Production and Characterization of Radiation-Sensitive Meiotic Mutants of *Coprinus cinereus*

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ABSTRACT

We have isolated four γ-ray-sensitive mutants of the basidiomycete *Coprinus cinereus*. When homozygous, two of these (*rad 3-1* and *rad 9-1*) produce fruiting bodies with very few viable basidiospores, the products of meiosis in this organism. A less radiation-sensitive allele of *RAD 3, rad 3-2*, causes no apparent meiotic defect in homozygous strains. Quantitative measurements of oidial survival of *rad 3-1;rad 9-1* double mutants compared to the single mutants indicated that *rad 3-1* and *rad 9-1* mutants are defective in the same DNA repair pathway. In the few viable basidiospores that are produced by these two strains, essentially normal levels of meiotic recombination can be detected. None of the mutants exhibits increased sensitivity to UV radiation. Cytological examination of meiotic chromosomes from mutant and wild-type fruiting bodies showed that *rad 3-1* homozygous strains fail to condense and pair homologous chromosomes during prophase I. Although *rad 9-1* strains are successful at chromosome pairing, meiosis is usually not completed in these mutants.

THE basidiomycete *Coprinus cinereus* is an excellent system for studies of meiosis. The process of meiosis in *C. cinereus* is long and naturally highly synchronous (RAJU and LU 1970; PUKKILA, YASHAR and BINNINGER 1984). Each mushroom cap contains 10^7–10^8 meiotic cells; therefore sufficient material for biochemical analysis can be obtained at any meiotic stage. All 13 chromosome pairs in the developing meiotic cells can be easily studied by light microscopy using rapid fixation and staining techniques (LU and RAJU 1970; PUKKILA and LU 1985), and electron microscopy has revealed the presence of classical, well-formed synaptonemal complexes (HOLM *et al.* 1981).

In addition to having a synchronous meiotic cycle, *C. cinereus* has a small nuclear genome (DUTTA 1974) with little or no interspersed repetitive DNA (WU, CASSIDY and PUKKILA 1983), two features that facilitate molecular cloning experiments. DNA-mediated cell transformation has recently been developed (BINNINGER *et al.* 1987), which will enable the cloning of genes by complementation of mutant phenotypes. Since genetic analysis is also well developed for this organism (LEWIS and NORTH 1974), the full panoply of classical and molecular genetic techniques can be applied to the dissection of meiotic pathways.

We wished to isolate mutants of *C. cinereus* that are defective in meiotic DNA metabolism. However, the differentiation of a vegetative dikaryon into a mature fruiting body with tetrads of spores involves meiosis and also other complex morphogenetic changes. There are therefore many mutations that could lead to lack of fruiting, or lack of spore formation, but that would not relate directly to DNA or chromosome functions during meiosis. In fact, even in *Saccharomyces cerevisiae*, a single-called organism, many of the genes expressed at high levels during meiosis may not be essential for this process (KABACK and FELDBERG 1985). In a multicellular organism such as *C. cinereus* this problem is likely to be exacerbated. Therefore, we chose to exploit the known correlation between radiation repair and meiosis in order to isolate mutants with defects in meiosis that are at the level of meiotic DNA metabolism. Reciprocal experiments with other organisms have shown that mutants isolated on the basis of meiotic deficiencies often exhibit mitotic radiation sensitivity (BAKER, CARPENTER and RIPOLL 1978) and that mutants isolated on the basis of sensitivity to radiation often have meiotic deficiencies, especially in recombination (BAKER *et al.* 1976; ORR-WEAVER and SZOSTAK 1985). In particular, mitotic sensitivity to ionizing radiation or alkylating agents is a good predictor of meiotic dysfunction, whereas mutants sensitive only to ultraviolet light and bulky adducts are rarely defective in meiosis (GAME 1983).

We therefore chose to isolate mutants defective in the repair of ionizing radiation, as a means of generating interesting meiotic mutants of *C. cinereus*. This approach has an additional advantage, in that radiation sensitivity can be monitored in the haploid phase of the organism's life cycle, thus facilitating the recovery of recessive mutants. In this paper we describe the

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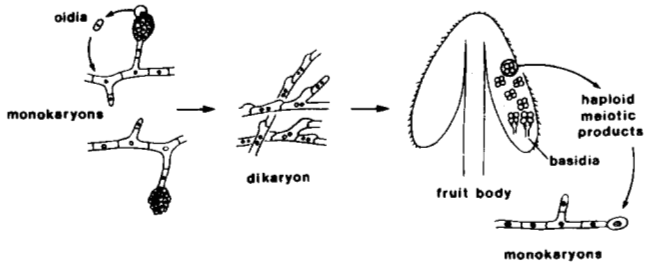

FIGURE 1.—Life cycle of *Coprinus cinereus*. Oidia are mitotically derived, haploid spores produced by monokaryons. They germinate to repeat the haploid portion of the life cycle. Two compatible monokaryons fuse and form stable dikaryons, which differentiate into fruiting bodies. Within the basidia, on the face of the gills of the fruiting body, the two nuclei of the dikaryon fuse and undergo meiosis. The four haploid nuclei, the products of meiosis, migrate into spores, on top of the basidia. These basidiospores germinate into monokaryons.

isolation and characterization of four mutants of *C. cinereus* that are sensitive to ionizing radiation, and we demonstrate that two of them have striking defects in meiosis.

