Allelic and Ectopic Interactions in Recombination-Defective Yeast Strains

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ABSTRACT

Ectopic recombination in the yeast Saccharomyces cerevisiae has been investigated by examining the effects of mutations known to alter allelic recombination frequencies. A haploid yeast strain disomic for chromosome III was constructed in which allelic recombination can be monitored using leu2 heteroalleles on chromosome III and ectopic recombination can be monitored using ura3 heteroalleles on chromosomes V and II. This strain contains the spo13-1 mutation which permits haploid strains to successfully complete meiosis and which rescues many recombination-defective mutants from the associated meiotic lethality. Mutations in the genes RAD50, SPO11 and HOP1 were introduced individually into this disomic strain using transformation procedures. Mitotic and meiotic comparisons of each mutant strain with the wild-type parental strain has shown that the mutation in question has comparable effects on ectopic and allelic recombination. Similar results have been obtained using diploid strains constructed by mating MATa and MATa haploid derivatives of each of the disomic strains. These data demonstrate that ectopic and allelic recombination are affected by the same gene products and suggest that the two types of recombination are mechanistically similar. In addition, the comparison of disomic and diploid strains indicates that the presence of a chromosome pairing partner during meiosis does not affect the frequency of ectopic recombination events involving nonhomologous chromosomes.

The majority of genetic recombination events in the yeast Saccharomyces cerevisiae involve like DNA sequences at identical positions on homologous chromosomes (allelic recombination). These events occur both in mitosis and in meiosis, with meiotic rates of recombination being several orders of magnitude greater than the corresponding mitotic rates (Esposito and Wagstaff 1981). Mitotic recombination is one of several pathways important for the repair of spontaneous DNA damage and mutations affecting this process usually alter the sensitivity of yeast strains to agents that damage DNA (reviewed by Friedberg 1988). In meiosis recombination serves an essential function since crossing over between homologous chromosomes is generally required for proper chromosome disjunction at the meiosis I reductional division. Mutations which lower meiotic recombination result in high levels of aneuploidy and greatly reduced spore viability (reviewed by Orr-Weaver and Szostak 1985). Mutations in the gene SPO13 cause a bypass of the first meiotic division and, consequently, are able to rescue some mutants from the lethality associated with meiotic recombination defects (Malone and Esposito 1981; Wagstaff, Klapholz and Esposito 1982).

Numerous genes affecting allelic recombination in yeast have been described. The well-known RAD genes, for example, were originally identified by the radiation-sensitive phenotype of mutants, and many of these genes have been shown to affect mitotic and meiotic recombination (reviewed by Friedberg 1988). Mutations in some genes such as RAD52 reduce both mitotic and meiotic recombination (Malone and Esposito 1980; Malone et al. 1988; Game et al. 1980; Prakash et al. 1989), while mutations in other genes can affect recombination in the two types of cell division differently. Mutations in RAD50, for example, confer a hyperrecombination phenotype in mitosis (Malone and Esposito 1981; Malone 1983), and yet rad50 strains are totally deficient in meiotic recombination (Game et al. 1980; Malone and Esposito 1981). Genes outside the RAD groups are also known to affect recombination. The SPO11 gene, for example, was initially identified by a mutation resulting in a conditional sporulation defect (Esposito and Klapholz 1981). It was shown subsequently to be essential for the induction of meiotic recombination and yet appears to have no effect on the frequency of mitotic recombination events (Klapholz, Waddell and Esposito 1985). Hop1 is another example of a meiosis-specific gene. Hop1 mutants display reduced frequencies of interchromosomal, but not of intrachromosomal, recombination during meiosis and it has been suggested that the gene product is involved in the pairing of homologous chromosomes (Hollingsworth and Byers 1989; Hollingsworth, Goetsch and Byers 1990).

