GENETIC EFFECTS OF UV IRRADIATION ON EXCISION-PROFICIENT AND -DEFICIENT YEAST DURING MEIOSIS

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ABSTRACT

The lethal and recombinational responses to ultraviolet light irradiation (UV) by excision-proficient (*RAD*$^+$) and deficient strains (*rad1*) of *Saccharomyces cerevisiae* has been examined in cells undergoing meiosis. Cells that exhibit high levels of meiotic synchrony were irradiated either at the beginning or at various times during meiosis and allowed to proceed through meiosis. Based on survival responses, the only excision repair mechanism for UV damage available during meiosis is that controlled by the *RAD1* pathway. The presence of pyrimidine dimers at the beginning of meiosis does not prevent cells from undergoing meiosis; however, the spore products exhibit much lower survival than cells from earlier stages of meiosis. The reduced survival is probably due to effects of UV on recombination. Meiotic levels of gene conversion are reduced only two to three times in these experiments; however, intergenic recombination is nearly abolished after a dose of 4 J/m^2 to the *rad1* strain. Exposure to 25 J/m^2 had little effect on the wild-type strain. Since normal meiotic reciprocal recombination is generally considered to involve gene conversion-type intermediates, it appears that unrepaired UV damage dissociates the two processes. These results complement those obtained with the *mei-9* mutants of Drosophila which also demonstrate a dissociation between gene conversion and reciprocal recombination. These results are consistent with molecular observations on the UV-irradiated *rad1* strain in that there is no excision of pyrimidine dimers or exchange of dimers during meiosis.

THE DNA repair mechanisms that have been identified in mitotically growing cells of the yeast *Saccharomyces cerevisiae* can be divided into two categories regarding their role in normal meiosis: essential or dispensable (COX and PARRY 1968; GAME and MORTIMER 1974; GAME *et al.* 1980). Since the excision repair pathway for ultraviolet light (UV) damage induced in yeast does not appear to be required for normal meiosis (SNOW 1968; DICAPRIO and HASTINGS, 1976; DOWLING 1981), it is possible to evaluate, as we have done, the role of the excision pathway in the repair of UV damage during meiosis. In addition, by using mutants of the *RAD1* gene, the effects of DNA damage on meiosis can be determined in the absence of excision repair. In the accompanying paper (RESNICK, STASIEWICZ and GAME 1983), we demonstrated that at the doses examined (2 J/m^2 or greater) there was no detectable excision of pyrimidine

dimers in *rad1* mutants regardless of when in meiosis the cells were irradiated or whether the cells were irradiated at the beginning and then allowed to continue through meiosis. After exposure to 4 J/m^2 (corresponding to 1600 pyrimidine dimers per diploid cell) the presence of pyrimidine dimers did not prevent the synthesis of large molecular weight DNA, and the average size synthesized was of the order of five times larger than the distance between dimers. As part of this study we have examined the genetic effects of unexcised DNA damage on the meiotic process in terms of viability of cells during meiosis, the production of haploid products (spores), viability of spores and recombination—either intragenic (conversion) or intergenic (reciprocal).

This study differs from previous reports (SIMCHEN, SALTS and PIÑON 1973; SALTS, SIMCHEN and PIÑON 1976; HOTTINGUER-DE MARGERIE and MOUSTACCHI 1979; MACHIDA and NAKAI 1980; KELLY and PARRY 1983) on the effects of DNA damage on meiotic events in yeast in that we have been able to compare the responses of wild-type to excision-defective strains under conditions in which we can also evaluate the molecular nature of events in terms of dimer excision and meiotic DNA metabolism (*i.e.*, amount of DNA synthesis and size of DNA synthesized) as cells proceed through meiosis. In addition we have utilized strains in which meiosis is relatively synchronous so that the timing of molecular and genetic events can be more closely monitored. As part of this genetic study we have adopted the "pull-back" procedure of SHERMAN and ROMAN (1963) to examine commitment to genetic events during meiosis. Using this method, we have been able to irradiate cells prior to the meiotic round of DNA synthesis, allow meiosis to continue and at various times plate cells to the appropriate medium to determine the genetic effects of DNA damage on cells proceeding through meiosis.

We have found that the presence of unexcised DNA damage during meiosis in *rad1* as compared with wild-type strains markedly affects meiotic genetic events. At low doses cells irradiated at the beginning of meiosis retain relatively high viability during subsequent stages as diploids but show a sharp decline in viability when haploidization occurs. Although meiotic intragenic recombination is decreased two- to threefold at these doses, reciprocal recombination, as measured by the appearance of live diploid recombinants, is nearly abolished. Therefore, it appears that DNA damage can have substantially greater effects on some meiotic related genetic processes than others.

MATERIALS AND METHODS

Yeast strains: Strains have been described in the accompanying paper (RESNICK, STASIEWICZ and GAME 1983).

Growth and sporulation: Procedures for growing and sporulating cells have been described in the accompanying paper. Following meiosis it was possible to obtain nearly pure populations of spores from cultures containing greater than 90% asci. Cells were treated with zymolase (RESNICK, STASIEWICZ AND GAME 1983), diluted in water and sonicated. This treatment resulted in a population of cells that was greater than 99% spores. The sonication also resulted in the disruption of spores so that less than 5% appeared in groups of two, three or four spores. For survival studies the cells were plated after appropriate dilution to YEPD (1% yeast extract, 2% peptone, 2% dextrose and 2% agar). To determine the extent of recombination, cells were plated to synthetic medium containing all of

the following except the component under study: 0.67% yeast nitrogen base, 2% glucose, 2% agar, 0.02 mg/ml of adenine sulfate, arginine, histidine, methionine, tryptophan and uracil, 0.03 mg/ml of leucine and lysine and 150 mg/ml of threonine. To detect canavanine-resistant strains, cells were plated to synthetic medium lacking arginine and containing 0.02 mg/ml of canavanine.

