RELATIONSHIPS BETWEEN A HYPER-REC MUTATION (*REM1*) AND OTHER RECOMBINATION AND REPAIR GENES IN YEAST

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ABSTRACT

Mutations in the *REM1* gene of *Saccharomyces cerevisiae* confer a semidominant hyper-recombination and hypermutable phenotype upon mitotic cells (GOLIN and ESPOSITO 1977). These effects have not been observed in meiosis. We have examined the interactions of *rem1* mutations with *rad6-1*, *rad50-1*, *rad52-1* or *spo11-1* mutations in order to understand the basis of the *rem1* hyper-rec phenotype. The *rad* mutations have pleiotropic phenotypes; *spo11* is only defective in sporulation and meiosis. The *RAD6*, *RAD50* and *SPO11* genes are not required for spontaneous mitotic recombination; mutations in the *RAD52* gene cause a general spontaneous mitotic Rec$^-$ phenotype. Mutations in *RAD50*, *RAD52* or *SPO11* eliminate meiotic recombination, and mutations in *RAD6* prevent spore formation. Evidence for the involvement of *RAD6* in meiotic recombination is less clear. Mutations in all three *RAD* genes confer sensitivity to X rays; the *RAD6* gene is also required for UV damage repair. To test whether any of these functions might be involved in the hyper-rec phenotype conferred by *rem1* mutations, double mutants were constructed. Double mutants of *rem1 spo11* were viable and demonstrated *rem1* levels of mitotic recombination, suggesting that the normal meiotic recombination system is not involved in producing the *rem1* phenotype. The *rem1 rad6* double mutant was also viable and had *rem1* levels of mitotic recombination. Neither *rem1 rad50* nor *rem1 rad52* double mutants were viable. This suggests that *rem1* causes its hyper-rec phenotype because it creates lesions in the DNA that are repaired using a recombination-repair system involving *RAD50* and *RAD52*.

THE study of mutants with increased levels of recombination in *Escherichia coli* has led to greater understanding of a number of genes involves in DNA metabolism. Mutations with an increased recombination (hyper-rec) phenotype in *E. coli* include lesions in the *polA*, *lig*, *uvrD*, *dut* and *dam* genes (KONRAD and LEHMAN 1975; ARTHUR and LLOYD 1980; TYE *et al.* 1977; MARINUS and KONRAD 1976; BALE, D'ALARCO and MARINUS 1979). In the presence of many of these mutations, the recombination that occurs appears to be essential. For example, double mutants of *polA* or *dam* and either *recA* or *recB* mutations are not viable (GROSS, GRUNDSTEIN and WITKIN 1971; MARINUS and MORRIS 1975). On the other hand, *recA uvrD* double mutants are viable but are no longer hyper-rec (ARTHUR and LLOYD 1980). All of these hyper-rec mutations cause nicks, gaps or breaks in the DNA, which presumably stimulate recombination. In addition

to their hyper-rec phenotype, most of these mutants also cause a hypermutable phenotype. In the case of the *polA* and *lig* mutations, this may be due to the induction of the SOS (error-prone) repair systems (KONRAD and LEHMAN 1975). In the case of *dam* mutations it appears to be due, at least in part, to the loss of methyl-directed mismatch repair (GLICKMAN and RADMAN 1980; PUKKILA *et al.* 1983). Second site revertants of *dam* mutations (*mutH*, *mutL*, *mutS*) that reduce the hyper-rec phenotype but increase the hypermutable phenotype of *dam* mutations are apparently defective in mismatch base repair (GLICKMAN and RADMAN 1980). Since hyper-rec mutations in *E. coli* have given considerable insight into recombination and repair processes, we have isolated and studied a hyper-rec mutation in *Saccharomyces cerevisiae* in the hope that it would be of similar utility in yeast.

The *REM1* gene was originally defined by the *rem1-1* allele isolated by GOLIN and ESPOSITO (1977). It was initially isolated as a mutation that conferred a hypermutable phenotype and was found to also increase mitotic recombination (GOLIN and ESPOSITO 1977). It caused no significant increase (or decrease) in meiotic recombination (GOLIN and ESPOSITO 1977). The mutant allele *rem1-1* was semidominant: heterozygous *rem1-1/REM1* diploids displayed approximately 50% of the increases in recombination and mutation found in homozygous *rem1-1/rem1-1* diploids. Using a direct screen for mutants affecting mitotic recombination, we isolated a mitotic hyper-rec mutation, *rem1-2*, which is allelic with *rem1-1*. Like *rem1-1*, it is hyper-rec, hypermutable and semidominant. One hypothesis to explain the hyper-rec phenotype of *rem1* mutations is that it leads to the appearance of the meiotic recombination system in mitotic cells. The frequency of meiotic recombination in yeast is several orders of magnitude greater than mitotic recombination. Thus, the presence of meiotic recombination functions in mitotic cells might lead to increased levels of mitotic recombination. Some support for this notion comes from the observation that *rem1* mutant strains have a distribution of crossover events that is intermediate between that which normally occurs in mitosis and that which occurs in meiosis (MALONE, GOLIN and ESPOSITO 1980). An alternative hypothesis is that the presence of *rem1* mutations leads to lesions in the DNA that stimulate recombination and produce mutations. We suppose that in yeast, as in *E. coli*, these recombinogenic defects might be nicks, gaps or breaks in the DNA. This second hypothesis also suggests the possibility that one (or many) of the known repair systems in yeast may be required for expression of the Rem^- phenotype. The experiments presented in this paper test these hypotheses by determining the effect of various Rec^- and repair defective mutations on strains containing *rem1*.

