Intrachromosomal Recombination in *Saccharomyces cerevisiae*: Reciprocal Exchange in an Inverted Repeat and Associated Gene Conversion

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ABSTRACT

Intrachromosomal gene conversion has not shown a strong association with reciprocal exchanges. However, reciprocal exchanges do occur between intrachromosomal repeats. To understand the relationship between reciprocal exchange and gene conversion in repeated sequences the recombination behavior of an inverted repeat was studied. We have found that in one orientation a single copy of the *kan*^{r} gene of the bacterial transposon Tn*903* flanked by part of the inverted repeats IS*903* does not give G418 resistance in *Saccharomyces cerevisiae*. A reciprocal exchange in the IS*903* repeats inverts the *kan*^{r} gene, which then gives G418 resistance in a single copy. Using this as a selection for intrachromosomal reciprocal exchange we have introduced multiple restriction site heterologies into the IS*903* repeats and examined the crossover products for associated gene conversions. Approximately 50% of crossovers, both in mitosis and meiosis, were associated with a gene conversion. This suggests that these crossovers result from an intermediate that gives a gene conversion in 50% of the events, that is, both reciprocal exchange and gene conversion between repeated sequences have a common origin. The data are most consistent with a heteroduplex mismatch repair mechanism.

GENETIC recombination between homologous sequences is one of the basic mechanisms for generating genetic diversity. Homologous recombination has been primarily studied as events that occur between single copy genes on homologous chromosomes. However, recent experiments have shown that homologous recombination also occurs between repeated genes (JACKSON and FINK 1981; JINKS-ROBERTSON and PETES 1986; KLEIN and PETES 1981; LICHTEN, BORTS and HABER 1987; LISKAY, STACHELEK and LETSOU 1984). The repeated genes may be organized as a tandem or inverted repeat on one chromosome or may be dispersed repeats.

Genetic recombinations between homologous genes in eukaryotes are reciprocal (crossing-over) and nonreciprocal (gene conversion). In the fungi, meiotic studies of recombination have shown that gene conversion events are associated with crossing-over in flanking regions approximately 50% of the time (FOGEL *et al.* 1979; HURST, FOGEL and MORTIMER 1972). After correction for incidental exchanges the association is calculated to be 35% (STADLER 1973) although there is a range of values (FOGEL, MORTIMER and LUSNAK 1981). The association of meiotic gene conversion with reciprocal exchange has led to the formulation of several models of recombination, all of which mechanistically link gene conversion and reciprocal exchange (HOLLIDAY 1964; MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). Mitotic gene conversion, although associated with reciprocal exchange, does not show such strong association as the meiotic events (ROMAN and JACOB 1958).

The association of gene conversion with reciprocal exchange is not obligatory. CARPENTER (1984) has described two mutations of *Drosophila melanogaster* that uncouple meiotic gene conversion from reciprocal exchange. Mutant strains show a tenfold decrease in reciprocal exchange without any detectable decrease in gene conversions. Conversely, using *Ascobolus immersus*, ROSSIGNOL and coworkers (1984) have found a class of meiotic reciprocal exchange events that are not associated with any detectable gene conversion in adjacent regions. They suggest that there may be a second pathway for these reciprocal exchange events. Intrachromosomal gene conversion between repeated genes has not been found to be associated with reciprocal exchange (JACKSON and FINK 1985; KLAR and STRATHERN 1984; KLEIN 1984). However, reciprocal exchanges do occur between repeated sequences.

Intrachromosomal gene conversion events appear to be mediated by the same pathways that are used in gene conversions between homologous chromosomes. Although this gene conversion does not show association with reciprocal exchange intrachromosomal reciprocal exchange still occurs. We were interested in examining reciprocal exchanges between repeated sequences to see if this recombination is associated with

gene conversion or is independent. In order to recover the products of an intrachromosomal exchange we have studied recombination in *Saccharomyces cerevisiae* of an inverted repeat. We have selected reciprocal exchanges as a subset of all the recombination events and then have determined whether these exchanges arose in association with a gene conversion. In this way we hoped to determine whether there is a major second recombination pathway in yeast.

MATERIALS AND METHODS

Media and growth conditions: Yeast strains were grown vegetatively at 30° and sporulated at 23°. YPD medium was used for nonselective growth (SHERMAN, FINK and HICKS 1981). G418 resistant segregants were selected on YPD plates supplemented with 0.5 mg/ml G418. Diploid strains were sporulated by first growing vegetatively in KP medium (50 mM potassium phthalate (pH 5), 1% potassium acetate, 0.17% yeast nitrogen base, 0.5% ammonium sulfate, 0.5% yeast extract and 1% Bacto-peptone). Cells were then transferred to KA medium (1% potassium acetate) supplemented with required amino acids and bases for sporulation.

Escherichia coli strains were grown at 37° in LB medium (1% Bactotryptone, 0.5% yeast extract, 1% NaCl). Plasmid bearing strains were grown in LB supplemented with 50 μg/ml ampicillin.

Plasmid constructions: Plasmid pJF2 was constructed by inserting the 1.1-kb *Hin*dIII *URA3* fragment of yeast into the *Hin*dIII site of pBR322, the 1.7-kb *Pvu*II fragment of the *E. coli* transposon Tn*903* (GRINDLEY and JOYCE 1980) into a filled-in *Bam*HI site of pBR322, and a 6-kb *Eco*RI fragment of yeast chromosome *III* into the *Eco*RI site of pBR322. The Tn*903* fragment contains the *kanamycin resistance* (*kan*^{r}) gene flanked by 360 bp of the inverted repeated sequences IS*903*. The cloned fragments of chromosome *III* used in this study were obtained from CAROL NEWLON and are contained in plasmids designated A6C and B9G (PALZKILL, OLIVER and NEWLON 1986). Plasmid pJF3 is identical to pJF2 with the exception of the chromosome *III* yeast fragment. The 6-kb *Eco*RI fragment was substituted with a 3-kb *Eco*RI fragment from an adjacent region of chromosome *III*. Plasmid pJF2-Mlu was constructed by a partial digestion of pJF2 with *Mlu*I to generate linear molecules. The linear molecules were recovered from an agarose gel and the *Mlu*I ends filled in with KLENOW fragment of DNA polymerase I. Ligation of the blunt ends generated pJF2-Mlu which has lost one *Mlu*I site.

