MEIOTIC RECOMBINATION AND SPORULATION IN REPAIR-DEFICIENT STRAINS OF YEAST

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ABSTRACT

A genetic system designed to monitor recombination and sporulation in various repair-deficient yeast strains was constructed. Variously heterozygous at seven or eight sites distributed across the genome, the system facilitated sensitive detection of changes in frequency or pattern of meiotic recombination. Ten *rad* mutants sensitive primarily to UV-irradiation and without terminal blocks in the sporulation process were studied. Seven were defective in excision repair (*rad1, rad2, rad3, rad4, rad10, rad14* and *rad16*), and three were defective in mutagenic repair (*rad5, rad9* and *rad18*). Individually, each mutant displayed behavior consistent with an orthodox meiosis including a wild-type meiotic recombination profile with respect to gene conversion, PMS and intergenic map distances. Accordingly, we conclude that these mutants are without major effect on meiotic heteroduplex formation or correction. However, certain combinations of excision-defective mutants with *rad18* exhibited marked ascosporal inviability. Tetraploids homozygous for *rad1* and *rad18* produce a large proportion of diploid spores containing a recessive lethal.

DNA metabolism is defined by the many processes in which DNA molecules participate, including replication, repair, recombination and mutagenesis. The analysis, isolation and characterization of particular mutants continue to uncover complex interrelationships among these processes. Our earlier studies on gene conversion led us to inquire concerning the relationship between DNA repair and meiosis in yeast. Mutations at several *rad* (radiation sensitive) loci in the *RAD3* and *RAD6* epistasis groups lead to increases in spontaneous and induced gene conversion rates, whereas mutations in the predominantly X-ray-sensitive *RAD52* epistasis group generally abolish both gene conversion and reciprocal exchange (reviewed in KUNZ and HAYNES 1981). Among the many radiation-sensitive mutants of yeast are several that generate only infrequent or inviable ascospores. The role of these genes in meiotic DNA metabolism is the subject of ongoing investigations (*e.g.*, GAME *et al.* 1980; PRAKASH *et al.* 1980). RESNICK, GAME and STASIEWICZ (1983) and RESNICK, STASIEWICZ and GAME (1983) found that UV-irradiated *rad1* strains, unable to excise pyrimidine dimers, display an almost complete lack of intergenic recombination and a significant decrease in gene conversion during meiosis. However, untreated cells were essentially normal in these experiments. This report concerns only mutant *rad* genes without terminal blocks in the sporulation process. We sus-

pected some of these mutant genes might exhibit subtle meiotic effects and, thus, be more suitable for study. For example, excision-defective *rad* mutants might impair or diminish meiotic heteroduplex correction and thus produce an increase in postmeiotic segregation (*PMS*) at the expense of gene conversions and restorations, even in the absence of UV-induced damage. This would be expected if the same gene products mediated both pyrimidine dimer excision and correction of normal but mismatched bases in meiotic heteroduplex DNA.

Excision-defective and other UV-sensitive mutants have been studied previously for possible meiotic effects by SNOW (1968), DICAPRIO and HASTINGS (1976) and others. These studies reported little if any effect on spore viability or meiotic recombination. But DICAPRIO and HASTINGS' (1976) study excepted, such efforts focused largely on reciprocal recombination rather than gene conversion. Here, we report detailed studies concerning the effects of several UV-sensitive mutants on reciprocal recombination, gene conversion, postmeiotic segregation and spore viability in unirradiated cells.

MATERIALS AND METHODS

Media and yeast strains: YEPD, GNA, synthetic complete, sporulation and omission media were described previously (CAMPBELL, FOGEL and LUSNAK 1975).

All *rad* mutants were derived from the strains listed in Table 1. The *rad5* and *rad9* mutants were kindly provided by LOUISE PRAKASH. All other *rad* mutants were derived from strains constructed by B. S. COX and others and supplied by the Berkeley Yeast Genetic Stock Center.

Procedures for genetic analysis: Standard procedures were used for mass mating, zygote isolation and ascus dissection by the plate dissection method (FOGEL *et al.* 1979). Tetrads were dissected directly onto GNA plates (solidified with 25 g of agar/liter) which subsequently served directly as master plates for replica platings onto various diagnostic media. The procedure preserved the integrity of the spore clones and allowed detection of PMS as half-sectored clones. All putative PMS spore cones were confirmed by sampling the master plate from the wild-type and mutant sides of the sectored clone and retesting on diagnostic media. Thus, tetrad segregations are reported as octads. Normal segregations are 4:4. Gene conversions are recorded as 6:2 or 2:6 and postmeiotic segregations as 5:3 or 3:5. The first integer in each ascal segregation class denotes the number of spores carrying the wild-type allele, and the second integer specifies the number of spores carrying the mutant marker. Reconstruction experiments, involving the formation of artificial sectors by micromanipulation, established the reliability of PMS detection for all markers used. Complementation tests were performed by spraying a thick suspension of tester cells from an atomizer onto a replica of the original master plate (FOGEL *et al.* 1979).

Expectations for tetrad spore survival classes were calculated from the binomial expansion $(p + q)^4$, where p is the proportion of viable spores and q is the proportion of inviable spores.

Radiation sensitivity: Radiation sensitivity was scored by a "double print" method. A replica-print of the master plate is immediately replica-plated onto a YEPD plate and this plate is irradiated. The protocol reduces the shielding of one cell by another. All radiation-sensitive phenotypes for the 18 *rad* alleles employed in this study were routinely scored by relative growth response following UV irradiation for 5 sec with a GE germicidal lamp which delivered 8 J/m^2/sec. In cases in which differential sensitivity to UV was employed in scoring the ascosporal clones of hybrids in which more than one *rad* gene was segregating, a lower dose, 5 sec at 0.7 J/m^2/sec, was used in addition. For scoring X-ray sensitivity of *rad18-2*, a total X-ray dose of 48 krads was delivered by a Machlett OEG 60 tube with a beryllium window. The X-ray apparatus was made available by ROBERT MORTIMER.

