radE*, a New Radiation-sensitive Locus in *Dictyostelium discoideum

By M. B. COUKELL AND A. M. CAMERON

Department of Biology, York University, 4700 Keele Street, Downsview,
Ontario, M3J 1P3, Canada

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Dictyostelium discoideum strain M28, which has been used widely in genetic studies, was found to carry a radiation-sensitive mutation. This allele, termed *rad-100*, was recessive in heterozygous diploids and mapped in linkage group III. Complementation analysis and survival studies on strains carrying *rad-100* suggested that this allele defines a new radiation-sensitive locus in *D. discoideum*, and this locus has been designated *radE*. *radE* strains were moderately sensitive to ultraviolet light (D_{10} 90 J m⁻²) and slightly sensitive to ¹³⁷Cs gamma rays (D_{10} 255 krad). *radE* strains also exhibited increased sensitivity to killing by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine but not by other alkylating agents such as ethyl methane-sulphonate or methyl methanesulphonate. The frequency of spontaneous methanol-resistant (*acrA*) mutants was approximately the same in cultures of *radE* and *radE*⁺ strains. However, when amoebae of these strains were irradiated with ultraviolet light, the frequency of induced mutants was significantly lower in cultures of the *radE* strain. Furthermore, when amoebae of wild-type strain NC4 were plated in the presence of caffeine after ultraviolet-irradiation, the survival curves were very similar to the curves obtained for amoebae of *radE* strains in the presence or in the absence of caffeine. These results suggest that the *radE100* mutation and caffeine interfere with an error-prone DNA repair pathway in *D. discoideum*.

INTRODUCTION

One class of genetic markers which have been used effectively in *Escherichia coli* (Van de Putte *et al.*, 1965) and in yeast (Cox & Parry, 1968) and which have recently been described in *Dictyostelium discoideum* (Welker & Deering, 1976) are mutations causing increased sensitivity to radiation. These markers are valuable because they generally have little effect on the growth of an organism under standard laboratory conditions, but can be easily detected by exposing the organism to an artificial source of radiation. Moreover, radiation-sensitive strains carrying mutations at different loci can often be distinguished by their characteristic responses to different types of radiation and/or to certain chemicals.

During a mutant hunt in this laboratory, Dr A. S. Tsang observed that one of our common laboratory stocks, strain HC91, was significantly more sensitive to ultraviolet (u.v.) irradiation than our other haploid stocks. To determine whether the u.v.-sensitive mutation in this strain could be a useful genetic marker and to establish the relationship of this allele to other radiation-sensitive alleles reported in *D. discoideum*, we have examined the sensitivity of strains carrying this mutation to radiation and to chemical mutagens, and the effect of this allele on the induction of mutations by u.v.-irradiation. We have also analysed the allele genetically and the results suggest that the mutation is at a new radiation-sensitive locus, *radE*.

METHODS

Chemicals. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was purchased from Aldrich Chemical Co., Milwaukee, Wis., U.S.A.; methyl methanesulphonate (MMS) from Eastman Kodak Co., Rochester, N.Y., U.S.A.; ethyl methanesulphonate (EMS) from Sigma; and caffeine from Baker Chemical Co., Phillipsburg, N.J., U.S.A. All culture media were purchased from Difco.

Strains and growth conditions. Strains used in this study are described in Table 1. All stocks were stored by desiccating the spores on silica gel (Perkins, 1962). Strains were grown clonally from spores on SM agar plates (Sussman, 1966) with *Enterobacter aerogenes* at 22 °C. Amoebae were scraped from the growth zone around a clone with an inoculating loop, suspended in 5.0 ml phosphate-buffered saline (PBS; Deering *et al.*, 1970) at room temperature, washed twice by centrifugation (500 g for 4 min) and finally resuspended in the same buffer at a concentration of 1×10^6 cells ml⁻¹. In some experiments, the amoebae were resuspended in PBS containing caffeine (filter-sterilized) at a final concentration of 0.15 mg ml⁻¹.