In addition to allelic interactions, recombination in
yeast can also involve like sequences at nonhomologous chromosomal locations. This type of recombination has been termed "ectopic" recombination and is thought to be evolutionarily important in the maintenance of sequence homogeneity within multigene families (Edelman and Gally 1970) and in the generation of chromosomal rearrangements. In addition, Carpenter (1987) has suggested that meiotic ectopic interactions may reflect a homology search important for homologous chromosome pairing. Ectopic recombination in yeast has been demonstrated between repeated sequences within a chromosome, between repeats on homologous chromosomes and between repeated sequences on nonhomologous chromosomes (for a review, see Petes and Hill 1988). The focus of the current study is recombination between artificially constructed repeats on nonhomologous chromosomes (heterochromosomal recombination). Heterochromosomal recombination events can, depending on the genomic positions of the interacting sequences, occur at frequencies similar to allelic recombination both in mitosis and in meiosis (Jinks-Robertson and Petes 1985, 1986; Lichten, Borts and Haber 1987; Lichten and Haber 1989). Like allelic gene conversion events, ectopic gene conversions are associated with reciprocal exchange (Jinks-Robertson and Petes 1986; Lichten, Borts and Haber 1987), resulting in reciprocal translocations between the relevant chromosomes. The association of allelic and ectopic gene conversion with crossing over indicates that the two types of recombination are mechanistically similar. In the present study we further investigate the mechanism of ectopic recombination by examining the effects of mutations in the genes RAD50, Spo11 and Hop1 on this process. These studies have utilized isogenic disomic and diploid strains, thus allowing the effects of meiotic chromosome pairing on heterochromosomal ectopic recombination also to be examined.

MATERIALS AND METHODS

**Media and growth conditions:** Yeast strains were grown and sporulated at 30°C. YPD (1% yeast extract, 2% Bactopeptone, 2% glucose, 2.5% agar) was used for nonselective growth. SD complete media (Sherman, Fink and Hicks 1982) missing one component was used to score nutritional requirements. SD-ura or SD-ura/leu strains were selected for by plating cells on medium containing 5-fluoro-orotic acid (for a review, see Chattoo et al. 1979) or a lithium acetate procedure (Ito et al. 1985). Strain SJR178 contains the radSO::hisC-URA3-hisG null allele and was constructed by L. Campeau. This plasmid was made by inserting the BamHI/HindIII-released hisG-URA3-hisG cassette from pNK51 at the BamHI site of the HOP1 coding sequence on plasmid pNH341. pNH341 was obtained from N. Hollingsworth and has a 5.2-kb BglII HOP1-containing fragment inserted into the BglII site of pUC7 (see Hollingsworth and Byers 1989). Plasmid pSR154 contains a Sp011 disruption and was constructed in three steps. First, the BamHI/HindIII hisG-URA3-hisG fragment from pNK51 was cloned into the BamHI site of pUC7 to give plasmid pSR159. Next a 2.7-kb HindIII fragment from plasmid pSO11-11 containing the SPO11 gene was cloned into the HindIII site of a modified pUC9 vector in which the EcoRI site had been deleted (plasmid pSR134) to give plasmid pSR138. pSO11-11 was obtained from R. E. Esposito and contains the 2.7-kb HindIII SPO11 fragment inserted into YCp50 (see Atcheson et al. 1987). Finally, the hisG-URA3-hisG cassette was excised from plasmid pSR132 by digestion with EcoRI and was inserted into a unique EcoRI site within the SPO11 gene on plasmid pSR138 to yield plasmid pSR154.

Plasmid pSR123 contains a lys2 gene disrupted with the ura3::NcoI allele (lys2::ura3::NcoI) and was constructed in this laboratory by L. Campeau. This plasmid was made by inserting a 5.5-kg BamHI fragment containing ura3::NcoI (from pSR93) into a unique BglII site within the LYS2 gene on plasmid pDP6 (Fleig, Primore and Philippson 1986) so that the LYS2 and URA3 sequences are transcribed in the same direction. Plasmid pSR91 contains a 5.5-kb BamHI URA3* fragment inserted into the BamHI site of pUC7. Plasmid pSR93 was derived from pSR91 by filling-in a unique NsiI site in the URA3 coding sequence with the Klenow fragment of DNA polymerase, creating an NsiI site.

**Strain constructions:** A complete list of the yeast strains used in this study is given in Table 1. Yeast transformations were performed using either a spheroplast procedure (Sherman, Fink and Hicks 1982) or a lithium acetate procedure (Ito et al. 1985). Strain SJR157 is a spo11-1 ura3-50 haploid strain that is disomic for chromosome III and was derived from strains JW168-7B and SJR156. Strain SJR178 contains the lys2::ura3::NcoI allele and was constructed by cotransformation of strain SJR157 with the replicating plasmid YEp24 (Boetsemen et al. 1979) and a DNA fragment from plasmid pSR123. Cells containing YEp24 were identified by selecting for Ura+ transformants; those incorporating the unselected linear fragment containing the lys2::ura3::NcoI allele were identified as Lys− colonies on a-amino-oacidic medium (Chattoo et al. 1979). Lys− transformants that had spontaneously lost YEp24 were selected by plating cells on medium containing 5-fluoro-oorotic acid.

