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Properties of spontaneous mitotic recombination occurring in the presence of the *rad52-1* mutation of *Saccharomyces cerevisiae*

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Summary

All major recombination pathways in the yeast *Saccharomyces cerevisiae* require the *RAD52* gene product. We have examined the effect of the *rad52-1* mutation on spontaneous mitotic recombination between heteroalleles, and found that prototrophs are produced at frequencies significantly above reversion. This residual recombination occurs at a relatively uniform level at all of the loci examined. To help understand the role that *RAD52* plays in mitotic recombination, we examined recombination between all pairwise combinations of six mutant alleles of the *LYS2* gene. The *rad52-1* mutation decreased the variation in amount of recombination between the various pairwise combinations as well as lowering the overall frequency of recombination. The reduced variation results in a different pattern of recombination in *rad52-1* cells than in wild type. One interpretation of these results is that the *RAD52* gene product, directly or indirectly, plays a role in the formation or the resolution of mismatches in heteroduplex DNA.

1. Introduction

The *RAD52* gene is apparently required for almost all types of genetic recombination in *Saccharomyces cerevisiae*. The *rad52-1* allele, isolated as an X-ray-sensitive mutation, is pleiotrophic and confers a variety of mutant phenotypes (reviewed in Game, 1983 and Haynes & Kunz, 1981). It is deficient in meiotic recombination at all loci on all chromosomes examined (Game *et al.* 1980; Prakash *et al.* 1980; Malone, 1983). As expected for a meiotic Rec$^-$ mutation, all spores formed following meiosis are inviable and do not germinate. The *rad52-1* allele also confers a Rec$^-$ phenotype for spontaneous mitotic recombination (Game *et al.* 1980; Prakash *et al.* 1980; Malone & Esposito, 1980). All current data support the contention that mitotic gene conversion is greatly reduced in *rad52-1*-containing strains. Consistent with this is the deficiency conferred by *rad52-1* for the switching of mating types in homothallic strains (Malone & Esposito, 1980). The effect of *rad52-1* on spontaneous mitotic crossing-over is less defined. Malone (1983) and Malone & Esposito (1980) found that recombinants formed by crossovers between homologous chromosomes were reduced 5- to 10-fold. Jackson & Fink (1981), however, found that intrachromosomal reciprocal events at a *his4* duplication were not reduced. Likewise, Prakash & Taillon-Miller (1981), as well as Zamb & Petes (1981), found that *rad52-1* did not inhibit sister-strand crossing-over. Orr-Weaver, Szostak & Rothstein (1981) examined the integration of non-replicating plasmids and argued that the relative frequency of integration was not reduced in *rad52-1* strains. Since a reciprocal crossover is required to insert a circular plasmid into a chromosome, their suggestion was that *rad52-1* did not affect mitotic crossing-over.

More recently, Haber & Hearn (1985) examined spontaneous mitotic recombination between the *his4* heteroalleles used by Jackson & Fink. However, Haber & Hearn examined *MATa/MATα* diploid recombination occurring between homologues with easily detectable outside markers, rather than at a duplication in haploids. They found that 84% of the events generating His$^+$ prototrophs in *rad52-1* strains were associated with exchange of outside markers. This is in contrast to 23% in Rad$^+$ cells. The events producing His$^+$ prototrophs were conversions; Haber & Hearn did not detect the reciprocal double *his4* mutant. They concluded that gene conversion did occur in the absence of *RAD52* and observed that its properties were markedly different from conversions occurring in wild-type cells. Not only was conversion often associated with exchange, but the pattern was different. On the basis of their observations, Haber & Hearn argued that the majority of the gene conversion events in *rad52-1* cells occurred by the formation of DNA heteroduplexes and mismatch repair.

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We have been interested in the role that *RAD52* plays in spontaneous mitotic recombination. We observed that the frequencies of prototroph formation at the different loci examined by Malone & Esposito for gene conversion were remarkably similar. If the prototrophs were due to recombination, and not reversion, the similar frequencies suggest that the residual recombination occurring in the absence of *RAD52* has very different properties from recombination occurring in its presence. One way to generate a uniform frequency of recombination between different pairs of mutant alleles is for recombination to no longer be dependent upon the distance between mutations, and for all heteroduplex DNA formed to be treated identically. To examine this question we looked at recombination between several different alleles at a single locus, *LYS2*. The data in this paper suggest that the *RAD52* gene product may play a role during spontaneous mitotic recombination in the formation and/or correction of mismatches.