MATERIALS AND METHODS

Strains and culture conditions: The life cycle of *Coprinus cinereus* is illustrated in Figure 1. The wild-type strain Java-6 (BINNINGER *et al.* 1987) was used for mutant isolation. Mutants were crossed and backcrossed to the standard Rad$^+$ strain Okayama-7 (WU, CASSIDY and PUKKILA 1983). Yeast-malt-glucose (YMG) medium (RAO and NIEDERPRUEM 1969) was used for all experiments except nutritional assays. Dikaryons were constructed by placing pieces of two monokaryons side by side on a YMG agar plate. The cultures were grown for 2 days at 37° and a subculture was then made from one side of the mated culture. This subculture was grown at 37° for 2 days to generate a dikaryon stock, which was then stored at 4°. Since nuclei, but not mitochondria, migrate during dikaryon formation (BAPTISTA-FERREIRA, ECONOMOU and CASSELTON 1983; MAY and TAYLOR 1988), this method ensured that a homogeneous culture would be used for fruiting. Dikaryon formation was checked by observation of colony morphology (dikaryotic cultures are fluffier and thicker than monokaryons) and by microscopic examination of hyphae for the presence of clamp connections (FINCHAM, DAY and RADFORD 1979). For fruiting, YMG dishes, plates, or slants were inoculated with a subculture from the stock dikaryon. This culture was grown at 37° until confluent. It was then induced to fruit by incubation at 25° under a 16 hr light-8 hr dark regime (PUKKILA, YASHAR and BINNINGER 1984).

To measure the effect of *rad* mutations on the frequency of meiotic recombination, *rad* mutant strains were crossed to both a *met 5* mutant strain (#220, derived from dikaryon N1/14 × SR54, supplied by J. NORTH) and to an *ade 8* mutant strain (Okayama-7) to generate Rad$^-$ Met$^-$ and Rad$^-$ Ade$^-$ strains. Compatible strains were crossed, and progeny were analyzed on minimal media supplemented with appropriate nutrients (MOORE and PUKKILA 1985).

Mutagenesis and screening: Oidia were isolated as previously described (ZOLAN and PUKKILA 1986) and irradiated in sterile water at a concentration of 6.5×10^7 oidia/ml. Ten milliliters of oidia in a 100 cm glass Petri dish were swirled gently during irradiation, which was from a GE 15 W germicidal lamp. The output of the lamp was 4.4 J/m^2/sec measured at a distance of 36 cm. Oidia were irradiated for a total of 132 J/m^2, diluted 10-fold, spread onto YMG plates, and incubated at 37° in darkness for 48 hr. This treatment resulted in 0.1% survival of irradiated oidia, relative to the survival of an unirradiated control sample. Colonies were transferred to gridded YMG plates, incubated at 37° for 48 hr, and kept at 4° until they were screened.

To screen for radiation-sensitive mutants, small chunks of mycelia (approximately 1 mm square) were transferred to YMG plates, which were then incubated at 37° overnight and irradiated with 40 krad of γ-rays, using a ^{137}Cs irradiator (J. L. Shepherd and Associates, Mark-1 model 68-A) with an output of 4.1 krad/min. This dose was determined to be optimal by an experiment in which chunks of nonmutagenized Java-6 were irradiated with various doses of γ-rays, and then incubated at 37° for 24 hr followed by growth at room temperature for 24 hr. Doses of 40 krad did not appreciably inhibit the growth of wild-type cultures. Petri dishes were turned continuously during irradiation, to ensure even treatment of the plates. Colonies that appeared significantly inhibited by the γ-ray treatment were further subcultured and tested for growth inhibition after 0, 10, 40 and 80 krad. Radiation-sensitive isolates and an unirradiated control were crossed to Okayama-7 to obtain compatible isolates for dikaryon formation, and progeny were further backcrossed to Okayama-7 to obtain healthy cultures. After the initial isolation of mutants, all gamma irradiations were from a ^{60}Co source operated by the Michigan Memorial Phoenix Project at The University of Michigan. This source consists of 16 rods of ^{60}Co, which are shielded in a well inside a concrete room. Stacks of plates are placed at appropriate distances from the source, which is then mechanically raised from the well. Because the plates do not rotate during the irradiation, they are placed at a minimum of 40 cm from the source. It was determined that at this distance the gradient of irradiation dose across the plates does not affect this qualitative screen. Cultures were examined after doses of 40, 60 and 80 krad, with growth compared to that of an unirradiated control.

Construction of double mutants: A *rad 3-1* monokaryon was crossed separately to *rad 9-1* and *rad 10-1* monokaryons. The resulting dikyarons were fruited, and radiation-sensitive basidiospore progeny from each cross were tested by complementation assays to determine whether they were single or double mutants. For example, each radiation-sensitive basidiospore isolate of the *rad 3-1* × *rad 9-1* cross was mated with compatible *rad 3-1* and *rad 9-1* strains. The resulting dikaryons were checked, by the chunk test, for radiation sensitivity. If both were sensitive (*i.e.*, if neither the *rad 3-1* nor the *rad 9-1* mutations were complemented) then that isolate was designated a double mutant. Each dikaryon was examined microscopically for the presence of clamp connections, to ensure that mating had occurred.

^{60}Co survival curves: Oidia were prepared at a concentration of approximately 5×10^7 cells/ml in sterile distilled water supplemented with penicillin (100 units/ml) and streptomycin (0.1 mg/ml; both from Sigma). One ml aliquots of this suspension were irradiated in 13 × 75 mm polypropylene tubes (Falcon) for approximately one hour at appropriate distances from the ^{60}Co source. Cultures were vortexed just prior to irradiation. Samples were diluted appropriately and triplicate 0.1-ml aliquots were spread onto YMG plates and incubated at 37° for 48 h. Plates were subsequently kept at room temperature, and were counted 3, 4 and 5 days after irradiation. After this time, no further colonies appeared on the plates. Total colony counts for each plate were used to calculate survival relative to that of an unirradiated control.