For crosses in which two UV-sensitive *rad* genes were heterozygous, segregants were scored according to some combination of (1) sensitivity to two different doses of UV, (2) X-ray sensitivity

TABLE 1

Strains

Designation	Genotype
X12-9B	*MATα ade2-1 rad1-1*
X12-6B	*MATa ade2-1 rad1-1*
X16-18C	*MATα ade2-1 rad1-1*
X16-9C	*MATa ade2-1 rad2-1*
X36B-3A	*MATα ade2-1 rad3-2*
X36B-3C	*MATa ade2-1 rad3-2*
X9B-7B	*MATα ade2-1 rad4-4*
X9B-7D	*MATa ade2-1 rad5-5*
ED86-56B	*MATα ade2-1 rad5-5*
ED86-60D	*MATa ade2-1 rad5-5*
ED85-11D	*MATα ade2-1 rad9-1*
ED85-22B	*MATa ade2-1 rad9-1*
X19-20B	*MATa ade2-1 rad10-1*
X19-20C	*MATa ade2-1 rad10-1*
JG 14	*MATα ade2-1 rad14-2*
X14-10A	*MATa ade2-1 rad16-1*
BC18	*MATα ade2-1 rad18-2 lys*
g683-1d	*MATa ade2-1 rad1-1 leu2 hom3-10 his1-7*
g683-3a	*MATα rad18-2 his1-1 trp2*
g721-2	*MATa/MATa leu2/+ ade2/+ can1-1/+ hom3/+ his1-7/his1-1 +/trp2*
g721-7	*MATα/MATα leu2/+ ade2/+ can1-1/+ hom3/+ his1-7/his1-1 +/trp2*
X3-4C	*trp1-1 ura3-1 MATα ade2-1 ade8-10 his2 met13 lys1-1 thr1*
X3-1A	*trp1-1 ura3-1 MATa ade2-1 ade8-10 his2 met13 lysl1-1 thr1*
ED66-20D	*trp1-1 ura3-1 MATα ade2-1 ade8-18 his2 met13 arg4-16 thr1 lys1-1*
ED66-20A	*trp1-1 ura3-1 MATa ade2-1 ade8-18 his2 met13 arg4-16 thr1 lys1-1*

(if one of the two markers was sensitive to X-rays as well as to UV) and (3) complementation testing. Any putative double-mutant segregant that was to be employed in further studies was confirmed as such by outcrosses to wild-type strains and subsequent observation of digenic segregation for UV sensitivity among the tetrads.

Control strains: For every *rad* mutant studied, otherwise comparable diploids homozygous for the *rad* mutation or the corresponding wild-type allele were derived from a parental hybrid heterozygous for the *rad* mutation. Either matings between segregants of the heterozygote or mitotic sectors provided the pair of diploids. To isolate reciprocal mitotic recombinants, cultures were grown to stationary phase and unbudded cells were spread at 50 cells/plate and X-rayed with a total dose of 10 krads to induce recombination. After colonies had grown, replicas of the plates were UV-irradiated and, subsequently, those colonies corresponding to prints half-sectored for UV resistance/sensitivity were further characterized. Thus, a closely related or isogenic wild-type control was included in the study of each mutant.

Selection of ochre suppressors of rad2-1: The *rad2-1* allele is a ochre mutation (J. GAME, personal communication). We selected lysine prototrophs from a diploid strain carrying *rad2-1* and *lys1-1*, a known ochre mutation, in homozygous condition. These lysine-independent derivatives had simultaneously acquired increased UV resistance, and the lysine independence and increased UV resistance cosegregated in subsequent tests.

Test for meiotic nondisjunction in a rad1-1 *and* rad18-2 *double mutant*: A zygotic clone of ED101 (*rad1-1/rad1-1, rad18-2/rad18-2*) was sporulated and 16 unselected tetrads were dissected. Thirty-eight spore clones survived and each was crossed to tester strains. In the resulting diploids, ten chromosomes carried heterozygous markers and could be monitored for aneuploidy. Ten tetrads were dissected for each mating. Segregations expected for a +/+/− trisome are mostly of the $4^+:0^-$ phenotypic class along with some segregations of the $3^+:1^-$ and $2^+:2^-$ types.

RESULTS

Single rad *mutants*: Seven *rad* mutants from the excision repair (*RAD3*) epistasis group (*rad1-1, rad2-1, rad3-2, rad4-4, rad10-1, rad14-2* and *rad16-1*) and three *rad* mutants from the *RAD6* epistasis group (*rad5-5, rad9-1* and *rad18-2*) (see KUNZ and HAYNES 1981 for review) were examined for meiotic effects. For each locus studied, a homozygous mutant diploid and wild-type control were derived from a diploid heterozygous for the *rad* mutant, sometimes by mating segregants and sometimes by mitotic recombination. The meiotic behavior of the strains was assayed by investigating sporulation, spore viability and recombination. For the latter, the following aspects were examined: gene to centromere map distance and frequency and pattern of gene conversion and postmeiotic segregation. Included in the strains were several genetic markers, distributed throughout the genome, which normally display high conversion frequencies (*i.e.*, 6–18%) but low PMS frequencies [*i.e.*, 0–3% of conversion tetrads depending on the marker (FOGEL *et al.* 1979)]. These markers were chosen to provide for the sensitive detection of increases in PMS frequency. For each pair of strains, the frequency and parity of conversion and PMS were evaluated, the second division segregation frequency was calculated for centromere-linked markers and the map distance was estimated for linked markers. The corresponding homozygous wild-type strain served as the control against which the homozygous mutant strain was compared for statistically significant differences. Complete data for two excision repair mutants, *rad1-1* and *rad4-4*, and two of the *RAD6* group, *rad9-1* and *rad18-2*, are presented in Tables 2–5, respectively. Table 6 summarizes the available data for all mutants. Each of the ten individual *rad* mutants examined exhibits apparently normal meiosis with respect to degree of sporulation, spore viability and meiotic recombination, including frequency and pattern of gene conversion and PMS. The high frequency of 0:4 and 4:0 segregations for *ade8* in the *rad18-2/rad18-2* diploid is attributed to mitotic recombination. The frequency of mitotic recombination is greatly increased in *rad18* strains (BORAM and ROMAN 1976).