2. Materials and Methods

(i) *Strains*

The relevant genotypes of *S. cerevisiae* strains are shown in Table 1. Strains were constructed by several backcrosses (at least three times) with wild-type laboratory strains in order to develop relatively isogenic backgrounds. Some of the strains used in backcrosses were K210-4A, K210-6D, K264-5B and K264-10D (kindly supplied by Dr S. Klapholz, University of Chicago). Standard techniques were used for sporulation, dissection, testing of auxotrophic requirements and prototrophic selection of diploids (Malone & Hoekstra, 1984). All strains contained the *ochre*-suppressible mutation *ade2-1* and at least one other suppressible auxotrophy such as *trp5-2*, *tyr1-1* or *met13-c* (Hawthorne & Leupold, 1974). These mutations were included to assay prototrophs arising from heteroalleles for the presence of suppressor mutations. The *lys2-1* and *lys2-2* alleles are *ochre*-suppressible mutations. If Lys^+ prototrophs occur by suppression rather than recombination, other *ochre*-suppressible mutations can be co-suppressed and detected in a replica plate assay (Malone, Golin & Esposito, 1981).

(ii) *Media*

Media recipes (lysine omission medium, etc.) have been described previously (Golin & Esposito, 1977). Liquid medium (YPD) is 1% yeast extract, 2% Bactopeptone and 2% dextrose. Solidified medium contains 1·8% Bactoagar (Difco). MMS plates, used to follow the segregation of *rad52-1*, are YPD medium containing 0·01% methyl methane sulphonate (Eastman Kodak). Strains containing *rad52-1* are sensitive to MMS.

(iii) *Isolation of* lys2 *mutations*

Mutations in the *LYS2* gene were selected using a modification of the procedure of Chattoo *et al.* (1979). Wild-type strains RM11-10D and RM13-128D were each inoculated into 40 ml of YPD and grown overnight to a concentration of 2×10^7 cells/ml (mid-exponential phase). Cells were pelleted and washed twice in sterile 0·2 M-NaH_2PO_4 buffer (pH 7·5). Cells were resuspended in 5 ml of phosphate buffer and 50 μl ethyl methane sulphonate (EMS, Eastman Kodak) added. The suspension was held at 24 °C for 45 min (corresponding to 81% survival). Preliminary EMS mutagenesis experiments indicated that this regimen generated a two log increase in forward mutation at *CAN1*. For the isolation of *lys2* mutations, aliquots of mutagenized cells were spread on supplemented minimal media containing 4 mg/ml α-aminoadipate (α-AA). Prior to EMS treatment, the starting cultures demonstrated a spontaneous α-AA resistance frequency of $4{\cdot}3 \times 10^{-6}$. After EMS treatment, the frequency of resistance rose to $6{\cdot}2 \times 10^{-4}$.

Approximately 600 α-AA-resistant colonies were picked to master plates and tested for their ability to grow on lysine omission medium. Concomitantly, these isolates were subjected to *lys2* complementation tests and allelism tests against known *lys2-1* and *lys2-2* mutations. Isolates that were unable to grow without added lysine, that did not complement the known *lys2* mutations, but that were able to undergo meiotic allelic recombination with both *lys2-1* and *lys2-2* were repicked to new master plates (44 isolates). Twenty-seven of these were in RM11-10D background and 17 were in RM13-128D.

All possible pairwise crosses were done, with diploids being selected on medium without homoserine or histidine. Diploids were subsequently replicated to lysine omission medium and the number of Lys^+ papillae counted. A wide range of responses varying from a few to several hundred papillae per 1 cm^2 diploid patch was observed. Eleven RM11-10D and 8 RM13-128D isolates from the pairwise crosses were chosen to pursue further. The 19 isolates were backcrossed with unmutagenized wild-type strains to remove the new *lys2* mutations from the mutagenized background. The diploids were sporulated, dissected and *lys2* mutations of both mating types isolated. Four *lys2* alleles which, when intercrossed, gave a wide range of mitotic papillation frequencies and demonstrated no papilla when homozygous were chosen, along with *lys2-1* and *lys2-2*, for subsequent use. These strains were subjected to two further clean-up backcrosses before being outcrossed to *rad52-1* strains. Following the recommendation of Dr J. K. Bhattacharjee (Miami University of Ohio), we have named the isolates *lys2-500*, *lys2-501*, *lys2-502* and *lys2-503* (using an allele-numbering scheme of Chattoo *et al.* 1979).

Table 1a. *Diploid strains:* LYS2 *and* RAD52 *genotypes*

	lys2-1	*lys2-2*	*lys2-500*	*lys2-501*	*lys2-502*	*lys2-503*
lys2-1	MH32	MH53	MH38	MH39	MH40	MH41	MH42
lys2-2	MH17	MH33	MH54	MH43	MH44	MH45	MH46
lys2-500	MH18	MH22	MH34	MH55	MH47	MH48	MH49
lys2-501	MH19	MH23	MH26	MH35	MH56	MH50	MH51
lys2-502	MH20	MH24	MH27	MH29	MH36	MH57	MH52
lys2-503	MH21	MH25	MH28	MH30	MH31	MH37	MH58

The strains listed in the upper right-hand portion of the table are *RAD52* diploids containing the *lys2* alleles designated by following a path along a row and down a column. The left-hand lower portion below the diagonal lists *rad52-1* diploid strains.