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**TABLE 1 Yeast strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>JW168-7B</td>
<td>Chromosome III disome. MATa/MATa his4::hisG-331/ his4A-25-leu2-1-leu2-27 cry1/Cry1; spo13-1+ads2</td>
</tr>
<tr>
<td>SJR156</td>
<td>MATa ura3-50 his3Δ1 can1-101</td>
</tr>
<tr>
<td>SJR157</td>
<td>Chromosome III disome. MATa/MATa leu2-1/leu2-27 ura3-50 spo13-1 can1-101 his3ΔA: hisG</td>
</tr>
<tr>
<td>SJR178</td>
<td>MATa/MATa leu2-1/leu2-27, ura3-50 spo13-1 can1-101 lys2::ura3::NcoI his3Δ1 hisG</td>
</tr>
<tr>
<td>SJR185</td>
<td>Same as SJR178 but rad50::his5G</td>
</tr>
<tr>
<td>SJR187</td>
<td>Same as SJR178 but hop1::his5G</td>
</tr>
<tr>
<td>SJR188</td>
<td>Same as SJR178 but spo11::his5G</td>
</tr>
</tbody>
</table>
structed in order to study the mitotic and meiotic effects of mutations in the genes.

Some subsequent construction of markers on chromosomes pSRl39 and pSR154, respectively. Following initial selection of Ura⁺ transformants, excision of the URA3⁺ gene from the disruption cassette by recombination between the flanking hisG direct repeats was detected by plating colonies on 5-FOA medium. The presence of the presumptive disruption in each strain was confirmed by Southern blot analysis (SOUTHERN 1975). At least two independently derived isolates of each disruption strain were used in the meiotic and mitotic experiments.

**Mitotic experiments:** Single colonies were inoculated into 5 ml of YPD and grown to approximately 2 x 10⁷ cells/ml. The cells were washed with H₂O and resuspended in 1 ml H₂O, vortexed vigorously, and plated at appropriate dilutions selectively on SD-leucine (SD-leu) and SD-ura and nonselectively on YPD plates. The recombination rate and standard deviation for each strain were calculated from the experimentally determined median number of prototrophic colonies according to LEA and COULSON (1948). The median value was determined using data from at least 10 independent cultures. For a statistical comparison of recombination levels in different strains, a Z-value was calculated by dividing the difference of the rates by the standard error of difference of the rates. Values were considered to be significantly different if P < 0.01.

**Meiotic experiments:** Single colonies were inoculated into 5 ml of YPA and were grown to approximately 10⁷ cells/ml. Cells were washed with 5 ml of H₂O and resuspended in 2 ml 2% potassium acetate. The suspensions were vortexed vigorously and plated at appropriate dilutions on SD-ura and SD-leu to determine the number of mitotically derived Ura⁺ and Leu⁺ recombinants, respectively, and on YPD to determine total cell numbers. The remaining cells were diluted to 5 ml in 2% potassium acetate and sporulated. After 4-7 days in sporulation medium, random spores were prepared using a glass bead-vortexing method (SHERMAN, FINK and HICKS 1982). Dilutions of the random spores were plated selectively on SD-leu and SD-ura, and nonselectively on YPD plates. Colonies were counted after 5 days.

**Isolation of haploid derivatives of disomic strains:** Mat-competent derivatives of the control, rad50, spo11 and hop1 disomic strains were isolated following treatment of exponentially growing cells with 100 µg/ml of the microtubule polymerization inhibitor methyl 2-benzimidazole carbamate (MBC; obtained from the Yeast Genetic Stock Center; WOOD 1982). In all cases, presumptive haploids were identified genetically by their ability to mate with and thus complement auxotrophic markers in tester haploid strains.