Irradiation conditions. For u.v.-irradiation 3.5 ml cell suspension was placed in a small plastic Petri dish (15 × 60 mm) and agitated gently under a 15 W germicidal lamp (General Electric). The incident dose rate was adjusted to 0.4 J m⁻² s⁻¹ as measured with a Latarjet dosimeter. At intervals, duplicate 0.2 ml samples were removed, diluted in PBS or in PBS plus caffeine and plated with bacteria on SM agar or on SM agar plus caffeine (0.15 mg ml⁻¹). The plates were incubated in the dark at 22 °C and plaques were counted as they appeared during the next 3 to 7 d.

For γ -irradiation, 1.3 ml cell suspension was placed in a glass test tube (15 × 150 mm) in an ice bath and exposed to a ¹³⁷Cs source in a Gammator M38-3 (Radiation Machinery Corp., Parsippany, N.J., U.S.A.). The dose rate was 2 krad min⁻¹. Duplicate samples were removed at intervals, diluted and plated with bacteria as described above.

Treatment with chemical mutagens. Cell suspension (2.0 ml) was placed in a 15 × 130 mm glass screw-capped tube and 0.05 to 0.10 ml mutagen was added to give the desired final concentration (MNNG, 0.68 mM; MMS, 44 mM; EMS, 200 mM); the tubes were then tightly sealed, placed in a rack at a 45° angle and shaken on a Gallenkamp orbital shaker (150 rev. min⁻¹) at 22 °C. At intervals, 0.1 ml samples were removed, added to 5.0 ml PBS at room temperature and centrifuged at 500 g for 4 min. The cells were washed twice more in 5.0 ml PBS and then diluted in PBS and plated in duplicate with bacteria on SM agar as described above. The solution of MNNG was prepared immediately before each experiment by adding 10 mg MNNG to 5.0 ml PBS and shaking at 22 °C for 90 min.

Frequency of methanol-resistant mutants. The frequency of methanol-resistant (*acrA*) mutants in u.v.-treated and in untreated cell populations was determined in the following way. Amoebae of each strain were collected from around 10 or more independent clones, washed, resuspended separately in PBS at 1×10^6 cells ml⁻¹ and irradiated with u.v. light (100 J m⁻²) as described above. A sample (1 ml) of each irradiated suspension was added to 5 ml of a suspension of *E. aerogenes* (1×10^{10} cells ml⁻¹ in PBS) and the cultures were shaken in 25 ml Erlenmeyer flasks at 22 °C. To determine the frequency of spontaneous mutants, a sample of untreated cells from each suspension was added to an identical bacterial suspension to give an initial concentration of viable cells approximately equal to that of the cultures receiving irradiated cells. All the cultures were then grown to early-stationary phase (1.0×10^7 to 1.5×10^7 cells ml⁻¹). This period of growth permitted the *radE*⁺ and *radE* cultures to complete approximately seven and nine generations, respectively, before being subjected to mutant selection. Each culture was then agitated vigorously and a sample was diluted in PBS and plated in duplicate with bacteria on SM agar plates to determine the viable count. Another sample was added to each of four SM agar plates containing 3% (v/v) methanol and spread with bacteria. Before addition to the selective plates, the cell concentration of each culture was adjusted to give 5 to 60 methanol-resistant mutants per plate. The total number of mutants on the four plates was recorded after 7 d at 22 °C and the frequency of mutants per viable cell was calculated for each clone.

Parasexual genetic analysis. Diploid strains DC1, DC115 and DC116 were obtained by shaking haploid strains carrying different temperature-sensitive growth (*tsg*) mutations together in buffer at 22 °C and selecting temperature-resistant clones on plates spread with *E. aerogenes* at 26.5 °C (Williams *et al.*, 1974; Coukell, 1975). Diploid strain DC124 was obtained by permitting strains HC91 and XP55 to fuse in buffer and selecting for clones capable of growing on lawns of *Bacillus subtilis* strain 36.1 at 26.5 °C as described by Newell *et al.* (1977). Diploid strains were identified by spore size and shape and by genetic markers (Sussman & Sussman, 1962; Katz & Sussman, 1972). All diploids were purified at least twice by clonal passage before they were used in an experiment. Methanol-resistant haploid segregants were selected and characterized as described previously (Coukell, 1975; Coukell & Roxby, 1977).