Double mutants: rad1-1 *and* rad18-2: The meiotic effect of blocking both major systems concerned with UV damage repair was investigated. From segregants of a strain (ED58) doubly heterozygous for *rad1-1* and *rad18-2*, we generated three diploids homozygous for both mutations and two homozygous wild-type diploids. The homozygous double-mutant strains displayed reduced ascospore viability (approximately 36%), compared with 95% ascospore viability in the wild-type controls. We have repeated this observation using several *rad1 rad18* strains of different genetic background (results not shown). This is in striking contrast to our earlier conclusion that homozygosis for *rad1* and *rad18* individually is without effect on meiosis. Representative data for such strains are presented in Table 7.

There is no correlation between the percentage of sporulated cells and *rad* genotype. All of the strains exhibit abundant sporulation.

Other double-mutant combinations: Given the meiotic effect of the *rad1 rad18* double mutant, we undertook to determine whether other double-mutant com-

TABLE 2

Meiotic segregations in unselected tetrads from a rad1-1/rad1-1 *diploid and isogenic wild type*

	Strain[a]																			
	X22Rr										X22RR									
	Ascus ratio									% pms	Ascus ratio									% pms
Marker	4:4	8:0	0:8	6:2	2:6	5:3	3:5	Other	%BCF	conv.	4:4	8:0	0:8	6:2	2:6	5:3	3:5	Other	%BCF	conv.
his2	91	0	0	10	8	0	0	0	16.5	0	102	0	0	6	9	0	0	0	12.8	0
met13	102	0	0	4	2	1	0	0	6.4	14.3	106	0	0	4	7	0	0	0	9.4	0
lys1-1	98	0	0	6	5	0	0	0	10.1	0	110	0	0	1	5	0	1	0	6.0	14.3
thr1	100	0	0	4	4	0	0	1[b]	9.2	20.0	111	0	0	1	5	0	0	0	5.1	0
trp1-1	109	0	0	0	0	0	0	0	0	0	117	0	0	0	0	0	0	0	0	0
ura3-1	109	0	0	0	0	0	0	0	0	0	115	0	0	2	0	0	0	0	1.7	0
*MAT***a**	109	0	0	0	0	0	0	0	0	0	117	0	0	0	0	0	0	0	0	0
%SDS *ura3*						13.8										17.4				
%Spore viability						93.4										94.8				

BCF, proportion of aberrant segregations; $\frac{\%\ \text{pms}}{\text{conv.}}$, proportion of aberrant segregations showing PMS.

[a] Strains X22Rr and X22RR represent reciprocal mitotic sectors from a +/*rad5* diploid strain.

[b] Aberrant 4+:4− segregation.

TABLE 3

Meiotic segregations in unselected tetrads from a rad4-4/rad4-4 *diploid and isogenic wild type*

	Strain[a]																			
	rad4-4/rad4-4 (X17Rr)										+/+ (X17RR)									
	Ascus ratio									% pms	Ascus ratio									% pms
Marker	4:4	8:0	0:8	6:2	2:6	5:3	3:5	Other	%BCF	conv.	4:4	8:0	0:8	6:2	2:6	5:3	3:5	Other	%BCF	conv.
ade8-10	88	1	1	5	4	0	0	0	9.3	0	75	0	12	5	4	0	0	0	10.7	0
his2	86	0	0	10	3	0	0	0	13.1	0	82	0	1	9	4	0	0	0	13.7	0
met13	91	1	1	3	3	0	0	0	6.2	0	89	0	0	3	4	0	0	0	7.3	0
thr1	88	1	0	4	6	0	0	0	10.2	0	84	1	1	5	5	0	0	0	10.6	0
lys1-1	94	1	0	2	2	0	0	0	4.1	0	90	0	0	3	3	0	0	0	6.2	0
trp1-1	99	0	0	0	0	0	0	0	0	0	96	0	0	0	0	0	0	0	0	0
ura3	96	0	0	2	1	0	0	0	3.1	0	96	0	0	0	0	0	0	0	0	0
*MAT***a**	97	0	0	0	2	0	0	0	2.0	0	95	0	0	0	1	0	0	0	1.0	0
%SDS for *ura3*						19.8										11.4				
%Spore viability						93.3										93.8				

[a] Strains X17Rr and X17RR represent reciprocal mitotic sectors from a +/*rad4-4* strain.

TABLE 4

Meiotic segregations in unselected tetrads from a rad9/rad9 *diploid and isogenic wild type*

	Strain[a]																			
	rad9-1/rad9-1 (ED99)										+/+ (ED100)									
	Ascus ratio									% pms	Ascus ratio									% pms
Marker	4:4	8:0	0:8	6:2	2:6	5:3	3:5	Other	%BCF	conv.	4:4	8:0	0:8	6:2	2:6	5:3	3:5	Other	%BCF	conv.
ade8-18	102	0	0	1	0	4	0	0	4.7	80.0	102	0	17	1	4	2	4	0	9.7	54.6
his2	95	2	1	10	9	0	0	0	16.7	0	102	0	0	15	13	0	0	0	21.5	0
met13	108	0	0	8	1	0	0	0	7.7	0	119	0	0	3	8	0	0	0	8.5	0
lys1-1	108	0	0	3	4	2	0	0	7.7	22.2	113	0	0	10	7	0	0	0	13.1	0
thr1	109	0	0	4	4	0	0	0	6.8	0	121	0	0	5	4	0	0	0	6.9	0
arg4-16	105	0	0	5	4	1	2	0	10.3	25.0	123	0	0	3	1	2	0	1	6.2	50.0
trp1-1	117	0	0	0	0	0	0	0	0	0	130	0	0	0	0	0	0	0	0	0
ura3	117	0	0	0	0	0	0	0	0	0	128	0	0	1	1	0	0	0	1.5	0
*MAT***a**	114	0	0	2	1	0	0	0	2.6	0	130	0	0	0	0	0	0	0	0	0
%SDS for *ura3*					10.3											9.4				
%SDS for *arg4-16*					26.7											27.6				
Map distance, *arg4-16-thr1* (cM)					12.4											10.0				
%Spore viability					95.6											97.8				

[a] ED99 and ED100 were constructed from segregants of a +/*rad9-1* diploid.

