# An Implanted Recombination Hot Spot Stimulates Recombination and Enhances Sister Chromatid Cohesion of Heterologous YACs During Yeast Meiosis

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ABSTRACT

Heterologous yeast artificial chromosomes (YACs) do not recombine with each other and missegregate in 25% of meiosis I events. Recombination hot spots in the yeast *Saccharomyces cerevisiae* have previously been shown to be associated with sites of meiosis-induced double-strand breaks (DSBs). A 6-kb fragment containing a recombination hot spot/DSB site was implanted onto two heterologous human DNA YACs and was shown to cause the YACs to undergo meiotic recombination in 5–8% of tetrads. Reciprocal exchanges initiated and resolved within the 6-kb insert. Presence of the insert had no detectable effect on meiosis I nondisjunction. Surprisingly, the recombination hot spots acted in *cis* to significantly reduce precocious sister-chromatid segregation. This novel observation suggests that DSBs are instrumental in maintaining cohesion between sister chromatids in meiosis I. We propose that this previously unknown function of DSBs is mediated by the stimulation of sister-chromatid exchange and/or its intermediates.

MEIOSIS consists of a single DNA replication followed by two consecutive nuclear divisions. Unique features of meiotic chromosome behavior are extensive pairing of homologous chromosomes, a high frequency of recombination, and an orderly "reductional" disjunction of each homolog pair in the first meiotic division (meiosis I); sister chromatids then separate from each other in meiosis II. In the yeast *Saccharomyces cerevisiae*, another meiosis-specific phenomenon has recently been described, namely, the induction of double-strand breaks (DSBs) at preferred sites in chromosomal DNA (SUN *et al.* 1989; CAO *et al.* 1990; KLECKNER *et al.* 1991; ZENVIRTH *et al.* 1992; WU and LICHTEN 1994). In several instances these DSB sites map to chromosomal regions associated with high frequencies of recombination ("hot spots"). One prominent DSB site (near *THR4* on chromosome *III*) was identified by its role in meiotic chromosome disjunction (GOLDWAY *et al.* 1993). When a region containing this site was present on a plasmid or on a chromosome fragment, meiotic disjunction fidelity of the two chromosome *III* homologs was perturbed. In addition, the presence of three different DSB sites has recently been shown to be associated with meiosis specific joint molecules that are formed primarily between intact non-sister chromatids in midprophase (SCHWACHA and KLECKNER 1994; COLLINS and NEWLON 1994). The observed joint molecules are thought to be DSB-mediated precursors of recombination. *Cis*-acting sequences thought to mediate meiotic chromosome pairing and recombination have been described in other organisms (VINCENT and JONES 1993; MCKIM *et al.* 1993, VILLENEUVE 1994). It is possible that meiotic DSB sites in *S. cerevisiae* mediate homologous chromosome pairing in addition to stimulating high levels of recombination.

We have examined the meiotic behavior of heterologous yeast artificial chromosomes (YACs) into which DSB-promoting regions were implanted. YACs are particularly effective tools for studying meiotic chromosome segregation, in that structural alteration of YACs is facilitated by straightforward genetic and molecular manipulations. Moreover, aneuploidy for the YACs does not affect spore viability. Homologous YAC pairs undergo normal meiotic segregation and recombine with each other at frequencies approaching those of native yeast chromosomes (SEARS *et al.* 1992). In contrast, heterologous YACs do not recombine and frequently missegregate in meiosis I. In 20–30% of tetrads dissected, heterologous YAC pairs either segregate to the same pole in meiosis I (nondisjunction in meiosis I, NDI) or one of the YACs undergoes precocious sister chromatid segregation (PSS). The decreased meiotic segregation fidelity of heterologous YACs prompted us to use them as test chromosomes for studying the meiotic effects of implanted DSB sites. We found that the presence of a DSB site implanted on each of the two YACs (370 and 230 kb each) enabled them to recombine with each other. Thus, the implanted DSB site behaved as a recombination hot spot. Unexpectedly, presence of the DSB site was also associated with a significant *cis*-acting reduction in precocious sister chromatid segregation never before attributed to a DSB site. Models of the mechanism by which this novel phenomenon occurs are discussed. The methods used to implant this particular DSB site onto the YACs are such that any test site of choice may be implanted onto our YACs using the reagents described

here. Potentially, this implantation method may be used to test candidate sequences involved in many aspects of meiotic (or mitotic) chromosome behavior.

## MATERIALS AND METHODS

**Strains and plasmids:** All *S. cerevisiae* diploid strains used in this study are of the following genotype: *MAT***a**/*MATα ura3-52/ura3-52 ade2-101/ade2-101 trp1Δ1/trp1Δ1 lys2-801/lys2-801 his3Δ200/his3Δ200 leu2-Δ1/leu2-Δ1* CEN6/ΔCEN6::*LEU2*-CEN11. YAC12 derivatives were generated from the previously described and characterized 370-kb long YAC12 (PAVAN *et al.* 1990; SEARS *et al.* 1992), which contains a human chromosome *2* DNA-derived backbone (W. G. KEARNS, personal communication). YAC21.4 derivatives were generated from the previously described YAC21.4 (MCCORMICK *et al.* 1989) that is 230 kb long and contains a human chromosome *21* DNA-derived fragment. Figure 1A describes the general structure of the YAC pairs analyzed. All YAC12-UH derivatives have *URA3* near the centromere on the short arm and *HIS3* on the distal end of the long arm and all YAC21.4-LT derivatives have *LYS2* near the centromere on the short arm and *TRP1* on the distal end of the long arm. Test sequences inserted into YAC target sites (A at 75 kb on YAC12, B at 225 kb on YAC12, or C at 140 kb on YAC21.4) were either a 3.6-kb *Bam*HI-*Bam*HI *ADE2* fragment or a 6.0-kb *Bgl*II-*Bgl*II fragment containing the recombination hot spot *his4-LEU2* (CAO *et al.* 1990). Implantation of test sequences into the YACs was done by transformation in the two following haploids: DS82 (haploid *MATα*) containing YAC12-UH and GS1-1 (haploid *MAT***a**) containing YAC21.4-LT.

The diploids with the heterologous YAC pairs (Table 1) were formed by mating haploids with various versions of the engineered YACs. DS101 contains the original YAC12-UH/YAC21.4-LT pair, with no inserts, and was formed by mating between DS82 and GS1-1. XG33 contains the YAC12-UH site A::*his4-LEU2*/YAC21.4-LT site C::*his4-LEU2* pair. XG36 contains the YAC12-UH site A::*ADE2*, site B::*his4-LEU2*/YAC21.4-LT site C::*his4-LEU2* pair. XG42 contains the YAC12-UH site B::*ADE2*/YAC21.4-LT site C::*ADE2* pair. XG43 contains the YAC12-UH site A::*ADE2*/YAC21.4-LT site C::*ADE2* pair. XG18 contains the YAC12-UH/YAC21.4-LT site C::*his4-LEU2* pair. XG65 is equivalent to XG18. XG74 contains only YAC21.4-LT and XG75 contains only YAC21.4-LT site C::*his4-LEU2*. DS161 contains YACWR.8-LT, a 390-kb mouse DNA-derived YAC (MCCORMICK *et al.* 1989; SEARS *et al.* 1992) and YAC12-UH with an 18-bp fragment, "IST" (for *I*nserted *S*equence *T*ag), inserted into a unique *Sph*I site in the subtelomeric Y′ region of the distal vector sequence.