UV irradiation: Methods for UV irradiation of cells are given in the accompanying paper (RESNICK, STASIEWICZ and GAME 1983). The sensitivity to UV was determined by plating cells to YEPD and irradiating them. Survival of cells that were irradiated and resuspended in KAC was determined at various times by plating to YEPD.

RESULTS

Meiosis in RAD$^+$ *and* rad1-1 *strains*: As described in the accompanying paper (RESNICK, STASIEWICZ and GAME 1983), the DNA metabolic events in the *RAD*$^+$ and *rad1-1* strains used in this study are comparable in terms of the timing of events and the rate of DNA synthesis. In addition sporulation kinetics are similar, with the maximum sporulation being greater than 90%. Using the pull-back method described by SHERMAN and ROMAN (1963), we have determined the commitment to meiotic intragenic recombination in a *his1* heteroallelic diploid (Figure 1). Recombination in the *RAD*$^+$ and *rad1-1* strains increases from less than 10^{-5} to greater than 5×10^{-3}/cell plated in a relatively synchronous pattern that follows the kinetics of DNA synthesis and sporulation. Intergenic recombination also increases in a synchronous fashion (see Figures 6 and 7). Similar to results reported by ESPOSITO and ESPOSITO (1974), the commitment to recombination begins shortly after the start of the meiotic round of DNA synthesis. Since survival remains essentially constant during this period and the levels of recombination are comparable, we conclude that the *RAD1* gene product is not required for a "normal" meiosis. These observations agree with previous findings (SNOW 1968; DICAPRIO and HASTINGS 1976; DOWLING 1981; RESNICK, STASIEWICZ and GAME 1983).

UV sensitivity of RAD$^+$ *and* rad1-1 *cells*: As expected, mitotically growing haploid and diploid *rad1-1* strains are considerably more sensitive than the wild type to UV irradiation (Figure 2). Both the haploids and the diploids are approximately 20 times more sensitive to the lethal effect of UV than the wild-type cells (Table 1) at the 10% level of survival. The *rad1-1* mutants are also more sensitive to the effects of UV on sporulation (sporulation is detected in this study by the appearance of asci containing at least two spores). Mitotic cells irradiated before resuspending in meiotic medium exhibit reduced sporulation. As shown in Figure 2, 10 J/m^2 to the *rad1-1* mutant results in a 30 to 50% reduction in sporulation, whereas the wild-type strain is unaffected. Since the *RAD*$^+$ strain is capable of excision repair during meiosis (see accompanying paper), it appears that unexcised damage markedly affects the meiotic process. The result that much smaller doses of UV (less than 4 J/m^2) have little effect on sporulation is consistent with the observations that there is considerable DNA synthesis, and the size of newly synthesized DNA is large after these doses to the *rad1-1* strain.

Unrepaired pyrimidine dimers also affect meiosis in the wild-type strain. As shown in Figure 2, a dose of 100 J/m^2 reduces sporulation by more than 50%;

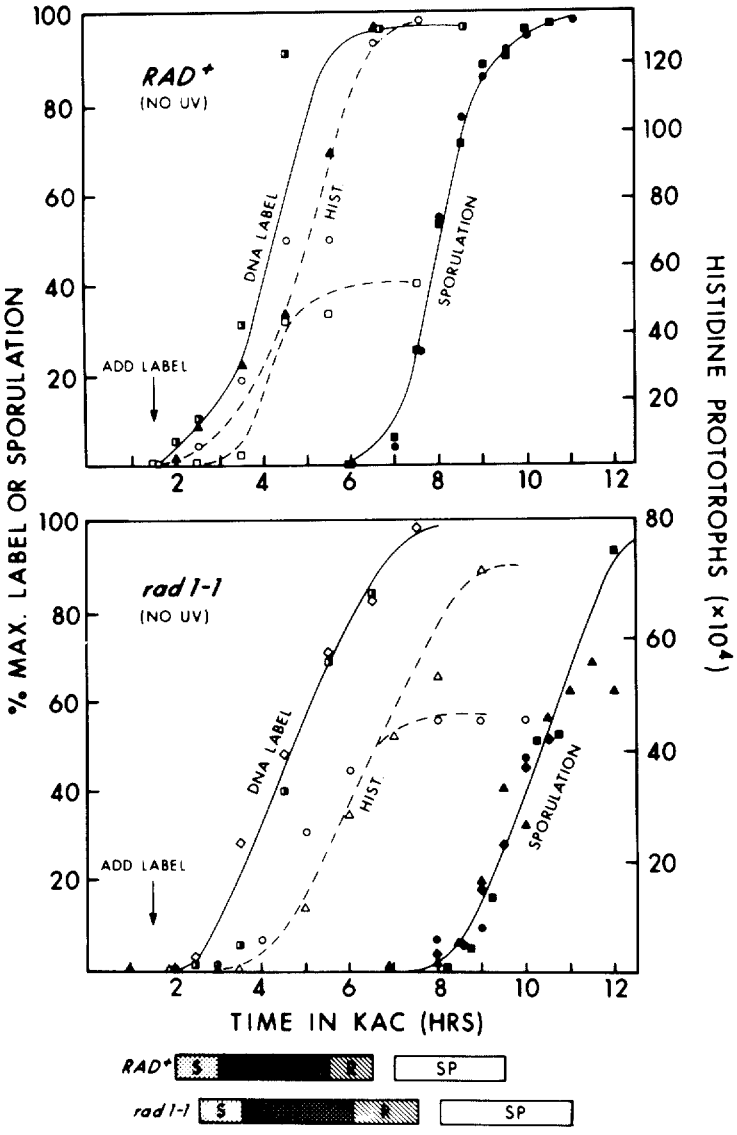

FIGURE 1.—DNA synthesis, recombination and sporulation in *RAD*$^+$ and *rad1-1* strains during meiosis. This figure corresponds in part to that of Figure 2 in the accompanying paper (RESNICK, STASIEWICZ and GAME 1983). Included in this figure is the commitment to intragenic recombination at the *HIS1* gene (*his1-7/his1-1*) that is observed as cells proceed through meiosis. The various symbols correspond to different experiments. The bars below the graphs identify the periods of DNA synthesis(s), recombination (R) and sporulation (SP).

removal of pyrimidine dimers is considerably reduced at this dose as compared with lower doses (*i.e.*, 25 J/m^2; RESNICK, STASIEWICZ and GAME 1983).

