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Three Additional Genes Involved in Pyrimidine Dimer Removal in *Saccharomyces cerevisiae*: *RAD7*, *RAD14* and *MMS19*

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Summary. The ability to remove ultraviolet (UV)-induced pyrimidine dimers from the nuclear DNA of yeast was examined in two radiation-sensitive *(rad)* mutants and one methyl methanesulfonate-sensitive *(mms)* mutant of the yeast *Saccharomyces cerevisiae*. The susceptibility of DNA from irradiated cells to nicking by an endonuclease activity prepared from crude extracts of *Micrococcus luteus* was used to measure the presence of dimers in DNA. The *rad7*, *rad14* and *mms19* mutants were found to be defective in their ability to remove UV-induced dimers from nuclear DNA. All three mutants belong to the same epistatic group as the other mutants involved in excision-repair. All three mutants show enhanced UV-induced mutations. The *rad14* mutant also shows epistatic interactions with genes in the other two UV repair pathways.

Introduction

In the yeast *Saccharomyces cerevisiae*, six genetic loci are now known to be involved in excision of ultraviolet light (UV)-induced pyrimidine dimers. These loci consist of *RAD1* (Prakash, 1975; Unrau, Wheatcroft and Cox, 1971; Waters and Moustacchi, 1974), *RAD2* (Resnick and Setlow, 1972), *RAD3*, *RAD4* (Prakash, 1977a; Reynolds, 1978), *RAD10* and *RAD16* (Prakash, 1977b). Although a mutation in any one of these six genes results in retention of pyrimidine dimers in the DNA following UV irradiation, the particular step of the dimer excision process which is defective is not known in any of the mutants. Another phenotype common to these mutants is that they exhibit enhanced UV-induced mutations at a wide variety of loci (Averbeck et al., 1970; Eckhardt and Haynes, 1977; Lawrence and Christensen, 1976; Resnick 1969; Zakharov, Kozina and Fedorova, 1970). This phenotype is also characteristic of excision-defective mutants in *Escherichia coli* (Ishii and Kondo, 1975; Kondo et al., 1970; Witkin, 1967). Thus far, all mutants known which enhance UV-induced mutations are those which are defective in excision of dimers.

In addition to the mutants described above, two other UV-sensitive mutants of yeast showed a somewhat increased mutation frequency following UV irradiation: *rad7* and *rad14* (Lawrence and Christensen, 1976). In addition, a mutant isolated as sensitive to the monofunctional alkylating agent, methyl methanesulfonate (MMS), *mms19*, (Prakash and Prakash, 1977) enhanced UV-induced reversion in diploids homozygous for *mms19* (Prakash and Prakash, unpublished results). We therefore decided to examine the ability of these three mutants, *rad7*, *rad14* and *mms19*, to remove pyrimidine dimers from their DNA. The results presented in this communication indicate that (1) *rad7*, *rad14* and *mms19* mutants are defective in dimer excision (2) they belong to the same epistatic group as the other excision-defective mutants of yeast, the *rad1* pathway, and (3) they show increased UV-induced mutations at a number of different loci.

Materials and Methods

Strains

The strains used for experiments on loss of endonuclease-sensitive sites contained the *tup2-107* gene which enables them to incorporate exogenous thymidine monophosphate into their DNA. The following strains were used: LP573-10A α *ade2 hs7 tup2-107 rad7*; LP572-10A α *ade1* and/or *ade2 tyr1 ura1 canR tup2-107 rad14* and LP564-2D α *ade2 met15-3 lys2-1 tyr1 tup2–107 mms19*. The *rad7* mutant was a deletion of *CYC1 (cyc1–13)* which also included the closely-linked gene, *rad7* and was obtained from Dr Chris Lawrence. The other *rad7* strains used and the *rad14* mutant were isolated by Cox and Parry (1968), while the *mms19* mutant was isolated by Prakash and Prakash (1977). The *tup2–107* was obtained from Dr. Reed Wickner

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Media

The following media were used: YPD. 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose, solidified with 2% Bacto-agar. Synthetic complete (SC) medium, used for viability determinations, consisted of 0.67% Difco yeast nitrogen base without amino acids, 2% dextrose, 2% Bacto-agar, adenine sulfate, L-arginine HCl, L-histidine HCl, L-methionine, L-tryptophan and uracil at 20 μg/ml, DL-homoserine at 100 μg/ml, L-isoleucine, L-leucine, L-lysine HCl and L-tyrosine at 30 μg/ml, L-valine at 150 μg/ml and L-phenylalanine at 50 μg/ml. Omission media used to score for revertants to prototrophy for arginine, histidine, lysine and tryptophan consisted of the above constituents lacking one of the supplements at a time and were designated SC minus arginine (SC-arg), SC minus histidine (SC-his), SC minus lysine (SC-lys) and SC minus tryptophan (SC-trp), respectively.

Detection of Dimers in DNA

The details of the procedures for labelling cells with tritiated thymidine-5′-monophosphate (^{3}H-dTMP), UV irradiation, preparation of spheroplasts, centrifugation in neutral CsCl gradients and alkaline sucrose sedimentation were as previously described (Prakash, 1975). The nuclease specific for DNA containing dimers was prepared from *Micrococcus luteus* (Miles Chemical Co., Elkhart, Ind.). The enzyme was partially purified according to a modification (Reynolds, 1976) of the method described by Carrier and Setlow (1970) and treatment with *M. luteus* extract was as described by Prakash (1977b). The enzyme preparation had no activity against unirradiated DNA but introduced nicks in DNA from UV irradiated cells. The nicks were detected by the slower sedimentation of irradiated DNA treated with *M. luteus* extract compared to non-enzyme treated DNA.

UV Irradiation

Mutation induction: cultures were grown in liquid YPD at 30° C to stationary phase, washed and plated on SC for viability determinations and on various omission media for determining UV-induced reversion of a particular marker. Cells were irradiated on the surface of plates with covers removed at a fluence rate of either 0.1 $Jm^{-2}s^{-1}$, or 1 $Jm^{-2}s^{-1}$, depending on the total fluence desired. The radiation source and its dosimetry are given in Lawrence et al. (1974). Plates were incubated in the dark at 30° C for 3–4 days before counting surviving colonies and 4–6 days before counting revertants on omission media.