Implantation vectors were constructed from cloned unique sequences (target sites A, B and C, Figure 1A) from interstitial locations on the YACs. The interstitial sequences were obtained from yeast strains containing deletion derivatives of YAC12 (75 and 225 kb; PAVAN *et al.* 1990) and YAC21.4 (140 kb; D. D. SEARS and P. HIETER, unpublished), generated by *Alu* fragmentation (VOLLRATH *et al.* 1988; PAVAN *et al.* 1990). The ends of the derivative YACs contain pBR322 sequences, including the gene $Amp^R$, and a yeast marker, *HIS3* or *TRP1*. Genomic DNA from the YAC-containing yeast cells was digested with *Sph*I, which is unique in the vector-derived sequences, located between $Amp^R$ and the telomere. Digested DNA was diluted, self-ligated and transformed into *Escherichia coli*. Recovered $Amp^R$ plasmids contained vector sequences as well as adjacent human DNA, extending to the first *Sph*I site. Human DNA sequences were subcloned and suitable pieces were inserted into the polylinker of pBlueScript $SK^+$, yielding the following set of implantation vectors (Figure 1B): pGS525 contains the 2.7-kb target site A cloned into the *Pst*I and *Xba*I polylinker sites, pGS534 contains the 1.4-kb target site B cloned into the *Pst*I and *Bam*HI polylinker sites, pGS681 contains the 2.6-kb target site C cloned into the *Eco*RV and *Bam*HI polylinker sites. A 3.6-kb *ADE2* fragment was inserted into human target restriction sites in the implantation vectors, to yield pGS611 (between two *Bgl*II sites in target site A), pGS612 (into a unique *Bgl*II site in target site B), and pGS749 (into a unique *Bam*HI site in target site C). The 6.0-kb *his4-LEU2* fragment was inserted into the same human target restriction sites in the implantation vectors as described above, to yield pGS790, pGS809 and pGS784, respectively.

**Insertion of test sequences into YACs:** Unique restriction sites mapped in the human DNA insert (*e.g.*, *Bgl*II and *Hpa*I shown in the target site B plasmid pGS534–see Figure 1B) may be used for the insertion of sequences to be implanted on the YACs. Implantation vectors with *ADE2* inserted into the hu-

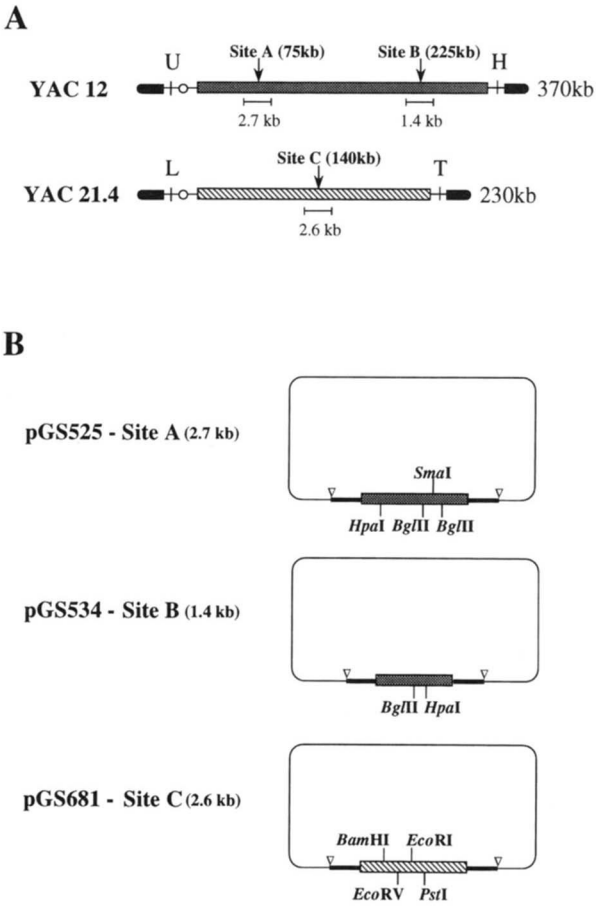


FIGURE 1.—The heterologous YAC12/YAC21.4 pair and implantation vectors, pGS525, pGS534 and pGS681, containing YAC12 target sites A, B and C, respectively. (**A**) Arrows point to the location of target sites A, B and C with the site's distance from the centromere in parentheses. Length of the target site fragments cloned into implantation vectors is shown below the corresponding site location. (**B**) Set of implantation vectors: pGS525 contains the 2.7-kb target site A, pGS534 contains the 1.4-kb target site B, and pGS681 contains the 2.6-kb target site C. Each target site is inserted into the pBlueScript polylinker (thick lines), $SK^+$ orientation. Restriction sites shown can be used to insert test sequences into cloned target sites. *Bss*HII sites (triangles) shown flanking the polylinker, as well as other unique sites in the polylinker, can be used to release the fragments prior to transformation.

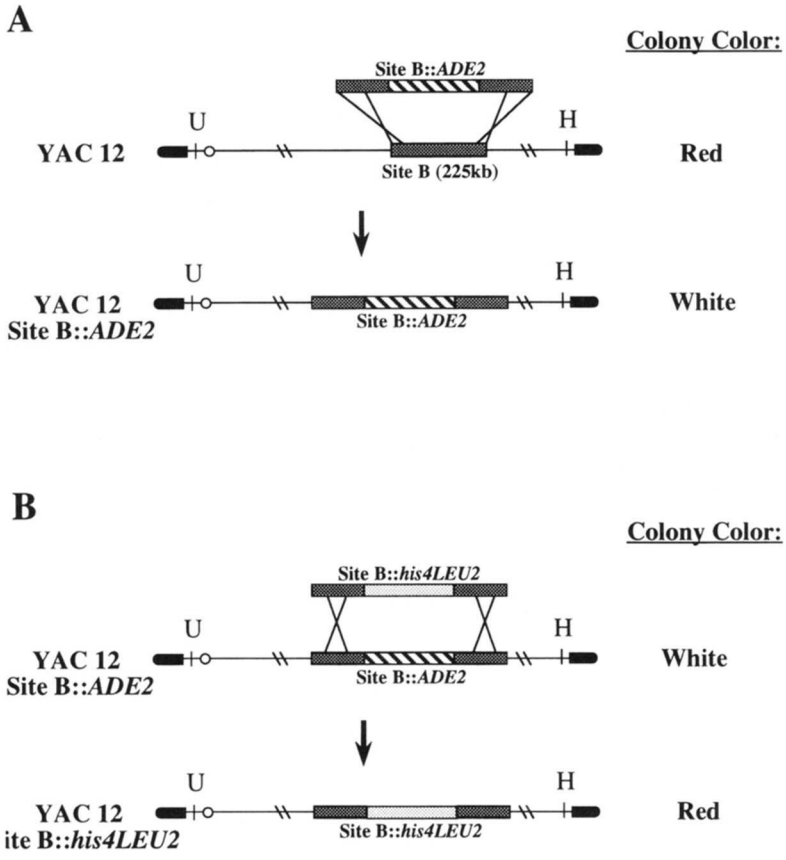


FIGURE 2.—Insertion of experimental sequences by transformation and homologous recombination through target site B sequences. (**A**) Insertion of *ADE2* into target site B. The site B::*ADE2* fragment from a derivative of implantation vector pG534 (pGS612) is transformed into a YAC12 containing haploid strain. Insertion of *ADE2* occurs via homologous recombination through the target site B sequences contained on YAC12 and in the fragment. Colony color changes from red to white upon insertion of *ADE2*. (**B**) Insertion of *his4-LEU2* into target site B. The site B::*his4-LEU2* fragment from a derivative of implantation vector pG534 (pGS809) is transformed into a YAC12-Site B::*ADE2* containing haploid strain. As in (A), insertion of *his4-LEU2* occurs via homologous recombination through the target site B sequences contained on YAC12 and in the fragment. Colony color changes from white to red upon replacement of *ADE2* with *his4-LEU2*.