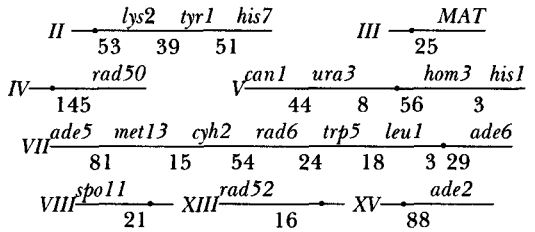
Recombination and repair pathways in yeast are complex, and many mutations affecting these processes have pleiotropic phenotypes (for reviews, see FOGEL, MORTIMER and LUSNAK 1981; ESPOSITO and WAGSTAFF 1981; HAYNES and KUNZ 1981). Recombination can occur in meiosis, and, at a lower frequency, in mitosis; the two recombination processes share some functions, whereas others are specific for meiotic recombination (see following data). HAYNES and KUNZ (1981) propose that dark repair functions can be loosely grouped into three major epistasis groups (or pathways): the *RAD3* group (primarily responsible for UV

excision repair), the *RAD52* group (primarily responsible for double-strand break repair and thought to be a recombination-repair pathway) and the *RAD6* group (an error-prone repair system).

The mutations used to study *rem1* were *rad50-1*, *rad52-1*, *spo11-1* and *rad6-1*; their phenotypes are summarized in Table 1. The *rad50* mutant phenotypes suggest that the *RAD50* gene may be a meiotic recombination function that is also used in mitotic repair processes. The properties of the *rad52-1* mutation suggest that the *RAD52* gene product may be a general recombination function in mitosis and meiosis; the repair defect of *rad52* mutants reflects the role of *RAD52* in recombination repair. The *rad6-1* mutation confers sensitivity to a large number of DNA damaging agents (UV, X-ray, methyl methane sulfonate (MMS), etc.); it is required for almost all induced mutagenesis, the implication being that it plays a central role in error-prone repair in yeast (PRAKASH 1974, 1976). Its role in meiotic recombination is less clear, although it does abolish sporulation (GAME *et al.* 1980; MALONE 1983). It seems certain that *RAD6* is required for repair and induced mutagenesis but not for spontaneous mitotic recombination.

MATERIALS AND METHODS

Yeast strains: The relevant genotypes of the strains used are shown in Table 2. Strains containing *rem1-1* and *rem1-2* were constructed by performing several backcrosses with *REM1* laboratory stocks in order to develop relatively isogenic backgrounds. The *rem1-1* mutation was obtained from JG25-26A, kindly supplied by JOHN GOLIN (University of Oregon). Some of the strains used in backcrosses were K264-5B and K264-10B (obtained from SUE KLAPHOLZ, University of Chicago); others were standard wild-type strains used in our laboratory. Many of the experiments described were performed using different (although related) genetic backgrounds in order to reduce the possibility that strain backgrounds affected the gene interactions; we found similar effects in all backgrounds. Linkage relationships of the genetic loci used are shown:



The Roman numerals refer to the chromosome number, and the numbers below the line refer to map distances between loci (MORTIMER and SCHILD 1980). Gene symbols are as defined by PLISCHKE *et al.* (1976); the position of the centromere is represented by a black circle.

Media and techniques: The recipes for all media used have been previously described (GOLIN and ESPOSITO 1977). Dropout media are synthetic complete media lacking a specific growth requirement (*e.g.*, URA dropout is complete media lacking uracil). MMS plates, used to follow segregation of *rad6-1*, *rad50-1* and *rad52-1*, are YPD plates containing 0.01% MMS (Eastman Kodak); strains containing these mutations do not grow on MMS plates. Standard techniques were used for sporulation, dissection, testing of auxotrophic requirements and prototrophic selection of diploids (ESPOSITO and ESPOSITO 1969; ESPOSITO *et al.* 1969). Segregation of *spo11-1* was followed by complementation tests with known *spo11-1* tester strains; the diploids formed were assayed for their ability to sporulate and/or for their level of meitoic chromosome segregation (KLAPHOLZ and ESPOSITO 1982). Segregation of *rem1-1* and *rem1-2* was followed by a mitotic recombination assay. Spore clones were crossed to

