MEIOTIC DNA METABOLISM IN WILD-TYPE AND EXCISION-DEFICIENT YEAST FOLLOWING UV EXPOSURE

MICHAEL A. RESNICK AND STANLEY STASIEWICZ

National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

JOHN C. GAME

Department of Genetics, University of California, Berkeley, California 94720

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ABSTRACT

The effects of UV irradiation on DNA metabolism during meiosis have been examined in wild-type (RAD^+) and mitotically defined excision-defective (*rad1-1*) strains of *Saccharomyces cerevisiae* that exhibit high levels of sporulation. The *rad1-1* gene product is not required for normal meiosis: DNA synthesis, RNA synthesis, size of parental and newly synthesized DNA and sporulation are comparable in RAD^+ and *rad1-1* strains. Cells were UV irradiated at the beginning of meiosis, and the fate of UV-induced pyrimidine dimers as well as changes in DNA and DNA synthesis were followed during meiosis. Excision repair of pyrimidine dimers can occur during meiosis and the *RAD1* gene product is required; alternate excision pathways do not exist. Although the rate of elongation is decreased, the presence of pyrimidine dimers during meiosis in the *rad1-1* strain does not block meiotic DNA synthesis suggesting a bypass mechanism. The final size of DNA is about five times the distance between pyrimidine dimers after exposure to 4 J/m^2. Since pyrimidine dimers induced in parental strands of *rad1-1* prior to premeiotic DNA synthesis do not become associated with newly synthesized DNA, the mechanism for replicational bypass does not appear to involve a recombinational process. The absence of such association indicates that normal meiotic recombination is also suppressed by UV-induced damage in DNA; this result at the molecular level is supported by observations at the genetic level.

MEIOSIS is a unique developmental stage in most diploid organisms that is characterized by extensive chromosomal interactions, genetic exchange and, ultimately, haploid products. Both the genetic and the biochemical nature of meiotic events have been examined at the level of genetic recombination, DNA changes and chromosomal modifications (*cf.* RASSMUSSEN and HOLM 1980; PUKKILLA 1977). In recent years it has been possible to identify some of the events using various meiotic and DNA repair-deficient mutants (BAKER *et al.* 1976). As part of a continuing effort to evaluate DNA repair functions during the life cycle of yeast, we are investigating the roles that mitotically identified repair functions might have in the meiotic process and how they provide

protection during meiosis. It is known that, in yeast (COX and PARRY 1968; GAME and MORTIMER 1974) and other organisms (see BAKER *et al.* 1976 for review), some genes that provide a radiation repair function in mitotic cells are essential for normal meiosis. Recently, the meiotic phenotypes of several meiosis-defective or meiotic-lethal X-ray-sensitive mutants in yeast have been characterized using interrupted meiosis experiments (GAME *et al.* 1980; PRAKASH *et al.* 1980) or molecular techniques (RESNICK *et al.* 1981).

In this report we examine the role of excision repair functions during meiosis. The *RAD1* gene product is required for excision of ultraviolet light (UV)-induced pyrimidine dimers from the DNA of mitotically growing cells; mutants defective in this gene are deficient in the incisional or earlier steps of repair (WILCOX and PRAKASH 1981), and a *rad1-1* mutant exhibits no dimer loss at very low doses (less than 0.5 J/m^2; RESNICK, BOYCE and COX 1981). Because events resembling aspects of excision repair have been implicated in meiosis from biochemical studies (HOWELL and STERN 1971) and from genetic studies (HOLLIDAY 1964), several workers have already studied spore viability and meiotic recombination in excision-defective strains. Strains mutated in the *RAD1* gene have been found to show wild-type frequencies of reciprocal meiotic recombination, gene conversion and postmeiotic segregation, as well as high spore viability (SNOW 1968; DICAPRIO and HASTINGS 1976; DOWLING 1981). However, DOWLING (1981) has observed a sharp reduction in spore viability in *rad1 rad18* double mutant strains, despite wild-type viability in each of the single mutants. Thus, the *RAD1* gene product may have a role in meiosis but does not appear to be implicated in mismatch repair.

We initiated this study with the purpose of determining (1) whether the *RAD1* gene product plays a role in meiotic DNA metabolism as judged by DNA synthesis and molecular weight studies; (2) whether UV-induced pyrimidine dimers are excised during meiosis in wild-type strains and whether other meiotic processes (e.g., the proposed mismatch correction system responsible for gene conversion) can excise dimers in the absence of *RAD1*-mediated excision repair; (3) the effect of unexcised pyrimidine dimers on meiotic DNA metabolism, particularly synthesis past dimers; (e) whether pyrimidine dimers might affect or be involved in molecular exchanges during meiosis.

Although it has been possible in previous studies with other organisms to examine either genetic or molecular changes during meiosis, it has not been possible to combine these two areas in one system. Utilizing genetically well-marked strains of yeast that exhibit high levels of meiotic synchrony (RESNICK *et al.* 1981) and sucrose gradient techniques that enable routine measurements of small changes in chromosomal DNA (RESNICK, BOYCE and COX 1981), we have been able to examine these phenomena throughout meiosis. Typically, cells are irradiated at the beginning of meiosis before DNA synthesis and the fate of pyrimidine dimers and their effects on meiosis are followed through the meiotic development cycle. If pyrimidine dimers in parental DNA are not excised, it should be possible to follow meiotic recombination at the molecular level since the dimers are expected to become associated with newly synthesized DNA (as analyzed mathematically by T. DARDEN and M. A. RESNICK,

unpublished). We present here results on DNA changes during meiosis of unirradiated and irradiated *RAD*$^+$ and *rad1-1* cells. The corresponding genetic changes are described by RESNICK *et al.* (1983).

MATERIALS AND METHODS

Strains

g690:	*MATa* / *MATα* ●	*leu2* / *LEU2*	*RAD* / *RAD*	*can1*^{r} / *CAN* ●	*HOM3* / *hom3-10*	*his1-1* / *his1-7*	*trp2* / *trp2*	*ADE4* / *ade4*	*CYH* / *cyh*^{r}
g694:	*MATa* / *MATα* ●	*leu2* / *LEU2*	*rad1-1* / *rad1-1*	*CAN* / *can1*^{r} ●	*hom3-10* / *HOM3*	*his1-7* / *his1-1*	*TRP2* / *trp2*	*ade2* / *ADE2*	

These strains are heterothallic and were constructed by three or more backcrosses to spores from the homothallic diploid strain SK1 which exhibits good meiotic synchrony (see KUENZI and ROTH 1974); appropriate spore isolates from a *RAD1/rad1* diploid were crossed to yield the g690 and g694 strains. Cells were stored on defined medium (synthetic complete: RESNICK 1969) rather than the more commonly used YEPD (1% yeast extract, 2% peptone, 2% dextrose).

Growth, sporulation and radioactive labeling: Procedures for growth and sporulation have been described (ROTH 1976; KUENZI and ROTH 1974). Briefly, cells were inoculated into supplemented presporulation media (SPS) containing 0.5% yeast extract, 1% peptone, 0.17% yeast nitrogen base without amino acids, 0.05 M potassium pthalate, 1% potassium acetate, 0.5% ammonium sulfate and tetracycline (10 μg/ml) and incubated in a shaking water bath (30°). To induce meiosis, logarithmically growing cells at 3–4 × 10^7/ml were rapidly washed two times with prewarmed sporulation media (KAC; 1% potassium acetate, tetracycline 10 μg/ml and one-fifth the standard concentration of the required amino acids). The cells were then suspended in KAC, sonicated briefly to disrupt clumps and adjusted to 2 × 10^7 cells/ml. Sterile 0.5% Nonidet P-40 (Bethesda Research Laboratories) was added at 1 μl/ml to reduce clumping and sticking to glassware. At 1.5 hr the KAC was adjusted to pH 6.0 using 0.25 M HCl.