man target sites were cut at polylinker sites flanking the human sequence, and fragments containing these inserts were transformed into haploid yeast strains with the appropriate target YAC. White, Ade$^+$ transformants were selected on SC-Ade plates (the parent strain is red, due to the *ade2-101* mutation). The one-step insertion of *ADE2* at target site B is shown in Figure 2. Insertions were verified by Southern hybridization. The resulting haploid strains, containing a YAC with *ADE2* at a given position, were used as implantation targets for insertion of the *his4-LEU2* site. Implantation vectors with *his4-LEU2* inserted into the human target sites were cut at polylinker restriction sites flanking the human sequence, and fragments containing these inserts were co-transformed with a selectable plasmid (*e.g.*, pRS424-Trp$^+$) into yeast strains bearing YACs with the appropriate *ADE2*-marked target site (Figure 2). Trp$^+$, red transformants were picked and the co-transforming plasmid was lost during growth on nonselective medium. Insertions were verified by Southern blots and by tetrad analysis.

**Pulsed-field gel electrophoresis and indentification of recombinant YACs:** Chromosome sized DNA embedded in agarose plugs was prepared as described (ROSE *et al.* 1990). All pulsed-field gel electrophoresis was done on an orthogonal field alternating gel electrophoresis (OFAGE) apparatus (CARLE and OLSON 1984). 1% agarose gels were run at 270 V for 14 h at 5° with a 14–22-s switch interval in 0.5 × TBE. Southern blots of OFAGE gels containing DNA from XG33 recombinant tetrads were hybridized individually with probes 1, 2, 5 and 6 (see Figure 4). Probes 1 and 2 are human DNA fragments corresponding to the ends of target site A. Probes 5 and 6 are human DNA fragments corresponding to the ends of target site C. Southern blots of OFAGE gels containing DNA from XG36 recombinant tetrads were hybridized individually with probes 3, 4, 5 and 6 (see Figure 4). Probes 3 and 4 are human DNA fragments corresponding to the ends of target site B.

**Media and genetic analysis:** Standard yeast media and genetic methods (ROSE *et al.* 1990) were employed in this study. Sporulation and tetrad analysis were performed as previously described (SEARS *et al.* 1992). NDI and PSS of the YACs were scored as previously described (SEARS *et al.* 1992). A spore colony containing two YACs was recognized as such by carrying the two centromere-linked markers, *LYS2* and *URA3*, as well as the markers at the distal ends of the long arms of the YACs, *HIS3* and *TRP1*. Each of these disomic YAC colonies was streaked on 5′-fluoro-orotic acid medium (BOEKE *et al.* 1984) and α-aminoadipate medium (CHATOO *et al.* 1979), to obtain derivative colonies that had lost the *URA3*- or *LYS2*-carrying YACs, respectively. These colonies with single YACs were then replicated onto selective media, to determine the markers carried by the YACs.

Linkage of IST and *HIS3* was determined by a modified spore colony lift procedure. Spore colonies were lifted from a tetrad dissection plate onto nylon filters and processed (BENTLEY *et al.* 1992). Filters were hybridized with an 18-nucleotide oligo-probe (complimentary to the IST sequence) at 61° for 3 hr and washed at 60° (BENTLEY *et al.* 1992). IST sequence is CCTGGAATTCGCCGCATG.

## RESULTS

**Heterologous YACs do not recombine with each other and segregate abnormally in meiosis I:** The two YACs employed in this study are made primarily of human DNA, see Figure 1A. YAC12 (PAVAN *et al.* 1990; SEARS *et al.* 1992) is 370 kb long and contains DNA derived from human chromosome *2*. YAC21.4 (MCCORMICK *et al.* 1989) is 230 kb long and contains DNA derived from human chromosome *21*. Each YAC contains the

**TABLE 1**

**Segregation and recombination of YAC12/YAC21.4 derivatives in isogenic strains**

| Strain | YAC12/YAC21.4 insert/insert | No. of tetrads | Meiosis I segregation | | | Recombinant tetrads (%) |
|---|---|---|---|---|---|---|
| | | | Normal (%) | PSS (%) | NDI (%) | |
| DS101 | None | 205 | 76.1 | 10.2 | 13.7 | 0 |
| XG33 | *his4LEU2/his4LEU2* | 207 | 78.3 | 6.2 | 15.5 | 5.3 |
| XG36 | *his4LEU2/his4LEU2* | 203 | 82.3 | 4.9 | 12.8 | 7.9 |
| XG42 | *ADE2/ADE2* | 191 | 73.8 | 10.5 | 15.7 | 0 |
| XG43 | *ADE2/ADE2* | 218 | 75.7 | 9.2 | 15.1 | 0 |
| XG18 | *-/his4LEU2* | 204 | 77.0 | 7.3 | 15.7 | 0 |
| XG65 | *-/his4LEU2* | 184 | 81.5 | 6.5 | 12.0 | 0 |

In strains XG33 and XG42 the inserts are in positions 75 kb and 140 kb (target sites A and C, see Figure 1A), in XG36 and XG43 the inserts are in positions 225 kb and 140 kb (target sites B and C) and in XG18 and XG65 the insert is only in YAC21.4, in position 140 kb (target site C).

centromere of chromosome *IV* of *S. cerevisiae* (CEN4), yeast telomeres (TEL) and two yeast genes as prototrophic markers, *URA3* or *LYS2* near the centromere on the short arm, and *HIS3* or *TRP1* at the distal end of the long arm. In addition, diploid strains to be tested are heterozygous on chromosome VI for a *LEU2/CEN11* replacement of CEN6, which unambiguously marks sister spores in tetrad analysis (SEARS *et al.* 1992).

Proper disjunction of the two YACs away from each other in meiosis I gives rise to tetrads in which two sister spore colonies are $Ura^+$ and the other two are $Lys^+$ (sister spores are designated by the centromere marker *LEU2* on chromosome *VI*). This segregation pattern is referred to as "normal" in Table 1 and was found in 74–82% of tetrads scored. Meiosis I abnormalities included in Table 1 are PSS and NDI. PSS is scored when the centromere marker of one YAC is present in nonsister spores, *i.e.*, one spore contains two different YAC centromere markers, two spores contain a single YAC each, and the fourth lacks a YAC (SEARS *et al.* 1992). NDI is scored when two sister spores contain both YAC centromere markers, *URA3* and *LYS2*, and the other two sisters are devoid of YACs. Tetrads absent of all markers on a particular YAC can result via premeiotic mitotic YAC loss or via meiotic loss of both sister chromatids. Tetrads in which one spore colony (out of the four) contains no YAC indicates either meiotic loss of a single YAC chromatid or nondisjunction meiosis II where two chromatids of the same YAC are contained in the same spore colony. Cases of YAC chromatid loss and nondisjunction meiosis II occurred with frequencies of less than 5% in the tetrads analyzed and are not included in Table 1 or in the analysis that follows, which focuses on the segregation of YACs in meiosis I. Reciprocal recombination, data also presented in Table 1, is scored when two spore colonies with parental YACs ($Ura^+His^+$ and $Lys^+Trp^+$) and two spore colonies with reciprocally recombinant YACs ($Ura^+Trp^+$ and $Lys^+His^+$) occur in a tetrad. DS101 contains the parental YAC12/YAC21.4 heterologous pair used for the studies described below (see Figure 1A) and exhibited 10% PSS and 14% NDI in the 205 tetrads analyzed (see Table 1). With the exception of two illegitimate recombinants described below, no reciprocal recombinants were observed in 205 tetrads of DS101.