Table 1b. *Details of strain genotypes*

Strain	Genotype
RM11-10D	*a tyr1-1 ura3-13 hom3 ade2-1 met13-c cyh2^R trp5-c leu1-c ade6*
RM13-128D	*α tyr1-1 can1^R ura3-13 his1 ade1-1 ade5 met13-c cyh2^R trp5-c leu1-12*
RM13, RM15, RM27	*a leu1-c trp5-c cyh2^R met13-c ura3-1 lys2-1 tyr1-2 his7-2 RAD52 ade2-1* *α leu1-12 trp5-2 CYH2^S met13-d ura3-13 lys2-2 tyr1-1 his7-2 RAD52 ade2-1*
RM41, RM42	*a leu1-c trp5-c cyh2^R met13-c ura3-1 lys2-1 tyr1-2 his7-2 rad52-1 ade2-1* *α leu1-12 trp5-2 CYH2^S met13-d ura3-13 lys2-2 tyr1-1 his7-1 rad52-1 ade2-1*
MH32	*a rad52-1 lys2-1 tyr1-1 his7-2 ura3-13 trp5-2 leu1-12* *α rad52-1 lys2-1 tyr1-1 his7-2 ura3-13 trp5-2 leu1-12*
MH33	*a rad52-1 lys2-2 tyr1-2 his7-1 ura3-1 trp5-c leu1-c* *α rad52-1 lys2-2 tyr1-2 his7-1 ura3-1 trp5-c leu1-c*

(iv) *Determination of mitotic recombination levels*

The procedure for determining mitotic recombination values was essentially as described in Malone & Hoekstra (1984). Single colonies from freshly constructed diploids were picked into 1 ml of YPD and cell concentration was determined by haemocytometer count. Ten ml of YPD was inoculated with 10^4 cells and the culture grown at 30°C with vigorous shaking to approximately 2×10^7 cells/ml. Each culture was initiated from an independent colony and for all diploids at least three (most often 6–10) cultures were measured. After harvesting by centrifugation, cells were washed twice in sterile phosphate buffer, sonicated briefly to disrupt clumps and plated on lysine omission and complete synthetic media. Plates were scored after three days of growth at 30°C.

(v) *DNA blot analysis*

The procedure for small-scale isolation of total yeast genomic DNA has been described (Hoekstra & Malone, submitted) DNA blotting, nick-translation and hybridization conditions are as described in Hoekstra & Malone (1985).

3. Results

(i) *Prototrophs produced at heteroallelic loci in* rad52-1 *are not due to reversion*

Malone & Esposito (1980) demonstrated that mitotic inter- and intragenic recombination between homologues in *rad52-1* strains were reduced compared to Rad$^+$ (i.e. *RAD52*) strains at all loci examined. Close examination of the data published by Malone & Espo-

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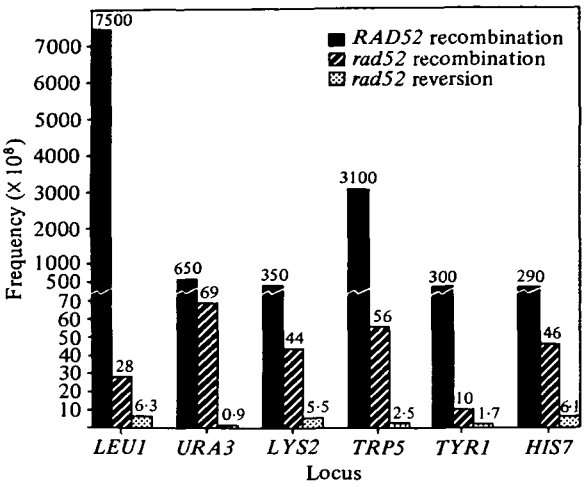

Fig. 1. Recombination and mutation frequencies at various loci in *RAD52* and *rad52-1* strains. Geometric mean recombination and reversion frequencies for strains RM13, RM15, RM27, RM41, RM42, MH32, and MH33 are presented. The Rad$^+$ values are from Malone & Hoekstra (1984), *rad52-1* frequencies are from Malone & Esposito (1980), and *rad52-1* reversion frequencies are from this work.