To prelabel cells before meiosis, (2-^{14}C)-uracil (56.5 mCi/μmol, 0.1 mCi/ml; New England Nuclear) was added (8–15 μC/ml) to SPS growing cells after adjusting the cell titer to 1–2 × 10^6 cells/ml; cells were grown to 2–3 × 10^7 cells/ml. The cells were then washed two times with media and suspended in the supplemented SPS at 1 × 10^7 cells/ml. Unlabeled uracil (20 μg/ml) was added, and the culture was incubated for another 1.5–2 generations. There is little appreciable change in total labeled DNA per milliliter of culture throughout meiosis. Cells were labeled during meiosis by adding 10–15 μCi/ml (6-^{3}H)-uracil (20 Ci/μmol, 1.0 mCi/ml; New England Nuclear) after a pH 6 adjustment, which enhances uracil uptake (MILLS 1974).

UV irradiation: Cells were diluted with prewarmed water to 5 × 10^6 cells/ml and irradiated in a rotating Petri dish with a germicidal lamp (model UVSL-58, Ultraviolet Products, Inc.). Cells were harvested and resuspended in prewarmed supplemented KAC containing Nonidet at 2 × 10^7 cells/ml. The output of the UV-lamp was monitored with a germicidal radiometer (model IL570, International Light, Inc.).

Measurements of DNA and RNA: Uptake of label into DNA and RNA was monitored using a modified technique of KUENZI and ROTH (1974). To determine label uptake in DNA, 0.25 ml of cells were added to 1.75 ml of 0.5 M NaOH containing 100 mg/ml of uracil and incubated overnight at 30°. The next day 0.55 ml of cold 50% trichloroacetic acid (TCA) was added, and the tube was placed on ice. The mixture was filtered through a 0.45-μm filter (Gelman) and rinsed with cold 0.01 M HCl. The filter was placed on a Hirsh funnel and rinsed again and then dried. To measure label uptake into RNA, 0.05 ml of cells was added to 0.5 ml of cold 10% TCA with 100 mg/ml of uracil and put on ice for 30–60 min. The mixture was filtered through a 0.45-μm filter and washed with cold 0.01 M HCl and dried.

UV-endonuclease treatment and sucrose gradient sedimentation: The procedures have been described by RESNICK, BOYCE and COX (1981). Cell samples (1–1.5 ml/gradient) were taken at various times during meiosis and washed two times with water and once with RM-1 (0.1 M Tris sulfate, pH 9.3; 0.01 M EDTA). The cells were then suspended in 1 ml of RM-1 and β-mercaptoethanol was added to 2%. The mixture was incubated for 10 min at 30°, washed with water and UV-endonuclease

buffer (0.05 M potassium phosphate, pH 6.5; 0.01 M EDTA) and resuspended in the UV-endonuclease buffer at 1×10^8 cells/ml. The cells (0.2 ml) were then added to the bottom of a polyallomar centrifuge tube followed by 20 μl of 10% Nonidet P-40 and 40 μl of Zymolase 5000 (10 mg/ml; Miles Laboratories, Inc.). The mixture was incubated for 5 min at 37° to lyse the cells and 10 min at 23°. To test for the presence of pyrimidine dimers, 35 μl of UV-endonuclease was added prior to the incubation at 23°. All manipulations after adding Zymolase were done with care to avoid disruption of cellular DNA. After incubation, 5–20% alkaline sucrose gradients (0.3 M NaOH, 0.02 M EDTA, 0.7 M NaCl) were formed beneath the lysate. Centrifugation was at 19 krpm in a Beckman SW55 rotor for 4 hr at 20°. Subsequent procedures and measurements of molecular weight have been described (RESNICK *et al.* 1981). The computer analysis of data is according to M. ROWLEY and M. A. RESNICK (unpublished results).

UV-endonuclease isolation: To detect the presence of pyrimidine dimers, the pyrimidine dimer specific activity (UV-endonuclease) from *Micrococcus luteus* extracts was utilized. The enzyme was isolated using a modified procedure of CARRIER and SETLOW (1970). Spray dried cells of *M. luteus* (10 g; Miles Laboratories, Inc.) were suspended in 200 ml of 0.01 M Tris, pH 8.0, and collected by centrifugation. Cells were resuspended in 450 ml of 0.01 M Tris, pH 8.0, and 0.2 M sucrose. After adding 60 mg of lysozyme (Boehringer Mannheim Biochemicals) the mixture was incubated at 30° for 45 min with occasional stirring. The lysate was placed in an ice bath and slowly stirred while a solution of 10% streptomycin sulfate (Boehringer Mannheim Biochemicals) was slowly added to give a final concentration of 1%; the lysate was stirred for 30–45 min. The lysate was then centrifuged at 9 krpm in a Sorvall SS-34 rotor for 40 min at 4°. Ammonium sulfate was slowly added to the supernatant to a final concentration of 65% while the mixture stirred in an ice bath. The mixture was centrifuged at 10 krpm for 30 min at 4°; the precipitate was suspended in 0.05 M phosphate buffer, pH 6.5, 0.01 M EDTA and 0.01 M β-mercaptoethanol. The enzyme was stored at −80°. Protein was determined to be 14 mg/ml based on the Bio Rad Protein Assay (Bio Rad Laboratories).

RESULTS

Meiotic events: To follow DNA synthesis during meiosis, ^{3}H-uracil is added at 1.5 hr after suspending cells in KAC which is prior to the beginning of the meiotic round of synthesis in strains with the SK1 background (RESNICK *et al.* 1981). Label incorporation begins approximately 30–60 min later and continues for about 3.5 hr in the *RAD1* and *rad1-1* strains (Figure 1). As a control we have used haploid cells and subjected them to the same growth conditions followed by incubation in KAC. Since label uptake into the nuclear DNA of the haploid cells was not observed, the DNA synthesized from 1.5 hr onward is specific to meiosis. The meiotic commitment to recombination (see accompanying paper) begins within 1 hr of the initiation of DNA synthesis and is completed by the first appearance of spores. Approximately 7 hr after the beginning of meiotic DNA synthesis, sporulation begins in both strains; greater than 95% of the cells sporulate. From these results we conclude that the absence of the mitotically identified pyrimidine dimer excision repair system does not affect DNA synthesis or genetic events during meiosis in unirradiated cells.

Chromosomal DNA during meiosis: Meiosis is a period of extensive genetic activity, and in some systems single-strand interruptions have been reported (HOTTA and STERN 1974; JACOBSON *et al.* 1975; KASSIR and SIMCHEN 1980; RESNICK *et al.* 1981). We have utilized alkaline sucrose gradient techniques to investigate the DNA of *RAD*$^+$ and *rad1-1* cells throughout meiosis. As was observed previously with *RAD*$^+$ strains (RESNICK *et al.* 1981), no significant changes were observed in parental DNA of the *rad1-1* strain during meiosis (Figure 2). The observed values of the number average molecular weight for single-strand DNA were between 1.3 and 1.6×10^8 daltons. These results are consistent with the

