RAD52-INDEPENDENT MITOTIC GENE CONVERSION IN *SACCHAROMYCES CEREVISIAE* FREQUENTLY RESULTS IN CHROMOSOMAL LOSS

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Manuscript received January 15, 1985
Revised copy accepted May 20, 1985

ABSTRACT

We have examined spontaneous, interchromosomal mitotic recombination events between *his4* alleles in both Rad$^+$ and *rad52* strains of *Saccharomyces cerevisiae*. In Rad$^+$ strains, 74% of the His$^+$ prototrophs resulted from gene conversion events without exchange of flanking markers. In diploids homozygous for the *rad52-1* mutation, the frequency of His$^+$ prototroph formation was less than 5% of the wild-type value, and more than 80% of the gene conversion events were accompanied by an exchange of flanking markers. Most of the *rad52* intragenic recombination events arose by gene conversion accompanied by an exchange of flanking markers and not by a simple reciprocal exchange between the *his4A* and *his4C* alleles. There were also profound effects on the kinds of recombinant products that were recovered. The most striking effect was that *RAD52*-independent mitotic recombination frequently results in the loss of one of the two chromosomes participating in the gene conversion event.

IN the past several years, it has become evident that mitotic recombination in *Saccharomyces cerevisiae* may occur by quite different mechanisms than are observed in meiotic cells. For example, some mutations that abolish meiotic recombination have much less effect on mitotic cells (reviewed by ESPOSITO and WAGSTAFF 1981). Of particular interest has been the *rad52-1* mutation, an X-ray-sensitive mutation that prevents cells from repairing double-strand breaks in chromosomes (RESNICK and MARTIN 1976; MALONE and ESPOSITO 1980; WEIFFENBACH and HABER 1981). In meiosis, the *rad52* mutation abolishes the recovery of viable meiotic recombinants (GAME *et al.* 1980; PRAKASH *et al.* 1980) and is defective in the formation of physically recombined DNA (BORTS *et al.* 1984). In mitotic cells, *rad52-1* does not prevent all forms of mitotic recombination but appears to be defective principally in recombination events that occur without an exchange of flanking markers. There is a general depression of both intergenic and intragenic recombination in *rad52* mitotic cells (MALONE and ESPOSITO 1980); however, neither unequal sister-chromatid exchange (ZAMB and PETES 1981; PRAKASH and TAILLON-MILLER 1981) nor

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the integration of a circular transforming plasmid by homologous recombination (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981) is impaired in *rad52-1* strains. The work of JACKSON and FINK (1981) provided a clear demonstration that *rad52-1* almost completely abolished gene conversion events that occurred without an exchange of flanking markers but had little effect on intragenic recombination accompanied by crossing over.

To examine the consequences of *rad52-1* on mitotic recombination in more detail, we have examined interchromosomal recombination events between two pairs of noncomplementing alleles of the *his4* locus. These markers were the same ones used by JACKSON and FINK (1981) in their study of intrachromosomal or unequal sister-chromatid recombination events. In JACKSON and FINK'S (1981) study, the reciprocal product generated by crossing over could not have been recovered. By examining interchromosomal recombination, we hoped to examine the fate of both participating chromatids in the recombination event. Furthermore, we could examine how interchromosomal recombination differed from intrachromosomal or unequal sister-chromatid recombination.

MATERIALS AND METHODS

Strains: The diploid strains used in these experiments are listed in Tables 1 and 3. These were constructed from haploid strains that resulted from a series of crosses involving strains 5799-4D (*MAT***a** *his4A-39, -260*) and 5965-20C (*MAT***a** *his4C-864, -1176 leu2-3*), which carry the same alleles used by JACKSON and FINK (1981). The *his4A* mutations are polar; thus, a *his4A HIS4C* strain is histidine requiring. His^+ recombinants must become *HIS4A HIS4C* (abbreviated *HIS4*). Because there are no readily scored markers distal to *his4* (MORTIMER and SCHILD 1980), we used recombinant DNA techniques to introduce dominant nutritional markers at the *HML* locus, 35 cM distal to *his4*. A *URA3*-containing insertion at *HML* was created by integrating a pBR322-*HMLα-URA3* plasmid (pJH24) at *HMLα* by transformation of strain DBY745 (*MATα ade1 leu2-3,112 ura3-52*) (S. STEWART and J. HABER, unpublished results). Strain K390 (*MATα hmlΔ::LEU2 mar1-1 leu2 his2 his3 trp1 can1 mal2*), provided by A. KLAR, contains a deletion of *HML* that was replaced by transformation with a segment containing the 2.3-kb *Sal*I-*Xho*I *LEU2* fragment (STRATHERN *et al.* 1982). Diploids of the following genotype were constructed:

$$\frac{HML\alpha::URA3}{hml\Delta::LEU2} \quad \frac{+}{his4C\text{-}864,1176} \quad \frac{his4A\text{-}39,260}{+} \quad \frac{\quad}{MAT\mathbf{a}} \quad \frac{\quad}{MAT\alpha}$$

where *HML* lies 35 cM distal to *his4C*. *his4C* is separated from *his4A* by approximately 250 base pairs (bp) of the *HIS4B*-coding region (DONAHUE, FARABAUGH and FINK 1982). *HIS4A* is 22 cM distal the centromere of chromosome *III*, whereas *MAT* lies 25 cM on the other chromosome arm (MORTIMER and SCHILD 1980).

