Meiotic Crossing Over Between Nonhomologous Chromosomes Affects Chromosome Segregation in Yeast

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Manuscript received August 27, 1996
Accepted for publication January 29, 1997

ABSTRACT

Meiotic recombination between artificial repeats positioned on nonhomologous chromosomes occurs efficiently in the yeast Saccharomyces cerevisiae. Both gene conversion and crossover events have been observed, with crossovers yielding reciprocal translocations. In the current study, 5.5-kb *ura3* repeats positioned on chromosomes V and XV were used to examine the effect of ectopic recombination on meiotic chromosome segregation. *Ura*+ random spores were selected and gene conversion vs. crossover events were distinguished by Southern blot analysis. Approximately 15% of the crossover events between chromosomes V and XV were associated with missegregation of one of these chromosomes. The missegregation was manifest as hyperploid spores containing either both translocations plus a normal chromosome, or both normal chromosomes plus one of the translocations. In those cases where it could be analyzed, missegregation occurred at the first meiotic division. These data are discussed in terms of a model in which ectopic crossovers compete efficiently with normal allelic crossovers in directing meiotic chromosome segregation.

During gamete formation in sexually reproducing organisms, the process of meiosis reduces the diploid chromosome number and the DNA content by one-half so that subsequent gamete fusion restores the correct amount of genetic material in the zygote. In the absence of such a specialized division, the genome content would double with each generation. The meiotic reduction of genetic material is achieved when a cell undergoes one round of chromosome replication followed by two successive nuclear divisions. The first of these two meiotic divisions (meiosis I or MI) is reductional, with homologous chromosomes disjoining and segregating to opposite poles of the spindle. The second meiotic division (meiosis II or MII) is equational and is formally analogous to a mitotic division, with sister chromatids moving to opposite poles of the spindle.

A key genetic feature that distinguishes meiosis from vegetative mitotic divisions is the induction of very high levels of meiotic recombination. The meiotic recombination events occur after DNA replication but before MI, and consist of both nonreciprocal gene conversions and associated crossovers. Meiotic crossing over not only generates novel, evolutionarily important combinations of alleles along a chromosome, but it also plays a critical role during gamete formation by facilitating the proper disjunction of homologous chromosomes at MI. Stable microtubule-mediated attachment of a chromosome to one pole of the MI spindle requires that it experience force in the opposing direction, and this opposing force is provided by attachment of the recombinationally linked homologue to the opposite pole of the spindle (Nicklas 1974). Chiasmata are the cytological manifestation of crossing over between homologues and in chiasmate organisms, mutations that eliminate or reduce meiotic crossing over are associated with the random segregation of homologues at MI. Available evidence thus indicates that crossing over generally is necessary for the proper disjunction of homologous chromosomes at MI (for reviews see Baker et al. 1976; Hawley 1988), although some organisms have a back-up distributive segregation system that can disjoin non-recombinant homologues (Nilsson-Tillgren et al. 1986; Hawley et al. 1993).

A second characteristic feature of meiosis is the formation of a distinct cytological structure that is absent in mitosis: the synaptonemal complex (SC; for a review see von Wettstein et al. 1984). The SC is a tripartite, proteinaceous structure that forms between paired homologous chromosomes before the first meiotic division. Given the similar temporal occurrence of genetic recombination and SC formation before MI, it was assumed for a number of years that SC formation facilitated recombination by bringing homologous chromosomes into close register. According to this view, meiotic recombination was absolutely dependent on and occurred after SC formation.

The simplistic view of the relation between SC formation and recombination has changed dramatically in recent years, due in large part to studies done in the yeast *Saccharomyces cerevisiae* (Atcheson and Esposito 1993; Hawley and Arbel 1993). An early indication...
that the dependence of recombination on SC formation between paired homologues might not be absolute was the observation in yeast that homologous sequences positioned on nonhomologous chromosomes recombine efficiently in meiosis (ectopic recombination; Jinks-Robertson and Petes 1985, 1986; Lichter et al. 1987). The occurrence of ectopic interactions suggested either that recombination could occur in the absence of SC, or that SC could form between short regions of homology embedded in nonhomologous chromosomes. Furthermore, it was suggested that such ectopic recombination events might reflect a genome-wide homology search that is responsible for chromosome pairing and that precedes mature SC formation (Smithies and Powers 1986; Carpenter 1987). Double-strand breaks (DSBs) appear to be the initiating event for meiotic recombination (Lichten and Goldman 1995) and detailed time-course analyses have shown that these breaks occur before SC formation (Padmore et al. 1992). In addition, meiotic recombination can occur in the absence of normal SC formation in some yeast mutants (Hollingsworth and Byers 1989; Rockmill and Roeder 1990).