Survival curves: cultures grown in liquid YPD at 30° C to stationary phase were washed and plated on YPD plates. Cells were irradiated with covers removed as described above. Plates were incubated in the dark at 30° C for 3–6 days before counting surviving colonies. Since the UV sensitivity of *rad* and *mms* strains can vary somewhat depending on the genetic background, care was taken to include the results obtained from genetically related strains for survival curves presented in Figures 5–8.

Results

Retention of Endonuclease-Sensitive Sites in the Nuclear DNA of rad7, rad14 and mms19 Mutants

The *rad7*, *rad14* and *mms19* mutants were irradiated with a fluence of 67 J/m^2 resulting in 7.3%, 0.27% and 11.5% survival, respectively. The nuclear DNAs obtained from unirradiated cells, irradiated cells, and irradiated cells allowed a period of dark repair for 4 h following UV irradiation were purified through neutral CsCl centrifugation and then sedimented in alkaline sucrose gradients with or without prior treatment with *M. luteus* extract. Breaks are not detected in nuclear DNA from UV irradiated cells since, when this DNA is sedimented in alkaline sucrose gradients without prior treatment with *M. luteus* extract, the profile obtained is like those obtained for DNA from unirradiated cells or from irradiated cells given a 4 h dark repair period and sedimented without prior enzyme treatment. The profiles from these three nuclear DNA samples were like that of DNA from unirradiated cells but treated with *M. luteus* extract prior to sedimentation (Fig. 1). The results for nuclear DNAs of *rad7* after treatment with *M. luteus* extract are given in Fig. 1. The irradiated DNA peaks between fractions 17–23 compared to unirradiated DNA which peaks between fractions 5–13, in the region of the T7 marker. The slower sedimentation observed

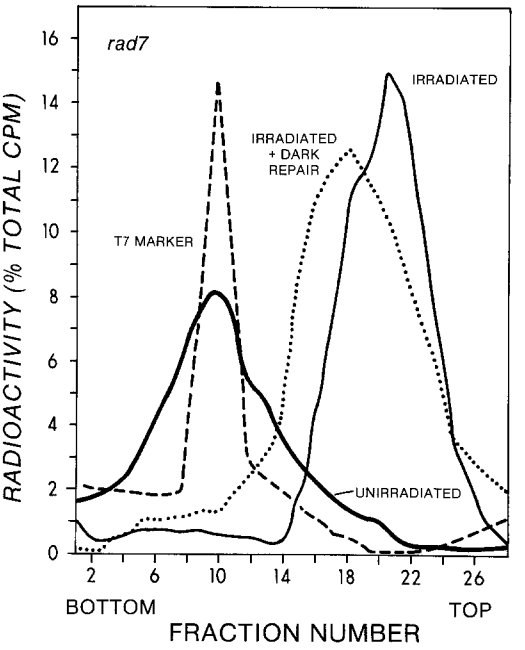


Fig. 1. Retention of endonuclease-sensitive sites in the nuclear DNA of the *rad7* strain irradiated at a fluence of 67 J/m^2 Alkaline sucrose gradient sedimentation patterns of nuclear DNA treated with 5 μl *M. luteus* nuclease: DNA from unirradiated cells (——); DNA from irradiated cells (——), DNA from irradiated cells given a 4 h dark incubation period in growth medium (...); T7 marker DNA (---). The nuclear DNAs obtained from unirradiated cells, from irradiated cells, or from irradiated cells given a 4 h dark incubation period in growth medium and sedimented in alkaline sucrose gradients without prior treatment with *M. luteus* nuclease gave profiles like that of unirradiated DNA treated with *M. luteus* nuclease prior to sedimentation, which sedimented to the same position as that of the T7 marker DNA

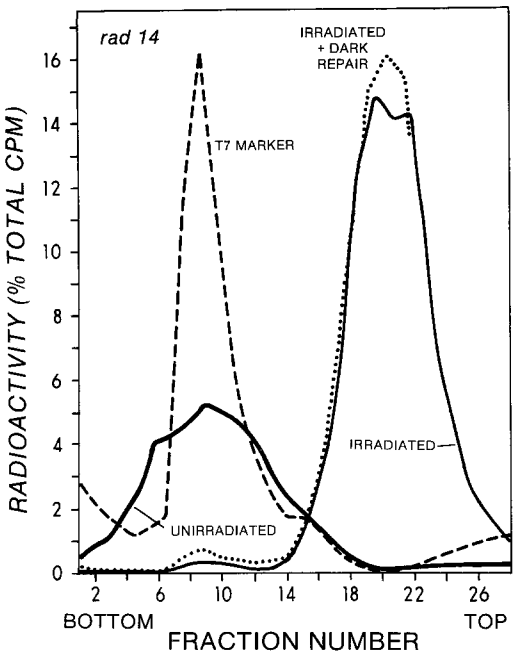


Fig. 2. Retention of endonuclease-sensitive sites in the nuclear DNA of the *rad14* mutant irradiated at a fluence of 67 J/m^2. Alkaline sucrose gradient sedimentation patterns of nuclear DNA treated with 5 µl *M. luteus* nuclease. Symbols are as in Fig. 1