The *rad52-1* allele was introduced by crossing haploid derivatives containing the appropriate markers with strain M298 (*MAT***a** *rad52-1 ade2-1*), provided by R. MALONE. ADZUMA, OGAWA and OGAWA (1984) have shown that the *rad52-1* allele is a missense mutation.

Genetic analysis: Cells were grown either on rich medium (YEPD) or synthetic complete plates lacking one or more amino acid or base at 30° (SHERMAN, FINK and HICKS 1982). Subcloned colonies of diploid cells were replica-plated to medium lacking histidine to select His^+ colonies. The *his4A-39,260* and *his4C-864,1176* alleles are noncomplementing double mutations that show virtually no reversion. We presume that all of the His^+ colonies arising in diploids heteroallelic for these loci arose by recombination. Only one His^+ colony was selected from each patch to ensure independence of the events. Radiation sensitivity of different strains was assayed by irradiating replica-plated colonies with a ^{60}Co γ-ray source. The rate of His^+ prototroph formation was

determined by a fluctuation test analysis of a minimum of ten independent samples (LURIA and DELBRÜCK 1943).

The linkage of other chromosome *III* markers to the His$^+$ recombinant allele and the identity of the remaining *his4* allele in the diploid were determined by tetrad analysis and subsequent complementation or allelism tests. A minimum of ten tetrads with four viable spores were analyzed to establish the linkage of *HIS4* to *HML* and *MAT*. The identity of *his4* alleles was determined both by complementation assays and from their ability to give rise to His$^+$ recombinants when crossed to Rad$^+$ tester strains. The *his4A-39,260* marker is a polar mutation that fails to complement *his4C* mutations (FINK 1966), whereas the *his4C-864,1176* mutations will complement nonpolar *his4A* mutations, such as *his4A-25*. *his4A,his4C* double mutants arising by gene conversion or recombination could be distinguished from *his4A* by crossing them to testers carrying the *his4C-864,1176* locus. The resulting diploids carrying *his4A* mutations will give rise to spontaneous or ultraviolet light-induced His$^+$ prototrophs, whereas those carrying *his4A,his4C* double mutants will not.

Diploids homozygous for *rad52* fail to sporulate; however, it was possible to determine the linkage of markers on chromosome *III* by taking advantage of the observation that *rad52* diploids spontaneously undergo loss of chromosome *III* at a frequency of 10^{-2} (MORTIMER, CONTOPOULOU and SCHILD 1982). Loss of one homologue of chromosome *III* from a nonmating *MAT***a**/*MAT*α diploid results in the formation of a monosomic derivative expressing either *MAT***a** or *MAT*α. Cells monosomic for chromosome *III* mate readily with Rad$^+$ cells of the opposite mating type so that complementation and allele testing for all markers on chromosome *III* (the various *his4* alleles as well as the *URA3* and *LEU2* insertions at *HML*) can be carried out. The validity of this approach in determining linkage of *MAT*, *his4* and *HML* alleles was demonstrated by examining linkage in several diploids whose genotype was known.

RESULTS

Analysis of intragenic HIS4 recombinants in Rad$^+$ diploids: Several related diploids were constructed with the following genotype:

$$\frac{HML\alpha::URA3}{hml\Delta::LEU2} \quad \frac{+}{his4C\text{-}864,1176} \quad \frac{his4A\text{-}39,260}{+} \quad \frac{\qquad}{MAT\alpha} \quad \frac{MAT\mathbf{a}}{MAT\alpha}$$

Approximately 360 independent colonies of diploids MH134, MH136 and MH150 were replica-plated to medium lacking histidine; from each patch one papillus was selected and purified for further testing. Diploid MH150 differed from the others in the linkage of the distal markers *URA3* and *LEU2* to the *his4A* and *his4C* alleles. In both arrangements, approximately 13% of the His$^+$ colonies were also auxotrophic for either *ura3* or *leu2* (Table 1A). The frequency of diploids homozygous for a distal marker is similar to that found for exchange events accompanying mitotic recombination at other loci (ESPOSITO 1978; ESPOSITO and WAGSTAFF 1981; ROMAN 1980). Three of the 362 His$^+$ recombinants had apparently suffered an associated chromosome loss event, as they were auxotrophic for either *ura3* or *leu2* and also expressed a single mating-type allele on the opposite side of the centromere. CAMPBELL and FOGEL (1977) and CAMPBELL, FOGEL and LUSNAK (1975) have previously noted that, in haploid strains disomic for chromosome *III*, chromosome losses accompanying recombination at *his4* occurred approximately 1% of the time. The significance of these chromosome losses will be discussed later, in view of the results obtained with *rad52-1*.

