortimer and Fogel 1974; King and Mortimer 1990). On the basis of these assumptions, we speculate that the interaction between chromosomes and the recombination machinery will follow second-order kinetics. Accordingly, larger chromosomes will provide larger targets for the recombination machinery and, on average, will be more likely to come in contact with this machinery and form crossovers earlier than small chromosomes. Once a chromosome receives a crossover, interference is initiated and begins to prevent reciprocal recombination bidirectionally from the site of each crossover, removing that DNA from the pool of sequences that can cross over again. As a result of early crossovers occurring preferentially on large chromosomes, large parts of large chromosomes are rendered unable to cross over again. At some point, small chromosomes successfully compete with the remaining recombination-proficient parts of the large chromosomes for recombinase. At this time, the ratio of recombinase to recombination-proficient DNA must be higher than at the beginning. Thus, what remains will initiate recombination at a higher rate than at early times and there will be less time for interference to reach a given point. These effects produce a higher average rate of recombination and less interference on whatever undergoes recombination late. Accordingly, the average rate of recombination is higher on the smaller chromosomes.

If synapsis is responsible for interference (Maguire 1977; Egel 1978), the model still applies except the rate-limiting step for crossover formation would be the nucleation of synapsis of homologous chromosomes. In this case, large chromosomes would be expected to synapse earlier based on their size, similar to the hybridization of large *vs.* small DNA molecules (Wetmur and Davidson 1968).

If crossover-induced release of tension is responsible for crossover interference, large chromosomes would be expected to exhibit more tension per unit length. Accordingly, a crossover would relieve more tension and cause more interference on the largest chromosomes.

Our model predicts that large chromosomes, on average, will form crossovers earlier than small chromosomes and that a crossover will affect more DNA on large chromosomes (*i.e.*, interference tracts will be larger) than on small chromosomes. It also predicts that altering the amount of crossover-forming machinery could either enhance or eliminate chromosome size-dependent control of recombination. Because our model is kinetically similar to that of King and Mortimer (1990), the computer simulations that were carried out and appear consistent with our results, must also apply.

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