TABLE 1

Properties of mutations used to study rem1

Mutation	Repair defect	Repair epistasis group	Spontaneous mitotic recombination between homologs	Meiotic recombination between homologs
rad6-1	UV sensitive[a, b] X ray sensitive[a]	*RAD6* error-prone repair[a, b]	Present[c, d]	Sporulation defective[e] Recombination defective?[e, f]
rad50-1	X ray sensitive[g]	*RAD52*-double-strand break repair[g]	Present[h]	Sporulation defective[e] Recombination deficient[e, h]
rad52-1	X ray sensitive[g]	*RAD52*-double-strand break repair[g]	Deficient[i, j]	Sporulation defective[e] Recombination deficient[e, h, i]
spo11-1	None[k]	None	Present[l, m]	Sporulation defective[l] Recombination deficient[l]

A review of the properties of repair genes in yeast is given in HAYNES and KUNTZ 1981. Some specific references are: [a] PRAKASH 1974; [b] PRAKASH 1976; [c] SAEKI, MACHIDA and NAKAI 1980; [d] R. E. MALONE, unpublished results; [e] GAME *et al.* 1980; [f] MALONE 1983; [g] GAME and MORTIMER 1974; [h] MALONE and ESPOSITO 1981; [i] PRAKASH *et al.* 1980; [j] MALONE and ESPOSITO 1980; [k] KLAPHOLZ 1980; [l] KLAPHOLZ and ESPOSITO 1982; [m] BRUSCHI and ESPOSITO 1982.

tester strains containing different heteroalleles and drug resistance markers; the resulting diploids were tested for mitotic recombination by replica plating to appropriate selective media. Diploids with the *rem1* mutation exhibit a 10- to 50-fold increase in recombinant papillae when compared with wild-type strains.

Isolation of rem1-2: The *rem1-2* mutation was isolated during a screen for mutations affecting spontaneous mitotic recombination. The diploid RM13 was mutagenized with ethyl methane sulfonate (Eastman Kodak) to a survivor level of 55% and the diploid cells plated on YPD (rich) medium. Approximately 1200 colonies were picked, and small patches were made on YPD "master" plates. After growth, each of these plates was then replicated to a series of dropout media to monitor mitotic gene conversion at the heteroallelic loci present in RM13. In addition, the masters were replicated to media containing either the drug canavanine sulfate (United States Biochemical Corporation) or cycloheximide (Sigma) in order to monitor reciprocal recombination levels (see following data). Twelve clones that exhibited mitotic hyper-rec phenotypes at all diagnostic loci were detected. After single-colony purification and retesting, six mutants retained their hyper-rec phenotype. The six strains were sporulated, and random spores were isolated. When the mutants were outcrossed to wild-type haploids, one of the six mutants gave rise to spores that conferred a hyper-rec phenotype, even though it was present in a heterozygous state. Subsequent analysis of the mutation showed that it segregated in a 2:2 fashion and was semidominant. The level of mitotic recombination in a heterozygote was approximately midway between the wild-type and the homozygous mutant strain. When *CAN1*^{s} mutant strains were replica plated to medium containing canavanine, more *can1*^{r} papillae were observed than in wild-type strains. This suggested that the mutant increased mutation rate, and it was crossed to *rem1-1*. Forty-five tetrads were examined, and all segregated 4:0 for the hyper-rec and hypermutable phenotype. From these data we conclude that the mutation is an allele of the *REM1* locus and have designated it *rem1-2*. Like *rem1-1*, analysis of *rem1-2* diploids showed no effect on meiotic map distances.

Determination of mitotic recombination frequencies: Single colonies from recently constructed diploids

TABLE 2

Genotypes of strains

Diploid	Relevant genotypes
MH1	a *rem1-2 spo11-1 ade2-1 lys2-1 tyr1-2 his7-1 CAN1^s ura3-1 + his1 + met13-c cyh2^r trp5-c leu1-c ade6* α *rem1-2 spo11-1 ade2-1 lys2-1 tyr1-2 his7-2 can1^r ura3-13 hom3 + ade5 met12-d CYH2^s trp5-2 leu1-12 +*
MH2	a *rem1-2 spo11-1 ade2-1 + tyr1-2 his7-1 CAN1^s ura3-1 + his1 + met13-c cyh2^r trp5-c leu1-c* α *rem1-2 spo11-1 ade2-1 lys2-1 tyr1-2 his7-2 can1^r ura3-13 hom3 + ade5 met13-d CYH2^s trp5-c leu1-12*
MH3	a *rem1-2 +* α *+ rad52-1*
MH4	a *rem1-2 ade2-1 lys2-2 tyr1-2 + CAN^s ura3-1 + met13-c rad6-1 trp5-48 leu1-c ade6* α *rem1-2 ade2-1 lys2-1 tyr1-2 his7-2 can1^r ura3-13 ade5 met13-d rad6-1 trp5-2 leu1-12 +*
MH5	a *rem1-1 +* α *+ rad52-1*
MH6	a *+ rad52-1* α *rem1-1 +*
MH7	a *+ rad52-1* α *rem1-1 +*
MH8	a *rem1-1 +* α *+ rad52-1*
MH9	a *rem1-2 +* α *+ rad52-1*
MH10	a *+ rad52-1* α *rem1-2 +*
MH11	a *+ rad52-1* α *rem1-2 +*
MH12	a *rem1-2 +* α *+ rad52-1*

