ISOLATION AND CHARACTERIZATION OF MMS-SENSITIVE MUTANTS OF *SACCHAROMYCES CEREVISIAE*

LOUISE PRAKASH AND SATYA PRAKASH

Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, Rochester, New York 14642

AND

Department of Biology, University of Rochester, Rochester, New York 14627

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ABSTRACT

We have isolated mutants sensitive to methyl methanesulfonate (MMS) in *Saccharomyces cerevisiae*. Alleles of *rad1*, *rad4*, *rad6*, *rad52*, *rad55* and *rad57* were found among these *mms* mutants. Twenty-nine of the *mms* mutants which complement the existing radiation-sensitive (*rad* and *rev*) mutants belong to 22 new complementation groups. Mutants from five complementation groups are sensitive only to MMS. Mutants of 11 complementation groups are sensitive to UV or X rays in addition to MMS, mutants of six complementation groups are sensitive to all three agents. The cross-sensitivities of these *mms* mutants to UV and X rays are discussed in terms of their possible involvement in DNA repair. Sporulation is reduced or absent in homozygous diploids of *mms* mutants from nine complementation groups.

PROCARYOTIC and eucaryotic organisms possess mechanisms which enable them to repair damage induced in their DNA by ionizing radiation, such as X rays, nonionizing radiation, such as ultraviolet (UV) light, and chemical agents. From work done mainly in procaryotic organisms, it has emerged that the pathways involved in DNA repair, mutation induction, genetic recombination and cell survival are interrelated. In *Escherichia coli*, where most of the investigations have focused, UV damage induced in DNA is repaired by several mechanisms (HANAWALT 1975; HAYNES 1975; HOWARD-FLANDERS 1968). Photoreactivation involves enzymatic splitting of the pyrimidine dimers *in situ* in the presence of visible light (see SETLOW 1966 for review). Excision-repair, the predominant repair pathway, occurs by enzymatic removal of pyrimidine dimers from the DNA and the resulting gap is sealed by repair synthesis using the opposite strand as a template (HANAWALT 1975). Excision-repair is mediated by many genes, including at least the *uvrA*, *uvrB*, *uvrC*, *polA* and *lig* loci (see HANAWALT 1975 for specific references). Dimers which escape excision-repair are repaired by processes operating before, during and after DNA replication. One of these "post-replication repair" processes is recombinational repair (RUPP *et al.* 1971) and another is error-prone repair which generates mutations (RADMAN 1975; WITKIN 1975). UV mutability is now believed to result from the in-

duction of error-prone repair of excision gaps or post-replication gaps and depends on at least the *recA*+ and *lex*+ functions (RADMAN 1975; WITKIN and GEORGE 1973; WITKIN 1974).

In the yeast *Saccharomyces cerevisiae*, mutants which conferred sensitivity primarily to UV were isolated by Cox and PARRY (1968). The mutants represent 22 distinct genetic loci (Cox and PARRY 1968); some of the mutants show cross-sensitivity to X rays and methyl methanesulfonate (MMS) (GAME and MORTIMER 1974; PRAKASH 1976b; ZIMMERMAN 1968). Four of the loci are known to be involved in dimer excision; these include *rad1* (UNRAU, WHEATCROFT and COX 1971; PRAKASH 1975; WATERS and MOUSTACCHI 1974), *rad2* (RESNICK and SETLOW 1972), *rad3* and *rad4* (GAME and COX 1972; PRAKASH unpublished results). Mutants selected for sensitivity to X rays have also been isolated in yeast (GAME and MORTIMER 1974; RESNICK 1969a; SNOW 1967). They represent eight distinct genetic loci designated *rad50* to *rad57*. In addition, the *rev1*, *rev2*, which is an allele of *rad5* (GAME and COX 1971), and *rev3* mutants, all of which reduce UV-induced reversion of the ochre-suppressible *arg4-17* allele, have been isolated. These mutants confer moderate sensitivity to UV and slight sensitivity to X rays (LEMONTT 1971).

The effects of genes *rad1* to *rad22* and the *rev* genes on UV-induced mutations (LAWRENCE and CHRISTENSEN 1976) and on chemically induced mutations (PRAKASH 1976a) have been extensively studied. Repair of UV damage and mutation induction by UV appear to require the functioning of three different pathways (Cox and GAME 1974; LAWRENCE and CHRISTENSEN 1976). Mutants defective in excision-repair show increased UV mutability (AVERBECK *et al.* 1970; LAWRENCE and CHRISTENSEN 1976; MOUSTACCHI 1969; RESNICK 1969b; and ZAKHAROV, KOZINA and FEDOROVA 1970), and increased mutability with nitroquinoline oxide (NQO) (PRAKASH 1976a). The *rad6*, *rad8*, *rad9*, *rad18*, *rev1*, *rev2* and *rev3* mutants lower UV-induced reversion and all seem to belong to a single error-prone repair pathway, distinct from the excision-repair pathway (Cox and GAME 1974; LAWRENCE and CHRISTENSEN 1976). The genes *rad50* to *rad57* seem to be involved in a third UV repair pathway (Cox and GAME 1974; LAWRENCE and CHRISTENSEN 1976). The effect of most of the *rad* genes on reversion induced by the chemical agents NQO, ethyl methanesulfonate (EMS), and HNO_2 has been studied (PRAKASH 1976a). Two genes, *rad6* and *rad9*, lower reversion induced by a wide variety of chemical agents, including NQO, EMS, HNO_2, nitrosoguanidine (NTG), MMS, diethyl sulfate (DES), dimethyl sulfate (DMS), nitrogen mustard (HN2), β-propiolactone, and tritiated uridine (PRAKASH 1974). HNO_2 reversion is lowered in *rad15* but increased in *rad18* mutants (PRAKASH 1976a). NQO reversion seems to be affected by the largest number of *rad* genes; *rad6*, *rad9*, *rad15*, *rad17*, *rad18*, *rev1*, *rev2* and *rev3* lower NQO reversion while *rad1*, *rad2*, *rad3*, *rad4*, *rad10* and *rad16* raise it (PRAKASH 1976a). The *rad10* mutant in our stocks is not an allele of *rad4* as reported (LAWRENCE and CHRISTENSEN 1976) and is indeed *rad10*.

Since the *rad* mutants of *S. cerevisiae* were selected for sensitivity to either UV or X rays, they may not represent mutations in all the genes concerned with

repair of DNA damage in general; they may represent only a limited sample of genes involved in DNA repair. In order to gain more understanding of the genetic and biochemical basis of DNA repair in *S. cerevisiae*, we have isolated mutants sensitive to the chemical MMS. These mutants represent an additional 22 complementation groups, many of which may be involved in DNA repair, as evidenced by cross-sensitivity to UV, X rays, or both, effects on UV- and EMS-induced mutations, and other properties.

MATERIALS AND METHODS

Strains. All mutants were derived from B-635, *a cyc1–115 his1–1 lys2 trp2.* Strains containing the *rad* mutations were isolated by Cox and PARRY (1968); *rev1*, *rev2* and *rev3* strains were isolated by LEMONTT (1971); *rad50* to *rad57* strains were obtained from DR. R. K. MORTIMER.

Media and chemicals. Methyl methanesulfonate was obtained from Eastman Organic Chemicals and was repurified by vacuum distillation at 10 to 15 mm Hg before use.