Screening haploid segregants for u.v. sensitivity. Since strains carrying the *radE100* mutation are only moderately u.v.-sensitive, the spot test described by Welker & Deering (1976) could not be used to test the haploid segregants for u.v. sensitivity. The following procedure was therefore developed. Each haploid

Table 1. Strains of *Dictyostelium discoideum* used

Diploids	Haploids	Relevant genotype*						Source or reference
		I	II	Linkage group	III	IV	VI	
NC4		<i>tsgE13 sprA1</i>			<i>radE100</i>	<i>bwnA1</i>		P. C. Newell, Katz & Sussmann (1972)
M28								Katz & Sussmann (1972)
TS12		<i>cycA1</i>	<i>whiA1 tsgD12</i>					Katz & Sussmann (1972)
HC91		<i>tsgE13 sprA1</i>	<i>acrA100</i>		<i>radE100</i>	<i>bwnA1</i>	<i>manA1</i>	Coukell (1975)
HPS1		<i>radA20</i>	<i>whiA1 acrA1 tsgD12</i>				<i>radB13</i>	D. L. Welker
HPS14		<i>cycA1</i>	<i>whiA1 acrA1 tsgD12</i>					D. L. Welker
XP55		<i>cycA5</i>		<i>bsgA5</i>				P. C. Newell
DC1	HC91/TS12	<i>tsgE13 + sprA1/+ + cycA1/+</i>	<i>+ acrA100/+ + whiA1 + tsgD12</i>	<i>radE100/+</i>		<i>bwnA1/+</i>		HC91 × TS12
DC115	HC91/HPS1	<i>tsgE13 sprA1/+ + + radA20</i>	<i>+ acrA100/+ + whiA1 acrA1 tsgD12</i>	<i>radE100/+</i>		<i>bwnA1/+</i>	<i>+ /manA1</i>	HC91 × HPS1
DC116	HC91/HPS14	<i>tsgE13 sprA1/+ + + cycA1</i>	<i>+ acrA100/+ + whiA1 acrA1 tsgD12</i>	<i>radE100/+</i>		<i>bwnA1/+</i>	<i>+ /radB13</i>	HC91 × HPS14
DC124	HC91/XP55	<i>tsgE13 sprA1/+ + + cycA5</i>	<i>acrA100/+</i>	<i>radE100/+ + bsgA5</i>		<i>bwnA1/+</i>		HC91 × XP55

* The genetic nomenclature is based on the system of Demerec *et al.* (1966). The relevant phenotypic traits are: *tsg*, unable to grow at 26.5 °C; *spr*, produces round spores; *cyc*, resistant to 500 µg cycloheximide ml⁻¹; *acr*, resistant to 3% (v/v) methanol; *whi*, produces white spores; *bwn*, produces brown pigment; *rad*, sensitive to u.v. light and/or γ rays; *bsg*, unable to grow on *B. subtilis*; *man*, lacks α-mannosidase-1. All mutant alleles are recessive except for *sprA1*.