TABLE 2—*Continued*

Diploid	Relevant genotypes
MH13	**a** *spo11-1 ade2-1 lys2-1 tyr1-2 his7-1 + his1 ade5 met13-c cyh2r trp5-c leu1-c ade6* α *spo11-1 ade2-1 lys2-2 tyr1-2 his7-1 hom3 + + met13-c cyh2r trp5-c leu1-c +*
MH14	**a** *spo11-1 ade2-1 lys2-1 tyr1-2 his7-1 + his1 ade5 met13-c cyh2r trp5-c leu1-c ade6* α *spo11-1 ade2-1 lys2-1 tyr1-2 his7-1 hom3 + ade5 met13-c cyh2r trp5-c leu1-c ade6*
MH15	**a** *rem1-2 ade2-1 + tyr1-1 his7-2 CAN1s ura3-13 ade5 met13-d trp5-2 leu1-c* α *rem1-2 ade2-1 lys2-2 tyr1-2 his7-1 can1r ura3-1 + met13-c trp5-c leu1-c*
RM13	**a** *ade2-1 + tyr1-1 CAN1s ura3-13 hom3 + + met13-c cyh2r trp5-c leu1-c ade6* α *ade2-1 lys2-1 + can1r ura3-1 + his1 ade5 + CYH2s trp5-2 leu1-12 +*
RM15	**a** *ade2-1 lys2-2 tyr1-2 his7-1 CAN1s ura-1 + met13-c cyh2r trp5-c leu1-c ade6* α *ade2-1 lys2-1 tyr1-1 his7-2 can1r ura3-13 ade5 met13-d CYH2s trp5-2 leu1-12 +*
RM27	**a** *HO ade2-1 lys2-1 tyr1-1 his7-2 can1r ura3-13 ade5 met13-d CYH2s trp5-2 leu1-12* α *HO ade2-1 lys2-2 tyr1-2 his7-1 CAN1s ura3-1 + met13-c cyh2r trp5-c leu1-c*
RM33	**a** *rem1-2 ade2-1 lys2-1 tyr1-2 his7-2 CAN1s ura3-1 + met13-c cyh2r tryp5-2 leu1-c ade6* α *rem1-2 ade2-1 lys2-2 tyr1-1 his7-1 can1r ura3-13 ade5 met13-dCYH2s trp5-2 leu1-12 +*
RM81	**a** *+ rad50-1* α *rem1-2 +*
RM82	**a** *rem1-2 +* α *+ rad50-1*
RM83	**a** *rem1-2 +* α *+ rad50-1*
RM92	**a** *rem1-2 +* α *+ rad6-1*
RM93	**a** *rem1-2 +* α *+ rad6-1*
RM94	**a** *rem1-2 +* α *+ spo11-1*
RM95	**a** *rem1-2 +* α *+ spo11-1*

were picked into 1 ml of sterile deionized water, and cell concentration was determined by hemacytometer count. Approximately 25 cells/ml were inoculated into 35 ml of YPD broth. The culture was grown at 30° with vigorous shaking until a cell concentration of approximately 2×10^7 cells/ml was reached. Each culture was inoculated from an independent colony. In most cases, several independent diploids were used. After they were harvested by centrifugation, cells were washed twice in an equal volume of sterile 0.2 M phosphate buffer (pH 7.5), sonicated briefly to disrupt clumps and plated at various dilutions on YPD, complete medium, dropout media lacking various auxotrophic requirements, complete medium containing cycloheximide or arginine dropout medium containing canavanine. Plates were scored after 3 days of incubation at 30°. To monitor mitotic gene conversion, we have measured the frequency of prototrophs in diploids containing pairs of auxotrophic alleles (*e.g.*, *his7-1/his7-2*). Such intragenic or heteroallelic recombination occurs primarily by gene conversion in yeast (ESPOSITO and WAGSTAFF 1981). To monitor mitotic crossing over, we measured the frequency of drug-resistant cells in diploids heterozygous for a recessive drug resistance locus. For example, a *CAN1^s/can1^r* diploid is sensitive to canavanine. A crossover event between the *CAN1* locus and the centromere can lead to a homozygous *can1^r/can^r* cell. Loss of the chromosome containing the dominant, sensitive allele would also generate a resistant cell. Where possible, we attempted to control for this by checking for expression of recessive alleles on the same chromosome as the drug-resistant locus. We examined both centromere-proximal recessive markers and recessive markers on the opposite arm wherever possible. In those strains that could be tested, none of 50 colonies (resistant to either drug) examined showed any evidence for chromosome loss.