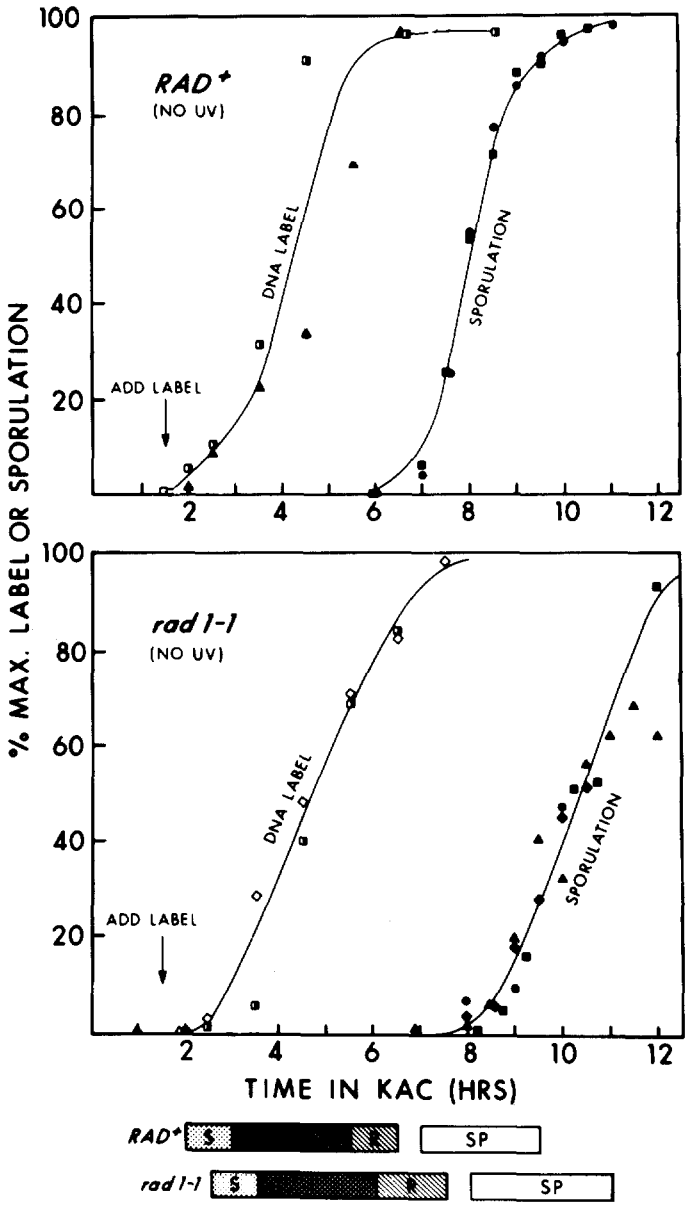

FIGURE 1.—Radioactive label uptake and sporulation in *RAD*$^+$ and *rad1-1* strains during meiosis. At 1.5 hr after resuspending cells in KAC (T = 1.5), ^{3}H-uracil was added and label uptake was measured as described in the MATERIALS AND METHODS. Sporulation was measured as the appearance of cells containing spores. The various symbols correspond to different experiments.

idea that possible interruptions are short-lived (RESNICK *et al.* 1981). Unlike the *rad52* mutant described in a previous study, which accumulates single-strand interruptions, the *rad1-1* mutant does not exhibit any interruptions in its parental DNA during meiosis.

As discussed in the previous section, the meiotic round of DNA synthesis

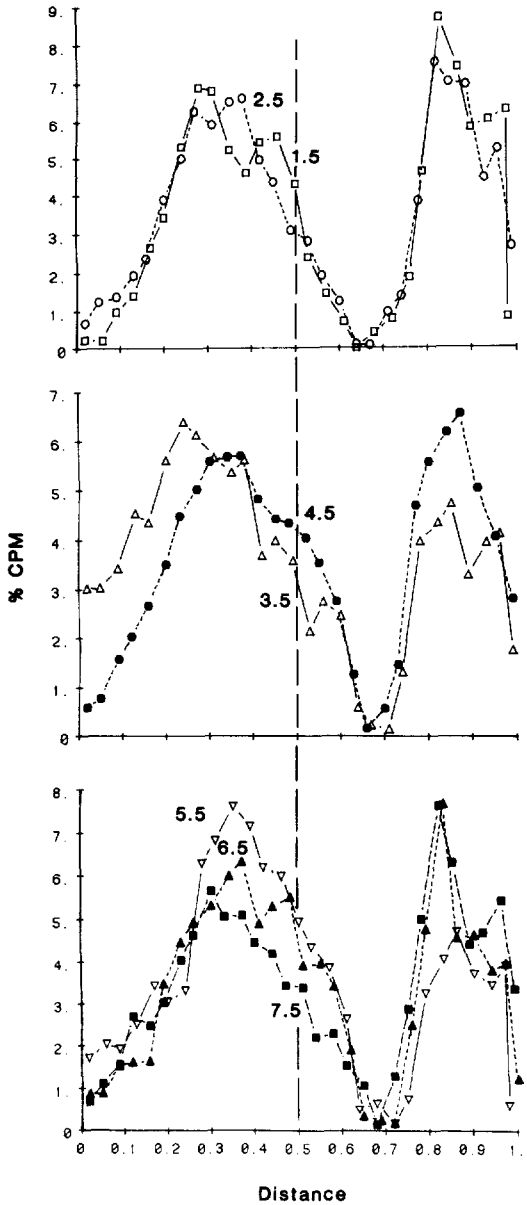

FIGURE 2.—Sucrose gradient sedimentation of *rad1-1* parental DNA during meiosis. Cells were prelabeled with ^{14}C-uracil as discussed in the MATERIALS AND METHODS. These were subsequently resuspended in KAC (at T = 0), and samples were taken at hourly intervals beginning at 1.5 hr (before the meiotic round of DNA synthesis; see Figure 1). The distribution of sizes of single-strand DNA was determined using alkaline sucrose gradient analysis (see MATERIALS AND METHODS); sedimentation was from right to left. The DNA peak at 0.3 corresponds to 2×10^8 daltons; the positions of bacteriophages T4 and T7 (0.6 and 0.125×10^8 single-strand molecular weight, respectively) sedimented under these conditions are presented in Figure 3. Results from different gradients are presented in the same figure (*i.e.*, 1.5 and 2.5 hr are in the top figure).

begins approximately 2.5 hr (T = 2.5 hr) after introducing cells into KAC. The newly synthesized chromosomal DNA of both the *RAD*$^+$ and the *rad1-1* strains was examined hourly after addition of ^{3}H-uracil to cells at T = 1.5 hr, and results were comparable (presented in Figure 3 are profiles obtained with *rad1*). During the first hour (T = 1.5–2.5 hr) the DNA is generally much smaller than the ^{14}C-labeled parental DNA and corresponds to intermediates in replication; by T = 3.5 hr the label is associated with DNA that is nearly chromosomal in size. Newly synthesized DNA in samples taken at later times is full sized (radioactivity in the upper fractions corresponds to mitochondrial DNA). These results indicate that the 3.5- to 4-hr period of DNA synthesis is not due to slow replication synthesis. Instead, the results are consistent with the synthesis of chromosomal size DNA in approximately 2 hr. A similar conclusion has been reached by JOHNSTON *et al.* (1982).

Effects of UV on sporulation and DNA synthesis: As described by RESNICK *et al.* (1983), UV affects sporulation more than it does survival. At doses of 100 J/m^2 to a *RAD*$^+$ and 10 J/m^2 to a *rad1-1* strain there is greater than 50% sporulation of cells irradiated before meiotic DNA synthesis as compared with survival of 20% and less than 5%, respectively. We have examined the extent of meiotic DNA synthesis at doses up to 25 J/m^2 in the wild-type (*RAD*$^+$) and 8 J/m^2 in *rad1-1* (Figure 4). It is clear that in the absence of the *RAD1* gene product, DNA synthesis can be greatly inhibited by low doses of UV. An exposure of 2 J/m^2 causes no marked effect; however, 4 J/m^2 decreases the rate and the final amount of synthesis. The rate of synthesis at 8 J/m^2 is 10–15% that of the control. These results are not due to a generalized degradation of DNA or shutting down of cellular activities. The amount of parentally labeled DNA remains constant in control and UV-irradiated cells throughout meiosis, and the rate and amount of RNA synthesis is also not markedly affected at doses up to 8 J/m^2 in *rad1-1* (data not shown). Therefore, it appears that large numbers of dimers can specifically inhibit total DNA synthesis during meiosis.