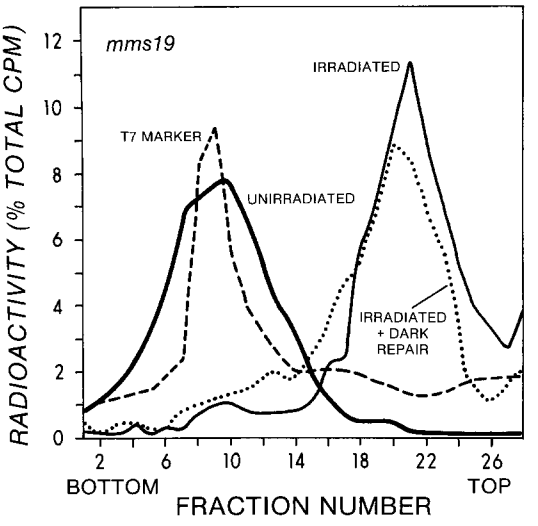


Fig. 3. Retention of endonuclease-sensitive sites in the nuclear DNA of the *mms19* mutant irradiated at a fluence of 67 J/m^2. Alkaline sucrose gradient sedimentation patterns of nuclear DNA treated with 5 µl *M. luteus* nuclease. Symbols are as in Fig. 1

for the DNA from irradiated cells indicates that this DNA contains endonuclease-sensitive sites, that is, pyrimidine dimers. Some of these endonuclease-sensitive sites are lost from the DNA, as seen by the faster sedimentation of DNA from irradiated cells of *rad7* which were given a 4 h dark repair period prior to extraction of DNA. DNA from these cells still sediments slower than DNA from unirradiated cells and peaks between fractions 14–22. Lower fluences were also used in order to see whether removal of pyrimidine dimers could occur. However, even when survival is 41.5% in the *rad7* mutant endonuclease-sensitive sites still remain in the DNA.

Results obtained for the *rad14* mutant are given in Fig. 2. The *rad14* mutant does not show any loss of endonuclease-sensitive sites from the DNA even after a 4 h dark repair period. The sedimentation pattern obtained for DNA from irradiated cells is very similar to that obtained for the DNA from irradiated cells given a 4 h dark repair period prior to spheroplast formation. Although survival in the *rad14* mutant was only 0.27%, a *rad6* mutant irradiated with a fluence of 67 J/m^2, which resulted in less than 0.01% survival, was able to remove all detectable endonuclease-sensitive sites under similar conditions (Prakash, 1977a). It thus seems that the *rad14* mutant is defective in the ability to remove pyrimidine dimers from UV irradiated DNA.

In some preliminary experiments on UV-induced reversion of *cyc1–115*, we noted that *mms19* showed enhanced reversion frequencies. The *mms19* mutant is sensitive to both MMS and UV but not X-rays. We therefore determined whether or not dimers could be excised from the DNA of *mms19* mutants. Figure 3 shows the results obtained. The sedimentation pattern obtained for DNA from irradiated cells and for DNA from irradiated cells given a 4 hour dark repair period prior to enzyme treatment shows that pyrimidine dimers remain in the nuclear DNA of *mms19*, since DNA from irradiated cells, whether or not a period of dark incubation occurred prior to enzyme treatment, peaks between fractions 17–23, far removed from DNA obtained from unirradiated cells.

All three mutants retain endonuclease-sensitive sites in their nuclear DNA under conditions where *RAD+* and several other *rad* mutants such as *rad6* and *rad9* (Prakash, 1977a) lose all detectable sites from their DNA. Therefore, we conclude that these three mutants, *rad7*, *rad14* and *mms19* are defective in their ability to remove UV induced pyrimidine dimers from their DNA.

UV-Induced Reversion

Reversion following UV irradiation was determined for various markers in these mutants. Table 1 A gives the results obtained for the reversion of the ochre

Table 1. UV-induced reversion of *lys2-1, trp2* and *arg4–17* in *MMS+* and *mms19* haploids

Strain	Gene	Fluence (J/m²)			
		0	2.5	5	10
A. Lysine-independent revertants (LYS+)/10^7 survivors					
B-635	*MMS+*	0 (100)	2 (100)	6 (99)	24 (100)
MD-55	*mms19*	1 (100)		74 (97)	210 (84)
LP1395-7C	*mms19*	6 (100)		195 (68)	442 (32)
B. Tryptophan-independent revertants (TRP+)/10^7 survivors					
B-635	*MMS+*	12 (100)		26 (91)	40 (100)
LP1395-3D	*mms19*	10 (100)		295 (57)	520 (32)
C. Arginine-independent revertants (ARG+)/10^7 survivors					
CL332-1B	*MMS+*	10 (100)	12 (100)	27 (88)	74 (92)
LP1395-3D	*mms19*	25 (100)	131 (83)	444 (57)	960 (32)

Numbers in parentheses indicate percent survival

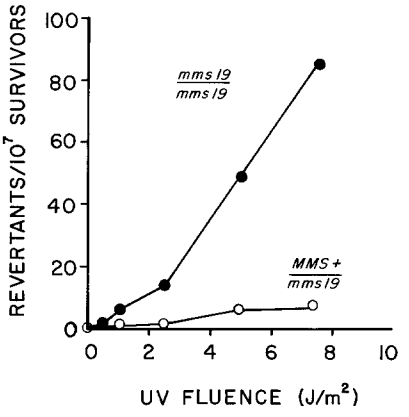


Fig. 4. UV-induced reversion of *cyc1–115* in *mms19/mms19* (●) or *mms19/MMS+* (○) diploids

suppressible allele *lys2-1* in *MMS+* and *mms19* haploids. Although spontaneous *LYS+* prototrophs in *lys2-1* mutants are mainly due to ochre suppressors, UV-induced revertants of the *lys2-1* allele are mainly due to site revertants (Lemontt, 1971). The results show that in *mms19* haploids, the frequency of lysine-independent *(LYS+)* revertants is much higher than in *MMS+* strains irradiated with the same fluence. A similar effect is seen for reversion of *trp2* to *TRP+* (Table 1B) and *arg4–17* to *ARG+* (Table 1C). Reversion in *mms19/mms19* homozygous diploids is also enhanced at the *cyc1–115* site (Fig. 4).

The enhanced mutation frequency following UV irradiation is quite substantial in *rad14* haploids, even though they are not particularly UV sensitive. The increase is observed both at the *his5-2* (Table 2A) and *arg4–17* alleles (Table 2B). UV-induced reversion of *arg4–17* is increased in diploids homozygous for *rad7* (Lawrence and Christensen, 1976).