TABLE 5

Meiotic segregations in unselected tetrads from rad18/rad18 *diploids and isogenic wild type*

	Strain[a]																			
	rad18-2/rad18-2 (H18Rr)										*rad18-2/+* (H18R+)									
	Ascus ratio									% pms	Ascus ratio									% pms
Marker	4:4	8:0	0:8	6:2	2:6	5:3	3:5	Other	%BCF	conv.	4:4	8:0	0:8	6:2	2:6	5:3	3:5	Other	%BCF	conv.
ade8-10	217	53	22	15	18	0	0	0	13.2	0	238	0	8	17	11	0	0	0	10.5	0
his2	263	1	0	26	35	0	0	0	18.8	0	237	1	1	19	16	0	0	0	12.9	0
met13	298	2	3	13	9	0	0	0	6.9	0	255	1	0	11	7	0	0	0	6.6	0
arg4-16	286	0	0	16	5	4	13	1[b]	12.3	47.5	247	0	0	8	9	1	8	1[c]	10.2	39.3
trp1-1	323	0	0	1	1	0	0	0	0.6	0	273	0	0	0	1	0	0	0	0.4	0
*MAT***a**	317	0	0	5	3	0	0	0	2.5	0	268	0	0	2	3	0	0	0	1.8	0
%SDS for *arg4-16*						27.0										26.8				
%Spore viability						90.0										91.4				

[a] These strains represent nonreciprocal mitotic sectors from a +/*rad18* heterozygous diploid.
[b] An aberrant 4+:4− segregation.
[c] An aberrant 2+:6− segregation.

TABLE 6

Meiotic behavior in various rad/rad *strains and their isogenic controls*

	rad1		*rad2*		*rad3*		*rad4*		*rad10*		*rad14*		*rad16*		*rad5*		*rad9*		*rad18*	
	rad/rad	+/+	*rad/rad*	+/+	*rad/rad*	+/+	*rad/rad*	+/+	*rad/rad*	+/+	*rad/rad*	+/+	*rad/rad*	+/+	*rad/rad*	+/+	*rad/rad*	+/+	*rad/rad*	+/+
No. of complete tetrads	109	117	89	91	99	50	99	96	61	68	47	49	108	112	95	67	117	130	325	274
Viable spores	93.4	94.8	92.7	92.7	88.9	68.0	93.3	93.8	82.2	85.7	94.5	92.5	91.7	94.4	90.0	88.0	95.6	97.8	90.0	91.4
Basic conversion																				
ade8-10			9.6	8.6	9.1	12.0	9.3	10.7	18.3	10.9	4.3	4.2	15.7	10.8					13.2	10.5
ade8-18															15.2	7.6	4.7	9.7		
his2	16.5	12.8	9.0	6.6	21.4	18.4	13.1	13.7	18.0	15.2	19.1	16.3	25.5	31.3	27.2	17.9	16.7	21.5	18.8	12.9
met13	6.4	9.4	13.5	5.5	12.1	26.0	6.2	7.3	6.6	17.9	8.5	6.1	13.1	10.7	2.1	9.0	7.7	8.5	6.9	6.6
arg4-16															8.6	4.5	10.3	6.2	12.3	10.2
thr1	9.2	5.1	12.4	11.0	8.1	10.2	10.2	10.6	11.5	7.4			7.4	6.3	5.4	9.0	6.8	6.9		
lys1-1	10.1	6.0	5.6	11.0	5.1	18.0	4.1	6.2	8.2	8.8	0	6.1	7.4	3.6	9.6	10.4	7.7	13.1		
trp1-1	0	0	0	1.1	0	4.0	0	0	3.3	0	0	0	1.9	0.9	0	0	0	0	0.6	0.4
ura3	0	1.7	0	3.3	4.0	0	3.0	0	1.6	1.5	0	2.0	3.7	0	2.1	0	1.5	0		
*MAT***a**	0	0	2.2	0	3.0	4.0	2.0	1.0	0	1.5	2.1	0	1.9	3.6	3.2	1.5	2.6	0	2.5	1.8
Genetic interval (cM)																				
arg4:thr1															16.0	11.2	12.4	10.0		
arg4:centromere															15.9	18.8	13.3	13.8	13.5	13.4
ura3:centromere	6.9	8.7	11.8	5.7	8.9	8.3	9.9	5.7	3.4	5.2	9.6	9.4	11.3	8.1	6.1	7.5	5.1	4.7		

TABLE 7

Meiotic segregations in unselected tetrads from a rad1-1/rad1-1 rad18-2/rad18-2 *diploid and related strains*

	Genotype				
	rad18-2 / *rad18-2* + / +	+ / + *rad1-1* / + *rad1-1*	*rad18-2* + / + *rad1-1*	+ + / + +	*rad18-2 rad1-1* / *rad18-2 rad1-1*
Strain	H18Rr	X22R	X58	ED61	ED60
Tetrads dissected	458	140	146	155	161
No. of complete tetrads	325	109	108	130	6
Viable spores	90.0	93.4	91.3	94.7	35.6

binations also showed meiotic effects where the component single mutants had none. As indicated, for each double-mutant combination, segregants from a doubly heterozygous *rad* progenitor strain were mated to produce several homozygous double-mutant strains and control wild-type strains.

Combinations of mutants from the same epistasis group were tested for meiotic effects. The *rad1-1/rad1-1 rad2-1/rad2-1* strain, with two mutations in the excision-defective epistasis group, exhibited a meiotic profile comparable to its isogenic control with respect to ascus production, ascospore viability and recombination (Table 8). The *rad5-5/rad5-5 rad18-2/rad18-2* strain, containing two mutations in the *RAD6* epistasis group, also displayed standard meiotic behavior (Table 9).

Six other double-mutant combinations, with component *rad* mutants from different epistasis groups, were constructed. Four (*rad2 rad5, rad1 rad9, rad2 rad9, rad16 rad18*) displayed normal meiotic behavior. Thus, merely combining a mutant from the excision repair group with one from the *RAD6* group does not generate a viability pattern comparable to that seen in the *rad1-1 rad18-2* double mutant. Two other combinations, *rad2-1 rad18-2* and *rad3-2 rad18-2*, did exhibit meiotic effects (Table 10). In these combinations, the meiotic phenotype is similar to that of the *rad1-1 rad18-2* combination, although the effect is somewhat less severe. Spore viability of *rad1-1 rad18-2* strains is approximately 40%, whereas for *rad2-1 rad18-2* and *rad3-2 rad18-2* the corresponding value is approximately 50%. The meiotic phenotype seems to require blocks in both the *RAD3* and the *RAD6* epistasis groups. In these experiments only *RAD18* from the *RAD6* epistasis group potentiates the effect. Whether this is generally true or depends on the particular alleles or loci used in this study is not clear.