UV irradiation survival curves: Oidia were suspended in sterile distilled water at a concentration of approximately 5×10^7 cells/ml. Eight milliliters were transferred to a glass Petri dish, a 1 ml control aliquot was taken, and the remaining oidia were irradiated. Irradiation was from one 8W Sylvania germicidal bulb, with an output of 0.4 J/m^2/sec at a distance of 90 cm. One-milliliter aliquots were taken at appropriate times, diluted, plated as for ^{60}Co survival curves, incubated for 48 hr in darkness at 37°, and counted as for the ^{60}Co irradiations. To examine photoreactivation, a 2-ml sample was taken after a UV dose of 96 J/m^2. One milliliter was then exposed to a GE F8T5 BLB bulb for 60 min. Both samples and an unirradiated control were then diluted, plated, and counted as for other survival curves.

Cytological methods: For light microscopy, cell walls were removed by digesting gill segments in 10 mg/ml Novozym 234 (Novo labs), 0.5 M mannitol, 0.05 M sodium maleate (pH 5.5) for 30 min at 37°. The spheroplasts were filtered through glass wool and pelleted in a variable speed microcentrifuge at 3000 rpm for 5 min. After one wash in 0.1 M MES (2-[*N*-morpholino]ethanesulfonic acid, Sigma), 0.001 M EDTA, 1 M sorbitol (pH 6.4) (DRESSER and GIROUX 1988), the spheroplasts were suspended in a small volume of the wash buffer (except 0.1 M sucrose replaced the sorbitol) and fixed by the addition of 4% formaldehyde (prepared from paraformaldehyde), 0.1 M sucrose, and the suspension was pipetted onto a poly-L-lysine-coated slide (Sigma type 1-B, 0.1%). The spheroplasts were allowed to settle for 10 min, and the slide was drained and then flooded with 0.4% Photoflo 200 (Kodak), and air dried. The slide was then stained with silver as described previously (PUKKILA and LU 1985).

For electron microscopy, small samples of fruiting bodies were fixed in 1% acrolein (Polysciences) in 0.05 M cacodylate buffer (pH 7.4) and the OsFeCN-uranium procedure (MCDONALD 1984) was then followed. The tissue was dehydrated and embedded in Epon-Araldite, and sections cut using a Sorvall MT2-B microtome were viewed and photographed using a Zeiss EM 10CA.

RESULTS

Mutant isolation and complementation: Oidia of Java-6, a wild-type strain of *C. cinereus*, were mutagenized with ultraviolet light. Twenty-five hundred colonies derived from surviving oidia were screened for sensitivity to ionizing radiation by irradiation of small chunks of mycelia (see MATERIALS AND METHODS; Figure 2). Of 27 candidates produced by the initial screening process, four have proven to be consistently radiation-sensitive by the criterion of the chunk test. Each of the four strains has been crossed and backcrossed five times to strain Okayama-7, a vigorous radiation resistant (Rad$^+$) strain. Okayama-7 was chosen because repeated backcrossing to this strain yielded consistently fertile fruiting bodies. The heterozygous dikaryons produced by crossing Okayama-7 to the radiation sensitive (*rad*) mutants were all Rad$^+$ in chunk test assays. Analysis of 100–200 random basidiospore progeny of Rad$^+$ × Rad$^-$ crosses showed that each *rad* mutation (designated *rad 3-1, rad 3-2, rad 9-1*, and *rad 10-1*) segregates as a single Mendelian factor.

Dikaryons were constructed for all possible pairs of

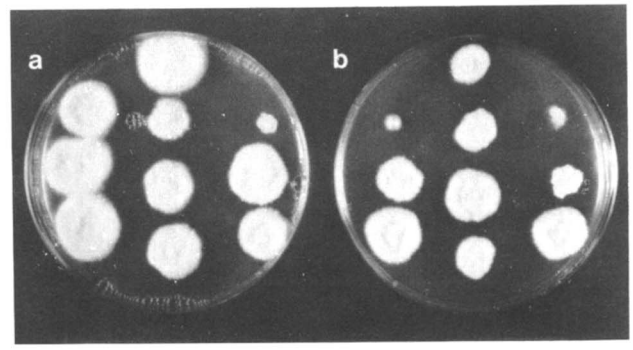

FIGURE 2.—Chunk test for the detection of radiation-sensitive mutants. Duplicate plates were unirradiated (a) or irradiated with 40 krad of γ-rays (b). A radiation-sensitive strain (upper left) is apparent.

TABLE 1

Radiation repair and tetrad formation in dikaryons

	rad 3-1		*rad 3-2*		*rad 9-1*		*rad 10-1*	
	Repair	Tetrads	Repair	Tetrads	Repair	Tetrads	Repair	Tetrads
rad 3-1	−	−	−	+	+	+	+	+
rad 3-2			−	+	+	+	+	+
rad 9-1					−	−	+	+
rad 10-1							−	+

rad mutants, to determine complementation relationships for radiation sensitivity and for fruiting. Each dikaryon was tested for radiation sensitivity by the chunk test, and each was fruited under standard conditions. Gill segments of each were examined for the presence of tetrads.

The *rad* mutations fell into three complementation groups (Table 1). *rad 3-1* and *rad 3-2* do not complement for radiation repair. All of the complementation studies were performed after mutants had been backcrossed twice to Okayama-7. The *rad 3-1, rad 3-2* complementation test was repeated after two more backcrosses to Okayama-7, and the result did not change.