The following media were used: YPD, 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose solidified with 1.8% Noble agar; YPG, 1% Bacto-yeast extract, 2% Bacto-peptone, 3% (v/v) glycerol and 1.8% Noble agar; SD, used for prototrophic selection of diploids, 0.67% Difco yeast nitrogen base without amino acids, 2% dextrose and 1.8% Noble agar. The sporulation media contained 2% potassium acetate, 0.1% dextrose, 0.25% Bacto-yeast extract, pH adjusted to 7.0, 1.8% Noble agar, and the following supplements present at 75 μg/ml: adenine sulfate, L-arginine-HCl, L-histidine-HCl, DL-homoserine, L-isoleucine, L-leucine, L-lysine-HCl, L-methionine, L-tryptophan, uracil and L-valine.

The YPD + MMS media was made as follows: after autoclaving 600 ml YPD media, the flask was allowed to cool to 45–50°C. Just before pouring, 2 ml sterile distilled H_2O was added to 0.21 ml redistilled MMS. The mixture was vortexed vigorously to dissolve the MMS and the dissolved MMS was then added to the YPD media. After thoroughly shaking the flask containing YPD + MMS, the plates were poured and used within about 20 hours; MMS-containing plates could not be used after 2 days because the MMS had decomposed sufficiently by then so that MMS-sensitive strains could no longer be distinguished from resistant strains.

Mutant isolation. Stationary cells of strain B-635, grown in liquid YPD, were washed and resuspended in sterile distilled water at a density of 5×10^4 cells/ml. Irradiation was carried out at a fluence of 125 Jm^{-2}, resulting in 2% survival. Cells were diluted and plated on YPG so that there were 50–70 colonies per plate. Survivors were plated on the glycerol medium to select against cytoplasmic petites which might have been induced by UV. The YPG plates were replica plated with velveteen onto YPD and YPD containing 0.035% MMS. All plates were incubated at 30° for two to three days. Colonies which did not grow on MMS-containing media were subcloned and retested for sensitivity to MMS by suspending in U-wells and transferring to YPD and YPD supplemented with 0.035% MMS by means of metal inoculators. A total of 80 MMS-sensitive mutants were obtained in this manner out of approximately 7000 clones examined. Thirty-eight of the mutants were examined in detail. Each of the 38 **a** *mms* strains was crossed to LP202–4C, *α cyc1–115 ade6 met15–3 can1*. Diploids obtained by prototrophic selection were sporulated and haploid segregants of the *α* mating type, carrying the particular *mms* gene and *ade6* and/or *met15–3* markers, were obtained by random spore methods. Sensitivity to MMS was scored by making suspensions of each strain in wells and transferring by means of metal inoculators to YPD plates and YPD + 0.035% MMS plates. After 2 days of incubation at 30°, *MMS+* strains form a visible spot of growth on the media whereas the *mms* strains show little or no growth (Figure 1).

MMS treatment (for survival curves). Cells were grown to stationary phase (2 to 3 days) in liquid YPD at 30°, washed in sterile distilled water and resuspended in 0.05 M potassium phosphate buffer, pH 7.0. The yeast suspensions, usually at 2 to 4×10^7 cells/ml, were incubated for 40 min at 30° with 0.5% MMS, which was prepared by adding 0.05 ml MMS to 10 ml of the cell suspension. At 10 min intervals, 1.5 ml cell suspension was removed and added to 1.5 ml

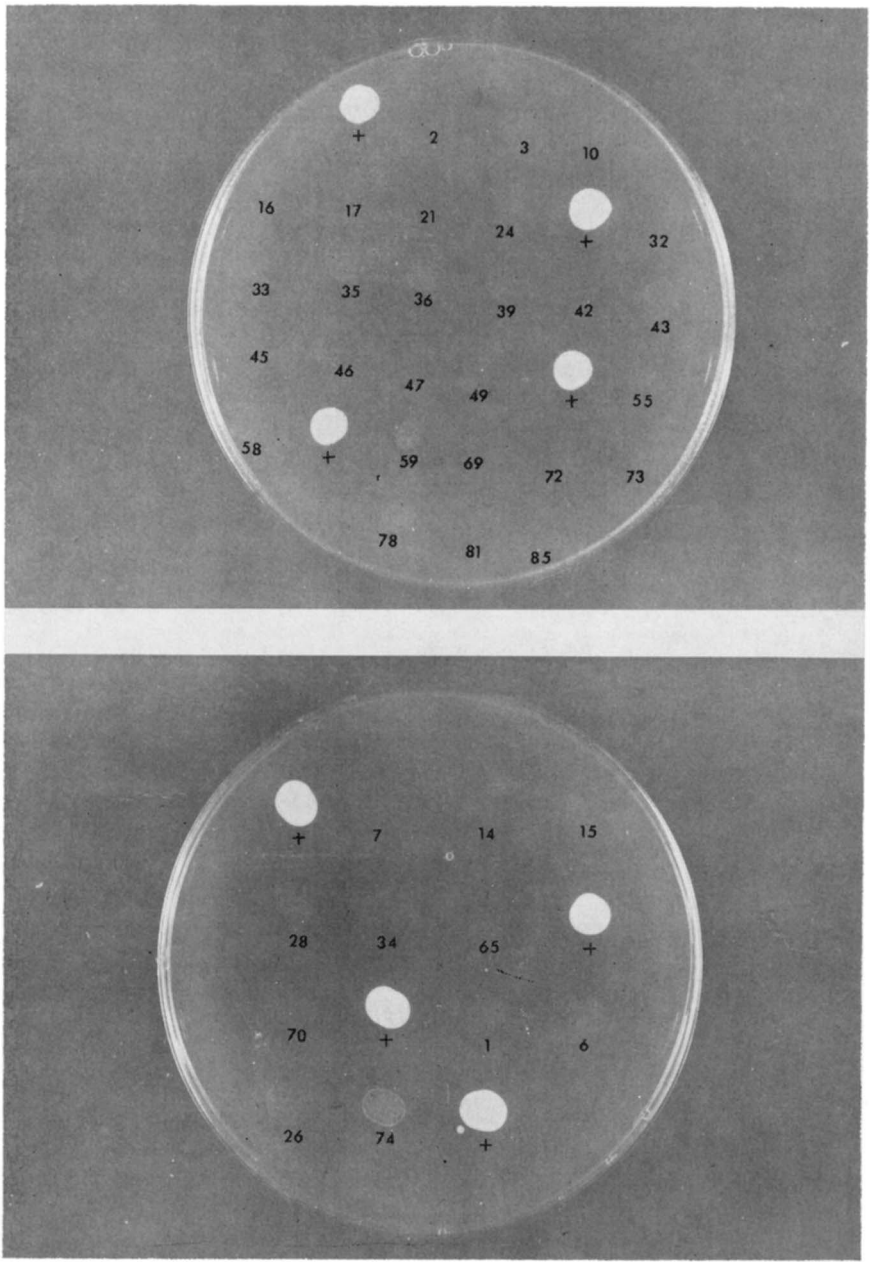


FIGURE 1.—The sensitivity of strain B-635 (*MMS*+) and mutants derived from it to growth on YPD + 0.035% MMS. Numbers refer to stock numbers (MD) of the MMS-sensitive mutants; + refers to B-635.

10% cold sodium thiosulfate. Appropriate dilutions were made and viability was determined after plating on YPG medium. Plates were incubated at 30° for 3–6 days before counting surviving colonies.

UV irradiation (for survival curves). Cells were plated on YPG media and irradiated with covers removed at a fluence rate of 1 Jm^{-2} s^{-1}. The radiation source and its dosimetry are given in LAWRENCE *et al.* (1974). Plates were incubated in the dark at 30° for 3–6 days before counting surviving colonies.

X-ray irradiation (for survival curves). Cells were plated on YPG media and exposed to X-irradiation on the surface of the petri dishes with covers removed. The source of X-irradiation was an OEG-60-T Machlett X-ray tube which was powered by a Picker Corporation custom-made X-ray generator. The X-ray unit was operated at 50 KVP and 25 ma, with only inherent filtration; the dose rate was 28 kilorad (KR) per min. Plates were incubated at 30° for 3–6 days before counting surviving colonies.