To examine the recombination events in greater detail we have analyzed

TABLE 1

HIS4 *recombinants in Rad*$^+$ *and* rad52 *diploids*

Strain	Diploid prototrophs			2n-1 prototrophs[a]	
	Leu$^+$ Ura$^+$	Leu$^+$ Ura$^-$	Leu$^-$ Ura$^+$	Leu$^+$ Ura$^-$	Leu$^-$ Ura$^+$
A. Rad$^+$ strain[b]					
MH134	58	2	6		
MH135	91	1	6		2 α
MH136	29		6		
MH150	138	15	5	1 α	
B. *rad52* strains[c]					
MH156	20	27	4	19 **a**	1 **a**
				1 α	11 α
MH160	15	32	17	23 **a**	7 α
MH163	13	15	6	13 **a**	4 **a**
				1 α	6 α

[a] The mating phenotype as well as the Leu and Ura phenotype is given for these 2n-1 strains.

[b] The genotypes of MH134, 135 and 136 were: $\frac{HML\alpha::URA3 \; + \; his4AMAT\mathbf{a}}{hm1::LEU2 \; his4C \; + \; MAT\alpha}$. The genotype of MH150 had reversed distal markers: $\frac{hm1::LEU2 \; + \; his4aMAT\mathbf{a}}{HML\alpha::URA3his4c \; + \; MAT\alpha}$. *HML*$\alpha$ is 35 cm distal to *his4C*, which is separated from *his4A* by approximately 250 bp of the *his4B*-coding region. *his4A* lies 22 cm from the centromere of chromosome *III*. The *MAT* locus is about 25 cm distal to the centromere on the opposite chromosome arm.

[c] The genotypes of MH156, 160 and 163 were: $\frac{HML\alpha::URA3 \; + \; his4AMAT\mathbf{a}}{hm1\Delta::LEU2 \; his4C \; + \; MAT\alpha} \; \frac{rad52\text{-}1}{rad52\text{-}1}$. MH160 was also homozygous for *lys2*, whereas MH163 was homozygous for *ade1*.

meiotic tetrads from 63 His$^+$ diploids. The identity of the recessive *his4* allele and the configuration of flanking markers was determined as described in MATERIALS AND METHODS. The 63 cases examined include all of the phenotypic classes found in Table 1, but classes homozygous for a distal marker are overrepresented. Not all exchange events accompanying gene conversions are detected as diploids homozygous for a distal marker. Because of the random segregation of recombined and parental chromatids (Figure 1), some exchange events will be undetected as His$^+$ Ura$^+$ Leu$^+$ diploids in which the distal markers are in nonparental configuration (reversed linkage). If most crossing over occurs during or after DNA replication (ESPOSITO 1978; ROMAN and FABRE 1983), the ratio of diploids homozygous for a distal marker and those with reversed linkage should be 1:1. Among 52 His$^+$ Ura$^+$ Leu$^+$ strains analyzed by tetrad analysis in Table 2, a total of six (11.5%) exhibited reversed linkage. Given that His$^+$ Ura$^+$ Leu$^+$ diploids represent 87% of the total set of His$^+$ prototrophs (see Table 1A), the proportion of diploids with reversed linkage is about 10% of the total. Thus, the proportion of diploids homozygous for a distal marker (12.9%) and those with reversed linkage are nearly equal; consequently, the majority of exchange events accompanying intragenic recombination must not be completed until after DNA replication.

Among diploids homozygous for one flanking marker, the more frequently recovered type was that homozygous for the marker distal of *his4A* (Table 1).

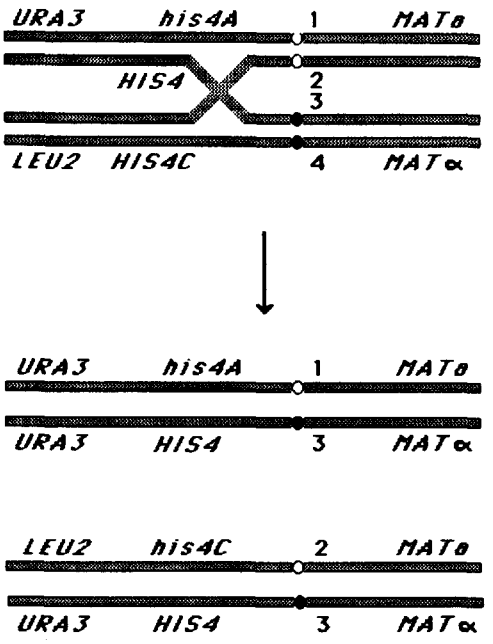

FIGURE 1.—Segregation of a chromosome carrying a His$^+$ allele. The *HIS4* allele is presumed to have arisen either by a gene conversion event with an associated exchange of flanking markers or by a simple reciprocal exchange in the region between the *his4C* and *his4A* markers. The event could have been initiated either during the G2 stage of the cell cycle or during the G1 stage, followed by DNA replication of the unresolved Holliday structure (ESPOSITO 1978). Assuming random segregation of mitotic sister centromeres, the *HIS4* allele linked to chromatid 3 should be recovered in a diploid homozygous for a distal marker or else in a diploid with nonparental configuration of flanking markers (reversed linkage).