RESULTS

The hyper-rec phenotype of rem1 *mutations does not depend upon the* SPO11 *meiotic recombination function*: To determine whether the hyper-rec phenotype of the *rem1-2* mutation was dependent upon meiotic recombination functions, we constructed two diploids that were heterozygous for *rem1-2* and *spo11-1*. Dissection of these diploids generated spores that were 88% viable (Table 3A). Analysis of the genotypes of the spores produced indicated that one-quarter of the segregants were *rem1-2 spo11-1* (Table 3B). Tetrad analysis also gave no indication of linkage. To determine the mitotic recombination phenotype of the double mutant, diploids homozygous for *rem1-2* and *spo11-1* were constructed containing a number of heteroallelic loci and two recessive drug resistance loci (to monitor gene conversion and crossing over, respectively; see MATERIALS AND METHODS). The data in Table 4 indicate that the *spo11-1* mutation does not eliminate the high levels of mitotic recombination caused by the *rem1-2* mutation. The double mutant exhibits an increase in recombination frequency at some loci compared with *rem1-2* alone (see DISCUSSION). These data also indicate that the *rem1-2* mutation stimulates recombination to about the same extent as the *rem1-1* allele.

Interactions between the rem1-2 *mutation and the* RAD50 *and* RAD52 *loci*: Because the hyper-rec effect of a *rem1* mutation was not prevented by inactivating a gene (*SPO11*) required for meiotic recombination, we examined the effect of the *rad52-1* mutation, since *rad52-1* eliminates both meiotic and mitotic recombination. We found, however, that the double mutant could not be constructed (Table 5). Diploids heterozygous for *rem1-2* (or *rem1-1*) and *rad52-1* had rather poor spore viability, and no double mutants have ever been detected. We infer that *rem1 rad52-1* strains are not viable.

Since the *SPO11* gene product (a meiotic Rec function) was not required for *rem1* strains, the lethality of the *rad52* double mutant could be most easily understood in terms of the mitotic defects conferred by *rad52*. To distinguish

TABLE 3

Analysis of $\frac{\text{rem1-2}}{\text{REM1}}\ \frac{\text{SPO11}}{\text{spo11-1}}$ *diploids*

A. Viability of spores produced

No. of diploids analyzed	Tetrad survival patterns (Viable:inviable)					
	4:0	3:1	2:2	1:3	0:4	% viable
2	21	11	1	1	0	88

B. Genotypes of spores produced

No. of spores analyzed	*rem1-2 SPO11*	*REM1 spo11-1*	*rem1-2 spo11-1*	*REM1 SPO11*
76	20	20	18	18

The diploids examined were RM94 and RM95.

between the mitotic recombination defect and the mitotic repair defect caused by *rad52-1*, we attempted to construct *rad50 rem1* strains. The *RAD50* gene is in the *RAD52* repair group, but *rad50* mutations do not eliminate spontaneous mitotic recombination. Thus, if the *rad50-1 rem1* double mutant were alive, it would suggest that the mitotic recombination defect of *rad52-1* was the reason that *rad52 rem1* strains were inviable. The data in Table 6 indicate that the *rad50 rem1* double mutant combination is lethal. We infer from this that it is the repair defect in the *RAD52* epistasis group, or repair pathway, that causes the inviability of both *rad50* and *rad52* with *rem1*.

The RAD6 *gene is not required for the hyper-rec phenotype of* rem1: The *RAD6* gene is not required for mitotic recombination but is essential for the repair of UV damage as well as damage by many chemical agents. Current data suggest that *RAD6* acts in a different epistasis group or repair pathway than do the *RAD50* and *RAD52* genes (HAYNES and KUNZ 1981). Therefore, we asked whether *rem1-2 rad6-1* double mutants were viable (Table 7). The double mutant was clearly alive, which allowed us to ask whether it was still hyper-rec. Table 8 reveals that a *rad6-1* mutation does not inhibit the hyper-rec phenotype of the *rem1-2* mutation. Thus, the *RAD6* pathway is neither required for viability nor recombination in *rem1* strains.

