Genetics of Radiation Sensitivity in the Slime Mould *Dictyostelium discoideum*

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(Received 15 May 1978)

Mutations leading to increased sensitivity to ^{60}Co gamma rays and/or ultraviolet light in the slime mould *Dictyostelium discoideum* have been assigned to eight loci on the basis of linkage and complementation tests. The linkages of these loci are: *radA*, linkage group I; *radB*, linkage group VI; *radC*, linkage group III; *radD*, linkage group IV; *radF*, linkage group IV; *radG*, linkage group III; *radH*, linkage group II; and *radI*, linkage group III. The *radC* mutants, which were isolated on the basis of increased sensitivity to ultraviolet light, are as resistant as their parental strains to ^{60}Co gamma rays, whereas all other mutants studied are sensitive to both ultraviolet light and ^{60}Co gamma rays. A new temperature-sensitive mutation, *tsgL606*, has been assigned to linkage group I.

INTRODUCTION

The cellular slime mould *Dictyostelium discoideum* is a useful eukaryotic organism for the study of DNA repair processes in vegetative and developing cells (Deering, 1975). Radiation-sensitive mutants, presumably defective in DNA repair, can be isolated with relative ease (Deering *et al.*, 1970) since this micro-organism normally exists as single haploid cells. Available genetic techniques based on the parasexual cycle of *D. discoideum* allow the linkage and complementation of these mutations to be determined (Welker & Deering, 1976). The molecular defects responsible for the radiation-sensitive phenotypes of these mutants can also be investigated (Khouri & Deering, 1973; Deering & Jensen, 1973, 1975; Guialis & Deering, 1976*a, b*). Finally, the developmental cycle of *D. discoideum* offers a unique opportunity to study DNA repair processes in differentiating cells.

A linkage map of genetic markers in *D. discoideum*, including those described for the first time in this report, is presented in Table 1. The linkage of the *radA20* and *radB13* mutations to linkage groups I and VI, respectively, has been described previously (Welker & Deering, 1976). We present here linkage and complementation results obtained for radiation-sensitive mutations which affect six additional loci in *D. discoideum*.

METHODS

Maintenance of stocks. *Dictyostelium discoideum* stocks were carried on agar plates in association with *Escherichia coli* B/r at 23 °C as described previously (Deering *et al.*, 1970). Long-term stocks were stored on silica gel (Perkins, 1962) or frozen as spores.

Strains. Haploid strains used in this work and their genotypes are listed in Table 2. The parental haploids used to construct diploid strains are given in Table 3.

Isolation of radiation-sensitive strains. The isolation of radiation-sensitive strains γ s1 to γ s21 after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) or 254 nm ultraviolet light (u.v.) has been described previously (Deering *et al.*, 1970).

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Table 1. *Linkage groups of Dictyostelium discoideum**

Linkage group	Markers
I	<i>acrB</i> , <i>aggB</i> , <i>cadA</i> , <i>cycA</i> , <i>devA</i> , <i>radA</i> [†] , <i>sprA</i> , <i>tsgE</i> , <i>tsgI</i> , <i>tsgL</i> [†]
II	<i>acrA</i> , <i>aggA</i> , <i>aggF</i> , <i>aggI</i> , <i>axeA</i> , <i>devB</i> , <i>radH</i> [†] , <i>sprB</i> , <i>tsgD</i> , <i>tsgF</i> , <i>tsgH</i> , <i>whiA</i>
III	<i>acrC</i> , <i>axeB</i> , <i>bsgA</i> §, <i>radC</i> [†] , <i>radG</i> [†] , <i>radI</i> [†] , <i>tsgA</i> , <i>tsgC</i>
IV	<i>aggJ</i> , <i>bwnA</i> , <i>ebrA</i> , <i>radD</i> [†] , <i>radF</i> [†] , <i>tsgB</i>
V [‡]	—
VI	<i>manA</i> , <i>radB</i> [†]
VII	<i>cobA</i>

* Adapted from Newell *et al.* (1977*b*); see also Coukell (1977).

[†] Linkage determined in this laboratory (see Results; also Welker & Deering, 1976).