Such homozygotes are indeed those expected from a simple reciprocal exchange between the proximal *his4A* and the distal *his4C* loci, followed by the segregation of one recombined and one parental chromatid. We cannot distinguish between simple crossovers between the alleles and gene conversion events with an associated exchange of flanking markers, because in such diploids one recovers only one of the two chromatids participating in the recombination event. However, it is worth noting that, among the small class of diploids exhibiting reversed linkage (class 2, Table 2), in which both crossover strands are recovered in the same cell, all His$^+$ recombinants appeared to have arisen by gene conversion rather than by simple crossovers. In no case did the His$^-$ chromosome carry the *his4A,his4C* double mutant expected for a simple exchange. The fact that one distal marker became homzygous more frequently than the other may reflect other aspects of the recombination event, such as allele-specific differences in mismatch repair of heteroduplex DNA or site-specific initiation of recombination.

Effect of rad52-1 *on intrachromosomal recombination between* his4 *alleles*: We conducted similar experiments selecting spontaneous His$^+$ papillae in diploids homozygous for the *rad52-1* mutation. The frequency with which His$^+$ recombinants arose was less than 5% of the frequency in Rad$^+$ strains. Fluctuation

TABLE 2

Analysis of His$^+$ recombinants in Rad$^+$ diploids

Class	Genotype			No.
A. Diploid MH134[2]				
1a	*URA3*	*HIS4*	*MAT***a**	10
	LEU2	*his4C*	*MATα*	
1b	*URA3*	*his4A*	*MAT***a**	4
	LEU2	*HIS4*	*MATα*	
1c	*URA3*	*HIS4*	(*MAT***a**)	1[b]
	LEU2	*HIS4*	(*MATα*)	
2a	*LEU2*	*his4A*	*MAT***a**	1
	URA3	*HIS4*	*MATα*	
2b	*LEU2*	*HIS4*	*MAT***a**	1
	URA3	*his4A*	*MATα*	
3a	*URA3*	*his4A*	*MAT***a**	5
	URA3	*HIS4*	*MATα*	
3b	*URA3*	*HIS4*	(*MAT***a**)	1[b]
	URA3	*HIS4*	(*MATα*)	
3c	*URA3*	*HIS4*	*MAT***a**	1
	URA3	*his4A, 4C*	*MATα*	
3d	*URA3*	*HIS4*	*MAT***a**	1
	URA3	*his4C*	*MATα*	
4a	*LEU2*	*his4A*	*MAT***a**	1
	LEU2	*HIS4*	*MATα*	
4b	*LEU2*	*his4C*	*MAT***a**	1
	LEU2	*HIS4*	*MATα*	
4c	*LEU2*	*HIS4*	*MAT***a**	1
	LEU2	*his4C*	*MATα*	
				28
B. Diploid MH150[2]				
1a	*LEU2*	*HIS4*	*MAT***a**	17
	URA3	*his4C*	*MATα*	
1b	*LEU2*	*his4A*	*MAT***a**	5
	URA3	*HIS4*	*MATα*	
2a	*URA3*	*his4A*	*MAT***a**	3
	LEU2	*HIS4*	*MATα*	
2b	*URA3*	*his4C*	*MAT***a**	1
	LEU2	*HIS4*	*MATα*	
3a	*LEU2*	*his4A*	*MAT***a**	4
	LEU2	*HIS4*	*MATα*	
3b	*LEU2*	*HIS4*	*MAT***a**	1
	LEU2	*his4A*	*MATα*	
4a	*URA3*	*his4C*	*MAT***a**	1
	URA3	*HIS4*	*MATα*	
4b	*URA3*	*HIS4*	*MAT***a**	2
	URA3	*his4A*	*MATα*	
4c	*URA3*	*HIS4*	*MAT***a**	1
	URA3	*his4C*	*MATα*	
				35

[a] The 63 His$^+$ diploids analyzed do not reflect the frequencies of different classes of events listed in Table 1.

[b] Linkage between *MAT* and *HML* was not determined.

tests based on 20 independent samples showed that the rate of His^+ prototroph formation in *rad52-1* diploids was 2.4×10^{-7} compared to a rate of 1.2×10^{-5} for Rad^+ diploids. The types of diploids recovered are listed in Table 1B. The types of His^+ diploids recovered in *rad52-1* strains are significantly different from those in Rad^+ strains (Table 1A). More than 75% of the *HIS4* cells were auxotrophic for either *ura3* or *leu2*, compared to 13% in the Rad^+ diploids. Furthermore, nearly one-third of the His^+ recombinants were apparently monosomic (2n-1) for chromosome *III*; these strains were auxotrophic either for *URA3* or *LEU2*, distal to *HIS4*, but also carried a single mating-type allele, located on the opposite side of the centromere. In Rad^+ strains, chromosome loss events accompanying gene conversion at *his4* occur rarely (Table 1A; CAMPBELL, FOGEL and LUSNAK 1975; CAMPBELL and FOGEL 1977); among *rad52* diploids they make up a major class of events. This very high frequency of chromosome loss among His^+ recombinants is not explained by the spontaneous rate of chromosome *III* loss in diploids homozygous for *rad52-1*, which is only approximately 10^{-2} (MORTIMER, CONTOPOULOU and SCHILD 1982).

We have analyzed the configuration of distal markers and *his4* alleles in these *rad52* His^+ strains (Table 3). All of the randomly selected His^+ diploids from strains MH160 and MH163 were analyzed, and the various classes are proportional to the larger sample listed in Table 1B. Although *rad52-1* strains fail to sporulate, we took advantage of rare spontaneous chromosome loss observed in *rad52* diploids to analyze the *HIS4* prototrophs. Monosomic diploids that had lost one or the other chromosome *III* homologues were used to establish linkage between the *his4* alleles and the distal markers (see MATERIALS AND METHODS).