The suppressibility of *rad2-1*, an ochre allele, was exploited to test further the correlation between ascospore viability and the mutant genotype. An ochre suppressor was derived in a *rad2-1/rad2-1 rad18-2/rad18-2* diploid. As predicted, this suppressed strain displays increased spore viability, *i.e.*, 80% (Table 9).

Further characterization of the rad1-1 rad18-2 *double mutant*: A detailed characterization of the *rad1-1 rad18-2* meiotic phenotype was undertaken with a view toward accounting for the double mutant's spore inviability. Approxi-

TABLE 8

Meiotic segregations in unselected tetrads from a rad1-1/rad1-1 rad2-1/rad2-1 *diploid and related strains*

	rad genotype		
	rad-1 / + + / *rad2-1*	*rad1-1 rad2-1* / *rad1-1 rad2-1*	+ + / + +
Strains	ED109	ED114, 116, 117	ED115
Complete tetrads	64 (80%)	122 (69.3%)	96 (81.4%)
Total tetrads	80	176	118
Viable spores	300 (93.8%)	630 (89.5%)	445 (94.3%)
% Conversion			
his2	15.6	17.4	15.6
met13	9.5	7.4	16.7
ade8-10	1.6	7.3	1.2
thr1	7.8	9.0	4.2
% SDS for *ura3*	12.7	9.2	8.6

TABLE 9

Meiotic analysis of rad5 *and* rad18

	rad genotype		
	rad5-5 / + + / *rad18-2*	*rad5-5 rad18-2* / *rad5-5 rad18-2*	+ + / + +
Strains	ED131	ED157, 158, 161	ED159, 160, 162
Complete tetrads	25 (83.3%)	85 (70.8%)	54 (90.0%)
Total tetrads	30	120	60
Viable spores	115 (95.8%)	423 (88.1%)	232 (96.7%)
Total spores	120	480	240
% Conversion			
his2	20.0	18.3	16.7
met13	8.0	13.8	11.1
arg4-16	8.0	7.0	9.3
Map distance (cM)			
arg4-16:thr1	8.7	10.8	9.3
ura3:centromere	4.0	4.2	7.5

mately 65% of the spores from a *rad1-1 rad18-2* diploid fail to generate visible colonies compared with 5% for controls. That this phenotype is a consequence of meiosis rather than vegetative lethality or a spore germination defect is shown by comparisons with the double heterozygote. If vegetative lethality or

TABLE 10

Meiotic analysis of rad2, rad3, *and* rad18

	rad genotype						
	rad2-1 / + + / *rad18-2*	*rad2-1 rad18-2* / *rad2-1 rad18-2*	+ / + + / +	*rad2-1 rad18-2 SUP°* / *rad2-1 rad18-2* / +	*rad3-2* / + + / *rad18-2*	*rad3-2 rad18-2* / *rad3-2 rad18-2*	+ / + + / +
Strains	ED128	ED147, ED148	ED149	ED196	ED183	ED199, ED200	ED202
Total tetrads dissected	20	200	59	20	59	80	20
% Viable spores	91.3	48.3	93.2	80.0	90.7	39.7	80.0

poor germination contributed to the spore inviability associated with *rad1-1 rad18-2* homozygous diploids, then this contribution should become evident in the tetrad analysis of doubly heterozygous diploids.

Spore viability in the double heterozygote was 95.3%. The inferred *rad* genotypes of the 11 inviable spores were randomly distributed, suggesting that functional *RAD1* and *RAD18* genes are not required for spore germination and vegetative growth but rather are required during the meiotic process itself. However, the possibility that the *RAD1* or *RAD18* gene products play some role in spore germination is not completely ruled out by this experiment, since some carry-over of the gene product from the heterozygous sporulating cell into the spores may occur.

In an analysis, in the double mutant, of the frequencies of tetrads containing 4, 3, 2, 1 and 0 viable spores these tetrad classes showed a binomial distribution, given the overall viability of 35–38%.

Microscopic observation showed that, of the spores that fail to proliferate into visible colonies, 75–95% nevertheless germinate and give rise to microcolonies containing an average of five to eight swollen, misshapen cells. Also, the clones from spores that do generate visible colonies grow more slowly than comparable clones from isogenic control strains, including *rad1-1/RAD1 rad18-2/RAD18* strains which yield 25% *rad1-1 rad18-2* segregants. All spores, regardless of whether they generate visible colonies, germinate after the same time interval on GNA medium.

Recombination was analyzed in *rad1-1/rad1-1 rad18-2/rad18-2* strains and control strains (Table 11). All strains, including the homozygous double mutant, exhibited at least 80% sporulation. Tetrads with four viable spores accounted for approximately 68% of all tetrads dissected except in the case of *rad1-1/rad1-1 rad18-2/rad18-2* strains. For the latter, tetrads with four and three viable spores were included in the tetrad analysis and together these accounted for only 4% of the tetrads dissected. Thus, the recombination analysis for *rad1-1/rad1-1 rad18-2/rad18-2* strains is based on a limited fraction of total tetrads. When three-spored data are included for tetrad analysis, the genotype of the fourth spore is inferred by assuming a $2^+:2^-$ segregation for each marker. The assumption is reasonable given the low conversion frequencies of the markers in these strains. Map distances for the two intervals, *MAT-leu1* and *hom3-trp2*, were calculated. Map distances do not differ significantly among the five *rad* genotypes. Most of the genetic markers scored normally display low conversion frequencies. However, the *rad1-1/rad1-1 rad18-2/rad18-2* strain ED60 carries several markers known to convert at high frequencies. In this hybrid, conversions were observed at *thr1, lys1, met13* and *arg4-16* (including PMS for the latter) (data not shown). Accordingly, we conclude that *rad1-1/rad1-1 rad18-2/rad18-2* strains are not correction or recombination deficient.

