Meiotic Recombination on Artificial Chromosomes in Yeast

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ABSTRACT

We have examined the meiotic recombination characteristics of artificial chromosomes in *Saccharomyces cerevisiae*. Our experiments were carried out using minichromosome derivatives of yeast chromosome *III* and yeast artificial chromosomes composed primarily of bacteriophage λ DNA. Tetrad analysis revealed that the artificial chromosomes exhibit very low levels of meiotic recombination. However, when a 12.5-kbp fragment from yeast chromosome *VIII* was inserted into the right arm of the artificial chromosome, recombination within that arm mimicked the recombination characteristics of the fragment in its natural context including the ability of crossovers to ensure meiotic disjunction. Both crossing over and gene conversion (within the *ARG4* gene contained within the fragment) were measured in the experiments. Similarly, a 55-kbp region from chromosome *III* carried on a minichromosome showed crossover behavior indistinguishable from that seen when it is carried on chromosome *III*. We discuss the notion that, in yeast, meiotic recombination behavior is determined locally by small chromosomal regions that function free of the influence of the chromosome as a whole.

SINCE they were originally described in 1983, linear artificial chromosomes have proven to be useful tools for studying telomere structure and function, and mitotic chromosome segregation (DANI and ZAKIAN 1983; MURRAY and SZOSTAK 1983; PLUTA *et al.* 1984; HIETER *et al.* 1985; KOSHLAND, KENT and HARTWELL 1985; MURRAY, SCHULTES and SZOSTAK 1986; SUROSKY, NEWLON and TYE 1986; MURRAY, CLAUS and SZOSTAK 1988). These studies have in part been successful because artificial chromosomes can be made to mimic the mitotic behavior of natural chromosomes. We have used artificial chromosomes to investigate meiotic chromosome segregation (DAWSON, MURRAY and SZOSTAK 1986). In some respects our studies have been successful for the opposite reason, *i.e.*, the artificial chromosomes do not mimic the meiotic behavior of natural chromosomes in every way. Natural chromosomes participate in meiotic crossing over with great consistency, and this crossing over is necessary to insure the disjunction of homologs at meiosis I. In the course of our experiments, we found that artificial chromosomes crossover at unusually low frequencies in meiosis, a characteristic which allowed us to investigate the fate of noncrossover homolog pairs in meiosis. Additionally, we were led to question the nature of this deficiency in recombination. Here we describe the meiotic recombination properties of artificial chromosomes, and of a minichromosome derivative of a natural yeast chromosome.

Studies in a variety of organisms have demonstrated that on a chromosomal scale, both the number and distribution of crossovers are regulated (JONES 1987; CARPENTER 1988; HAWLEY 1988). Recently, fine-scale mapping of crossover events in yeast has shown that frequencies can vary dramatically over distances as small as one thousand base pairs (LARKIN and WOOLFORD 1984; COLEMAN *et al.* 1986; SYMINGTON and PETES 1988; SYMINGTON *et al.* 1991). Our results suggest that, in yeast, the recombination behavior of the sequences in a particular small interval is largely determined by the sequences in that interval. Effects from the sequences in adjacent intervals were shown to be minimal in our experiments.

MATERIALS AND METHODS

Strains, media and genetic methods: Media were prepared as described in SHERMAN, FINK and LAWRENCE (1979). *Saccharomyces cerevisiae* strains are described in Table 1. DD35, DD117, DD97 and DD114 were derived from the same parental strain and are isogenic except for differences evident in the genotypes presented in Table 1. DL98, DL112 and DL72 are isogenic strains derived from the strains used by NICOLAS *et al.* (1989). Chromosome *VIII* of DL98 and DL112 has a 2060-bp *Hpa*I-*Hpa*I deletion which removes the entire *ARG4* coding region. This deletion was introduced to eliminate ectopic interactions between the *ARG4* alleles on chromosome *VIII* and the artificial chromosomes.

Recombination data from all yeast linear plasmids (YLps; these constructs may also be referred to as artificial chromosomes), 72-kbp derivatives of chromosome *III* (mini *III*s) and chromosome constructs were obtained by tetrad analy-

TABLE 1

Strains used in this study

Strain	Genotype	Source
DD35	**a**/α, *trp1/trp1, ura3/ura3, his3-11, 15/his3-11, 15, ade1/ADE1, arg4-17/ARG4, leu2-3, 112/leu2-3, 112, lys2/LYS2*. YLp45 [*LEU2, trp1* (*Xba*I site fill-in), *URA3, his3* (*Hin*dIII site fill-in)], YLp49 [*leu2* (400-bp deletion), *TRP1, ura3* (*Nco*I site fill-in), *HIS3*].	DAWSON, MURRAY and SZOSTAK (1986)
DD117	*a*/α, *trp1/trp1, ura3/ura3, his3-11, 15/his3-11, 15, ade1/ADE1, arg4-17/ARG4, leu2-3, 112/leu2-3, 112, lys2/lys2*. YLp45 [*LEU2, trp1* (*Xba*I site fill-in), *URA3, his3* (*Hin*dIII site fill-in)], YLp55 [*leu2* (*Cla*I site fill-in), *TRP1, ura3* (*Nco*I site fill-in), *HIS3*].	This study
DD97	*a*/α, *trp1/trp1, ura3/ura3, his3-11, 15/his3-11, 15, ade1/ADE1, arg4-17/ARG4, leu2-3, 112/leu2-3, 112, lys2/lys2*. Mini *III* [*URA3, leu2-3, 112, trp1* (*Xba*I site fill-in)]. Mini *III* [*ura3* (*Nco*I site fill-in), *LEU2 TRP1*].	This study
DD114	**a**/α, *trp1/TRP1, ura3/ura3, his3-11, 15/his3-11, 15, ade1-1/ADE1, arg4-17/ARG4, LEU2/leu2-3, 112, lys2/lys2, can1/CAN1, HIS3::PGK1/PGK1*	This study
DL98	**a**/α, *trp1/trp1, ura3/ura3, his3-11, 15/his3-11, 15, ade1/ADE1, arg4* (*Hpa*I deletion)/*arg4* (*Hpa*I deletion), *leu2-3, 112/leu2-3, 112, lys1/lys1*. YLp56 [*LEU2, trp1* (*Xba*I site fill-in). *URA3, ARG4, his3* (*Hin*dIII site fill-in)], YLp60 [*leu2* (*Cla* site fill-in), *TRP1, ura3* (*Nco*I site fill-in) *arg4-RV* (AT removal at *Eco*RV), *HIS3*].	This study
DL112	**a**/α, *trp1/trp1, ura3/ura3, his3-11, 15/his3-11, 15, ade1/ADE1, arg4* (*Hpa*I deletion)/*arg4* (*Hpa*I deletion), *leu2-3, 112/leu2-3, 112, lys1/lys1*. YLp56 [*LEU2, trp1* (*Xba*I site fill-in), *URA3, ARG4, his3* (*Hin*dIII site fill-in)], YLp61 [*leu2* (*Cla*I site fill-in), *TRP1, ura3* (*Nco*I site fill-in) *arg4-Bg* (*Bgl*II site fill-in), *HIS3*].	This study
DL72	**a**/α, *trp1/trp1, ura3/ura3, his3-11, 15/his3-11, 15, ade1/ADE1, arg4-RV* (AT removal at *Eco*RV)/*ARG4, leu2-3, 112/leu2-3, 112, lys1/lys1*, *Hin*dIII fragment of *URA3* inserted at the *Apa*I site upstream of 12.5-kbp sequence on chromosome *VIII*/*Bam*HI fragment of *HIS3* inserted at the *Sna*BI site downstream of 12.5-kbp sequence on chromosome *VIII* (see Figure 4c)	This study