Sporulation. Diploids which were heterozygous or homozygous for a particular *mms* gene were grown on YPD plates for 1–2 days at 30° and then replica-plated with velveteen onto sporulation plates. After 5 days of incubation, cell suspensions were made and the number of asci was determined by microscopic examination. Two-, three-, and four-spored asci were grouped together.

RESULTS

Allelism with rad *mutants and number of complementation groups of* mms *mutants.* The MMS sensitivity of the **a** MMS-sensitive mutants described in this paper is shown in Figure 1. Lack of growth on a YPD plate containing 0.035% MMS clearly distinguishes the MMS-sensitive mutants from the *MMS+* parent strain. The allelism of the 38 MMS-sensitive mutants to existing radiation-sensitive mutants, the *rad* and *rev* mutants, was determined by making appropriate crosses and isolating diploids by prototrophic selection. For crosses involving mutants of *rad1* to *rad22*, *rev1* and *rev3*, diploids were tested for both MMS sensitivity, as described above, and for UV sensitivity, by exposing cells on the surface of YPD plates to fluences of 50 Jm^{-2} and 100 Jm^{-2}. For crosses involving mutants *rad50* to *rad57*, diploids were tested for both MMS sensitivity and for X-ray sensitivity, by exposing cells on the surface of YPD plates to 50 KR. Plates were incubated at 30° except for crosses involving the temperature sensitive *rad55* allele, which were incubated at 25°. For the complementation tests, the appropriate diploid homozygous for a particular *rad* gene was included as a control for every cross. The allelism of MMS-sensitive mutants with *rad* mutants is given in Table 1. Four of the MMS-sensitive mutants did not complement *rad52*; these consist of MD-7, MD-14, MD-28 and MD-70. Thus, these four

TABLE 1

Allelism of MMS sensitive mutants with rad *and* rev *mutants in* S. cerevisiae

MD-72 = *rad1* (*rad1–20*); MD-78 = *rad4* (*rad4–10*); MD-65 = *rad6* (*rad6–3*)
MD-7, MD-14, MD-28, MD-70 = *rad52* (*rad52–3*, *52–4*, *52–5*, and *52–6* respectively)
MD-15 = *rad55* (*rad55–6*); MD-34 = *rad57* (*rad57–6*)

Allele numbers in parentheses refer to the *rad* allele number given to these MMS sensitive mutants.

mutants are considered to be alleles of *rad52*. In addition, MD-15 was found to be an allele of *rad55* and MD-34 was found to be allelic to *rad57*. The strains carrying mutations in the genes *rad50* to *rad57* were all found to be sensitive to MMS. Three other MMS-sensitive mutants were allelic to *rad* mutants. One was MD-65, allelic to *rad6*. What was perhaps unexpected was to find alleles of *rad1* and *rad4*, both of which are involved in dimer excision (UNRAU, WHEATCROFT and COX 1971; WATERS and MOUSTACCHI 1974; PRAKASH 1975; PRAKASH unpublished results), among our MMS-sensitive mutants; MD-72 is allelic to *rad1* while MD-78 is allelic to *rad4*. Both MD-72 and MD-78 show enhanced UV-induced reversion (PRAKASH and PRAKASH unpublished results), a phenotype expected of excision-defective mutants (AVERBECK *et al.* 1970; LAWRENCE and CHRISTENSEN 1976; MOUSTACCHI 1969; RESNICK 1969b).

In order to determine the number of complementation groups present among the MMS-sensitive mutants which complemented the *rad* and *rev* mutants, the **a** MMS-sensitive strains were crossed to the α MMS-sensitive strains. Diploids obtained by prototrophic selection were transferred to YPD and YPD + 0.035% MMS plates. The appropriate homozygous MMS-sensitive diploid was always included as a control on each plate. Table 2 gives the results of these complementation tests. There are few allelic identities among these 29 mutants; however, there is one group containing four mutants: MD-1, MD-6, MD-26, and MD-58 are all noncomplementing with each other and therefore are alleles of one locus. Three mutants, MD-3, MD-32 and MD-69 fall into one complementation group. Two other complementation groups were found to each contain two mutants: MD-24 is allelic to MD-81 and MD-10 is allelic to MD-74. The remaining 18 mutants complemented all other mutants and may represent 18 distinct loci. Table 3 gives the locus and allele designation of these MMS-sensitive mutants.

Sensitivities of the mutants to MMS, UV and X rays. Survival curves after treatment with MMS, UV and X rays were obtained for 24 MMS-sensitive mutants representing 22 complementation groups. Several experiments were performed on B-635, the parent strain from which the mutants were derived, to determine how much variability exists from experiment to experiment. As can be seen in Figure 2, there is good reproducibility in replicate experiments. Figures 2A and 2B gives the results for MMS and UV survival curves. Both curves are similar in that each has a shoulder. The X-ray survival curve for B-635 (Figure 2C) is typical of that obtained for haploid strains; it has no shoulder but exhibits a tail (GAME and MORTIMER 1974).

(1) *Mutants sensitive only to MMS.* Five mutants, *mms2–1*, *mms4–1* (Figure 3), *mms1–4* (Figure 4), *mms5–2* (Figure 5) and *mms22–1*, are sensitive only to MMS. Two of the mutants, *mms2–1* (MD-2) and *mms22–1* (MD-85), give survival curves which are like that of the *MMS*+ strain, but they were classed as MMS-sensitive mutants since they do not grow when spotted on MMS-containing media (Figure 1). MMS survival curves are obtained by relatively short treatments with MMS of cells suspended in buffer. On the other hand, strains spotted on MMS-containing media must divide in the presence of MMS.

TABLE 2

Results of complementation tests among the 29 MMS-sensitive mutants (MD strains) which are not allelic to the rad *or* rev *mutants of* S. cerevisiae

1	2	3	6	10	16	17	21	24	26	32	33	35	36	39	42	43	45	46	47	49	55	58	59	69	73	74	81	85	MD strain #
S	+	+	S	+	+	+	+	+	S	+	+	+	+	+	+	+	+	+	+	+	+	S	+	+	+	+	+	+	*1*
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*2*
		S	+	+	+	+	+	+	+	S	+	+	+	+	+	+	+	+	+	+	+	+	+	S	+	+	+	+	*3*
			S	+	+	+	+	+	S	+	+	+	+	+	+	+	+	+	+	+	+	S	+	+	+	+	+	+	*6*
				S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S	+	+	*10*
					S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*16*
						S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*17*
							S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*21*
								S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S	+	*24*
									S	+	+	+	+	+	+	+	+	+	+	+	+	S	+	+	+	+	+	+	*26*
										S	+	+	+	+	+	+	+	+	+	+	+	+	+	S	+	+	+	+	*32*
											S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*33*
												S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*35*
													S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*36*
														S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*39*
															S	+	+	+	+	+	+	+	+	+	+	+	+	+	*42*
																S	+	+	+	+	+	+	+	+	+	+	+	+	*43*
																	S	+	+	+	+	+	+	+	+	+	+	+	*45*
																		S	+	+	+	+	+	+	+	+	+	+	*46*
																			S	+	+	+	+	+	+	+	+	+	*47*
																				S	+	+	+	+	+	+	+	+	*49*
																					S	+	+	+	+	+	+	+	*55*
																						S	+	+	+	+	+	+	*58*
																							S	+	+	+	+	+	*59*
																								S	+	+	+	+	*69*
																									S	+	+	+	*73*
																										S	+	+	*74*
																											S	+	*81*
																												S	*85*

+ = diploid not sensitive; S = diploid sensitive.

