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Characterization of an *Escherichia coli* Mutant (*radB101*) Sensitive to γ and uv Radiation, and Methyl Methanesulfonate

NEIL J. SARGENTINI[1] AND KENDRIC C. SMITH

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

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After *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis of *Escherichia coli* K-12 (*xthA14*), an X-ray-sensitive mutant was isolated. This sensitivity is due to a mutation, *radB101*, which is located at 56.5 min on the *E. coli* K-12 linkage map. The *radB101* mutation sensitized wild-type cells to γ and uv radiation, and to methyl methanesulfonate. When known DNA repair-deficient mutants were ranked for their γ-radiation sensitivity relative to their uv-radiation sensitivity, their order was (starting with the most selectively γ-radiation-sensitive strain): *recB21*, *radB101*, wild type, *polA1*, *recF143*, *lexA101*, *recA56*, *uvrD3*, and *uvrA6*. The *radB* mutant was normal for γ- and uv-radiation mutagenesis, it showed only a slight enhancement of γ- and uv-radiation-induced DNA degradation, and it was ~60% deficient in recombination ability. The *radB* gene is suggested to play a role in the *recA* gene-dependent (Type III) repair of DNA single-strand breaks after γ irradiation and in postreplication repair after uv irradiation for the following reasons: the *radB* strain was normal for the host-cell reactivation of γ- and uv-irradiated bacteriophage λ; the *radB* mutation did not sensitize a *recA* strain, but did sensitize a *polA* strain to γ and uv radiation; the *radB* mutation sensitized a *uvrB* strain to uv radiation.

INTRODUCTION

A major factor in the rapid accumulation of knowledge on the repair of radiation-damaged DNA has been the availability of numerous DNA repair-deficient mutants of *Escherichia coli* K-12 [reviewed in Ref. (*1*)]. In order to further our studies on the repair of ionizing-radiation-induced damage in *E. coli* [e.g., Ref. (*2*)], we have undertaken a program of isolating new ionizing-radiation-sensitive mutants. This report identifies one such mutant and describes the chromosomal map location of its mutation (*radB101*) and the phenotype associated with the mutation when transduced into a wild-type strain.

MATERIALS AND METHODS

Bacterial Strains

The strains of *E. coli* used are listed in Table I. Transductions and conjugations were accomplished generally as described by Miller (*9*).

[1] Author to whom correspondence should be addressed.

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TABLE I

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

Stanford Radiology number	*Relevant genotype*	*Other genotype*[b]	*Source or derivation*[b]
SR96	+	Hfr H *thyA deo thi*	Hfr H No. 1, F. Bonhoeffer
SR144	*polA1*	F$^-$ *thyA36 deo(C2?) lacZ53 rha-5 rpsL151* λ^-	JG138, J. Gross
SR192	*lexA101*	F$^+$? *metE thyA36 deo(C2?) lacZ53 rpsL151* λ^-	DY99 (*5*)
SR248	+	F$^-$ *leuB19 metE70 thyA36 deo(C2?) lacZ53 malB45 rha-5 bioA2 rpsL151* λ^-	KH21, R. B. Helling
SR250	*uvrB5*	As SR248 but Mal$^+$ Bio$^+$	DY145 (*5*)
SR255	*recB21*	As SR749 but *deoB16*	(*6*)
SR263	*endA101*	F$^-$ *argA103 pheA97 thyA301 bio-87*	KMBL1788, A. K. Ganesan
SR349	*uvrA6*	As SR248 but Mal$^+$	(*7*)
SR359	+	Hfr 1 *metA mel*	S108, R. Schmitt
SR392	+	As SR248 but Met$^+$	DY174 (*8*)
SR393	*uvrD3*	Same as SR392	DY175 (*8*)
SR629	+	Same as SR349	(*7*)
SR669	*recA56*	Hfr P045 *ilv-318 thr-300 srlA300*::Tn*10*(Tc^r) *rpsE300*	JC10240, A. J. Clark
SR675	+	F$^-$ *leuB6 thr-1 lacY1 thi-1 tonA21 supE44* λ^-	C600 r$^+$m$^+$, A. C. Y. Chang
SR715	+	F$^-$ *trpC22*::Tn*10*(Tc^r) *tna* λ^-	W3110 *trpC22*, C. Yanofsky
SR749	+	F$^-$ *argE3 his-4 leuB6 proA2 thr-1 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 tsx-33 rpsL31 supE44* λ^-	AB1157, ECGSC
SR750	*xthA14*	As SR749 but *metE70 rha-6*	NH5016, ECGSC
SR857	+	F$^-$ *his-68 trp-45 tyrA2 purC50 gal-6 lacY1 malA1 mtl-2 xyl-7 thi-1 tonA2 tsx-70 rpsL125 supE44?* λ^r λ^-	H677, ECGSC
SR860	+	F$^-$ *argH1 purI66 ara-13 gal-6 lacY1 mtl-2 xyl-7 thi-1 nadB4 tonA2 rpsL9 supE44* λ^r	PA3306, ECGSC
SR865	+	F′ *lac*$^+$/*lacY thi?*	JC2625, A. J. Clark
SR884	*recF143*	As SR749 but Mtl$^+$ Xyl$^+$ *tnaA300*::Tn*10*(Tc^r) *sup*$^+$	JC12334, A. J. Clark