sis. Minichromosomes and artificial chromosomes show some variability in copy number. Only tetrads with two copies of each chromosome of interest were included in the analysis. In strains carrying the shortest YLps, DD35 and DD117, 35% and 41% of the four spore viable tetrads showed 2:2 segregation for both. YLps bearing the 12.5-kbp insert in DL98 and DL112 showed 2:2 segregation in 74% of the four spore viable tetrads and the mini *III*s in DD97 both segregated 2:2 in 97% of the four spore viable tetrads.

In the cases of nondisjunction or precocious sister chromatid disjunction of YLps or mini *III*s, spores which contained more than one chromosome were streaked to single colonies on YPD plates and replica plated to appropriate media to screen for loss of one chromosome. The markers on the remaining chromosome could then be determined. Tetrads with inviable spores were excluded from the results. Spore viability was greater than 90% in all experiments.

Construction of artificial chromosomes and minichromosomes: The construction and general structures of the artificial chromosomes and mini *III*s have been described previously (DAWSON, MURRAY and SZOSTAK 1986; MURRAY, SCHULTES and SZOSTAK 1986). Alterations of genomic DNA and artificial chromosome DNA were accomplished using one and two step gene replacement techniques (ROTHSTEIN 1983; BROWN and SZOSTAK 1983).

YLp56 was constructed by first cloning a 12.5-kbp *Apa*I-*Sna*BI fragment containing the *ARG4* gene (−7.3 kbp to +5.2 kbp relative to the *ARG4* translation start site, see Figure 1 and Figure 2 legend of NICOLAS *et al.* 1989), into a plasmid carrying the 4.4-kbp *Bcl*I fragment of bacteriophage λ (positions 9361–13,820 on the bacteriophage λ map; Figure 1a). The flanking λ sequences were then used to target integration by homology into YLp45 (Figure 2).

YLp57 was built in two steps. First, YLp55 (Figure 2) was modified in the same fashion as YLp56 by addition of the 12.5 kbp (the *ARG4* allele was again carried in this construct) to give YLp101. Second, YLp57 was generated by targeting pNS309 into this YLp. pNS309 contains the 3.2-kbp *Pst*I fragment of *ARG4* bearing an *arg4-RV* allele cloned into the *Pst*I site in the polylinker of pMLC28, and a 1.1-kbp *URA3* fragment cloned into the *Bam*HI site of the polylinker (SCHULTES and SZOSTAK 1990). pNS309 was cut with *Bgl*II (which cuts within *ARG4*) and targeted to recombine with the *ARG4* gene on the YLp by homologous recombination. Transformants were selected by their ability to grow on media deficient for uracil. Ura$^-$ derivatives which had lost the integrated pNS309 were selected on 5-fluoroorotic acid (BOEKE, LACROUTE and FINK 1984). These were screened by growth phenotype and Southern analysis for retention of the *arg4-RV* allele (see strain list for remaining markers carried on these YLps).

The other YLps used in these experiments (YLp60 and YLp61) were built by sequential modification of YLp57. YLp57 was modified with the 3.2-kbp *Pst*I fragment of *ARG4* by homologous recombination to create YLp62. YLp60 was generated by targeting pNS309 into YLp62 in the same fashion as YLp57 (see preceding paragraph).

YLp61 was generated by modification of YLp62 using the same procedure as for YLp60, except that pNS314 was used. This plasmid carries the 1.1-kbp *URA3* fragment at the *Bam*HI site in the polylinker of pMLC28 and the 3.2-kbp *Pst*I fragment bearing an *arg4-Bg* allele at the *Pst*I site of the polylinker (SCHULTES and SZOSTAK 1990).

The construction of the mini *III*s used in this study is described elsewhere (MURRAY and SZOSTAK 1986). The 54-kbp interval of contiguous sequences from chromosome *III* extends from *LEU2* to an *Eco*RI site ~4.5 kbp to the right of the *PGK1* gene (Figure 3a). This end of the mini *III* is punctuated by the *TRP1* gene. The left end of the minichromosome from *LEU2* to the telomere is composed of sequences from *HMR*, pBR322 and *URA3*.

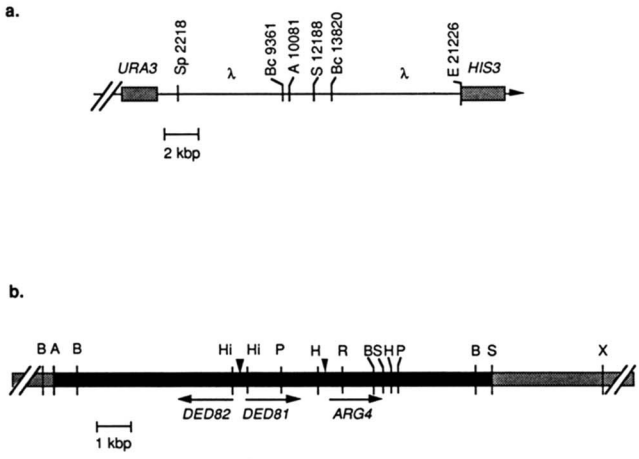

FIGURE 1.—(**a**) Restriction map of the right arm of the YLp. Bacteriophage DNA is represented by a thin line. Yeast sequences are represented by filled bars. Lambda map positions are shown next to restriction sites. (**b**) Restriction map of chromosome *VIII* including the 12.5-kbp region used in our experiments. The black region is the *Apa*I-*Sna*BI 12.5-kbp fragment. Contiguous sequences of chromosome *VIII* are represented by stippled bars. Arrowheads between the *Hin*dIII sites and the *Hpa*I and *Eco*RV sites represent the double-strand break sites described by SUN *et al.* (1989). Arrows beneath the chromosome represent the direction of transcription of the designated genes. A = *Apa*I, B = *Bgl*II, Bc = *Bcl*I, E = *Eco*RI, H = *Hpa*I, Hi = *Hin*dIII, *P* = *Pst*I, R = *Eco*RV, S = *Sna*BI, X = *Xho*I SP = *Sph*I.