The observation that two nonrecombinant YACs disjoin from each other 70–80% of the time, rather than segregate randomly, is consistent with previous evidence that a distributive disjunction mechanism exists in *S. cerevisiae* (see DISCUSSION for references), and that nonrecombinant artificial chromosomes are incorporated into the distributive pool. However, it was possible that undetected recombination events occurring within the homologous 2–3-kb region of vector sequence, distal to the prototrophic markers, may be responsible for the proper disjunction of these heterologous nonrecombinant YACs. The Tn*3* β-lactamase gene ($Amp^R$), contained in this vector sequence, has been shown to be a hot spot for meiotic recombination and gene conversion when inserted into a yeast chromosome (STAPLETON and PETES 1991). A copy of the $Amp^R$ gene is located between the prototrophic marker and the telomere on each end of the YACs. $Amp^R$ sequences could potentially stimulate recombination between heterologous YACs which would subsequently be responsible for the observed increase in "normal" meiosis I disjunction (75–80%) over that expected for random segregation (*i.e.*, 50% "normal" and 50% "abnormal"). To address this possibility, we constructed a heterologous YAC pair that includes a YAC12 derivative which contains an 18-bp IST, inserted into the subtelomeric region of the long arm, approximately 2.4 kb distal to the *HIS3* prototrophic marker and approximately 300 bp distal to $Amp^R$. Recombination events within $Amp^R$ sequences are detected by linkage of the flanking *TRP1* and IST markers.

Meiotic segregation data of this heterologous YAC pair (contained in DS161, 78 tetrads analyzed, data not shown) were not significantly different from those observed for DS101 (see Table 1). Linkage of IST and *HIS3* was determined by tetrad analysis and colony hybridization (see MATERIALS AND METHODS). IST was linked to *HIS3* in all 59 tetrads exhibiting normal meiosis I segregation, and no recombination event was detected.

We conclude that recombination within the telomeric $Amp^R$ sequences is not responsible for the high figures of proper segregation observed for heterologous YAC pairs (75–80% compared to the expected 50% for random segregation). Thus the nonrecombinant, heterologous YACs are disjoined via a distributive disjunction mechanism.

**Insertion of experimental sequences into the YACs:** To insert experimental sequences into YAC12 and YAC21.4, we cloned small interstitial regions from within each YAC, to be used as insertion target sites (described in MATERIALS AND METHODS). The resulting "implantation" vectors each contain one of the cloned target sites shown in Figure 1A. The implantation vectors for target sites A, B and C (pGS525, pGS534 and pGS680) are shown in Figure 1B. To generate "YAC recipients" for implantation analyses, *ADE2* was cloned into each implantation vector. Target site:: *ADE2*-containing restriction fragments were transformed into YAC bearing haploid strains, to generate *ADE2* YAC insertions via homologous recombination through the target site sequence (Figure 2A). The resulting haploid strains, with a YAC harboring *ADE2* at a given position (see Figure 2), serve as recipients for the implantation of any test sequences one wishes to be analyzed in meiosis. The presence of *ADE2* in our strains (which are normally red due to the *ade2-101* mutation) causes them to be white. Thus, *ADE2* serves as a color marker for its subsequent replacement by transformed test sequences cloned into a target site fragment of the implantation vectors (Figure 2B).

**Insertions of a recombination hot spot into YAC12 and YAC21.4:** A recombination hot spot sequence was cloned into each of the implantation vectors. Implantation of the hot spot sequence into YAC target sites was achieved as described in MATERIALS AND METHODS (see also Figure 2B). We have inserted into the YACs the 6-kb *Bgl*II-*Bgl*II test fragment, *his4-LEU2*, that contains the *LEU2* gene inserted near *HIS4* (CAO *et al.* 1990). This chromosomal region was reported to contain two meiosis-induced DSB sites (CAO *et al.* 1990) and has been shown to mediate the formation of joint molecules between non-sister chromatids in meiotic prophase (SCHWACHA and KLECKNER, 1994). The *his4-LEU2* sequence was inserted at two alternative target sites on YAC12 (sites A and B), 75kb or 225kb from the centromeric end of the YAC, and at target site C on YAC21.4, 140 kb from the centromeric end (see Figures 1A and 4A). All *his4-LEU2* target inserts were designed to have the same orientation relative to the centromeres of the YACs. The locations of YAC target sites were deduced from the sizes of the deletion derivative YACs, described in MATERIALS AND METHODS, and were verified by the sizes of recombinant YACs described below, measured by pulsed-field gel electrophoresis.

**Nonhomologous YACs with inserted recombination hot spots recombine with each other:** We have constructed a series of YAC12/YAC21.4 isogenic diploids with or without the *his4-LEU2* test insert at target sites A and C, or B and C (Figures 1A and 4A), and control strains containing *ADE2* inserted at the same locations. These strains were sporulated and subjected to tetrad analysis (Table 1). For each strain analyzed, approximately 200 tetrads with four viable spores were scored in which each YAC segregated $2^+:2^-$.

Strikingly, in Table 1, only the two strains with *his4-LEU2* inserts on each of two nonhomologous YACs yielded reciprocally recombinant tetrads. Eleven such recombinant tetrads were obtained from strain XG33 and 16 from XG36. Two spore colonies with the parental YACs ($Ura^+His^+$ and $Lys^+Trp^+$) and two spore colonies with the reciprocal recombinant YACs ($Ura^+Trp^+$ and $Lys^+His^+$) were present in each recombinant tetrad. All spore colonies from recombinant tetrads were subjected to a two-stage physical analysis. Chromosome-sized DNA was prepared in agarose blocks and separated by pulsed-field gel electrophoresis. Photographs of the ethidium-bromide stained OFAGE gels showed that spore colonies with non-parental (recombinant) YAC markers contained YAC DNA molecules of new sizes (Figure 3). The new sizes were as expected from a reciprocal exchange occurring within the *his4-LEU2* inserts between one chromatid of YAC12 and one chromatid of YAC21.4 (Figure 4). From diploid XG33, with *his4-LEU2* inserts at target sites A and C, the size of the $Ura^+Trp^+$ YAC is 165 kb and the $Lys^+His^+$ YAC is 435 kb (Figure 4B). From diploid XG36, with *his4-LEU2* inserts at target sites B and C, the size of the $Ura^+Trp^+$ YAC is 315kb and the $Lys^+His^+$ YAC is 285 kb (Figure 4C).