TABLE 3

Locus and allele numbers of the MMS-sensitive strains

Locus and allele no.	MD stock no.	Locus and allele no.	MD stock no.
mms1–1	1	*mms9–1*	33
mms1–2	6	*mms10–1*	35
mms1–3	26	*mms11–1*	36
mms1–4	58	*mms12–1*	39
mms2–1	2	*mms13–1*	42
mms3–1	3	*mms14–1*	43
mms3–2	32	*mms15–1*	45
mms3–3	69	*mms16–1*	46
mms4–1	10	*mms17–1*	47
mms4–2	74	*mms18–1*	49
mms5–1	24	*mms19–1*	55
mms5–2	81	*mms20–1*	59
mms6–1	16	*mms21–1*	21
mms7–1	17	*mms22–1*	85
mms8–1	73		

This difference may account for the discrepancy obtained in these two methods of scoring mutants as MMS-sensitive.

(2) *Mutants sensitive to both MMS and UV*. Eight mutants exhibited varying degrees of sensitivity to UV compared with the *MMS+* strain. These 8 mutants represent 6 complementation groups since one group, *mms3*, was represented by three alleles. The alleles *mms3–1* (Figure 6A), *mms3–2* (Figure 6B) and *mms3–3* (Figure 6C), as well as the mutants *mms6–1* (Figure 7), *mms18–1* (Figure 8) and *mms19–1* (Figure 9), are sensitive to UV but give X-ray survival

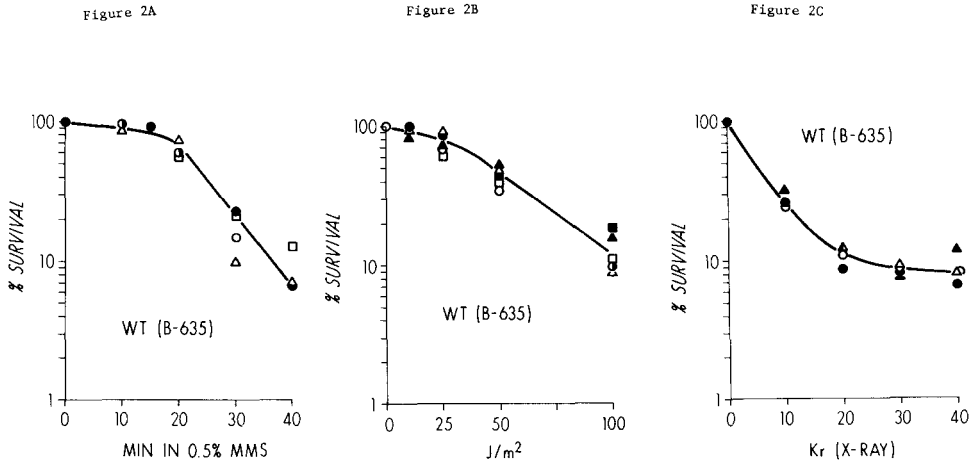


FIGURE 2.—The survival curves obtained for B-635, the parent strain, after treatment with (A) 0.5% MMS (B) UV irradiation (C) X-ray irradiation.

Figure 3

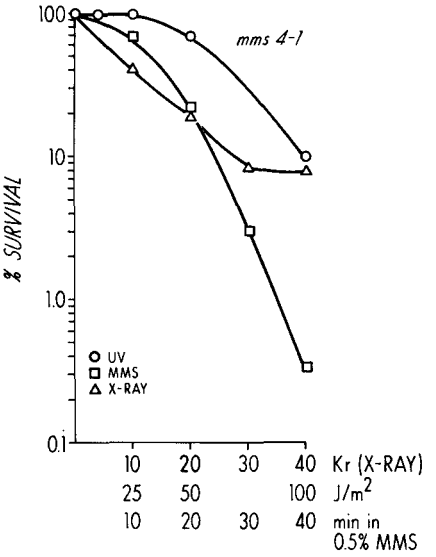


Figure 4

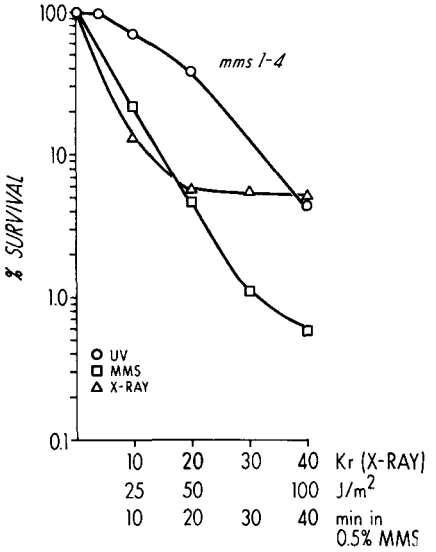
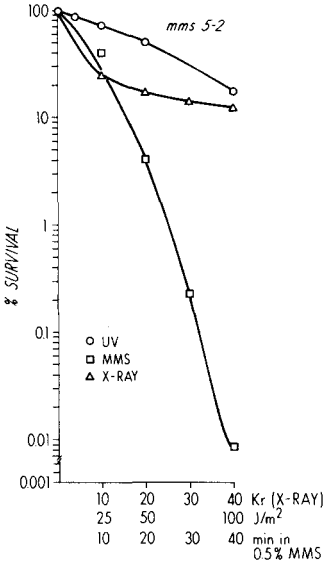


Figure 5



FIGURES 3–5.—Survival curves obtained for the *mms* mutants in response to MMS (□), (○) and X rays (△); mutants sensitive only to MMS.

FIGURE 3 (top)—*mms4–1*.

FIGURE 4 (bottom left)—*mms1–4*.

FIGURE 5 (bottom right)—*mms5–2*.

curves characteristic of the *MMS*+ strain. The remaining two mutants in this class, *mms13–1* and *mms10–1*, are not as sensitive to UV as the other mutants. The *mms13–1* mutant is slightly UV-sensitive only at high fluences (Figure 10) and exhibits a shouldered survival curve. The shoulder is absent in the survival curve obtained for *mms10–1* (Figure 11); nevertheless, *mms10–1* is only slightly

Figure 6A

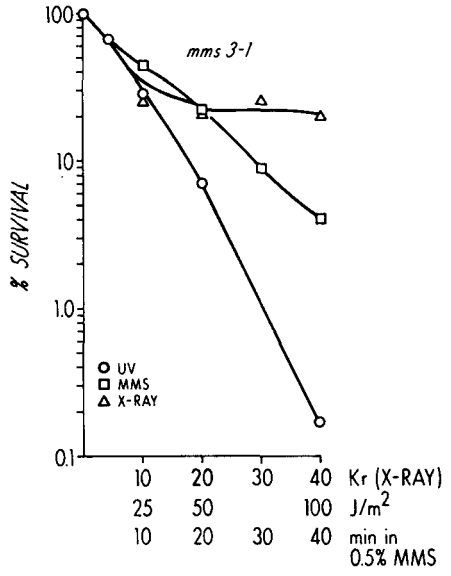


Figure 6B

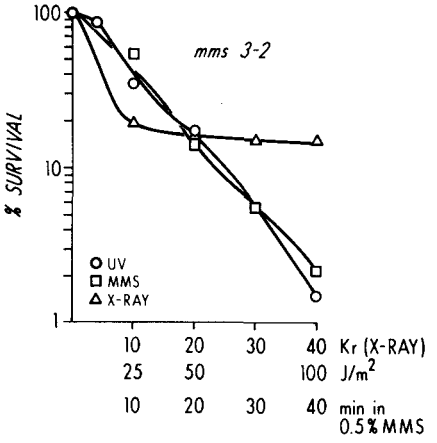


Figure 6C

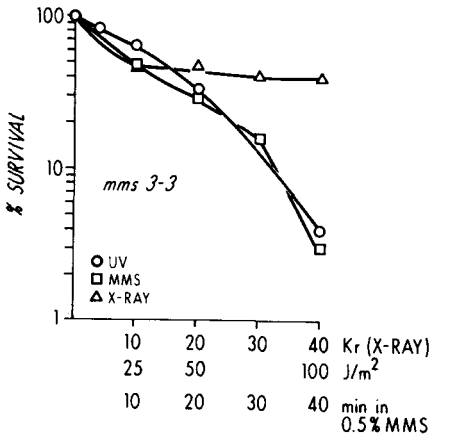


FIGURE 6.—Survival curves obtained for the *mms* mutants in response to MMS (□), UV (○) and X rays (△); mutants sensitive to both MMS and UV.