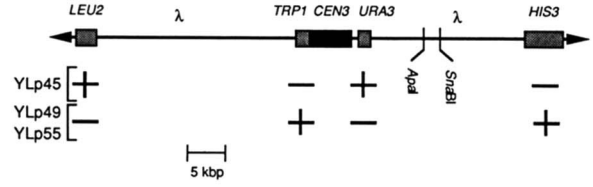

FIGURE 2.—Scale drawing of artificial chromosomes used in these studies. The structure of the artificial chromosomes from DD35 and DD117 are shown. In both strains, one artificial homolog is *LEU2*$^+$:*trp1*$^-$:*URA3*$^+$:*his3*$^-$, and the other is *leu2*$^-$:*TRP1*$^+$:*ura3*$^-$:*HIS3*$^+$.

Construction of chromosome *III* alleles: On chromosome *III*, the 54-kbp interval over which we measured recombination extends from *LEU2* on the left to a *Bgl*II site ~4 kbp to the right of the *PGK1* gene. The *HIS3* gene was cloned into the *Bgl*II site of the *PGK1* flanking sequences carried on a plasmid. An *Eco*R1 fragment from this plasmid containing *HIS3* and flanking sequences from the *PGK1* region was targeted by homology onto chromosome *III* using the single-step gene replacement technique (ROTHSTEIN 1983).

Construction of *URA3* and *HIS3* chromosome *VIII* homologs: *URA3* insertion at the *Apa*I site upstream of *ARG4* on chromosome *VIII* was accomplished by cloning the 1.1-kbp *Hin*dIII fragment of *URA3* into the *Apa*I site of a 1.5-kbp *Bgl*II fragment from the region upstream of *ARG4* (see Figures 1 and 4c). The resulting construct was targeted into an *arg4-RV* allelic chromosome *VIII* by homologous recombination.

The *HIS3* insertion at the downstream *Sna*BI site was generated in the same fashion as the *URA3* insertion at *Apa*I. A 1.7-kbp *Bam*HI fragment of *HIS3* was cloned into the *Sna*BI site of a 3.3-kbp *Bgl*II-*Xho*I fragment from downstream of *ARG4* (see Figures 1 and 4c). The construct was targeted onto an *ARG4* allelic chromosome *VIII* by homologous recombination.

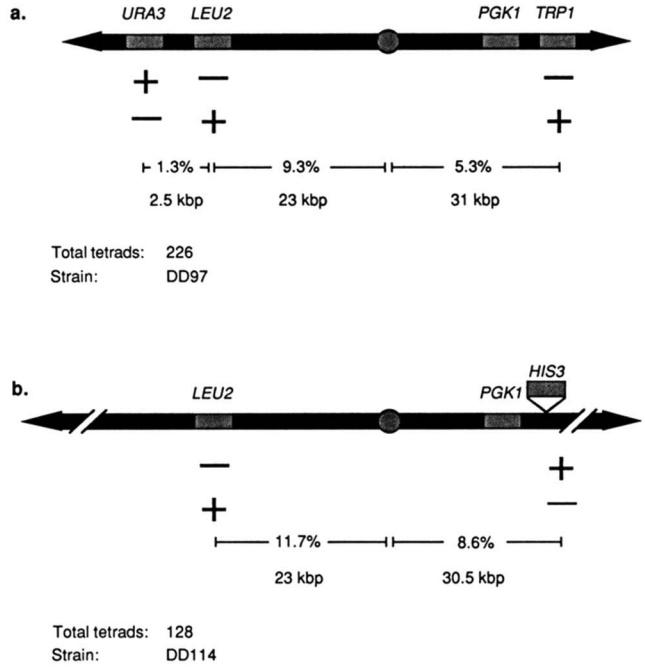

FIGURE 3.—Crossing over in a 54-kbp interval of chromosome *III* on a 72-kbp minichromosome. (**a**) The structure of the mini *III*s is shown. The mini *III*s were constructed as described in the MATERIALS AND METHODS. A diploid carrying the mini *III* homologs was sporulated and tetrad analysis used to determine recombination frequencies. The crossover frequencies for the intervals from *URA3* to *LEU2*, *LEU2* to *CEN3*, and *CEN3* to *TRP1* are included along with the number of tetrads scored in the experiment. All tetrads showed either zero or one crossover except one which showed single crossovers in intervals I and III. (**b**) Crossing over in a 54-kbp interval of chromosome *III* in its natural environment. The construction of these chromosomes is described in the METHODS AND MATERIALS. Except for one tetrad in which single crossovers occurred in intervals I and II, all tetrads showed either zero or one crossover in these intervals. The frequencies of crossing over obtained in the experiments shown here (20.4%, 26 crossovers in 128 tetrads in DD114) and (16.2%, 33 crossovers in 226 tetrads in DD97) are not significantly different as determined by a *G* test of independence ($G = 1.879$, $P > 0.1$).

Statistical and mapping procedures: *G* tests were used to measure statistical significance (SOKAL and ROHLF 1969). Percent crossing over was used as a measure of recombinational activity. The values presented indicate the total number of crossovers observed in a given interval, divided by "total tetrads," multiplied by 100. "Total tetrads" is the set of four-spore viable tetrads that showed 2:2 segregation for both chromosomes being examined. Tetrads showing more than a single crossover on the chromosomes being examined were rare and are noted in the appropriate figure legends. The increase in crossover frequency that would result if positive interference was relieved was approximated by calculating the number of single crossover tetrads that would be expected in the absence of interference (SNOW 1979). We used the equation $p(1) = 2kx(1 - e^{-2x})/e2^{kx} - 1$. x is the mean exchange frequency per chromatid observed in our experiments and k is the level of interference estimated by SNOW (1979) for the *LEU2-MAT* interval of chromosome *III* ($k = 0.429$) or the *CEN8-CUP1* interval of chromosome *VIII* ($k = 0.232$).