Plasmids pJF3-MBH-1 and pJF3-MBH-2 each contain three heterologous sites in the IS*903* sequences. The first of these, at *Mlu*I, was created as described above. The remaining two were made by oligonucleotide mutagenesis as described below. A *Bgl*II site was created by changing a G nucleotide to a T. A *Hpa*I site was created by changing an A nucleotide to a T and a second A nucleotide 3 bases away to a C. pJF3-MBH-1 and pJF3-MBH-2 differ as to which IS*903* sequence carries the *Bgl*II and *Hpa*I sites.

Mutagenesis: Site-directed mutagenesis was performed using cloned fragments of the IS*903* sequences in M13mp10 with 20-base oligomers that contained the desired mutations to prime DNA synthesis on the single stranded M13 templates. Template preparation and reaction conditions were as described by KUNKEL, ROBERTS and ZAKOUR (1987). The method of KUNKEL (1985) was used to enrich for mutants among the progeny bacteriophage recovered after transfection of the *in vitro* reaction products into *E. coli* host strain JM101.

TABLE 1

Strains

Strain	Genotype and comments
270-32B	**a** *his3-200 ura3-52 trp1-281 ino1-13* non-reciprocal translocation of the distal portion of the left arm of chromosome *III* onto chromosome *I*
DBY 745	*α leu2-3,2-112 ade2-101 ura3-52*
DBY 785	**a** *trp1 ura3-52*
304-40C	*α ura3-1 met4 his4 gal2*
260-1A	*α ura3-52 rad52-1*
KW1	270-32B with pJF2 integrated by transformation between *HIS4* and *HML*
KW2	270-32B with pJF3 integrated by transformation between *HIS4* and *HML*
KW2-10D	*α trp1-281 rad52-1* with pJF3 on chromosome *III* spore segregant from KW2 × 260-1A
KW4	270-32B with pJF2-Mlu integrated by transformation between *HIS4* and *HML*
KW5	Spore segregant from KW4 × DBY 745; normal chromosome *I*; pJF2-Mlu on chromosome *III*
KW6	304-40C with pJF3-MBH-1 integrated by transformation between *his4* and *HML*
KW7	304-40C with pJF3-MBH-2 integrated by transformation between *his4* and *HML*
KW11	KW1 × DBY 745
KW14	KW5 × 304-40C
KW15	KW6 × DBY 785
KW18	KW7 × DBY 785

Yeast strains: The strains used are described in Table 1. All strains which contain integrated plasmids were constructed by transformation with linear plasmid DNA as described by SHERMAN, FINK and HICKS (1981). The *URA3* gene was used for selection of stable transformants. Southern blot analysis of strain KW4 indicated the presence of an extra copy of the distal left arm of yeast chromosome *III*. Orthogonal-field alternation gel electrophoresis (CARLE and OLSON 1984) showed that chromosome *I* was greatly increased in size in this strain. This strain apparently carried a nonreciprocal translocation of part of chromosome *III* on chromosome *I*. After crossing strain KW4 with a strain carrying a normal chromosome *I*, strain DBY745, a spore segregant was recovered that lacked the extra copy of chromosome *III* information, but still carried the transformed chromosome *III* from strain KW4.

Mitotic experiments: Single colonies were suspended in 100 μl water. Appropriate dilutions were plated on YPD and G418 plates. Colonies were counted after 2 days. Rates were calculated using the median method of LEA and COULSON (1948). Independent G418-resistant segregants were selected by patching single colonies onto YPD plates and after overnight growth replica-plating onto G418 plates. A single G418-resistant colony from each patch was picked for Southern analysis.

Meiotic experiments: Single colonies were inoculated into YPD and grown overnight. An aliquot of 30 μl of the overnight culture was used to inoculate 25 ml KP medium. After growth to a cell density of 2×10^7 cells/ml, the cells were transferred to KA medium. An aliquot was removed to determine the background mitotic frequency of G418

resistance. The remaining cells were sporulated for three days at 23°. Random spores were prepared according to DAVIDOW and BYERS (1984) and appropriate dilutions plated on YPD and G418 plates. Colonies were counted after 2 days.

DNA preparation: Plasmid DNA was prepared according to Clewell and Helinski (1970). Yeast chromosomal DNA was isolated as described by SHERMAN, FINK and HICKS (1981). Double-stranded M13 replicative form DNA was prepared as described by MANIATIS, FRITSCH and SAMBROOK (1982).

DNA sequence analysis: The *Bam*HI-*Sma*I fragment from pJF2-Mlu, which contains the filled in *Mlu*I site, and the analogous fragment from pJF3-MBH-1, were subcloned into M13mp10. The IS*903* portion of the insert was sequenced by the dideoxy chain termination method (SANGER, NICKLEN and COULSON 1977).

Southern analysis: Yeast chromosomal DNA was digested with the indicated restriction enzymes using the conditions recommended by the manufacturer. Chromosomal DNA fragments were fractionated by agarose gel electrophoresis. DNA fragments were transferred to nitrocellulose filters according to SOUTHERN (1975). The filters were hybridized with radioactive probe. ^{32}P-labeled pJF2 or pJF3 was prepared according to the method of FEINBERG and VOGELSTEIN (1984). Hybridization was performed in 6 × SSC, 1 × Denhardt's, 0.25 M NaH_2PO_4 pH 6, and 30 mM tetrasodium pyrophosphate at 65° for 16 hr. Hybridized fragments were visualized by autoradiography.

Conversion events of the *Bgl*II and *Hpa*I sites were determined by hybridization of Southern blots with oligonucleotide probes. The filters described above were stripped to remove the hybridized pJF3 probe by treatment with 50% formamide, 5 × SSC, 1 × Denhardt's for 30 min at 70°. Approximately 7 ng of the appropriate 20-base oligomer was ^{32}P-labeled with T4 polynucleotide kinase according to standard procedure (MANIATIS, FRITSCH and SAMBROOK 1982) and precipitated with ethanol to remove the unincorporated label. The filters were hybridized with the ^{32}P-labeled oligomers as described above with the exception that the *Bgl*II probe was hybridized at 51° and the *Hpa*I probe at 49°. The filters were washed in 1 mM Tris (pH 7.4), 1% sarkosyl for 5 min at room temperature. This was followed by four 5-min washes in 1 mM Tris (pH 7.4) at room temperature.

Hybridized fragments were visualized by exposure to x-ray film for 1–2 days at −70°.