UV sensitivity of RAD$^+$ *and* rad1-1 *cells during meiosis*: There have been several reports on the changes in UV sensitivity of cells during meiosis (SALTS, SIMCHEN and PIÑON 1976; SIMCHEN, SALTS and PIÑON 1973; HOTTINGUER-DE

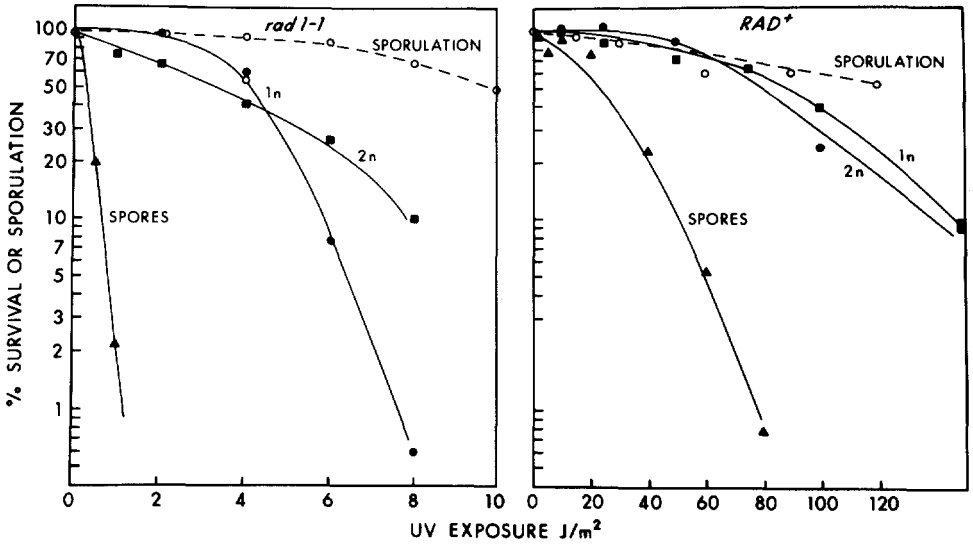

FIGURE 2.—Effects of UV on survival and sporulation of *RAD*$^+$ and *rad1-1* strains. Haploid and diploid cells were grown in presporulation medium plated to YEPD and irradiated. To determine effects on sporulation, cells were irradiated after growth in presporulation medium and resuspended in sporulation medium (KAC); the extent of sporulation was determined 24 hr later. To measure spore response to UV, asci from an unirradiated culture (> 95% asci) were disrupted, and the essentially pure population of spores was plated to YEPD and irradiated.

TABLE 1

Relative UV sensitivity of rad1-1 vs. RAD$^+$ *strains*[a]

Endpoint	Factor of increase
Diploid survival	18
Haploid survival	22
Sporulation of UV-irradiated diploid cells	12
Survival of UV-irradiated spores	75

[a] Based on a comparison of doses between *rad1-1* and *RAD*$^+$ strains at the 10% level of survival.

MARGERIE and MOUSTACCHI 1979); the changes in dose response vary within a factor of two (two sets of conditions are compared on the basis of increase or decrease in dose required to yield comparable levels of survival, *i.e.*, the dose reduction factor). Because of increased synchrony, we have been able to examine changes in sensitivity as cells proceed completely through meiosis to the final sporulated state. The UV sensitivity of *rad1-1* cells in meiosis including the period of DNA synthesis remains comparable with that of mitotic cells (Figure 3). These results are consistent with the observation that no other excision repair mechanism arises during meiosis in *rad1-1* strains (RESNICK, STASIEWICZ and GAME 1983). However, at later times corresponding to the beginning of sporulation and haploidization the UV damage has a greater effect.

A similar response is found for the wild-type cells (Figure 4) in that the greatest sensitivity occurs as cells become asci. Although differences in dose