[‡] Recent experiments indicate that the marker *tsgC* which had previously been assigned to linkage group V is instead linked to the markers *tsgA* and *radC* on linkage group III (Ratner & Newell, 1978; K. L. Williams & D. L. Welker, unpublished data).

§ A re-evaluation of the linkage of the *bsgA* locus places this marker on linkage group III (Ratner & Newell, 1978) and not on linkage group V as suggested previously (Newell *et al.*, 1977*b*).

Strains $\Delta\gamma 1$ and $\Delta\gamma 3$ were isolated from strain $\Delta X3$ following mutagenesis with MNNG ($80 \mu\text{g ml}^{-1}$; K & K Laboratories, Plainview, N.Y., U.S.A.) for 30 min at room temperature. Strain $\Delta X3$ had a survival of 10 to 20% following this treatment. Cells surviving the MNNG treatment were plated on agar plates in association with *E. coli* B/r to yield 20 to 60 plaques per plate. These plaques were then replica-plated on to plates prespread with *E. coli* B/r using velveteen as described by Deering *et al.* (1970). The first replica was retained as a source of unirradiated stocks while the master plate was irradiated with 200 krad ^{60}Co gamma rays and a second replica was produced from the irradiated master plate. Those plaques which grew on the first set of replica plates but not on the second were re-screened for sensitivity to gamma rays, cloned if more sensitive than the parental strain, and stored for future use.

Strains TW8 and HPS104 were selected on the basis of sensitivity to 254 nm u.v. from TS12 and NP2, respectively, following mutagenesis with MNNG ($100 \mu\text{g ml}^{-1}$) for 30 min at room temperature. The survival of TS12 and NP2 after this treatment was about 5%. Strain TW8 was isolated by the velveteen replica-plating technique using 50 J m^{-2} u.v. in place of the gamma-ray irradiation. Strain HPS104 was isolated using sterile toothpicks to transfer cells from individual plaques to two replica plates. One replica was retained for stocks and the other was exposed to 50 J m^{-2} u.v. Clones which grew on the unirradiated replicas but not on the u.v.-irradiated plates were re-screened for u.v. sensitivity and cloned for stocks if more sensitive than the parental strains.

Selection of diploids. Haploids to be fused were treated using modifications of the technique of Williams *et al.* (1974a). The haploids were incubated at 10^7 amoebae ml^{-1} in a 1:1 ratio for 17 h at 23°C in either phosphate-buffered saline (PBS) (Deering *et al.*, 1970) or 40 mM-phosphate buffer [containing (per litre): $0.5 \text{ g MgCl}_2 \cdot 6\text{H}_2\text{O}$, $1.5 \text{ g Na}_2\text{HPO}_4$, $4 \text{ g KH}_2\text{PO}_4$, 1.5 g KCl ; pH 6.4] with or without streptomycin sulphate (B grade, Calbiochem; $500 \mu\text{g ml}^{-1}$).

The majority of the diploids used in this work were selected on the basis of temperature resistance (Loomis, 1969), after haploids bearing non-allelic recessive temperature-sensitive mutations were allowed to fuse. Diploids were also selected on the basis of simultaneous radiation and temperature resistance (Welker & Deering, 1976) or on the basis of simultaneous temperature resistance and ability to grow on *Bacillus subtilis* strain 36.1 (Newell *et al.*, 1977a).

Selection of drug-resistant segregants. Methanol-resistant segregants were selected from the heterozygous diploids by plating approximately 10^5 diploid spores on plates containing either 2 or 3% (v/v) methanol (Williams *et al.*, 1974b). Cells from plaques which appeared after 4 to 5 d incubation were transferred to a second set of methanol-containing plates and grown for stocks. Cycloheximide-resistant segregants were selected on plates containing $500 \mu\text{g}$ cycloheximide ml^{-1} (Katz & Sussman, 1972).