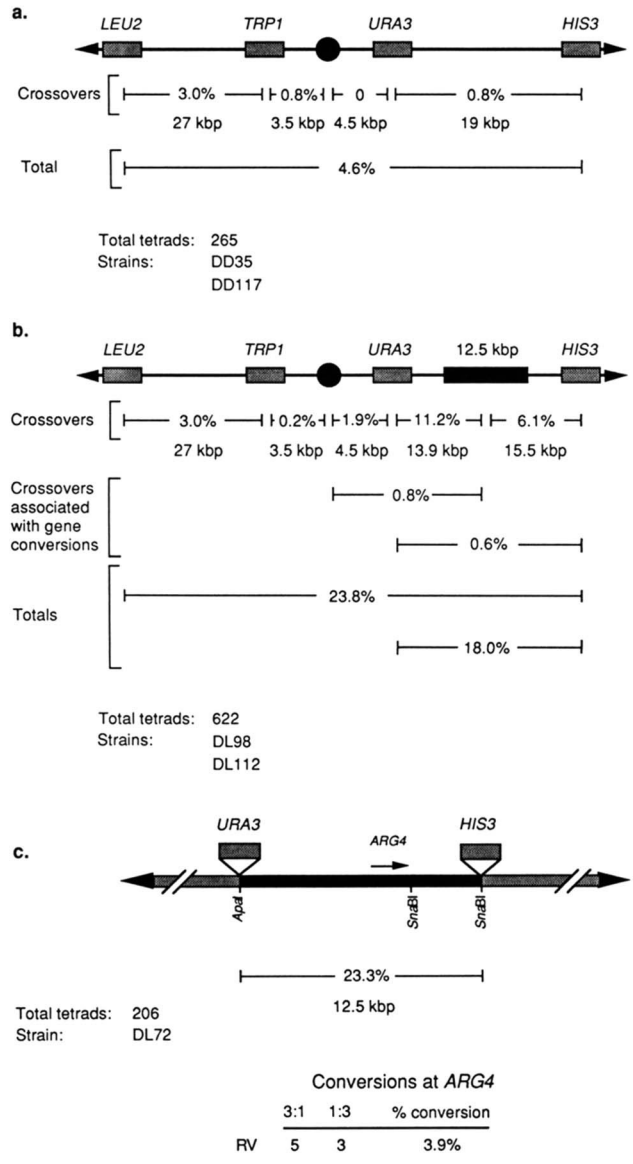

FIGURE 4.—Crossover frequencies for artificial chromosomes bearing the 12.5-kbp insert. All data are expressed as the percentage of total tetrads with crossovers in the intervals indicated. All of the strains used are heterozygous for the centromere linked marker *ADE1*, consequently we were able to deduce the segregation of the artificial chromosome centromeres, and could therefore divide the chromosomes into genetic intervals which include the centromere. The chromosomes are not drawn to scale. The physical distances between markers are shown below the genetic distances. (**a**) For comparison we show crossover data from artificial chromosomes without the 12.5-kbp insert. Recombination data from two experiments, DD35 (119 tetrads) and DD117 (146 tetrads) are combined. None of the tetrads showed multiple crossovers. (**b**) Summary of crossover data for YLps bearing the 12.5-kbp insert. These data are combined from two experiments with the isogenic strains DL98 and DL112 which differ only in which *ARG4* allele is carried on the YLps. Gene conversion data from these experiments are summarized in Figure 5. A breakdown of all the recombinant tetrads observed in these experiments is presented in Figure 6, a and b. Crossovers that occurred in conjunction with gene conversions and hence could not be assigned to a specific interval are indicated. The total of all crossovers that could be mapped to the *URA3* to *HIS3* interval was 18.0%. Of the scored tetrads, 501 (80.5%) showed correct disjunction of the YLps, 69 (11.1%) showed precocious sister disjunction, and 52 (8.4%) showed meiosis I nondisjunction. We analyzed crossing over on the YLps in all of these tetrads. All but two crossover tetrads fell in the correct disjunction category. The remaining two (Figure 6, a and b, class A) showed meiosis I nondisjunction. (**c**) Crossovers and gene conversions that occurred between *URA3* and *HIS3* inserts. *URA3* and *HIS3* were inserted on separate homologs. The *URA3* homolog has an *arg4-RV* allele so that gene conversions can be monitored. Most of the tetrads showed either zero or one crossover. Four tetrads showed two crossovers in the *URA3* to *HIS3* interval.

RESULTS

Recombination of yeast linear plasmids with bacteriophage λ DNA backbones: We have studied the recombination behavior of artificial chromosomes composed largely of bacteriophage λ DNA. The artificial chromosomes carry yeast elements known to be required for the normal replication and segregation of linear chromosomes. These include the centromere from chromosome *III* (*CEN3*), Tetrahymena telomeres modified *in vivo* by the addition of yeast telomeric sequences, and *ARS1* (for *a*utonomously *r*eplicating *s*equence), an origin of DNA replication. Additionally, each artificial chromosome carries yeast genes whose functions can easily be monitored by assaying for growth on the appropriate media [see DAWSON, MURRAY and SZOSTAK (1986) and MATERIALS AND METHODS for descriptions of the construction of the artificial chromosomes). The frequency of crossing over between artificial homologs was initially determined using the pair shown in Figure 2. Both chromosomes carry four yeast genes; *LEU2*, *TRP1*, *URA3* and *HIS3*. These two chromosomes are identical except that in one, mutations were introduced into *LEU2* and *URA3*, and in the other, mutations were introduced into *TRP1* and *HIS3* (Figure 2). The phenotypes conferred by these chromosomes make it straightforward to detect crossovers between them along their entire length. We introduced these model chromosomes into a diploid strain, induced sporulation and examined their meiotic recombination properties by tetrad analysis. In these experiments the artificial chromosomes exhibited crossovers in about 4.6% of all meioses, giving the 54-kbp *LEU2-HIS3* interval a genetic map length of about 2.3 centimorgans (cM) (Figure 4a and DAWSON, MURRAY and SZOSTAK 1986). The majority (8 of 12) of the crossovers were in the left arm of the chromosome between *LEU2* and *TRP1*.