segregant was inoculated (using a sterile toothpick) on to a grid on a SM agar plate, previously spread with *E. aerogenes*, and incubated at 22 °C. When the plaques were 10 to 15 mm in diameter amoebae were scraped from the growth zone around each plaque and transferred to 5.0 ml PBS at room temperature to give a final concentration of 0.3×10^6 to 2.0×10^6 cells ml⁻¹. The suspensions were agitated vigorously and a sample of each was diluted and plated in duplicate on SM agar with bacteria to determine the viable count. A 3.5 ml sample of each suspension was then transferred to a small Petri dish, irradiated with u.v. light (100 J m⁻²) as described above, diluted in PBS and plated in duplicate on SM agar. Plaques were counted on both sets of plates over the next 3 to 7 d, and the percentage of cells surviving was calculated for each segregant. Under these conditions, the values obtained for strains NC4 (*radE*⁺) and M28 (*radE*) in eight experiments were 69 ± 11 (range 50 to 82) and 14 ± 7 (range 5 to 23), respectively. Therefore, segregants exhibiting more than 50% survival were scored as *radE*⁺ while segregants showing less than 30% survival were scored as *radE*. A few segregants which initially gave survival values between 30 and 50% were re-analysed to establish their correct phenotype.

RESULTS

Sensitivity of strains HC91 and M28 to u.v.-irradiation

The u.v. survival curves obtained for strain HC91 and the original wild-type parent of this strain, NC4, are shown in Fig. 1. As reported by Freim & Deering (1970), strain NC4 was quite resistant to u.v.-irradiation at low doses, with a 10% survival dose (D_{10}) of about 190 J m⁻². On the other hand, the survival curve for strain HC91 lacked the broad shoulder characteristic of strain NC4 and the D_{10} for this strain was only 90 J m⁻². Since strain HC91 was isolated as a spontaneous methanol-resistant derivative of strain M28 (Katz & Sussman, 1972; Coukell, 1975), the u.v. sensitivity of strain M28 was also examined; the survival curve for this strain was identical to that of HC91 (Fig. 1). The M28 stock used in these experiments was obtained from Dr E. R. Katz (via Dr P. C. Newell) in 1971 and spores of this strain have been stored desiccated on silica gel since that time. Therefore, this u.v.-sensitive allele was probably introduced into strain M28 along with the t_2^s (*tsgE13*) and *sp*^R (*sprA1*) alleles during MNNG mutagenesis (see Katz & Sussman, 1972). This new radiation-sensitive allele was initially designated *rad-100*.

The u.v. survival curves for strains of *D. discoideum* carrying the radiation-sensitive alleles *radA20* (strain HPS1) and *radB13* (strain HPS14) (Welker & Deering, 1976) are also shown in Fig. 1. Under the same conditions, these strains had D_{10} values of 8 and 2 J m⁻² respectively; therefore, strains carrying the *rad-100* allele are considerably less u.v.-sensitive than strains carrying *radA20* or *radB13*.

Sensitivity of strains carrying rad-100 to γ-rays and to chemical mutagens

To further characterize the *rad-100* mutation, the relative viabilities of amoebae of strains NC4 and M28 were determined after exposure to γ-rays and to various alkylating agents. Strain M28 was only slightly more sensitive to γ-rays than strain NC4 (Fig. 2). The D_{10} values for these strains under our experimental conditions were 255 and 275 krad, respectively.

When amoebae of strains NC4 and M28 were incubated in the presence of EMS (200 mM) or MMS (44 mM), the survival curves for the two strains were similar, except that the M28 cells appeared to be slightly more sensitive than the wild-type cells to short exposure to MMS (Fig. 3*a, b*). However, when amoebae of the same strains were incubated with MNNG (0.68 mM), the cells carrying the *rad-100* mutation were consistently more sensitive to the mutagen (Fig. 3*c*). Despite attempts to maintain constant conditions, the MNNG survival curves for both strains varied considerably between experiments. Nevertheless, in every experiment, the viability of M28 cells in the presence of MNNG was lower than the viability of strain NC4. The major difference between the MNNG survival curves for these two strains was the absence of a shoulder on the M28 curve (Fig. 3*c*), as in the u.v. survival curves (Fig. 1).

Fig. 1

Fig. 2

Fig. 1. Survival curves after u.v.-irradiation for various radiation-resistant and radiation-sensitive haploid strains: $\circ$, NC4; $\bullet$, TS12; $\square$, HC91; $\blacksquare$, M28; $\triangle$, HPS1; $\blacktriangle$, HPS14. Each curve is representative of results obtained in two or more experiments.