Mechanism of spore inviability in rad1-1 rad18-2 *homozygotes*: To test the hypothesis that nondisjunction might account for the ascospore inviability observed in homozygous *rad1-1 rad18-2* diploids, surviving spores from such a strain, ED101, of genotype *MAT***a**/*MATα ade2-1/ade2-1 thr1/THR1 rad1-1/*

TABLE 11

Recombination in rad1,18 *double mutant and isogenic control strains*

	All strains of genotype $\frac{a}{\alpha}\ \frac{leu2}{+}\ \frac{hom3\text{-}10}{+}\ \frac{his1\text{-}7\ +}{+\ his1\text{-}1trp2}\ \frac{+}{+}$				
	rad genotype				
	$\frac{rad1}{+}\ \frac{+}{rad18}$	$\frac{rad1}{rad1}\ \frac{rad18}{rad18}$	$\frac{+}{+}\ \frac{+}{+}$	$\frac{rad1}{+}\ \frac{rad18}{rad18}$	$\frac{rad1}{rad1}\ \frac{rad18}{+}$
Strains	ED126	ED135, 136	ED138, 139, 140	ED155, 153	ED156, 154
No. of complete tetrads	89	7 + 32 3-spored	88	40	27
Map distance (cM)					
matα:leu2	19.5	26.9	17.6	34.6	16.7
hom3:trp2	30.1	41.0	29.9	27.5	21.2
No. of *his* prototrophs	1	0	1	0	0
No. of conversions					
leu2	4	0	0	0	0
hom3-10	1	0	0	0	0
trp2	0	0	1	0	0
met1	3	0	0	1	0

rad1-1 rad18-2/rad18-2 were outcrossed. From 20 tetrads dissected, 38 spores produced visible colonies. Each was mated with a haploid tester carrying ten marked chromosomes. The resulting diploids were sporulated and ten tetrads were dissected for each. Spores disomic for any of the ten marked chromosomes in the nondisjunction tester would be detected by trisomic segregation ratios for the marker carried on the chromosome present in extra copy. There are three types of segregations for a trisomic +/+/− hybrid: $4^+:0^-$, $3^+:1^-$ and $2^+:2^-$, with the first phenotypic class predominating. No extra chromosomes were detected in 38 tested spore clones. The results clearly eliminate nondisjunction as the primary mechanism responsible for ascospore inviability in the *rad1-1 rad18-2* strains. The results do not specifically eliminate chromosome loss as the cause of spore inviability, but they do show that the surviving spores do not inherit chromosomes lost from inviable spores. The fact that inviable spores are capable of some residual growth and division also suggests that they are not nullisomic for any whole chromosomes.

Tetraploid analysis: The behavior of tetraploids homozygous for *rad1-1* and *rad18-2* was examined. Rare matings were selected by replica plating mixtures of two diploids, one of which was homozygous for *leu2* and heterozygous for *trp2* and the other homozygous for *trp2* and heterozygous for *leu2*, onto synthetic medium lacking both leucine and tryptophan. The resulting tetraploids thus carried a single copy of $LEU2^+$ and $TRP2^+$ and three mutant alleles of each. The centromere-linked *LEU2* was used to identify first division segregations. The diploid parents exhibited approximately 40% viable spores. Of the 30 isolates, 26 gave segregations consistent with tetraploidy, whereas ascospore viability was much improved in these tetraploids.

One such tetraploid was also employed in a larger study. The tetraploid exhibited 88% spore viability. Segregations for *leu2, trp2, hom3, his1* and *MAT* were those expected for their respective tetraploid configurations. Colonies from 14 tetrads containing four *MAT***a**/*MAT*α diploids were induced to sporulate and ten tetrads were dissected from each colony. Thus, 56 spore colonies were analyzed in all. Colonies lacking a single chromosome should show the segregation of a recessive lethal superimposed on the normally poor spore viability displayed by *rad12 rad18* homozygotes. In this analysis spore colonies, from which none of the ten dissected tetrads showed more than two surviving spores, were taken to harbor a recessive lethal. Given the overall spore viability in this experiment of 44%, we expect four to five spore colonies to display such a survival pattern. In fact, 21 such colonies are observed. Seven tetrads had two spore colonies that segregated a recessive lethal. In six of these tetrads, *LEU2* segregated 2:2 and could be compared with the recessive lethal: in five cases they displayed a ditype segregation, suggesting a first division segregation of the lethal. Of the remaining tetrads, two had no spore segregating a recessive lethal, four had a single such spore and one tetrad had three (Table 12). In contrast, dissection of eight tetrads from a related RAD^+ tetraploid yielded no recessive lethality in any spore clone, and spore viability averaged 89%.

TABLE 12

Segregation of recessive lethality in tetraploids

	No. of spores/tetrad segregating a recessive lethal[a]				
	0	1	2	3	4
No. of tetrads from *rad1*, *rad18* tetraploid	2	4	7	1	0
No. of tetrads from *RAD*$^+$ tetraploid	8	0	0	0	0

[a] Ten tetrads were dissected from each spore colony. If no tetrad had more than two surviving spores, the colony was assumed to segregate a recessive lethal.

DISCUSSION

Meiotic recombination and spore viability were examined in yeast strains mutant at loci involved in excision and repair processes. Without exception there were no significant recombinational differences between each of the single *rad* mutants and closely related wild-type control strains.

The present study is a broadly comprehensive survey of the meiotic phenotypes displayed by yeast repair mutants. Previous studies examined gene conversion, postmeiotic segregation and reciprocal recombination at only a few sites for a limited number of radiation-sensitive mutants. COX and PARRY (1968) evaluated the sporulation capacity of UV-sensitive *rad* mutants and found that only *rad6* was blocked in ascus production. GAME *et al.* (1980) later showed that diploids homozygous for *rad6* completed premeiotic DNA replication but failed to generate meiotic gene convertants. SNOW's (1968) study of meiotic recombination in *rad* mutants estimated recombination in random ascospore samples taken from strains heteroallelic at *his1* and heterozygous at the flanking sites *thr3* and *arg6*. Compared with the control, meiotic recombination in the *rad* mutants was found not to differ significantly with respect to intragenic and intergenic recombination.

