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Characterization of a New Radiation-Sensitive Mutant, *Escherichia coli* K-12 *radC102*

ISRAEL FELZENSZWALB,[1] NEIL J. SARGENTINI, AND KENDRIC C. SMITH[2]

Department of Radiology, Stanford University School of Medicine, Stanford, California 94305

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A new radiation-sensitive mutant, *radC*, has been isolated. The *radC* gene is located at 81.0 min on the *Escherichia coli* K-12 linkage map. The *radC* mutation sensitized cells to uv radiation, but unlike most DNA repair mutations, sensitization to X rays was observed only for rich medium-grown cells. For cells grown in rich medium, the *radC* mutant was normal for γ-radiation mutagenesis, but showed less uv-radiation mutagenesis than the wild-type strain; it showed normal amounts of X- and uv-radiation-induced DNA degradation, and it was ~60% deficient in recombination ability. The *radC* strain was normal for host cell reactivation of γ- and uv-irradiated bacteriophage λ; the *radC* mutation did not sensitize a *recA* strain, but did sensitize a *radA* and a *polA* strain to X and uv radiation and a *uvrA* strain to uv radiation. Therefore, we suggest that the *radC* gene product plays a role in the growth medium-dependent, *recA* gene-dependent repair of DNA single-strand breaks after X irradiation, and in postreplication repair after uv irradiation.

INTRODUCTION

The rapid accumulation of knowledge concerning DNA repair has come from studying DNA repair-deficient mutants of *Escherichia coli* (*1, 2*). Our laboratory has embarked on a program to isolate new radiation-sensitive mutants of *E. coli* in the hope of discovering new pathways of DNA repair and of better characterizing known pathways. After *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) mutagenesis of an *E. coli* K-12 strain, a number of X-ray-sensitive mutants were isolated (*3*). These mutants have been designated *rad* (for *rad*iation resistance). Two of the *rad* mutations have been characterized; *radA*, mapping at 99.6 min (*4*), and *radB*, mapping at 56.5 min (*3*). The present study characterizes a third mutant strain, *radC*, whose radiation-sensitizing mutation maps at 81.0 min.

MATERIALS AND METHODS

Bacterial strains. The strains of *E. coli* used are listed in Table I. Transductions and conjugations were accomplished using the procedures of Miller (*8*).

Media. SMM was 0.4% glucose–salts medium (*9*), supplemented with required L-amino acids at 1 m*M* and thiamine · HCl at 0.5 μg/ml. SMM was solidified with Difco Noble agar (*10*) at 1.6%. YENB was yeast

[1] On leave from the Instituto de Biologia, Universidade do Estado do Rio de Janeiro, 20551 Rio de Janeiro, Brazil.

[2] To whom correspondence should be addressed.

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TABLE 1

Strains of *E. coli* K-12 Used[a]