Effects on meiosis: Fruiting of homozygous dikaryons indicated that *rad 3-1* and *rad 9-1* produce greatly reduced numbers of viable spores (Table 2; Figure 3). These results, originally obtained after two backcrosses to Okayama-7, were again observed after five backcrosses to the wild-type strain. *rad 9-1* always produces white fruiting bodies with approximately 10^4–10^5 fewer viable spores than wild-type strains. *rad 3-1* fruiting bodies contain variable numbers of spores, with some fruiting bodies producing nearly 10% of the number seen on wild-type gills. Repeated fruitings of a given isolate vary in the actual numbers of spores produced. However, the viability of spores produced by *rad 3-1* is always low. Direct comparisons indicate that those few *rad 3-1* spores that are produced are never more than about 1% as viable as the more numerous spores from wild-type strains (Table 2).

TABLE 2

Basidiospore production by homozygous *rad* mutants

Strain	Tetrad formation	Spore viability	
		%	*n*
Rad$^+$	+	83	10^2
rad 3-1	−	1.1	10^4
rad 3-2	+	58	10^2
rad 9-1	−	4.9	10^3
rad 10-1	+	38	10^2

Viability tests were performed on strains that had been backcrossed five times to strain Okayama-7. Basidiospores were collected and counted using a hemocytometer, the concentrations were adjusted, three aliquots were plated, and the values were averaged.

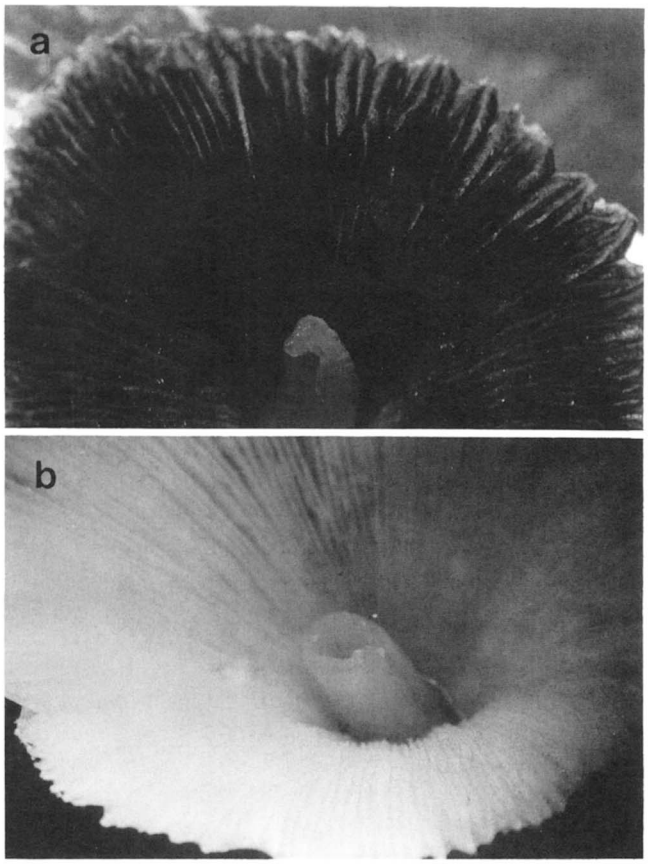

FIGURE 3.—Caps of wild-type (a) and *rad 3-1* (b) fruiting bodies.

Examination of gill segments of *rad 3-1* strains revealed the presence of scattered spores, in groups of two or three, but few or no tetrads.

In order to determine whether the repair defects and the meiotic defects in *rad 3-1* and *rad 9-1* result from a single gene mutation in each strain, we isolated progeny of crosses between *rad 3-1* or *rad 9-1* and Okayama-7. Each isolate was tested for radiation sensitivity, and each was crossed to a compatible *rad 3-1* (for the *rad 3-1* × Okayama-7 progeny) or *rad 9-1* (for the *rad 9-1* × Okayama-7 progeny) strain. For the *rad 3-1* progeny, 30 radiation-sensitive isolates were examined and all of these produced pale fruiting bodies when crossed to another *rad 3-1* strain, indicating reduced numbers of spores. Twenty progeny were radiation-resistant, and all of these produced wild-type appearing fruiting bodies when crossed to a *rad 3-1* strain. For *rad 9-1*, 22 radiation-sensitive progeny were fruited, and all generated white fruiting bodies when crossed to a *rad 9-1* strain. Fifty radiation-resistant progeny were examined, and all of these produced wild-type appearing fruiting bodies when crossed to a *rad 9-1* strain. Therefore, the radiation-sensitive and sporeless phenotypes of *rad 3-1* and *rad 9-1* are most likely pleiotropic effects of single gene mutations in these strains.

rad 3-2, which does not complement *rad 3-1* for radiation repair, does produce tetrads when homozygous. After five backcrosses to strain Okayama-7, the viability of *rad 3-2* spores was 70% of that of comparable wild-type spores (Table 2). The *rad 3-1* × *rad 3-2* heterozygous dikaryon exhibits the same fruiting phenotype as the *rad 3-2* homozygous dikaryon. It is possible that *rad 3-1* and *rad 3-2* are mutant in different genes, which do not show complementation unless they are in the same nucleus, as has been demonstrated for certain developmental mutations of *Schizophyllum commune* (KLEIN and DEPPE 1985). However, analysis of 500 progeny of a cross between *rad 3-1* and *rad 3-2* yielded no radiation-resistant recombinants. Therefore, *rad 3-1* and *rad 3-2* are either mutations in tightly linked genes, or they are mutations in the same gene. Their lack of complementation indicates that they probably are allelic.

Quantitative defects in repair and pathway analysis: The chunk test (Figure 2) is a rapid and reliable way to screen for radiation-sensitive mutants. However, it does not allow the phenotypes of different mutants to be compared. Quantitative measurements of the radiation sensitivities of *rad 3-1* and *rad 3-2* (Figure 4) indicated that *rad 3-2* is significantly less sensitive to ionizing radiation than is *rad 3-1*. To reduce their colony-forming ability to the same extent, *rad 3-2* requires twice as much gamma radiation as *rad 3-1*.