Fig. 2. Survival curves after γ -irradiation for strains NC4 ($\circ$) and M28 ($\bullet$). Each curve is representative of results obtained in three experiments.

Fig. 3. Survival curves for strains NC4 ($\circ$) and M28 ($\bullet$) after incubation with 200 mM-EMS (a), 44 mM-MMS (b) and 0.68 mM-MNNG (c). Each point in (a) and (b) is an average of values obtained in two experiments. In (c), the surviving fractions $\pm$ s.d. were calculated from data obtained in three experiments.

Effect of caffeine on the u.v. sensitivity of wild-type and rad-100 strains

Since Freim & Deering (1970) reported that the presence of caffeine at 0.15 mg ml^{-1} eliminated the shoulder from u.v. survival curves of wild-type amoebae, we examined its effect on strains NC4 and M28. The survival curve for strain NC4 in the presence of caffeine was superimposable on the curve for strain M28 in the absence of caffeine (Fig. 4). When

Fig. 4

Fig. 5

Fig. 4. Effect of caffeine (0.15 mg ml^{-1}) on the survival curves after u.v.-irradiation for strains NC4 ($\circ$, $\bullet$, $\blacktriangle$) and M28 ($\square$, $\blacksquare$, $\blacktriangledown$). Amoebae were diluted and plated in the absence ($\circ$, $\square$; data from Fig. 1) and in the presence ($\bullet$, $\blacktriangle$, $\blacksquare$, $\blacktriangledown$; two experiments) of caffeine.

Fig. 5. Survival curves after u.v.-irradiation for various diploid strains: $\circ$, DC1; $\bullet$, DC115; $\square$, DC116. Each curve is representative of results obtained in two or more experiments.

u.v.-irradiated amoebae of strain M28 were diluted and plated in the presence of caffeine at the same concentration, there was no detectable effect on cell viability at doses up to 75 J m^{-2} . However, at higher doses, the viability of the mutant cells was reduced slightly (Fig. 4). Under our experimental conditions, caffeine at 0.15 mg ml^{-1} had no effect on the plating efficiency of either strain; however, at higher concentrations, the plating efficiencies of both strains were decreased.

Effect of the rad-100 allele on the frequency of acrA mutants

To determine if the presence of the *rad-100* allele affects the frequency of spontaneous or u.v.-induced mutations, the frequency of methanol-resistant mutants was measured in untreated and in u.v.-treated cultures of strains carrying the *rad*⁺ or *rad-100* allele. Previous genetic studies (Williams *et al.*, 1974; Coukell, 1975) have established that methanol-resistance in *D. discoideum* results from mutation at a single locus, *acrA*. Table 2 presents the results obtained with two *rad*⁺ strains (NC4 and XP55) and one *rad-100* strain (M28). The frequency of spontaneous *acrA* mutants was approximately 1×10^{-6} for all three strains, in agreement with an earlier report by Mosses *et al.* (1975). When amoebae of the *rad*⁺ strains were irradiated with u.v. light (100 J m^{-2}) and then permitted to grow in a bacterial suspension to express the induced mutations, the frequency of *acrA* mutants increased 60- to 65-fold in cultures of both strains. On the other hand, the frequency of mutants in cultures of strain M28 after the same treatment was stimulated only 14-fold. Thus, strain M28 which carries the *rad-100* allele appears to be less susceptible to u.v.-induced mutation than *rad*⁺ strains.