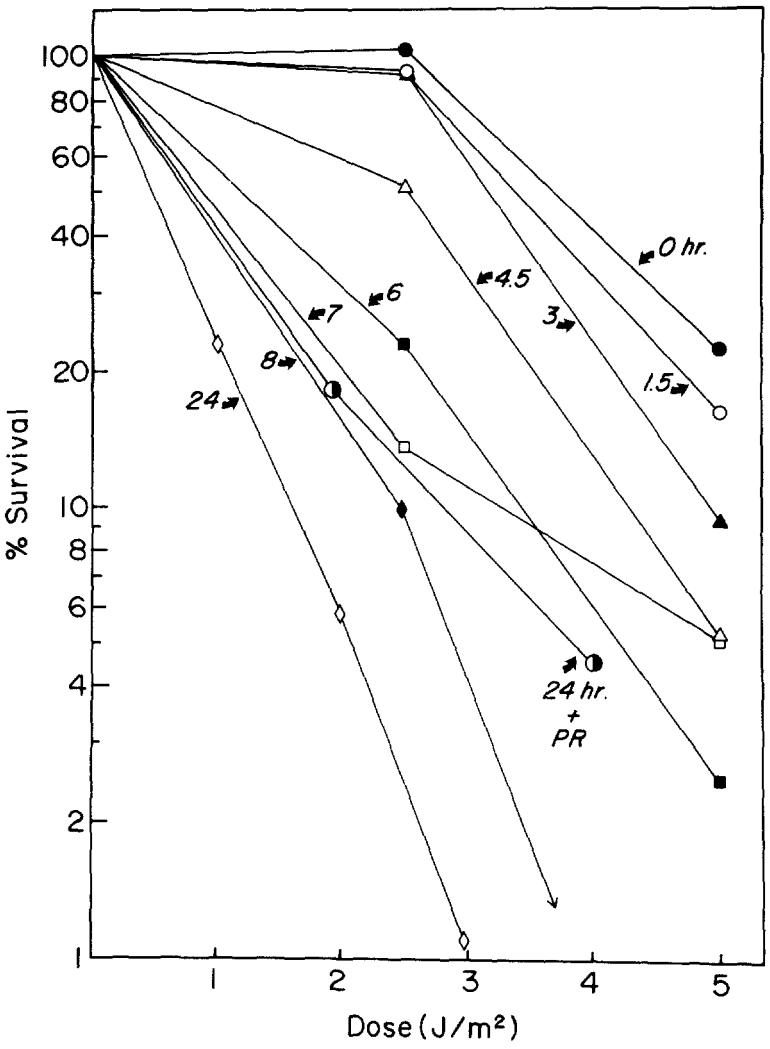

FIGURE 3.—Survival of *rad1-1* cells irradiated at various times of sporulation. Cells were grown in presporulation medium and resuspended in sporulation medium. At various times cells were diluted and plated to YEPD and irradiated; more than 95% of the cells at 24 hr were asci. After UV irradiation of asci at 24 hr, some of the plates were exposed to black light to determine whether the damage could be photoreactivated (24 hr + PR).

response occur during meiosis as shown by others (SIMCHEN, SALTS and PIÑON 1973; HOTTINGUER-DE MARGERIE and MOUSTACCHI 1979), there is a high level of resistance throughout meiosis for *RAD*$^+$ strains which is presumably due to excision repair (compare Figures 3 and 4).

Surprisingly, haploid spore products are much more sensitive to UV than either haploid or diploid vegetatively growing cells (Figures 2, 3, and 4). Both wild-type and *rad1-1* spores are at least three to four times more sensitive. Since the *rad1-1* spores are greater than 70 times more sensitive to UV than *RAD*$^+$

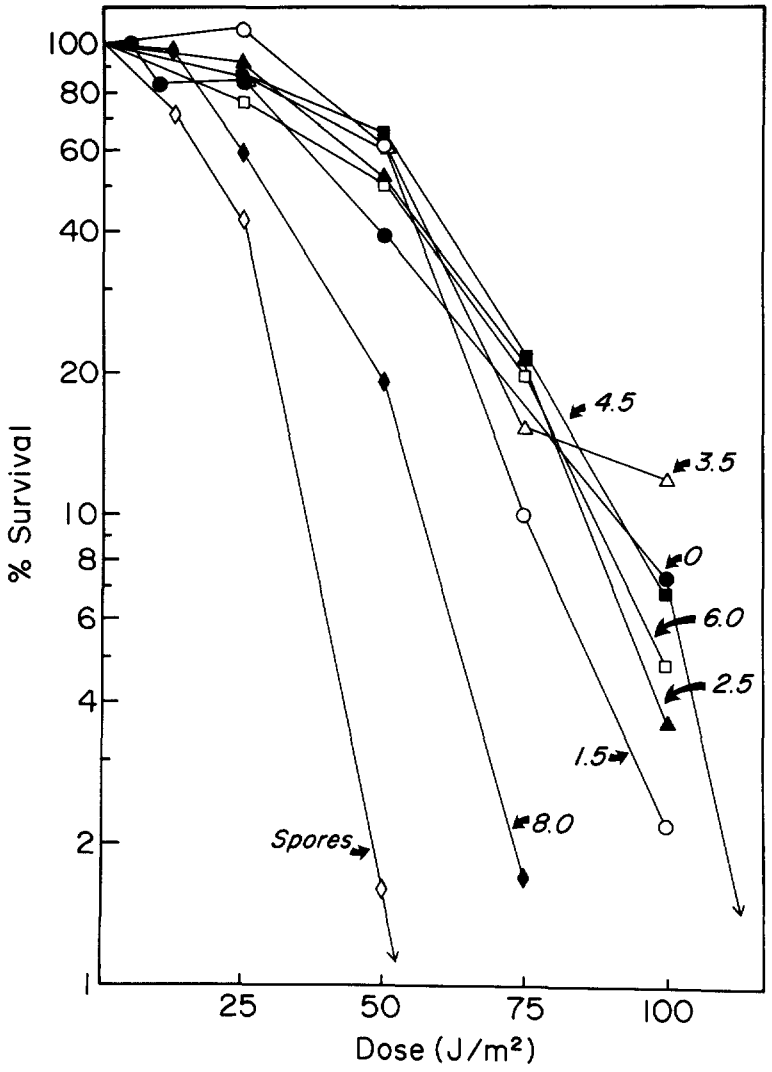

FIGURE 4.—Survival of *RAD*$^+$ cells irradiated at various times of sporulation. Cells were grown in presporulation medium and resuspended in sporulation medium. At various times cells were diluted and plated to YEPD and irradiated. At 24 hr the sporulated population was disrupted so as to yield an essentially spore population (see MATERIALS AND METHODS); the spores were diluted and irradiated.

spores, excision repair is functional and is essential in protecting wild-type spores from UV irradiation. A major component of damage in the spores is pyrimidine dimers since exposure to near UV light after UV treatment of the *rad1-1* strain increases survival (see Figure 3). This effect, which is presumably due to photoreactivation of pyrimidine dimers can reduce the UV dose effect by more than a factor of two.