TABLE I—*Continued*

Stanford Radiology number	*Relevant genotype*	*Other genotype*[b]	*Source or derivation*[b]
SR922	+	As SR248 but Thy$^+$	(7)
SR923	*recA56*	Same as SR922	Same as SR922
SR977	*xthA14 radB101*	As SR749 but *metE70 trpC22*::Tn*10*(Tc^r) *rha-6*	SR993 × P1::Tn*9*cts · SR715, select Tc^r
SR990	+	Hfr Hayes V *pheA*:: Tn*10*(Tc^r) *pro*XIII *lac thi*	NK6024, N. Kleckner
SR991	+	As SR248 but *pheA*::Tn*10*(Tc^r)	SR248 × P1::Tn*9*cts · SR990, select Tc^r
SR993	*xthA14 radB101*	As SR749 but *metE70 rha-6*	SR750, MNNG, select γ radiation sensitive
SR1015	*radB101*	As SR248	SR991 × P1::Tn*9*cts · SR993, select Phe$^+$
SR1016	+	As SR860 but *pheA*::Tn*10*(Tc^r)	SR860 × P1::Tn*9*cts · SR990, select Tc^r
SR1026	*radB101*	As SR248	SR991 × P1::Tn*9*cts · SR1015, select Phe$^+$
SR1059	+	As SR248	SR991 × P1vir · SR1026, select Phe$^+$
SR1060	*radB101*	Same as SR1059	Same as SR1059
SR1065	+	As SR248 but *srlA300*::Tn*10*(Tc^r)	SR1059 × P1::Tn*9*cts · SR669, select Tc^r
SR1066	*recA56*	Same as SR1065	Same as SR1065
SR1067	*radB101*	As SR248 but *srlA300*::Tn*10*(Tc^r)	SR1060 × P1::Tn*9*cts · SR669, select Tc^r
SR1068	*radB101 recA56*	Same as SR1067	Same as SR1067
SR1069	+	As SR248 but Met$^+$	SR1059 × P1::Tn*9*cts · SR144, select Met$^+$
SR1070	*polA1*	Same as SR1069	Same as SR1069
SR1071	*radB101*	As SR248 but Met$^+$	SR1060 × P1::Tn*9*cts · SR144, select Met$^+$
SR1072	*radB101 polA1*	Same as SR1071	Same as SR1071
SR1073	+	As SR248 but Met$^+$	SR248 × P1::Tn*9*cts · SR144, select Met$^+$
SR1074	*polA1*	Same as SR1073	Same as SR1073
SR1092	*uvrB5*	As SR248 but *pheA*::Tn*10*(Tc^r) Mal$^+$ Bio$^+$	SR250 × P1::Tn*9*cts · SR990, select Tc^r