FIGURE 6a (top)—*mms3–1*.

FIGURE 6b (bottom left)—*mms3–2*.

FIGURE 6c (bottom right)—*mms3–3*.

Figure 7

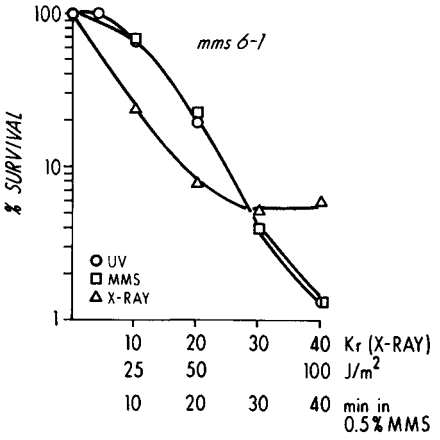


Figure 8

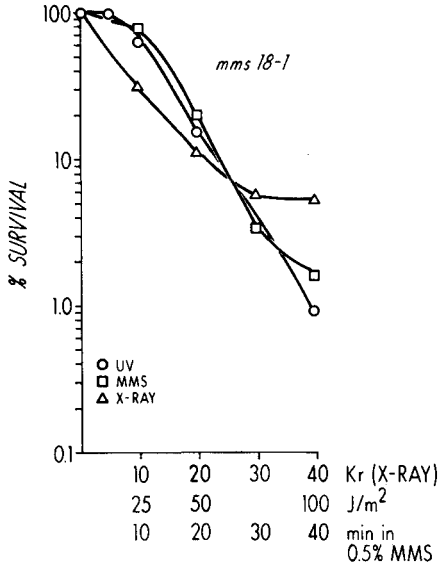
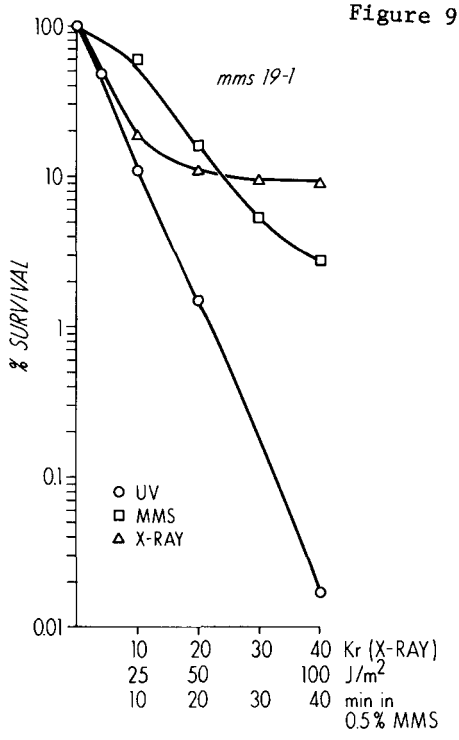


Figure 9



FIGURES 7–9.—Survival curves obtained for the *mms* mutants in response to MMS (□), UV (O) and X rays (△); mutants sensitive to both MMS and UV.

FIGURE 7 (top)—*mms6–1*.

FIGURE 8 (bottom left)—*mms18–1*.

FIGURE 9 (bottom right)—*mms19–1*.

Figure 10

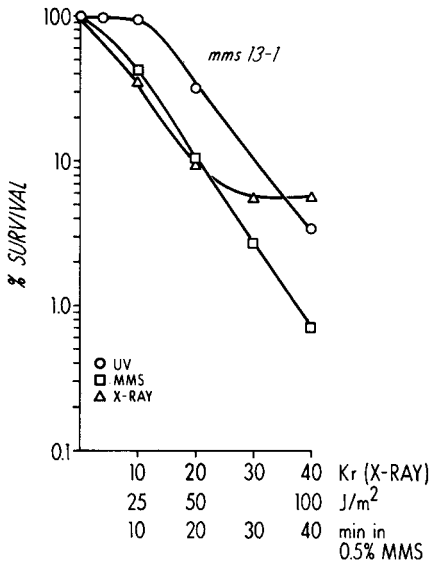
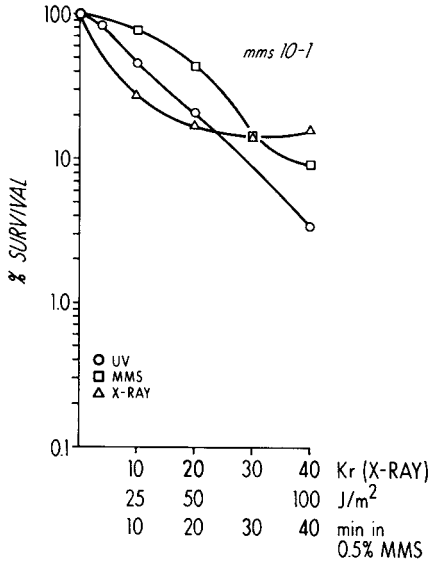


Figure 11



FIGURES 10 and 11.—Survival curves obtained for the *mms* mutants in response to MMS (□), UV (○) and X rays (△); mutants sensitive to both MMS and UV.

FIGURE 10 (left)—*mms13–1*.

FIGURE 11 (right)—*mms10–1*

UV-sensitive. The *mms10–1* (MD-35) mutant, like the *mms2–1* and *mms22–1* mutants described above, is sensitive to MMS only when grown in the presence of MMS (see Figure 1 and Figure 11).

(3) *Mutants sensitive to both MMS and X rays.* This class consists of five mutants and the extent of sensitivity to X rays covers a wide range of responses. The *mms9–1* mutant gives a survival curve in which the resistant tail is eliminated (Figure 12) and is quite X-ray sensitive. The other mutants in this group, *mms14–1* (Figure 13), *mms16–1* (Figure 14), *mms20–1* (Figure 15) and *mms8–1* (Figure 16), exhibit varying degrees of X-ray sensitivity; however, the resistant tail is still present in all of these survival curves.