**RESULTS**

**Strain constructions:** Strain SJR178 was constructed in order to study the mitotic and meiotic effects of mutations in the genes RAD50, SPO11 and HOP1 on both allelic and ectopic recombination. The relevant features of this strain are illustrated in Figure 1. SJR178 is a haploid strain which is disomic for chromosome III; one copy of chromosome III contains the MATa and leu2-27 alleles while the other copy contains MATα information and the leu2-1 allele. Heterozygosity at the MAT locus is required for cells to initiate the meiotic cycle; presence of the leu2 heteroalleles allows allelic recombination events to be monitored by measuring the frequency of Leu⁺ prototrophic colonies. Strain SJR178 also contains ura3 heteroalleles which can be used to monitor ectopic heterochromosomal recombination by measuring the frequency of Ura⁺ prototrophic colonies. The ura3-50 allele is at the URA3 locus on chromosome V and the ura3ΔNcoI allele is at the LYS2 locus on chromosome II. Each ura3 gene is transcribed towards its respective centromere. Finally, SJR178 contains the spo13-1 mutation which results in a bypass of the meiosis 1 reductional division after induction of meiotic recombination (MALONE and ESPOSITO 1981; WAGSTAFF, KLAPHOLZ and ESPOSITO 1982). This bypass rescues some strains defective in meiotic recombination from the lethality associated with the random disjunction of homologous chromosomes at meiosis I and, in addition, allows haploid strains to successfully complete meiosis and form viable haploid spores.

**Figure 1.**—Genetic features of strain SJR178.
Three isogenic derivatives of strain SJR178 were constructed using transformation procedures in order to assess the effects of recombination defects on both allelic and ectopic recombination (see MATERIALS AND METHODS for details of strain constructions and characterizations). SJR185, SJR187 and SJR188 contain disruptions of the RAD50, HOPl and SPO11 genes, respectively, with bacterial hisG sequences. The RAD50, HOPl and SPO11 genes are on chromosomes XIV, IX and VIII, respectively (MORTIMER et al. 1989). Similar attempts to construct a rad52 disomic strain consistently yielded strains which were near-diploid with respect to chromosome number. This spontaneous diploidization was probably due to the high frequency of chromosome loss reported previously in rad32/rad52 diploid strains (MORTIMER, CONTOPoulos and SCHILD 1981). In addition to the ploidy problem, one of the leu2 heteroalleles used to monitor allelic recombination exhibited a greatly increased reversion frequency which precluded productive recombination measurements in the rad52 near-diploid strains (data not shown).

There is evidence that, at least in the case of meiotic intrachromosomal recombination, absence of a chromosomal pairing partner may have a profound effect on recombination frequencies (WAGSTAFF et al. 1985). Since the chromosomes involved in the ectopic interactions in the disomic strains described above do not have a homologue for meiotic pairing, the validity of results obtained with these strains was questionable. We therefore constructed diploid strains isogenic to the control and each of the mutant disomic strains (see MATERIALS AND METHODS). The diploid strains have allowed us to examine the effect of chromosome pairing on ectopic recombination events involving nonhomologous chromosomes and to determine the effect of repeat copy number on the frequency of ectopic interactions.

Mitotic recombination in mutant strains: The mitotic frequencies of Leu+ and Ura+ prototrophic colonies representing allelic and ectopic recombination events, respectively, were measured in at least ten independent cultures for the control disomic strain SJR178 and each of the isogenic mutant strains. Recombination rates were then calculated using the experimentally determined median frequency (LEA and COULSON 1948) and are given in Table 2. Recombination rates for allelic and ectopic interactions in the isogenic diploid strains were similarly determined and are also presented in Table 2. With the exception of the rad50 strains (see below), the results obtained in the disomic and diploid strains were comparable. While this result was expected for allelic recombination, it was somewhat surprising that the increase in the dosage of the ura3 genes from two in the disomes to four in the diploids did not cause a concomitant increase in the ectopic recombination rates. One possible explanation for the lack of a dosage effect is that DNA damage in the diploid strain is more likely to be recombinationally repaired using information from the homologous chromosome rather than the nonhomologous chromosome. Such a preference would presumably reflect the extent of absolute sequence homology.

Rad50 mutations are known to confer a hyperrecombination phenotype in mitosis (MALONE and ESPOSITO 1981; MALONE 1983). While the rad50::hisG mutation caused a 6.6-fold increase in the allelic recombination rate between the leu2 heteroalleles in the disomic strain, the ectopic recombination rate between the ura3 heteroalleles remained at control levels. The allelic but not the ectopic recombination rate in the rad50 diploid was the same as that in the disome. In fact, the rad50 diploid was the only diploid in which there was a significant gene dosage effect on ectopic recombination.