Although recombination frequencies vary somewhat for different regions of the genome (LARKIN and WOOLFORD 1984; COLEMAN *et al.* 1986; SYMINGTON and PETES 1988; SYMINGTON *et al.* 1991), the observed crossover frequency of 23 kbp/cM is much lower than would be expected for most similarly sized intervals of yeast DNA. The average correlation between physical and genetic map lengths is about 3.6 kbp/cM (MORTIMER and SCHILD 1985). Measurements of the genetic map length of the 54-kbp *LEU2-*

PGK1 interval of chromosome *III*, which includes *CEN3* (the centromere carried on the artificial chromosomes) yield a value of about 5.4 kbp/cM (MORTIMER and SCHILD 1985). Thus, these artificial chromosomes undergo crossing over at frequencies that are four to seven times less than average for equivalent lengths of DNA in natural yeast chromosomes.

The structural differences between the artificial chromosomes and their natural counterparts suggested two possible explanations for the lower level of crossing over exhibited by the artificial chromosomes. One obvious difference between the artificial chromosomes and natural chromosomes is overall length. The artificial chromosomes are about 62 kbp long, while natural yeast chromosomes range from 240 to 1640 kbp (CARLE and OLSON 1985). If efficient homologous pairing, which may be a prerequisite for normal crossing over, requires extensive stretches of homology, then the low levels of recombination seen with artificial chromosomes could be explained by their much shorter length. Alternatively, the length of the artificial chromosomes might be adequate for pairing and crossing over, but being composed primarily of non-yeast DNA, they may lack sequences conducive to normal levels of crossing over.

Recombination on minichromosomes derived from chromosome *III*: We tested the model that the low level of crossing over on the artificial chromosomes could be attributed to their short lengths. Our approach was to measure the crossover frequency on minichromosomes (mini *III*s) similar in size to the YLps described above. The mini *III*s are about 72 kbp in length and carry a 54-kbp interval of contiguous sequences from chromosome *III* including the centromere. If normal crossing over requires chromosome-sized lengths of homology, we reasoned that the crossover frequency on the mini*III*s would be depressed relative to the values obtained when the interval was carried in its natural environment on chromosome *III*. The mini *III* homologs each carry the portion of chromosome *III* that extends from *LEU2* through *CEN3* to an *Eco*RI site about 4.5-kbp centromere distal to *PGK1*, at which the *TRP1* gene was integrated (Figure 3a and MURRAY, SCHULTES and SZOSTAK 1986). Each mini *III* also carries the *URA3* gene at its left end. Mutations were introduced into the two mini *III*s to allow the detection of crossovers between them (Figure 3a). Tetrad analysis revealed crossovers between *LEU2* and *TRP1* in 14.6% of all meioses, for a genetic map distance of 7.3 cM. This recombination frequency was within the range of published values for the *LEU2-PGK1* interval on chromosome *III* (MORTIMER and SCHILD 1985). For a more accurate comparison, we measured the frequency of crossing over for this interval of chromosome *III* in the same strain used for the mini *III* measurements. To this end, we inserted the *HIS3* gene into the *Bgl*II site ~4.0-kbp centromere distal from *PGK1* (Figure 3b). The site of insertion of the *HIS3* gene on the natural chromosome is about 500 bp from the site at which *TRP1* is inserted on the right arm of the mini *III*. The frequency of crossing over for the *LEU2-HIS3* interval was 20.3% or about 10.2 cM (Figure 3b). The crossover frequency measurements for this interval of chromosome *III*, obtained from full-length chromosome and mini *III* experiments, proved to be statistically indistinguishable (see Figure 3 legend).

Crossing over between YLps bearing 12.5-kbp inserts from chromosome *VIII*: If the YLps are deficient for sequences which promote or mediate recombination, then addition of recombinogenic sequences to the YLps might be expected to augment their levels of recombination. To test this possibility, we inserted 12.5 kbp of DNA from chromosome *VIII* into the right arms of the YLps described above (Figures 1 and 4b). This fragment contains the *ARG4* region which has been particularly well characterized for its gene conversion properties (FOGEL *et al.* 1979; NICOLAS *et al.* 1989; SCHULTES and SZOSTAK 1990). The effects of this insertion on both crossing over and gene conversion between the YLps were examined using two similar strains. The raw data from these crosses are tabulated in Figure 6. In this section we describe the effects of the insert on crossing over. Data on gene conversion is presented in the following section.

The YLps bearing the insert showed markedly higher levels of crossing over than their insert-free counterparts (Figures 4 and 6). Overall, the crossover frequency of the artificial chromosomes carrying chromosome *VIII* DNA was about fivefold higher than the chromosomes without this DNA (23.8% *vs.* 4.6%, Figure 4, a and b, respectively). The majority of the crossovers were confined to the *URA3-HIS3* region (see Figure 4b and Figure 6) which contained the chromosome *VIII* DNA. The increase in crossing over in this interval was about 22-fold (0.8% *vs.* 18.0%, Figure 4, a and b). There was no increase in the frequency of crossing over in the left arm of the artificial chromosomes.

The enhanced levels of crossing over which resulted from the insertion of the chromosome *VIII* segment suggested that the recombinogenic properties of a relatively small region of the genome can be transferred with it to a new locale. We tested this notion by examining whether the crossover levels of the YLps, attributable to the insert, are characteristic of the 12.5-kbp interval when it is in its native environment. To accomplish this, the *HIS3* gene was inserted at one end of the 12.5-kbp interval on one copy of chromosome *VIII* while *URA3* was inserted at the other end of the interval on another copy of chro-

mosome *VIII*. A diploid bearing these versions of chromosome *VIII* was sporulated and crossing over was measured between *HIS3* and *URA3* (Figure 4c). The value we obtained, 23.3%, was statistically indistinguishable from the 18.0% value attributed to the presence of the 12.5-kbp interval on the YLp (48/206 *vs.* 115/622; $G = 2.5$, $P > 0.1$).

Gene conversion at the *ARG4* locus on the YLps: Previous characterizations of recombination at *ARG4* revealed features which allowed us to use gene conversion as a second measure of the natural state of recombination of the 12.5-kbp interval when it is carried on the artificial chromosomes. Gene conversion at the *ARG4* locus shows polarity (FOGEL *et al.* 1979; NICOLAS *et al.* 1989; SCHULTES and SZOSTAK 1990). That is, when gene conversion is measured for various alleles of *ARG4*, high conversion frequencies are seen for alleles which map to the 5′ end of the gene and conversion frequencies drop for alleles which map increasingly toward the 3′ end.