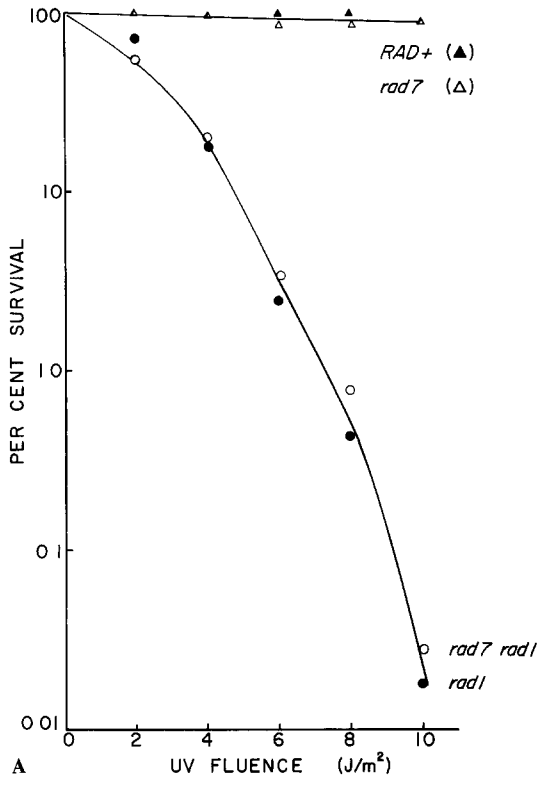
UV sensitivity in radx rad7, radx rad14 and radx mms19 Double Mutant Combinations

Since UV sensitivity of a *rad* or *mms* mutant can vary in different strains due to differences in genetic background, whenever double mutant combinations were constructed, the single mutants obtained from the same cross were used to obtain the data given in Figures 5–8.

Table 2. UV-induced reversion of *his5-2* and *arg4–17* in *RAD+* and *rad14* haploids

Strain	Gene	Fluence (J/m²)				
		0	2.5	5	7.5	10
A. Histidine-independent revertants (HIS+)/10^7 survivors						
CL332-1B	*RAD+*	4 (100)	13 (100)	34 (88)	57 (90)	90 (92)
LP1397-8A	*rad14*	2 (100)	434 (100)	759 (100)	882 (100)	1,245 (73)
B. Arginine-independent revertants (ARG+)/10^7 survivors						
CL332-1B	*RAD+*	10 (100)	12 (100)	27 (88)	36 (90)	74 (92)
LP1397-8A	*rad14*	3 (100)	68 (100)	187 (100)	306 (100)	674 (73)

Numbers in parentheses indicate percent survival



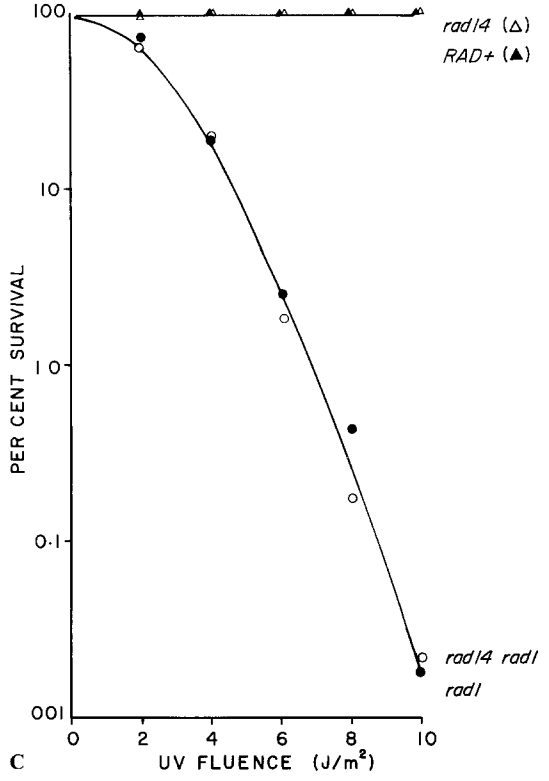


Fig. 5A–C. Survival curves following UV irradiation of **A** *RAD1+ RAD7+*, (▲); *RAD1+ rad7*, (△); *rad1 RAD7+*, (●); and *rad1 rad7*, (○) haploids. **B** *RAD1+ MMS19+*, (▲); *RAD1+ mms19*, (△); *rad1 MMS19+*, (●); and *rad1 mms19*, (○) haploids. **C** *RAD1+ RAD14+*, (▲), *RAD1+ rad14*, (△), *rad1 RAD14+*, (●); and *rad1 rad14* (○) haploids. The points on each survival curve in Figure 5 are the averages of data obtained from two to four different strains