Excision of pyrimidine dimers during meiosis in RAD$^+$: The excision of UV-induced pyrimidine dimers in yeast is a rapid process in logarithmically growing cells (REYNOLDS and FRIEDBERG 1981). To examine dimer excision during meiosis and the effect of a *rad1-1* mutation, we assayed for UV-endonuclease-sensitive (UES) sites using an *M. luteus* extract. The extract has no activity toward unirradiated mitotic or meiotic DNA (data not shown). As shown in Figure 5 the irradiation of RAD$^+$ cells at T = 1.5 hr results in several UES sites per molecule. [There is also some reduction in the size of the large molecular weight DNA after high doses of UV (REYNOLDS and FRIEDBERG 1981).] Subsequent incubation in the meiotic medium results in a loss of all detectable sites by 3 hr after a dose of 25 J/m^2, which corresponds to approximately 100% survival. In the absence of treatment with UV-endonuclease, no breaks (over those induced by UV directly) are observed in the parental DNA throughout the repair period. Thus, we conclude that the repair of pyrimidine dimers in cells undergoing meiosis is a rapid process that does not result in the accumulation of single-strand gaps due to repair or meiosis itself. As in the case of mitotic cells (WHEATCROFT, COX and HAYNES 1975), excision of dimers appears to become

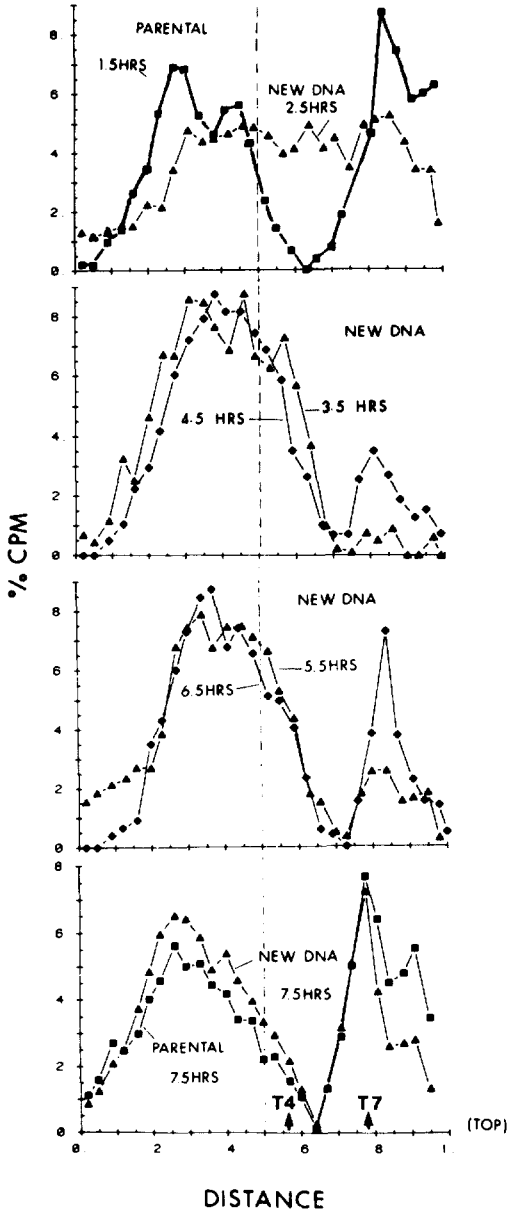

FIGURE 3.—The size distribution of newly synthesized DNA during meiosis in *rad1-1*. At 1.5 hr after resuspension of cells in meiotic medium ^{3}H-uracil was added. The distribution of sizes of newly synthesized single-strand DNA was determined using alkaline sucrose gradient analysis (see MATERIALS AND METHODS). Results from different gradients are presented in the same figure. For purposes of comparison, the distribution of parental DNA is presented in the top figure.

saturated after high doses of UV. After exposure to 100 J/m^2, corresponding to 30% survival, excision is slow, and by 3 hrs after irradiation less than 50% of the sites are removed.

Fate of pyrimidine dimers in rad1-1: To determine whether *RAD1* is involved

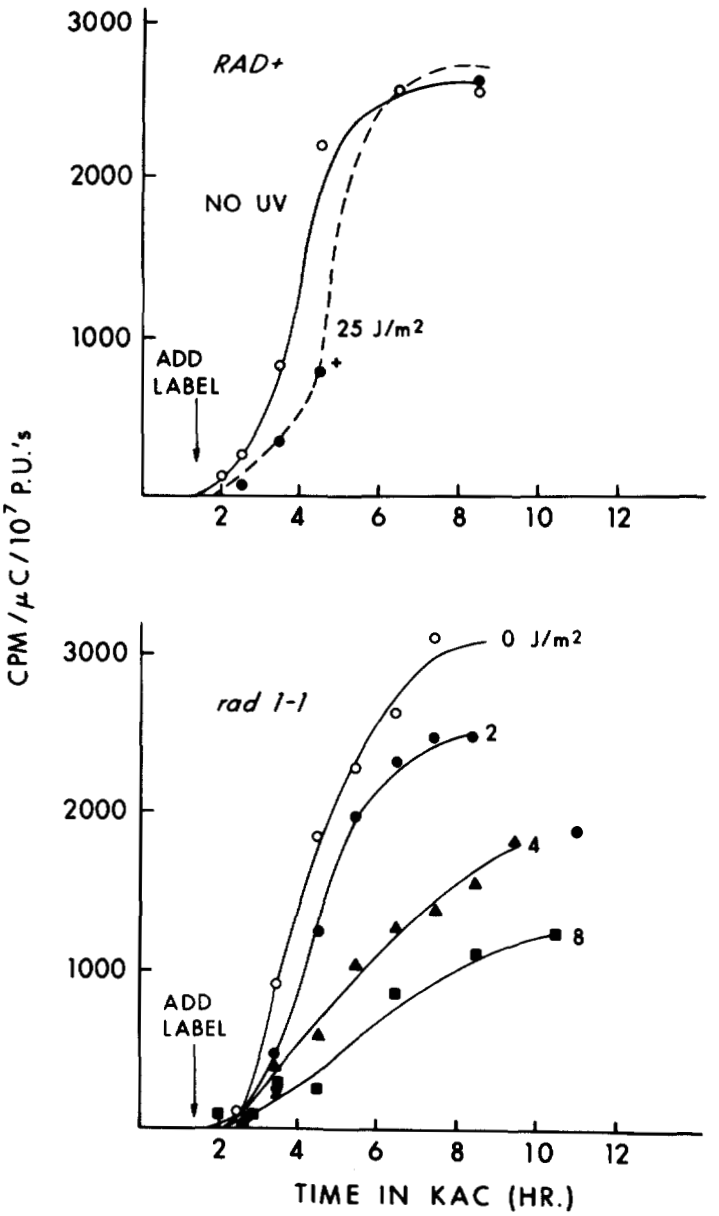

FIGURE 4.—Postirradiation DNA synthesis in meiotic cells. Cells were suspended in sporulation medium at 0 hr. At 1.5 hr they were diluted, irradiated, and resuspended in KAC, at which time ^{3}H-uracil was added (see MATERIALS AND METHODS). The data are expressed as counts per minute in DNA per microcurie of ^{3}H-uracil added per 10^{7} plating units when the label was added.

in excision repair in meiotic cells, rad1-1 cells were irradiated at various stages of meiosis, and UES sites were analyzed as before. Results are shown (Figure 6) for 4 and 8 J/m^2 irradiation at 1.5 hr after introducing cells into KAC (before DNA synthesis). In the experiment with 4 J/m^2, the UV-irradiated parental DNA (^{14}C) is reduced in size from 1.4×10^{8} to 0.33×10^{8} daltons after UV-

