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 disjoint 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 CYCS3 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.
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 bisection 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). Ascii were dissected and analyzed as previously described (Sherman et al. 1986). Tetrad data from the small number of three viable-spored ascii were consistent with data from four viable-spored ascii 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 pLFZ73, as previously described (Guacci and Kaback 1991). This plasmid was constructed from plasmid pVG57 (Guacci and Kaback 1991) by inserting the 2.2-kb BglII fragment from the YAL049 region of chromosome I and a 1.6-kb SphI telomere containing fragment from pKR56 (a gift from V. Zakian). Cleavage of this plasmid with BamHI resulted in a 10-kb linear centromere-containing minichromosome with telomeres at both ends that was introduced into yeast strain VG37B. 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 BamHI and 0.8-kb Pml-Stul 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 EcoRI target fragment from plasmid pYY67 (Stensaas et al. 1989) was inserted in the Yip55-based plasmid containing the 5' half of the HIS3 gene. The 5.0-kb target fragment contains the PH011 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 BglII fragment in the BglII site at the right end of the PH011 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 Hpal 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" BamHI site of plasmid pVG59 (Guacci and Kaback 1991). A ClaI-SphI 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 (Rimpe et al. 1993; Barton and Kaback 1994) and in heterozy-
TABLE 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB1</td>
<td>MATα CDC24 CDC19 [YLPVG47 URA3] adel::His3 pho11::LEU2 TRP1 leu2-3,112 his3-11,1 ura3-1</td>
</tr>
<tr>
<td></td>
<td>MATαcdc24-4 cdc19-1Δ [YLPVG47 URA3] ADE1 PHO11 trp1 leu2-3,112 his3-11,1 ura3-1</td>
</tr>
<tr>
<td>DB1R</td>
<td>MATα CDC24 CDC19 adel::His3 pho11::LEU2 TRP1 leu2-3,112 his3-11,1 ura3-1</td>
</tr>
<tr>
<td></td>
<td>MATαcdc24-4 cdc19-1Δ ADE1 PHO11 trp1 leu2-3,112 his3-11,1 ura3-1</td>
</tr>
<tr>
<td>JM31</td>
<td>MATα [YLPVG59 URA3] iARG4 fun30::LEU2 ADE1 0 TRP1 leu2-3,112 his3-11,15 ura3-1 met10</td>
</tr>
<tr>
<td></td>
<td>MATα[YLPVG59 URA3] 0 fun30::ADE1 phIS3 trp1 leu2-3,112 his3-11,15 ura3-1 MET10</td>
</tr>
<tr>
<td>JM31R</td>
<td>MATα [YLPVG59 URA3] 0 fun30::ADE1 phIS3 trp1 leu2-3,112 his3-11,15 ura3-1 MET10</td>
</tr>
<tr>
<td>JM32</td>
<td>MATα CDC24 [YLPVG59 URA3] 0 fun30::ADE1 phIS3 trp1 leu2-3,112 his3-11,15 ura3-1 MET10</td>
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<tr>
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<td>MATαcdc24-5 [YLPVG59 URA3] iARG4 fun30::LEU2 adel 0 TRP1 leu2-3,112 his3-11,15 ura3-1 met10</td>
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<td>JL51</td>
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<tr>
<td></td>
<td>MATα iHis3 0 [YLPVF273URA3] cdc24-4 fun30::LEU2 adel phleu2-3,112 his3 ura3-1 arg4</td>
</tr>
<tr>
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<tr>
<td>DH11</td>
<td>MATα adel cdc15-1 pho11::LEU2 [URA3 His3-Tx I] TRP1 leu2-3,112 his3-11,15 ura3-1</td>
</tr>
<tr>
<td></td>
<td>MATα ADE1 CDC15 PHO11 [URA3 His3-Tx I] trp1 leu2-3,112 his3-11,15 ura3-1</td>
</tr>
<tr>
<td>DH19</td>
<td>MATα adel cdc15-1 pho11::LEU2 TRP1 leu2-3,112 his3-11,15 ura3-1</td>
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<td>MATα ADE1 CDC15 PHO11 trp1 leu2-3,112 his3-11,15 ura3-1</td>
</tr>
<tr>
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<td>MATα cdc24 cdc19-1Δ fun30::URA3 His3-Tx I] trp1 leu2-3,112 his3-11,15 ura3-1</td>
</tr>
<tr>
<td>DH13</td>
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</tr>
<tr>
<td>CAB21</td>
<td>MATα fun30::LEU2 ADE1 [URA3 HIS3-Tx II] TRP1 leu2-3,112 his3 CDC27 PET9</td>
</tr>
<tr>
<td></td>
<td>MATα fun30::LEU2 adel [URA3 HIS3-Tx II] trp1 leu2-3,112 his3 cdc27-1 pet9</td>
</tr>
<tr>
<td>CAB22</td>
<td>MATα fun30::LEU2 adel [URA3 HIS3-Tx II] TRP1 leu2-3,112 his3 CDC27 pet9</td>
</tr>
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<td>MATα fun30::LEU2 ADE1 [URA3 HIS3-Tx II] trp1 leu2-3,112 his3 cdc27-1 PET9</td>
</tr>
<tr>
<td>CAB25</td>
<td>MATα fun30::LEU2 ADE1 trp1 leu2-3,112 his3 CDC27 pet9</td>
</tr>
<tr>
<td>CAB36</td>
<td>MATα adel pho11::ADE2 [URA3 HIS3-Tx II] TRP1 adel101 trp1 leu2-3,112 his3 his3</td>
</tr>
<tr>
<td>CAB38</td>
<td>MATα adel pho11::ADE2 [URA3 HIS3-Tx II] del101 trp1 leu2-3,112 his3 his3</td>
</tr>
</tbody>
</table>