RESULTS

Selection of intrachromosomal reciprocal exchanges: Strain KW1 contains a single copy of the bacterial transposon Tn*903* integrated into chromosome *III* of yeast (Figure 1). The kanamycin resistance gene (*kan*^{r}) of Tn*903* (NOMURA, YAMAGISHI and OKA 1978) provides resistance to the antibiotic G418 in eukaryotes. The *URA3* gene of pJF2 was used to select for the transformation event which inserted pJF2 into chromosome *III* flanked by a duplication of the targeted yeast sequences to give strain KW1. The *kan*^{r} gene is flanked by 360 bp of the inverted IS*903* sequences. Since this construction has only a fragment of the IS*903* sequences which encode transposase the yeast transformants do not express transposase. This single copy of the *kan*^{r} gene does not provide resistance to strains grown on medium containing 0.5 mg/ml G418. However, strains carrying more than one copy of this *kan*^{r} gene are resistant to 0.5 mg/ml G418.

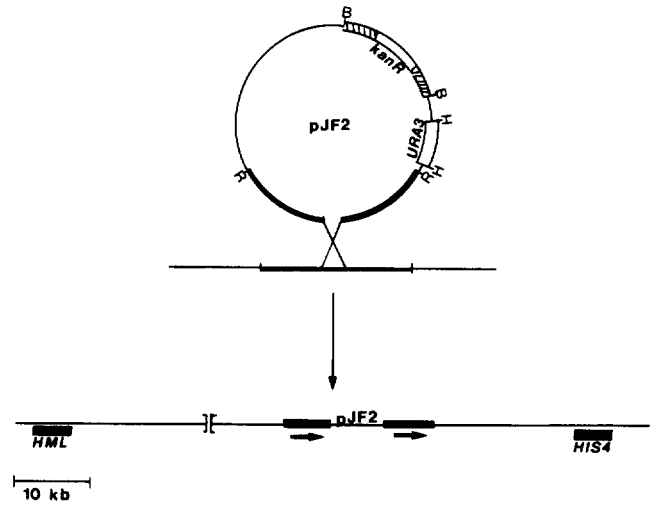

FIGURE 1.—Construction of strain KW1. Restriction enzyme sites in pJF2 are: B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI. *Thin line*, pBR322 sequences; *thick line*, 6-kb *Eco*RI fragment of yeast chromosome *III*. Plasmid pJF2 was digested with *Sac*I and used to transform a *ura3* strain to *URA3*. The integration of the plasmid by recombination with the homologous region results in a direct duplication of the chromosome *III* fragment. The approximate location of the insertion on chromosome *III* is shown.

G418-resistant segregants of KW1 occur at a frequency of 4.3×10^{-6} during vegetative growth. The structure and copy number of the *kan*^{r} insert was analyzed by Southern blots. The most frequent event leading to G418 resistance was a reciprocal exchange between the IS*903* inverted repeats. This recombination event inverts the *kan*^{r} gene, placing the 5′ end near the *Sal*I site of pBR322. Examples of the hybridization pattern produced by the inversion are shown in Figure 2. For reasons that are not clear this inverted orientation allows a more efficient expression of the *kan*^{r} gene in yeast. Transcription of the *kan*^{r} gene may be increased in the inverted orientation by the proximity of a cryptic promoter or enhancer or a UAS activity in pBR322 between the *Bam*HI and *Pvu*II sites. Orientation specific expression of the yeast *HIS3* gene inserted into pBR322 at the *Bam*HI site has been reported (STRUHL 1981). A cryptic pBR322 promoter in this region has also been reported to allow expression of a yeast *RAD7* gene that lacks its own promoter (PEROZZI and PRAKASH 1986). Expression of the yeast *HO* gene lacking URS1 has also been attributed to regulatory sequences in pBR322 (RUSSELL *et al.* 1986). The starting orientation where the single copy *kan*^{r} gene does not lead to G418 resistance will be referred to as the OFF orientation. The crossover product resulting in the inverted orientation of the *kan*^{r} gene and G418 resistance will be referred to as the ON orientation.

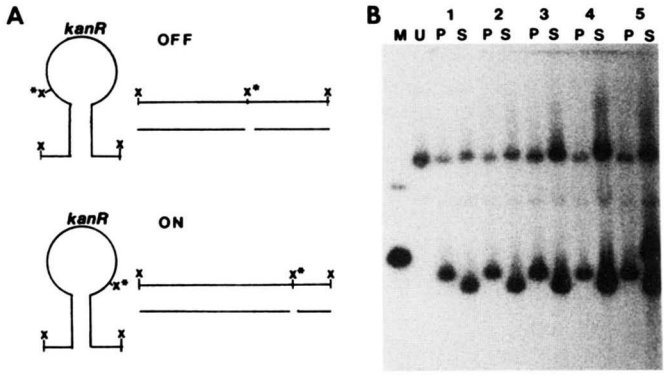

FIGURE 2.—Southern analysis of G418 resistant segregants of strain KW1. DNA was prepared from unselected and G418 resistant segregants containing a single copy of pJF2. Samples were digested with *Xho*I (×), which cuts once in pJF2, fractionated on an 0.7% agarose gel, transferred to nitrocellulose and hybridized with ^{32}P-labeled pJF2. A, Schematic illustration of the restriction patterns generated by *Xho*I digestion of DNA from the KW1 parent strain (OFF) and from a segregant carrying an inverted *kan*^{r} gene (ON). The stem-loop represents the paired inverted IS*903* repeats flanking the *kan*^{r} gene. The starred *Xho*I site (×*) changes position with respect to the flanking *Xho*I sites in the inverted ON orientation. The unlabeled lines to the right of the stem-loop drawings represent the *Xho*I bands shown in the autoradiogram in part B. B, Autoradiogram of a representative Southern blot. All samples were digested with *Xho*I. Lane M contains pJF2 DNA. Lane U, untransformed 270-32B. The upper band hybridizes to the yeast chromosome *III* fragment. The fainter lower band hybridizes to *URA*3. Lanes marked P, unselected KW1 segregants. Lanes marked S, 1–4, G418 resistant segregants containing inverted *kan*^{r} genes. A slight shift up of the top band and a shift down of the bottom band are evident in comparison with the unselected segregants. Lane 5S, G418 resistant segregant that has undergone unequal sister chromatid exchange between the direct repeats flanking pJF2. A *Xho*I fragment which comigrates with linear pJF2 is detected. The position of the bottom band indicates that one copy of *kan*^{r} is inverted.

Characterization of events leading to G418 resistance: As described above, most of the G418 resistant segregants were the result of a crossover in the inverted IS*903* sequences. Since all the products of the repeat recombination are recovered in such an event this construction would be useful for further studies on the association of reciprocal exchange and gene conversion in intrachromosomal repeats. We therefore were concerned that this recombination should have properties similar to those described for homologous recombination.