Characterization of segregants. The radiation sensitivity of most segregants was screened by spotting cells from the segregant clones on to agar plates and exposing them to u.v. (Welker & Deering, 1976). Usually a fluence of 50 J m^{-2} was used; however, for the *radC35* and *radC44* mutations a fluence of 75 J m^{-2} was used since strains with these mutations are more resistant to u.v. than are other radiation-sensitive strains. The presence of the *radH18* mutation was determined by irradiating spores from segregant clones in PBS with a gamma-ray dose of 200 krad and then plating portions of the spore suspension on plates that had been prespread with bacteria. This was necessary for the segregants of DPS49 and DPS50 because the u.v. screening procedure was not reliable at the fluence required to screen for the *radH18* mutation (100 J m^{-2}).

Cycloheximide and methanol resistance were determined using agar plates containing $500 \mu\text{g}$ cycloheximide ml^{-1} and 2% methanol, respectively. Temperature resistance was determined using plates incubated at the restrictive temperature (27°C). The presence of α -mannosidase-I activity was screened using *p*-nitrophenyl- α -D-mannopyranoside as described by Free *et al.* (1976). Spore colour was ascertained by visual inspection of fruiting bodies. The ability to grow using *B. subtilis* as a food source was determined by incubation on agar plates in association with *B. subtilis* strain 36.1 (Newell *et al.*, 1977a). Diploid segregants, presumably arising as mitotic recombinants, were identified on the basis of their phenotypes and spore sizes using a model B Coulter counter (Sackin & Ashworth, 1969).

Irradiation. A germicidal lamp was used as a source of 254 nm u.v. at incident intensities of 0.3 to $3.5 \text{ J m}^{-2} \text{ s}^{-1}$. A ^{60}Co Gammacell 200 (Atomic Energy of Canada) was used as a source of gamma rays for these experiments; irradiation was at dose rates of 6 to 24 krad min^{-1} .

For u.v. survival curves, vegetative cells were washed from agar plates after growing overnight in association with *E. coli* B/r. The bacteria were removed by repeated differential centrifugation ($150 g$ for 2 min) and the cells were resuspended at 1×10^6 to $2 \times 10^6 \text{ ml}^{-1}$ in PBS as determined using either a haemocytometer or a Coulter counter. The cell suspension (5 ml) in a 90 mm glass Petri dish was rotated at 80 rev. min^{-1} during irradiation. Cells were irradiated with gamma rays in suspension in PBS with aeration as described by Deering *et al.* (1970). Following irradiation, appropriate dilutions were plated with *E. coli* B/r (Freim & Deering, 1970). Survival was determined from the total number of plaques which appeared after 5 to 7 d incubation at 23°C .

Table 2 (*cont.*)

Strain*	Parent	rad	acr	axe	bsg	bwn	cyc	man	tsg	whi	cob
			Radiation-sensitive strains constructed in this work								
HPS1	DPS1	A20	A1	?	+	+	+	A1	D12	A1	+
HPS14	DPS2	B13	A1	?	+	+	A1	+	D12	A1	+
HPS22	DPS3	A22	A1	B1	+	+	+	+	D12	A1	+
HPS42	DPS5	I24	A1	B1	+	+	A1	+	D12	A1	+
HPS50	DPS6	B13	+	A1,B1	+	+	A1	+	A1	+	+
HPS52	HPS1	A20	A1	?	+	+	-600	A1	D12	A1	+
HPS53	DPS7	A20	+	A1,B1	+	+	-600	A1	A1	+	+
HPS64	DPS17	C44	A604	A1,B1	+	+	+	+	A1	+	+
HPS65	DPS21	G5	A1	+	+	+	A1	+	D12	A1	+
HPS66	DPS22	F21	A1	+	+	+	A1	+	D12	A1	+
HPS67	DPS24	F21	A605	A1,B1	+	+	+	+	A1	+	+
HPS68	DPS23	D14	A605	A1,B1	+	+	+	+	A1	+	+
HPS80	DPS25	C44	+	A1,B1	+	+	A1	+	A1	+	+
HPS84	DPS21	G5	A1	+	+	+	A1	+	A1	+	+
HPS87	DPS52	G5	A605	A1	+	A1	A1	+	D12	A1	?
HPS203	HPS104	C44	A604	A1,B1	+	+	+	+	A1	+	+
HPS208	HPS256	H18	A609	+	+	+	+	+	L606	+	+
HPS256	γs18	H18	+	+	+	+	+	+	L606	+	+

* 1, Rothman & Alexander (1975); 2, Williams *et al.* (1974b); 3, Kessin *et al.* (1974); 4, Katz & Sussman (1972); 5, Williams & Newell (1976); 6, Free *et al.* (1976); 7, Newell *et al.* (1977a).