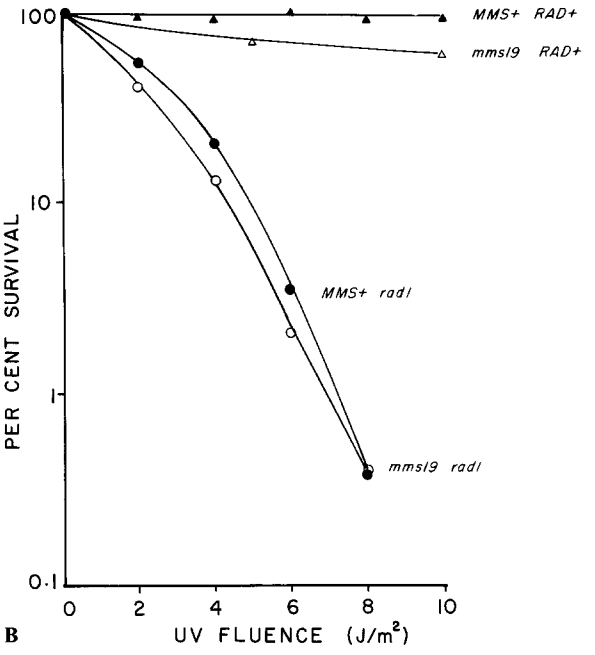
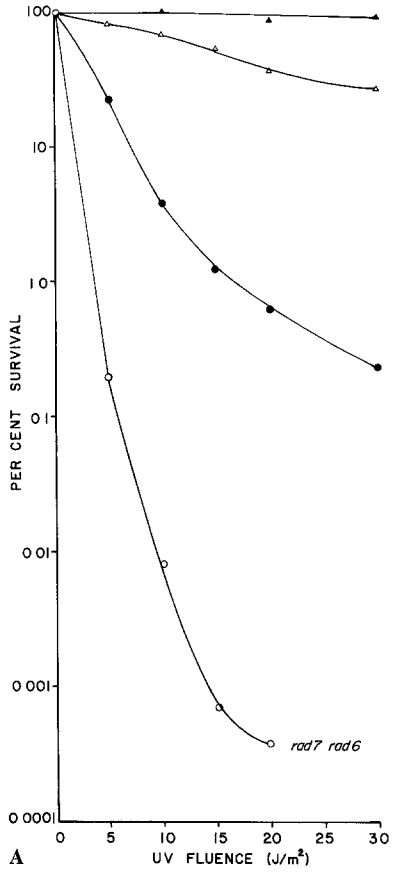


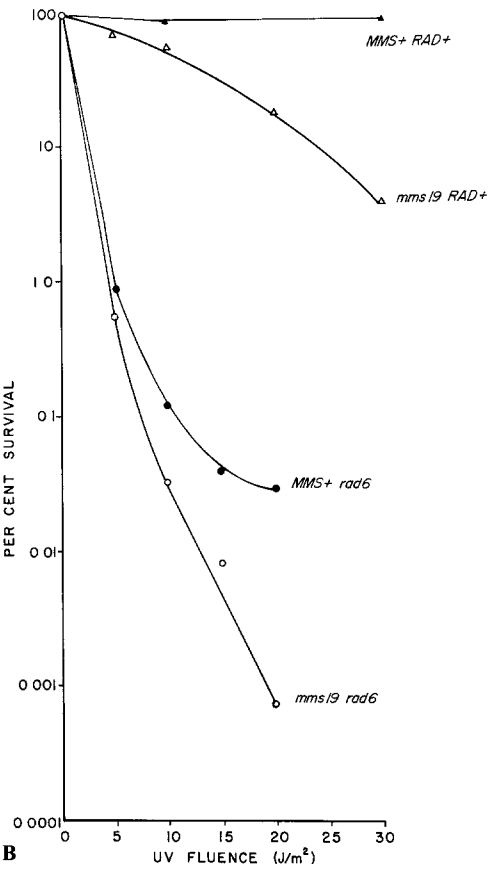
Table 3. Recombination between *mms19* and *rad* mutants of the excision-defective epistatic group

Cross	Tetrads			Wild-type recombinants/ Total spores
	PD	NPD	T	
mms19 × rad1	1	2	5	
mms19 × rad2	2	0	1	7/30
mms19 × rad3	3	2	6	18/63
mms19 × rad4	0	1	0	4/24
mms19 × rad7				11/44
mms19 × rad10	1	0	2	2/17
mms19 × rad14				3/15
mms19 × rad16	1	2	4	

Combinations With rad1

In order to determine whether these three mutants, *rad7*, *rad14* and *mms19*, belong to the same epistatic group as the other excision-defective mutants of yeast, double mutant combinations were made by crossing *rad1* with *rad7*, *rad14* and *mms19*. Survival following UV irradiation was determined for the single and double mutants generated from these crosses. All three mutants, *rad7*, *mms19* and *rad14* show epistatic interactions with *rad1* (Fig. 5A, B and C, respectively). In all three cases, the double mutants are no more sensitive than the *rad1* single mutant.





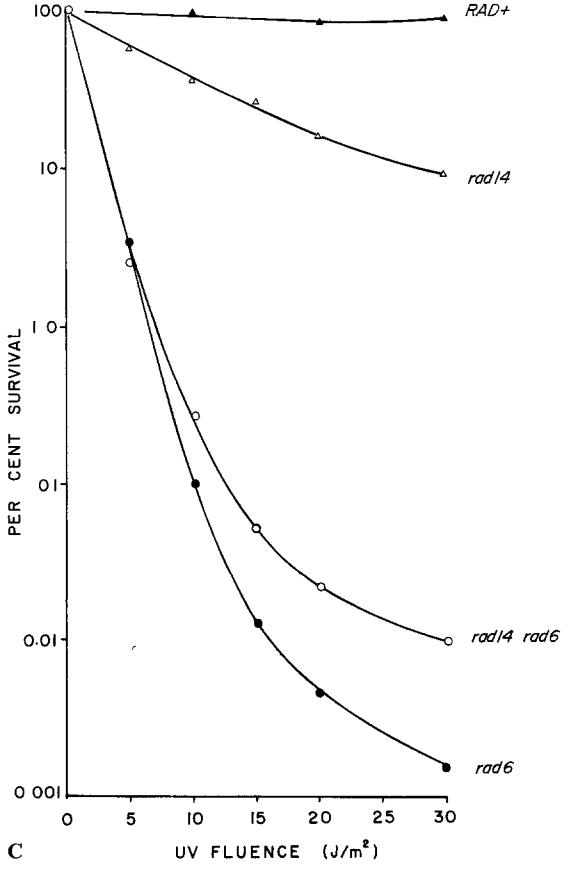


Fig. 6A–C. Survival curves following UV irradiation of **A** *RAD6+ RAD7+*, (▲); *RAD6+ rad7*, (△); *rad6 RAD7+*, (●); and *rad6 rad7*, (○) haploids. **B** *RAD6+ MMS19+*, (▲); *RAD6+ mms19*, (△); *rad6 MMS19+*, (●); and *rad6 mms19*, (○) haploids. **C** *RAD6+ RAD14+*, (▲), *RAD6+ rad14*, (△); *rad6 RAD14+*, (●); and *rad6 rad14*, (○) haploids. Each point on the survival curves of the *mms19 rad6* and *rad14 rad6* double mutants are averages obtained from two different strains

The *mms19* mutant, which was isolated as a mutant sensitive to MMS, represents a mutation in a new gene involved in excision repair which has not been identified previously. Diploids constructed between *mms19* and other excision-defective *rad* mutants *rad1*, *rad2*, *rad3*, *rad4*, *rad7*, *rad10*, *rad14* and *rad16* are all UV-resistant, showing that *mms19* complements these genes. In addition, data obtained from tetrads and random spores from these crosses show that *mms19* recombines with all of these genes (Table 3) and therefore defines a new locus.