If the SC is not essential for meiotic recombination, then what is its precise role in meiosis? While this issue has not yet been fully resolved, there is evidence that the SC may impact on sister chromatid cohesion and hence may be important for chiasma maintenance (Magain 1990; Miyazaki and Orr-Weaver 1994). Engbrecht et al. (1991) have presented evidence that crossovers occurring in the absence of SC in yeast fail to direct the disjunction of homologous chromosomes at MI, presumably because they fail to mature into chiasmata. There is also evidence from yeast that the SC is important for the phenomenon of interference, in which a crossover inhibits the occurrence of additional crossovers in nearby genetic intervals (Sym and Roeder 1994).

While it is clear that crossovers between homologues are important for disjunction at MI, data from Drosophila females indicate that irradiation-induced meiotic crossovers ("interchanges") between nonhomologous chromosomes likewise can direct chromosome disjunction. The resulting gametes have been reported to contain only one of the two translocation products, indicating an efficient segregation of the interchange chromosomes to opposite poles of the MI spindle (Parker 1969; Parker and Williamson 1976). The cosegregation of reciprocally translocated chromosomes into the same haploid product of meiosis is frequent in yeast (Jinks-Robertson and Petes 1986; Lichten et al. 1987; Goldman and Lichten 1996), however, leading to the speculation that reciprocally recombined nonhomologous chromosomes may assort randomly with respect to one another in this organism. We report here a detailed analysis of the impact of meiotic ectopic recombination on subsequent chromosome segregation in yeast. We find that ectopic crossing over between nonhomologous chromosomes is accompanied by high levels of missegregation of the chromosomes bearing the recombination substrates. These results are discussed in terms of the relation between meiotic crossing over and chromosome disjunction at MI.

**MATERIALS AND METHODS**

**Strains, media and growth conditions:** Diploid strains were obtained by mating SJR52 (MATa his3::ura3-50 ura3-50 leu2-3,112 his4 trpl100 ade2 met8-1,107) and SJR59 (MATa/ MATa his3::ura3-50 ura3-50 leu2-3,112 his4 trpl100 ade2 met8-1,107) with haploid MAI7a spores derived from SJR59 (MATa/ MATa his3::ura3-50 ura3-50 leu2-3,112 his4 trpl100 ade2 met8-1,107) and SJR59-13b (MATa his3::ura3-50 ura3-50 CEN-LEU2 leu2-3,112 his4 trpl100 met8-1,107). The following three spore strains were used: SJR59-6b (his3::ura3-50 ura3-50 CEN-LEU2 leu2-3,112 his4 trpl100 can1-101), SJR59-11d (his3::ura3-50 ura3-50 CEN-LEU2 leu2-3,112 his4 trpl100 can1-101) and SJR59-13b (his3::ura3-50 ura3-50 CEN-LEU2 leu2-3,112 his4 trpl100 can1-101).

Diploid strains were grown vegetatively at 30°C and were sporulated at room temperature. Standard yeast media and genetic techniques were used (Sherman 1991).YPD (1% yeast extract, 2% Bacto-peptone, 2% dextrose; 2.5% Bacto-agar) was used for nonselective growth. Recombinants were selected and nutritional markers scored on synthetic complete (SC) drop-out plates, which were made by supplementing synthetic minimal medium (0.17% yeast nitrogen base without amino acids and ammonium, 0.5% NH4SO4, 2% dextrose, 2.5% agar) with all but the one relevant amino acid or base.

**Isolation and genetic characterization of Ura+ recombinants:** Diploid strains were grown to ~2 x 10^7 cells/ml in 50 ml YPA at 30°C. Cells were washed with H2O and appropriate volumes plated on SC-ura and YPD to assess the mitotic frequency of Ura+ recombinants. The remaining cells were resuspended in sporation medium at a density of ~1 x 10^7 cells/ml and incubated for 4–5 days at room temperature. For random spore analysis, spored cells were incubated in spore pretreatment buffer (0.1 M β-mercaptoethanol in 20 mM EDTA, 0.2 M Tris-HCl, pH 9) for 10 min at room temperature, followed by a 1 hr incubation in 10% (v/v) glusolase at room temperature. Following brief centrifugation, cells were resuspended in 0.1% Triton X-100. Tetrads were disrupted by vortexing in the presence of glass beads. Random spores thus prepared were plated on SC-ura to select recombinants and on YPD to determine the total number of viable spores. A given spored culture was not used for subsequent analyses unless the mitotic frequency of Ura+ recombinants was at least 10 times greater than the corresponding mitotic frequency. The frequency of Ura+ spores in all spored cultures was ~5 x 10^5.

Meiotic Ura+ recombinants were purified nonselectively on YPD and a single colony of each was patched to a master YPD plate and used for subsequent phenotypic and physical analyses. Nutritional markers were scored by replica-plating to appropriate drop-out media and mating type was assessed.
To determine if crossing over had occurred between the glass bead lysis procedure of Hoffmann and Winston (1987).

Physical characteristics of Ura" recombinants: Chromosomal DNAs were isolated from Ura" recombinants using the glass bead lysis procedure of Hoffmann and Winston (1987). To determine if crossing over had occurred between the ura3 repeats on chromosomes V and XV, DNAs were digested with EcoRI and run on a 0.5% agarose gel. Following transfer to Hybond-N (Amersham), filters were probed with a 1.2-kb restriction fragment containing the URAS locus. The presence of either a 9- and/or a 20-kb restriction fragment was diagnostic of a crossover event (see Figure 1).