Table 3. *Diploid strains constructed in this work*

Strain	Parental haploids		Strain	Parental haploids	
DPS1	γ s20	XM1	DPS32	HPS66	HPS68
DPS2	γ s13	XM1	DPS36	HPS104	TW8
DPS3	A γ 1	XM1	DPS37	HPS64	TW8
DPS4	γ s14	XM101	DPS38	HPS66	X36
DPS5	A γ 3	XM1	DPS39	x2	XM1
DPS6	HPS14	x2	DPS40	HPS201	TS12
DPS7	HPS52	x2	DPS42	HPS84	XP55
DPS17	HPS203	TS12	DPS46	TW8	x2
DPS20	HPS22	HPS53	DPS47	HPS1	HPS53
DPS21	γ s5	XM1	DPS49	HPS208	XP55
DPS22	γ s21	x9	DPS50	HPS209	HPS256
DPS25	HPS104	XM1	DPS51	HPS42	XP55
DPS26	TW8	HPS204	DPS52	γ s5	HPS204
DPS27	HPS68	HPS256	DPS53	HPS42	HPS87
DPS28	HPS65	HPS256	DPS54	HPS87	TW8
DPS29	HPS66	HPS256	DPS55	HPS42	HPS80

Fig. 1

Fig. 2

Fig. 1. Survival curves following u.v. irradiation for several radiation-resistant haploid strains: ●, NC4; ○, TS12; ■, NP2; □, NP7; ▲, XM1; △, HR7; ◆, HPS204; ◇, XP55.

Fig. 2. Survival curves following u.v. irradiation for diploids constructed from radiation-resistant parental haploids: ●, DPS39 (x2/XM1); ○, DPS40 (HPS201/TS12). The survival curve for NC4 (---) is included for comparison.

RESULTS

Response of radiation-resistant and -sensitive strains to u.v. and gamma-ray irradiation

Responses to u.v. irradiation similar to the response of the wild-type strain NC4 have been observed in a number of haploid strains derived from it. As shown in Fig. 1, NC4 as well as several strains bearing many of the commonly used genetic markers such as acriflavin resistance, cycloheximide resistance, temperature sensitivity and spore colour mutations has a 10% survival dose (D_{10}) of 155 J m⁻². Strain HR7 with a D_{10} of 135 J m⁻² was slightly less resistant than NC4 to u.v. Thus, the genetic markers in these strains, with the possible exception of those in HR7, do not affect resistance to u.v.

Fig. 3. Survival curves following u.v. (a) and gamma-ray (b) irradiation for type 1 radiation-sensitive strains: $\bullet$, $\gamma s13$ (*radB13*); $\circ$, $\gamma s20$ (*radA20*); $\blacksquare$, $\lambda\gamma 1$ (*radA22*); $\square$, $\lambda\gamma 3$ (*radI24*). Survival curves for NC4 (---) are included for comparison.

Fig. 4. Survival curves following u.v. (a) and gamma-ray (b) irradiation for type 2 radiation-sensitive strains: $\bullet$, $\gamma s5$ (*radG5*); $\circ$, $\gamma s14$ (*radD14*); $\blacksquare$, $\gamma s18$ (*radH18*); $\square$, $\gamma s21$ (*radF21*). Survival curves for NC4 (---) and $\gamma s13$ (.....) are included for comparison.