Previous studies have demonstrated that the genes SPO11 and HOPl are meiosis-specific and have no effect on mitotic recombination (KLAPHOLZ, WADDELL and ESPOSITO 1985; HOLLINGSWORTH and BYERS 1989). In the current study, disruption of the HOPl gene had no statistically significant effect on the rate of allelic or ectopic recombination in the disomic or diploid strain. Allelic recombination was reduced slightly in the diploid spo11 strain (0.01 < P < 0.05) but not in the disomic strain. Disruption of SPO11 had no effect on ectopic recombination in either the disomic or diploid strain.

Effects of recombination defects on meiotic recombination: Previous studies have demonstrated that spo13 mutations rescue rad50, spo11 and hop1 strains from the meiotic lethality associated with reduced recombination (MALONE and ESPOSITO 1981; KLAPHOLZ, WADDELL and ESPOSITO 1985; HOLLINGSWORTH and BYERS 1989). To confirm this in our strains, spore viability was measured by dissecting dyads from the control disomic and diploid strains and from each of the mutant strains. Spore viability was comparable (70–80%) in all strains (data not shown). Allelic and ectopic meiotic recombination frequencies for the disomic and diploid strains are presented in Table 3 as mean frequencies of prototrophic spores. The means were derived from at least four independent experiments per strain and in each experiment, the mitotic frequency of prototrophs in the culture prior to sporulation was also measured. While the mean mitotic frequencies are also given in Table 3, it should be emphasized that these values exhibited considerable fluctuation and are much less accurate than the rates given in Table 2 that were calculated using median frequencies. They are nevertheless included since they provide an indication of
Allelic and Ectopic Recombination

**TABLE 2**
Mitotic recombination rates

<table>
<thead>
<tr>
<th>Assay</th>
<th>Disome (X 10⁻²)</th>
<th>Diploid (X 10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allelic (Leu⁺)</td>
<td>Ectopic (Ura⁺)</td>
</tr>
<tr>
<td></td>
<td>3.1 ± 0.5 (1.0)</td>
<td>2.6 ± 0.4 (1.0)</td>
</tr>
<tr>
<td>Control</td>
<td>17 18</td>
<td>1.1</td>
</tr>
<tr>
<td>rad50</td>
<td>3.6 2.9 0.6</td>
<td>3.5 3.4</td>
</tr>
<tr>
<td>spo11</td>
<td>2.9 26 9.0</td>
<td>3.1 15</td>
</tr>
<tr>
<td>hop1</td>
<td>4.9 2400 490</td>
<td>2.4 250</td>
</tr>
<tr>
<td></td>
<td>21.0 19.0 0.9</td>
<td>10.7 11.3</td>
</tr>
<tr>
<td></td>
<td>2.6 2.5 1.0</td>
<td>3.1 3.2</td>
</tr>
<tr>
<td></td>
<td>3.0 32.6 10.8</td>
<td>1.9 37.2</td>
</tr>
</tbody>
</table>

Values given are the recombination rate ± the standard deviation calculated according to LEA and COUSON (1948). Numbers in parentheses are the rate normalized to those in the appropriate control strain.

*P < 0.01.

**TABLE 3**
Induction of meiotic recombination

<table>
<thead>
<tr>
<th>Assay</th>
<th>Allelic (Leu⁺ X 10⁻⁶)</th>
<th>Ectopic (Ura⁺ X 10⁻⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mitotic Meiotic Inductiona</td>
<td>Mitotic Meiotic Inductiona</td>
</tr>
<tr>
<td>A. Disomes</td>
<td>1.5 1100 730</td>
<td>4.6 250</td>
</tr>
<tr>
<td>Control</td>
<td>17 18</td>
<td>1.1</td>
</tr>
<tr>
<td>rad50</td>
<td>3.6 2.9 0.6</td>
<td>3.5 3.4</td>
</tr>
<tr>
<td>spo11</td>
<td>2.9 26 9.0</td>
<td>3.1 15</td>
</tr>
<tr>
<td>hop1</td>
<td>4.9 2400 490</td>
<td>2.4 250</td>
</tr>
<tr>
<td>B. Diploids</td>
<td>21.0 19.0 0.9</td>
<td>10.7 11.3</td>
</tr>
<tr>
<td>Control</td>
<td>2.6 2.5 1.0</td>
<td>3.1 3.2</td>
</tr>
<tr>
<td>rad50</td>
<td>3.0 32.6 10.8</td>
<td>1.9 37.2</td>
</tr>
</tbody>
</table>

*a Induction is the ratio of the meiotic frequency to the mitotic frequency.

the level of induction of meiotic recombination.