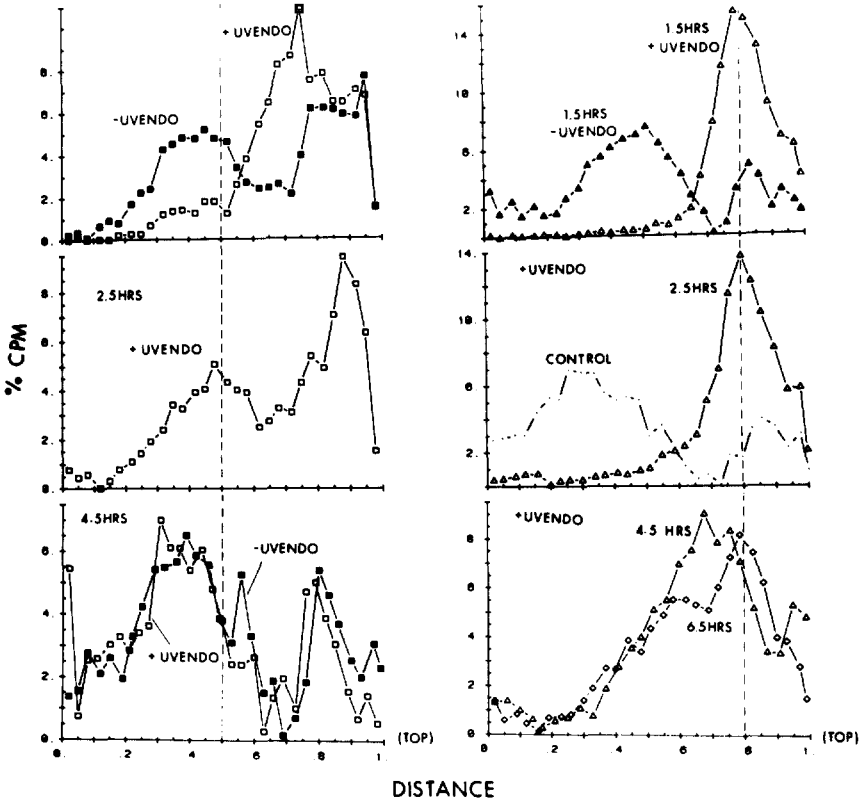

FIGURE 5.—Removal of UES sites from parental DNA after UV irradiation of meiotic *RAD*$^+$ cells. Cells that were prelabeled in their DNA were suspended in sporulation medium at T = 0. At 1.5 hr the cells were UV irradiated and resuspended in sporulation medium. Samples were lysed, treated with UV-endonuclease and sedimented on alkaline sucrose gradients as described in the MATERIALS AND METHODS. For comparison, an unirradiated control incubated with the UV-endonuclease is included in the middle right figure. The figures on the left correspond to 25 J/m^2 exposure and those on the right to 100 J/m^2. (Results from different gradients are presented on the same figure).

endonuclease treatment. Since a dose of 4 J/m^2 is expected to produce an average of approximately one dimer per 10^7 daltons, about one-quarter of the dimers are recognized by the UV-endonuclease [UES sites equal (1.44/0.33) − 1) per 1.44×10^8 daltons], as reported previously for mitotic cells (RESNICK, BOYCE and COX 1981). There is no significant change in the sucrose gradient profiles of UV-endonuclease-treated DNA obtained from cells incubated in KAC up to 7 hr after irradiation. Similar results on the stability of UES sites were obtained with cells irradiated with 8 J/m^2 after 1.5 hr in KAC (Figure 6) or when cells were irradiated 40 min before introducing them into KAC or at 5.5 hr in KAC. The latter observation excludes the possibility of a unique mechanism appearing later in meiosis that might be inhibited by UV damage introduced at an early stage. Irradiation at 5.5 hr results in UES sites being produced in both parental and newly synthesized DNA. We conclude that no other excision repair mechanisms arise during meiosis (such as mismatch repair) which can substitute for

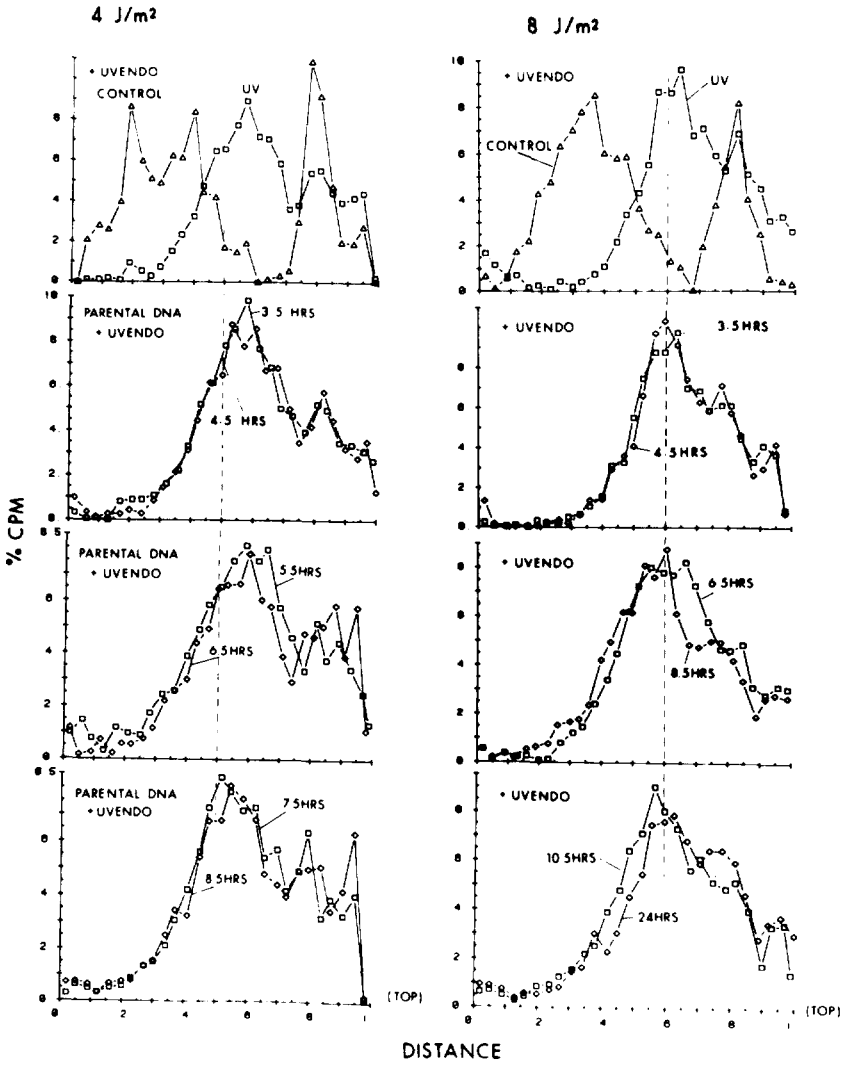

FIGURE 6.—The stability of UES sites during meiosis in a *rad1-1* strain. Cells were irradiated at T = 1.5 hr after suspension in KAC and sampled at various times (*i.e.*, T = 3.5, 4.5 hr, etc). Samples were prepared for gradient analysis and incubated with UV-endonuclease as described in MATERIALS AND METHODS to determine the disposition of UES sites in parental DNA. The figures to the left correspond to a dose of 4 J/m^2 and those on the right to 8 J/m^2; these were separate experiments and, therefore, the controls are slightly different. Because of low sporulation (about 30%) after 8 J/m^2, cells could be examined at much later times (up to 24 hr).

the *RAD1* excision repair pathway. The observations on the stability of UES sites corresponds well with the survival results presented in the accompanying paper in that survival remains constant until the late stages of meiosis.

We have also investigated the size of parental DNA after UV exposure and subsequent incubation in KAC, since it is possible that the presence of DNA damage might affect recombination so as to lead to an accumulation of recom-

binational intermediates. Within the limits of alkaline sucrose gradient analysis, we could detect no changes in the parental DNA without UV-endonuclease treatment when *rad1-1* cells were incubated in KAC up to 7 hr after irradiation (they were exposed to 4 J/m^2 at 1.5 hr in KAC; data not shown).

Pyrimidine dimers and DNA synthesis: Since pyrimidine dimers remained in parental DNA of the *rad1-1* mutant throughout meiosis, the effects of dimers on the integrity of meiotic DNA synthesis could be examined. As shown in Figure 4, the amount and rate of DNA synthesis decreases with increasing UV doses; the decreases could be due to pyrimidine dimers acting as blocks to synthesis or alternatively causing delays in DNA chain elongation. To test this, cells were irradiated at T = 1.5 hr and ^{3}H-uracil was then added. Samples were taken during meiosis and analyzed for the size distribution of newly synthesized DNA.