TABLE I—*Continued*

Stanford Radiology number	*Relevant genotype*	*Other genotype*[b]	*Source or derivation*[b]
SR1104	+	As SR248 but *tna300*::Tn*10*(Tc^r)	SR248 × P1vir · SR884, select Tc^r
SR1105	*recF143*	Same as SR1104	Same as SR1104
SR1107	+	F$^-$ *argA his pheA gal lac grpE280 rpsL*	K2809, H. Uchida
SR1108	+	As SR248 but Mal$^+$	SR248 × P1kc · SR192, select Mal$^+$
SR1109	*lexA101*	Same as SR1108	Same as SR1108
SR1110	*uvrB5*	As SR248 but Mal$^+$ Bio$^+$	SR1092 × P1vir · SR1026, select Phe$^+$
SR1111	*uvrB5 radB101*	Same as SR1110	Same as SR1110
SR1156	*xthA14 radB101*	As SR749 but *metE70 trpC22*::Tn*10*(Tc^r) *thyA rha-6*	SR977, select Tmp^r
SR1163	+	As SR248 but Thy$^+$	SR248 × P1::Tn*9*cts · SR255, select Thy$^+$
SR1164	*recB21*	Same as SR1163	Same as SR1163

[a] Genotype nomenclature is that used by Bachmann and Low (*3*).

[b] "Same as . . . " indicates that the strain is a cotransductant or coconjugant with the indicated strain. Tc^r indicates that isolates were tetracycline resistant. MNNG indicates mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Trimethoprim-resistant (Tmp^r) mutants, i.e., *thyA* mutants, were selected by the method of Stacey and Simson (*4*). Phe$^+$, Met$^+$, Thy$^+$, and Bio$^+$ indicate that the isolates no longer required phenylalanine, methionine, thymine, or biotin, respectively, for growth. Mal$^+$, Mtl$^+$, Xyl$^+$ indicate that the isolates could use maltose (at 0.2%), mannitol, or xylose, respectively, in place of glucose. ECGSC indicates that the strain was received from the *E. coli* Genetic Stock Center.

Media

MM was a 0.4% glucose–salts medium (*10*) containing thiamine · HCl at 1 μg/ml. For strains SR749, SR750, and SR993, MM was supplemented (SMM) with L-arginine, L-histidine, L-leucine, L-methionine, L-proline, and L-threonine (all at 1 m*M*). For strains SR349, SR392, SR393, SR629, SR922, SR923, SR1059, SR1060, SR1073, SR1074, SR1104, SR1105, SR1108, SR1109, SR1163, and SR1164, MM was supplemented with thymine at 10 μg/ml, D-biotin at 1 μg/ml, and L-leucine and L-methionine at 1 m*M*. For nonspecified strains, MM was supplemented with only the minimal requirements for growth; i.e., amino acids at 1 m*M*. For strain SR263 and its derivatives, thymine was used at 50 μg/ml. The *purC* and *purI* deficiencies were satisfied by using adenine at 200 μg/ml; for *nadB*, diphosphopyridine nucleotide (Worthington Biochemical Corp.) was used at 5 μg/ml. Recombinant selection media were MM supplemented as above but deficient in a single normally required sup-

plement, as appropriate. Mutant-selection media: glu-0 was the SMM for strain SR1059 but containing filter-sterilized lactose (0.4%) in place of glucose; glu-300 and glu-2000 were glu-0 also containing glucose at 300 or 2000 μg/ml, respectively; leu-0 and leu-15 were the SMM for strain SR1059 but deficient in leucine or containing leucine at 15 μg/ml, respectively. Media were solidified by adding Noble agar (Difco) at 1.6%. LBt [modified from Ref. (*9*)] was tryptone (Difco) at 1%, yeast extract (Difco) at 0.5%, NaCl at 1%, and thymine at 10 μg/ml. YENB was nutrient broth (Difco) at 0.8% and yeast extract at 0.75%. YENB agar was nutrient agar (Difco) at 2.3% and yeast extract at 0.75%. LCTG-1% (*11*) was tryptone at 1%, yeast extract at 0.5%, NaCl at 1%, Bacto agar (Difco) at 1%, and, after autoclaving, glucose at 0.1%, $CaCl_2$ at 2 m*M*, and thymine at 10 μg/ml. LCTG-0.7% was LCTG-1% modified to contain Bacto agar at 0.7%. PB was Na_2HPO_4 at 5.83 g/liter and KH_2PO_4 at 3.53 g/liter, pH 7.0. PBS was 0.05 *M* KH_2PO_4 and 0.1 *M* NaCl, adjusted to pH 7.3 with NaOH.