Survival curves following u.v. irradiation of diploids constructed by fusion of two radiation-resistant haploids differed only slightly from those obtained with the wild-type haploid NC4. These diploids had about the same D_{10} as NC4 but were more sensitive than NC4 at fluences below the D_{10} and more resistant than NC4 at fluences above the D_{10} (Fig. 2). These minor differences presumably are a consequence of the diploidy of the cells but their basis is not understood at present. Similar survival curves were obtained using heterozygous diploids constructed by fusion of a radiation-sensitive haploid and a radiation-resistant haploid for both u.v. and gamma-ray irradiation (Welker & Deering, 1976).

The 10 radiation-sensitive mutations used in these studies can be classified into three types on the basis of their radiation-sensitive phenotypes. Mutants of type 1 [$\gamma s13$ (*radB13*), $\gamma s20$ (*radA20*), $\lambda\gamma 1$ (*radA22*) and $\lambda\gamma 3$ (*radI24*)] are, in comparison with the wild-type strain NC4, very sensitive to both u.v. and gamma rays. These strains have no shoulder on either the u.v. or gamma-ray survival curves (Fig. 3; see also Deering *et al.*, 1970). Mutants of type 2 [$\gamma s5$ (*radG5*), $\gamma s14$ (*radD14*), $\gamma s18$ (*radH18*) and $\gamma s21$ (*radF21*)] have u.v. and

Fig. 5. Survival curves following u.v. (a) and gamma-ray (b) irradiation for type 3 radiation-sensitive strains: ●, TW8 (*radC35*); ■, HPS104 (*radC44*); ▲, HPS64 (*radC44*). Gamma-ray survival curves for the parental radiation-resistant strains TS12 (○) and NP2 (□) are included for comparison, as are survival curves for NC4 (---).

gamma-ray sensitivities intermediate between the response of the wild-type strain and more sensitive type 1 strains (Fig. 4). Type 3 mutants [TW8 (*radC35*) and HPS104 (*radC44*)] are sensitive to u.v. but are as resistant as their parental strains to gamma rays (Fig. 5). Both type 2 and type 3 strains have shoulders on their u.v. and gamma-ray survival curves, with apparently exponential declines at higher doses.

The sensitivity of some strains bearing these radiation-sensitive mutations varied somewhat depending on the genetic background in which the mutations were expressed. For example, HPS64, a derived *radC44* strain, had a u.v. sensitivity similar to that of TW8, a *radC35* strain, while the originally isolated *radC44* strain, HPS104, was more sensitive than TW8 (Fig. 5). Though HPS104 might conceivably contain a second radiation-sensitive mutation which could lead to its increased u.v. sensitivity, no evidence for such a mutation was found in the linkage analysis of the *radC44* mutation (Table 4). Analogous relatively small changes in sensitivity have also been observed for strains bearing the *radA20*, *radC35*, *radD14*, *radF21*, *radG5* and *radH18* mutations.

Genetic analyses of radiation-sensitive mutations

The linkage of the *radA20* and *radB13* mutations to linkage groups I and VI, respectively, has been described previously (Welker & Deering, 1976).

The *radA22* mutation of $\Delta\gamma 1$ was assigned to the same locus as the *radA20* mutation on the basis of the results of linkage and complementation tests. The *radA22* mutation was linked to linkage group I on the basis of its segregation relative to the linkage group I marker *cycA1* (Table 4). The *radA22* mutation affects the same locus as the *radA20* mutation since the heterozygous *radA20/radA22* diploid, DPS20, had the same u.v. sensitivity as the *radA20/radA20* diploid, DPS47. These diploids had u.v. sensitivities similar to the sensitivity of the *radA20* haploid strain, γ S20 (Fig. 6). The u.v. sensitivities of DPS20 and DPS47 were not the result of dominant radiation-sensitive mutations since the *radA20/+* and *radA22/+* diploids DPS1 and DPS3 (Fig. 6) were as resistant as diploids DPS39 and DPS40, which were constructed by fusion of pairs of radiation-resistant haploids (Fig. 2). Thus, both the *radA20* and *radA22* mutations were recessive.