In those crossover recombinants that contained EcoRI fragments diagnostic of a normal copy of chromosome V as well as the V-XV translocation (15- and 20-kb fragments, respectively; see Figure 1), the division at which missegregation occurred was determined by examining the CEN5 sequences present. The diploids from which meiotic recombinants were derived were heterozygous for an insertion of the LEU2 gene adjacent to CEN5 (Jinks-Robertson and Petes 1986). To assess the identities of centromeres, genomic DNAs were digested with BamHI and Southern blots were probed with a 1.5-kb BamHI/BglII RAD52-containing fragment (from pSR14; Jinks-Robertson and Petes 1986). A 3.7-kb CEN5-homologous fragment was diagnostic of CEN5, while a 5.9-kb fragment was diagnostic of CEN5-LEU2.

The presence of more than one copy of chromosome V and/or XV in Ura" recombinants classified as simple gene conversion events (13-kb plus 16-kb EcoRI fragments; see Figure 1) was assessed by Southern blot analysis. DNAs were digested simultaneously with BamHI and BglII and Southern blots of the BamHI/BglII digests were probed with 32P-labeled sequences specific for chromosomes V, XV, and II. Chromosomes V and XV were represented by 4.2- and 5.1-kb fragments, respectively, when probed with a 1.2-kb URA3 fragment; chromosome II was represented by a 2.1-kb fragment when probed with a 1.5-kb EcoRV fragment containing the 3' end of the LYS2 gene (see Fleg et al., 1986). Following hybridization using a mixture of the 1.2-kb URA3 and 1.5-kb LYS2 probes, the amounts of probes hybridizing to the 2.1-, 4.2-, and 5.1-kb genomic fragments were quantitated by scanning the autoradiographs with a densitometer. The hybridization signals for all three fragments within a lane were normalized to that for the 2.1-kb fragment, thus making the chromosome II-specific fragment an internal standard for all DNA samples. As a control to confirm that an altered chromosome dosage could be detected, EcoRI-digested DNA from the diploid strain SJR59, which has URA3 sequences inserted on only one copy of chromosome XV, was included on all Southern blots.

Representative Ura" recombinants containing translocation chromosomes were examined by Southern blot analysis for possible disomy of chromosomes other than V and XV. Genomic DNAs were digested with BamHI plus BglII as described above, and Southern blots containing these digest were probed with 32P-labeled DNAs specific for chromosomes II, VIII, IX, and XIII. The following DNA fragments were used as probes and detected the indicated chromosome-specific fragments: a 4.9-kb BamHI/BglII HOP1-containing fragment (from pNH241; see Hollingsworth and Byers 1989) detected a 4.9-kb fragment from chromosome IX; a 3.4-kb HindIII SPO13-containing fragment (from pSPO1319; see Wang et al., 1987) detected a 6.7-kb fragment from chromosome VIII; a 1.5-kb BamHI/BglII RAD52-containing fragment (see Schuld et al., 1983) detected a 1.5-kb BamHI/BglII fragment from chromosome XIII; and a 1.5-kb EcoRV fragment containing the 3' end of the LYS2 gene (see Fleg et al., 1986) detected a 2.1-kb fragment from chromosome II. Following hybridization using a mixture of all four probes, the intensities of bands on the Southern blots were quantitated as described above, normalizing fragments to the amount of label in the 2.1-kb chromosome II-specific fragment.

RESULTS

Strains used to assess the effect of ectopic recombination on meiotic chromosome segregation: Diploid yeast strains were constructed to determine what effect, if any, meiotic ectopic recombination has on chromosome segregation during meiosis. The diploid strains were derived by mating haploid strain SJR52 to three related haploid strains of opposite mating type (SJR59-6b, SJR59-11d and SJR59-13b; see MATERIALS AND METHODS). These strains have several features that are relevant to the present analysis. First, the strains are homozygous for the ura3-50 allele at the URA3 locus on chromosome V and are also homozygous for an insertion of the ura3-3 allele into the HIS3 locus on chromosome XV (a description of the his3::ura3-3 allele is given in Jinks-Robertson and Petes, 1986). The presence of the homozygous ura3-3 insertion at HIS3 allows physical detection of all copies of chromosomes V and XV using a URA3-specific probe. As shown in Figure 1, the ura3 alleles on chromosomes V and XV are located on 13- and 16-kb EcoRI restriction fragments, respectively. Both ura3 alleles are transcribed toward their respective centromeres so that crossing over yields monocentric reciprocal translocations.