Strain number	*Relevant genotype*	*Other genotype*	*Source or derivation*
SR47	*recA56*	Hfr PO45 *ilv-318 thr-300 thi-1 rel-1 rpsE* λ^-	JC5088, J. Foulds
SR114	*uvrA6*	F^- *argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44* λ^-	AB1886, S. Linn
SR248	+	F^- *leuB19 metE70 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151* λ^-	KH21, R. B. Helling
SR380	+	HfrR4 PO10 *argF58 argI61 serB28 thr-25 purA54 tonA49*	PC0950, ECGSC
SR420	+	HfrKL16 PO45 *thi-1 rel-1* λ^-	KL16, A. J. Clark
SR749	+	Same as SR114	AB1157, ECGSC
SR750	*xthA14*	Same as SR114 but *metE70 rha-6*	NH5016, ECGSC
SR776	*radA100*	Same as SR114 but Thr^+	Ref. (5)
SR788	*polA5*	F^- *lacZ53 thyA36 deo(C2?) rpsL151* λ^-	CM4050, ECGSC
SR841	+	Same as SR420 but *gyrA upp pncA*	SR420, spont. Nal^r, Azu^r, Anc^r
SR859	+	HfrH PO1 *glyA6 thi-1 relA1* λ^-	AT2457, ECGSC
SR865	+	F′ *lac*$^+$*/lacY thi?*	JC2625, A. J. Clark
SR885	+	Same as SR114 but Mtl^+ Xyl^+ *tnaA300*::Tn*10*(Tc^r) *sup*$^+$ λ^s, $S13^s$	JC12337, A. J. Clark
SR941	*xthA14 radC102*	Same as SR114 but *metE70 rha-6*	SR750, MNNG
SR960	+	F^- *ilvA700*::Tn5(Kn^r) *thyA deo* λ^r	CBK007, K. J. Shaw
SR1119	+	F^- *deoC araD139 (lac)U169 malE7*::Tn5(Kn^r) *f16B relA rpsL*	T5M7, T. Silhavy
SR1167	+	F^- *argH1 cysE52 hisG1 leuB6 thr-1 trp-1 ara-13 gal-6 lacY1 malA1 mtl-2 xyl-7 thi-1 tonA2 rpsL9* λ^r λ^-	JM70 ECGSC
SR1168	+	F? *gltC10 metB pyrE zib-205*::Tn*10*(Tc^r) *lac thi rpsL* λ^-	BW229 ECGSC
SR1179	+	Same as SR1167 but *gltC10 pyrE zib-205*::Tn*10*(Tc^r)	SR1167 × Plvira·SR1168, select Tc^r
SR1185	+	Same as SR114 but *gltC10 pyrE zib-205*::Tn*10*(Tc^r)	SR749 + Plvira·SR1168, select Tc^r
SR1186	+	Same as SR114	SR1185 + Plvira·SR941, select Pyr^+
SR1187	*radC102*	Same as SR114	Same as SR114

SR1216	+	Same as SR114 but *ilvA700*::Tn*5*(Kn^r)	SR1186 × P1::Tn*9cts*·SR960, select Kn^r
SR1217	*radC102*	Same as SR1216	SR1187 × P1::Tn*9cts*·SR960, select Kn^r
SR1218	+	Same as SR114 but *metE70*	SR1216 × P1::Tn*9cts*·SR248, select Ilv$^+$
SR1219	*radC102*	Same as SR1218	SR1217 × P1::Tn*9cts*·SR248, select Ilv$^+$
SR1221	*polA5*	Same as SR114	SR1218 × P1::Tn*9cts*·SR788, select Met$^+$
SR1225	*radC102 polA5*	Same as SR114	SR1219 × P1::Tn*9cts*·SR788, select Met$^+$
SR1228	+	Same as SR114 but *malE7*::Tn*5*(Kn^r)	SR1186 × Plvir·SR1119, select Kn^r
SR1229	*radC102*	Same as SR1228	SR1187 × Plvir·SR1119, select Kn^r
SR1231	*uvrA6*	Same as SR114	SR1228 × P1::Tn*9cts*·SR114, select Mal$^+$
SR1235	*radC102 uvrA6*	Same as SR114	SR1229 × P1::Tn*9cts*·SR114, select Mal$^+$
SR1236	+	Same as SR114 but *thyA*	SR1186, select Tmp^r
SR1237	*radC102*	Same as SR114 but *thyA*	SR1187, select Tmp^r
SR1240	*recA56*	Same as SR114	SR1236 × SR47, select Thy$^+$
SR1241	*radC102 recA56*	Same as SR114	SR1237 × SR47, select Thy$^+$
SR1269	+	Same as SR114 but Thr$^+$	SR1186 × P1::Tn*9cts*·SR776, select Thr$^+$
SR1270	*radA100*	Same as SR1269	Same as SR1269
SR1271	*radC102*	Same as SR1269	SR1187 × P1::Tn*9cts*·SR776, select Thr$^+$
SR1272	*radC102 radA100*	Same as SR1269	Same as SR1271