Since the absence of excision repair was observed to enhance greatly the UV

sensitivity of cells taken at various times during meiosis, we examined the effect of unexcised pyrimidine dimers induced at the early stages of meiosis on the viability of cells as they proceeded through meiosis. As shown in the accompanying paper, pyrimidine dimers do not permanently block DNA synthesis. Mitotically growing *rad1-1* cells were irradiated with 4 J/m^2 (about 50% survival), suspended in KAC and survival was measured at various times (Figure 5). Initially, the number of plating units increases due to completion of mitotic divisions. Survival is essentially constant from 2–8 hr, which included the entire period of DNA synthesis (see Figure 1; RESNICK, STASIEWICZ and GAME 1983). However, survival begins to decrease rapidly shortly before the appearance of spores. By 24 hr there is a fourfold decrease in survival over that at 12 hr. Therefore, it appears that, although the cells can tolerate a large number of pyrimidine dimers throughout much of meiosis, the lethal effect of pyrimidine dimers is much greater in the late stages of meiosis—possibly during reductional or equational segregation of chromosomes. Wild-type cells irradiated with a considerably higher dose (25 J/m^2) at the beginning of meiosis retain their resistance to this dose throughout meiosis, presumably due to excision of pyrimidine dimers.

Effects of UV on meiotic recombination: As expected, exposure of mitotic cells to UV increases recombination (Figures 6 and 7). We examined whether the presence of UV damage at the beginning of (or prior to) meiosis would affect the subsequent increases in meiotic recombination (see Figure 1). The appearance of canavanine resistant cells (*can*^{R}) under our conditions primarily signals a commitment to reciprocal recombination between the centromere and the *CAN1* locus at early stages of meiosis and to haploidization at later stages. We used a low concentration of canavanine (20 μg/ml) in our CAN plates, which, with our strains described here, circumvents the block in expression of diploid *can*^{r} recombinants reported by GAME *et al.* (1980). The *RAD*$^+$ cells from unirradiated cultures begin to show a commitment to homozygosis soon after the initiation of DNA synthesis, long before the appearance of spores (Figure 6). Exposure to UV at a dose that leads to little lethality (25 J/m^2; Figure 4) delays the first appearance of *can*^{r} cells. Similar results are observed for commitment to intragenic recombination (Figure 6). The rates of appearance of recombinants and the final levels are comparable to those in the controls for both types of recombination. From these results we conclude that nonlethal UV exposure of wild-type cells undergoing meiosis leads to few changes in the levels of recombination, as also described by SALTS, SIMCHEN and PIÑON (1976), presumably because excision repair occurs rapidly at nonlethal doses to a wild-type strain (RESNICK, STASIEWICZ and GAME 1983).

In the absence of excision repair, a much lower UV dose at the beginning of meiosis markedly affects viability and the subsequent round of meiotic recombination. Exposure to 4 J/m^2 before suspending cells in meiotic medium (KAC) decreases the final level of commitment to meiotic intragenic recombination among the survivors by a factor of two to three times (Figure 7). Even so, there is still a considerable increase due to the meiotic process as indicated by the levels of recombination observed soon after irradiation and 4–6 hr later in KAC.

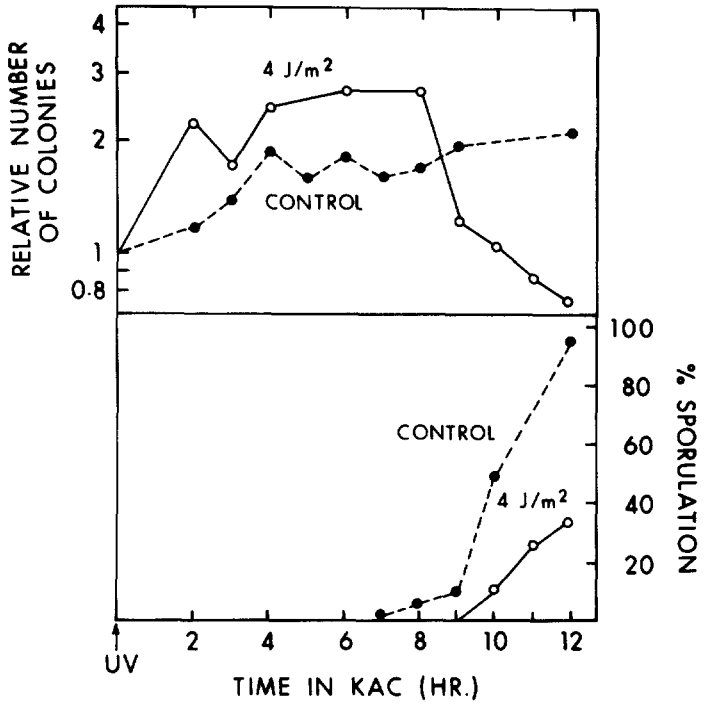

FIGURE 5.—Effect of UV exposure at the beginning of meiosis on *rad1-1* cells undergoing meiosis. Cells were grown in presporulation medium, suspended in water, irradiated (4 J/m^2) and resuspended in sporulation medium (KAC). They were subsequently plated to YEPD. The control was subjected to the same procedure except that it was not irradiated. The percentage of sporulation of the UV-irradiated and control cells is described in the lower figure.