When the results with 4 J/m^2 in Figure 7 are compared with those in Figure 3 (no UV), it is clear that the synthesis of large molecular weight DNA is affected by UV. Within 1 hr (T = 2.5 hr, Figure 4) after adding label to unirradiated cells in KAC, nearly one-third of the DNA is large molecular weight (in the bottom 50% of the gradient). By 3 hr the label associated with large DNA has a profile comparable to that of the parental DNA. At 1 hr after irradiation, very little DNA is observed in the large molecular weight region (data not shown). By 2 hr after irradiation (3.5 hr in KAC) only about 20% of the counts are in large DNA and the peak of the profile is in the range of 0.6×10^8 daltons. At 5.5 hr the DNA reaches a maximum of 0.5×10^8 daltons. For comparison the results obtained after treating the cellular DNA from the UV-irradiated cells with UV-endonuclease are presented in the upper graph of Figure 7. From these results we conclude that, although there is a delay in DNA synthesis, the eventual size of the newly synthesized DNA is about five times larger than the distance between pyrimidine dimers. Similar results were obtained after exposure to 2 or 8 J/m^2 in that the size of the DNA synthesized was much larger than the distance between pyrimidine dimers. Since small molecular weight intermediates do not appear to accumulate, pyrimidine dimers do not act as absolute, or even temporary blocks, to DNA synthesis.

Pyrimidine dimers in parental DNA and recombination: Pyrimidine dimers induce recombination in *E. coli*; GANESAN (1974) demonstrated that pyrimidine dimers in parental DNA become associated with newly synthesized DNA. Such exchanges are not observed with UV-irradiated, mitotically growing yeast cells (RESNICK, BOYCE and COX 1981), even though UV induces genetic recombination in mitotic cells. Because the level of genetic exchange is high in meiosis (MORTIMER and SCHILD 1980; also see RESNICK *et al.* 1983), it should be possible to detect such exchanges at the molecular level in UV-irradiated meiotic cells by observing the reassortment of pyrimidine dimers after UV (T. DARDEN and M. A. RESNICK, unpublished results). When reciprocal recombination occurs, one-half of the exchanges will result in parental DNA becoming covalently linked with newly synthesized DNA (Figure 8). If pyrimidine dimers are present in the parental DNA that is exchanged, the apparent size of the newly synthesized DNA will appear smaller after UV-endonuclease treatment.

Results are shown for a *rad1-1* strain at 6 hr after UV (T = 7.5 hr; Figure 9).

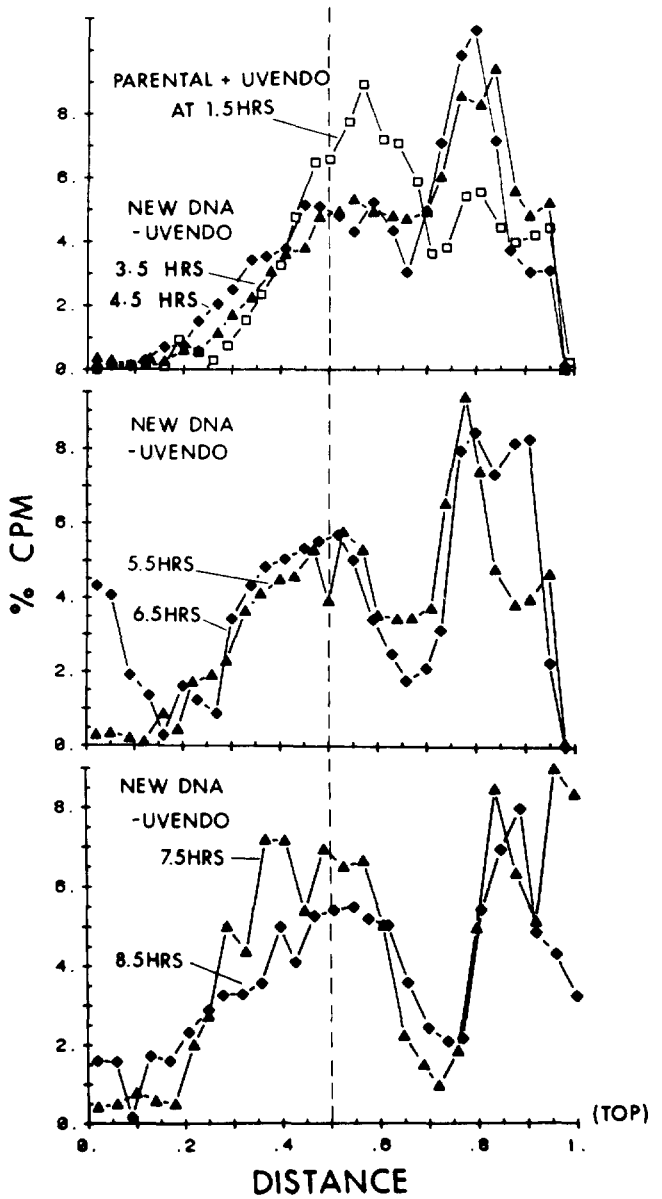

FIGURE 7.—Postirradiation DNA synthesis in *rad1-1* cells undergoing meiosis. Cells that were suspended in KAC at T = 0 hr were UV irradiated at T = 1.5 hr; they were resuspended in KAC containing ^{3}H-uracil (15 μCi/ml). The size of newly synthesized DNA (meiotic DNA synthesis) was examined using alkaline sucrose gradient analysis (see MATERIALS AND METHODS). For purposes of comparison, a graph of parental DNA (which contains pyrimidine dimers) treated with UV-endonuclease is also included in the top figure.

Cells labeled with ^{14}C-uracil were irradiated prior to meiotic DNA synthesis (T = 1.5 hr) and subsequently incubated with ^{3}H-uracil. There was no difference in the profiles of newly synthesized DNA which was either treated or not treated with the UV-endonuclease. This observation was made at earlier times

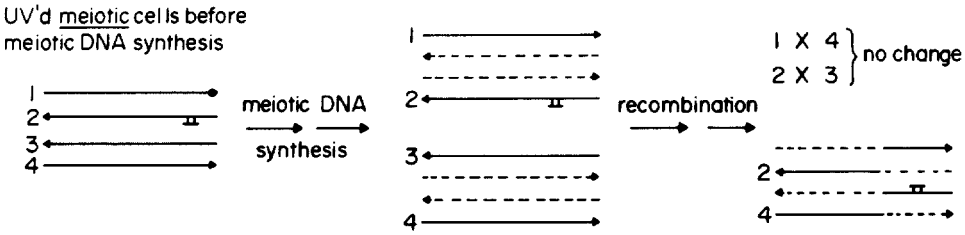

FIGURE 8.—A diagram for the redistribution of pyrimidine dimers between parental and newly synthesized DNA due to recombination. Pyrimidine dimers are induced in parental DNA prior to the meiotic round of DNA synthesis (*i.e.*, at T = 1.5 hr) and DNA synthesis proceeds past the pyrimidine dimers (*cf.* Figure 7). If meiotic reciprocal recombination occurs, strands containing pyrimidine dimers can become associated with newly synthesized strands. When treated with UV-endonuclease, the length of DNA strands containing pyrimidine dimers (in the parental portion) will be shortened; therefore, the apparent size of newly synthesized DNA can be decreased. In this example a recombination event between chromosomes 1 and 4 or 2 and 3 will not lead to the pyrimidine dimers becoming associated with newly synthesized DNA. However, recombination between 2 and 4 causes parental DNA containing a pyrimidine dimer to become associated with newly synthesized DNA. The apparent length of newly synthesized DNA will be decreased by UV-endonuclease treatment.