Parasexual genetic analysis of the rad-100 allele

To determine whether the *rad-100* allele is recessive or dominant in heterozygous (+/*rad-100*) diploids, the sensitivity of strain DC1 to u.v.-irradiation was examined. Strain DC1 is a diploid constructed by fusing strain HC91 with the u.v.-resistant strain, TS12

Table 2. Effect of u.v.-irradiation on the frequency of *acrA* mutants in *rad*⁺ and *rad-100* strains of *D. discoideum*

Strain	Genotype	No. of clones examined	U.v. treatment	10 ⁶ × Frequency of <i>acrA</i> mutants per viable cell ± s.d.	Relative stimulation
NC4	<i>rad</i> ⁺	10	—	1.0 ± 0.4	63
		10	+	62.8 ± 11.8	
XP55	<i>rad</i> ⁺	10	—	1.2 ± 0.6	65
		10	+	78.3 ± 22.2	
M28	<i>rad-100</i>	15	—	1.1 ± 0.6	14
		15	+	15.0 ± 7.1	

(see Table 1; Fig. 1). The u.v. survival curve for strain DC1 (Fig. 5) was very similar to the curve for strain TS12; therefore, the *rad-100* allele is recessive.

Although the u.v. survival curves for strains carrying *rad-100* were quite different from the curves for strains carrying the radiation-sensitive alleles *radA20* and *radB13* (Fig. 1), complementation tests were carried out between these strains to determine if the *rad-100* mutation mapped at the *radA* or *radB* locus. Survival curves for the resulting *rad-100/radA20* (strain DC115) and *rad-100/radB13* (strain DC116) diploids are presented in Fig. 5. The results indicated that the *rad-100* allele complements the other two radiation-sensitive mutations; thus, *rad-100* does not reside at *radA* or *radB*. This mutation is also not allelic with the recently identified *radC35*, *radD14*, *radF21*, *radG5*, *radH18* or *radI24* mutations (Welker & Deering, 1978; D. L. Welker, personal communication). Therefore, the *rad-100* mutation defines a new radiation-sensitive locus, *radE*, and the allele in strains M28 and HC91 has been designated *radE100*.

To determine if the *radE* locus maps in linkage groups I, II or IV, methanol-resistant segregants of diploid DC1 (see Table 1) were selected as described previously (Coukell, 1975; Coukell & Roxby, 1977). The methanol-resistant, haploid segregants were then grouped into four classes (1 to 4) according to their genotypes, and the strains in each class were screened for the presence of the *radE*⁺ or *radE100* allele as described in Methods. The results are summarized in Table 3, expt 1. Since both *radE*⁺ and *radE100* strains were found among the haploid segregants, the *radE* locus cannot be in linkage group II; if *radE* was in group II, linked to *acrA*, then all of the haploid segregants would have been radiation-sensitive. In addition, *radE* would appear not to be in linkage groups I or IV, since the *radE100* allele segregated independently of *sprA* and *bwnA*, markers for groups I and IV, respectively (see Table 1).

To determine if the *radE* locus is in linkage group III, strain HC91 was fused with strain XP55 which carries the *bsgA5* allele (inability to grow on *B. subtilis*) in group III (Ratner & Newell, 1979). Clones of the resulting diploid (DC124) were then segregated on methanol-agar plates and the methanol-resistant, haploid segregants were screened for the *bsgA* marker by inoculating each strain on to a SM agar plate spread with a lawn of *B. subtilis*. Following incubation at 22 °C, the haploid segregants exhibiting little or no growth (*bsgA* strains) and the segregants exhibiting growth properties comparable to strain HC91 (*bsgA*⁺ strains) were examined for sensitivity to u.v.-irradiation. All of the *bsgA* segregants were u.v.-resistant while all of the *bsgA*⁺ segregants were u.v.-sensitive (Table 3; expt 2). These results suggest that the *radE100* allele is linked to the *bsgA* locus in linkage group III.