We measured the conversion frequencies of two *ARG4* alleles on the YLps. One, *arg4-RV*, located at the 5′ end of the gene showed gene conversion frequencies between 6.0% and 8.2% in its natural chromosome environment (NICOLAS *et al.* 1989; SCHULTES and SZOSTAK 1990). The other, *arg4-Bg*, maps to the 3′ end of the gene and showed conversion frequencies between 0.4% and 2.3% in previous studies (NICOLAS *et al.* 1989; SCHULTES and SZOSTAK 1990). In separate experiments we examined strains carrying one $ARG4^+$ YLp and one YLp bearing either the *arg4-RV* or the *arg4-Bg* allele. The *arg4-RV* allele showed 4.5% conversion and the *arg4-Bg* allele converted at a frequency of 0.6% (Figure 5, a and b, respectively; the data from these experiments are tabulated in Figure 6, a and b, respectively). The value we obtained for the *arg4-RV* allele on the YLps is not significantly different from the previously published values for conversion of this allele when it is carried on chromosome *VIII* (12/269 *vs.* 62/838; $G_{adj} = 3.06$, $P > 0.05$ with Yate's correction). Conversion events at both alleles showed parity (Figure 6), and 25% of the *arg4-RV* conversions (3 of 12) were associated with crossovers between *URA3* and *HIS3* (Figure 6). We have also measured the conversion frequency of the *arg4-RV* allele when it is carried on chromosome *VIII* (Figure 4c). In this experiment we observed 3.9% conversion of the *arg4-RV* allele, a value which is statistically indistinguishable from value we obtained with the YLps (8/206 *vs.* 12/269: $G_{adj} = 0.03$, $P > 0.1$ with Yates correction). However, it is significantly lower than previously published values. Our measurements of gene conversion for *arg4-RV* on the natural chromosome (without insertions of *HIS3* and *URA3*) are in keeping with previously published values (data not shown) suggesting that the *HIS3* and *URA3* heterologies depress the conversion levels at *ARG4* on chromosome *VIII*.

DISCUSSION

The experiments described here demonstrate that YLps composed mainly of bacteriophage λ DNA show low levels of meiotic crossing over. Our experiments with minichromosomes show that the small size of the YLps is not the cause of their low levels of crossing over. Instead, it seems that the YLps do not carry sequences that support or mediate crossing over in yeast. Such sequences may directly or indirectly promote either pairing or recombination itself. In our experiments with the YLps we also measured conversion of the yeast genes they carried (*LEU2*, *TRP1*, *URA3*, *HIS3*). It is worth noting that the conversion frequencies of these genes were not remarkably low (1.0–2.5% in most experiments; data not shown). Assuming these levels of conversion are indeed "normal" it may be that the artificial chromosomes are adequate substrates for the homology searches that have been proposed to occur early in meiosis and may lead to gene conversions (CARPENTER 1987; SMITHIES and POWERS 1986; ENGEBRECHT, HIRSCH and ROEDER 1990), but deficient in some later step which prevents them from experiencing crossing over. Meiotic crossovers have been shown to be necessary for insuring meiosis I disjunction of homologs, yet in previous experiments (DAWSON, MURRAY and SZOSTAK 1986) we demonstrated that crossovers experienced by the YLps (without the 12.5-kbp *ARG4* insert) do not provide this insurance. The fact that the crossovers on the YLps are both unusually rare and functionally different from chromosomal meiotic crossovers is consistent with the possibility that they are not generated by the same pathway as most chromosomal crossovers. Alternatively, the YLp crossovers that do occur may be completely normal but the YLps may be deficient in some other feature required for crossovers to insure disjunction.

The recombination characteristics of the YLps changed dramatically when they were modified by the insertion of with 12.5-kbp of DNA from chromosome *VIII*. The recombination characteristics of the 12.5-kbp locus when carried on the YLps mimicked those obtained for the same region on chromosome *VIII*. Our results suggest that the signals which dictate the recombination behavior of the 12.5-kbp fragment are intrinsic to the interval and act autonomously.

Crossing over and gene conversion for the 12.5-kbp region were examined on artificial chromosomes and in its natural context on chromosome *VIII*. We examined two aspects of gene conversion; frequency and polarity. Genetic studies of chromosome *VIII* suggest that there are at least two sites for the initiation of gene conversion near *ARG4* in the 12.5-kbp