Both allelic and ectopic recombination rates were greatly elevated in meiosis relative to mitosis in the control disomic strain SJR178 (Table 3A). As expected, disruption of the SPO11 and RAD50 genes eliminated the induction of allelic meiotic recombination. The induction of ectopic recombination in meiosis was similarly abolished in the spo11 and rad50 strains. In the hop1 disomic strain, allelic meiotic recombination was greatly reduced relative to the control strain, but there was still a residual 10-fold induction. The same was true of ectopic recombination in this strain. A meiotic effect of the hop1 mutation on ectopic recombination was not expected since the gene product is thought to be involved in the pairing of homologous chromosomes (HOLLINGSWORTH and BYERS 1989).

The allelic and ectopic meiotic results obtained with the diploid strains (Table 3B) are essentially the same as those obtained with the disomic strains, although there is a slight dosage effect evident if one compares ectopic recombination in the hop1 disomic and diploid strains. The similarity in the ectopic results obtained with the disomic and diploid strains indicates that chromosomal pairing does not inhibit meiotic interactions between nonhomologous chromosomes.

**DISCUSSION**

The RAD50 gene has a pleiotropic role in yeast, affecting both mitotic recombination/repair and meiotic recombination. Rad50 mutants exhibit increased sensitivity to X-ray damage (FRIEDBERG 1988), have increased levels of spontaneous mitotic recombination (MALONE and ESPOSITO 1981; MALONE 1983), are completely deficient in the induction of allelic meiotic recombination (GAME et al. 1980; MALONE and ESPOSITO 1981) and do not develop a typical tripartite meiotic synaptonemal complex (ALANI, PADMORE and KLECKNER 1990). Like allelic interchromosomal recombination, intrachromosomal recombination in rad50 mutants is elevated in mitosis and greatly reduced in meiosis (GOTTLIEB, WAGSTAFF and ESPOSITO 1989). The RAD50 gene has been sequenced (ALANI, SUBBIAH and KLECKNER 1989) and although its precise function is not known, it has recently been suggested that the encoded protein is involved in a chromosomal homology search necessary for mitotic DNA repair, meiotic recombination, and chromosome pairing (ALANI, PADMORE and KLECKNER 1990). In both the disomic and diploid strains examined in this study, disruption of RAD50 completely abolished both allelic and ectopic recombination in meiosis. In agreement with previous observations, we found a mitotic stimulation of allelic recombination in the disomic and diploid strains. A similar mitotic elevation of ectopic recombination was observed in the diploid strain but not in the disomic strain. While the discrepancy could be indicative of a mechanistic difference in allelic and ectopic recombination in the disomic strains, we do not believe that such a conclusion is warranted. MALONE and ESPOSITO (1981) have reported differences in the degree of stimulation of allelic recombination by rad50-1, with elevation values ranging from 1.5-fold to 6.1-fold for different pairs of heteroalleles. The difference seen in the present study between allelic and ectopic recombination in the disomic strains may well be an artifact of the particular alleles employed. It should be noted that the only strains exhibiting an ectopic gene dosage effect in comparisons of the disomes and diploids were
the rad50 strains; this observation may be relevant to
the function of the RAD50 protein. It would be inter-
esting to examine whether mitotic recombination be-
tween naturally occurring repeats such as Ty exhibits
a similar dosage dependency in rad50 strains.

The spo11 gene is meiosis-specific and has no effect
on mitotic recombination. Spo11 mutations com-
pletely abolish the induction of meiotic recombination
between homologues (Klapolz, Wadell and Es-
posito 1985) and reduce intrachromosomal recom-
bination (Wagstaff et al. 1985). In the mitotic ex-
periments reported here, neither allelic nor ectopic
recombination is affected by spo11 in the disomic
strain; there is a slight reduction of allelic but not of
ectopic recombination in the diploid. Meiotic induc-
tion of both types of recombination is completely
abolished in the spo11 disomic and diploid strains.