The genetic system designed to assess meiotic recombination in the current study facilitated the detection of changes in PMS frequency. PMS is construed as the lack of correction in meiotic heteroduplex DNA (FOGEL *et al.* 1979). In principle, we might suppose that the correction process and excision repair share common reactions. If the excision defective *rad* mutants were correction defective, homozygous mutant strains would be expected to exhibit an increase in PMS, *i.e.*, 5:3 and 3:5 tetrads, with a concomitant decrease in 6:2 and 2:6 tetrads. In the total absence of correction the expected number of 5:3 and 3:5 tetrads should increase by twice the characteristic number of 6:2 and 2:6 segregations since half of the correction events, on the average, result in restoration to the parental configuration. Thus, we were especially concerned with the sensitive detection of increases in PMS. Our strategy was to monitor several sites known to exhibit high conversion but low PMS. For example, *his2* typically exhibits an approximate basic conversion frequency (BCF—total aberrant segregations/total tetrads) of 18% but PMS events are rare (FOGEL *et al.* 1979). If correction were blocked in some particular *rad* background, then we

would expect to encounter about 36 PMS spore clones in an unselected sample of 100 tetrads.

Ten mutants, including seven that were excision defective, were examined with respect to PMS. At the nine heterozygous sites monitored, none differed significantly from their respective wild-type controls. This result is consistent with findings reported by others. DICAPRIO and HASTINGS (1976) studied PMS at the *SUP6* site in *S. cerevisiae*. Diploids homozygous for *rad18-1, rad2-6, rad3-2, rad4-4, rad16-1* or *rad18-1* did not exhibit significant increases in PMS compared with controls. In the yeast *Schizosaccharomyces pombe*, the *rad2* mutant confers sensitivity to UV. MUNZ and LEUPOLD (1979) found the frequency of PMS unchanged in this mutant background. HOLLIDAY and DICKSON (1977) found that *Ustilago maydis* strains mutant at the *uvs3* locus, and defective in excision repair, likewise show no PMS effect. Thus, deficiencies in excision repair need not cause a deficiency in heteroduplex correction. This may reflect a lack of involvement of pyrimidine dimer repair enzymes in meiotic heteroduplex correction or it may reflect the nature of the *rad* mutant alleles we studied, which may not affect all of the functions of the repair enzyme(s). *RAD3*, as shown by NAUMOVSKI and FRIEDBERG (1983) and HIGGINS *et al.* (1983), performs a vital function in yeast in addition to its role in dimer excision; consequently, UV-sensitive alleles of this gene must still perform some cellular function which may or may not be involved in meiotic heteroduplex repair. Loci that regulate this process were recently identified by WILLIAMSON and FOGEL (1980). These *pms* mutants alter the frequency and extent of correction, whereas the frequency of conversional initiation remains unchanged. Moreover, the *pms* mutants are not sensitive to UV or X-rays.

Rad mutants in a third UV epistasis group, believed to be involved in recombinational repair (HAYNES and KUNZ 1981), are mostly defective in the overall meiotic process and produce inviable spores (GAME and MORTIMER 1974) and so could not be studied here. These mutants are also sensitive to X-rays. Many of the mutants in this group are unable to form meiotic recombinants (GAME *et al.* 1980; MALONE and ESPOSITO 1981). More surprisingly, *rad6-1*, a mutant in the *rad18* UV epistasis group, is also defective in meiosis and is unable to sporulate (COX and PARRY 1968) or recombine, although premeiotic DNA synthesis does occur. Diploids homozygous for *rad6* may form a normal synaptonemal complex and must be defective in some other precondition for genetic recombination (KUNDU and MOENS 1982).

Although *RAD6* is the only locus in its UV epistasis group that is so far known to be required for successful meiosis, our observation that double mutants involving *rad18* and various excision-defective mutations have reduced spore viability due to genetic lethality suggests that *RAD18* may play some role in meiosis. Two other mutants in this epistasis group (*rad5* and *rad9*) show no meiotic phenotype when combined with excision-defective mutations.

Approximately 50 *rad1-1/rad1-1 rad18-2/rad18-2* yeast hybrids were examined in this study. All exhibited severe ascospore inviability, in contrast to the observation that hybrids singly homozygous for *rad1-1* or *rad18-2* displayed about 90% spore viability. The rationale for constructing such a double mutant derives from the observation that, although each single mutant is substantially

UV sensitive (COX and PARRY 1968), the response of the double mutant with respect to UV sensitivity is strongly synergistic (COX and GAME 1974). This and other data (UNRAU, WHEATCROFT and COX 1971; LAWRENCE and CHRISTENSEN 1976) indicate that the two *rad* mutants operate in different but interacting repair systems. Unlike each of the single mutants alone, *rad1 rad18* double-mutant strains irradiated during S-phase are unable to integrate newly synthesized DNA into a high molecular weight form during postreplication incubation (DICAPRIO and COX 1981). We were curious to determine whether the mitotic interaction between *rad1* and *rad18* was paralleled meiotically. Indeed, the meiotic response of the *rad1 rad18* double mutant can be viewed as a possible synergistic interaction between meiotic effects which in each mutant alone are too slight to be detected genetically.

Analysis of a tetraploid homozygous for *rad1* and *rad18* indicates that recessive lethals occur at a significant rate. More than one-third of the diploid spore clones analyzed contained a recessive lethal. Since the tetraploid generated tetrads with one and three lethal-containing spores, we conclude that the lethals did not arise during vegetative growth: such events (*e.g.*, mitotic chromosome loss) should produce only tetrads in which two spores are normal and two segregate a recessive lethal. Rather, the distribution of lethal-containing spore clones among the tetrads appears to fit a binomial distribution, although the sample size is small. This is consistent with the spore viability pattern in tetrads derived from diploid *rad1 rad18* strains, which fit the same distribution.

The extremely poor spore viability in tetrads from lethal-containing spore colonies precluded determination of first or second division segregation of the lethals. Therefore, we are unable to ascertain whether the lethality results from chromosome loss or from large deficiencies or lethal point mutations. In the tetrads derived from the tetraploid meiosis, we observed a high first division segregation frequency in those cases when two lethal-containing spores were present. This suggests that whole chromosomes are being lost. However, the limited sample size makes this notion quite tentative. However, it is clear that spore lethality in *rad1 rad18* strains results from heritable genetic defects.

Approximately one-third of the diploid spores carried recessive lethals. This is considerably less than the approximately 60% lethality observed among haploid spores. A possible interpretation postulates that spontaneous genetic damage arises during meiosis, which in wild type can be repaired by either the *RAD3* or *RAD6* pathways. In the absence of these pathways, some damage might be repaired in diploid spores by a recombinational repair pathway, perhaps mediated by genes in the *RAD52* epistasis group (GAME 1983). However, in haploid spores replication would have to occur prior to recombinational repair, and such replication might serve to "fix" the damage in both copies of the DNA or be lethal in itself, so that G2 repair in haploids might be impossible. DICAPRIO and COX (1981) suggest that *rad1 rad18* strains are uanble to repair gaps in newly synthesized DNA opposite pyrimidine dimers. We do not know whether similar gaps occur in meiotic cells, but this would be consistent with our results.