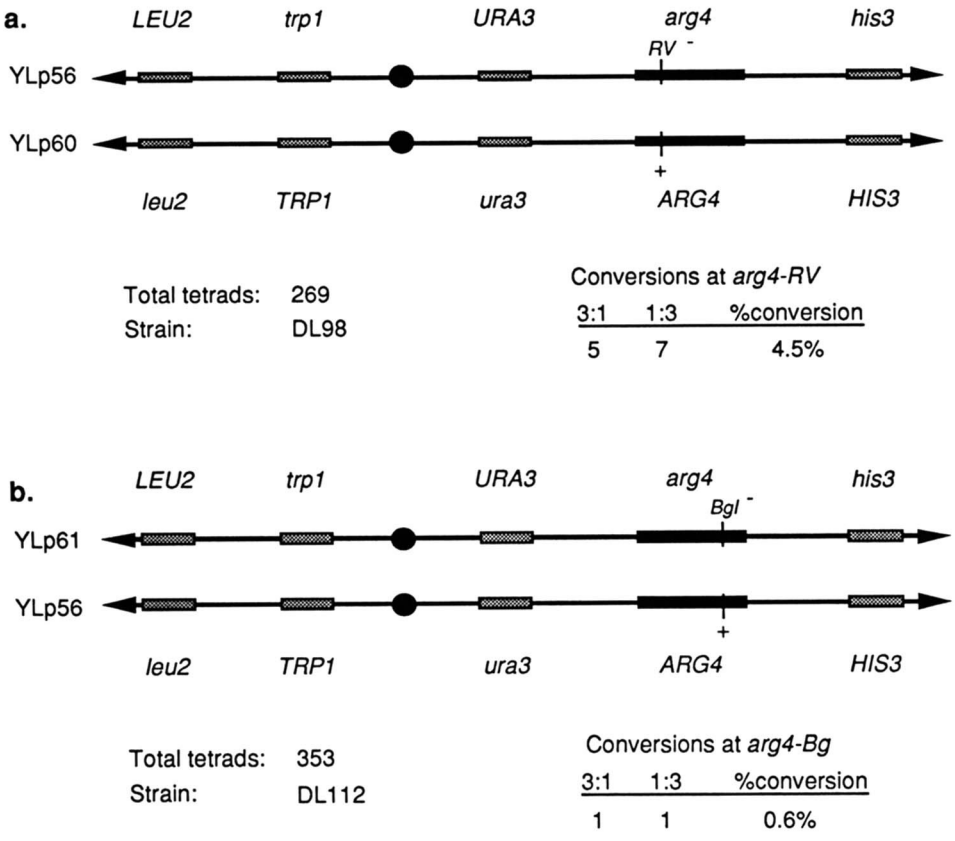

FIGURE 5.—Gene conversion of *ARG4* on the YLps. The structures of the artificial chromosomes used to measure gene conversion at *ARG4* are shown along with summaries of the gene conversion data. (**a**) The homologs used to measure gene conversion at the *Eco*RV site (260 bp from initiation of translation) include a chromosome with the *Eco*RV mutation (see MATERIALS AND METHODS) and a homolog with a wild-type copy of *ARG4*. (**b**) The chromosomes used to measure gene conversion at *Bgl*II (1274 bp from the initiation of transcription) include one homolog with the *arg4-Bg* allele (described in MATERIALS AND METHODS) and another with the wild type allele.

interval (FOGEL *et al.* 1979; NICOLAS *et al.* 1989; SCHULTES and SZOSTAK 1990, see Figure 4b). Both have been shown to map to a region at which double strand breaks occur in meiosis (SUN *et al.* 1989; CAO, ALANI and KLECKNER 1990). Deletion of the site nearest to *ARG4* greatly reduces the conversion frequency and abolishes polarity across the *ARG4* gene (NICOLAS *et al.* 1989). Our results demonstrate that the frequency and distribution of conversion events at *ARG4* are largely unaffected by transfer of the locus on the 12.5-kbp piece of DNA to the YLps. Similar results were obtained when the 12.5-kbp interval was transferred to the *URA3* locus on chromosome *V* (B. DE MASSY and A. NICOLAS, personal communication). The sequences outside of the 12.5-kbp interval on chromosome *VIII* would seem to have only minor effects on conversion at the *ARG4* locus. These results are not startling when one considers the frequency with which ectopic gene conversion events occur. In at least two cases, moving a gene from its natural location to a position on a different chromosome did not greatly reduce the frequency with which the displaced gene recombined with the copy remaining at the original location (JINKS-ROBERTSON and PETES 1985; LICHTEN, BORTS and HABER 1987). Our results add to the evidence that extensive tracts of homology are not necessary for frequent recombinogenic interactions between pairs of genes.

The crossover behavior of the YLps and mini *III* chromosomes provided insight into the phenomenon of interference, and the role of crossing over in ensuring meiotic disjunction. Two of our experiments compare the crossover behavior of an interval in its native environment to that seen in a different environment. First, we compared the frequency of crossing over across the 12.5-kbp interval in both the YLp and natural settings. The values we obtained were indistinguishable. Second, the frequency of crossing over across a 54-kbp interval of chromosome *III* remained unchanged when that interval was transferred to a minichromosome. The fact that the frequency of crossing over did not increase when the 12.5- and 54-kbp intervals were transferred from the natural chromosomes is consistent with the notion that their recombination frequencies are not normally greatly affected by positive interference from crossovers in adjacent intervals. The levels of interference in the region of chromosome *III* carried on the mini *III* and a large region of chromosome *VIII* which includes the *ARG4* locus have been estimated (SNOW 1979). If the levels of positive interference determined by SNOW were imposed on the chromosome *VIII* and *III* intervals only by crossovers occurring outside of those intervals, we calculate that we would see increases in crossing over in the chromosome *VIII* interval from 23.3% to about 35%, and in the chromosome *III* interval from 20.3% to about 34% when those intervals were moved to interference free environments