We examined 17 independent G418 resistant segregants of KW1 by Southern analysis. Of these, 16 had a single copy *kan*^{r} gene in the inverted ON orientation. The remaining segregant had two tandem copies of the vector insert, the result of an unequal sister chromatid exchange between the flanking direct repeats of the target yeast chromosome *III* sequence. One of the *kan*^{r} genes in this duplication was inverted into the ON orientation.

Although the region of chromosome *III* where pJF2 is integrated has not been reported to have any stimulatory effects on recombination, we inserted the *kan*^{r} gene into a different region of chromosome *III*. Strain KW2 carries pJF3 at a site adjacent to the site of integration of strain KW1. The frequency of G418 resistant segregants in KW2, 4.1×10^{-6}, is not significantly different from the frequency in strain KW1, 4.3×10^{-6}. The spectrum of events leading to G418 resistance in KW2 are identical to those of KW1 (data not shown).

Yeast strains homozygous for the *rad52* mutation are defective in the repair of double strand breaks. Such strains are also deficient in reciprocal exchange and gene conversion in both mitotic and meiotic growth (GAME *et al.* 1980; JACKSON and FINK 1981). To test whether the reciprocal crossover in the IS*903* sequences requires the *RAD52* gene product strain KW2-10D, which carries the *kan*^{r} gene on chromosome *III* and the *rad52-1* allele, was examined for the segregation of G418 resistant colonies. The frequency of G418 resistant segregants was less than 5×10^{-8}, a reduction of at least 100-fold from the *RAD52* strain. Thus we conclude that the reciprocal exchange in the IS*903* sequences requires the *RAD52* gene product. The few G418 resistant segregants that were recovered from the *rad52-1* strain were examined by Southern blot analysis and found to contain inverted *kan*^{r} genes.

Recombination in yeast is greatly increased in meiosis. To determine whether the reciprocal exchange in the IS*903* sequences also showed this meiotic increase, random spore analysis was performed on diploid strain KW11. The meiotic frequency of G418 resistance was determined to be 1.4×10^{-4}, an increase of 40-fold from the mitotic rate. Most of the G418 resistant segregants are the result of a crossover in the IS*903* sequences to the ON orientation.

Reciprocal recombination in the inverted repeat IS*903* is sensitive to the *rad52-1* mutation and increases in meiosis. By these criteria the recombination resembles chromosomal homologous recombination.

Association of intrachromosomal reciprocal exchange with gene conversion: *a) Conversion in singly marked repeats.* Allelic gene conversion is associated with reciprocal recombination of flanking markers approximately 50% of the time. However, there are cases of dissociation of gene conversion from reciprocal exchange (CARPENTER 1984; JACKSON and FINK 1985; KLAR and STRATHERN 1984; KLEIN 1984; ROSSIGNOL *et al.* 1984). To determine whether the reciprocal exchanges in the IS*903* sequences occur in association with gene conversion or whether this reciprocal recombination might be mediated by a separate pathway, we introduced a heterology into one of the two identical IS*903* sequences to allow us to monitor gene conversion events associated with the reciprocal exchange. A *Mlu*I site in one of the IS*903* sequences

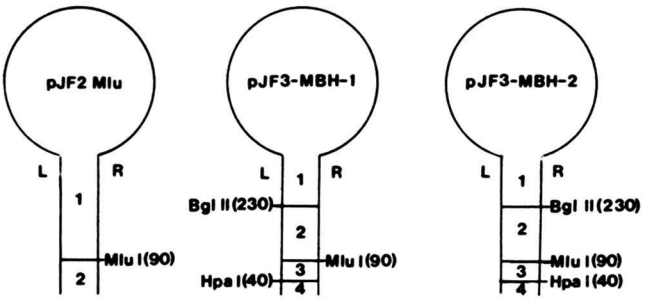

FIGURE 3.—Positions of heterologous sites in the IS*903* repeats. Unique restriction sites used to monitor conversion events between the IS*903* repeats flanking the *kan*^{r} gene are shown. The plasmids which carry the altered IS*903* sequences are indicated at the top of each stem. Strains KW5 and KW14 contain pJF2-Mlu; KW6 and KW15 contain pJF3-MBH-1; KW7 and KW18 contain pJF3-MBH-2. The number in parentheses is the exact position of each restriction site from the base of the stem which is 360 bp. L refers to the IS*903* segment nearest the 5′ end of the *kan*^{r} gene in the OFF orientation, R refers to the IS*903* segment at the 3′ end of *kan*^{r}. In segregants which have an inverted *kan*^{r} gene but no associated conversion event, the restriction site(s) above the crossover site will change position (from L to R or vice versa). This allows the position of the crossover to be localized to one of two regions (in pJF2 Mlu) or four regions (in pJF3-MBH-1 and 2). The regions are indicated in the center of each stem.

was destroyed by filling in the sticky ends. DNA sequencing confirmed that this was a 4-bp insertion.

The position of this mutation in the IS*903* sequence is shown in Figure 3. The *kan*^{r} gene carrying this heterology was introduced into yeast to create strain KW5. G418 resistant segregants were recovered and analyzed by Southern blots to confirm that they resulted from a crossover in the IS*903* sequences to the ON orientation. Gene conversion of the *Mlu*I site gives diagnostic patterns on Southern blots when chromosomal DNA is digested with *Mlu*I (see Figure 4). The association of reciprocal exchange with conversion was determined in both mitosis and meiosis.

Southern analysis of 56 independent G418 resistant segregants from vegetative growth showed conversion of the *Mlu*I site 32% of the time. The conversions showed parity. Analysis of 52 G418 resistant random spores showed a 23% association of gene conversion in meiosis. Again the gene conversions showed parity. The data are summarized in Table 2. They show that, at least some of the time, the reciprocal exchange events are associated with gene conversions.