The rem1 *mutation does not reverse the meiotic defect of either* spo11-1 *or* rad6-1: The *spo11-1* mutation has the meiotic phenotype of reduced sporulation and greatly reduced spore viability (≤ 1%) (KLAPHOLZ and ESPOSITO 1982). The *rad6-1* mutation totally eliminates sporulation (GAME *et al.* 1980). For both mutations it has been proposed that the meiotic defect is a deficiency in genetic recombination. All data for *spo11-1* confirm the defect, whereas the available data for *rad6-1* suggest that its primary lesion may not be in recombination (MALONE 1983). We wondered whether the increased recombination levels in mitosis exhibited by *rem1* mutant strains might allow productive sporulation in the presence of *rad6-1* or *spo11-1* mutations. Doubly mutant diploids were exposed to sporulation medium and examined for sporulation and spore viability

TABLE 4

Spontaneous mitotic recombination frequencies in spo11 *and* rem1 *diploids*

Diploid genotype	No. of cultures	Recombination frequency × 10^{5}[a]								
		Intragenic							Intergenic	
		lys2-1/*lys2-2*	*tyr1-1*/*tyr1-2*	*his7-1*/*his7-2*	*ura3-1*/*ura3-13*	*met13-c*/*met13-d*	*trp5-c*/*trp5-2*	*leu1-c*/*leu1-12*	*can1*^{r}/*CAN1*^{r}	*cyh2*^{r}/*CYH2*^{r}
+/+	14	0.40	0.30	0.36	0.51	4.2	3.1	3.1	22	41
rem1-1[b]/*rem1-1*	10	3.8(9.6)	4.0(13)		7.5(15)	55.1(13)	30.4(9.8)	44.6(15)	160(7.3)	
rem1-2/*rem1-2*	3	8.1(20)	4.2(14)	8.5(24)	10(20)	28(6.7)	26(8.4)	69(22)	180(8.2)	320(7.8)
rem1-2 spo11-1/*rem1-2 spo11-1*	10		2.8(9.3)	2.7(7.5)	23(45)	81(19)	340(110)	200(65)	790(36)	2800(68)

[a] Values given are geometric mean frequencies. The numbers within parentheses indicate the relative increase over wild-type frequencies.
[b] The *rem1-1* recombination frequencies are taken from GOLIN 1979.

TABLE 5

Analysis of $\frac{\text{rem1}}{\text{REM1}}\ \frac{\text{RAD52}}{\text{rad52}}$ *diploids*

A. Viability of spores produced

Diploid	Tetrad survival patterns: Viable:inviable 4:0	3:1	2:2	1:3	0:4	% Viable
rem1-1 / *REM1* *RAD52* / *rad52-1* diploids						
MH5	6	24	9	1	0	72
MH6	0	3	7	0	0	58
MH7	4	19	12	5	0	64
MH8	2	4	2	2	0	65
Total	12	50	30	8	0	67
rem1-2 / *REM1* *RAD52* / *rad52-1* diploids						
MH3	4	25	18	2	0	66
MH9	0	3	6	1	0	55
MH10	3	11	5	1	0	70
MH11	8	22	16	2	0	69
MH12	2	2	4	1	1	58
Total	17	63	49	7	1	66

B. Genotypes of spores produced

Diploid genotype	Total no. of spores analyzed	No. of viable spores	Viable spore genotype: *rem1 RAD52*	*REM1 rad52-1*	*rem1 rad52-1*	*REM1 RAD52*
rem1-2 / *REM1* *RAD52* / *rad52-1*	548	375	124	118	0	133
rem1-1 / *REM1* *RAD52* / *rad52-1*	400	260	101	84	0	75

Diploids heterozygous for *rem1* and *rad52* were sporulated and dissected by micromanipulation. After 3 days, spores were examined for viability. Viable spores were tested for *rem1* and *rad52* as described in text.

(Table 9). The *rem1* hyper-rec phenotype, even though it elevates mitotic recombination as much as 25-fold, does not overcome the meiotic defects of either mutation.

DISCUSSION

The *rem1-1* and *rem1-2* mutations cause increased frequencies of spontaneous mitotic recombination (a hyper-rec phenotype). One possibility for the increase in recombination is the induction of meiotic recombination functions that are

TABLE 6

Analysis of $\frac{\text{rem1-2}}{\text{REM1}}\ \frac{\text{RAD50}}{\text{rad50-1}}$ *diploids*

A. Viability of spores produced

	Tetrad survival patterns					
	Viable:inviable					
Diploid	4:0	3:1	2:2	1:3	0:4	% Viable
RM81	0	7	3	0	0	68
RM82	5	17	10	0	1	69
RM83	1	9	13	3	0	58
Total	6	33	26	3	1	65

B. Genotypes of spores produced

Diploid genotype	Total no. of spores analyzed	No. of viable spores	Viable spore genotype			
			rem1-2 RAD50	*REM1 rad50-1*	*rem1-2 rad50-1*	*REM1 RAD50*
$\frac{\textit{rem1-2}}{\textit{REM1}}\ \frac{\textit{RAD50}}{\textit{rad50-1}}$	276	166	56	55	0	53

Diploids heterozygous for *rem1-2* and *rad50-1* were sporulated and dissected by micromanipulation. After 3 days, spores were examined to determine viability. Viable spores were picked and tested for the presence of *rem1* and *rad50-1* as described in MATERIALS AND METHODS.