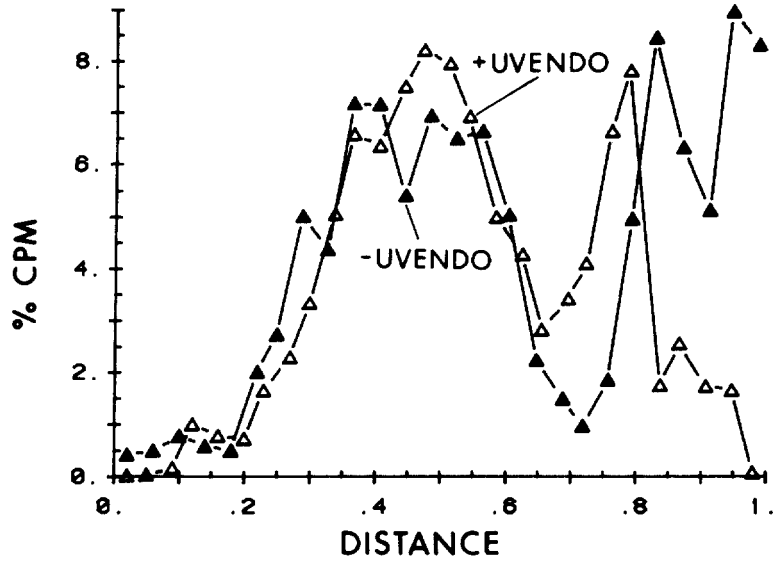

FIGURE 9.—The absence of association of UES sites with DNA synthesized during meiosis (see Figure 8). Cells that were in meiotic medium (KAC) for 1.5 hr (T = 1.5 hr) were diluted, exposed to 4 J/m^2 and resuspended in KAC containing ^{3}H-uracil (15 μCi/ml). At various times they were harvested and prepared for alkaline sucrose gradient analysis. One portion was treated with UV-endonuclease (see MATERIALS AND METHODS). Results from two different gradients were combined. This figure is for a sample taken at 7.5 hr; comparable results were found at earlier times and with other doses.

after UV and in experiments with exposures of 2 and 8 J/m^2. Thus, it can be concluded that at the limits of detection in these experiments (see DISCUSSION) covalent associations of parental and newly synthesized DNA that might arise during meiotic recombination do not occur.

DISCUSSION

The genetic and cytological effects of DNA damage on cells undergoing meiosis have been the subject of investigations in several single cell and whole animal and plant systems. However, it has not been possible previously to examine within the same organism all of the following aspects of DNA damage on meiotic cells: amount of damage, DNA synthesis, DNA repair, genetic control of repair, haploidization, lethality and recombination. In this and the accompanying paper we have examined all of these in cells undergoing meiosis after UV exposure; by utilizing gentle lysis techniques, high molecular weight chromosomal DNA could be isolated throughout meiosis enabling the study of pyrimidine dimer damage at sufficiently low doses to allow sporulation.

We have compared the meiotic response of the *rad1-1* excision-defective strain with a repair-proficient strain in the absence of radiation. There are no significant differences between these strains in viability, intragenic and intergenic recombination, DNA synthesis, levels of sporulation, and germination of haploid products. These results are consistent with genetic results reported by SNOW (1968), DICAPRIO and HASTINGS (1976) and DOWLING (1981). There are also no apparent changes in the profiles of DNA obtained from these strains. Since the *rad1-1* mutation does not appear to be leaky in terms of excision at low doses (0.2 J/m^2; RESNICK *et al.* (1981), we conclude that the *RAD1* gene product is not required for any of these processes. However, as will be discussed later, it is essential in the protection of cells undergoing meiosis from the damaging effects of UV and presumably other agents causing related damage.

As shown by RESNICK *et al.* (1983) the effects of UV damage are greatly increased in meiotic cells lacking the *RAD1* gene product. Since wild-type cells irradiated during meiosis remove UES sites and the *rad1* mutant does not, we conclude that the sensitivity is due to a lack of pyrimidine dimer excision. Although the *rad1* cells may not be able to excise pyrimidine dimers, there is an ability to tolerate this damage during the early stages of meiosis. Many of the characteristics of meiosis such as DNA synthesis, intragenic recombination and sporulation are only slightly affected by 2 J/m^2 at the beginning of meiosis. After 4 J/m^2—corresponding to approximately 1600 pyrimidine dimers per diploid cell—there is a considerable amount of DNA synthesis and sporulation. It is, therefore, clear that yeast cells can tolerate a limited amount of DNA damage during meiosis. At least four possible mechanisms for tolerating the DNA damage can be proposed.

(1) The damage is neutral, *i.e.*, at the low doses being used it codes correctly; (2) There are secondary excision mechanisms, such as mismatch repair, to remove small amounts of damage; (3) Replication may occur, but the damage causes interruptions; therefore, subsequent events that might require synthesis could proceed (such as sporulation); (4) Recombination repair mechanisms as proposed for *E. coli* (GANESAN 1974; WEST, CASSUTO and HOWARD-FLANDERS 1981) might allow a means for "bypassing" the effects of pyrimidine dimers during meiotic DNA synthesis.

The first possibility is unlikely since the effects of the damage are, in fact, detected in spores. As shown by RESNICK *et al.* (1983) spores that arise from

UV-irradiated cells undergoing meiosis exhibit lower survival than haploid cells or unsporulated diploid cells plated soon after irradiation. Hence, the damage may disrupt some aspect of the meiotic process (e.g., chromosome segregation) or it may itself be lethal if inherited by a haploid spore. The second possibility is excluded since no excision of pyrimidine dimers is detected; a loss of damage equivalent to 0.2 J/m^2 (RESNICK *et al.* 1981) would have been detected in these experiments.

The third possibility has been examined by following meiotic DNA synthesis after irradiation. A low dose of 2 J/m^2 has no apparent effect on rate or amount of synthesis. Exposure to 4 J/m^2 results in a decreased rate of synthesis. The reduced rate is also reflected by a delay in the appearance of large molecular weight DNA (Figure 7). However, unlike the situation with mitotic cells (RESNICK, BOYCE and COX 1981; DICAPRIO and COX 1981; PRAKASH 1981), there is no accumulation of small molecular weight DNA (comparable to the distance between pyrimidine dimers) at early times of synthesis after 2 or 4 J/m^2. Thus, although they slow synthesis, pyrimidine dimers do not appear to act as temporary blocks to synthesis. If they did, the number average molecular weight would initially be approximately 10^7. These results also differ from observations with other mitotic eukaryotic systems deficient in excision repair. Both mammalian cells in culture (LEHMAN 1978) and Drosophila cells from mutants lacking excision repair (BOYD and SETLOW 1976) accumulate small molecular weight DNA. The absence of such intermediates may indicate differences between mitotic and meiotic cells in the replication of damaged DNA. There does, however, appear to be an effect on the final molecular weight in that it is less than for the parental DNA. The molecular weight of DNA synthesized after 2 and 4 J/m^2 is about 75 and 35% of the parental DNA, respectively.

A recombinational "bypass" mechanism was investigated by following the disposition of pyrimidine dimers during meiosis. Recombination of large regions would lead to a reassociation of pyrimidine dimers with newly synthesized DNA (see Figure 8). In addition, the reassociation of pyrimidine dimers would result in the parental DNA having a higher molecular weight (after UV-endonuclease treatment) than it has immediately after the UV treatment. After doses of up to 8 J/m^2 to premeiotic cells, we detected no changes in the size of parental DNA treated with UV-endonuclease regardless of when in meiosis the cells were sampled. Similarly, we observed that the newly synthesized DNA did not contain any UES sites.

These results are comparable to earlier observations with mitotic cells in that recombination involving large regions could not be detected. Since exchanges between homologous chromosomes would also be detected, it appears that reciprocal recombinational events between homologues are also absent. One explanation for the lack of molecular recombination events could be a limited ability for detection. As shown in Figure 8 only one-half of the reciprocal recombination events would be observable. However, because meiotic recombination is high in yeast, there should be detectable exchanges of parental DNA containing UES sites if recombination is not inhibited. For example, since the genetic map is about 5000 cM in length (MORTIMER and SCHILD 1980), correspond-

ing to about 100 reciprocal exchanges/cell, and the DNA content after meiotic DNA synthesis is approximately 4×10^{10} daltons, there should be an average of about one recombinational event/10^8 daltons. After a dose of 4 J/m^2 there are about two UES sites/10^8 daltons. Based on calculations developed by T. DARDEN and M. A. RESNICK (unpublished results), the expected DNA profile for newly synthesized DNA after UV-endonuclease treatment would be considerably different from that observed in Figure 9 (shifted toward smaller molecular weights) if meiotic recombination were occurring at normal frequencies. Thus, the nature of the bypass mechanism remains undetermined indicating that the mechanism is more complex than described in these possibilities. For example, although there may be a mechanism of bypass replication, the temporary presence of interruptions could distort the meiotic recombinational process.