[a] Genotype nomenclature is that used by Bachmann and Low (*6*). Nal^r, Azu^r, Anc^r, Kn^r, Tc^r, and Tmp^r indicate that isolates were resistant to nalidixic acid, 6-azauracil, 6-aminonicotinamide, kanamycin, tetracycline, and trimethoprim, respectively. Pyr$^+$, Ilv$^+$, Met$^+$, Thy$^+$, and Thr$^+$ indicate that isolates no longer required uracil, isoleucine–valine, methionine, thymine, and threonine, respectively, for growth. Mal$^+$ indicates that the isolates could use maltose (at 0.2%) in place of glucose. MNNG indicates mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Tmp^r mutants, i.e., *thyA* mutants, were selected by the method of Stacey and Simson (*7*). ECGSC indicates that the strain was received from the *E. coli* Genetic Stock Center at Yale University. All strains were shown not to be P1 lysogens by the method described elsewhere (*3*).

extract (Difco) at 0.75% and nutrient broth (Difco) at 0.8%. YENB agar was yeast extract at 0.75% and nutrient agar (Difco) at 2.3%. YENBG and phosphate buffer (PB) have been described (*5*). ΔYENB plates were SMM plates without histidine (His-0) but containing YENB at 1.5% (v/v). The media used to determine recombination ability were SMM agar deficient in the appropriate amino acid or containing lactose or L-arabinose in place of glucose; all contained streptomycin sulfate at 200 μg/ml.

Preparation of cells. Logarithmic-phase cells were prepared by diluting stationary-phase cells 1:50 (for SMM-grown cells) or 1:100 (for YENB-grown cells) into homologous medium and shaking at 37°C. Cells were harvested by membrane filtration (Millipore HAWP, 0.45-μm pore size) at an optical density at 650 nm (OD_{650}) of 0.4 (Zeiss PMQ II spectrophotometer), which was reached after three or more cell population doublings. These cells were washed and resuspended in PB at an OD_{650} of 0.1, i.e., 2×10^7 colony-forming units (CFU)/ml for YENB-grown cells, or 1×10^8 CFU/ml for SMM-grown cells.

Irradiation and survival determination. Methods for γ (^{137}Cs) (*3*), X (50 kVp) (*5*), and uv (primarily at 254 nm) (*11*) irradiations were as previously described. Cells were aerated during the irradiations unless otherwise noted. CFU/ml were determined by diluting cells appropriately in PB, plating them in duplicate on YENB or SMM, and incubating them for 24–48 hr. Then the colonies were counted and the cell survival was calculated.

Radiation mutagenesis. Cells were prepared as for the survival studies, except that the cells were harvested by centrifugation at 5000*g* for 6 min, washed twice, and resuspended in PB at an OD_{650} of 10 for γ irradiation and an OD_{650} of 0.1 for uv irradiation. Gamma-irradiated cells (0.2 ml) were spread (in triplicate) without dilution on His-0 or ΔYENB plates to assay for His^+ mutants, or with dilution on ΔYENB plates to assay for survivors. Ultraviolet-irradiated cells were concentrated 100-fold by centrifugation and plated as above. The survival data for uv-irradiated cells were determined using nonconcentrated cells. Plates were incubated at 37°C for 2 (survivors) or 3 (mutants) days. The radiation-induced mutant frequency was calculated as described previously (*11*).

DNA degradation assay. DNA was labeled by growing cells at 37°C for at least four generations in YENB containing [*methyl*-^{3}H]thymidine at 10 μCi/ml (Amersham, 47.5 Ci/mmol). Cells were then filter-harvested, washed, resuspended in nonradioactive YENB at 37°C, and incubated for at least one generation time. Cells were then filter-harvested, washed, and resuspended in PB at an OD_{650} of 1.0 for X irradiation and at an OD_{650} of 0.1 for uv irradiation. After X irradiation, cells were diluted 1:20 into nonradioactive YENB. After uv irradiation, cells were concentrated 10-fold by centrifugation and then diluted 1:20 into nonradioactive YENB. After various incubation times at 37°C, the amount of undegraded DNA was determined as before (*3*).