Quantitative measurements of survival also allowed the analysis of epistatic interactions among the *rad* mutants. Since multiple DNA repair pathways exist in all organisms (HANAWALT *et al.* 1979; FRIEDBERG 1985), we were interested to know whether the *rad* mutants we had isolated were defective in one or more repair pathways. In particular, we wished to know whether those mutations that lead to meiotic defects (*rad 3-1* and *rad 9-1*) were in the same repair pathway, and whether a mutation that does not cause an apparent meiotic defect (*rad 10-1*) was in a different repair pathway. If two *rad* mutants are defective in the same repair pathway, then one of the two mutant genes should be epistatic to the other. That is, the sensitivity

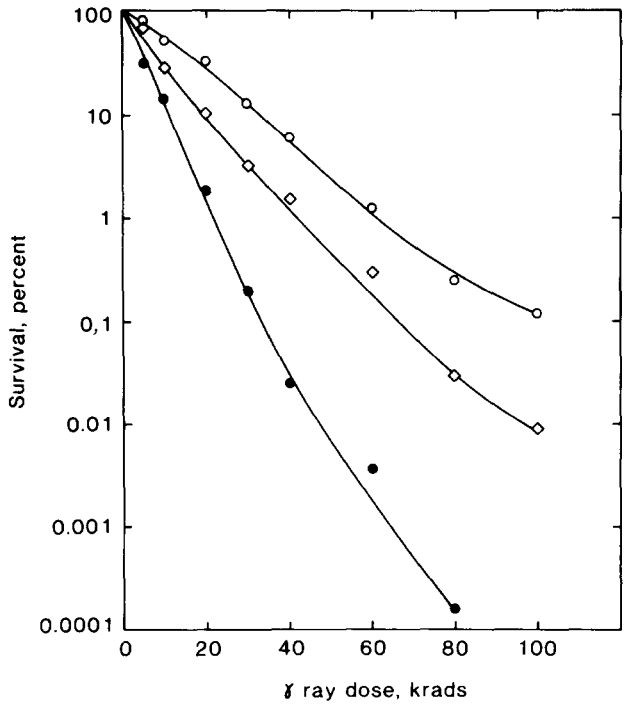

FIGURE 4.—Oidial survival after irradiation with gamma rays. Suspensions of oidia were irradiated, diluted, and plated, and survival was determined relative to that of an unirradiated control sample of each strain. (○), wild-type congenic control; (●), *rad 3-1*; (◇), *rad 3-2*.

of a double mutant should be the same as that of one of the single mutants (BRENDEL and HAYNES 1973; HAYNES and KUNZ 1981; GAME 1983). If the two genes are in different repair pathways, then the double mutant should be more sensitive than either single mutant, and this enhanced sensitivity will be either additive or synergistic (BRENDEL and HAYNES 1973; GAME 1983).

The double mutants *rad 3-1;rad 9-1* and *rad 3-1;rad 10-1* were constructed as described in MATERIALS AND METHODS. The gamma-radiation sensitivities of these strains were compared with those of the single mutants from which the double mutants were derived. Our results (Figure 5) showed both synergistic and epistatic interactions between pairs of *C. cinereus* repair genes. The *rad 3-1;rad 10-1* double mutant is significantly more sensitive than either single mutant (Figure 5a). In contrast, the *rad 3-1;rad 9-1* double mutant has exactly the same sensitivity as the *rad 3-1* single mutant (Figure 5b).

Sensitivity to UV light: None of the *rad* single mutants or double mutants is sensitive to UV light, either by the criterion of a modified chunk test (not shown) or as measured by oidial survival (Figure 6). In addition, all of the *rad* mutants are proficient in photoreactivation. After a UV dose of 96 J/m^2, the oidial survival of all strains was enhanced two- or three-fold when irradiated cells were exposed to photoreactivating light for 60 min.

Effect on recombination: The effect of each *rad* mutation on intergenic recombination was measured by examining the recombination frequency between *ade 8* and *met 5*, two genes on linkage group I (LEWIS and NORTH 1974). None of the *rad* mutations is linked to either of these markers. Dikaryons were constructed that were homozygous for a given *rad* mutation and heterozygous for the two nutritional markers. The experiment was performed using *rad* strains and a wild-type control, each of which had been backcrosssed to Okayama-7, an *ade 8* mutant, three times. Similar levels of meiotic recombination were observed in all of the crosses (Table 3). The *rad 3-1* result is from an analysis of only 104 spores, which were all the viable spores that could be recovered from 18 fruiting bodies, and the deviations in both *rad 3-1* and *rad 3-2* are slight, especially in comparison to those found for other meiotic mutants (BAKER *et al.* 1976). It is possible that these results were caused by slight differences in genetic background unrelated to the *rad* mutations.

Cytological characterization: Since strains homozygous for *rad 3-1* or *rad 9-1* have obvious defects in spore production (Figure 3), it was of interest to characterize meiotic chromosome behavior in these fruiting bodies. Examination of basidia from *rad 9-1* fruiting bodies after fixation, squashing, and silver staining (PUKKILA and LU 1985) revealed no abnormalities in the timing or extent of chromosome pairing, whereas basidia with fully paired chromosomes were not detected in *rad 3-1* fruiting bodies (results not shown). A procedure that was more gentle and rapid was then adopted for examination of the *rad 3-1* nuclei, as described in Materials and Methods. *rad 3-1* basidial protoplasts were prepared and allowed to settle before staining. Typical nuclear contents are shown in Figure 7, a and b. It appears that the chromosomes are largely unpaired (more than 13 silver stained rods are apparent), and not as condensed as the control Rad$^+$ chromosomes are at the time of nucleolar fusion, or become during meiotic prophase (Figure 7, c and d).