The ura3-3 and ura3-50 alleles can recombine by either gene conversion or crossing over to produce Ura" segregants. A simple gene conversion event involving the ura3-50 and ura3-3 alleles does not alter the sizes of the parental 13- and 16-kb EcoRI fragments. In contrast, since EcoRI cuts outside of the homologous regions on chromosomes V and XV, crossing over between the heteroalleles yields novel URA3-homologous EcoRI fragments of ~9 and 20 kb (Figure 1). Given the relative positions of the mutations in the ura3-3 and ura3-50 alleles (5' and 3' ends of the gene, respectively), one would expect the URA3 allele to be on the 9-kb translocation fragment (chromosome XV) rather than on the 20-kb translocation fragment (chromosome V). In a previous study, the location of the URA3 allele was mapped in translocation-bearing spores; in eight of nine recombinants examined, the URA3 allele was linked to CEN15 and, therefore, was on the 9-kb EcoRI fragment (Jinks-Robertson and Petes 1986). The identities of the chromosomes inferred from Southern analysis were confirmed by CHEF analysis of representative genomic DNAs (data not shown).

A second important feature of the diploids used in this study is heterozygosity for a LEU2 insertion adjacent to the centromere of chromosome V. In meiotic haploid recombinants containing two copies of CEN5, Southern blot analysis can be used to determine if one or both types of centromere are present. Presence of either CEN5 only or CEN5-LEU2 only is diagnostic of nondisjunction.
A. 

![Diagram](image1)

**Figure 1.**—Crossing over between *ura3* heteroalleles produces novel restriction fragments that can be detected by Southern blot analysis. (A) Thin and thick lines correspond to chromosomes V and XV, respectively; circles indicate centromeres. The open boxes correspond to the 5.5-kb BamHI fragment that was inserted into the *HIS3* locus on chromosome XV to yield the *his3*: *ura3*-3 allele. The horizontal arrows below the boxes indicate the position and extent of the *URA3* coding sequence. * indicates the positions of the 5' and 3' mutations in the *ura3*-3 and *ura3*-50 alleles, respectively (FALCO et al. 1983). Vertical arrows above the chromosomes correspond to genomic EcoRI restriction sites flanking the *ura3* sequences; the sizes of the resulting *URA3*-homologous fragments are indicated. (B) Genomic DNAs were digested with EcoRI and probed with *URA3*-homologous sequences. Lanes 1 and 2 contain only the fragments corresponding to the V:XV and XV:XV translocations; lanes 3, 4, and 6 contain fragments diagnostic of chromosomes V and XV; lanes 7 and 8 contain fragments diagnostic of both normal plus only one of the translocation chromosomes; and lanes 9 and 10 contain fragments diagnostic of both translocations plus one of the normal chromosomes. Lane 5 contains DNA isolated from a diploid heterozygous for the reciprocal translocation.

at meiosis II, while presence of both *CEN5* and *CEN5-LEU2* is diagnostic of meiosis I missegregation.

**Ectopic crossing over is associated with missegregation of chromosomes V and XV:** Approximately 350 Ura+ meiotic recombinants derived from each of the three related diploid strains were classified individually as being the result of either gene conversion or crossing over based on Southern blot analysis of EcoRI-digested genomic DNAs. The results of the Southern analysis are summarized in Table 1. Of the 1095 Ura+ recombinants analyzed, 996 contained only the restriction fragments diagnostic of chromosomes V and XV, and thus were classified as simple gene conversion events. The remaining 99 Ura+ recombinants contained at least one of the translocation chromosomes. The most common class of crossover recombinants (84 of the 99 crossover recombinants) was the reciprocal translocation class, in which both the V:XV and XV:V translocation chromosomes were present and the normal V and XV chromosomes were absent. In addition to the reciprocal translocation class of Ura+ recombinants, however, ~15% of the crossover recombinants contained restriction fragments diagnostic of three different chromosomes. These aberrant segregants contained either both the V:XV and XV:V translocations plus a normal copy of chromosome V or XV, or they contained a normal copy of both V and XV plus only one of the two possible translocations (see Figure 1). Such hyperploid segregants were noted in earlier studies (JINKS-ROBERTSON and PETES 1986; LICHTEN et al. 1987), but were not pursued further.

The numbers and types of aberrant segregants recovered are summarized in Table 2. In principle there are four classes of recombinants, each containing three different chromosomes: class 1 contains chromosomes V, XV and XV:V; class 2 contains chromosomes V, XV and V:XV; class 3 contains chromosomes V, V:XV and XV:V; and class 4 contains chromosomes XV, V:XV and XV:V. The first two classes contain a normal copy of chromosome V, a normal copy of chromosome XV and one of the two translocation chromosomes (either V:XV or XV:V). Classes 3 and 4 each contain both translocation chromosomes (V:XV and XV:V) plus either a normal copy of chromosome V or a normal copy of chromosome XV. Classes 1 and 4 correspond to missegregation of *CEN15*-linked sequences while classes 2 and 3 correspond to missegregation of *CEN5*-linked sequences. It is important to note that seven class 1 and eight class 5 recombinants were detected, but no examples of class 2 or class 4 recombinants were obtained (see DISCUSSION). Also, no spores were identified that contained all four possible chromosomes. **Aberrant segregation occurs at MI:** For the class 3 aberrant segregants in which *CEN5* sequences failed to segregate properly, it was possible to determine at which meiotic division missegregation occurred. As noted above, the starting diploid strains were heterozygous for an insertion of *LEU2* sequences adjacent to *CEN5*. If missegregation occurred at MI, then the normal chromosome V and the V:XV translocation would have been derived from homologues rather than from sister chromatids. One, therefore, should see restriction fragments diagnostic of both *CEN5* and *CEN5-LEU2*. If,
TABLE 1