HOP1, like spo11, is a meiosis-specific gene; hop1
mutations reduce the level of recombination between
homologous chromosomes, but have no effect on in-
trachromosomal recombination involving direct re-
peats (Hollingsworth and Byers 1989). The syn-
aptic complex (SC) is absent in hop1 mutants and
the protein localizes to paired chromosomes during
meiosis (Hollingsworth, Goetsch and Byers
1990). Based on these observations, it has been sug-
gested that hop1 is involved in the meiotic pairing of
homologous chromosomes, possibly as a component
of the SC. Hollingsworth and Byers (1989) pre-
dicted that ectopic recombination, like intrachromo-
osomal recombination, might be hop1-independent.
In our experiments with the hop1 disomic and diploid
strains, this is clearly not the case. If the hop1 gene
product is a component of the SC, then the SC, or
some precursor of the SC, must be important for
ectopic recombination. Since hop1 affects allelic and
ectopic recombination similarly, it may be that it is
important for the homology search/check hypothe-
sized by Carpenter (1987; see below) to precede
formation of extended SC. It should be noted that
mutations in the RED1 gene are phenotypically similar
to those in hop1. In meiosis, red1 strains lack exten-
sive SC and are somewhat deficient in both allelic and
ectopic recombination (Rockmill and Roeder
1990).

Although the studies done to date reveal no clear
differences between allelic and ectopic recombination,
differences may yet be found. It has been proposed
that two rounds of recombination may occur in
meiosis (Carpenter 1987). The first round is inde-
pendent of chromosome pairing per se and would
produce all of ectopic recombination and a portion of
the allelic recombination. This round of recombi-
nation would be of the gene conversion type only and
would be involved in determining whether an inter-
action is acceptable (extended sequence homology
between homologous chromosomes) or unacceptable
(by limited sequence homology between nonhomol-
ogous chromosomes). An acceptable interaction
would result in the the zippering up of the SC to bring
chromosomes completely into register. Another
round of recombination, dependent upon the com-
plete alignment of the homologs, would contribute to
the remainder of the allelic recombination, including
all reciprocal exchanges. If this scenario is correct, it
should be possible to isolate mutations that differen-
tially affect allelic and ectopic recombination. It
should be noted that meiotic ectopic interactions be-
tween nonhomologous chromosomes in yeast fre-
frequently result in reciprocal translocations (Jinks-
Robertson and Petes 1986; Lichter, Borts and Haber
1987). We are currently examining whether the res-
olution of an interaction as a crossover in yeast is
dependent upon the absolute length of the interacting
sequences as the above model would predict.

Wagstaff et al. (1985) reported that the frequency
of intrachromosomal recombination involving direct
repeats was elevated approximately 10-fold in a hap-
loid meiosis system relative to the meiotic frequencies
observed in a standard diploid system (Jackson
and Fink 1985). They proposed that the difference in
the haploid and diploid meiotic results could be explained
by competition between inter- and intrachromosomal
events for some limiting component of the recombi-
nation machinery. The presence of a pairing partner
in diploid strains would thus result in an apparent
suppression of intrachromosomal interactions. In the
experiments reported here, the levels of meiotic het-
brochromosomal recombination were indistinguishable
in the disomic and diploid strains. Thus, in contrast
to what has been found for intrachromosomal recom-
bination, the presence of a potential chromosomal
pairing partner has no effect on ectopic recombination
events involving nonhomologous chromosomes. Al-
though our results with heterochromosomal recom-
bination are difficult to reconcile with the intrachro-
mosomal observations, it should be noted that intrachro-
mosomal recombination has been shown by
mutational analysis to be different in some respects
from normal allelic recombination (Aguilera
and Klein 1988). The apparent lack of competition be-
tween hetero- and interchromosomal recombination
reported here is consistent with the hypothesis that
ectopic interactions reflect a homology search that is
necessary for and precedes the end-to-end synopsis of
homologous chromosomes.

In summary, we have found that allelic and ectopic
recombination events respond similarly to mutations
in the genes RAD50, spo11 and hop1. It should be
noted that similarities are found in a mutant defective
in recombinational DNA repair (rad50 mutant) as well
as in mutants that are defective only in meiotic recom-
bination (spo11 and hop1 mutants). Combined with
the earlier findings that ectopic and allelic recombination are induced in meiosis and that both ectopic and allelic gene conversion events are associated with reciprocal exchanges (Jinks-Robertson and Petes 1986; Lichter, Borts and Haber 1987), the current data support the hypothesis that the two types of recombination are mechanistically similar and are promoted by similar gene functions.

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LITERATURE CITED


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