Other techniques. The methods used for mutagenesis with MNNG and for the determination of host cell reactivation and recombination ability are described elsewhere (*3*).

RESULTS

Strain SR750 (*xthA*) was mutagenized with MNNG (*3*), and a radiation-sensitive (*rad*) derivative was isolated (SR941). To locate the mutation(s) causing the radiation sensitivity of strain SR941, it was mated with an Hfr strain, SR380, to produce a "gradient of transmission" (*8*). An analysis of recombinants selected for arginine prototrophy and screened for methionine, mannitol, and xylose markers and for γ-radiation sensitivity indicated that the location of a radiation-sensitizing mutation in strain SR941 was at about 82 min on the *E. coli* K-12 linkage map (data not shown).

Further mapping was performed by transductional crosses using a bacteriophage P1 lysate of strain SR941 as the DNA donor and using genetic markers in the vicinity of the approximate map location. These experiments yielded the result that a radiation-sensitizing mutation was not located close to the *metE, ilvA,* and *xyl* loci (data not shown). In a reciprocal cross using strain SR941 as the recipient, the γ-radiation resistance marker was found to be linked 2% of the time with the *tnaA* locus [SR941 × P1 · SR885 (*tnaA*::Tn*10*)]. Based on these data, strain SR1179 (*cysE pyrE*) was transduced with P1vira propagated on strain SR941. The cotransduction data obtained

TABLE II

Calculation of the *radC* Gene Chromosomal Map Position from Cotransduction Frequency Data

Selected phenotype in the transduction[a]	*Cotransduction frequency (%) of* radC *gene with selected marker*[b]	*Calculated distance between* radC *gene and selected marker (min)*[c]	*Map position of selected marker (min)*[d]	*Calculated position of the* radC *gene (min)*[e]
$PyrE^+$ (79)	81	0.1	81.3	81.2
$CysE^+$ (60)	65	0.3	80.5	80.8
Tc^r (51)	2	1.5	82.7	81.2

[a] Strain SR941 (*radC*) was transduced with Plvira·SR885(*tnaA*::Tn*10*) selecting for tetracycline resistance (Tc^r). Strain SR1179 (*pyrE cysE*) was transduced with Plvira·SR941(*radC*), selecting for uracil or cysteine prototrophy ($PyrE^+$ or $CysE^+$, respectively). The bracketed values are the numbers of transductants used.

[b] Transductants were picked and isolated on the same type of medium on which they were selected. The Rad phenotype was determined by growing isolated transductants overnight in YENBG, diluting 10^{-2} in PB, γ-irradiating them with 40 krad, and plating diluted cells on YENB. The cotransduction frequency was calculated by dividing the number of Rad^- transductants by the bracketed value in the first column, and multiplying by 100.

[c] The distances listed were calculated according to Wu (*12*) as equal to $L(1 - F^{1/3})$ where L is the length of DNA carried by the transducing bacteriophage, i.e., 2.0 min (*13*) and F is the frequency of cotransduction divided by 100.

[d] Map positions from Bachmann and Low (*6*).

[e] Map positions were calculated by adding or subtracting (as appropriate) the distance between the *radC* locus and the selected marker, to or from (as appropriate) the selected marker map location.

with the markers (*pyrE* and *cysE*) suggest that the map position of a mutation (called *radC102*) is at 81.0 min on the linkage map (Table II). Figure 1 shows the map position of *radC*.