The physical analysis of the recombinant YACs suggests that they resulted from recombinational events within the *his4-LEU2* inserts contained on the parental YACs, as depicted in Figure 4, B and C. An alternative interpretation could be, however, that the *his4-LEU2* inserts had only served to align the two YACs and that recombination had occurred at another point on the aligned YACs, equidistant from either *his4-LEU2* insert. To distinguish between these two possibilities, pulsed-field gels were blotted onto membranes, and hybridized separately to four unique-sequence probes obtained from each side of the two YAC target sites that flank the *his4-LEU2* test insert, diagrammed in Figure 4. If exchange had occurred entirely within the insert, a recombinant YAC was expected to hybridize to one probe derived from YAC12 and one from YAC21.4, according to the markers it carried. The other recombinant YAC in the same tetrad was expected to hybridize to the other two probes, one derived from each parental YAC. Of the XG33 tetrads, 24 out of 25 recombinant YACs examined in this manner hybridized to the expected probe combinations, *i.e.*, one probe from each parental YAC, as shown in Figure 4B. One recombinant did not strongly hybridize to any probe, possibly due to low levels of DNA

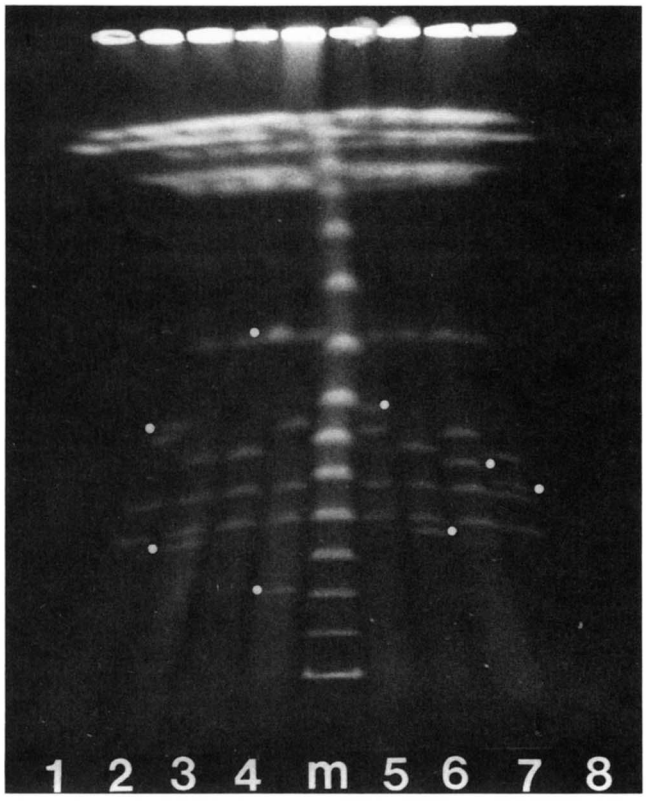


FIGURE 3.—OFAGE gel of recombinant YACs. The ethidium bromide-stained OFAGE gel shows the separation of chromosome-sized DNA from four spores in a recombinant tetrad of XG33 (lanes 1–4) and of XG36 (lanes 5–8). YAC bands are marked with white dots and represent either parental-sized YACs (lanes 1, 2, 5 and 6) or recombinant YACs (lanes 3, 4, 7 and 8). Lane 1, UH, 370 kb; lane 2, LT, 230 kb; lane 3, LH, 435 kb; lane 4, UT, 165 kb; lane 5, UH, 370 kb; lane 6, LT, 230 kb; lane 7, UT, 315 kb; lane 8, LH, 285 kb. m, λ phage DNA concatamer ladder as size marker.

prepared from this strain for pulsed-field gel analysis. Of the XG36 tetrads, 19 out of 19 recombinant YACs examined hybridized to the expected probe combination shown in Figure 4C. These additional results confirm that the reciprocal recombination events scored above did initiate *and* resolve completely within the 6-kb *his4-LEU2* insert.

**YACs with inserted recombination hot spots show reduced PSS:** Spore colonies that contained both YACs, *i.e.*, had the phenotype $Ura^+Lys^+$, resulted from NDI or from PSS. For each of these colonies the two YACs were separated on 5′-fluoro-orotic and α-aminoadipate plates, and their markers were determined (see MATERIALS AND METHODS). None of these YACs was recombinant for the parental YAC markers, indicating that NDI and PSS occurred in cells in which the YACs had not recombined with each other.

Table 1 contains meiosis I segregation characteristics of the YACs with and without the *his4-LEU2* hot spots. NDI is not significantly affected by the inclusion of inserts in the YACs. In spite of having the recombination hot spot (*his4-LEU2*) on both heterologous YACs, or on YAC21.4 only, NDI still occurs in 12–16% of the tetrads. However, the presence of *his4-LEU2* on the YACs had a marked effect on PSS. When the insert is present on both YACs, the frequency of PSS is 5–6%, compared to 10% in the strains without the *his4-LEU2* insert [$\chi^2_{[1]}$ = 26.1 ($P$ = 0.025–0.001) for the comparison of strains XG33 + XG36 *vs.* DS101 + XG42+XG43; XG33 did not differ significantly from XG36 ($\chi^2_{[1]}$ = 0.35) and DS101, XG42 and XG43 did not differ from each other ($\chi^2_{[2]}$ = 0.24)]. This reduction could not result from reciprocal exchange between the YACs because the expected level of PSS among the nonrecombinant tetrads generated would be 9% (92–95% nonrecombinant tetrads multiplied by 10% PSS). Moreover, strains with the *his4-LEU2* insert on one YAC only (XG18 and XG65) also showed a reduction in PSS frequency, though this reduction was not quite significant [$\chi^2_{[1]}$ = 2.63 for the comparison XG18 + XG65 *vs.* DS101 + XG42 + XG43 ($P$ = 0.11); XG18 did not differ from XG65 in PSS frequency ($\chi^2_{[1]}$ = 0.1)]. The reduction in PSS observed in strains XG18 and XG65, however, appeared to exist only for YAC21.4, which carries the *his4-LEU2* insert, and not for YAC12 (see below, and Table 2). The presence of *ADE2* homology on the two YACs (XG42 and XG43) did not contribute to a reduction in PSS frequency. These results suggest that the insert acted in *cis*, to reduce the PSS frequency of the YAC that carried it, irrespective of the other YAC.

Two lines of evidence support the interpretation that a *cis* effect of reducing precocious sister chromatid segregation is induced by the presence of the *his4-LEU2* recombination hot spot. First, when the PSS tetrads from strains XG18 and XG65 were characterized as to which of the two YACs had undergone precocious segregation, we found a marked tendency for the YAC without the *his4-LEU2* insert to precociously divide in meiosis I (Table 2), compared to the second, *his4-LEU2*-containing YAC in the same strains (compare also PSS frequencies of individual YACs to those given for DS101, XG42 and XG43). Thus there was a significant reduction in PSS level for YAC21.4 in strains XG18 + XG65 (2.6%) compared to DS101, XG42, XG43 (pooled, 6.0%), $\chi^2_{[1]}$ = 6.3 ($P$ = 0.025–0.01). A similar comparison with DS101 alone gave $\chi^2_{[1]}$ = 5.1 ($P$ = 0.025–0.01). On the other hand, PSS levels of YAC12 in these strains remained constant. Second, we examined the segregation behavior of YACs in two additional strains, which contain only one YAC each (Table 2). Strain XG74 contains only YAC21.4, without an insert, whereas XG75 contains the same YAC21.4 with an *his4-LEU2* insert at target site C. The two strains are isogenic to each other and to the strains whose meiotic segregation data are presented in Table 1. As can be seen in Table 2, the presence of the *his4-LEU2* insert on the monosomic YAC also resulted in a reduction of PSS frequency (compared to the isogenic strain with the same monosomic YAC, but without an *his4-LEU2* insert). The reduction was similar to that