These defects in meiotic chromosome behavior were also observed using the electron microscope. Rad$^+$ fruiting bodies in mid or late pachytene were scanned and the presence of paired lateral elements was recorded. As illustrated in Figure 8a, paired lateral elements are obvious in both tangential and cross sections of the bivalents. In mid-pachytene, 96 of 103 nuclei contained more than two paired segments (37 of these contained more than six paired segments). Even at late pachytene, 40 of the 79 nuclei examined contained more than two paired segments. In contrast (Figure 8b), only a limited number of correctly spaced lateral elements were observed in sections from *rad 3-1* fruiting bodies sampled at four distinct times,

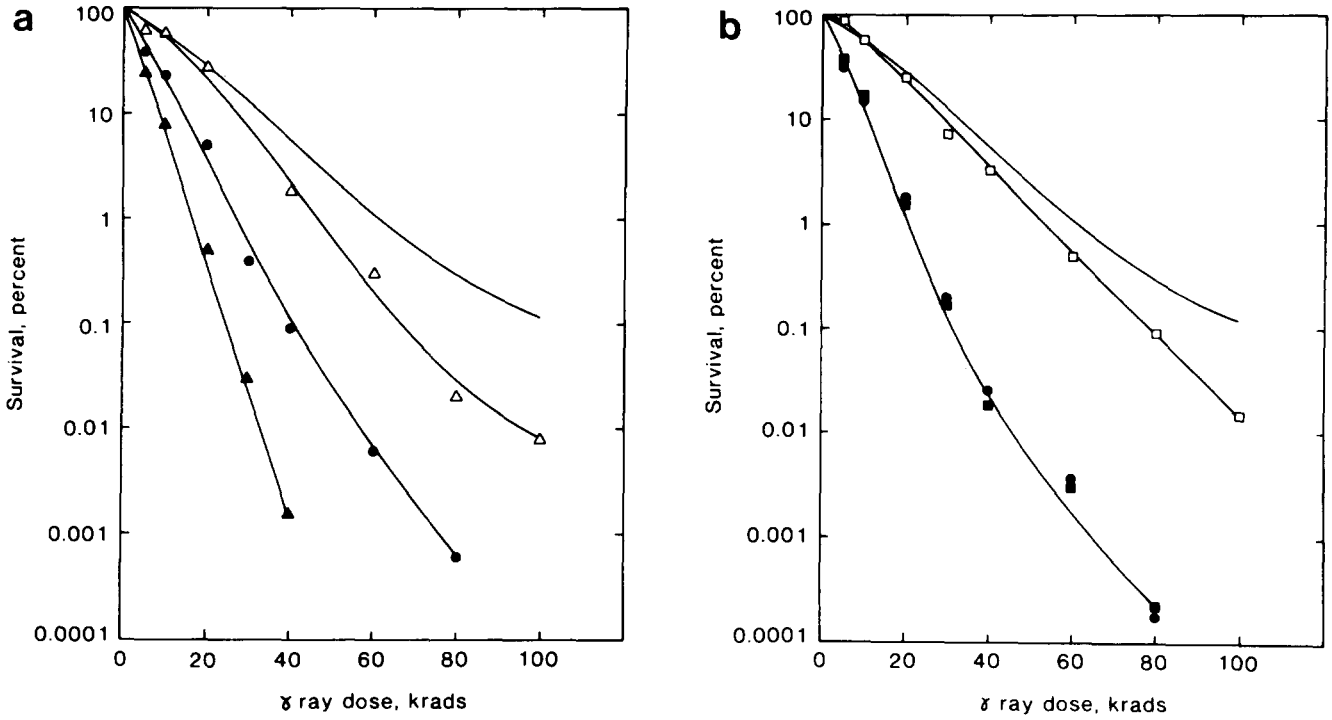

FIGURE 5.—Pathway analysis of *rad* mutations. Oidia were treated as described in the legend to Figure 4. In both panels, the data for the wild-type control are the same as those shown in Figure 4, and are represented by the plain line. Each panel contains data for three mutant strains whose oidial survival was determined at the same time, in order to facilitate comparisons among these strains. Panel a: (●), *rad 3-1*; (△), *rad 10-1*; (▲), *rad 3-1;rad 10-1* double mutant. Panel b: (●), *rad 3-1*; (□), *rad 9-1*; (■), *rad 3-1;rad 9-1* double mutant.

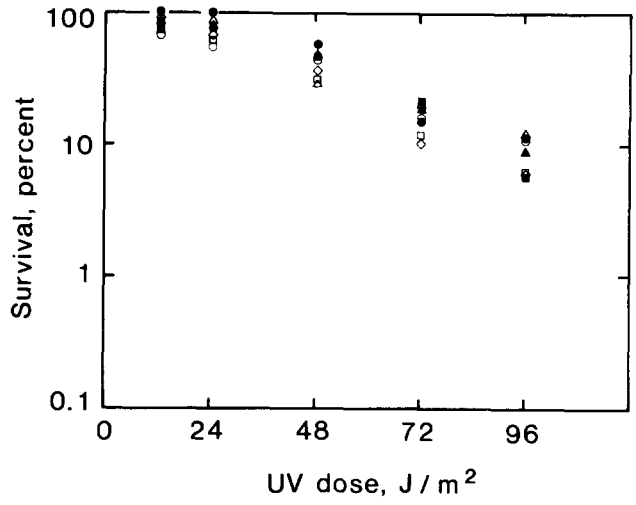

FIGURE 6.—Oidial survival after irradiation with UV light. Suspensions of oidia were irradiated with 254 nm UV light, diluted, and plated. Survival was determined relative to that of an unirradiated control sample of each strain. Symbols are the same as those used in Figures 4 and 5.