The *radC* mutation was transduced into the $xthA^+$ strain, SR1185, to produce strains SR1186 (wild type) and SR1187 (*radC*). Relative to the wild-type strain, the *radC* mutant was sensitive to X rays (Fig. 2a) and to uv radiation (Fig. 2b) when grown in and plated on YENB, but was not sensitive to X rays (Fig. 2a), and showed less sensitization to uv radiation (Fig. 2b) if grown in and plated on minimal medium. These data indicate that the *radC* mutation caused a deficiency in a phenomenon called "medium-dependent resistance" (MDR) (*5*) to X radiation, and a minor effect on MDR to uv radiation (see Discussion). We also tested the effect of the *radC* mutation on another medium effect for uv-irradiated cells, "minimal medium recovery" (MMR) (*14*) (see Discussion). The *radC* mutation elicited the phenomenon

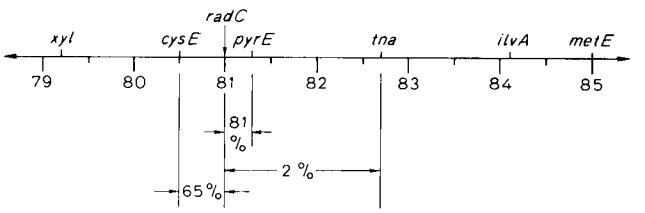

FIG. 1. Order of genetic loci in the region from *xyl* to *metE* on the *E. coli* K-12 chromosome. The frequency of cotransduction between *radC* and other markers is shown (taken from Table II). The position of each of the genes (in terms of minutes) except *radC* (which was calculated to be at 81.0 min) is from Bachmann and Low (*6*).

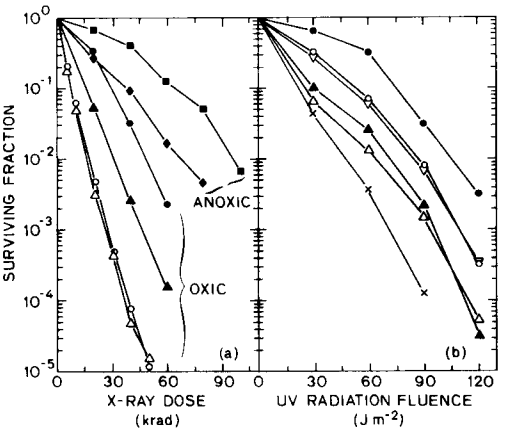

FIG. 2. Radiation survival of wild-type and *radC* cotransductant strains of *E. coli*. Cells grown to logarithmic phase in YENB or SMM were treated with X (a) or uv (b) radiation before plating on YENB or SMM, respectively. X irradiation was either oxic (air) or anoxic (N_2). Symbols are: wild type (SR1186) YENB (●, ■), SMM (○); *radC* (SR1187) YENB (▲, ◆), SMM (△). For the MMR experiments (b), SMM-grown wild-type (SR1186) cells (▽) and *radC* (SR1187) cells (×) were plated on YENB. To visualize MMR one should compare curves ○ and ▽ for the wild-type strain, and curves △ and × for the *radC* strain. Points are the means of data from two experiments.

of MMR, i.e., the uv-irradiated minimal medium-grown cells showed higher survival on minimal medium plates than on YENB plates. These data are summarized in Table III.

To determine in which repair systems the *radC* gene might function, mutations affecting known DNA repair processes were added to the *radC* strain. The *radC* mutation sensitized the *polA* (Fig. 3b) and *radA* (Fig. 3c) strains, but not the *recA* strain (Fig. 3a), to X rays. Data for minimal medium-grown cells (Fig. 3d) will be discussed below. Also the *radC* mutation sensitized the *polA* (Fig. 4b), *radA* (Fig. 4d), and *uvrA* (Fig. 4c) strains, but not the *recA* (Fig. 4a) strain, to uv radiation.

Since repair mechanisms can exhibit both error-free and error-prone DNA repair modes (*15, 16*), the *radC* mutant was tested for γ- and uv-radiation mutagenesis. The *radC* mutant was normal for γ-radiation mutagenesis whether irradiated under oxic (Table IV) or anoxic conditions (data not shown), but showed less uv-radiation mutagenesis than the wild-type strain (Table IV).

Radiation-sensitive mutants can exhibit a deficiency in recombination ability (*17*) and show abnormal radiation-induced DNA degradation (*18, 19*). The *radC* mutant was found to be ~60% deficient in recombination ability (Table V), but it exhibited normal levels of X- and uv-radiation-induced DNA degradation (Fig. 5).