Results of Southern analysis of Ura+ meiotic recombinants

<table>
<thead>
<tr>
<th>Diploid</th>
<th>Total Ura+ analyzed</th>
<th>Gene conversion</th>
<th>Crossing over</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJR52 × SJR59-6b</td>
<td>385</td>
<td>350</td>
<td>35</td>
</tr>
<tr>
<td>SJR52 × SJR59-11d</td>
<td>314</td>
<td>285</td>
<td>29</td>
</tr>
<tr>
<td>SJR52 × SJR59-13b</td>
<td>396</td>
<td>361</td>
<td>35</td>
</tr>
<tr>
<td>Totals</td>
<td>1095</td>
<td>996 (91%)</td>
<td>99 (9%)</td>
</tr>
</tbody>
</table>

on the other hand, nondisjunction of CEN5 sequences occurred at MI, then chromosomes V and V:VX would be sisters and their centromeres should be identical. All eight of the class 3 recombinants contained both CEN5 and CEN5-LEU2 sequences (data not shown), indicating exclusively MI missegregation. Since there are no known differences in the CEN15 sequences in the starting diploids, it was not possible to determine at which meiotic division missegregation occurred in the class 1 recombinants.

Ectopic gene conversion is not strongly associated with chromosome missegregation: Since ectopic crossing over between chromosomes V and VX is accompanied by a high level of missegregation for these chromosomes, it was important to determine whether the resulting aneuploidy was unique to the crossover class of recombinants or if it was a characteristic of gene conversion events as well. Missegregation associated with ectopic gene conversion would result in Ura+ spores being disomic for either chromosome V or VX. Possible disomy for these chromosomes was assessed by quantitative Southern blot analysis (Figure 2A). Briefly, the amounts of a URA3 probe hybridizing to a chromosome V-specific and a chromosome VX-specific restriction fragment were quantitated relative to the amount of a LYS2 probe hybridizing to a chromosome II-specific fragment. One case of apparent disomy for chromosome V was found in 101 Ura+ gene convertants analyzed. While this may indicate an association between ectopic gene conversion and chromosome missegregation, the association is clearly much weaker than that observed in the crossover class of recombinants (1/101 vs. 15/99; χ² = 13.67, P < 0.001). Although double crossovers would be expected to be very rare, it is possible that the aneuploid recombinant classified as a gene conversion event may have been the result of a double crossover.

Failure to detect missegregation of chromosomes other than V and VX in the aberrant segregants: The above data indicate that ectopic crossing over interferes with the segregation of chromosomes V and VX. To assess whether missegregation is limited to the chromosomes involved in ectopic events, or if it is a more general phenomenon affecting all chromosomes, the relative dosages of genes on four other yeast chromosomes were examined by quantitative Southern blot analysis. Chromosomes II, VIII, IX and XIII were detected using probes from the LYS2, SPO13, HOP1 and RAD52 genes, respectively. Analysis of 22 translocation-bearing Ura+ recombinants failed to detect altered dosage of any of the four additional chromosomes examined (Figure 2B). If it is assumed that the 7.5% missegregation observed for chromosomes V and VX individually (15% total missegregation for these two chromosomes; see above) represents the average missegregation for each of the four other chromosomes examined, then one would have expected to observe 6.6 examples of missegregation among the 88 additional chromosomes examined. Chi-square analysis indicates that the frequency of missegregation differs significantly for those chromosomes participating in ectopic crossing over vs. those chromosomes not involved in ectopic interactions (χ² = 7.1; P < 0.05). CHEF analysis of aberrant segregants also failed to detect additional chromosome imbalances as judged by the intensity of ethidium bromide staining (data not shown). We conclude that missegregation is specifically associated with ectopic crossing over and does not reflect a more general problem with chromo-

TABLE 2

Aberrant Ura+ translocation-bearing segregants

<table>
<thead>
<tr>
<th>Class</th>
<th>Chromosomes present</th>
<th>No. of recombinants</th>
<th>Missegregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V + VX + VX:V</td>
<td>7</td>
<td>CEN15 (division not known)</td>
</tr>
<tr>
<td>2</td>
<td>V + VX + V:VX</td>
<td>0</td>
<td>CEN5</td>
</tr>
<tr>
<td>3</td>
<td>V + V:VX + VX:V</td>
<td>8</td>
<td>CEN5 (8/8 MI)</td>
</tr>
<tr>
<td>4</td>
<td>VX + V:VX + VX:V</td>
<td>0</td>
<td>CEN15</td>
</tr>
</tbody>
</table>

ªChromosomes present were assessed by Southern blot analysis of EcoRI-digested genomic DNAs probed with URA3-specific sequences.