The *radC35* and *radC44* mutations of strains TW8 and HPS104, respectively, were also assigned to a single locus. Heterozygous *radC35/radC44* diploids, DPS36 and DPS37, had

Table 4. Linkage analysis of methanol-resistant haploid segregants of diploids heterozygous for radiation sensitivity

Parental diploid	Radiation sensitive mutation	Radiation resistance or sensitivity	Linkage group					
			I Cyc, Cyc ⁺	II Acr, Acr ⁺	I, III Tsg, Tsg ⁺	III Esg, Esg ⁺	IV Ewn, Ewn ⁺	VI Man, Man ⁺
Dps3	<i>radA22</i>	Resistant Sensitive	40 0 0 77	40* 0 77* 0				21 19 47 30
Dps17	<i>radC44</i>	Resistant Sensitive	57 47 47 48	104* 0 95* 0	0 104 95 0			
Dps26	<i>radC35</i>	Resistant Sensitive		66* 0 63* 0	66 0 0 63		31 35 30 33	
Dps4	<i>radD14</i>	Resistant Sensitive	22 64 18 48	86* 0 66* 0			86 0 0 66	33 53 26 40
Dps38	<i>radF21</i>	Resistant Sensitive		35* 0 41* 0			35 0 0 41	
Dps42	<i>radG5</i>	Resistant Sensitive		56* 0 48* 0		56 0 0 48		
Dps49	<i>radH18</i>	Resistant Sensitive	0 0 30 33	0 0 63* 0	0 0 33 30	0 0 50 13		
Dps50	<i>radH18</i>	Resistant Sensitive	38 33 0 0	71* 0 0 0	33 38 0 0	43 28 0 0		
Dps51	<i>radI24</i>	Resistant Sensitive		35* 0 21* 0		35 0 0 21		

* *acrA* selected by methanol resistance.

Fig. 6. Survival curves following u.v. irradiation for *radA* diploids: ●, DPS1 (*radA20/+*); ■, DPS3 (*radA22/+*); ▲, DPS20 (*radA20/radA22*); ◆, DPS47 (*radA20/radA20*). The survival curve for $\gamma s20$ (*radA20*) (---) is included for comparison.

u.v. sensitivities similar to the sensitivity of the *radC35* haploid $\tau w8$. The *radC35* and *radC44* mutations were recessive since the *radC35/+* and *radC44/+* diploids, DPS46 and DPS17, had u.v. sensitivities similar to those of diploids constructed from pairs of radiation-resistant haploids. In addition, both the *radC35* and *radC44* mutations were linked to linkage group III on the basis of their segregation relative to the temperature-sensitive mutation *tsgA1* which had previously been assigned to linkage group III (Kessin *et al.*, 1974). With the screening procedure used, it was not possible to distinguish between temperature sensitivities resulting from different temperature-sensitive mutations; thus diploids were constructed in such a way that the *tsgA1* mutation was the only temperature-sensitive mutation transmissible to the segregant haploids. These diploids were constructed by fusion of a *tsgD12* haploid with an *acrA*, *tsgA1* haploid. Since the *acrA* and *tsgD12* markers are both on linkage group II, but in these diploids physically on separate chromosomes, the *tsgD12* marker was preferentially excluded from the methanol-resistant (*acrA*) segregants of these diploids, thus allowing the *tsgA1* mutation to serve as a marker for linkage group III. The linkage data obtained from segregants of DPS17 (*radC44/+*) and DPS26 (*radC35/+*) are presented in Table 4.

The *radD14* and *radF21* mutations were both linked to linkage group IV on the basis of their segregation relative to the spore colour marker, *bwnA1*, which was previously assigned to linkage group IV (Katz & Sussman, 1972; Williams *et al.*, 1974b). All radiation-sensitive haploid segregants of diploids constructed from radiation-sensitive, not brown, and radiation-resistant, brown parental haploids were not brown, while all radiation-resistant haploid segregants were brown. The linkage data obtained from methanol-resistant segregants of DPS4 (*radD14/+*) and DPS38 (*radF21/+*) are presented in Table 4. Since the radiation-sensitive phenotypes which result from the *radD14* and *radF21* mutations are identical and both mutations are on linkage group IV, the complementation of these mutations was tested. The *radD14/radF21* diploid, DPS32, was as resistant to u.v. as the diploids constructed from pairs of radiation-resistant haploids. Thus the *radD14* and *radF21* mutations affect different loci on linkage group IV.