DISCUSSION

The *xthA* background (strain SR750) was used in our search for new radiation-sensitive mutants, as discussed elsewhere (*3*). This approach aided the isolation of the *radC* mutation, because it sensitized the *xthA* strain 2.6-fold to X rays while it sensitized the wild-type strain by only 1.6-fold (sensitization factors are the D_1 ratios,

TABLE III

Dose Yielding 1% Survival (D_1) from X or uv Radiation Survival Curves for Cells Grown in or Plated on Rich (R) or Minimal (M) Medium

Strain	*X radiation*			
	R → R (krad)	*M → M (krad)*	*MDR*	*%MDR*
SR1186 (+)	49	18	2.72	100
SR1187 (*radC*)	31	16	1.94	54
SR1269 (+)	46	14	3.28	100
SR1270 (*radA*)	35	12	2.91	83
SR1271 (*radC*)	30	14	2.14	50
SR1272 (*radC radA*)	18	10	1.80	35

	uv radiation					
	R → R ($J\ m^{-2}$)	*M → M ($J\ m^{-2}$)*	*M → R ($J\ m^{-2}$)*	*MDR*	*%MDR*	*MMR*
SR1186 (+)	105	87	85	1.20	100	1.02
SR1187 (*radC*)	71	63	48	1.12	65[a]	1.31

Note. Data sources: Figs. 2a, 2b, 3c, and 3d. Nomenclature: For example, M → R means cells grown in minimal medium and plated on rich medium. Calculations: MDR (discussed in the text) is the R → R value divided by the M → M value. %MDR is the (MDR − 1) value for the mutant strain divided by the (MDR − 1) value for the appropriate wild-type strain (listed directly above the mutant); then multiply by 100. MMR (discussed in the text) is the M → M value divided by the M → R value; a value greater than 1.0 indicates MMR.

[a] This value is difficult to interpret because the strain also shows MMR.

i.e., "wild-type"/*radC* mutant, where D_1 is the dose killing 99% of the cells). One explanation of these data is that the *radC* gene product is an exonuclease that overlaps the function of the *xthA* gene product (exonuclease III). However, since *Exo*III is thought to be involved in *polA*-dependent excision repair of ionizing radiation-induced DNA damage (*20, 21*), it is possible that any gene involved in *recA*-dependent repair (i.e., *polA*-independent repair) will show a greater involvement in X-ray survival for *xthA* cells.

X-ray D_1 values (from Fig. 2a, YENB-grown and plated cells) were used to calculate the oxygen enhancement ratio (OER), i.e., D_1 (anoxic irradiation)/D_1 (oxic irradiation). The OER was 2.0 (96 krad/49 krad) for the wild-type strain and 2.2 (68 krad/31 krad) for the *radC* strain. We conclude that the *radC* mutation sensitizes cells slightly more to lesions induced by X rays in the presence of oxygen than in its absence.

Wild-type *E. coli* K-12 cells grown to logarithmic phase in YENB are more resistant to X rays and uv radiation than are cells grown in SMM. This enhanced survival capability is called MDR and is a *recA lexA*-dependent DNA repair phenomenon (*5*). A *radA* mutation blocks MDR partially after X irradiation and completely after uv irradiation (*3*). Diver *et al.* (*4*) suggested that there are at least two pathways of MDR for X-ray survival, but only the *radA*-dependent pathway is also involved in uv radiation survival.