(4) *Mutants sensitive to MMS, UV and X rays.* This category consists of six mutants which exhibit sensitivity to all three agents: MMS, UV and X rays. Like the mutants in the other phenotypic classes, the mutants in this class show varying responses to UV and X rays. The *mms12–1* mutant is sensitive to UV and X rays (Figure 17); the *mms7–1* (Figure 18), *mms 11–1* (Figure 19) and *mms17–1* (Figure 20) mutants are X-ray sensitive even though the resistant tail is still present, but they all exhibit slight to moderate UV sensitivity. The *mms21–1* mutant, on the other hand, is fairly UV sensitive and gives a UV survival curve lacking a shoulder while it is somewhat sensitive to X rays

Figure 12

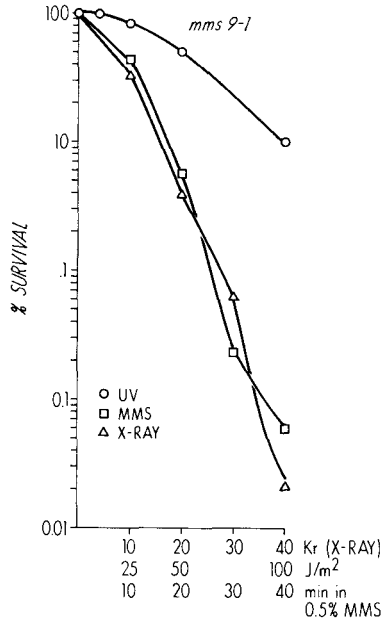


Figure 13

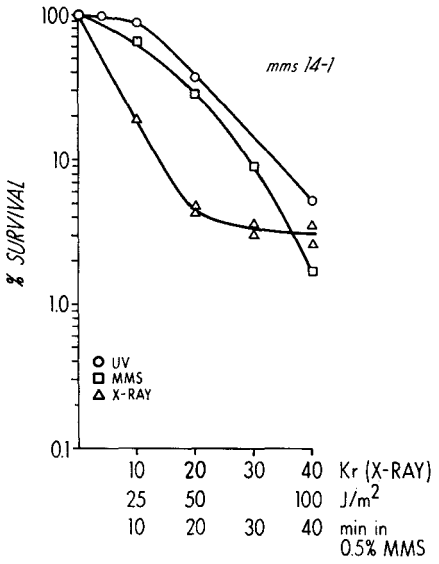
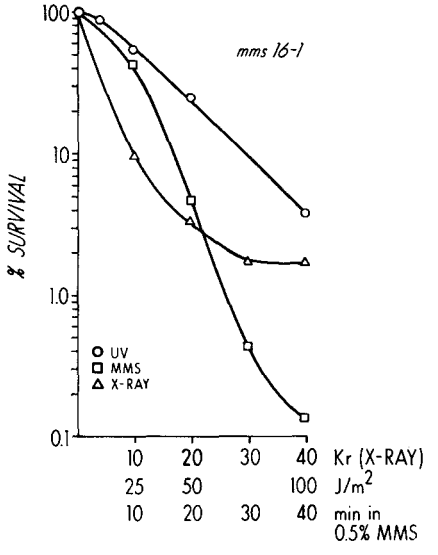


Figure 14



FIGURES 12–14.—Survival curves obtained for the *mms* mutants in response to MMS (□), UV (○) and X rays (△); mutants sensitive to both MMS and X rays.

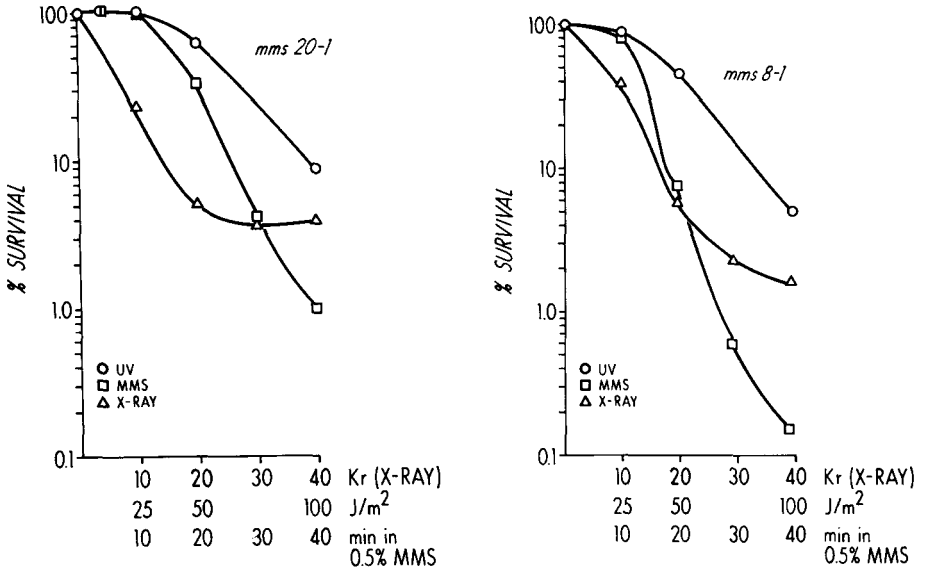
FIGURE 12 (top)—*mms9–1.*

FIGURE 13 (bottom left)—*mms14–1.*

FIGURE 14 (bottom right)—*mms16–1.*

Figure 15

Figure 16



FIGURES 15 and 16.—Survival curves obtained for the *mms* mutants in response to MMS (□), UV (O) and X rays (△); mutants sensitive to both MMS and X rays.

FIGURE 15 (left)—*mms20–1*.

FIGURE 16 (right)—*mms8–1*.

(Figure 21). The *mms15–1* mutant is very sensitive to X rays and gives a survival curve lacking a resistant tail; however, it is only slightly UV-sensitive (Figure 22).

The responses to MMS, UV and X rays exhibited by mutants at these 22 loci indicate that 11 of them confer sensitivity to at least UV or X rays while 6 of them appear to confer sensitivity to all three agents.

Sporulation. Since it is known that in bacteria DNA repair and recombination share common steps (see HANAWALT and SETLOW 1975 for review), the effect of the *mms* genes on sporulation ability was measured. Although a deficiency in sporulation does not in itself reflect a deficiency in recombination ability, deficiency in recombination may cause reduced sporulation ability. Since sporulation ability varies greatly in different strains, isogenic strains were constructed in order to minimize any genetic background effects. This was accomplished by crossing each α *mms* strain to the parent strain, B-635, from which the mutants were derived, such that a series of diploids heterozygous for a particular *mms* gene was constructed. The sporulation efficiency in these heterozygous diploids was compared to that in the diploids homozygous for the particular *mms* gene. These two diploids are isogenic in that they each contain the same α strain, while

Figure 17

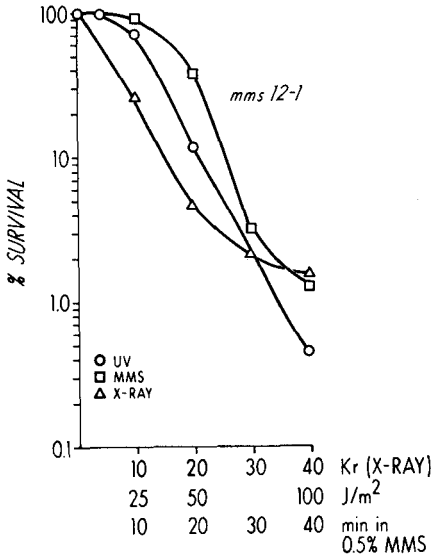
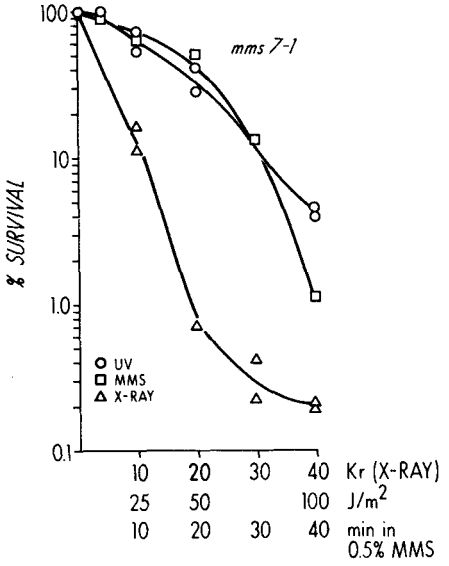
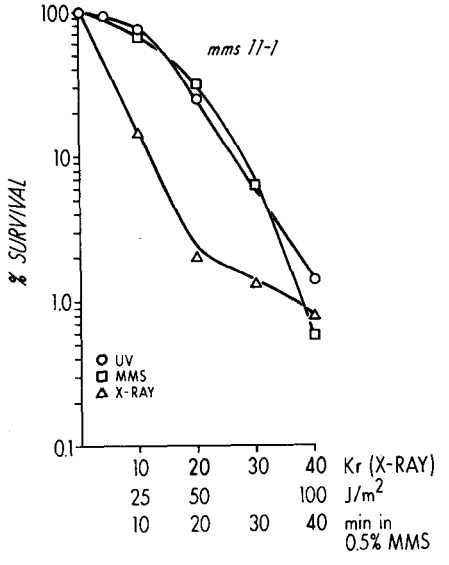


Figure 18

Figure 19





FIGURES 17–19.—Survival curves obtained for the *mms* mutants in response to MMS (□), UV (O) and X rays (△); mutants sensitive to MMS, UV and X rays.