The genotypes of His^+ diploids arising in *rad52-1* strains revealed several significant differences compared to Rad^+ recombinants: (1) more than 80% of the gene conversion events were accompanied by an exchange of flanking markers, (2) the ratio between diploids homozygous for a distal marker and those with reversed linkage was 7:1, (3) the "missing" diploids with reversed linkage were apparently replaced by a large class of 2n-1 strains monosomic for chromosome *III* and (4) there was a seven-fold decrease in the ratio between diploids homozygous for the marker distal to *his4A* and those homozygous for the marker distal to *his4C*. These data are examined in detail below.

Most rad52 *His^+ colonies have undergone an exchange of flanking markers*: Of 152 *rad52* diploids analyzed, only 24 (16%) appear to have occurred without an exchange of flanking markers (classes 1a, 5b and 5d; Table 3). Among the 54 cases in which chromosome *III* loss had apparently accompanied the formation of wild-type *HIS4* allele (class 5), only five contained the parental arrangement of flanking markers. Moreover, among *rad52* diploids homozygous for a distal marker (classes 3 and 4), in which an exchange must have accompanied the gene conversion event, 68 of the 70 His^+ diploids contained the *HIS4* protrophic allele on a chromosome with a recombined configuration of markers (the exceptions are class 4C). Thus, the vast majority of spontaneous intragenic recombinants occurring in a *rad52-1* background resulted in an

TABLE 3

Analysis of His$^+$ recombinants in a diploid homozygous for rad52

Class[a]	Genotype			No.
1a	*URA3*	*HIS4*	*MAT***a**	18
	LEU2	*his4C*	*MATα*	
1b	*URA3*	*his4A*	*MAT***a**	1
	LEU2	*HIS4*	*MATα*	
2a	*LEU2*	*his4C*	*MAT***a**	4
	URA3	*HIS4*	*MATα*	
2b	*LEU2*	*HIS4*	*MAT***a**	2
	URA3	*his4A*	*MATα*	
2c	*LEU2*	*his4A*	*MAT***a**	3
	URA3	*HIS4*	*MATα*	
3a	*LEU2*	*HIS4*	*MAT***a**	47
	LEU2	*his4C*	*MATα*	
4a	*URA3*	*HIS4*	*MATα*	1
	URA3	*his4C*	*MAT***a**	
4b	*URA3*	*his4A*	*MAT***a**	20
	URA3	*HIS4*	*MATα*	
4c	*URA3*	*HIS4*	*MAT***a**	2
	URA3	*his4C*	*MATα*	
5a	*LEU2*	*HIS4*	*MAT***a**	36
5b	*URA3*	*HIS4*	*MAT***a**	4
5c	*URA3*	*HIS4*	*MATα*	13
5d	*LEU2*	*HIS4*	*MATα*	1
				152

Data from two independent diploids, MH160 and MH163, of genotype: $\frac{HML\alpha::URA3 \; + \; his4AMAT\mathbf{a} \quad rad52\text{-}1}{hm1\Delta::LEU2\; his4C \; + \; MAT\alpha \quad rad52\text{-}1}$

[a] Various genotypic classes are equivalent to those listed in Table 2.

exchange of flanking markers on the strand that was converted to wild-type information.

rad52 *recombinants are frequently associated with chromosome loss*: It is evident that we did not find a 1:1 ratio between diploids homozygous for a distal marker and those exhibiting reversed linkage, as we had found among Rad$^+$ diploids. There were 70 diploids homozygous for a distal marker (as judged from the presence of two mating-type alleles and both a His$^+$ and a His$^-$ allele) but only nine diploids with distal markers in nonparental linkage (Table 3, classes, 2, 3, and 4). None of the reversed linkage cases contained a recessive *his4A,4C* double mutant, indicating that these intragenic recombinants were the result of gene conversion with an associated crossover rather than a simple reciprocal exchange.

The notable absence of diploids with reversed linkage seems to coincide with the appearance of a large class of monosomic strains containing only a recom-

bined chromosome *III*. Diploids with reversed linkage result from gene conversion events in which both recombined participating chromatids segregate during the mitosis to the same daughter cell. The monosomic strains could be explained as cases in which one of the two participating chromatids was unable to segregate (or replicate) and was therefore lost. Indeed, if one adds up the 49 monosomic strains with a recombined chromosome *III* (classes 5a and 5c) with the nine instances in which diploids with reversed linkage were recovered (class 2), the total (58) is similar to the number of diploids homozygous for a distal marker (70). Thus, the appearance of monosomic strains may occur at the expense of diploids that should inherit both recombined chromosomes participating in the exchange event.