Combinations With rad6

Double mutants of *rad7 rad6* and *mms19 rad6* are much more UV sensitive than the *rad6* single mutants (Fig. 6A and B, respectively), indicating that both *rad7* and *mms19* do not belong to the epistatic group involving *rad6*. The *RAD6+* gene is required for UV-induced mutations (Lawrence and Christensen, 1976;

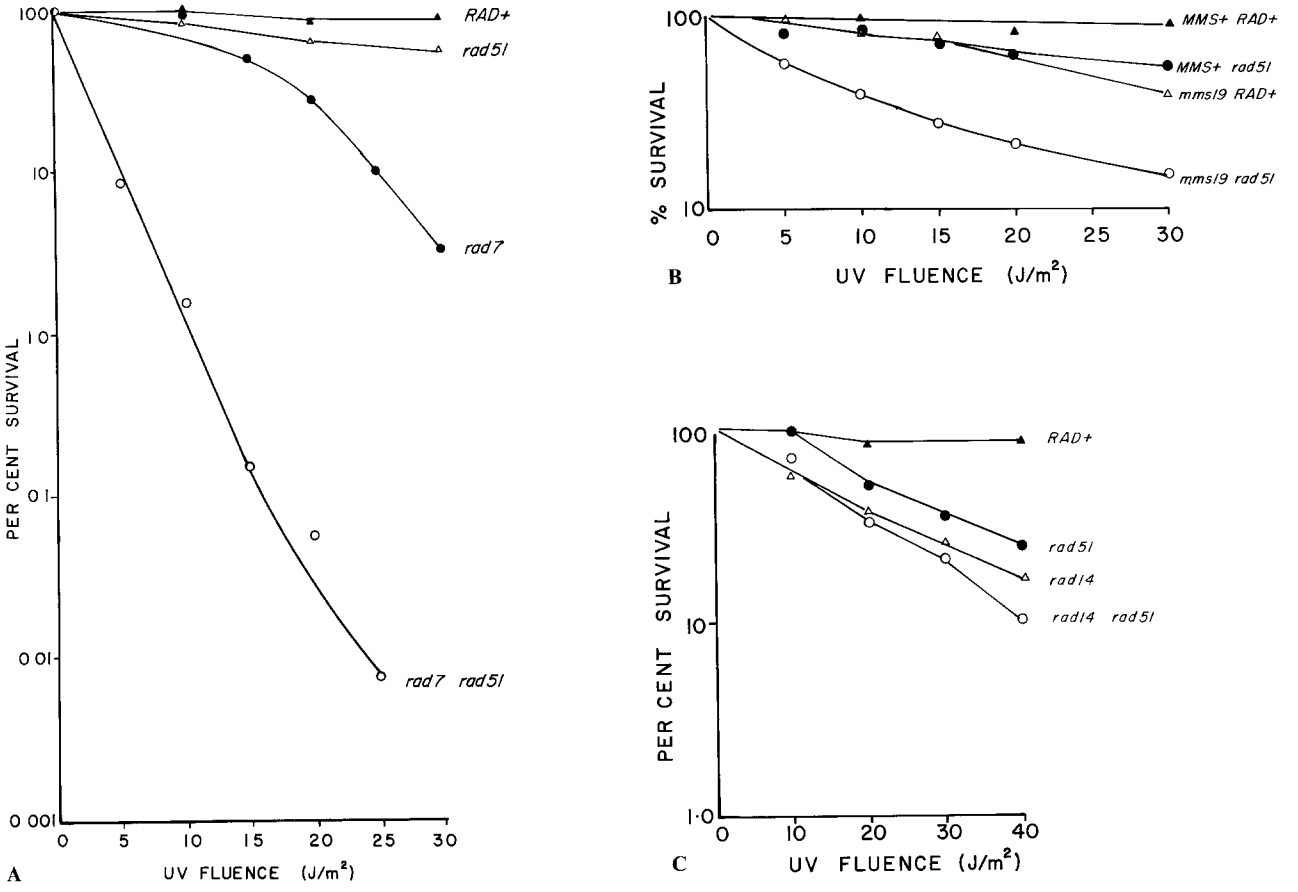


Fig. 7 A–C. Survival curves following UV irradiation of **A** *RAD51+ RAD7+*, (▲); *rad51 RAD7+*, (△); *RAD51+ rad7*, (●), and *rad51 rad7*, (○) haploids. **B** *RAD51+ MMS19+*, (▲); *RAD51+ mms19*, (△); *rad51 MMS19+*, (●), and *rad51 mms19*, (○) haploids. **C** *RAD51+ RAD14+*, (▲); *RAD51+ rad14*, (△); *rad51 RAD14+*, (●); and *rad51 rad14*, (○) haploids. Each point on the survival curves in Fig. 7 are averages obtained from two different strains

Cox and Game, 1974). On the other hand, the *rad14 rad6* double mutant is not more sensitive than the *rad6* single mutant (Fig. 6C), suggesting that *rad14* belongs to the *rad6* epistatic group as well.