Test for Bacteriophage P1 Lysogeny

Strains to be tested were grown overnight in LBt and separated from their culture supernatants by sedimentation at 5000*g* for 6 min. Samples (0.1 ml) of supernatant (undiluted and a 100-fold dilution) were mixed with 0.1-ml volumes of overnight culture of strain SR675 (LBt supplemented with $CaCl_2$ at 5 m*M*), held 20 min at 37°C, mixed with 2.5-ml volumes of R-top agar, and poured over R-agar base plates (*9*). The presence of plaque-forming units (PFU), which indicated lysogeny, was determined after overnight incubation at 37°C.

Mutagenesis with N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG)

Strain SR750 (*xthA14*) was mutagenized with MNNG (Aldrich Chemical Co.) generally as described by Miller (*9*). A 5-ml logarithmic-phase LBt culture was harvested by sedimentation at 5000*g* for 6 min, washed twice in 0.1 *M* sodium citrate buffer at pH 5.5, and resuspended in 4 ml of citrate buffer. A 0.4-ml volume of a freshly made MNNG solution (1 mg/ml in citrate buffer) was added to the cells and the mixture was held at 37°C for 20 min before pelleting the cells, washing them once with PB, resuspending them in 10 ml of LBt, and incubating them overnight. Mutagenized cells were spread on YENB to get 200 colonies per plate. When the colonies became visible, they were replica-plated onto YENB and γ-irradiated. After incubation at 37°C, colonies that appeared to be more γ-radiation sensitive were picked from nonirradiated master plates and saved for further testing.

Radiation Survival Determination

Cells were cultured overnight in SMM, diluted 50-fold into SMM, and shaken at 37°C until an optical density at 650 nm (OD_{650}) of 0.5 (Zeiss PMQ II spectrophotometer) was attained. Cells were then filter-harvested (Millipore HAWP, 0.45 μm pore size), washed, and resuspended in PB at an OD_{650} of 0.2, i.e., $\sim 1 \times 10^8$ colony-forming units/ml. Cells were then irradiated, diluted in PB, and plated, in triplicate, on SMM. After 2 days at 37°C, colonies were counted and the radiation survival was calculated.

Irradiation

Gamma irradiation (^{137}Cs) was accomplished using an 8000-Ci Mark I, model 25 irradiator (J. L. Shepherd and Associates). Cells were bubbled with air for 5 min or with N_2 for 15 min (for anoxic irradiation) before irradiation. Cell suspensions were irradiated in a 25 × 100-mm Corex tube (mounted in a Lucite support) with continued bubbling. The dose rate was 4.68 krad/min as determined by a ferrous sulfate method (*12*). Ultraviolet irradiation, using an 8-W General Electric germicidal lamp (G8T5) emitting primarily at 254 nm, was accomplished as previously described (*13*). The fluence rate was ~0.8 J m^{-2} sec^{-1}, as determined with an International Light germicidal photometer (IL-254).

Methyl Methanesulfonate (MMS) Treatment

Logarithmic-phase cells were prepared as above, washed, and resuspended in PBS at an OD_{650} of 0.05. A 24.5-ml volume of these cells (at 37°C) was added to 0.5 ml of MMS (Aldrich Chemical Co.) (dissolved in dimethyl sulfoxide) to yield a final concentration of 0.025 *M*. The mixture was shaken slowly at 37°C. Samples were withdrawn and diluted 10-fold or more with PBS containing 0.1% sodium thiosulfate before plating as above. The 10-fold dilution into thiosulfate solution effectively inactivated the unreacted MMS (data not shown).

Mutagenesis Determination

Cells were prepared as for the radiation survival determination. However, 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 or 0.2 for uv irradiation. Cells were irradiated as above, except that the γ-radiation dose rate was 1.56 krad/min. Gamma-irradiated cells (0.1 ml) were plated without dilution (in quadruplicate on glu-0 or glu-300 to assay Lac^+ mutants or with dilution (in triplicate) on glu-300 to assay survivors. Ultraviolet-irradiated cells were concentrated 100-fold by centrifugation and plated (0.1 ml) without dilution (in quadruplicate) on glu-0 or glu-2000 to assay Lac^+ mutants, or (0.2 ml) on leu-0 or leu-15 to assay Leu^+ mutants, or with dilution (in triplicate) on glu-300 and leu-15 to assay survivors. The surviving-fraction data for uv-irradiated cells were determined on nonconcentrated cells. Plates were incubated at 37°C for 2 (survivors) or 3 (mutants) days. The radiation-induced mutant frequency was calculated as previously described (*13*).