If gene conversion results from repair of heteroduplex DNA, and repair to the parental genotype (restoration events) is as likely as repair to the donor genotype (conversion events), then gene conversion should be detected near the site of a reciprocal exchange approximately 50% of the time. The double-strand break repair model of gene conversion posits that every initiated event results in a successful gene conversion. Since the length of the IS*903* sequences, 360 bp, is well within the range of measured conversion tracts (FOGEL, MORTIMER and LUSNAK 1981;

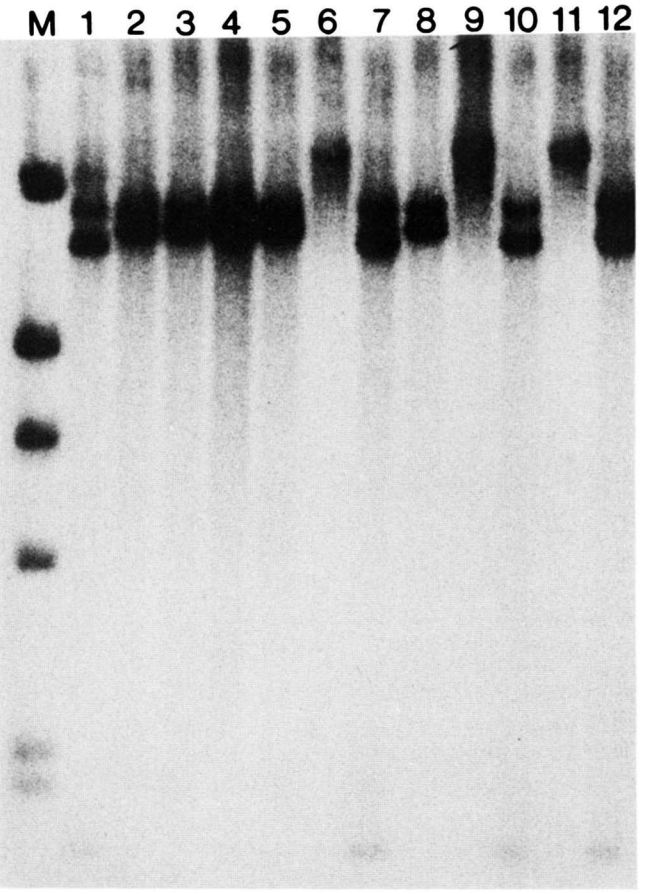

FIGURE 4.—Southern analysis of G418 resistant segregants of strain KW5. DNA samples were digested with either *Xho*I (data not shown) or *Mlu*I, fractionated on 0.5% agarose gels, transferred to nitrocellulose and hybridized with ^{32}P-labeled pJF2-Mlu. The *Xho*I blot confirmed that all G418 resistant segregants shown contain inverted *kan*^{r} genes, indicating that a reciprocal exchange between the IS*903* repeats has occurred. The *Mlu*I blot, shown above, detects conversions associated with the reciprocal exchange. Lane M contains *Hin*dIII-digested λ DNA as molecular weight markers. From top to bottom, the sizes are 23, 9.4, 6.6, 4.4, 2.3 and 2 kb. Lane 1, KW1 parent, which contains an *Mlu*I site in each IS*903* repeat. Three bands are detected: a 1.52-kb fragment containing the *kan*^{r} gene and two large fragments containing the flanking pJF2 Mlu sequences. Lane 2, KW5 parent, which contains one *Mlu*I site in the R-IS*903* (see Figure 3). Lanes 6, 9 and 11, KW5 convertants which contain no *Mlu*I sites in the IS*903* repeats. Lanes 7, 10 and 12, KW5 convertants which contain two *Mlu*I sites. Lanes 3, 4, 5 and 8, KW5 segregants in which the *Mlu*I site remained unconverted. The crossovers that inverted the *kan*^{r} gene occurred above the *Mlu*I site as indicated by a restriction pattern that is indistinguishable from the uninverted parent (lane 2).

GOLIN and ESPOSITO 1984) we expected that most, if not all, of the reciprocal exchanges were the result of resolution of a gene conversion. Thus, we should see close to 50% associated conversion with a heteroduplex repair mechanism or 100% associated conversion with a gap repair mechanism of conversion. One interpretation of the data in Table 2 is that in the IS*903* sequences the conversion tracts are short, perhaps being limited in length when the conversion event reaches the edge of homology of the repeat. To

TABLE 2

Recombination events between IS*903* repeats in strain KW5

	Reciprocal exchange without conversion[a]		Reciprocal exchange with conversion[b]		Reciprocal exchange and unequal sister chromatid exchange
	Region 1	Region 2	+*Mlu*I site	−*Mlu*I site	
Mitotic	38	0	10	8	0
Meiotic[c]	37	2	6	6	1

[a] The positions of regions 1 and 2 are shown in Figure 3.

[b] Conversion is detected by Southern analysis by the presence of 2 (+*Mlu*I site) or no (−*Mlu*I site) *Mlu*I sites in the IS*903* repeats. See Figure 4 for an example.

[c] Random spore analysis using KW14 diploid. For details see MATERIALS AND METHODS.

determine the length of the conversion tracts and to get a more accurate measurement of the number of crossovers that were associated with conversion we introduced additional heterologies into the IS*903* sequences.

b) Conversion in multiply marked repeats. Strains KW6 and KW7 were constructed to permit a detailed study of conversion tract lengths and conversion frequencies associated with the crossover in the IS*903* inverted repeats. Two additional heterologies, one on each side of the *Mlu*I site, were introduced by oligonucleotide directed mutagenesis (KUNKEL 1985). The mutations create a *Bgl*II site and a *Hpa*I site in one repeat of the IS*903* sequences. The positions of these sites are shown in Figure 3.

G418 resistant segregants of KW6 and KW7 were selected from vegetative and meiotic growth and analyzed by Southern blots. First we confirmed by a *Xho*I digestion that the resistant segregants had undergone a reciprocal exchange in the IS*903* sequences to the inverted ON orientation and assayed for the presence of the *Mlu*I site in each repeat of the IS*903* sequence by restriction digestion of the chromosomal DNA with *Mlu*I. Next we examined the repeats for the presence of the *Bgl*II and *Hpa*I sites by oligonucleotide hybridization to the same nitrocellulose filters used previously to show that a crossover in the IS*903* sequences had occurred. Representative autoradiograms are shown in Figure 5.

We analyzed 42 mitotic G418 resistant segregants from KW6 and 48 mitotic G418 resistant segregants from KW7. Five of the segregants from KW7 contained no detectable alteration or inversion of the *kan*^{r} gene and were not analyzed further. Forty-eight meiotic G418 resistant segregants each from KW15 and KW18 were analyzed. Two of the segregants from KW15 resulted from an unequal sister chromatid exchange between the duplicated target yeast sequences. These were not analyzed further. The remaining G418 resistant segregants from the mitotic and meiotic experiments all contained a single *kan*^{r} gene in the inverted ON orientation. They are summarized in Table 3 and Figure 6.