Change in the nature of exchange events: Another striking feature of these data was a marked change in the proportion of His^+ recombinants homozygous for each of the distal markers. Among Rad^+ diploids, the ratio of those homozygous for the marker distal to *his4A* compared to that distal to *his4C* was 33:8 (Table 1A). In *rad52-1* diploids this ratio was nearly reversed, with only 56 diploids homozygous or hemizygous for the marker distal to *his4A* (Ura^+ Leu^-) and 131 cases homozygous or hemizygous for *LEU2*, distal to *his4C* (Table 1B). Thus, there must be some fundamental difference in the types of recombination events that were recovered in Rad^+ and *rad52* diploids. It should be pointed out that the simplest recombination event to generate a His^+ recombinant, namely, a crossover between the *his4C* and *his4A* alleles, would generate Ura^+ Leu^- homozygotes. In fact, it was the opposite class of Ura^- Leu^+ homozygotes that were more frequently recovered from *rad52* strains. These latter intragenic recombinants must have arisen from gene conversions involving mismatch repair of a heteroduplex region of DNA. Consequently, although *RAD52*-independent His^+ prototrophs are usually associated with exchange events, they are not simple intragenic crossovers; rather, they are most often gene conversions with an associated crossing over.

DISCUSSION

RAD52-independent recombination events are distinctly different from the types of recombination events found in Rad^+ strains. First, the frequency of His^+ recombinants associated with an exchange of flanking markers increased from 23% in Rad^+ strains to 84% in *rad52* diploids. This result is quite similar to that obtained by JACKSON and FINK (1981) studying intrachromosomal or sister-chromatid recombination events of the same *his4* alleles. Second, truly reciprocal exchanges of flanking markers do not occur as frequently in *rad52* strains as in Rad^+ diploids. In wild-type diploids, the number of His^+ recombinants homozygous for a distal marker and those exhibiting reversed linkage were nearly equal, as expected from the random segregation of chromatids during mitosis. In contrast, only 6% of the His^+ recombinants exhibited reversed linkage in *rad52* strains, whereas 45% were homozygous for a distal marker. This marked reduction in the expected class of reversed linkage recombinants suggests that many of the *RAD52*-independent events were not reciprocal exchanges. Third, there was a striking increase in the number of

2n-1 diploids, hemizygous for chromosome *III*, nearly all of which (49 of 54) were recombined for the flanking *HML* and *MAT* markers. The fact that *rad52*-defective strains cannot repair broken chromosomes (RESNICK and MARTIN 1976; WEIFFENBACH and HABER 1981) suggests that the mechanism of generating a His^+ prototroph in *rad52* strains may also lead to the loss of the other participating chromatid.

One further indication that *RAD52*-independent interchromosomal recombination is distinctive comes from an examination of the exchange events accompanying gene conversion. Although the *his4A* allele appears to be preferentially converted to wild type in both Rad^+ and *rad52* diploids (as determined in those cases in which no crossing over has occurred), the ratio of diploids homozygous for the marker distal to *his4A* relative to those distal to *his4C* was more than 3:1 in Rad^+ strains but less than 1:2 in *rad52* diploids. Thus, there must be some fundamental difference in either the initiation or resolution of recombination intermediate Rad^+ and *rad52* strains.

The *rad52-1* mutation did not completely eliminate gene conversion events, either with or without an exchange of flanking markers. Sixteen percent of these His^+ colonies were gene conversions without any exchange of flanking markers. Another 6% of the total were His^+ diploids with reversed linkage. This latter class might have arisen by a simple reciprocal exchange between the *his4A* and *his4C* alleles, but in all nine cases the genotypes of these diploids indicated that they had arisen by a *bona fide* gene conversion rather than a simple exchange (Table 3, class 2). Furthermore, the majority of diploids homozygous for a distal marker (Table 3, class 2) are most readily understood as gene conversion events and not as simple exchanges between the *his4* alleles. Thus, excluding the 2n-1 diploids (from which no conclusions may be drawn), the vast majority of *RAD52*-independent events must have involved the mismatch repair of heteroduplex DNA. We recognize that the *rad52-1* allele is a missense mutation (ADZUMA, OGAWA and OGAWA 1984) so that some of the events we recovered could be due to a residual, low level of *RAD52* gene product activity. However, recent gene disruption experiments (D. SCHILD, personal communication; M. RESNICK, personal communication) indicate that a null allele of *rad52* is no more radiation sensitive than the *rad52-1* allele. It remains possible that other aspects of *rad52* activity are not as deficient as radiation sensitivity.

A model for rad52*-independent mitotic recombination*: To explain the distinctive features of *RAD52*-independent recombination, we have considered the model shown in Figure 2. We show only those events in which an exchange of flanking markers accompanies the formation of a His^+ allele. Mitotic recombination is shown occurring at the G2 stage of the cell cycle; however, very similar conclusions would be drawn from mitotic recombination events initiated in G1 (ESPOSITO 1978). We assume that all mitotic recombination events observed in *rad52* diploids must be initiated by single-strand breaks (MESELSON and RADDING 1975), as double-strand breaks fail to yield viable recombinants in *rad52* strains (ORR-WEAVER, SZOSTAK and ROTHESTEIN 1981; WEIFFENBACH and HABER 1981). We propose that, although the invading DNA strand can