FIGURE 17 (top)—*mms12–1.*

FIGURE 18 (bottom left)—*mms7–1.*

FIGURE 19 (bottom right)—*mms11–1.*

Figure 20

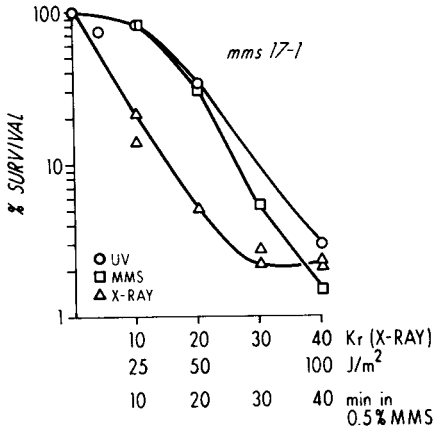


Figure 21

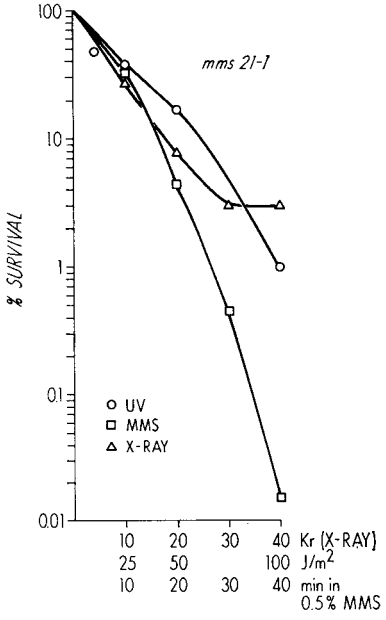
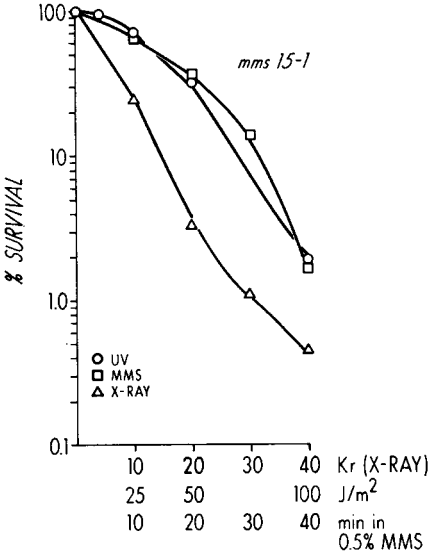


Figure 22



FIGURES 20–22.—Survival curves obtained for the *mms* mutants in response to MMS (□), UV (○) and X rays (△); mutants sensitive to MMS, UV and X rays.

FIGURE 20 (top)—*mms17–1.NN*

FIGURE 21 (bottom left)—*mms21–1.*

FIGURE 22 (bottom right)—*mms15–1.*

the **a** strains differ only by the mutation(s) induced by UV used for isolating the mutants. Results are given in Table 4. There is considerable variation in the sporulation ability of different heterozygous controls and it is most likely due to differences in their genetic background. Sporulation is reduced or absent in homozygous diploids of *mms3–1*, *mms3–2* but not the allele *mms3–3* and is also reduced or absent in *mms4–1*, *mms7–1*, *mms9–1*, *mms12–1*, *mms13–1*, *mms14–1*, *mms17–1* and *mms21–1*. We cannot make any statements about the effects of *mms15–1*, *mms18–1*, *mms19–1*, *mms20–1* and *mms22–1* since the *MMS+/mms* heterozygotes involving these genes exhibit very low sporulation. It then appears that *mms* mutants belonging to 9 complementation groups reduce the sporulation ability when present in the homozygous state.

DISCUSSION

We have isolated mutants belonging to 22 complementation groups which confer sensitivity to the monofunctional alkylating agent methyl methanesulfonate. In addition to mutants at these new loci, we isolated one allele each of *rad6*, *rad55*, *rad57*, *rad1* and *rad4* and four alleles of *rad52*. Mutants at the *RAD6*, *RAD52*, *RAD55* and *RAD57* loci are known to be sensitive to both X rays and

TABLE 4

Effect of genes conferring sensitivity to MMS on sporulation

mms gene	*MMS+/mms* (% sporulation)	Total no. cells counted	*mms/mms* (% sporulation)	Total no. cells counted
1–4	8.63	278	7.41	324
2–1	31.76	821	37.87	771
3–1	8.71	815	0	700
3–2	8.75	560	0.98	814
3–3	4.14	821	4.96	705
4–1	9.61	853	0.13	750
5–1	41.08	324	27.78	234
6–1	17.51	1108	15.90	1000
7–1	17.83	808	3.16	697
8–1	6.25	320	5.67	300
9–1	18.67	900	0.54	926
10–1	5.96	520	7.00	557
11–1	11.10	1100	23.11	900
12–1	22.95	671	0	780
13–1	33.04	805	0.88	800
14–1	20.52	853	7.20	750
15–1	3.13	638	0	700
16–1	7.75	400	8.56	222
17–1	6.22	546	0	800
18–1	0.50	400	0	556
19–1	2.46	609	0	600
20–1	1.96	306	0	300
21–1	53.26	768	0	363
22–1	0.33	301	0	200

MMS (present results; PRAKASH 1976b; ZIMMERMAN 1968). Mutants in either the *RAD1* (PRAKASH 1975; UNRAU, WHEATCROFT and COX 1971; WATERS and MOUSTACCHI 1974), *RAD2* (RESNICK and SETLOW 1972), *RAD3* and *RAD4* loci (PRAKASH unpublished results) are known to be defective in excision of UV-induced pyrimidine dimers. By selecting directly for sensitivity to MMS, alleles of the *RAD1* and *RAD4* loci were obtained, which implies that these loci may also play a role in repair of MMS damage to DNA.

The total number of genetic loci represented by these *mms* mutants may be less than 22, since complementation could occur between some pairs of allelic mutants. In spite of the limitations involved in determining the number of genes from information on complementation, we have estimated the number of genetic loci which may be involved in conferring MMS sensitivity by the maximum likelihood estimator:

$$\frac{N}{\hat{n}} = \sum_{J=\hat{n}-k+1}^{\hat{n}} 1/J \qquad \text{(LEWONTIN and PROUT 1956, equation 6)}$$

where N is the total number of mutants studied, $\hat{n}$ is the estimated number of loci and k is the observed number of complementation groups. The maximum likelihood estimator is sufficient; all of the information relating to $\hat{n}$ in the sample is contained in k. The estimated number of gene loci ($\hat{n}$) by this method is 48. However, such estimates of the number of genes depend on the genetic constitution of the strain and the experimental methods used in mutant isolation and therefore are of limited use. The variance of the maximum likelihood estimator is given by:

$$\sigma^2\hat{n} \simeq \frac{n}{e^{N/n} - (1 + N/n)} \qquad \text{(LEWONTIN and PROUT 1956, equation 24)}$$

The standard deviation of our estimated number of gene loci is about 15.