Whereas there is a meiotic increase in intragenic recombination after irradiation, no such increase is found for intergenic recombination. Intergenic recombination was measured by the presence of red colonies on nonselective medium (YEPD) or *can*^{r} colonies on selective medium. As shown in Figure 7, exposure to 4 J/m^2 reduces the appearance of these colonies during meiosis to levels comparable to those soon after irradiation. Since intragenic recombination occurs at high levels (albeit reduced as compared with the unirradiated controls), we conclude that the presence of DNA damage during meiosis has affected intergenic recombination to a much greater extent than intragenic recombination, as judged by the appearance of live recombinants. However, precise interpretation of the data in Figure 7 is difficult due to the fact that, at later times, many of the red and *can*^{r} colonies in the unirradiated culture arise by haploidization rather than recombination. Hence, the appearance of red and *can*^{r} colonies at later times after UV actually monitors the effects of UV on both recombination and haploidization. In the irridated culture, most haploids will be inviable, due to the decrease in viability observed when irradiated cells undergo sporulation. Therefore at times of 8 hr or later a greater relative effect of UV is expected on the frequency of red and *can*^{r} colonies than on histidine

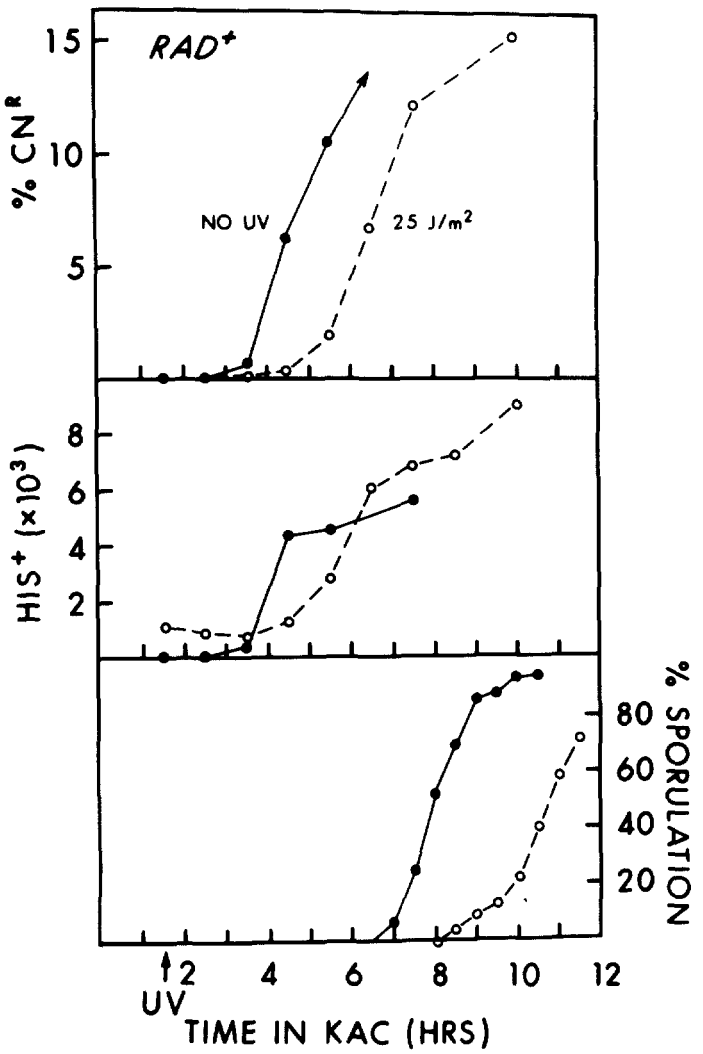

FIGURE 6.—The effect of UV exposure at the beginning of meiosis on meiotic recombination in a *RAD*$^+$ strain. Cells were grown in presporulation medium, suspended in water, exposed to 25 J/m^2 and resuspended in sporulation medium (KAC). They were subsequently plated to medium containing canavanine to determine the appearance of *CAN*^{r} cells; these could have arisen by reciprocal recombination or haploidization. To determine the frequency of intragenic recombination at the *HIS1* locus, the cells were also plated to medium lacking histidine and to YEPD. The percentage of sporulation of the UV-irradiated and control cells is described in the lower figure.

prototrophs, since the latter arise only via recombination and not by haploidization alone. Moreover, viable diploid recombinants may appear only transiently in the culture, since if left longer in sporulation medium many will commit to haploidization and, thus, lethality. A possible transient appearance of recombinants should apply to both intragenic and intergenic recombination, and so the absence of any intergenic recombinants at early times clearly indicates a greater effect of UV on these than on histidine prototrophs.