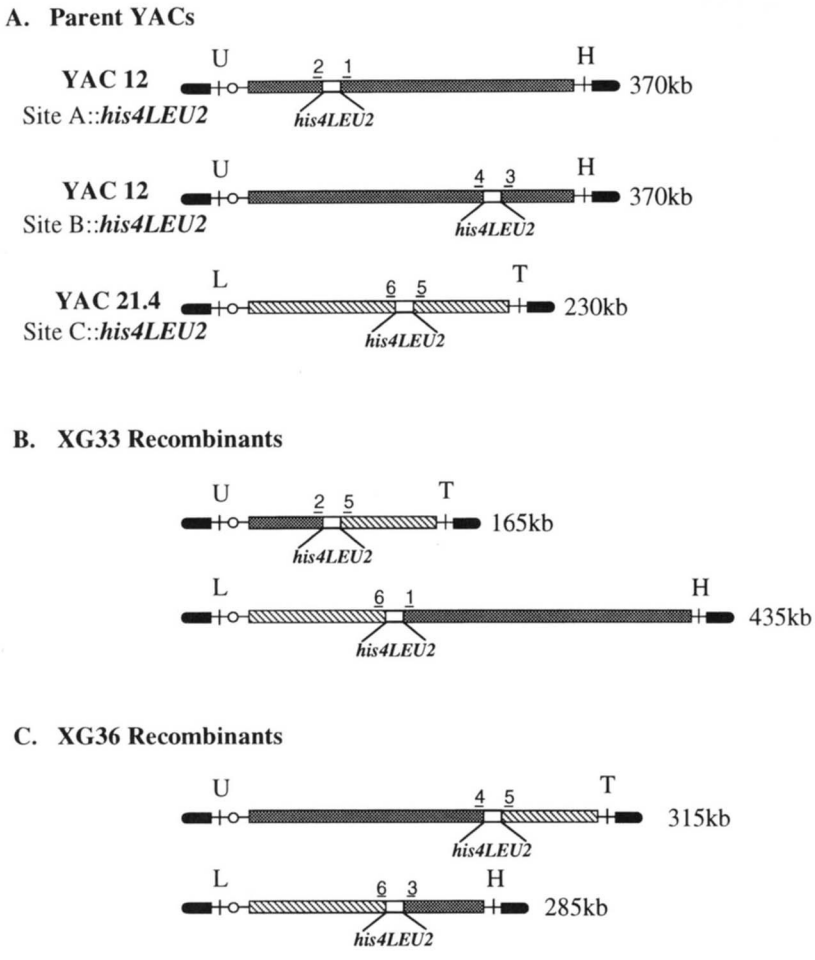


FIGURE 4.—Parental YACs with *his4-LEU2* inserts and recombinant YACs generated from recombination in XG33 and XG36 diploids. (**A**) Parental YACs that contain the *his4-LEU2* insert at either site A, B or C. (**B**) Structure of recombinant YACs, 165-kb UT and 435-kb LH, generated from exchange events occurring within the *his4-LEU2* sites, inserted into target sites A and C, in the XG33 diploid. (**C**) Structure of recombinant YACs, 315-kb UT and 285-kb LH, generated from exchange events occurring within the *his4-LEU2* sites, inserted into target sites B and C, in the XG36 diploid. Small, numbered lines above the YACs correspond to probes used to confirm that the boundaries of recombintation initiation and resolution lie completely within the *his4-LEU2* site. Recombinant YACs hybridized to two probes, one probe from each parental YAC on opposite sides of the corresponding target site. For example, 165-kb UT recombinant YACs from XG33 hybridized to probe 2 (from the proximal side of target site A) and to probe 5 (from the distal side of target site C). The 435-kb LH recombinant YACs from the same tetrad hybridized conversely to probes 1 and 6.

**TABLE 2**

**Distribution of precocious sister-chromatid separation among YACs**

| Strain | YAC12 site A or B | YAC21.4 Site C | YAC12 PSS (%) | YAC21.4 PSS (%) | No. of tetrads |
|---|---|---|---|---|---|
| DS101 | No insert | No insert | 3.9 | 6.3 | 205 |
| XG42 | *ADE2* | *ADE2* | 3.2 | 7.3 | 191 |
| XG43 | *ADE2* | *ADE2* | 4.6 | 4.6 | 218 |
| XG33 | *his4LEU2* | *his4LEU2* | | 6.2[a] | 207 |
| XG36 | *his4LEU2* | *his4LEU2* | | 4.9[a] | 203 |
| XG18/ XG65 | No insert | *his4LEU2* | 4.4 | 2.6 | 388 |
| XG74 | – | No insert | – | 4.8 | 207 |
| XG75 | – | *his4LEU2* | – | 1.6 | 187 |

[a] PSS of YAC12 *vs.* YAC21.4 could not be distinguished in these diploids.

observed for strains containing two heterologous, *his4-LEU2*-containing YACs (Table 2), but was only of borderline significance due to limited sample sizes [PSS in XG75 *vs.* XG74 gave $\chi^2_{[1]} = 3.2$ ($P = 0.1$–$0.05$); however, a comparison of PSS in XG75 *vs.* PSS of YAC21.4 in strains XG74, DS101, XG42 and XG43 together (pooled) gave $\chi^2_{[1]} = 5.5$ ($P = 0.025$–$0.01$)].

**Rare illegitimate recombination events between nonhomologous YACs:** Rare spore colonies with YACs of non-parental marker combinations were obtained in the tetrad analysis of strains that did not contain the *his4-LEU2* inserts on both YACs (strains in Table 1, except XG33 and XG36, and some additional isogenic strains of which smaller samples of tetrads were dissected and analyzed). In total, we encountered 11 such cases among 1219 tetrads with four viable spores (0.90%) and three cases among 319 asci with three viable spores (0.94%). The DNA of the spore colonies of these unusual tetrads was separated by pulsed-field gel electrophoresis and in several cases a YAC of a non-parental length was associated with the unusual marker combination. In these tetrads, even spore colonies with parental combinations of YAC markers occasionally contained a YAC of non-parental length. In all but two of the tetrads, one or two YAC chromatids were lost. "Reciprocal" non-parental YACs were found only in two tetrads: In the first, the YAC lengths (in kilobases) were 230 (LT–*LYS2*, *TRP1*), 310 (UH–*URA3*, *HIS3*), 270 (LH) and 275 (UT). The first YAC is of parental length but the other three are not, and the total length of the four YACs is only 1085 kb, instead of the expected 1200 kb (230 + 230 + 370 + 370 = 1200 kb). The second tetrad with "reciprocal" non-parental YAC markers consisted of one spore without a YAC, another with the parental 230-kb (LT) YAC and the remaining two spores had YACs of lengths 105 kb (UT)

and 435 kb (LH). The lengths of the latter two, although they have reciprocal marker combinations, do not add up to the expected 600 kb, as expected from a genuine reciprocal exchange. Thus in all cases YAC material was lost. This suggests that recombination events that lead to the formation of new non-parental YACs also created fragments, or YAC derivatives that were nonfunctional during the subsequent meiotic or mitotic divisions, and hence were lost. In several cases it appears that more than two YAC chromatids were involved in the recombination event, for instance the first example described above. Involvement of more than two chromatids in the recombination event may be related to the observed loss of chromatids.