The association of conversion with reciprocal exchange in the IS*903* sequences is approximately 50% in both mitosis and meiosis (Table 4). The majority of the conversions involve only one of the three sites. We do not believe that this means that the average conversion tract is considerably shorter than 360 bp. Rather, as will be discussed in more detail later, it is a result of the nonrandom placement of the heterologous sites in the IS*903* sequences.

Most of the co-conversion events, 23 of 30 when the data for all the strains in both mitosis and meiosis are considered together, are best explained by mismatch repair of asymmetric heteroduplex. These events are called continuous conversion events (AHN and LIVINGSTON 1986). Seven discontinuous conversion events were observed in strain KW7. They are diagrammed in Figure 6. Segregants 1–4 can be explained by mismatch repair of symmetric heteroduplex. Mitotic gene conversion events that are best explained by a symmetric heteroduplex intermediate have been observed by others (ESPOSITO and WAGSTAFF 1981; ROMAN and FABRE 1983; AHN and LIVINGSTON 1986). Segregants 5–7 have a middle site that remains heterozygous while sites on both sides are converted. Independent repair events of asymmetric heteroduplex can explain this.

Although the mitotic (20 of 52) and meiotic (10 of 45) co-conversion frequencies (Table 3 and Figure 6) appear to be different, the difference is not statistically significant, $P > 0.1$.

Frequency of reciprocal exchange: Table 4 shows the frequency of reciprocal exchange in the IS*903* sequences. KW6 and KW7, each containing three heterologous sites, show a 5–10-fold lower rate of reciprocal exchange than KW5 which has only one heterology. This reduced rate is more pronounced in mitosis than meiosis. The reduction may be due to strain background or homology requirement for reciprocal exchange. We have consistently observed a higher rate in different spore segregants in the cross from which KW5 was derived and so believe that the reduction may be due to a requirement for a minimum length of continuous homology for a reciprocal exchange. Multiple heterozygosities have been reported to lower gene conversion frequencies, presumably by lowering the efficiency of heteroduplex formation (NICOLAS and ROSSIGNOL 1983). Multiple heterozygosities can lead to second repair-induced recombination events (R. BORTS and J. HABER, personal communication). In the IS*903* sequences this may be manifest as second reciprocal exchange events that return the *kan*^{r} gene from the inverted ON orientation to the OFF orientation and are undetected by selection on G418.

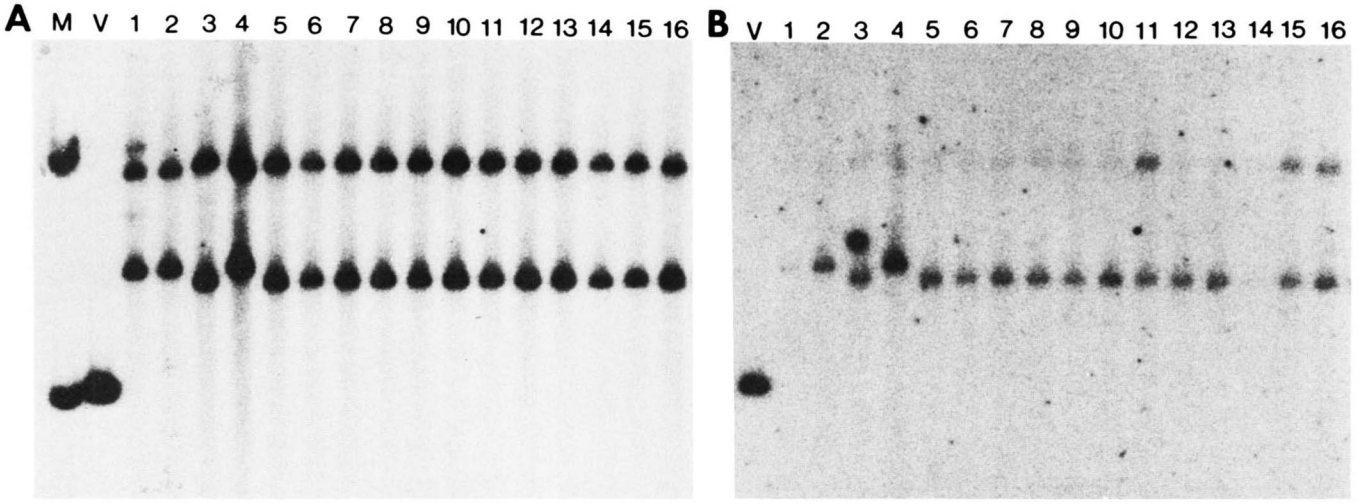

FIGURE 5.—Southern analysis of G418 resistant segregants of strain KW7. DNA samples were digested with *Xho*I, fractionated in an 0.5% agarose gel and transferred to nitrocellulose. A, Autoradiogram of a filter hybridized with ^{32}P-labeled pJF3-MBH-2. Lane M contains *Hin*dIII-digested λ DNA as molecular weight markers. The 23- and 9.4-kb fragments are shown. Lane V contains linear pJF3-MBH-2 DNA which is 10.1 kb in length. Lane 1, KW2 parent. Lane 2, KW7 parent. Lanes 3–16, G418 resistant segregants of strain KW7. All except lane 4 contain inverted *kan*^{r} genes. B, The filter shown in panel A, was stripped and rehybridized to a ^{32}P-labeled oligonucleotide which contains the *Hpa*I site present in the R-IS*903*. Lanes 11, 15 and 16, KW7 convertants which contain two *Hpa*I sites. Lane 14, KW7 convertant which contains no *Hpa*I sites. Lanes 3, 5–10, 12 and 13, KW7 segregants in which the *Hpa*I site remained unconverted. The crossovers that inverted the *kan*^{r} genes occurred above the *Hpa*I site as there was no change in migration of any of the restriction bands of the segregants relative to the uninverted parent (lane 2).