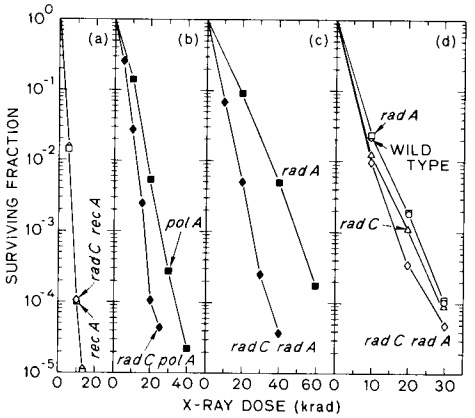

FIG. 3. X-radiation survival of *E. coli* strains. The cells were grown to logarithmic phase in and plated on YENB. Symbols are: (a) *recA* (SR1240) (□), *radC recA* (SR1241) (◇); (b) *polA* (SR1221) (■), *radC polA* (SR1225) (◆); (c) *radA* (SR1270) (■), *radC radA* (SR1272) (◆); (d) the cells were grown to logarithmic phase in and plated on SMM. Symbols are: wild type (SR1269) (○), *radA* (SR1270) (□), *radC* (SR1271) (△), *radC radA* (SR1272) (◇). Points are the means of data from two experiments.

After uv irradiation, *uvr* mutants exhibit a higher survival when grown on minimal rather than on rich medium plates (*14*). This process, called minimal medium recovery (MMR) (*14*), has also been observed for *uvrA* (*9*), *lon* (*22*), *ruvB* (*23*), *rer* (*24*), prestarved *recA*, and *recC* (*9*) and *ssb* (T. V. Wang and K. C. Smith, *Photochem. Photobiol.*, in press) mutants, but not for wild-type strains (*9*).

We have used D_1 values to calculate the role of the *radC* mutation in MDR, as well as in MMR (Table III). This analysis suggests several points: (1) *radC* is on a different MDR pathway than *radA*, because the *radA radC* combination blocks more X-ray MDR than either single mutation. (2) There are likely to be more than two

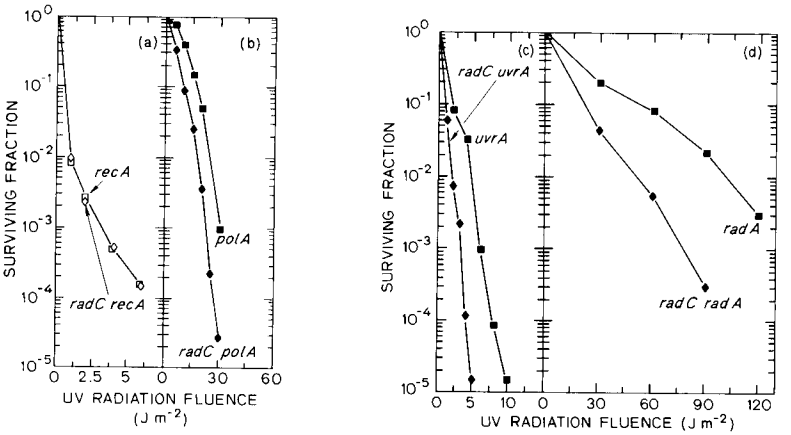

FIG. 4. Ultraviolet radiation survival of *E. coli* strains. Cells were grown to logarithmic phase in and plated on YENB. Symbols are: (a) *recA* (SR1240) (□), *radC recA* (SR1241) (◇); (b) *polA* (SR1221) (■), *radC polA* (SR1225) (◆); (c) *uvrA* (SR1231) (■), *radC uvrA* (SR1235) (◆); (d) *radA* (SR1270) (■), *radC radA* (SR1272) (◆). Points are the means of data from two experiments.

TABLE IV

Gamma and uv Radiation Mutagenesis (*his-4* → His^+) in Wild-Type (SR1186) and *radC* (SR1187) Strains[a]

		Surviving fraction		*Mutants per 10^8 survivors*	
Mutagen	*Dose*	*SR1186*	*SR1187*	*SR1186*	*SR1187*
γ radiation (oxic)	2 krad	0.90	0.80	1.2	1.5
	4	0.80	0.55	1.8	3.4
	6	0.72	0.40	4.4	4.3
	8	0.68	0.30	6.4	7.8
	10	0.60	0.24	9.9	9.9
uv radiation	5 J m^{-2}	0.90	0.60	3.5	0.5
	10	0.85	0.45	8.2	1.5
	15	0.80	0.30	18.0	4.7
	20	0.68	0.10	24.9	8.1

[a] Data are the means of four experiments.

pathways of X-ray MDR, because the *radC radA* double mutant still shows a significant amount of X-ray MDR. (3) The *radC* mutant shows MMR. Since even rich medium-grown cells show MMR (*14*), the uv-radiation MDR observed in the *radC* mutant is probably the result of an antagonistic interaction between the processes causing MMR and MDR. Therefore, uv-radiation MDR for the *radC* mutant, or for other mutants showing MMR, will be difficult to interpret.