Table 3. Segregation analysis of diploid strains heterozygous for the *radE100* allele

Expt	Diploid	Selected marker	Haploid segregant classes								Total
1	DC1	<i>acrA</i>	1		2		3		4		111
			<i>tsgE sprA cycA⁺ bwnA⁺</i>	<i>tsgE sprA cycA⁺ bwnA</i>	<i>tsgE⁺ sprA⁺ cycA bwnA⁺</i>	<i>tsgE⁺ sprA⁺ cycA bwnA</i>	<i>radE⁺ radE100</i>				
			<i>radE⁺ radE100</i>	<i>radE⁺ radE100</i>	<i>radE⁺ radE100</i>						
			9	7	30	26		9	0	22	
2	DC124	<i>acrA</i>	1		2		Total				60
			<i>bsgA⁺</i>	<i>bsgA</i>	<i>radE⁺ radE100</i>						
			<i>radE⁺ radE100</i>	<i>radE⁺ radE100</i>							
			0	24		36	0	60			

DISCUSSION

In two earlier genetic studies on *D. discoideum* (Welker & Deering, 1976, 1978), mutations resulting in increased sensitivity to radiation were assigned to eight different loci in five linkage groups: group I, *radA*; group II, *radH*; group III, *radC*, *radG* and *radI*; group IV, *radD* and *radF*; and group VI, *radB*. Strains carrying a mutation at any of these loci, except *radC*, were sensitive to both u.v. light and γ -rays, while *radC* mutants were only sensitive to u.v.-irradiation. In the present study, a new radiation-sensitive allele in *D. discoideum* has been analysed. Like mutations at the *radC*, *radG* and *radI* loci, this new allele (*rad-100*) was found to be recessive in heterozygous diploids and it mapped in linkage group III. However, unlike *radG* and *radI* mutants, strains carrying the *rad-100* allele were only slightly sensitive to γ -rays and complementation tests indicated that *rad-100* does not map at *radC* (D. L. Welker, personal communication). Therefore, *rad-100* would appear to map at a new radiation-sensitive locus in linkage group III; this has been named *radE*.

When amoebae of the wild-type strain NC4 were diluted and plated in the presence of caffeine after u.v.-irradiation, the survival curve for this strain was very similar to the curve obtained for the *radE* strain in the presence or absence of caffeine. Thus, after u.v.-irradiation, *radE*⁺ amoebae in the presence of caffeine behave as phenocopies of *radE* cells. This finding suggests that the *radE*⁺ gene product normally functions in a caffeine-sensitive repair pathway. The effect(s) of caffeine on cell survival after treatment with different chemical or physical agents has been studied in a number of lower and higher eukaryotic cells (Fabre, 1972; Hannan & Nasim, 1977; McCormick *et al.*, 1972; Kihlman *et al.*, 1974). These studies revealed that in most eukaryotic cells caffeine appears to inhibit post-replication recombinational repair rather than other repair systems. Therefore it is possible that in *D. discoideum* the *radE*⁺ gene product is a component of a post-replicative repair pathway.

In addition to being sensitive to u.v. light, *radE* strains were also more sensitive than *radE*⁺ strains to killing by MNNG. On the other hand, the survival curves were comparable for *radE*⁺ and *radE* strains exposed to other monofunctional alkylating agents such as EMS or MMS. MNNG and u.v. light have also been observed to be more effective than EMS at inducing mutations in *D. discoideum* (Liwerant & Pereira Da Silva, 1975; Coukell, unpublished results). In the present study, u.v. light was less effective at inducing *acrA* mutations in a *radE* strain than in *rad*⁺ strains. Together, these observations suggest that some u.v.- and MNNG-induced DNA damage is repaired by a pathway other than the one involved in repairing EMS- and MMS-induced damage and that the former pathway is 'error-prone'. Moreover, the presence of caffeine or a mutation at the *radE* locus would appear to interfere with the normal function of this pathway.

Due to the relatively modest u.v. sensitivity of *radE* strains and the fact that there are other markers in linkage group III (including other *rad* loci), mutations at the *radE* locus are probably not particularly valuable as genetic markers. However, employed with other *rad* mutations to construct double and triple mutants, *radE* mutations might be useful in attempting to elucidate the various DNA repair pathways in *D. discoideum*.

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