TABLE 7

Analysis of $\frac{\text{rem1-2}}{\text{REM1}}\ \frac{\text{RAD6}}{\text{rad6-1}}$ diploids

A. Viability of spores produced

	Tetrad survival patterns					
	Viable:inviable					
No. of diploids analyzed	4:0	3:1	2:2	1:3	0:4	% Viable
2	22	8	1	0	0	92

B. Genotypes of spores produced

No. of spores analyzed	*rem1-2 RAD6*	*REM1 rad6-1*	*rem1-2 rad6-1*	*REM1 SPO11*
56	19	14	12	11

The diploids analyzed were RM92 and RM93. After sporulation and dissection, viable spores were tested for the presence of *rem1-2* and *rad6-1* as described in the text.

not normally present (at least in high levels) during mitosis. For example, modification of an operator (or promoter) for a positive regulator of meiotic Rec functions could lead to semidominant production of those functions during mitosis. (In this hypothesis, an *ad hoc* explanation of the increased mutation frequency caused by *rem1* is that it would be due to the presence of unusual

TABLE 8

Spontaneous mitotic recombination in rem1 rad6 *double mutants*

Diploid genotype	No. of cultures	Mean recombination frequency × 10^5				
		ura3-1 / *ura3-13*	*met13-c* / *met13-d*	*trp5-c* / *trp5-2*	*leu1-c* / *leu1-12*	*can1^r^* / *CANL^s^*
rem1-2 *rad6-1* / *rem1-2* *rad6-1*	3	4.9 (9.6)	72 (17)	900 (290)	32 (10)	980 (45)

Mitotic recombination frequencies are the geometric mean of the three cultures. The values in parentheses are the relative increase over wild-type recombination frequencies. For wild-type and *rem1* frequencies refer to Table 4. The diploid used in these experiments was MH4.

TABLE 9

Sporulation of rem1 spo11 *and* rem1 rad6 *double mutants*

Diploid genotype	No. diploids examined	% Sporulation	Spores	
			% Viable	No. examined
rem1-2 / *rem1-2*	5	69	82	290
rad6-1 / *rad6-1*	4	0.2	0	10
spo11-1 / *spo11-1*	2	34	0	120
rad6-1 *rem1-2* / *rad6-1* *rem1-2*	2	<0.2	0	3
spo11-1 *rem1-2* / *spo11-1* *rem1-2*	2	38	0	100

The degree of sporulation was determined by microscopic examination of at least 150 cells per diploid. Asci were then dissected, and viability of the spores was determined after 3 days. The diploids were made from segregants of intercrosses of diploids heterozygous for *rem1-1*, *rem-2* and *spo11-1* or *rad6-1*.

DNA metabolic enzymes during mitosis.) Since the *spo11-1* mutation did not prevent the hyper-rec phenotype of *rem1*, we feel that induction of the meiotic recombination system by *rem1* mutations is unlikely. Of course, it is possible that meiotic functions other that *SPO11* are utilized in the enhancement of recombination caused by *rem1*. Nonetheless, it is true that the "normal" complete meiotic recombination system cannot be responsible for the increased level of mitotic recombination conferred by *rem1*.

An alternative explanation of the *rem1* hyper-rec phenotype is that DNA lesions are created that cause induction of repair "system(s)." When these repair systems act on the lesions, they lead to the production of recombinants. (The hypermutable phenotype of *rem1* mutations would then be explainable by simply

assuming that the lesions were also mutagenic.) There are at least three repair systems, pathways or "epistasis groups" in yeast; several of these pathways apparently overlap, and no clear scheme has emerged that allows all of the repair mutants to be unambiguously classified. However, most mutants can be placed into three categories as discussed in the introduction of this paper. We have utilized repair mutants that fall into two of these categories. The *RAD50* and *RAD52* genes are in the "double-strand break" repair pathway, whereas the *RAD6* gene is in the error-prone repair pathway (HAYNES and KUNZ 1981). It is reasonable to assume that double-strand breaks are repaired via a recombination-repair mechanism. Recently, SZOSTAK *et al.* (1983) have proposed a model for yeast recombination that incorporates a double-stranded break as central intermediate. Although their model addressed the properties of meiotic recombination, it was motivated by data obtained from mitotic studies of plasmids containing double-strand breaks. It should be noted that cells that have been transformed with plasmid DNA containing double-stranded breaks may utilize recombination processes resembling those in cells containing chromosomal double-strand breaks caused by radiation.