Based on the interactions of the *radC* mutation with known DNA repair mutations, we can associate the *radC* gene with certain genetic pathways of DNA repair. Since the *radC* mutation sensitized the *polA* (Fig. 3b) strain to X rays, but not the *recA* strain (Fig. 3a), the *radC* gene should be involved in the growth medium-dependent

TABLE V

Effect of the *radC* Mutation on Genetic Recombination Ability[a]

		Experiment			
Marker selected	*Hfr strain*	*1*	*2*	*3*	*Mean*
Thr^+	SR859	0.33	0.48	0.46	0.42
Ara^+	SR859	0.22	0.47	0.51	0.40
Leu^+	SR859	0.32	0.44	0.44	0.40
Pro^+	SR859	0.30	0.60	0.47	0.46
His^+	SR841	0.37	0.38	—	0.37

[a] Recipient strains SR1186 (wild type) and SR1187 (*radC*) were mated simultaneously in each experiment with the same Hfr and F′ cultures. The recipient strains were mated with the F′ donor strain (SR865) to determine each recipient's ability to take up donor DNA; the *radC* strain showed a 25% deficiency in the uptake of donor F′ (scoring for Lac^+ Sm^r recombinants). F′ uptake data were used to normalize recombinant data. Data presented are the ratios (*radC*/wild type) of "normalized" recombinants per milliliter obtained for each selected phenotype after 45 min of conjugation.

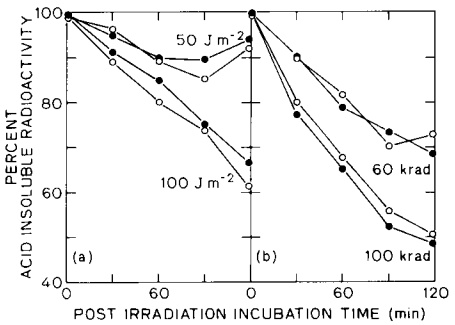

FIG. 5. Radiation-induced DNA degradation in wild-type and *radC* strains of *E. coli*. Cells were tested for their ability to degrade their radioactively labeled DNA after treatment with 0, 50, or 100 J m^{-2} of uv radiation (a) or 0, 60, and 100 krad of X radiation (b). All points are the percentage of counts in the samples, relative to the nonirradiated samples at the same incubation time. Symbols are: wild type (SR1186) (●); *radC* (SR1187) (○). Points are the means of data from two experiments.

(*recA*-dependent) repair of DNA single-strand breaks produced by ionizing radiation (*25*). Since the *radC* mutation sensitizes the *uvrB* strain to uv radiation (Fig. 4c) and causes no deficiency in host cell reactivation (data not shown), the *radC* gene product is most likely involved in postreplication repair. The recombination deficiency seen in the *radC* mutant (Table V) is consistent with this proposition. The *radC* mutant could also be deficient in long patch excision repair after uv irradiation, since all of the mutations that inhibit postreplication repair have also been shown (when tested) to inhibit long patch excision repair (*2*).

Multiple genetic pathways and mechanisms for uv-radiation mutagenesis have been demonstrated (*11, 26, 27*). The decreased uv-radiation mutability of the *radC* mutant (Table IV) suggests that the *radC* gene is involved in one (or more) of the DNA repair pathways that must be responsible for uv-radiation mutagenesis.

Studies are underway to further characterize the role of the *radC* gene product in DNA repair, and to understand the molecular nature of the multiple processes involved in MDR.

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