The *radG5* and *radI24* mutations were assigned to linkage group III on the basis of their segregation relative to the marker *bsgA5*. Recent experiments indicate that the *bsgA5*

and *tsgC7* markers are linked to the markers *tsgA1* and *radC44* (Ratner & Newell, 1978; K. L. Williams & D. L. Welker, unpublished data) rather than defining a fifth linkage group. All radiation-sensitive segregants of diploids constructed from a *radG5* or *radI24* haploid able to grow using *B. subtilis* as a food source and a radiation-resistant haploid unable to grow on *B. subtilis* were able to grow in association with this bacterium while the radiation-resistant haploid segregants were not. The linkage data obtained with segregants of DPS42 (*radG5*/+) and DPS51 (*radI24*/+) are presented in Table 4. The *radG*, *radI* and *radC* mutations affect different loci on linkage group III since the diploids DPS53 (*radG5*/*radI24*), DPS54 (*radG5*/*radC35*) and DPS55 (*radI24*/*radC44*) are as resistant to u.v. as diploids constructed from pairs of radiation-resistant haploids.

The *radH18* mutation was assigned to linkage group II on the basis of its segregation relative to mutations at the *acrA* locus which was previously assigned to linkage group II (Williams *et al.*, 1974*b*). All methanol-resistant haploid segregants of DPS49 were radiation-sensitive as expected if the *radH18* and *acrA609* mutations of HPS208 were physically linked (Table 4). Conversely, all the methanol-resistant haploid segregants of DPS50 were radiation-resistant, as expected if the *radH18* and *acrA610* mutations were on the same linkage group but brought into the diploid from the parental haploids HPS256 and HPS209, respectively.

The *tsgL606* mutation of HPS256 (and HPS208) was assigned to linkage group I on the basis of its segregation relative to the marker *cycA1*. The haploid segregants of DPS49 and DPS50 were either temperature-sensitive, cycloheximide-sensitive or temperature-resistant, cycloheximide-resistant as expected if *tsgL606* were on linkage group I. The *tsgL606* mutation also segregated independently of the *bsgA5* mutation, as expected if the *tsgL* and *bsgA* loci are on separate linkage groups. Of the 38 temperature-resistant segregants of DPS50, 18 grew on *B. subtilis* and 20 did not, while 10 of the 33 temperature-sensitive segregants of DPS50 grew on *B. subtilis* and 23 did not.

DISCUSSION

In addition to the radiation-sensitive mutations characterized in this work, Dr M. B. Coukell has studied an independently isolated mutation, *radE*, found in strain m28 (Katz & Sussman, 1972). This mutation is not allelic with any of the other radiation-sensitive mutations described here (M. B. Coukell, personal communication; D. L. Welker & R. A. Deering, unpublished results). Therefore, the radiation-sensitive mutations so far studied in *D. discoideum* identify nine complementation groups involved with DNA repair in this organism.

All radiation-sensitive mutations that have been genetically characterized are recessive, as are most other characterized mutations in *D. discoideum*. The recessive nature of these mutations is a requirement for the selection of heterozygous diploids on the basis of simultaneous radiation and temperature resistance since the diploid must be more resistant than the parental radiation-sensitive strain (Welker & Deering, 1976). The recessive nature of the radiation-sensitive mutations studied suggests that a gene product is lacking or decreased in these mutants, rather than the possible alternative that the sensitivity reflects an excess of a gene product. The latter might be expected to be dominant.