sito reveals recombination frequencies for various heteroallelic loci ranging from $1{\cdot}0 \times 10^{-7}$ to $6{\cdot}9 \times 10^{-7}$ (Fig. 1). The greatest variation between any of the heteroalleles in *rad52-1* strains is only a 6·9-fold difference in recombination frequency (*LEU1* versus *TYR1*). Compared to this relatively low range of fluctuation between loci, Rad$^+$ strains had recombination frequencies ranging from $2{\cdot}9 \times 10^{-6}$ to $7{\cdot}5 \times 10^{-5}$ (*HIS7* versus *LEU1*). This is approximately a 26-fold difference. The Rad$^+$ recombination frequencies (Fig. 1) are taken from the larger data set described by Malone & Hoekstra (1984). The data used include those described by Malone & Esposito (1980). In addition to the general suppression of intragenic recombination and the relatively *uniform* amount of heteroallelic recombination in *rad52-1* cells, the recombination that occurs has a pattern different from Rad$^+$ strains. It was necessary to demonstrate that prototrophic colonies arising in *rad52-1* homozygous diploids from heteroallelic loci were recombinants rather than revertants. Reversion frequencies for the auxotrophic alleles present in the Malone & Esposito *rad52-1* strains were measured in homoallelic diploids. As shown in Fig. 1, the frequency of recombination in the *rad52-1* background averages almost 10-fold greater than the combined reversion frequency for both input alleles comprising a given heteroallelic pair. In other words, heteroallelic intragenic mitotic recombination (gene conversion) in *rad52-1* strains does occur, and the level of recombination is almost 10 times greater than mutation. On the basis of these observations, we feel there is low-level recombination occurring in *rad52-1* strains of *Saccharomyces cerevisiae*. The recombination events display a more uniform distribution than those occurring in wild-type strains.

(ii) *Isolation and characterization of* lys2 *mutations*

Because the heteroalleles examined in Fig. 1 are in different genes on different chromosomes, at varying distances from chromosomal landmarks (centromeres and telomeres, for example), we felt it necessary to examine a set of mutations carefully along a defined genetic interval. The fluctuation from locus to locus in Rad$^+$ strains could reflect different probabilities of recombination occurring at different loci, rather than a variation in the recombination mechanism itself. If so, the reduction of conversion at different loci in *rad52-1* strains to a more uniform level might only represent a uniform probability of initiation at all loci. This hypothesis is opposed to one proposing a mechanism in *rad52-1* strains which was distance- and heteroduplex-independent. To determine whether the observed uniformity reflects events occurring within a locus, rather than being dependent on recognition of loci *per se*, we examined recombination between several alleles within the *LYS2* gene.

LYS2 mutations, defective in α-aminoadipate reductase, were selected on the basis of resistance to α-AA in the absence of lysine (Chattoo *et al.* 1979). From initial characterization a set of six alleles (*lys2-1*, *lys2-2*, *lys2-500*, *lys2-501*, *lys2-502* and *lys2-503*) were chosen for use in precise measurements of recombination levels. The criteria used for choosing these alleles were: (i) intercrosses of the isolates creating heteroallelic diploids gave a wide range of Lys$^+$ papillae in Rad$^+$ cells; and (ii) sibling crosses, creating homozygous *lys2* diploids, gave no revertant colonies in a simple replica-plate assay. For the four alleles isolated, formation of Lys$^+$ revertants by this replica-plate assay was extremely low, indicating that the levels of reversion and suppression for these alleles would not significantly affect our analysis in wild-type strains. UV-induced mitotic recombination and meiotic recombination experiments using all pairwise combinations of the 6 *lys2* alleles were consistent with their being located at different positions along the *LYS2* gene (data not shown).

Although the four mutations generated for this study are EMS-induced and likely to be single base-pair changes, we examined the *LYS2* genomic region for gross structural changes (such as deletions or insertions). (The *LYS2*-containing plasmid, p1-L13, was kindly supplied by S. Carl Falco, E. I. DuPont deNemours and Co., Wilmington, Delaware.) Our reasoning for examining the mutations by Southern blot analysis is based on the observation that a TY element insertion, such as the insert contained in *ura3-52*, can stimulate recombination several-fold (M. F. H. and R. E. M., unpublished observation). A Southern blot and restriction map of p1-L13 is given in Fig. 2. Based on this analysis we detect no gross DNA alteration in any of the 6 *lys2* alleles.