TABLE 3

Single site and continuous conversions associated with reciprocal exchange between IS*903* repeats in strains KW6 and KW7[a,b]

	*Hpa*I site		*Mlu*I site		*Bgl*II site		*Hpa*I + *Mlu*I		*Mlu*I + *Bgl*II		*Hpa*I + *Mlu*I + *Bgl*II		No conversion	Total
	L	R	L	R	L	R	L	R	L	R	L	R		
KW6 mitotic	0	1 (2)	1	0 (2)	3	11 (33)	0	0 (0)	0	4 (10)	2	2 (10)	18 (43)	42
KW15 meiotic	2	0 (4)	0	0 (0)	4	14 (39)	0	1 (2)	0	1 (2)	0	3 (7)	21 (46)	46
KW7 mitotic	0	0 (0)	0	1 (3)	9	6 (42)	0	1 (3)	2	0 (5)	2	0 (5)	15 (42)	36
KW18 meiotic	0	0 (0)	0	0 (0)	5	10 (32)	1	0 (2)	1	1 (4)	1	1 (4)	28 (58)	48
														172

[a] L and R indicate the direction of conversion. In L events the sequence present in the left copy of IS*903* in the parent is now found in both IS*903* repeats. In R events the sequence of the parental right copy is found in both IS*903* repeats.
[b] Numbers in parentheses indicate the percent of total events in each class.

Distribution of recombination events: The position of the crossover events is nonrandomly distributed (Tables 2 and 5). In strain KW5 all of the mitotic events that involved a crossover that was not associated with a conversion occurred in region 1 (see Figure 3). Region 1 is 75% of the IS*903* length. Of the meiotic crossovers that were not associated with a conversion, 95% occurred in region 1. When the position of the crossover in the multiply marked IS*903* is considered, most of these events are in region 1 of the IS*903* sequence (see Figure 3) between the *Bgl*II site and the loop encoding the *kan*^{r} gene (Table 5). If the crossovers associated with conversion and the crossovers not associated with conversion represent equal probability of mismatch repair of a heteroduplex to the restored or converted genotype (HASTINGS 1984), then preferential resolution of the recombination intermediate in region 1 suggests that many of the events proceed unidirectionally along the IS*903* sequence toward the loop region.

The fact that the *Hpa*I site, which is only 40 bp from the end of the IS*903* sequence, is involved in 19% of the conversion events shows that conversions can result with only a very short paired region on one side. We propose that these events are initiating in the 40-bp region and that heteroduplex formation proceeds along the IS*903* sequence toward the loop region. Although the minimum length of perfect homology required for reciprocal exchange has not been measured in yeast, in *E. coli* it is 23–90 bp (SHEN and HUANG 1986).

Direction of the conversion events: Conversion events in yeast normally show parity (FOGEL, MORTIMER and LUSNAK 1981). In the experiments reported here the *Mlu*I heterology shows parity of conversion (see Table 2), but the *Bgl*II heterology in the IS*903*

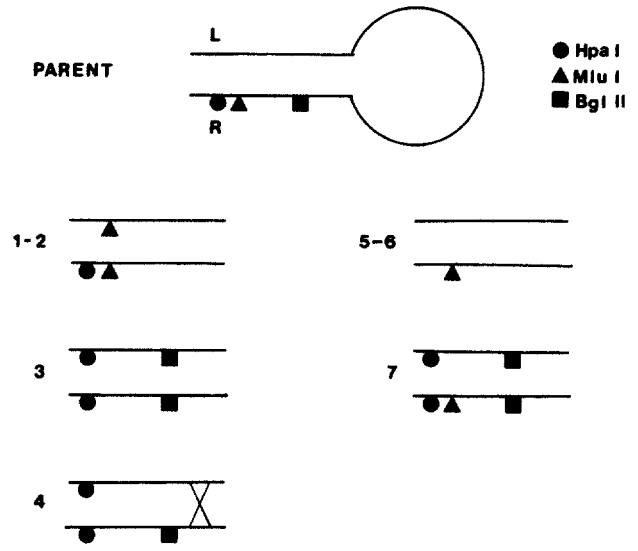

FIGURE 6.—Discontinuous conversion events associated with reciprocal exchange between the IS*903* repeats in strain KW7. The IS*903* repeats of convertants 1–7 are shown in the same orientation (relative to flanking markers) as the parent. Convertants 1–4 have undergone conversions in both directions. One site (or two) has received information from L-IS*903* and another has received information from R-IS*903*. The simplest explanation for these events is mismatch repair of symmetric heteroduplex. The unconverted *Bgl*II site in 4 allows the position of the crossover to be determined. Convertants 5–7 have undergone conversions of both the *Hpa*I and *Bgl*II sites, while the *Mlu*I site between these sites remained unconverted. This pattern would be expected from independent mismatch repair on both strands of an asymmetric heteroduplex.

TABLE 4

Summary of recombination of events in strains with heterologous IS*903* repeats

	Reciprocal exchange frequency[a,b]	Associated conversion (%)
KW5 mitotic	1.4×10^{-6}	32
KW14 meiotic	7.0×10^{-5}	23
KW6 mitotic	5.0×10^{-7}	58
KW15 meiotic	2.3×10^{-5}	54
KW7 mitotic	1.5×10^{-7}	65[c]
KW18 meiotic	5.7×10^{-5}	42

[a] Mitotic reciprocal exchange frequencies given are the median frequencies of G418 resistance from five independent colonies for each strain. The reciprocal exchange rates, calculated using the median method of LEA and COULSON (1948) are: KW5, 2.6×10^{-7}; KW6, 5.9×10^{-8} and KW7, 1.9×10^{-8}.

[b] Meiotic reciprocal exchange frequencies are the proportion of total spores on YPD that are resistant to G418.

[c] Includes both continuous and discontinuous conversion events.

sequence shows disparity of conversion in both mitosis and meiosis in strain KW6 and KW15 (see Table 3). The ratio of conversion events where both IS*903* sequences have the *Bgl*II site to conversions events where the site is lost is 5:17 or 0.29 in KW6. Meiotic G418 resistant segregants of KW15 have a conversion ratio of 4:18 or 0.22. These are significant at $P < 0.05$. No significant disparity was observed in KW7 and KW18. KW6 and KW15 differ from KW7 and KW18 in the placement of the *Bgl*II site with respect to the *Mlu*I site in the IS*903* sequence (see Figure 3). This suggests that the context of a site can be important in determining the direction of a conversion event.

DISCUSSION

We have used a system that allows a selection of intrachromosomal reciprocal exchange events to determine whether these crossovers are associated with a gene conversion event. Using a repeat that is marked at three sites with heterozygous restriction enzyme markers we have found that mitotic reciprocal exchange in an inverted repeat has a 60% association with conversion. In meiosis the association is 50%. Similar observations on homologous meiotic crossovers have been made by R. BORTS and J. HABER (personal communication). These data are consistent with a heteroduplex mismatch repair mechanism of gene conversion (MESELSON and RADDING 1975). They can also be explained by a double strand break repair model (SZOSTAK *et al.* 1983) if the assumption is made that in the inverted repeat IS*903* half of the gaps that initiate recombination are so small as not to cover any of the heterozygous markers. However, we believe this not to be the case because, as we argue below, the average conversion tracts are close to the length of the IS*903* sequence. The most important point, however, is that the data reported here are most easily interpreted as a mechanistic linkage of reciprocal exchange with gene conversion and do not provide any evidence for an independent pathway of reciprocal exchange.