Combinations With rad51

The third pathway involved in repair of UV damage in yeast is thought to be an error-free recombinational repair system controlled by *RAD51* and other loci (Lawrence and Christensen, 1976; Prakash et al., 1979). Double mutants consisting of *rad7* and *rad51* are clearly much more UV-sensitive than either single mutant (Fig. 7A). The *mms19* mutation also increases the UV sensitivity of *rad51*, although the effect is not as great (Fig. 7B). The *rad14* mutation, however, does not appear to affect the sensitivity of *rad51* (Fig. 7C). The *rad7* and *mms19* mutants both belong only to the *rad1* epistatic group and not to either *rad6* or *rad51*. The *rad14* mutant, on the other hand belongs to all three epistatic groups. Since the *rad14* mutant is only moderately UV-sensitive, one could conclude that rather than belonging to all three repair

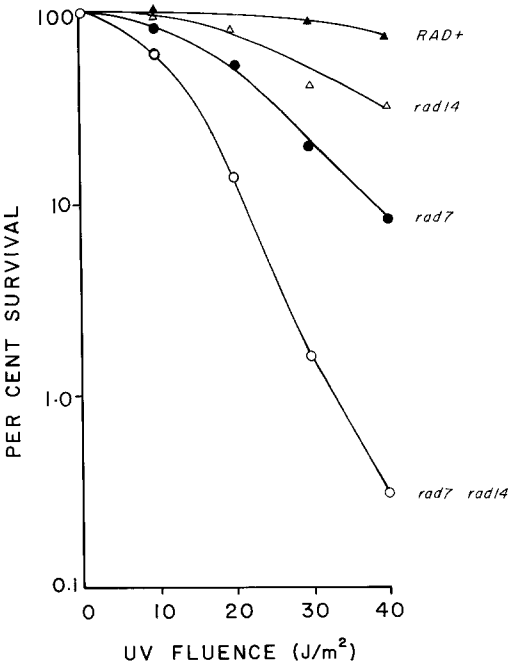


Fig. 8. Survival curves following UV irradiation of *RAD7+ RAD14+*, (▲); *RAD7+ rad14*, (△); *rad7 RAD14+*, (●); and *rad7 rad14*, (○) haploids. The points on each survival curve are the averages obtained from three different strains

pathways for UV damage in yeast, *rad14* belongs to none of them. Several lines of evidence argue against this. First, enhanced UV-induced mutation and defective pyrimidine dimer excision are observed in *rad14* mutants. Second, the *rad7 rad14* double mutants are much more UV sensitive than either single mutant alone (Fig. 8). If the *rad14* mutation were not playing a role in repair of UV damage, the sensitivity of the *rad7 rad14* double mutant would not be greater than either single mutant. The increased sensitivity of *rad7* when combined with *rad14* can be interpreted by supposing that *rad14* does indeed play a role in all three repair pathways; thus, the sensitivity of *rad7* is increased in the presence of the *rad14* mutation because the *rad6* and/or *rad51* pathways are partially blocked.

Discussion

The results presented here show that mutants of the *RAD7*, *RAD14* and *MMS19* loci are defective in the removal of UV-induced pyrimidine dimers from the nuclear DNA of yeast and that these genes belong to the epistatic group involved in the excision of pyrimidine dimers. This brings the total number of loci involved in excision of dimers to nine; these loci consist of *RAD1*, *RAD2*, *RAD3*, *RAD4*, *RAD7*, *RAD10*, *RAD14*, *RAD16* and *MMS19*. It is interesting to note that *mms19*, which was isolated as a mutant sensitive to MMS, is involved in dimer excision. In fact, one allele of *RAD1* (designated as *rad1–20)* and one allele of *RAD4* (designated as *rad4–10*) were obtained in our collection of MMS-sensitive mutants. The diploids *rad1/rad1–20* and *rad4/rad4–10* were found to be MMS-sensitive. Some of the other excision defective mutants are reported to be sensitive to the lethal effects of other chemicals such as nitrous acid (Zimmermann, 1968). Mutants in nine genes of the *RAD1* epistatic group show enhanced UV-induced reversion compared to *RAD+* strains but the extent of enhancement varies in different mutants and for different loci.

Although nine genes are involved in dimer excision in yeast, it is unlikely that all nine loci will be structural genes for a UV-endonuclease (s). Bacteriophage T4 has a UV-endonuclease coded for by a single gene, the *v* gene (Friedberg and King, 1971; Yasuda and Sekiguchi, 1970). In *E. coli,* it has recently been demonstrated that the *uvrA* and *uvrB* loci are the structural genes for the UV-endonuclease (Seeberg, 1978). In humans, five complementation groups for dimer excision have been identified from cell fusion studies (Kraemer et al., 1975), but it has not yet been established which genes code for the UV-endonuclease(s). Extracts prepared from human fibroblasts obtained from individuals without xeroderma pigmentosum (XP) are able to excise dimers from irradiated *E. coli* DNA as well as from human unfractionated irradiated cell-free preparations, where the DNA is presumably present as chromatin. However, extracts prepared from fibroblasts of patients with XP and belonging to complementation group A can remove dimers from purified DNA but not from chromatin (Mortelmans et al., 1976). These results suggest that UV-endonuclease activity is present in extracts from complementation group A fibroblasts but cannot excise pyrimidine dimers from chromatin. XP cells of complementation group A may be defective in a protein factor, which is required for the incision activity of UV endonucleases on chromatin. Some of the excision-defective mutants of yeast may have defects in such protein(s). Recently, it has been reported that in human fibroblasts following UV irradiation, more repair synthesis occurs in the staphylococcal nuclease-sensitive linker regions than in the nuclease-resistant core regions (Smerdon and Lieberman, 1978). The regions of repair synthesis which were originally nuclease-sensitive later become nuclease-resistant and appear in core DNA. Nucleosome rearrangement seems to accompany repair of UV damage and may depend on a variety of proteins. Defects in any such proteins may also lead to reduced excision ability.