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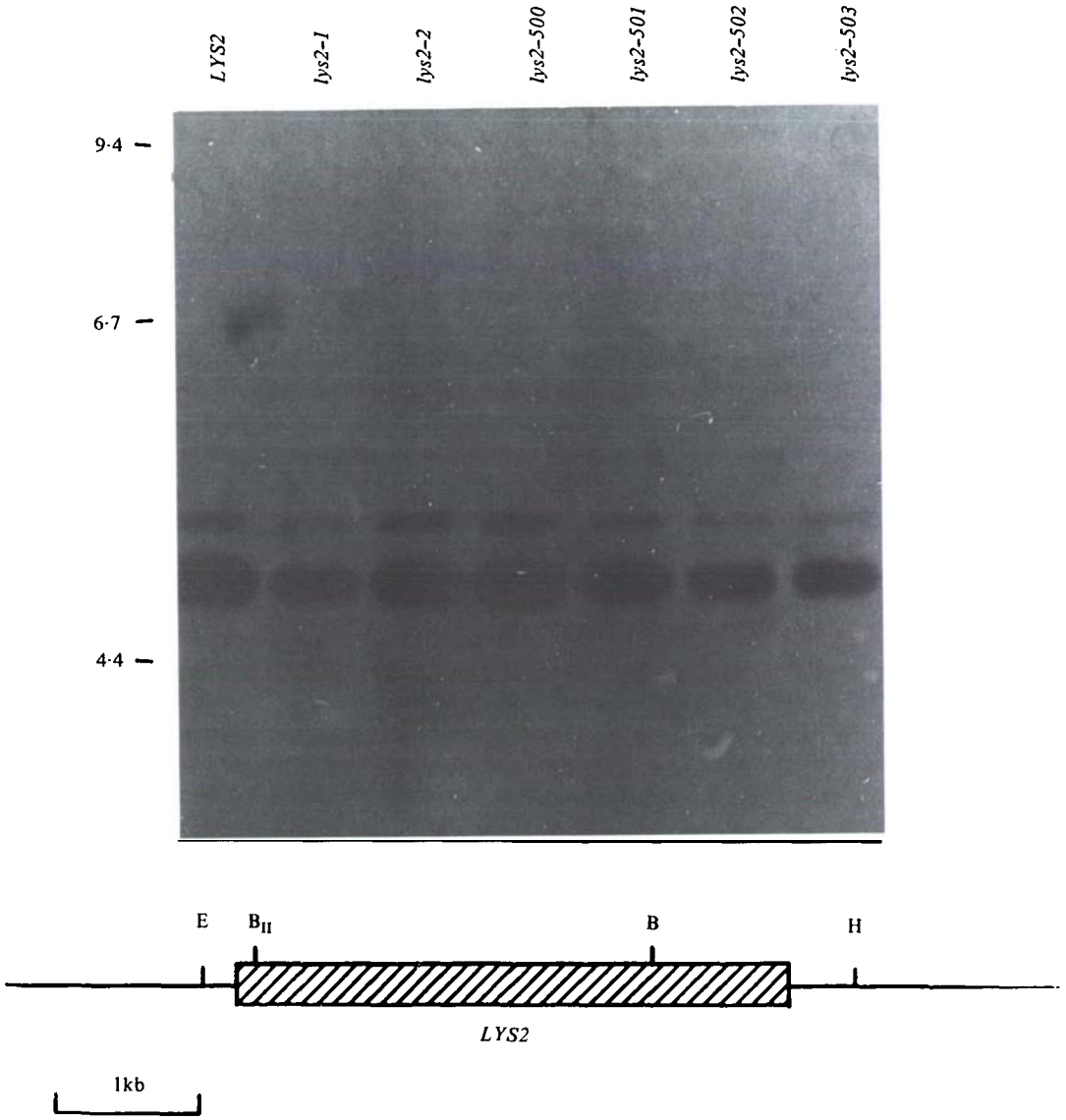

Fig. 2. Southern analysis of new *lys2* mutations. Approximately 3 μg of total yeast DNA were digested with Bgl II, run in 0·8% agarose overnight, and blotted to nitrocellulose, and probed with an EcoRI-HindIII fragment containing *LYS2* from p1-L13. The numbers above the lanes refer to the *lys2* allele number.

(iii) *Spontaneous mitotic recombination at* LYS2 *in* RAD52 *and* rad52-1 *backgrounds*

Figure 3A shows a histogram representation of recombination levels for all 15 pairwise combinations of *lys2* alleles in wild-type strains. (Recombination frequencies, rather than rates, are presented in order to facilitate direct comparison with the work of Malone & Esposito (1980).) As in Fig. 1, relative recombination levels between heteroallelic pairs fluctuated greatly. However, in this case heteroalleles are confined to a small genetic interval, and the variation in levels is more likely to reflect the mechanism of the exchange event occurring at *LYS2* rather than the probability of the event occurring at the locus. The largest difference in recombination frequencies is 69-fold (*lys2-503/lys2-2* versus *lys2-500/lys2-2*) with a range of $3{\cdot}7 \times 10^{-6}$ to $2{\cdot}5 \times 10^{-4}$. It should be noted that at least 50 to 100 Lys^+ colonies per culture were picked and retested in all experiments. This was done in part because *lys2-1* and *lys2-2* are *ochre* alleles, capable of being suppressed by tRNA mutations. Where necessary, corrections were made for suppression, but in most cases suppressors occurred in less than 5–10% of the putative recombinants in wild-type strains. (All strains contained multiple diagnostic suppressible auxotrophic mutations (see Materials and Methods).)