ªThe meiotic division at which missegregation of CEN5-containing chromosomes occurred was determined using a CEN5-linked LEU2 polymorphism. See text for details.
some metabolism in those cells experiencing ectopic events.

DISCUSSION

Diploid yeast strains containing ura3 alleles on chromosomes V and XV were used to study the effect of ectopic recombination on meiotic chromosome segregation. Meiotic recombination between the heteroalleles was detected by selecting for Ura+ random spores, and each recombination event was classified as either a gene conversion or a crossover by Southern blot analysis (Figure 1). Given the relative orientations of the mutations in the ura3 alleles used in this study, one would predict that the URa3 allele should reside on the XV:V translocation chromosome. Approximately 10% of 1095 Ura+ spores analyzed contained the XV:V translocation either with or without the reciprocal V: XV translocation, indicating meiotic crossing over between the ura3 alleles. Of the 99 crossover recombinants isolated, 85% contained only the restriction fragments diagnostic of the V:XV and XV:V translocations. Recombinants in this reciprocal translocation class were genetically balanced and contained a haploid complement of genes that normally reside on chromosomes V and XV. The remaining 15% of the crossover recombinants were hyperploid as a result of meiotic chromosome missegregation. Given that the frequency of meiotic missegregation for an average yeast chromosome is in the range of 10^{-2}–10^{-4} (Goldway et al. 1993; Sora et al. 1982), the amount of missegregation associated with ectopic crossing over is extraordinary. An examination of simple gene conversion events revealed that missegregation is associated specifically with the crossover class of recombinants, indicating that it is not simply a consequence of an inappropriate meiotic interaction between nonhomologous chromosomes. Additional analysis also indicated that there is a specific problem with the segregation of the chromosomes containing the ectopic substrates rather than a more general problem with chromosome segregation in those cells experiencing ectopic exchange.

The genetically unbalanced, hyperploid crossover recombinants contained either a normal chromosome in addition to both the V: XV and XV: V reciprocal translocations, or contained normal copies of both chromosomes V and XV and only one of the two translocation chromosomes. While, in principle, four classes of Ura+ unbalanced recombinants are possible, only two of these four classes were detected: seven recombinants in the V + XV + XV:V class and eight recombinants in the V + V: XV + XV:V class (Table 2). Failure to detect the V + XV + V: XV class was not surprising because, as noted above, the wild-type URa3 allele is expected to reside on the XV:V chromosome rather than on the V: XV chromosome. While the reason for the absence of the V + V: XV + XV: V class is not readily apparent, we suggest below that this anomaly can be explained by competition between allelic and ectopic crossovers for directing chromosome segregation. Alternatively, the XV + V: XV + XV: V imbalance may be associated with inviability or with poor germination/growth.

In the case of the eight recombinants in the V + V: XV + V:V class, analysis of a GEN5-linked marker demonstrated that missegregation occurred at MI. There are two types of MI missegregation that can occur: homologue nondisjunction and precocious sister chromatid segregation (PSS). With homologue nondisjunction, the chromosome V homologues (one of which has the translocation-bearing chromatid) would segregate to the same pole at MI and then segregation of sister chromatids would occur normally at MII. With PSS, one homologue would segregate to one pole of
the MI spindle while the sisters comprising the other homologue would undergo a MI-like equational division and segregate to opposite poles. In MI the centromere-associated sisters would segregate equationally while the free nonsister chromatid would segregate randomly. Although it is straightforward to distinguish homologue nondisjunction from PSS when using artificial chromosomes (Dawson et al. 1986; Sears et al. 1992), it is not possible to do so with the system used here.

The purpose of the present study was to determine what impact, if any, meiotic crossing over between nonhomologous chromosomes has on subsequent chromosome segregation. Our data can be considered in the context of two general models relating ectopic recombination to meiotic segregation: (1) ectopic crossovers have no impact on segregation and (2) ectopic crossovers are equivalent to and thus compete with allelic crossovers in directing meiotic segregation. The first model assumes that ectopic crossovers do not develop into stably maintained chiasmata and hence are completely ineffective in directing meiotic chromosome segregation. According to this model, homologues would disjoin normally at MI and the segregation of the translocation-bearing chromatids with respect to each other would appear random. As illustrated in Figure 3A, the reciprocal translocation products would be expected to cosegregate into the same meiotic product one-quarter of the time. While this model predicts the formation of the predominant, reciprocal translocation class of spores, it cannot account for the high frequency of missegregation associated with ectopic crossing over between nonhomologous chromosomes.

Whereas the first model assumes that ectopic crossovers have no effect on meiotic chromosome segregation, the second model assumes that they are functionally equivalent to allelic crossovers, resulting in the formation of multivalents instead of the normal bivalents. The cytological behavior of multivalents in translocation heterozygotes has been comprehensively reviewed by Rickards (1983), and the discussion that follows relies heavily on these cytological observations. It is important to note, however, that whereas the cytological studies examined the meiotic consequences of preformed chromosome rearrangements, the studies reported here examine the impact on meiotic chromosome behavior of the process that actually generates the rearrangement.