These observations on recombination agree with the genetic results described by RESNICK *et al.* (1983). Commitment to meiotic gene conversion is decreased and reciprocal recombination events are essentially absent at a dose of 4 J/m^2. Sporulation occurs, but the spores are largely inviable. Thus, we conclude that the toleration of pyrimidine dimers (with respect to DNA synthesis and spore formation) during meiosis is not due to a recombinational type of repair. On the contrary, the presence of this DNA damage depresses meiotic genetic recombination and expected molecular recombination.

Therefore, although UV has been well established as being an inducer of mitotic genetic recombination between homologous chromosomes in several systems including yeast and sister chromatid exchange as is measured cytologically in mammalian cells (for review see RESNICK 1979), the presence of unexcised damage markedly depresses the high levels of reciprocal recombination observed meiotically. Related observations have been made by SALTS, SIMCHEN and PIÑON (1976) who found that UV exposure at certain times appears to reverse commitment to recombination during interrupted meiosis experiments in *RAD*$^+$ cells. γ-Ray damage has also been shown under some circumstances to depress meiotic recombination in Chlamydomonas (LAWRENCE and HOLT 1970) and to reduce chiasma frequency in Lilium (LAWRENCE 1961) and Tradescantia (LAWRENCE 1961) when irradiation occurs just before the start of meiosis. The depression in recombination by UV damage in yeast is not due to a lack of DNA synthesis, nor is it associated with the single-strand interruptions found in *rad52* mutants that lack the ability to form live meiotic recombinants (RESNICK *et al.* 1981). It is not clear whether the effect is on recombination directly or whether it is on a process such as chromosome pairing which is itself a prerequisite for recombination. Even so, cells can continue through meiosis as shown here and by RESNICK *et al.* (1983), but resulting spores are largely inviable. These observations, although restricted mainly to the excision-defective strain, may relate to events in a wild-type strain for those cases in which damage remains unexcised.

LITERATURE CITED

BAKER, B. S., A. T. C. CARPENTER, M. S. ESPOSITO, R. E. ESPOSITO, L. SANDLER, 1976 The genetic control of meiosis. Annu. Rev. Genet. **10**: 53–134.

BOYD, J. B. and R. B. SETLOW, 1976 Characterization of postreplication repair in mutagen-sensitive strains of *Drosophila melanogaster*. Genetics **84:** 507–526.

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

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

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

DICAPRIO, L. and P. J. HASTINGS, 1976 Postmeiotic segregation in strains of *Saccharomyces cerevisiae* unable to excise pyrimidine dimers. Mutat. Res. **37:** 137–146.

DOWLING, E., 1981 Meiotic recombination and sporulation in repair-deficient strains of yeast. Ph.D. Thesis, University of California, Berkeley.

GAME, J. C. and R. K. MORTIMER, 1974 A genetic study of X-ray sensitive mutants in yeast. Mutat. Res. **24:** 281–292.

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

GANESAN, A., 1974 Persistence of pyrimidine dimers during post-replication repair in ultraviolet light-irradiated *Escherichia coli* K12. J. Mol. Biol. **87:** 103–119.

HOLLIDAY, R., 1964 A mechanism for gene conversion in fungi. Genet. Res. **5:** 282–304.

HOTTA, Y. and H. STERN, 1974 DNA scission and repair during pachytene in *Lilium*. Chromosoma **46:** 279–296.

HOWELL, S. H. and H. STERN, 1971 The appearance of DNA breakage and repair activities in the synchronous meiotic cycle of *Lilium*. J. Mol. Biol. **55:** 357–378.

JACOBSON, G. K., R. PIÑON, R. E. ESPOSITO and M. S. ESPOSITO, 1975 Single-strand scissions of chromosomal DNA during commitment to recombination at meiosis. Proc. Natl. Acad. Sci. USA **72:** 1887–1891.

JOHNSTON, L. H., D. H. WILLIAMSON, A. L. JOHNSON and D. J. FENNEL, 1982 On the mechanism of premeiotic DNA synthesis in the yeast *Saccharomyces cerevisiae*. Exp. Cell Res. **141:** 53–62.

KASSIR, Y. and G. SIMCHEN, 1980 Single-strand scissions of nuclear yeast DNA occur without meiotic recombination. Curr. Genet. **2:** 79–85.

KUENZI, M. and R. ROTH, 1974 Timing of mitochondrial DNA synthesis during meiosis in *Saccharomyces cerevisiae*. Exp. Cell Res. **85:** 377–382.

LAWRENCE, C. W., 1961a The effect of the irradiation of different stages in microsporogenesis on chiasma frequency. Heredity **16:** 83–89.

LAWRENCE, C. W., 1961b The effect of radiation on chiasma formation in *Tradescantia*. Radiat. Bot. **1:** 92–96.

LAWRENCE, C. W. and P. D. HOLT, 1970 The effect of gamma radiation and alpha particles on gene recombination in *Chlamydomonas reinhardi*. Mutat. Res. **10:** 545–555.

LEHMANN, A., 1978 Workshop summary: replicative bypass mechanism in mammalian cells. pp. 485–488. In: *DNA Repair Mechanisms*, Edited by P. C. HANAWALT, E. C. FRIEDBERG, and C. F. FOX. Academic Press, Inc., New York.

MILLS, D., 1974 Effect of pH on adenine and amino acid uptake during sporulation in *Saccharomyces cerevisiae*. J. Bacteriol. **112:** 180–185.

MORTIMER, R. K. and D. SCHILD, 1980 Genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44:** 519–571.

PRAKASH, L., 1981 Characterization of postreplication repair in *Saccharomyces cerevisiae* and effects of *rad6*, *rad18*, *rev3*, and *rad52* mutations. Mol. Gen. Genet. **184:** 471–478.

PRAKASH, S., L. PRAKASH, W. BURKE and B. A. MONTELONE, 1980 Effects of the *RAD52* gene on recombination in *Saccharomyces cerevisiae*. Genetics **94:** 31–50.

PUKKILLA, P. J., 1977 Biochemical analysis of genetic recombination in eukaryotes. Heredity **39:** 193–217.

RASMUSSEN, S. W. and P. B. HOLM, 1980 Mechanics of meiosis. Hereditas **93:** 187–216.

RESNICK, M. A., 1969 Genetic control of radiation sensitivity in *Saccharomyces cerevisiae*. Genetics **62:** 519–531.

RESNICK, M. A., 1979 The induction of molecular and genetic recombination in eukaryotic cells. Adv. Radiat. Biol. **8:** 175–217.

RESNICK, M. A., J. BOYCE and B. COX, 1981 Postreplication repair in *Saccharomyces cerevisiae*. J. Bacteriol. **146:** 285–290.

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

RESNICK, M. A., J. N. KASIMOS, J. C. GAME, R. J. BRAUN and R. M. ROTH, 1981 Changes in DNA during meiosis in a repair-deficient (*rad52*) of yeast. Science **212:** 543–545.

REYNOLDS, R. J. and E. C. FRIEDBERG, 1981 Molecular mechanisms of pyrimidine dimer excision in *Saccharomyces cerevisiae*: incision of ultraviolet-irradiated deoxribonucleic acid *in vivo*. J. Bacteriol. **146:** 692–704.

ROTH, R., 1976 Temperature-sensitive yeast mutants defective in meiotic recombination and replication. Genetics **83:** 675–686.

SALTS, Y., G. SIMCHEN and R. PIÑON, 1976 DNA degradation and reduced recombination following UV irradiation during meiosis in yeast (*Saccharomyces cerevisiae*). Mol. Gen. Genet. **146:** 55–59.

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

WEST, S. C., E. CASSUTO and P. HOWARD-FLANDERS, 1981 Heteroduplex formation by rec A protein: polarity of strand exchanges. Proc. Natl. Acad. Sci. USA **78:** 6149–6153.

WHEATCROFT, R., B. COX and R. H. HAYNES, 1975 Repair of UV-induced DNA damage and survival in yeast. I. Dimer excision. Mutat. Res. **30:** 209–218.

WILCOX, D. R. and L. PRAKASH, 1981 Incision and post-incision steps of pyrimidine dimer removal in excision defective mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **148:** 618–623.

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