Figure 3B represents the recombination frequencies for the same heteroalleles as in Fig. 3A, in a *rad52-1* background. (Note the scale differences between Fig. 3A and 3B.) For all heteroallelic pairs, the reduction in recombination frequencies in *rad52-1* cells is of similar magnitude to that observed in Fig. 1. The decrease is comparable to the average reduction of approximately 10- to 50-fold reported by Prakash *et al.* (1980) and Malone & Esposito (1980). While there is variation between the geometric mean recombination frequencies given in Fig. 3B

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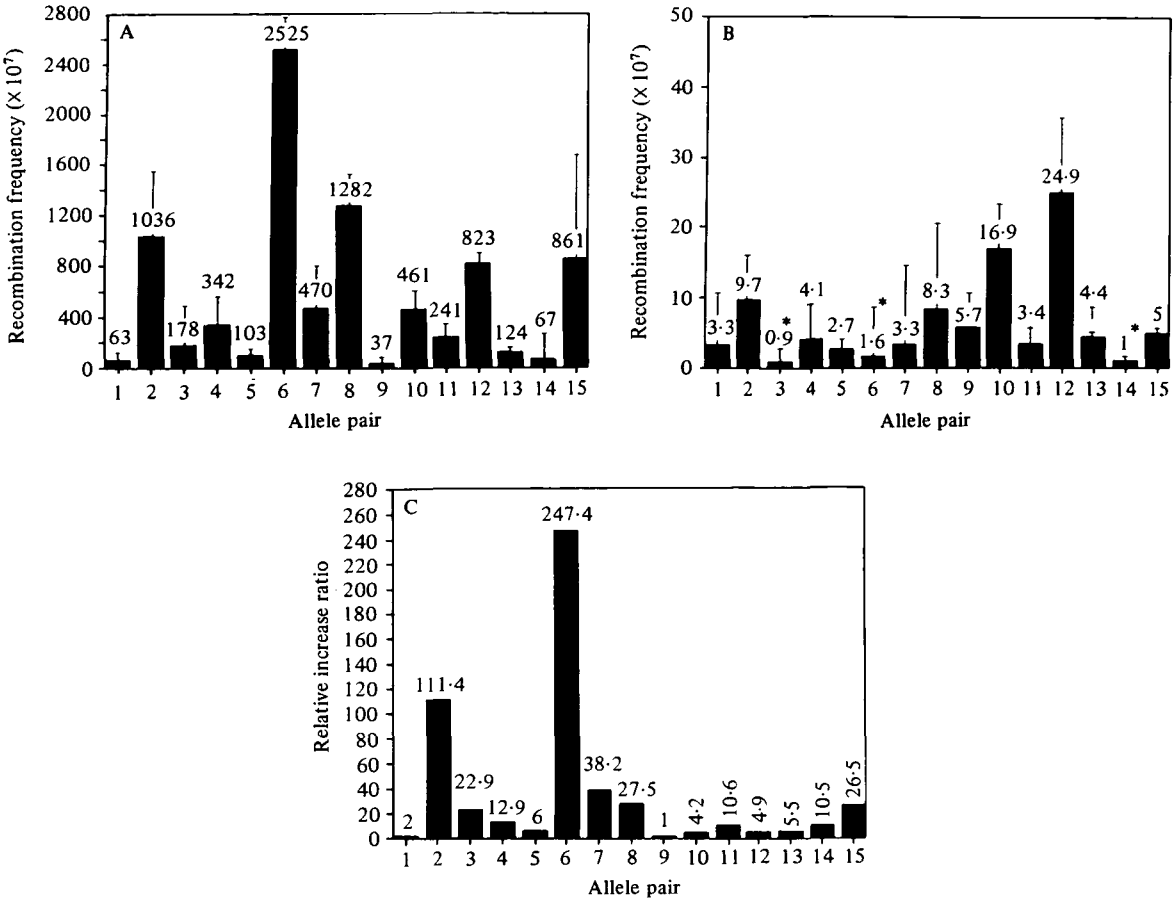

Fig. 3. Spontaneous mitotic recombination at *LYS2* in *RAD52* and *rad52-1* strains. The data represent geometric mean recombination frequencies for *RAD52* (A) and *rad52-1* (B) strains for all pairwise combinations of the 6 *lys2* alleles. The data in Fig. 3C are the *RAD52/rad52-1* ratios, for each heteroallelic pair, normalized to the lowest ratio. If the *rad52-1* mutation caused only a reduction of recombination, the values would all be 1·0. The allelic pairs are as follows: (1) *lys2-1/lys2-2*, (2) *lys2-1/lys2-500*, (3) *lys2-1/lys2-501*, (4) *lys2-1/lys2-502*, (5) *lys2-1/lys2-503*, (6) *lys2-2/lys2-500*, (7) *lys2-2/lys2-501*, (8) *lys2-2/lys2-502*, (9) *lys2-2/lys2-503*, (10) *lys2-500/lys2-501*, (11) *lys2-500/lys2-502*, (12) *lys2-500/lys2-503*, (13) *lys2-501/lys2-502*, (14) *lys2-501/lys2-503*, and (15) *lys2-502/lys2-503*. Strains used are listed in Table 1 (MH17–MH30 and MH38–MH52).