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References

Averbeck, D., Laskowski, W., Eckardt, F., Lehmann-Brauns, E.: Four radiation sensitive mutants of *Saccharomyces*. Survival after UV- and X-ray-irradiation as well as UV-induced reversion rates from isoleucine-valine dependence to independence. Mol. Gen. Genet. **107**, 117–127 (1970)

Carrier, W.L., Setlow, R.B.: Endonuclease from *Micrococcus luteus* which has activity toward ultraviolet-irradiated deoxyribonucleic acid: purification and properties. J. Bacteriol. **102**, 178–186 (1970)

Cox, B.S., Game J.: Repair systems in *Saccharomyces*. Mutat. Res. **26**, 257–264 (1974)

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

Eckardt, F., Haynes, R.H.: Induction of pure and sectored mutant clones in excision-proficient and deficient strains of yeast. Mutat. Res. **43**, 327–338 (1977)

Friedberg, E.C., King, J J.: Dark repair of ultraviolet-irradiated deoxyribonucleic acid by bacteriophage T4: purification and characterization of a dimer-specific phage-induced endonuclease. J. Bacteriol. **106**, 500–507 (1971)

Ishii, Y., Kondo, S.: Comparative analysis of deletion and base-change mutabilities of *Escherichia coli* B strains differing in DNA repair capacity (wild-type, *uvrA*$^-$, *polA*$^-$, *recA*$^-$) by various mutagens. Mutat. Res. **27**, 27–44 (1975)

Kondo, S., Ichikawa, H., Iwo, K., Kato, T.: Base-change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities. Genetics **66**, 187–217 (1970)

Kraemer, K.H., De Weerd-Kastelein, E.A., Robbins, J.H., Keijzer, W., Barrett, S.F., Petinga, R A., Bootsma, D.: Five complementation groups in xeroderma pigmentosum. Mutat. Res. **33**, 327–340 (1975)

Lawrence, C.W., Christensen, R.· UV mutagenesis in radiation sensitive strains of yeast. Genetics **82**, 207–232 (1976)

Lawrence, C.W., Stewart, J.W., Sherman, F., Christensen, R.: Specificity and frequency of ultraviolet-induced reversion of an iso-l-cytochrome *c* ochre mutant in radiation-sensitive strains of yeast. J. Mol. Biol **85**, 137–162 (1974)

Lemontt, J.· Mutants of yeast defective in mutation induced by ultraviolet light. Genetics **68**, 21–33 (1971)

Mortelmans, K., Friedberg, E.C., Slor, H., Thomas, G., Cleaver, J.E.: Defective thymine dimer excision by cell-free extracts of xeroderma pigmentosum cells. Proc. Natl. Acad Sci. U.S.A. **73**, 2757–2761 (1976)

Prakash, L.. Repair of pyrimidine dimers in nuclear and mitochondrial DNA of yeast irradiated with low doses of ultraviolet light. J. Mol Biol. **98**, 781–795 (1975)

Prakash, L.: Repair of pyrimidine dimers in radiation-sensitive mutants *rad3, rad4, rad6,* and *rad9* of *Saccharomyces cerevisiae* Mutat. Res. **45**, 13–20 (1977a)

Prakash, L.: Defective thymine dimer excision in radiation-sensitive mutants *rad10* and *rad16* of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **152**, 125–128 (1977b)

Prakash, L., Prakash, S : Isolation and characterization of MMS-sensitive mutants of *Saccharomyces cerevisiae*. Genetics **86**, 33–55 (1977)

Prakash, S., Prakash, L., Burke, W., Montelone, B.A.: Effects of the *rad52* gene on recombination in *Saccharomyces cerevisiae*. Genetics (1979)

Resnick, M.: Genetic control of radiation sensitivity in *Saccharomyces cerevisiae*. Genetics **62**, 519–531 (1969)

Resnick, M., Setlow, K.: Repair of pyrimidine dimer damage induced in yeast by ultraviolet light. J. Bacteriol. **109**, 979–986 (1972)

Reynolds, R.: Excision repair in *Saccharomyces cerevisiae*. Ph. D. thesis. University of Tenessee, Knoxville, Tenn. (1976)

Reynolds, R.J.: Removal of pyrimidine dimers from *Saccharomyces cerevisiae* nuclear DNA under nongrowth conditions as detected by a sensitive, enzymatic assay. Mutat. Res. **50**, 43–56 (1978)

Seeberg, E.: Reconstitution of an *Escherichia coli* repair endonuclease activity from the separated *uvr A+* and *uvr B+/uvrC+* gene products. Proc. Natl. Acad. Sci. U.S.A. **75**, 2569–2573 (1978)

Smerdon, M.J., Lieberman, M.W.. Nucleosome rearrangement in human chromatin during UV-induced DNA repair synthesis. Proc. Natl. Acad. Sci. U.S.A. **75**, 4238–4241 (1978)

Unrau, R., Wheatcroft, R., Cox, B.S.: The excision of pyrimidine dimers from DNA of ultraviolet irradiated yeast. Mol. Gen. Genet. **113**, 359–362 (1971)

Waters, R., Moustacchi, E.: The disappearance of ultraviolet-induced pyrimidine dimers from the nuclear DNA of exponential and stationary phase cells of *Saccharomyces cerevisiae* following various post-irradiation treatments. Biochim. Biophys. Acta **353**, 407–419 (1974)

Witkin, E.M.: Mutation-proof and mutation-prone modes of survival in derivatives of *Escherichia coli* B differing in sensitivity to ultraviolet light. Brookhaven Symp. Biol. **20**, 17–55 (1967)

Yasuda, S., Sekiguchi, M.: Mechanism of repair of DNA in bacteriophage. II. Inability of ultraviolet-sensitive strains of bacteriophage in inducing an enzyme activity to excise pyrimidine dimers. J. Mol. Biol. **47**, 243–255 (1970)

Zakharov, I A , Kozina, T.N., Fedorova, I.V.. Effets de mutations vers la sensibilité au rayonnement ultraviolet chez la levure. Mutat. Res. **9**, 31–39 (1970)

Zimmermann, F.K.: Sensitivity to methyl methanesulfonate and nitrous acid of ultraviolet light-sensitive mutants in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **102**, 247–256 (1968)

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