If, in addition to the ectopic crossover between chromosomes V and XV, an allelic crossover occurs between both the V homologues and the XV homologues, then all four chromosomes would be recombinationally linked and thus would be expected to form a chain quadrivalent similar to those that arise in translocation heterozygotes. Formation of an alternate (1/1/1/1) chain quadrivalent is depicted in Figure 3B, where chromosomes V and XV are numbered 1-4. The ectopic crossover targets the nonhomologous chromosomes 2 and 3 to disjoin while allelic crossing over between the homologues 1/2 and 3/4 causes 1 to disjoin from 2 and 3 to disjoin from 4. The net result would be segregation of 1 and 3 to one pole and segregation of 2 and 4 to the other pole. While the exclusive occurrence of an alternate segregation pattern is clearly contradicted by our results (it neither accounts for the frequent cosegregation of the reciprocal translocations nor does it explain the exchange-associated missegregation), it should be noted that a 1/1/1/1 orientation is only one of several possible orientations that a chain quadrivalent can assume. As shown in Figure 3B, a 1/2/1 orientation would give rise to the observed balanced translocation class. In addition, the orientations with three centromeres directed toward one pole and one centromere toward the other pole (3/1, 1/1/2 and 2/1/1 and 1/3 orientations) would give rise to aberrant segregations of the types observed. Although cytological data from a variety of organisms indicate that it is not possible to make a general prediction concerning the relative frequencies of these latter types of chromosome orientations (see Rickards 1983), the simplistic assumption that 3/1, 1/1/2 and 1/3 orientations are equally likely (note that 2/1/1 would not be predicted to produce a viable Ura+ product) makes the absence of the XV + V: XV + XV:V class difficult to explain. The underrepresentation of this class can be rationalized, however, if one assumes that one of the homologous pairs frequently fails to recombine (perhaps because of interference imposed by the ectopic crossover), resulting in the formation of a trivalent and a univalent instead of a chain quadrivalent.

Figure 4 presents the predicted segregation patterns for chromosomes in trivalent/univalent configurations. All crossovers within the trivalent are assumed to be functionally equivalent and thus to compete for establishment of the first bipolar attachment. The univalent does not participate in recombination with its homologue and is assumed to segregate independently of the chromosomes in the trivalent. In Figure 4A chromosome XV is the univalent; in Figure 4B chromosome V is the univalent. While both trivalent/univalent configurations can give rise to the balanced V:XV + XV:V class, the types of hyperploid spores predicted are related to which chromosome is the univalent. When chromosome XV is the univalent, two types of Ura+ hyperploid spores could be produced: V + XV + XV:V and XV + V:XV + XV:V. Since (1) the latter hyperploid class was not observed and (2) one cannot derive the observed V + V:XV + XV:V class from this trivalent/univalent pattern, we suggest that XV is rarely the univalent. In contrast, when V is the univalent, the two hyperploid classes one would predict could arise are exactly the two hyperploid classes that we observed: the V + XV + XV:V class and the V + V:XV + XV:V class. The assumption that V is most often the univalent when a trivalent/univalent arises is, therefore, most consistent
A. Ectopic crossover does not impact on chromosome segregation

![Diagram showing chromosome segregation patterns with and without ectopic crossing over.](image)

B. Ectopic crossover plus allelic crossovers between both pairs of homologs leads to the formation of a chain quadrivalent

![Diagram showing chromosome alignment and segregation patterns with ectopic and allelic crossovers.](image)

**FIGURE 3.**—Models of the impact of ectopic crossing over on meiotic chromosome segregation. The possible segregation patterns of chromosomes V and XV are shown in both a full and abbreviated form. In the full form, black and gray lines correspond to chromosomes V and XV, respectively. Chromosomes are shown after premeiotic DNA synthesis and sister chromatids are attached at their centromeres (circles). The black boxes correspond to the ectopic *ura3* recombination substrates. In the abbreviated form, the fully white and black circles correspond to chromosomes containing structurally identical sister chromatids; the half-black circles correspond to chromosomes composed of structurally dissimilar sister chromatids (one normal and one translocation) resulting from ectopic crossing over. The possible orientations of the chromosomes on the MI spindle are indicated by the vertical arrows, along with the chromosome constitutions of resulting Ura+ segregants after completion of MII. Note that the *URA3* allele is expected to reside on the XV translocation and that, for simplicity, only MI has been illustrated. (A) Ectopic crossovers do not develop into chiasmata and, therefore, do not impact normal homologue disjunction. Segregation of the translocation chromatids will appear random. (B) Ectopic crossovers develop into chiasmata and are functionally equivalent to allelic crossovers, resulting in the formation of a chain quadrivalent at MI. All possible orientations in which either two chromosomes segregate to each pole or three chromosomes segregate to one pole and one chromosome to the other pole are illustrated.

with the data. Since chromosome XV is twice the size of chromosome V, an allelic crossover in addition to the ectopic crossover may be more likely to occur between the XV homologues than between the V homologues. Thus, from strict size considerations, one might expect V to be the univalent more often than XV.