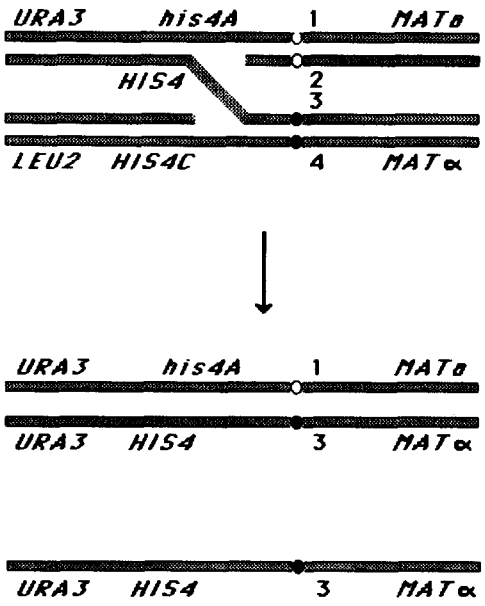

FIGURE 2.—A model accounting for *RAD52*-independent, spontaneous His$^+$ recombinants. We presume that recombination must be initiated by invasion of a single strand of DNA (MESELSON and RADDING 1975) rather than a double-strand break repair (SZOSTAK *et al.* 1983), because *rad52* mutants are unable to repair double-strand breaks. In the absence of *RAD52* gene function, a nonreciprocal structure is generated, either because *RAD52* gene function is necessary to rejoin strands of DNA that are cut during the formation of a recombinant or because *RAD52* is required to stabilize some other intermediate. The broken chromatid (2) will be extremely unstable and will be lost, so that the chromatid carrying the *HIS4* allele will be recovered either as a diploid homozygous for a distal marker or as a monosomic (2n-1) strain containing a single chromosome *III*. A class of diploids with reversed linkage (see Figure 1) will be eliminated.

be ligated to the resident strand of the same polarity, the joining of the opposite set of nonparental strands is prevented in *rad52* diploids, possibly because the two DNA molecules cannot isomerize to form a symmetrical Holliday structure. Thus, one of the two chromatids participating in the recombination event will not be rejoined, creating a broken chromosome that cannot be repaired without the *RAD52* function. Depending on the segregation of the His$^+$ chromosome with one or the other nonsister centromere, one would expect to recover either diploids homozygous for a flanking marker or 2n-1 monosomic diploids in which the second participating chromatid was mitotically unstable and lost. The class of diploids with reversed linkage would be eliminated, as such diploids depend on the recovery of both intact participating chromatids in the same cell after mitosis. A similar picture was offered by HOLLIDAY *et al.* (1976) to account for the effects of the *rec-1* mutation in Ustilago.

It should be noted that all of the chromosome loss events associated with the formation of His$^+$ prototrophs appear to have resulted from the loss of an entire homologue rather than from the loss of all or part of one chromosome arm. There were no cases in which a diploid expressed one mating type but

was still heterozygous for the *URA3* and *LEU2* inserts at *HML*. Conversely, all of the diploids apparently homozygous for *URA3* or *LEU2* contained two viable chromosome *III* homologues, as evidenced by the spontaneous formation of both *MAT***a**- and *MAT*α-mating derivatives arising from *rad52*-induced chromosome loss. If these diploids had been hemizygous for part of the left arm, only monosomic diploids containing the intact homologue would have been detected. Finally, we note from our previous work on the healing of broken chromosomes (WEIFFENBACH and HABER 1981) that, with *rad52* diploids containing a broken chromosome *III*, we failed to recover "healed" derivatives homozygous for the distal portion of one arm.

We have considered an alternative model in which the basic mechanism to generate prototrophs is the same in both Rad$^+$ and *rad52* strains, but there is a very high probability of breaking (and losing) either DNA strand during recombination in *rad52* cells. Although such a model might account for the 95% reduction in the frequency of His$^+$ prototrophs and also for the high proportion of monosomic derivatives, it does not account for the marked change in the proportion of His$^+$ chromosomes that are associated with an exchange event (25% in Rad$^+$ strains but more than 80% in *rad52* diploids). A "random break" model would also not account for the change in frequency of diploids homozygous or hemizygous for the marker distal to *his4A vs.* the marker distal to *his4C*.

It is tempting to suggest that *RAD52*-independent recombination also occurs as a subset of all recombinants recovered in Rad$^+$ diploids. For example, it is possible that the recombination-associated chromosome loss that is seen at a very low level in Rad$^+$ diploids (CAMPBELL, FOGEL and LUSNAK 1975; CAMPBELL and FOGEL 1977) arises from the same mechanism that appears to be prevalent in *RAD52*-independent recombination. These chromosome loss events would only constitute a significant class when *RAD52*-dependent recombination was eliminated. Furthermore, it is possible that gene conversion events without an exchange of flanking markers are *RAD52* dependent, whereas events associated with an exchange of flanking markers are *RAD52* independent (JACKSON and FINK 1981). In this view, exchange-associated recombination may occur via a pathway quite distinct from the formation of gene convertants without exchange. However, it is equally possible that *RAD52*-independent events arise from the same intermediates of recombination used in *RAD52*-dependent recombination and that these structures are resolved to give the unusual recombinants found in *rad52* strains only when the *RAD52* gene product is missing.

We should point out that there are two observations that cannot readily be explained by the model shown in Figure 2. First, ORR-WEAVER, SZOSTAK and ROTHSTEIN (1981) observed that the integration of transformed, circular plasmids by homologous recombination occurs in *rad52* strains at nearly wild-type levels. Similar results were reported by SCHERRER, MANN and DAVIS (1982). It is possible that, in this respect, integration of a plasmid at its homologous chromosomal location is not analogous to intragenic recombination events between homologous chromosomes.