($2{\cdot}7 \times 10^{-7}$ versus $24{\cdot}9 \times 10^{-7}$), almost all the recombination values are within one standard deviation of each other. The differences between each of the 15 *lys2* pairwise combinations in the *rad52-1* background are not apparently significant (1-way ANOVA, $F = 1{\cdot}002$, D.F. = 14, $P = 0{\cdot}462$). A similar statement cannot be made for the *RAD52* data in Fig. 3A. Therefore, the recombination that occurs along the length of *LYS2* in *rad52-1* is apparently homogeneous, uniform, and relatively independent of the mutant alleles present. It should be noted that 3 of the 15 heteroallelic pairs in the *rad52-1* background generate prototroph levels that are not significantly greater than reversion levels (these 3 are marked by an asterisk in Fig. 3B). Disregarding these 3 heteroallelic pairs, the largest difference in recombination frequencies for *rad52-1* strains among the *lys2* heteroalleles is 9·2-fold, a value similar to the 6·9-fold fluctuation observed by Malone & Esposito (1980) for inter-locus comparisons.

Figure 3C is a histogram plot which directly compares the relative amount of recombination for a given heteroallelic pair in wild type versus *rad52-1*. To generate this comparative figure, we have taken the ratio of *RAD52/rad52-1* recombination for a given heteroallelic pair and normalized the value to the lowest ratio, which has been given a value of one. This plot demonstrates that the spectrum of recombination in *rad52-1* cells is drastically altered from wild type. If the heteroallelic pattern of recombination in *rad52-1* were simply a reduction of the same distribution in wild type, then the expected values for Fig. 3C would all be unity. This is clearly not the case.

Evidence that the selected Lys$^+$ colonies for each heteroallelic pair in *rad52* mutants can be attributed to recombination is taken from the data in Table 2 and Fig. 3. In 12 of 15 cases the level of prototrophs arising from a heteroallelic pair (Fig. 3) is higher than the level of homoallelic reversion (Table 2) in the *rad52-1* background. It is interesting to note that the occurrence of mutation in *rad52-1* is elevated relative to wild type for 4 of the 6 alleles. There is approximately a 5- to 10-fold average increase in reversion rates in *rad52-1* strains. This agrees favourably with previous

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Table 2. *Frequency of reversion to prototrophy*

Diploid genotype	Reversion frequency × 10^7					
	lys2-1 / *lys2-1*	*lys2-2* / *lys2-2*	*lys2-500* / *lys2-500*	*lys2-501* / *lys2-501*	*lys2-502* / *lys2-502*	*lys2-503* / *lys2-503*
rad52-1 / *rad52-1*	1·8 ±2·6	3·8 ±4·6	1·8 ±2·1	1·0 ±1·3	2·4 ±0·8	1·5 ±3·4
RAD52 / *RAD52*	0·1 ±0·02	1·3 ±3·6	0·3 ±0·9	2·4 ±0·7	0·1 ±0·8	3·4 ±4·7

The data represent geometric mean reversion frequencies of 7 and 4 cultures per diploid for *rad52-1* and *RAD52* strains, respectively. The diploids used are MH32–MH37 and MH53–MH58.

observations by Prakash *et al.* (1980) that *rad52-1* strains are slightly hyper-mutable. One interpretation of the increase in spontaneous mutation is that lesions which are normally processed by recombination–repair are channelled to another (error-prone) repair pathway.

From the wild-type mitotic recombination frequencies (Fig. 3A) it is possible to calculate a map of the *LYS2* gene indicating the relative positions of the 6 *lys2* mutations examined (Fig. 4). The best map order for the *lys2* alleles appears to be: *lys2-503*, *lys2-2*, *lys2-1*, *lys2-501*, *lys2-502*, *lys2-500*. Precise alignment of these mutations on the *LYS2* gene will require gap rescue analysis (Orr-Weaver *et al.* 1983) and/or nucleotide sequencing.

