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 *MAT***a** and *MATα* 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.* 1980), 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°. YPD (1% yeast extract, 2% Bacto-peptone, 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 markers. SD-uracil (SD-ura), for example, is missing uracil. For sporulation, cells were grown in YPA (1% yeast extract, 2% Bacto-peptone, 1% potassium acetate) and sporulated in 2% potassium acetate supplemented with required amino acids. Plasmid-containing *Eschericha coli* strains were grown in LB (1% yeast extract, 0.5% Bacto-tryptone, 1% NaCl) supplemented with 50 μg/ml ampicillin.

Plasmid constructions: Plasmid pNKY51 (ALANI, CAO and KLECKNER 1987) was the source of the *hisG-URA3-hisG* cassette used to disrupt the relevant yeast genes. The plasmid pNKY83 contains the *rad50::hisG-URA3-hisG* null allele and was obtained from N. KLECKNER (ALANI, SUBBIAH and KLECKNER 1989). Plasmid pSR139 contains a disruption of the *HOP1* gene and was constructed by inserting the *Bam*HI/*Bgl*II-released *hisG-URA3-hisG* cassette from pNKY51 at the *Bam*HI site within the *HOP1* coding sequence on plasmid pNH34-1. pNH34-1 was obtained from N. HOLLINGSWORTH and has a 5.2-kb *Bgl*II *HOP1*-containing fragment inserted into the *Bgl*II site of pIC7 (see HOLLINGSWORTH and BYERS 1989). Plasmid pSR154 contains a *SPO11* disruption and was constructed in three steps. First, the *Bam*HI/*Bgl*II *hisG-URA3-hisG* cassette fragment from pNKY51 was cloned into the *Bam*HI site of pUC7 to give plasmid pSR132. Next a 2.7-kb *Hin*dIII fragment from plasmid pSPO11-11 containing the *SPO11* gene was cloned into the *Hin*dIII site of a modified pUC9 vector in which the *Eco*RI site had been deleted (plasmid pSR134) to give plasmid pSR138. pSPO11-11 was obtained from R. E. ESPOSITO and contains the 2.7-kb *Hin*dIII *SPO11* fragment inserted into YCp50 (see ATCHESON *et al.* 1987). Finally the *hisG-URA3-hisG* cassette was excised from plasmid pSR132 by digestion with *Eco*RI and was inserted into a unique *Eco*RI site within the *SPO11* gene on plasmid pSR138 to yield plasmid pSR154.

Plasmid pSR123 contains a *LYS2* gene disrupted with the *ura3ΔNcoI* allele (*lys2::ura3ΔNcoI*) and was constructed in this laboratory by L. CAMPEAU. This plasmid was made by inserting a 5.5-kb *Bam*HI fragment containing *ura3ΔNcoI* (from pSR93) into a unique *Bgl*II site within the *LYS2* gene on plasmid pDP6 (FLEIG, PRIDMORE and PHILIPPSEN 1986) so that the *LYS2* and *URA3* sequences are transcribed in the same direction. Plasmid pSR91 contains a 5.5-kb *Bam*HI *URA3*$^+$ fragment inserted into the *Bam*HI site of pUC7. Plasmid pSR93 was derived from pSR91 by filling-in a unique *Nco*I site in the *URA3* coding sequence with the Klenow fragment of DNA polymerase, creating an *Nsi*I 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.* 1983). Strain SJR157 is a *spo13-1 ura3-50* haploid strain that is disomic for chromosome *III* and was derived from strains JW168-7B and SJR156. Strain SJR178 contains the *lys2::ura3ΔNcoI* allele and was constructed by cotransformation of strain SJR157 with the replicating plasmid YEp24 (BOTSTEIN *et al.* 1979) and a linear DNA fragment from plasmid pSR123. Cells containing YEp24 were identified by selecting for Ura$^+$ transformants; those incorporating the unselected linear fragment containing the *lys2::ura3ΔNcoI* allele were identified as Lys$^-$ colonies on α-aminoadipate medium (CHATTOO *et al.* 1979). Lys$^-$ transformants that had spontaneously lost YEp24 were selected by plating cells on medium containing 5-fluoro-orotic acid

TABLE 1

Yeast strains

Strain	Description
JW168-7B	Chromosome III disome. *MATα/MAT***a** *his4B-331/his4A-25 leu2-1/leu2-27 cry1/CRY1; spo13-1 ade2 cyh2 lys2 tyr1 ura3; URA3* in rDNA (WAGSTAFF *et al.* 1985)
SJR156	*MAT***a** *ura3-50 his3Δ1 can1-101*
SJR157	Chromosome III disome. *MATα/MAT***a** *leu2-1/leu2-27 ura3-50 spo13-1 can1-101 his3Δ1 his4*
SJR178	*MATα/MAT***a** *leu2-1/leu2-27 ura3-50 spo13-1 can1-101 lys2::ura3ΔNcoI his3Δ1 his4*
SJR185	Same as SJR178 but *rad50::hisG*
SJR187	Same as SJR178 but *hop1::hisG*
SJR188	Same as SJR178 but *spo11::hisG*

(5-FOA; BOEKE, LACROUTE and FINK 1984). Strains SJR185 (*rad50::hisG*), SJR187 (*hop1::hisG*) and SJR188 (*spo11::hisG*) were derived from strain SJR178 by transformation with appropriate linear DNA fragments from plasmids pNKY83, pSR139 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 cells 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 2×10^7 cells/ml. The cells were washed with H_2O and resuspended in 1 ml H_2O, 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 10^7 cells/ml. Cells were washed with 5 ml of H_2O 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 the 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: Mating-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. A 2:2 segregation of the mating type alleles in crosses with tester haploids was confirmation that one copy of chromosome *III* in the disomic parent had indeed been lost. The meiotic segregations of markers on chromosomes *II* and *V* were also checked to confirm that the haploids used for subsequent construction of diploid strains contained only one copy of these chromosomes. For each strain at least two independent isolates of each mating type were used for constructing diploid 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 *MAT***a** 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 I 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.