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 [YLPVF273 URA3] for bisection III. R indicates an isogenic strain produced by reconstituting chromosome I. The two chromosome I–I 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.

* CDC19 is also known as PYK1.

gates 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 ascis, 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)/2PD + 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 SP07 or FUN24, markers that both map near to, but on the opposite side of, the chromosome I centromere (Mortimer and Schidl 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, $NPD_{expected} = PD + NPD + TT = 1/2 [1 - TT/(PD + NPD + TT)] - (1 - 3TT/2(PD + NPD + TT))^2$ (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 all physical distances 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 IH153-TRP1 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-PH011 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-
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 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

<table>
<thead>
<tr>
<th>Gene pair and interval size</th>
<th>Chromosome and size</th>
<th>Strain</th>
<th>PD (FDS)</th>
<th>NPD</th>
<th>TT (SDS)</th>
<th>cM</th>
<th>cM/kb</th>
<th>C</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>ade1-pho11::LEU2 64 kb</td>
<td>Bi I 90 kb</td>
<td>DB1</td>
<td>1.05</td>
<td>0.87</td>
<td>1.16</td>
<td>0.82</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ade1-pho11::LEU2 64 kb</td>
<td>Bi II 135 kb</td>
<td>JM31/JM32</td>
<td>0.44</td>
<td>0.64</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ade1-pho11::LEU2 64 kb</td>
<td>Bi I 90 kb</td>
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<td>0.29</td>
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</tbody>
</table>

**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 ascis 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, r² = 0.32), indicating that k for a given size interval is lower, and thus interference
TABLE 3
Homologues that fail to cross over

<table>
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<th>Interval</th>
<th>Chromosome</th>
<th>Uncorrected</th>
<th>Corrected</th>
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<td>CEN1-ADE1-PHO11</td>
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<td>Full length</td>
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<td>13.0</td>
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</tr>
<tr>
<td>iHIS3-iTRP1</td>
<td>Bisection III—60 kb</td>
<td>23.6</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>Full length</td>
<td>41.1</td>
<td>30.9</td>
</tr>
</tbody>
</table>

The percentage of asci that failed to crossover (the E₀ class) was determined from individual tetrads for the intervals shown. Uncorrected E₀ values are the percent of asci that had only PD and FDS tetrads for all marker pairs. The corrected E₀ 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 ascis 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. In 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 (r² = 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).

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.

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 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.
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 (Müller 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 Müller (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

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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 later. 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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