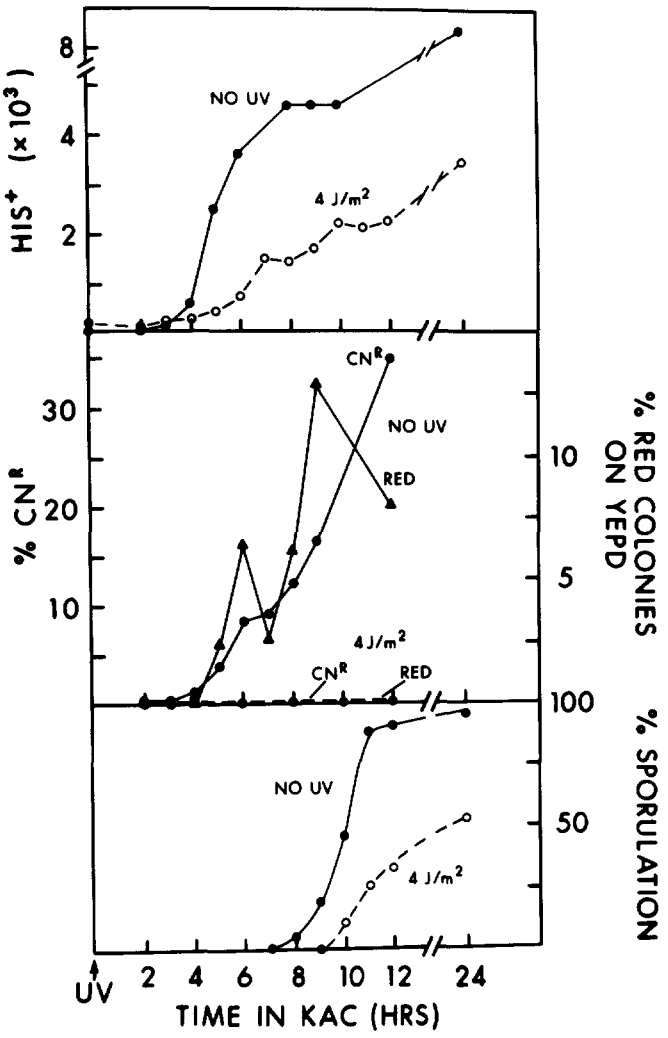

FIGURE 7.—The effect of UV exposure at the beginning of meiosis on meiotic recombination in a *rad1-1* strain. Cells were grown in presporulation medium, suspended in water, exposed to 4 J/m^2 and resuspended in sporulation medium (KAC). They were subsequently plated to medium containing canavanine to determine the appearance of *CAN*^{r} cells; these could have arisen by reciprocal recombination or haploidization. Cells were also plated to YEPD. Red colonies appearing on YEPD were due to the appearance of the recessive *ade2* allele and could have been due to reciprocal recombination or haploidization. To determine the extent of intragenic recombination at the *HIS1* locus, the cells were also plated to medium lacking histidine. Results are expressed as recombinants per survivor. The percentage of sporulation of the UV-irradiated and control cells is described in the lower figure.

We examined this possibility more rigorously by measuring the frequency of intergenic recombinants among histidine prototrophs at various times from irradiated and unirradiated cultures. We reasoned that histidine prototrophs at very early times would represent UV-induced recombinants but at later times would represent cells that had committed to meiotic recombination but had not

yet committed to haploidization and lethality. Hence, if transient viable intergenic meiotic recombinants occurred, we would expect them to be detected among meiotic histidine prototrophs.

Table 2 shows combined data for the frequency of homozygosis for several markers in diploid histidine prototrophs from cultures with and without UV. There is little or no increase in the irradiated culture, at least until 13 hr, whereas a significant increase is seen at early times in the unirradiated culture. Although the number of colonies examined was not large, these results support those described for colonies not selected as histidine prototrophs.

DISCUSSION

In this and the accompanying paper (RESNICK, STASIEWICZ and GAME 1983) we confirm previous findings (SNOW 1968; DICAPRIO and HASTINGS 1976; DOWLING 1981) that the excision repair function identified by the *RAD1* gene is not required for normal meiosis. However, as we have shown, this repair capability is necessary to protect both vegetatively growing cells and cells undergoing meiosis from the effects of UV radiation.

Based on a comparison of UV survival and dimer excision capabilities of *rad1-1* and RAD^+ strains (RESNICK, STASIEWICZ and GAME 1983), it is clear that the excision repair system is the major mechanism for removing pyrimidine dimers throughout meiosis. Since wild-type spores are considerably more resistant to UV than those of the *rad1-1* strain, the excision repair mechanism must also be capable of functioning in spores or as spores germinate. Similarly, the ability to photoreactivate damage is also present in spores.

Although *rad1* cells are considerably more UV sensitive than RAD^+ cells, they can still tolerate DNA damage during early meiosis (Figures 3 and 5). A dose of 4 J/m^2 would be expected to produce approximately 1600 dimers per diploid genome (RESNICK and SETLOW 1972; UNRAU, WHEATCROFT and COX 1972). The high survival is probably associated with other modes of recovery such as the ability to synthesize DNA past these lesions as shown in the accompanying paper. It seems unlikely that the enzymatic system proposed to be responsible for mismatch correction leading to meiotic gene conversion (HOLLIDAY 1964) is also capable of recognizing pyrimidine dimers, since, if it were, survival would be expected to increase in the *rad1* strain during or after the recombination stages of meiosis. The opposite is, in fact, observed (Figures 3 and 5). Moreover, no loss of dimers is observed from meiotic DNA in the *rad1* strain.

The decrease in viability observed (Figure 5) when *rad1* cells irradiated early in meiosis reach the later stages and undergo sporulation could be due to segregation of recessive lethal events or the effects of pyrimidine dimers on chromosome disjunction as will be discussed. The former explanation is unlikely since haploid and diploid survivals are comparable in vegetative cells at these doses. Neither reason can explain the much greater sensitivity of spores (compared with early meiotic or vegetative cells) when irradiated as spores (Figure 3). The D_{37} (dose to kill to 37% survival level in exponential survival range) for spores is only 0.4 J/m^2; if the induction of pyrimidine dimers is comparable to

TABLE 2

Frequency of homozygosis for recessive markers in histidine prototrophs from sporulating cultures of the rad1-1 *strain with and without UV radiation*

Time in sporulation medium (hr)	After 4 J/m^2 UV at T = 1.5 hr			Without UV radiation		
	No. of diploid histidine prototrophs tested	No. of recombinants	Recombination (%)	No. of diploid histidine prototrophs tested	No. of recombinants	Recombination (%)
0				50	0	
2	59	3	5.1			
3	60	3	5.0	52	2	3.8
4	110	5	4.5	69	4	5.8
5	60	2	3.3	52	7	13.5
6	61	2	3.3	47	15	31.9
7	59	3	5.1	23	2	8.7
8	57	3	5.3			
10	61	5	8.2			
13	62	8	12.9			