## DISCUSSION

**The experimental system:** A pair of homologous YACs behaves in meiosis as any other pair of yeast chromosomes (SEARS *et al.* 1992), namely homologs recombine at a rate typical of yeast chromosomes, homologous centromeres segregate away from each other in meiosis I, and sib-centromeres separate in meiosis II. Heterologous YAC pairs, on the other hand, did not undergo recombination (SEARS *et al.* 1992). Additionally, heterologous YACs disjoined from each other normally in only 75–80% of tetrads. Two main patterns of malsegregation were found in the remaining tetrads: NDI and PSS. Meiotic segregation of the two heterologous YACs reported here, YAC12 and YAC21.4, was essentially the same as that reported by SEARS *et al.* (1992). The high rate of malsegregation in meiosis I may be attributed to the lack of homology and/or the absence of recombination events between the YACs. The two heterologous YACs probably do not pair with each other in meiotic prophase and fail to form a standard, tripartite synaptonemal complex (SC) (LOIDL *et al.* 1994). Pairing and synapsis appear to be required for reciprocal exchange (reviewed by HAWLEY 1988), and the subsequently formed chiasmata hold the homologs together until they are properly disjoined in anaphase I (reviewed by ROEDER 1991). However, synapsis and formation of SC by themselves may be important for some aspects of segregation fidelity, irrespective of chiasma formation. It is possible that in many meioses, the two heterologous YACs missegregate because they have not paired, not because they lack chiasmata. Distributive disjunction, an alternative chromosome segregation mechanism, is likely to govern the segregation of nonrecombinant heterologous YACs and is discussed below.

The absence of recombination between heterologous YAC pairs, and their high frequency of meiosis I missegregation, provide a suitable background against which one can test DNA sequences that are expected to affect meiotic chromosome behavior. For this purpose we have developed implantation vectors that allow us to insert sequences at two interstitial locations on YAC12 (sites A and B) and one location on YAC21.4 (site C). By similar methodology, sequences may also be inserted at other sites and near the ends of the YACs. These reagents add considerably to the versatility of YAC marker chromosome manipulation that allows for the detailed study of meiotic and mitotic chromosome behavior (HIETER *et al.* 1990).

**Why do the nonhomologous YACs segregate better than expected?** Heterologous YAC pairs disjoin from each other in meiosis I at a frequency considerably higher than 50%, which would be expected for two chromosomes that segregate independently of each other. This improved segregation (75–80% normal meiosis I) could be attributed to an alternative mechanism that regulates meiotic segregation of achiasmate nonhomologous chromosomes. Distributive disjunction was analyzed extensively in *Drosophila melanogaster* females (for reviews see GRELL 1976; CARPENTER 1991; HAWLEY *et al.* 1993) and also documented in *S. cerevisiae* (DAWSON *et al.* 1986; MANN and DAVIS 1986; KABACK 1989; GUACCI and KABACK 1991; LOIDL *et al.* 1994; SEARS *et al.* 1992; SEARS 1994). If such a mechanism exists in yeast, our YAC segregation data, and data reported by others (see references above), strongly indicate that it is only partly effective.

An alternative explanation of the improved segregation of nonhomologous YAC pairs is that they do pair and/or recombine with each other at the ends, which contain homologous, albeit short plasmid sequences. Bacterial plasmid DNA (pBR322) inserted into yeast chromosomes have been reported to serve as meiotic recombination hot spots (STAPLETON and PETES 1991). This possibility was shown to be unlikely by analysis of a subtelomeric YAC marker that allows the detection of recombinants within the distal vector sequences of heterologous YACs. No recombination was observed in 59 tetrads scored as "normal" segregation. If recombination between the two YACs had occurred within the extreme terminal telomere repeats, however, it would not have been detected. Nevertheless, we speculate that recombination and chiasmata occurring at the extreme ends would not be effective in holding the two YACs together until anaphase I, because the short distance between such a chiasma and the end of the chromosome may not provide sufficient sister-chromatid cohesion. It is the cohesion between sister chromatids that is thought to keep chiasmata from falling apart until chromosome separation. Achiasmate pairing between the ends of heterologous YACs remains a possible force that ensures their disjunction, but this needs to be investigted cytologically.

**Inserted recombination hot spots are functional on the YACs:** The *his4-LEU2* chromosomal region, also known as *HIS4-LEU2*, was reported to behave as a recombination hot spot on chromosome *III* of *S. cerevisiae* and to contain two meiosis-induced DSB sites (CAO *et al.* 1990). When inserted into our heterologous YACs of 370 and 230 kb, this small 6-kb chromosomal fragment is sufficient to cause the YACs to recombine with each other at high rates. Diploids XG33 and XG36 gave

27 recombinant tetrads out of 410 tetrads (Table 1). These recombination data reflect a genetic distance of 3.3 cM contributed by the insert, which corresponds to 0.55 cM/kb. Compared to the average genomic value of 0.34 cM/kb (MORTIMER *et al.* 1989), the *his4-LEU2* insert on a heterologous YAC pair behaves as a recombination hot spot, especially if one takes into account that recombinogenic activity may be limited to small subregions near the two DSB sites. In previous experiments, a recombination-promoting effect was observed when a 12.5-kb fragment containing the *ARG4* recombination hot spot was inserted onto a pair of short (50 kb), metacentric yeast artificial chromosomes (ROSS *et al.* 1992). However, it is not clear to us whether the observed increase in recombination was due to the recombinogenic activity of the *ARG4* hot spot or to alleviation of the recombination-repressing effect of the centromere by increasing the chromosome arm length by 50%.

Recombination between the YACs in our experiments was reciprocal. Recombinant YACs in each case were of the sizes expected from the positions of *his4-LEU2* insertion on the YACs and from the exchange event having occurred in the inserts (Figures 3 and 4). The inserts served as the actual sites of exchange initiation and resolution, rather than serving merely as sites of alignment. This was demonstrated by every recombinant YAC hybridizing to two out of four "border" probes, one from each parental YAC target site, prepared from cloned target site sequences bracketing the recombinogenic inserts (Figure 4).

Isogenic strains that had the *his4-LEU2* insert on only one YAC in the heterologous pair did not yield any recombinant YACs (Table 1). This result could mean either that DSBs are required on both YACs in order to stimulate recombination, or that the insert is also required for homology between the otherwise heterologous YACs, to allow for recombination if a double-strand break has occurred. The latter is more likely because the proportion of molecules of natural yeast chromosomes found to be broken at each DSB site per meiosis is low (ZENVIRTH *et al.*, 1992), and is not compatible with a requirement for DSBs to occur on both DNA molecules when they initiate exchange. Existing DSB recombination models (SZOSTAK *et al.* 1983) also suggest that a DSB on only one of the partner molecules initiates exchange.

How much homology is required around the DSB site for an exchange to be materialized? In the experiments reported here the homology was approximately 6kb and in similar experiments, with the same pair of heterologous YACs, the inserted homology that yielded recombinant YACs was 3.5 kb (V. DROR and G. SIMCHEN, unpublished). Further experiments with this system are required to determine the minimal homology, embedded in heterologous chromosomes, that is required for exchange in meiosis.