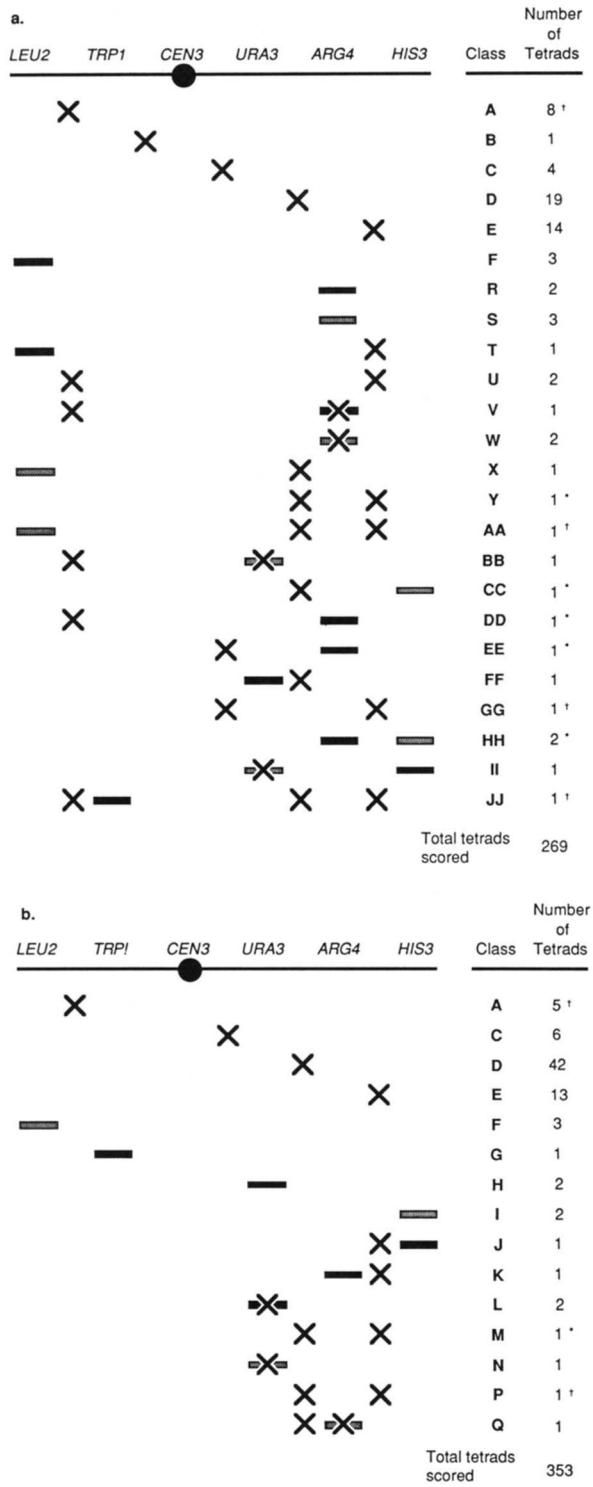

FIGURE 6.—Recombination data from meiosis of diploids with the artificial chromosomes. (**a**) Recombination data from a diploid with an *arg4-RV* allele on one artificial chromosome and *ARG4* on the other. (**b**) Recombination data from a diploid carrying artificial chromosomes with *arg4-Bg* end *ARG4* alleles. Data from these experiments are summarized in Figures 4 and 5. Each unique class of recombination is given a letter designation A-JJ. Crossovers are denoted by a bold × that is centered between and below the markers where the crossover occurred. Gene conversions are denoted by a bar that falls directly beneath the marker converted. A solid bar is used for $1^+:3^-$ conversions and a stippled bar for $3^+:1^-$ conversions. An × over a bar indicates that the converted chromatid has a crossover between the markers flanking the converted marker. Conversion associated crossovers can not be assigned a position to one side or the other of the converted marker. In the column designated Number of Tetrads, an asterisk (*) is used to indicate two-strand double crossovers (DCO) and other multiple recombination events that involve the same chromatids. Classes marked by a cross (†) are described below. Arbitrarily we refer to the *TRP1*$^+$, *HIS3*$^+$ chromatids as chromatids 1 and 2; and the *LEU2*$^+$, *URA3*$^+$, *ARG4*$^+$ chromatids as chromatids 3 and 4. Class A contains tetrads with crossovers between *LEU2* and *TRP1*. In each experiment (panels a and b) one class A tetrad showed meiosis I nondisjunction for the artificial chromosomes. Class P can be explained by a three strand DCO involving chromatids 1, 2 and 3. The class AA tetrad experienced a three-strand DCO involving chromatids 1, 3 and 4, and a gene conversion of *LEU2* on chromatid 1. The class GG tetrad experienced a three-strand DCO involving chromatids 1, 3 and 4. Class JJ can most easily be explained by a three-strand DCO of chromatids 1, 2 and 4, a crossover between *LEU2* and *TRP1* on chromatids 2 and 3, and a conversion of *TRP1* on chromatid 1.

(see MATERIALS AND METHODS for calculations). Among the possible explanations of our results is that the effects of interference are strongest over short distances (relative to the size of the intervals we have studied). If this is true, then most interference imposed on any particular small region of a natural chromosome would be due to crossovers which occur within that region, and crossovers which occurred a small distance from the region would provide little interference. Therefore, moving an interval out of its native environment would not necessarily remove it from the primary sources of positive interference. This notion leads to the prediction that high levels of interference would not be seen when measured over moderately sized genetic intervals in yeast. This was the result obtained when recombination was examined over six genetic intervals (centromere to *CUP1*) of chromosome *VIII*, a region which comprises about one third of the genetic map length of the chromosome (MORTIMER and FOGEL 1974). These results in yeast are in contrast to those obtained in a host of other organisms in which chiasma interference has been extensively studied. In many of these organisms a crossover imposes complete interference over large intervals that comprise 10 to 50% of the length of the chromosome (reviewed by JONES 1987).

Recent work has revealed significant differences among yeast chromosomes with respect to the average density of exchange per unit length of DNA (MORTIMER *et al.* 1989; KABACK, STEENSMA and DE JONGE 1989). Shorter chromosomes have a higher average level of cM/kbp than the longest ones (0.6 *vs.* 0.3 cM/kbp). This difference has been interpreted as the manifestation of a regulatory mechanism that ensures a minimum number of exchanges per chromosome (at least one) required for proper meiosis I chromosome segregation. The mechanism of control of crossover frequency remains a mystery. Many models propose that crossover frequency is in part controlled by

the number and/or efficacy of potential recombination initiation sites on each chromosome. Alternatively, variations in crossover frequencies on different chromosomes might be explained by crossover interference. By this model, each chromosome would have a probability approaching 1.0 of experiencing its initial crossover. Interference acting over long distances would diminish the probability of subsequent crossovers occurring on the same chromosomes. The above models are not mutually exclusive (for discussion see KING and MORTIMER 1990). While our results prove neither model, they are entirely consistent with the notion of initiation site number and/or efficacy as a regulator of crossover frequency.

A second property of crossing over which we considered was the effect of crossovers on meiotic disjunction. Our earlier experiments demonstrated that crossovers between the YLps do not ensure their disjunction (DAWSON, MURRAY and SZOSTAK 1986). In contrast, crossovers attributable to the 12.5-kbp interval appeared normal in the sense that they enhanced proper meiotic segregation. In the experiment described in Figure 4, noncrossover tetrads showed 10% nondisjunction (50 of 485 noncrossover pairs nondisjoined) whereas those with crossovers in the right (12.5-kbp insert bearing) arm showed 0.0% nondisjunction (0 of 122 crossover pairs nondisjoined; Figure 6, all crossover classes except A and DD). Interestingly, even in YLps bearing the 12.5-kbp insert, crossovers in the left arm did not ensure correct disjunction (14%, 2 of 14 crossover pairs nondisjoined Figure 6, classes A and DD). This intriguing result suggests that crossovers that are associated with the 12.5-kbp insert ensure correct disjunction while those that occur in the left arm do not. Experiments addressing this phenomenon will be presented elsewhere.

Considered together, the results of our analysis of recombination on a 12.5-kbp interval of chromosome *VIII* and a 54-kbp interval of chromosome *III* suggest that it is regulated locally rather than from afar. This conclusion is in keeping with the fine scale analysis of recombination of chromosome *III* described by SYMINGTON and colleagues (1991). Their results demonstrate that recombination events are distributed nonuniformly in the *LEU2* to *CEN3* interval, and that small deletions primarily alter recombination in the immediate adjacent environment. The recombination behavior of a collection of *X* chromosomes bearing homologous inversions led to the similar conclusion that in *Drosophila* there are discrete *cis*-acting elements which specify regional crossover characteristics (SZAUTER 1984).

Our results demonstrate that the YLps themselves are recombinationally quiet but can support normal recombination of the yeast sequences they carry. Because of these characteristics the YLps may be useful vectors for examining the recombination behavior of small intervals of DNA in yeast. crossing over between homologous inserts in YAC vectors has recently been suggested as a way of mapping contigs within genomic YAC libraries (CELLINI, LACATENA and TOCCHINI-VALENTINI 1991). Our results indicate this may be a viable strategy.

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