The meiotic phenotypes of *rad* mutant combinations characterized in the

present study underscore the complex relationship among DNA metabolism functions. Double-mutant combinations were found to display effects on the meiotic process where the component single mutants did not, thus revealing the involvement of the latter in meiosis. Although DNA metabolism is particularly complex, the significance of the double-mutant effects reported here is probably applicable to any genetic analysis. That is, the conclusion that a negative result for a single mutant precludes its involvement in the process under examination must be a tentative one.

In summary, although we cannot conclude that the *rad* mutants studied here play a role in meiotic recombination as such, some of them function in the repair of spontaneous DNA damage incurred during meiosis.

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LITERATURE CITED

BORAM, W. R. and H. ROMAN, 1976 Recombination in *Saccharomyces cerevisiae*: a DNA repair mutation associated with elevated mitotic recombination. Proc. Natl. Acad. Sci. USA **73:** 2828–2832.

CAMPBELL, D. A., S. FOGEL and K. LUSNAK, 1975 Mitotic chromosome loss in a disomic haploid of *Saccharomyces cerevisiae*. Genetics **79:** 383–396.

COX, B. S. and J. C. GAME, 1974 Repair systems in *Saccharomyces*. Mutat. Res. **26:** 257–264.

COX, B. S. and J. M. PARRY, 1968 The isolation, genetics and survival characteristics of ultraviolet light sensitive mutants in yeast. Mutat. Res. **6:** 37–55.

DICAPRIO, L. and B. S. COX, 1981 DNA synthesis in UV-irradiated yeast. Mutat. Res. **82:** 69–85.

DICAPRIO, L. and P. J. HASTINGS, 1976 Postmeiotic segregation in strains of *Saccharomyces cerevisiae* unable to excise pyrimidine dimers. Mutat. Res. **37:** 137–140.

FOGEL, S., R. MORTIMER, K. LUSNAK and F. TAVARES, 1979 Meiotic gene conversion: a signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **43:** 1325–1341.

GAME, J. C., 1983 Radiation sensitive mutants and repair in yeast. pp. 109–137. In: *Yeast Genetics: Fundamental and Applied Aspects*, Edited by J. F. T. SPENCER, D. M. SPENCER and A. R. W. SMITH. Springer Verlag, New York

GAME, J. C. and R. K. MORTIMER, 1974 A genetic study of X-ray sensitive mutants in yeast. Mutat. Res. **124:** 281–292.

GAME, J. C., T. J. ZAMB, R. J. BRAUN, M. RESNICK and R. M. ROTH, 1980 The role of radiation (*rad*) genes in meiotic recombination in yeast. Genetics **94:** 51–68.

HAYNES, R. H. and B. A. KUNZ, 1981 DNA repair and mutagenesis in yeast. pp. 371–414. In: *The Molecular Biology of the Yeast Saccharomyces*, Edited by J. STRATHERN, J. BROACH and E. W. JONES. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

HIGGINS, D. R., S. PRAKASH, P. REYNOLDS, R. POLAKOWSKA, S. WEBER and L. PRAKASH, 1983 Isolation and characterization of the *RAD3* gene of *Saccharomyces cerevisiae* and inviability of *rad3* deletion mutants. Proc. Natl. Acad. Sci. USA **80:** 5680–5684.

HOLLIDAY, R. and J. M. DICKSON, 1977 The detection of post meiotic segregation without tetrad analysis in *Ustilago maydis*: evidence that a mutation defective in excision of pyrimidine dimers can repair mismatched bases in hybrid DNA. Mol. Gen. Genet. **153:** 331–335.

KUNDU, S. C. and P. B. MOENS, 1982 The ultrastructural phenotype of the radiation sensitive mutant *rad6* in yeast. Chromosoma **87:** 125–132.

KUNZ, B. A. and R. H. HAYNES, 1981 Phenomenology and genetic control of mitotic recombination in yeast. Annu. Rev. Genet. **15:** 57–89.

LAWRENCE, C. W. and R. CHRISTENSEN, 1976 UV mutagenesis in radiation-sensitive strains of yeast. Genetics **82:** 207–232.

MALONE, R. E. and R. E. ESPOSITO, 1981 Recombinationless meiosis in yeast. J. Mol. Cell Biol. **1:** 891–901.

MUNZ, P. and U. LEUPOLD, 1979 Gene conversion in nonsense suppressors of *Schizosaccharomyces pombe.* Mol. Gen. Genet. **170:** 145–148.

NAUMOVSKI, L. and E. C. FRIEDBERG, 1983 A DNA repair gene required for the incision of damaged DNA is essential for viability in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **80:** 4818–4821.

PRAKASH, S., L. PRAKASH, W. BURKE and B. MONTELONE, 1980 Effects of the *rad52* gene on recombination in *Saccharomyces cerevisiae.* Genetics **94:** 31–50.

RESNICK, M. A., J. C. GAME and S. STASIEWICZ, 1983 Genetic effects of UV irradiation on excision-proficient and -deficient yeast during meiosis. Genetics **104:** 603–618.

RESNICK, M. A., S. STASIEWICZ and J. C. GAME, 1983 Meiotic DNA metabolism in wild-type and excision-deficient yeast following UV exposure. Genetics **104:** 583–601.

SNOW, R., 1968 Recombination in ultraviolet-sensitive strains of *Saccharomyces cerevisiae.* Mutat. Res. **6:** 409–418.

UNRAU, P., R. WHEATCROFT and B. S. COX, 1971 The excision of pyrimidine dimers from DNA of ultraviolet irradiated yeast. Mol. Gen. Genet. **113:** 359–362.

WILLIAMSON, M. and S. FOGEL, 1980 Correction defective (*cor*) mutants that alter the frequency and length of correction tracts in meiotic hybrid DNA (abstract). In: *Tenth International Conference of Yeast Genetics and Molecular Biology.* Louvain-La-Neuve, Belgium.

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