Inserted sequence homology without DSB potential is probably not sufficient for recombination between the two heterologous YACs. This may be concluded from the absence of recombinant YACs among the meiotic progeny from strains in which *ADE2* was inserted on the two YACs (*e.g.*, XG42). Thus we suggest that a minimal length of homology, as yet undetermined, is required for recombination of heterologous YACs, as well as a DSB in one of the inserted homologies. An experiment that will demonstrate these minimal requirements for exchange will consist of inserted homologies on the two nonhomologous YACs, with a DSB site only in one of the two inserts.

**Rare illegitimate recombination between YACs:** In contrast to the reciprocal recombinant YACs that were obtained at a reasonably high frequency among meiotic progeny of strains that contained YACs with the *his4-LEU2* inserts, the other strains produced rare, non-parental YACs that appeared to have arisen from illegitimate, nonreciprocal exchanges. Fourteen such cases were found in 1538 tetrads and the YACs were characterized genetically and by pulsed-field gel electrophoresis. YAC material was lost in all 14 tetrads. In 12 tetrads, only two or three YACs (instead of the possible four) could be observed among the spore colonies. In the remaining two tetrads, the total added length of the four YACs was considerably shorter than the 1200 kb expected to result from recombination between four YAC chromatids, two of size 370 kb and two of 230 kb. In several cases it appears that more than two YAC chromatids were involved in the recombination event. Involvement of more than two chromatids in the recombination event may be the rule among these rare cases and may be related to the observed loss of chromatids. It is possible that repetitive sequences in the human DNA backbone of these YACs may be involved in the "illegitimate" exchanges, for instance *Alu* or LINE elements. The exact nature of these events needs to be further investigated. Their very low frequency, however, suggests that the heterologous YACs are not intimately paired in most meioses (except in strains with the *his4-LEU2* insert on both YACs). Had they been paired, LINE and *Alu* elements could perhaps provide homology for illegitimate exchanges that were initiated by naturally occurring DSBs at preferred sites on the human DNA YACs (D. ZENVIRTH, S. KLEIN and G. SIMCHEN, in preparation).

**The inserted recombination hot spot does not affect levels of NDI:** One would expect to observe an improvement in the meiosis I disjunction fidelity of two heterologous YACs resulting from recombination and chiasma formation between them (5–8% of tetrads scored). Indeed, all tetrads that were scored as reciprocally recombinant segregated normally in meiosis I (and none of the NDI and PSS tetrads contained recombinant YACs). However, the presence of inserted recombination hot spots did not have a noticeable effect on the overall frequency of YAC NDI (Table 1). The

expected reduction in NDI due to YAC recombination is rather small (15% reduced to 14%), however, and could not be detected in the tetrad samples analyzed. It was possible that pairing of the YACs, mediated by hot spot inserts, could in itself improve their disjunction in meiosis I, even in the absence of recombination. If the frequency of YAC pairing through *his4-LEU2* sites is considerably higher than the frequency of YAC exchange at these sites, the lack of disjunction improvement implies that pairing without exchange has little effect on chromosome disjunction in meiosis I. It has been suggested that only those recombination events that occur within the context of a synaptonemal complex and result in the formation of stable chiasmata are capable of ensuring meiosis I disjunction fidelity (reviewed in HAWLEY and ARBEL 1993).

**A role for DSBs in preventing precocious sister chromatid segregation:** YACs containing the recombination hot spot insertion clearly exhibited reduced levels of precocious sister chromatid segregation in meiosis I (see Table 1). This effect was observed in strains with a *his4-LEU2* insert on one or both YACs, suggesting that it was not due to insert-mediated pairing of the YACs. Diploids in which only one of the two YACs contained the insert showed a reduction in PSS frequency for the *his4-LEU2*-containing YAC only, whereas PSS frequency for the other YAC was unchanged. Furthermore, a monosomic YAC strain containing the insertion of *his4-LEU2* into the YAC also resulted in a reduced PSS frequency (Table 2). Our interpretation of these data is that the *his4-LEU2* insert acts in *cis* to reduce precocious sister chromatid segregation of the YAC that contains it. The results suggest that DSBs, sister chromatid exchanges (SCE) resulting from DSBs, and/or intermediate structures of sister chromatid exchange, may be instrumental in maintaining chromatid cohesion. Experiments with circular chromosomes have shown that exchanges between sister chromatids are common in meiosis in *S. cerevisiae* (SUN *et al.* 1991). A circular artificial chromosome that consisted largely of phage lambda DNA showed a lower rate of SCE than a circular chromosome derived from yeast chromosome *III*. However, when the *ARG4* recombination hot spot and DSB region was inserted into the λ DNA circle, SCE increased considerably (SUN *et al.* 1991). Although SCE occurs at low levels in yeast meiosis (HABER *et al.* 1984; JACKSON and FINK 1985; GAME *et al.* 1989; GAME 1992), evidence of increased SCE was found in situations where pairing is absent (WAGSTAFF *et al.* 1985; SUN *et al.* 1991). This increase is presumably due to the lack of physical constraints on SCE normally thought to be exerted by the tripartite synaptonemal complex. In light of cytological observations of unsynapsed nonhomologous chromosomes (LOIDL *et al.* 1994), we believe that neither the heterologous YAC pairs nor the YAC monosomes used in this study become synapsed in a tripartite synaptonemal complex and thus SCE within them is potentially uninhibited. Sister chromatid exchanges could not be observed in our experiments, but we regard SCEs or related events, stimulated in *cis* by the *his4-LEU2* insert, as the most plausible mechanism that maintains cohesion between the unpaired YAC sister chromatids in meiosis I.

Another molecular mechanism suggested for sister chromatid cohesion was that of catenation between the sister molecules following DNA replication of a multireplicon chromosome (MURRAY and SZOSTAK 1985). Such catenation needs to be resolved by a topoisomerase-like activity, in mitosis as well as in meiosis I. The maintenance of sister chromatid cohesion until anaphase I would require meiosis-specific regulation of this activity (its repression until anaphase I). We suggest that the sister chromatids are held together by molecular events known to occur regularly between non-sister chromatids in meiosis, namely DSBs that evolve into exchanges or their intermediates, without invoking any new mechanism. Furthermore, a common mechanism, shared by SCE and by inter-homolog exchange, is more economical with regard to cellular resources and makes sense from the evolutionary point of view (previously suggested by SUN *et al.* 1991). We propose that DSBs and recombination events that result from them lead to two different components of a functional chiasma: an exchange between non-sister chromatids holds the two homologs and rare exchanges between sister chromatids contribute to the cohesion that is needed to prevent the chiasma from resolving prior to anaphase I. It should be emphasized, however, that another aspect of sister chromatid cohesion in meiosis, namely sib-centromere cohesion (MAGUIRE 1990), is not explained by DSB metabolism. Sib-centromeres remain as one functional unit in meiosis I and attach by spindle fibers to one pole of the spindle. Failure of sib-centromere cohesion is expected to lead to precocious sister chromatid segregation (SUJA *et al.* 1992; SEARS *et al.* 1995). We know that centromeric regions are not especially rich in DSB sites (ZENVIRTH *et al.* 1992; S. KLEIN, D. ZENVIRTH and G. SIMCHEN, unpublished) and that gene conversion and recombination near the centromere are suppressed (LAMBIE and ROEDER 1986, 1988). Clearly, other cohesive forces at the centromere, as well as DSB site-induced cohesion along the chromosome arms, are required for proper disjunction of sister chromatids.

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