The inviability of *rem1 rad50-1* and *rem1 rad52-1* double mutants strongly indicates that the *RAD52* "pathway" is indispensible in the presence of *rem1* mutations. If this pathway is one that acts by a recombinational mechanism, this is consistent with the hyper-rec phenotype of *rem1* and lends credence to the contention that *rem1* leads to lesions in the DNA that must be repaired for the cell to survive. We presume, therefore, that the increased levels of recombination observed in *rem1* mutants are associated with repair and are essential. This is similar to the observations made in *E. coli* for *lig*, *polA* and *dam* mutations (KONRAD and LEHMAN 1975; MARINUS and MORRIS 1975; BALE, D'ALARCO and MARINUS 1979). It is interesting to note that, although both *RAD50* and *RAD52* are required for X-ray repair, *RAD50* is not necessary for spontaneous mitotic recombination. This suggests that the putative recombination event that takes place in recombination-repair may not be equivalent to "normal" spontaneous mitotic recombination. Consistent with this idea is the observation that *RAD50* is required for *induced* mitotic recombination (SAEKI, MACHIDA and NAKAI 1980). It is tempting to speculate that the difference between X-ray recombination-repair and normal spontaneous mitotic recombination may be the difference between recombination initiated by double-strand breaks caused by X rays and recombination initiated by other means such as single-strand nicks or unbroken homologous strand invasion (MESELSON and RADDING 1975; CASSUTO *et al.* 1981; ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981). If the requirement in *rem1* mutant strains for the *RAD52* repair pathway were due to the creation of double-strand breaks, then DNA from *rem1* strains should have a smaller average molecular weight than wild-type DNA. We are currently analyzing DNA from *rem1* mutants with a variety of physical techniques.

A third hypothesis to explain the effect of *rem1* mutations on recombination is that it leads to the induction of a completely new recombination system. A precedent for this kind of event exists in *E. coli*, in which the *sbcA* and *sbcB* mutations create new recombination pathways (CLARK 1973). We feel that this

third possibility is less likely, because strains containing a *rem1* mutation do require *RAD50*, *RAD52* and presumably the entire recombination-repair pathway. Additionally, *rem1* provided no help to *spo11-1* cells in meiosis; if *rem1* turned on a new Rec pathway, it might well supplement the recombination defect in *spo11-1* cells.

The data in Table 3 indicate that more mitotic recombination occurs in a *rem1 spo11* diploid than in the presence of *rem1* alone. Note that, with the exception of the *tyr1* and *his7* loci, all other loci exhibit frequencies two- to tenfold higher in the double mutant. KLAPHOLZ and ESPOSITO (1982) have found that *spo11-1* has little or no effect on mitotic recombination. BRUSCHI and ESPOSITO (1982) suggest that *spo11-1* may specifically increase mitotic crossing over but not intragenic recombination. Although it is unclear whether the *rem1* and *spo11* mutations are acting synergistically, it *is* clear that double-mutant strains do exhibit a hyper-rec phenotype. Thus, the *SPO11* function, which is required for meiotic recombination to occur, is not necessary for the hyper-rec phenotype of *rem1* (Table 4).

Although the hyper-rec phenotype exhibited by the *rad6-1 rem1-2* double mutant is consistent with the meiotic recombination system not being induced by *rem1* mutations, it does not provide strong support for this conclusion. Although GAME *et al.* (1980) suggested that *RAD6* was required for meiotic recombination, subsequent evidence indicates that it may be required for some other aspect of meiosis (MONTELONE, PRAKASH and PRAKASH 1981; MALONE 1983). The viability of the *rad6-1 rem1-2* double mutant gives us the opportunity to test whether the hypermutability of *rem1* strains is dependent upon the *RAD6* error-prone repair system. We are testing this by analyzing mutation rates in the double mutant.

In conclusion, we feel that mutating the *REM1* locus in yeast leads to the expression of a new or altered function(s) that, in turn, may lead to lesions in the DNA. We propose that these lesions lead to breaks in the DNA that, if not repaired by the *RAD52* recombination-repair pathway, cause the cells to die. The semidominance of the two *rem1* mutant alleles would occur if the mutant allele positively controls a "new" function that leads to lesions. Alternatively, it could be due to a mutant enzymatic function that can compete with the wild-type product in *rem1/REM1* heterozygotes. Finally, the *REM1* gene product may be a component of a multienzyme complex. The *rem1*-defective product would still be able to form the complex but would confer mutant properties upon it. Complexes containing *rem1* product and complexes containing *REM1* product could compete equally well. The ability of a single mutation to be semidominant, mutagenic and recombinogenic has significant portents for a number of interesting problems in higher eukaryotic systems. Perhaps one of the most relevant is the relationship between mutagenesis and carcinogenesis in mammalian systems. A single event creating a mutation such as *rem1* would allow not only an increased frequency of mutations (most of which are recessive) but would also cause them to become homozygosed by mitotic recombination.

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