beginning after the completion of karyogamy and ending when the paraphyses had expanded (this is a morphological change in the underlying sterile cells of the gill, which occurs at the time of metaphase I in Rad$^+$ fruiting bodies) (PUKKILA, YASHAR and BINNINGER 1984). The number of paired segments neither increased nor decreased over this time period. About 12% of the 681 nuclei examined had one or two paired segments, but less than 2% had more than two

TABLE 3

Effects of *rad* mutations on intergenic recombination

Strain	Percent recombination	*n*
Rad$^+$	15.6	499
rad 3-1	9.6	104
rad 3-2	24.4	491
rad 9-1	14.8	508
rad 10-1	17.3	514

Recombination between *ade 8* and *met 5* was determined for a cross between a *rad x*; *met 5* strain and a *rad x*; *ade 8* strain. All strains were constructed using *rad* mutants or a wild-type control that had been backcrossed to Okayama-7 three times.

paired segments. All of the 100 *rad 9-1* nuclei examined had paired segments. We conclude that the *rad 3-1* mutants are unable to complete meiotic chromosome condensation and pairing, whereas the defect in *rad 9-1* nuclei does not appear to involve these processes. Results from serial sectioning and three-dimensional reconstruction confirming the presence of unpaired lateral elements in the *rad 3-1* strains will be presented elsewhere (P. J. PUKKILA, M. E. ZOLAN and W. HANTON, in preparation).

DISCUSSION

We have described four recessive radiation-sensitive mutants of *C. cinereus*, which define three complementation groups for DNA repair (Table 1). Two of the mutants, *rad 3-1* and *rad 9-1*, produce fruiting

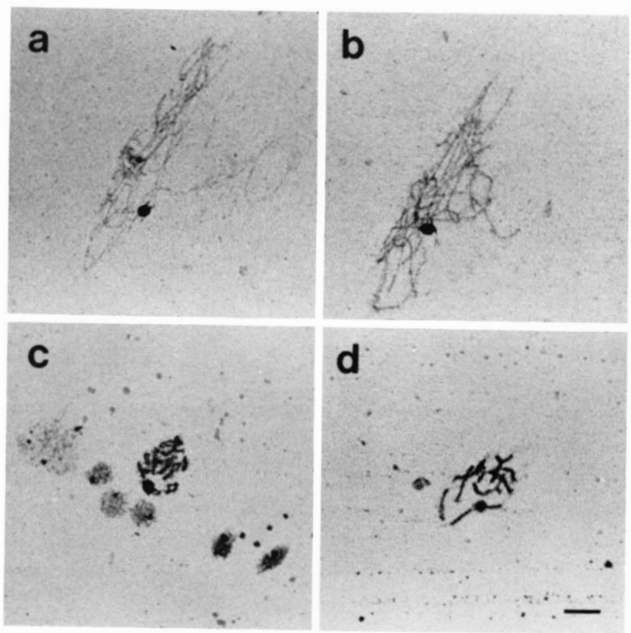

FIGURE 7.—Light micrographs of Coprinus chromosomes in a *rad 3-1* homozygote (a and b) and in Rad$^+$ congenic control homozygotes (c and d). Bar indicates 5 μm.

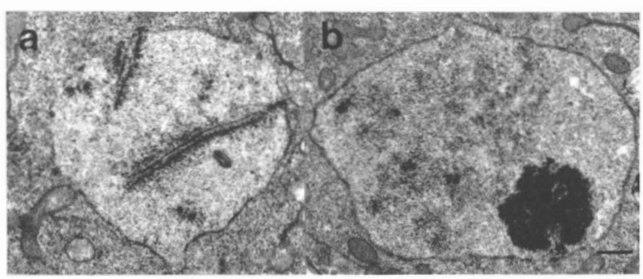

FIGURE 8.—Electron micrographs of Coprinus nuclei from a Rad$^+$ congenic control strain (a) and a *rad 3-1* strain (b). Bar indicates 0.5 μm.

bodies with vastly reduced numbers of viable spores (Table 2). The radiation-sensitive and basidiosporeless phenotypes of *rad 3-1* and *rad 9-1* cosegregate through meiosis, and have remained unchanged after five backcrosses of these mutants to a Rad$^+$ strain. These results imply that homozygous dikaryons of *rad 3-1* and *rad 9-1* are defective in meiosis. Although lack of spore formation can of course result from defects in processes other than meiosis *per se*, the fact that *rad 3-1* and *rad 9-1* are also clearly repair mutants implies that their primary defects are in the metabolism of DNA. In addition, microscopic examination of *rad 3-1* homozygous dikaryons indicates that its chromosomes do not pair during prophase I of meiosis.

Although *rad 3-1* and *rad 3-2* do not complement for radiation repair, they have different fruiting phenotypes; *rad 3-2* homozygous dikaryons produce tetrads of viable spores, whereas those of *rad 3-1* do not. It is possible that *rad 3-1* and *rad 3-2* are nonallelic, but that the two gene products must be within the same nucleus in order to complement, a condition satisfied by meiosis but not by a vegetative dikaryon. However, our finding of no recombinants among 500 progeny indicates with 95% certainty that the two mutations are not more than 0.3 cM apart (STEVENS 1942). It is likely that *rad 3-1* and *rad 3-2* are different mutations of the same gene; the fact that *rad 3-2* exhibits much better oidial survival than *rad 3-1* (Figure 4) implies that *rad 3-2* has a less severe defect in the *RAD 3* gene product.