The data reported here indicate that the process of ectopic crossing over exerts an immediate effect on MI chromosome segregation, the primary manifestation of which is a high level of MI missegregation of the chromosomes harboring the recombination substrates. While both the chain quadrivalent and trivalent/univalent alignment patterns can account for the types of hyperploid spores observed in this study, accounting for the observed ratios of spore types is more difficult. These ratios likely reflect not only the probabilities of incidental allelic crossovers but also the relative occurrence and stability of various chromosome orientations once a quadrivalent or trivalent/univalent has formed. In spite of these uncertainties, the data indicate that ectopic crossovers can compete effectively with allelic crossovers in directing segregation, and hence ectopic crossovers can be assumed to develop into functioning chiasmata.

It has been argued that only those crossovers oc-
CROSSING OVER AND SEgregation

A. V+V: XV+XV: V trivalent plus XV univalent

1. Translocations disjoin

2. Vs disjoin

B. XV+V: XV+XV: V trivalent plus V univalent

1. Translocations disjoin

2. XVs disjoin

Figure 4.—Meiosis I segregation patterns predicted from the formation of a trivalent and a univalent. Symbols are as described in Figure 3. The gray vertical arrows on either side of the univalents indicate random segregation at MI. Note that the URA3 allele is expected to reside on the XV:V translocation; those Ura' spores expected to be viable are boxed. For simplicity, only MI has been illustrated. In B1, 1/2 of the MI products resulting from the boxed MI segregation pattern will contain the XV:V translocation (along with a normal V and XV) and hence will be Ura'. In B2, only 1/4 of the MI products resulting from the boxed MI segregation pattern will contain both the XV:V translocation and the VXV translocation (along with a normal V) and hence will be Ura'. If one makes the simplifying assumption that the MI segregation patterns in B1 and B2 are equally likely, then there should be twice as many spores in the V + XV + XV:V class as there are in the V + V: XV + XV:V class. The observed 1:1 ratio (seven in the V + XV + XV:V class and eight in the V + V: XV + XV:V class) is not a statistically significant deviation from this expectation.

Curing in the context of SC develop into functional chiasmata in yeast (Engebrecth et al. 1991). Chiasma stability depends on crossover-distal sister chromatid cohesion (Maguire 1990), which in turn may be facilitated by the SC. There is no reason to suspect that ectopic recombination interferes with SC formation between the participating homologues or that normal sister chromatid cohesion might be compromised by ectopic interactions. Carpenter (1987) suggested that there may be a minimal length of homology necessary to support meiotic crossing over between ectopic substrates; if enough homology exists, then SC would form to stabilize the interaction. An interesting issue concerns the importance, if any, of SC at the actual site of an ectopic crossover event. While it is not feasible technically to address such localized SC formation, there are several interesting issues that could be addressed by simply changing the locations or sizes of the interacting repeats: (1) does the centromere proximity of an ectopic exchange influence its ability to impact chromosome segregation, (2) does the size of an ectopic substrate influence its ability to impact chromosome segregation, and (3) does an ectopic exchange interfere with the occurrence of allelic exchanges on the same chromosome?

Drosophila gametes derived from meioses with induced interchanges between nonhomologous chromosomes have been found to contain only one of the two possible translocation products (Parker and Williams 1976; Hawley 1988). In contrast, the experiments reported here and earlier (Jinks-Robertson and Petes 1986; Lichten et al. 1987) clearly demonstrate that reciprocally recombined nonhomologous chromosomes frequently cosegregate during yeast meiosis. While the identification of only half-translocations in the Drosophila experiments may seem to be at odds with the yeast data, it should be noted that the Drosophila experiments involved interactions between a compound X and a free chromosome 4 and generally were not complicated by the occurrence of additional allelic cross-
over events. In a situation where a given pair of homologues is likely to be involved in allelic as well as ectopic interactions, the relevant chromosomes may assume pairing configurations similar to those documented in translocation heterozygotes, resulting in frequent mis-segregation. Ectopic interactions during meiosis thus have the potential to be deleterious to organisms with genomes containing abundant repetitive DNA.

We acknowledge the expert technical contributions of Miyono Hendrix and Kimberly Pfafcan and the helpful comments of the members of the laboratory. A special thanks goes to Alastair Goldman for many insightful, enlightening discussions and for comments on the manuscript. This work was supported by the National Institutes of Health grant GM-38464 to S.J.R.

LITERATURE CITED


BAKER, B. S., A. T. C. CARPENTER, M. S. ESPOSITO, R. E. ESPOSITO and HENDRIX and KIMBERLY Pwrm and the helpful comments of the manuscript. This work was supported by the National Institutes of Health grant GM-38464 to SJ-R.