4. Discussion

In this report we have examined spontaneous, intragenic mitotic recombination in *rad52-1* strains. The *RAD52* gene product is one of the more interesting recombination and repair functions in yeast. Strains with mutations in *RAD52* are X-ray-sensitive, and *rad52-1* homozygous diploids exhibit greatly reduced frequencies of mitotic inter- and intragenic recombination between homologues (reviewed in Game, 1983). The *rad52-1* mutation also confers a reduction in the recovery of viable recombinants after meiosis (Game *et al.* 1980; Prakash *et al.* 1980), and strains containing it are partially defective in the production of physically recombined DNA in meiosis (Borts *et al.* 1984; Haber, personal communication). The *RAD52* gene has been cloned, sequenced and a conceptual translation of the coding region proposed (Schild *et al.*, 1983*a*, *b*; Adzuma, Ogawa & Ogawa, 1984). The gene could encode a 56-KDa protein. Interestingly, Resnick and coworkers have demonstrated that *rad52* strains lack a 70-KDa endonuclease (Chow & Resnick, 1983; Resnick *et al.* 1984), leading to the proposal that the *RAD52* gene is a control function for the endonuclease. The *rad52-1* allele has been sequenced by Adzuma *et al.* (1984) and carries a mis-sense mutation at codon 90 of 504 codons in total. The *rad52-1* allele confers a phenotype for repair and meiotic recombination essentially identical to a gene disruption created by Schild *et al.* (1983*b*). We conclude that it is likely that strains containing *rad52-1* have little functional gene product present.

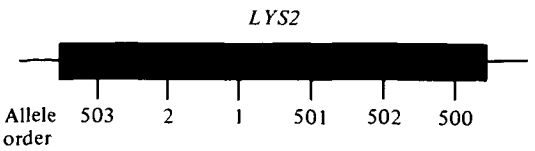

Fig. 4. Allele order of the *LYS2* mutations. A consensus order of alleles along the *LYS2* gene was determined from the geometric mean recombination frequencies for all pairwise combinations of *lys2* mutations in a Rad^+ background. While the distances between different combinations are not strictly additive, the consensus order is consistent for all combinations.

The data presented here indicate that a low level of mitotic recombination occurs in *rad52-1* mutant strains. Two hypotheses to explain this observation are: (i) there is another recombination pathway in $Rad52^-$ cells, or (ii) the *rad52-1* mutant is leaky, and a small amount of functional *RAD52* product is present. We favour the former hypothesis for three reasons. First, whenever tested, the properties of the *rad52-1* mutation are similar to *rad52* gene disruptions created *in vitro* and transplaced into the chromosome (Schild *et al.* 1983*b*). Secondly, published data suggest that, in some instances, recombination in the form of crossing-over can occur in *rad52-1* strains (Jackson & Fink, 1981; Klein & Petes, 1981; Orr-Weaver *et al.* 1981; Prakash & Taillon-Miller, 1981; Zamb & Petes, 1981). Third, if the *rad52-1* mutation were leaky, the simplest expectation would be that the rank order of recombination at heteroallelic pairs would remain the same, although the amount of recombination would be reduced. In other words, frequencies would be reduced but the distribution would be the same. We note, however, that even if the second hypothesis were true, it would imply that low levels of *RAD52* gene product alter the mechanism of recombination events, not simply their frequency.

Examination of recombination in *rad52-1* strains

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along a defined genetic interval, *LYS2*, has provided an interpretation of the original observations of Malone & Esposito (1980). Recombination levels are greater than mutation levels for 12 of 15 heteroallelic pairs examined in this study and for all heteroalleles examined by Malone & Esposito (1980). The levels of recombination in *rad52-1* strains are relatively uniform compared to wild type. Comparison between heteroallelic recombination at loci on different chromosomes indicates that there is only about a 7-fold variation in frequencies. Recombination between different heteroalleles along a small genetic interval demonstrates at most a 9-fold range in frequencies. Using the same pairs of heteroalleles, wild-type strains show a 70-fold range in recombination frequencies. One interpretation of this observation is that all heteroduplexes containing heteroallelic mismatches are formed with equal probability. Furthermore, each mismatch is repaired equivalently. This interpretation is based on a single-strand exchange mechanism like that proposed by Meselson & Radding (1975) rather than a double-strand break model (Resnick, 1976; Szostak *et al.* 1983). If a background *RAD52*-independent recombination system is generating the recombinants, it has very different properties from the *RAD52*-dependent system. If, on the other hand, the altered recombination is due to a low level of the *RAD52* product present in *rad52-1* cells, the amount of gene product is affecting the mechanism of the event. Either way, the data suggest that *RAD52* plays a role, directly or indirectly, in the formation and/or correction of mismatches.

How can a single mutation in a recombination–repair function lead to a change both in the level and the distribution of spontaneous mitotic gene conversion? If *rad52-1* blocks the major mitotic recombination pathway, any recombinants formed are likely to do so by a secondary route. Owing to its X-ray sensitivity (Game *et al.* 1980; Prakash *et al.* 1980), inability to survive mating-type interconversion (Malone & Esposito, 1980), and lack of gapped plasmid integration (Orr-Weaver *et al.* 1981), the *rad52-1* mutation is believed to inactivate a function involved in double-strand break repair (recombination–repair). A cell attempting recombination by a double-strand break mechanism would likely become inviable in the absence of *RAD52*. Consistent with the proposal of Haber & Hearns (1985), it seems that a *RAD52*-independent pathway might proceed by a mechanism like that proposed by Meselson & Radding (1975).

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