The examination of oidial survival (Figures 4 and 5) implies that *rad 9-1, rad 10-1* and *rad 3-2* are not very γ-ray sensitive. It is puzzling that all four mutants are radiation-sensitive by the criterion of the chunk test, but that only *rad 3-1* demonstrates a significantly reduced level of oidial survival in response to γ-radiation. It is possible that the underlying metabolic differences between quiescent oidia and growing mycelia lead to repair of DNA damage by nonidentical mechanisms in the two cell types. Alternately, the chunk test and oidial survival may measure quite different aspects of repair proficiency. For oidial survival curves, oidial suspensions in water are irradiated and subsequently diluted and plated. Thus, survival reflects the ability of a uninucleate spore to germinate and form a colony. In the chunk test, multicellular bits of mycelia are irradiated, and growth during the 48 hr following irradiation is compared to the growth of an unirradiated control. Therefore, a strain that is inhibited but not killed by gamma rays would be scored as sensitive by a chunk test but might be barely more sensitive than the wild-type control when oidial survival is measured. The fact that our screen, done by a chunk test, yielded *rad 3-1* and *rad 9-1*, two interesting meiotic mutants, means that this method is useful and adequate for our purposes.

Quantitative analysis revealed that the *rad 3-1;rad10-1* double mutant is significantly more radiation-sensitive than either single mutant (Figure 5a). This implies that *rad 3-1* and *rad 10-1* are defective in two different repair pathways. However, it is also possible that the two mutations are in the same repair pathway and each reduces the level of its gene product incompletely (GAME and COX 1972). It has been shown in *Caenorhabditis elegans* (HARTMAN and HERMAN 1982) and in *Drosophila melanogaster* (BAKER, CARPENTER and RIPOLL 1978; BAKER and SMITH 1979) that many gene products with repair functions also have other essential mitotic roles. Therefore, it is possible that strains with null mutations of some *rad* genes would not have been recovered in our screen, which required mitotic viability in the absence of irradiation (see MATERIALS AND METHODS). If null mutants of these genes are viable, as is the case for the yeast genes *rad 50, rad 52*, and *rad 54* (SCHILD *et al.* 1983), then gene product relationships may be determined more clearly. All of the *rad* mutants can

function as hosts in DNA-mediated cell transformation (M. E. ZOLAN and J. LAING, unpublished observation). This will allow the eventual cloning of these genes and the construction of defined disruptions (BINNINGER 1987), which will allow the characterization of null phenotypes.

In contrast to the result for the *rad 3-1;rad 10-1* double mutant, the *rad 3-1;rad 9-1* mutant is exactly as sensitive as the *rad 3-1* single mutant (Figure 5b). This strongly implies that *rad 3-1* and *rad 9-1* are defective in the same repair pathway, or are part of a multiprotein repair complex, and is striking because both mutants are defective in meiosis as well. Experiments with *rad* mutants of *S. cerevisiae* led to the hypothesis that in an epistatic interaction the sensitivity of the double mutant will be equal to that of the most sensitive single mutant (BRENDEL and HAYNES 1973; HAYNES and KUNZ 1981; GAME 1983). None of these studies was performed using known null mutations, however. The phenotype of the *rad 3-1;rad 9-1* double mutant could be that of the most sensitive single mutant, or that of the one that is the least leaky, or that of the one that acts first in the repair pathway.

The fact that none of the *rad* mutants is sensitive to ultraviolet light makes them analogous to the yeast *rad 50* series of mutants, which predominantly exhibit X-ray sensitivity, but are only slightly sensitive to ultraviolet light (COX and GAME 1974). This is in contrast to *rec-1* of *Ustilago*, which is sensitive to both X-rays and ultraviolet light (HOLLIDAY *et al.* 1976). The amount of photoreactivation seen in our strains is consistent with that previously reported for *C. cinereus* (RAHMAN and COWAN 1974).

Recombinants were observed in basidiospore progeny of homozygous dikaryons of all of the *rad* mutants (Table 3). This result is especially striking for *rad 3-1* and *rad 9-1*, which produce so few spores overall. *rad 3-1* is defective in meiosis I; meiotic nuclei from wild-type fruiting bodies contain thirteen synapsed chromosomes, whereas in those from *rad 3-1* fruiting bodies the meiotic process has clearly broken down (Figures 7 and 8). Our results imply that the *rad 3-1* gene product is required for an early step in meiosis, up to and including the specific pairing of homologues, and that in its absence pairing rarely occurs. If the pairing process were equally aberrant in the meiocytes that produced the small number of viable spores, it would appear that either recombination occurs before the completion of synaptonemal complex formation in *Coprinus*, or that the relatively uncondensed chromosomes allow normal levels of recombination in these mutants. High frequencies of "ectopic" recombination between genes on nonhomologous chromosomes have been reported in yeast (JINKS-ROBERTSON and PETES 1985; 1986; LICHTEN, BORTS and HABER 1987), which also indicates that recombination need not be dependent on the completion of homologous chromosome synapsis. Our data indicate that the meiotic defect in *rad 9-1* is subsequent to recombination and pairing. Both of these mutants are defective in the repair of damage induced by ionizing radiation in mitotic cells. The ease of cytological observation in Coprinus should facilitate analysis of additional alleles of these genes, and aid in elucidating the common mechanisms underlying chromosome behavior during repair and meiosis.

We have used the phenotype of radiation sensitivity as a screen for interesting meiotic mutants of *C. cinereus*, because this organism is ideally suited to studies of meiosis, and we expect that further study of these mutants will help us to understand this fundamental cellular process. We have also observed that defects in meiosis in this basidiomycete result in sporeless, but otherwise healthy, fruiting bodies. Therefore, this technique may be useful in the construction of sporeless strains of commercially important basidiomycetes, whose copious spore production causes respiratory problems that currently make large-scale growth of these mushrooms unfeasible.

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