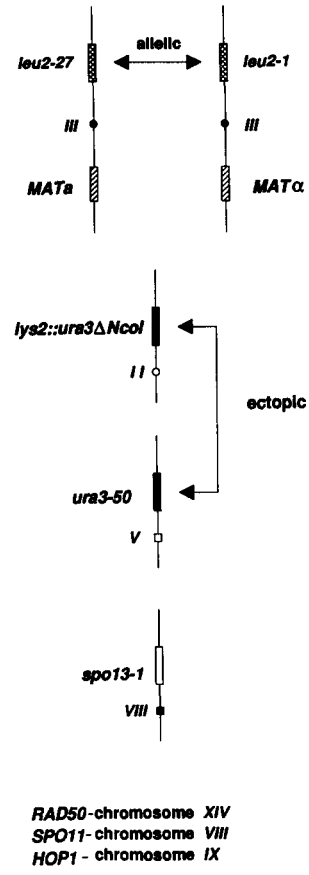

FIGURE 1.—Genetic features of strain SJR178.

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*, *HOP1* and *SPO11* genes, respectively, with bacterial *hisG* sequences. The *RAD50*, *HOP1* 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 *rad52/rad52* diploid strains (MORTIMER, CONTOPOULOU 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 *HOP1* 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 *HOP1* 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

TABLE 2

Mitotic recombination rates

Assay	Disome (× 10^7)		Diploid (× 10^7)	
	Allelic (Leu$^+$)	Ectopic (Ura$^+$)	Allelic (Leu$^+$)	Ectopic (Ura$^+$)
Control	3.1 ± 0.5 (1.0)	2.6 ± 0.4 (1.0)	3.0 ± 0.6 (1.0)	3.2 ± 0.5 (1.0)
rad50	*20.4 ± 3.0 (6.6)	2.4 ± 0.4 (1.0)	*25.4 ± 4.4 (8.4)	*9.5 ± 1.3 (2.9)
spo11	2.5 ± 0.5 (0.8)	2.4 ± 0.4 (1.0)	1.4 ± 0.2 (0.5)	2.9 ± 0.5 (0.9)
hop1	2.2 ± 0.4 (0.7)	3.0 ± 0.5 (1.2)	4.2 ± 0.8 (1.4)	3.2 ± 0.5 (1.0)

Values given are the recombination rate ± the standard deviation calculated according to LEA and COULSON (1948). Numbers in parentheses are the rate normalized to those in the appropriate control strain.
*$P < 0.01$.

TABLE 3

Induction of meiotic recombination

Assay	Allelic (Leu$^+$ × 10^6)			Ectopic (Ura$^+$ × 10^6)		
	Mitotic	Meiotic	Induction[a]	Mitotic	Meiotic	Induction[a]
A. Disomes						
Control	1.5	1100	730	4.6	250	54
rad50	17	18	1.1	5.8	5.9	1.0
spo11	3.6	2.9	0.8	3.3	3.4	1.0
hop1	2.9	26	9.0	3.1	15	4.8
B. Diploids						
Control	4.9	2400	490	2.4	250	104
rad50	21.0	19.0	0.9	10.7	11.3	1.0
spo11	2.6	2.5	1.0	3.1	3.2	1.0
hop1	3.0	32.6	10.8	1.9	37.2	19.6

[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 interesting to examine whether mitotic recombination between 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 completely abolish the induction of meiotic recombination between homologues (KLAPHOLZ, WADDELL and ESPOSITO 1985) and reduce intrachromosomal recombination (WAGSTAFF *et al.* 1985). In the mitotic experiments 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 induction 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 intrachromosomal recombination involving direct repeats (HOLLINGSWORTH and BYERS 1989). The synaptonemal 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 suggested that *HOP1* is involved in the meiotic pairing of homologous chromosomes, possibly as a component of the SC. HOLLINGSWORTH and BYERS (1989) predicted that ectopic recombination, like intrachromosomal 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 hypothesized 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 extensive 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 independent of chromosome pairing *per se* and would produce all of ectopic recombination and a portion of the allelic recombination. This round of recombination would be of the gene conversion type only and would be involved in determining whether an interaction is acceptable (extended sequence homology between homologous chromosomes) or unacceptable (only limited sequence homology between nonhomologous 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 complete 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 differentially affect allelic and ectopic recombination. It should be noted that meiotic ectopic interactions between nonhomologous chromosomes in yeast frequently result in reciprocal translocations (JINKS-ROBERTSON and PETES 1986; LICHTEN, BORTS and HABER 1987). We are currently examining whether the resolution 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 haploid 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 recombination 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 heterochromosomal recombination were indistinguishable in the disomic and diploid strains. Thus, in contrast to what has been found for intrachromosomal recombination, the presence of a potential chromosomal pairing partner has no effect on ectopic recombination events involving nonhomologous chromosomes. Although our results with heterochromosomal recombination are difficult to reconcile with the intrachromosomal observations, it should be noted that intrachromosomal 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 between 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 synapsis 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 recombination (*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; LICHTEN, 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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