The recombination events that we have observed in the IS*903* sequences appear to initiate randomly. To make this statement we assume that once the recombination event is initiated, it proceeds unidirectionally. The preferential position of the crossovers (Tables 2 and 5) suggest that this is the case. Most of the crossovers are resolved in the region near the loop (see Figure 3). Thus, if all conversions of the *Hpa*I site represent initiation events in region 4 (see Figure 3), 19% of the conversions initiated in this region. Region 4 is 40 bp of 230 bp or 17% of the IS*903* sequence that could give a conversion by our model. We take 230 bp (total length of IS*903* minus region 1) as the region where detectable conversion events can occur. Events that initiate in region 1 will not give a conversion as there are no sites to monitor conversion above region 1. Similarly, 12% of the conversions initiated in region 3 which is 22% of the physical length and 69% of the conversions initiated in region 2 which is 61% of the physical length of the IS*903* sequence that can give a conversion. Similar results are obtained when the mitotic and meiotic data are considered separately.

With a random site of initiation but a nonrandom

TABLE 5

Positions of crossovers in IS*903*[a]

	Unassociated with conversion				Region[b]					
					Associated with conversion					
	1	2	3	4	1	1 or 2	2 or 3	1, 2 or 3	2, 3 or 4	3 or 4
KW6 mitotic	14	4	0	0	0	14	1	4	0	1
KW15 meiotic	18	2	0	1	0	18	0	1	1	2
KW7 mitotic[c]	11	3	1	0	1	18	1	4	1	0
KW18 meiotic	21	5	1	1	0	15	0	2	1	0

[a] In conversion events involving all three heterologous sites the position of the crossover could not be determined. These are not included in this table.
[b] Regions are diagrammed in Figure 3.
[c] Includes both continuous and discontinuous conversion events.

site of resolution of the recombination most of the conversion events will look like single site conversions, as indeed we have found. The *Bgl*II site is involved in 69% of the single site conversions in the multiply marked IS*903* sequence (Table 3). With random initiation but a unidirectional extension of the recombination intermediate, most of the conversions cover an average length of 135 bp (the start of region 2 is 270 bp from the base of the loop as shown in Figure 3).

It is not clear why the direction of migration of the heteroduplex or other recombination intermediate is unidirectional. This may reflect a topological constraint for unwinding in a stem loop structure. It should be pointed out that this is different from polarity of gene conversion since we should be able to detect conversion events that spread in both directions from an initiation site. If, indeed, one says that the data presented here show polarity of conversion, then most of the crossovers must occur at the high conversion (*Bgl*II) side.

AHN and LIVINGSTON (1986) have measured mitotic conversion tracts in a repeated sequence carried on a plasmid. They found the average conversion length to be 0.5 kb. R. BORTS and J. HABER (personal communication) have estimated meiotic conversion tracts to be 1.5 kb ± 1.2 kb. The length of sequence over which we are monitoring gene conversion (360 bp) is smaller. Thus many of the events that initiate in regions 3 and 4 should be co-conversion events. Examination of Table 3 shows that this is the case. In fact most events continue into region 1. *Mlu*I + *Bgl*II co-conversions and *Hpa*I + *Mlu*I + *Bgl*II co-conversions are more frequent than *Hpa*I and *Mlu*I single site conversions.

We have observed instances of events that we interpret as repair of symmetric heteroduplex. These events were only noted in mitosis and only in one strain, KW7. Why strain KW6, which carries the same heterozygous sites, does not show this is unclear. However, the absence of symmetric heteroduplex formation in the KW6 IS*903* sequence may be related to the disparity of conversion of the *Bgl*II site which is not seen in KW7.

We have observed that recombination in the IS*903* sequences is similar to homologous recombination in several features. The mitotic recombination events are two to three orders of magnitude less frequent than the meiotic events. Although the mitotic strains are haploid, intrachromosomal recombination still occurs at a significant frequency. In fact, we have observed that the mitotic frequency of G418 resistance is tenfold higher in haploid strains than in diploid strains with one copy of the *kan*^{r} gene. Suppression of meiotic intrachromosomal recombination by a homologous chromosome has been noted (JACKSON and FINK 1985; WAGSTAFF *et al.* 1985).

In spite of the fact that two copies of the *kan*^{r} gene in the OFF orientation lead to G418 resistance and the presence of direct repeats of the target yeast chromosomal sequence of 3–6 kb that flank the *kan*^{r} gene, we have found few examples of unequal sister chromatid exchange. We do not believe that there is any selective growth disadvantage for such duplications based on our initial transformations in which we recovered strains with multiple tandem integrated copies of the vector. Most of the unequal sister chromatid exchange events that we recovered were accompanied by inversion of at least one copy of the *kan*^{r} gene. Whether this reflects a true concerted event or a selection bias is not clear at this time.

Intrachromosomal gene conversion has not been found to show a strong association with reciprocal exchange (JACKSON and FINK 1981; KLEIN and PETES 1981; KLEIN 1984). These experiments imposed no selection on the length of the conversion event and the resolution of the conversion intermediate. The events reported in this paper are selected as reciprocal exchanges. When a subset of the interactions between repeats, the reciprocal exchanges, are examined, 50% are associated with conversion.

The frequency of reciprocal exchange in this study is 10- to 100-fold lower than reported intrachromo-

somal gene conversion events. This may be related to the short length of the repeated sequence used in these experiments as compared to other systems. We have observed that a 2.5-fold increase in the length of the repeat sequence leads to a 10-fold increase in crossovers in mitosis and a 20-fold increase in meiosis (K. K. WILLIS and H. L. KLEIN, unpublished observations). A more important factor could be the ratio of conversion events to reciprocal exchanges. In recent experiments we have found that the unselected meiotic conversion frequency in the IS*903* sequences is approximately 100-fold higher than the reciprocal exchange frequency (K. K. WILLIS and H. L. KLEIN, unpublished observations). This means that most interactions between repeats are conversions that are resolved as noncrossover. A small percentage of the interactions are reciprocal exchanges and these are associated with conversion. These observations point to one major recombination activity between repeated sequences located on one chromosome that has its origin as a gene conversion event.

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