Histidine prototrophs were picked randomly from his$^-$ plates restreaked on complete medium and stamped to appropriate test media. Colonies that were *trp2*, *leu2* or *CAN1*^{s} were classified as recombinants between the marker concerned and the centromere, and pooled data for these three intervals are given above. *CAN1*^{s} homozygotes were readily detected on CAN plates by the absence of resistant papillae, which are very frequent in heterozygous colonies. To distinguish recombination from haploidization, only those colonies that were heterozygous for mating type (as judged by ability to sporulate) were included in this analysis. In the unirradiated culture, most histidine prototrophs from samples plated at 7 hr or later were haploid, due to commitment to sporulation, and those that were diploid were probably uncommitted to meiosis, so that the apparent recombination frequency among diploids declined at late times. In the irradiated culture few, if any, live haploids were found, even after 13 hr in sporulation medium.

that in vegetative cells, this corresponds to only eight dimers/cell. A possible explanation for the sensitivity of spores may relate to need for transcription during germination. If many genes must be "turned on" before DNA synthesis can begin or if a considerable amount of DNA synthesis must occur before alternative repair systems (e.g., postreplicational) can operate, then the presence of dimers in essential genes would prevent or greatly reduce their transcription so that functional gene products would not be formed (RESNICK and HOLLIDAY 1971; SWENSON and SETLOW 1964). This situation contrasts with haploid cells irradiated during vegetative growth. In this case, cells would be expected to have gene transcripts and gene products at the time of irradiation so that there could be continued growth.

As expected (see review, RESNICK 1979), UV increased the levels of recombination in vegetatively growing cells. When cells proceeded through meiosis, a further sharp increase in intragenic recombination occurred (Figure 7); this increase was presumably due mostly to gene conversion. Therefore, commitment to meiotic levels of recombination is possible in cells having considerable DNA damage, although the frequency of meiotic prototrophs is reduced when compared with the unirradiated controls. It is not clear whether this represents an actual decrease in recombination or is a consequence of the inviability of the haploid products in the irradiated culture. If irradiated cells that are committed

to meiotic recombination continue through meiosis the cells will die, resulting in a decreased frequency of prototrophs per viable cell plated at late times. The extent of DNA damage that still permits the observed recombinant frequency can be approximated using the genetic map described by MORTIMER and SCHILD (1980). The genetic length of chromosome *V*, which contains the *HIS1* gene, is approximately 200 cM, and the total genetic map is in the range of 5000 cM. When a linear correspondence of genetic to physical distance is assumed, this is 1/25 of the total DNA. A dose of 4 J/m^2 would, therefore, be expected to produce approximately 60 pyrimidine dimers in the homologous chromosomes. Based on this calculation and the genetic results, homologous chromosome interactions as evidenced by intragenic recombination are not prevented by extensive DNA damage during meiosis.

Given the observation of high levels of meiotic intragenic recombination from cells irradiated at the beginning of meiosis, the absence of an increase in intergenic recombination is surprising. These results correlate well with an absence of expected molecular exchange events (RESNICK, STASIEWICZ and GAME 1983). In light of previous observations of a strong relationship between gene conversion and reciprocal recombination events (FOGEL *et al.* 1979), it appears that UV damage may dissociate the two types of meiotic recombination. These results are particularly interesting in light of the observations by CARPENTER (1982) with meiotic and repair-deficient mutants of Drosophila. She demonstrated that *mei-218* and *mei-9* mutants have normal levels of gene conversion while crossover events are greatly reduced. Although the *mei-9* mutant of Drosophila and *rad1* mutant of yeast are both defective in excision repair, the absence of an effect of the *rad1* mutation on normal meiosis could be due to defects in different parts of a commonly evolved repair pathway. In Lilium (LAWRENCE 1961a) and Tradescantia (LAWRENCE 1961b) γ-irradiation just before the start of meiosis has been shown to depress chiasma frequency, so the effect of preexisting DNA damage on meiotic recombination may be a general one. The presence of DNA damage in the repair-deficient mutant of yeast also leads to a change in the spectrum of recombination events. Although there are several possibilities, the following brief explanations are offered in light of current models for meiotic chromosomal and recombinational interactions.

1. An early stage of recombination may involve a strand invasion and displacement mechanism (*i.e.*, the Aviemore model, MESELSON and RADDING 1975); an intermediate would be a Holliday-type structure. Since one of the driving forces is DNA synthesis, interruptions or slowing down by pyrimidine dimers might prevent the formation of a complete Holliday structure and the possibility for isomerization and reciprocal events to occur.

2. Synaptonemal complexes appear in yeast soon after the onset of meiotic DNA synthesis (ZICKLER and OLSON 1975; BYERS and GOETSCH 1975). They act to bring and maintain homologous chromosomes in close association during the zygotene and pachytene stages of meiosis, and they presumably enable recombination to occur. If the UV damage prevents their efficient synthesis or the ability to maintain pairing of homologous chromosomes, initiating events in recombination might be possible, whereas subsequent reciprocal exchanges may be prevented.

Regardless of the mechanisms of recombination that are affected by UV, these results demonstrate that normal patterns of meiotic recombination are altered considerably by the presence of unexcised pyrimidine dimers during meiosis. It is possible that these observations may extend to other types of damage in that there may be classes of lesions that only moderately affect gene conversion and abolish reciprocal recombination events. The absence of reciprocal events may account for the increased lethality observed as UV-irradiated cells proceed through meiosis. If reciprocal recombination is associated with normal disjunction, then its loss due to the presence of UV damage would lead to considerable aneuploidy and a subsequent decrease in survival when cells undergo haploidization.

Finally, the maintenance of the excision repair function throughout meiosis may relate to a necessity to withstand environmental insults. The natural habitat of Saccharomyces includes the surfaces of fruits and vegetables which may experience considerable sunlight. With a decrease in moisture content and nutrients, the cells enter into meiosis and form spores. If there is exposure to sunlight, DNA damage may be produced which would have to be removed for subsequent growth to occur. As previously shown (RESNICK 1970) excision repair and photoreactivation mechanisms in mitotically growing yeast cells confer considerable resistance to sunlight-induced damage. This damage is largely composed of pyrimidine dimers.

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