The u.v.-sensitive, gamma-ray-sensitive type 1 and type 2 mutants have comparable sensitivities to u.v. and gamma rays. The ratio of the D_{10} of the radiation-sensitive mutants to the D_{10} of their parental radiation-resistant strains for both u.v. and gamma-ray irradiation are given in Table 5. The correspondence between the D_{10} ratios for u.v. and gamma rays for the type 1 and type 2 mutants implies that the repair pathways affected by these mutations are capable of functioning in the repair of either u.v.- or gamma-ray-induced DNA damage to similar extents. On the other hand, the ratios for the type 3, *radC* mutants are not similar for u.v. and gamma-ray irradiation since the *radC* mutations preferentially

Table 5. *Ratio of the 10% survival dose of radiation-sensitive mutants to the 10% survival dose of their radiation-resistant parental strains for u.v. and gamma-ray irradiation*

Strain	Mutation	Linkage group	Ultraviolet light		⁶⁰ Co gamma rays	
			<i>D</i> ₁₀ (J m ⁻²)	Ratio*	<i>D</i> ₁₀ (krad)	Ratio*
Type 1						
γs20	<i>radA20</i>	I	6.5	0.04	9.3	0.03
Δγ1	<i>radA22</i>	I	6.5	0.04	9.3	0.03
γs13	<i>radB13</i>	VI	1.5	0.01	4.3	0.01
Δγ3	<i>radI24</i>	III	5.3	0.03	5.7	0.02
Type 2						
γs14	<i>radD14</i>	IV	17.5	0.11	40.0	0.13
γs21	<i>radF21</i>	IV	17.5	0.11	40.0	0.13
γs5	<i>radG5</i>	III	25.0	0.16	50.0	0.17
γs18	<i>radH18</i>	II	49.0	0.32	70.0	0.23
Type 3						
tw8	<i>radC35</i>	III	42.7	0.28	280.0	1.00
hps104	<i>radC44</i>	III	27.0	0.17	280.0	1.00

* *D*₁₀ mutant strain/*D*₁₀ resistant parental strain. The parental 10% survival doses were 155 J m⁻² for u.v. irradiation and either 280 krad (for type 3 mutants) or 300 krad (for type 1 and type 2 mutants) for gamma-ray irradiation.

affect the repair of u.v.-induced DNA damage. Results of alkaline sucrose gradient analyses of the DNA from u.v.-irradiated cells suggest that the *radC* mutations affect the incision step of an excision repair pathway in *D. discoideum* (Welker & Deering, 1978*a*).

Typical patterns of segregation were obtained in the linkage analyses discussed here. In some cases the ratios of segregant classes were not 1:1. Presumably these ratios varied due to clonal growth effects or marker compatibility effects (Newell *et al.*, 1977*b*). The best segregation patterns were obtained with freshly cloned diploid populations. In most cases some diploids were also obtained in the procedure for selecting haploid segregants. In these, a recombinational event or new mutation presumably occurred on the linkage group bearing the drug resistance marker used in the selection. Such segregants were identified on the basis of their phenotypes and their diploidy was confirmed by measurements of spore size using a Coulter counter.

For some mutations the selection of diploids on the basis of simultaneous radiation and temperature resistance has been successfully used to obtain diploids between temperature-sensitive strains bearing known genetic markers and unmarked radiation-sensitive, temperature-resistant strains (Welker & Deering, 1976; this work). However, the use of u.v. or gamma rays in the selection of diploids could have potentially deleterious effects, such as the production of translocations or new mutations. In particular, one clone of *DPS4* obtained using this procedure gave patterns of segregation which suggested that a translocation or new mutation leading to chromosome incompatibility had occurred involving linkage groups IV and VI. A second clone of *DPS4* gave the expected pattern of segregation for markers on these linkage groups (Table 4). While it cannot be shown that a translocation occurred as a result of the isolation procedure, caution should be exercised in the use of this technique.

It is now possible to construct radiation-sensitive strains to meet the needs of the experimenter. In particular, axenic strains bearing a number of the radiation-sensitive mutations have been constructed. Double mutant haploids have also been constructed in order to determine whether the radiation-sensitive mutations affect the same or different repair pathways (Welker & Deering, 1978*b*).

We wish to thank M. B. Coukell, P. C. Newell, D. I. Ratner and K. L. Williams for communicating their results prior to publication. We thank M. Sheely and S. Howell for their technical assistance. This investigation was supported by Public Health Services Research Grant GM-16620 from the National Institute of General Medical Sciences.

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