A second observation is that *rad52* does not prevent the recovery of the reciprocal products of spontaneous unequal sister-chromatid mitotic recombination in the tandemly repeated yeast ribosomal DNA (rDNA) genes (ZAMB and PETES 1981; PRAKASH and TAILLON-MILLER 1981). However, KEIL and ROEDER (1984) have recently shown that rDNA contains a specific mitotic "hot spot" that may stimulate recombination in rDNA by another pathway that may not be *RAD52* dependent.

The existence of a recombination pathway in which only one of the two participating chromosomes is recovered is consistent with observations in several other organisms. For example, BERG and GALLANT (1971) and SARTHY and MESELSON (1976) argued that some exchange events in bacterial cells were not reciprocal. Among eukaryotes, the study by HOLLIDAY *et al.* (1976) on *rec1-1* strains of *Ustilago maydis* suggested that one of the two participating chromatids is often broken or lost during mitotic recombination. HOLLIDAY (1984) has reviewed other evidence supporting the existence of multiple mitotic recombination pathways in Ustilago.

Comparison of intra- and interchromosomal gene conversion events: Recently, there have been several studies that have been interpreted to argue that intrachromosomal gene conversion events may differ from interchromsomal events in both meiotic and mitotic cells (KLAR and STRATHERN 1984). Gene conversions of alleles contained in relatively short (2–3 kb) regions of homology were almost never accompanied by an exchange of flanking markers. It seems more likely that this constraint is not a feature of an intrachromosomal (*vs.* interchromosmal) gene conversion event but reflects the fact that these conversion events occurred between regions containing short amounts of homology. First, exchanges associated with intrachromosomal gene conversion seem to occur more frequently when the alleles are contained within regions of longer homology. For example, the *his4A* and *his4C* alleles studied by JACKSON and FINK (1981) were located within duplications of 24 kb, and between 12 and 25% of the conversion events were associated with exchange. Some of these events may have resulted from simple crossovers between the two alleles, but our data suggest that many His^+ recombinants arose by gene conversions, even when there was an exchange of flanking markers. Conversely, exchanges of flanking markers occur rarely, even in interchromosomal gene conversions when the alleles are contained in regions of limited homology (KLAR and STRATHERN 1984; MUNZ *et al.* 1984; J. E. HABER unpublished observations).

We find very little difference between conversions of the *his4* alleles whether they are located on the same chromosome or on opposite homologues. In our interchromosomal events, 77% of the events occurred without an exchange of flanking markers, whereas during intrachromosomal events, between 88 and 75% of the prototrophs occurred without exchange, depending on the intrachromosomal orientation of the *his4* alleles (JACKSON and FINK 1981). Furthermore, the effect of the *rad52-1* mutation on gene conversion and recombination appears to be quite similar. Depending on the orientation of markers in the intrachromosomal experiment, *rad52-1* His^+ recombinants were associated by an exchange of flanking markers between 97 and 92% of the time (JACKSON and

FINK 1981). In our interchromosomal experiment 84% were accompanied by an exchange.

It must be remembered that our experiments were carried out in *MAT***a**/*MATα* diploids, whereas JACKSON and FINK used haploid strains. Because there are differences in the level (and possibly in the spectrum) of recombination events in *MAT***a**/*MATα* strains *vs.* those expressing only one mating type (FRIIS and ROMAN 1968; ESPOSITO *et al.* 1982), some of the small differences between intra- and interchromosomal studies of *his4* recombination may depend on the cell's mating type. It is also possible that the somewhat more extreme differences seen in the intrachromosomal events might also reflect a difference in the time during the cell cycle when recombination occurs. Previous studies have shown that mitotic recombination can occur both in the G1 as well as the G2 stage of the cell cycle (ESPOSITO 1978; FABRE 1978; GOLIN and ESPOSITO 1981; ROMAN and FABRE 1983). A large proportion of the *RAD52*-independent events observed in JACKSON and FINK'S intrachromosomal experiment appears to have occurred in G2, by unequal sister-chromatid exchange. For most events, however, we cannot determine when during the cell cycle mitotic recombination occurred.

As a general observation, we note that, if most mitotic recombination was both initiated and resolved prior to DNA replication, then all exchange events would be manifested as diploids with reversed linkage, because diploids homozygous for a distal marker can only arise if events are resolved (but not necessarily initiated) after DNA replication (ESPOSITO 1978; ROMAN and FABRE 1983) (see Figure 1). The fact that in Rad^+ diploids we found as many cases homozygous for a distal marker as those with distal markers in nonparental configuration argues that the resolution of crossing over associated with His^+ prototroph formation must generally occur after DNA replication. The data for *rad52* strains also favor this interpretation.

We are grateful to RHONA BORTS, KATHY KARRER, RALPH KIEL and SHIRLEEN ROEDER for their detailed comments on this manuscript. The advice and criticisms of MICHAEL LICHTEN have been invaluable. GERALD FINK and AMAR KLAR kindly provided strains. This work was supported by National Institutes of Health grant GM20045.

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Communicating editor: D. BOTSTEIN