It has been suggested that at least three pathways for dark repair of DNA exist in yeast (BRENDEL and HAYNES 1973; COX and GAME 1974; GAME and COX 1973; LAWRENCE and CHRISTENSEN 1976). One pathway is involved in excision repair of UV and nitrogen mustard (HN2) damage, the second pathway repairs MMS and X-ray damage, and the third pathway is involved in the repair of damage caused by all four of these agents (BRENDEL and HAYNES 1973). If a pathway for repair of both MMS and X-ray damage exists, then all MMS-sensitive mutants involved in repair of MMS damage should be sensitive to X rays and all X-ray sensitive mutants involved in repair of X-ray damage should be sensitive to MMS. The fact that we have 5 mutants which confer sensitivity only to MMS suggests that the repair of MMS damage and X-ray damage may involve common steps, but some steps may be unique to MMS damage. Alternatively, these 5 mutants may not be involved in repair of MMS damage but show increased sensitivity only to MMS due to some alteration in the membrane which renders the cells more permeable to MMS and therefore more sensitive to this agent. Until further experiments are carried out, these two alternatives cannot be distinguished.

The second class of interest consists of 8 mutants representing 6 genetic loci.

The characteristic feature of these mutants is that although they are as resistant to X rays as the wild-type parent strain, they show sensitivity to both MMS and UV. Like the mutants sensitive only to MMS, these mutants sensitive to MMS and UV represent a new phenotypic class. In organisms where X-ray sensitive mutants have been isolated and then tested for MMS sensitivity, the X-ray-sensitive mutants have been found also to be MMS-sensitive. The reverse is also true, *i.e.*, mutants selected for MMS-sensitivity and tested for response to X-rays have been shown to be X-ray sensitive. In *E. coli*, *recA* mutants (KONDO *et al.* 1970), *lex* mutants (MOUNT, LOW and EDMISTON 1972) and *polA* mutants (KONDO *et al.* 1970) are sensitive to X-rays, MMS and UV. We have found that the yeast mutants selected for sensitivity to X rays (*rad50* to *rad57*) are all MMS sensitive (present results). One of the *mms* mutants in this class, *mms19–1*, increases UV-induced reversion (PRAKASH and PRAKASH unpublished results), and although it is not allelic to any of the known genes involved in excision of pyrimidine dimers, it may nevertheless still be involved in the excision-repair pathway. One locus in this group, *mms3*, is represented by three alleles, one of which, *mms3–3*, seems even more resistant to X rays than the parent strain. Since the specific defect in these mutants is not known, the role they play in repair processes is not known, but their sensitivity to both UV and MMS suggests that there may be some steps common to repair of UV and MMS damage which are not shared by pathways which act on X-ray induced damage. If indeed excision-repair is somehow involved, as suggested by our isolation of *rad1* and *rad4* alleles (Table 1) among the *mms* mutants, then the same enzymes might be involved in dimer excision and in excision of alkylation products. The major product found after alkylation of either whole cells or free DNA with MMS is 7-methyl guanine (BROOKES and LAWLEY 1960, 1961), but this product is apparently not enzymatically excised from DNA (PRAKASH and STRAUSS 1970; LAWLEY and ORR 1970). One of the minor alkylation products, O^6-methyl guanine is enzymatically excised from DNA (LAWLEY and ORR 1970). Thus, the possibility does exist that in yeast, the same enzymes which excise UV-induced dimers may also excise O^6-methylguanine from damaged DNA. Endonuclease II of *E. coli* excises O^6-methylguanine from alkylated DNA and a similar enzymatic activity has been found in yeast (FRIEDBERG, HADI and GOLDTHWAIT 1969). However, endonuclease II has not been tested for its ability to act on DNA containing UV-induced pyrimidine dimers.

The five mutants (*mms9-1*, *mms14-1*, *mms 16-1*, *mms20-1* and *mms8-1*) which show MMS and X-ray sensitivity may participate in the same repair pathway as the *rad50* to *rad57* mutants, which are also MMS and X-ray sensitive. The last class of mutants, which are sensitive to UV and X rays, in addition to MMS, may play a role in the same repair pathway as *rad6* and *rad18*, which are also sensitive to all three agents (BRENDEL and HAYNES 1973; COX and GAME 1974). However, until further work is done with these *mms* mutants, we cannot be certain of what role they actually play in the repair of damaged DNA. There may be considerable overlap in repair pathways, even though a particular pathway may deal primarily with one type of damage over another.

The number of genes either directly or indirectly involved in DNA repair in yeast seems to be large. In addition to the 30 or so *rad* and *rev* loci, we now have 22 *mms* loci. Probably, many of these *mms* genes are involved in the repair of damaged DNA and other related metabolic pathways, since they show cross sensitivities to UV and/or X rays and also affect EMS- and UV-induced reversion (PRAKASH and PRAKASH, unpublished results). In addition, four of these *mms* mutants show spontaneously occurring increased mitotic segregation of homozygosity to canavanine resistance (*canr/canr*) from heterozygous canavanine sensitive (+/*canr*) diploids. This increase may be due to either deletions or increased mitotic recombination. The remaining loci did not have any effect on the frequency of mitotic segregation of homozygosity to canavanine resistance (PRAKASH and PRAKASH, in preparation). The involvement of the *MMS* genes in DNA repair may be due to a direct effect of the enzyme(s) made by these genes or due to the regulatory effect of these genes. The repair potential of the cell can also be affected by changes in chromosomal proteins which may make the chromosomes more accessible to the action of these agents. Mutations in proteins analogous to protein X, a protein in *E. coli* which is believed to stabilize damaged DNA (GUDAS and PARDEE 1976), might also reduce the repair capacity of the cell. It is also possible that the effects of some of these genes are more indirect than those already pointed out. Some *mms* mutants may represent mutations in genes whose enzyme products are involved in modifications of DNA, such as methylases, such that the unmodified DNA is more prone to nucleolytic degradation. The *dam-3* mutation in *E. coli*, which results in reduced adenine methylase activity and in a 5-fold reduction in 6-methyl adenine residues in the DNA of *E. coli* K12 or $\phi\lambda$, confers increased sensitivity to MMS, UV and mitomycin C (MARINUS and MORRIS 1975). Pyrimidine auxotrophs in Ustilago have been shown to be UV sensitive (Moore 1975). However, our *mms* mutants are not auxotrophic for adenine, guanine, uracil or cytosine.

Mutants belonging to nine of the *mms* complementation groups reduce the sporulation capacity of diploids, when present in the homozygous state (Table 4), which suggests that the functions of these genes are indispensable in meiosis and sporulation. In addition to being sensitive to MMS, all of the mutants which lower or abolish sporulation are also sensitive to UV or X rays or both in haploids, except for *mms4-1*. Of the 22 UV sensitive *rad* mutants previously isolated, only the *rad6* mutant abolishes sporulation (Cox and PARRY 1968). However, many of the X-ray sensitive *rad* mutants seem to affect sporulation or spore viability. In *rad51*, *rad52*, and *rad55* homozygotes, sporulation is reduced or absent and spore viability seems to be reduced in *rad50*, *rad52*, *rad53*, *rad55* and *rad57* (GAME and MORTIMER 1974). The mutants *rad6* and *rad50* to *rad57* are all sensitive to MMS and affect sporulation or spore viability. It seems that in selecting for MMS-sensitive mutants, one is also enriching for mutant genes whose functions are indispensable in sporulation.

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Corresponding editor: R. E. ESPOSITO