Host-Cell Reactivation (HCR) Determination

This procedure has been modified from that of Rothman and Clark (*11*). Bacteriophage λvir stock was diluted 80-fold in 0.01 *M* $MgSO_4$ to yield ~1 × 10^7 PFU/ml for uv irradiation or 9-fold in nutrient broth (Difco) (final concentration = 4%) to yield ~1 × 10^8 PFU/ml for γ irradiation. Irradiations were as above except that γ-irradiated suspensions were not bubbled. After irradiation, the phage was stored at 4°C and the same irradiated phage preparation was used in all of the HCR experiments. The host cells were grown overnight in LBt, harvested by centrifugation (5000*g* for 6 min), washed twice, and resuspended in one-half volume of $MgSO_4$.

Irradiated phages (0.1 ml, diluted in $MgSO_4$) were mixed with host cells (0.2 ml). After 20 min at 37°C, 2.5 ml of LCTG-0.7% (at 45°C) was added to each phage–host mixture (in duplicate) and the contents were poured onto an LCTG-1% plate. The PFU were counted after an overnight incubation at 37°C, and survival (as a result of HCR) was determined.

Recombination Ability Determination

Donor and recipient cells were shaken overnight at 37°C in LBt, diluted 100-fold in LBt, and cultured to an OD_{650} of 0.3–0.4. A 0.25-ml volume of donor cells was added to a 5-ml volume of recipient cells at 37°C and the mixture was shaken slowly. All combinations of donor and recipient strains were used concurrently in each experiment. After 45 min of incubation, 0.2 ml of conjugation mixture and nonmated control cultures were diluted 10-fold with PB and vortexed vigorously for 10 sec. Afterwards, further dilutions were made and 0.1-ml volumes were plated (in duplicate) on either glu-0 or leu-0 plates [supplemented with streptomycin sulfate (Sigma) at 200 μg/ml] as appropriate for Lac^+ or Leu^+, respectively, exconjugant (or F'-ductant) selection.

DNA Degradation Assay

An overnight SMM culture was diluted 200-fold into SMM modified to contain thymine at 2μg/ml and [*methyl*-^{3}H]thymine (Amersham, 30 Ci/mmole) at 2 μCi/ml, and shaken for four population doublings at 37°C to an OD_{650} of 0.2. These radioactive cells were filter-harvested, washed, resuspended in warm SMM, and incubated until an OD_{650} of 0.5 was attained. These "chased" cells were filter-harvested, washed, resuspended in the mineral salts portion of SMM at an OD_{650} of 0.2, and either γ- or uv-irradiated. These cells were mixed with an equal volume of SMM modified to contain twice the normal concentration of glucose, thiamine · HCl, and supplements, and incubated. At graded incubation times, duplicate (quadruplicate for nonirradiated, nonincubated samples) 0.2-ml samples of culture were placed on Whatman No. 17 paper rectangles (1.75 × 1.25 cm) supported on steel pins; the rectangles had previously been saturated with 10% TCA and dried, and then each moistened with 0.2 ml of thymine at 1 mg/ml and dried. At the end of the experiment, the rectangles were washed thrice with 5% TCA and once with 95% ethanol (10-min cycles), dried, and placed in Omnifluor (New England Nuclear), and their radioactivity was determined with a liquid scintillation spectrometer.

RESULTS

In a preliminary screening, 272 colonies were scored as presumptive radiation-sensitive mutants of strain SR750 (*xthA*) among ~25,000 MNNG-treated survivors, and they were cloned and saved for further testing. These putative mutants were designated *rad* (for radiation resistance). Gamma and uv-radiation survival curves were produced for each *rad* strain. One strain, SR993, was selected for further study because it was relatively more sensitive to γ radiation than to uv radiation (cf. Figs. 1a and b). That is, for γ radiation the D_1 (a dose killing 99% of the cells) for

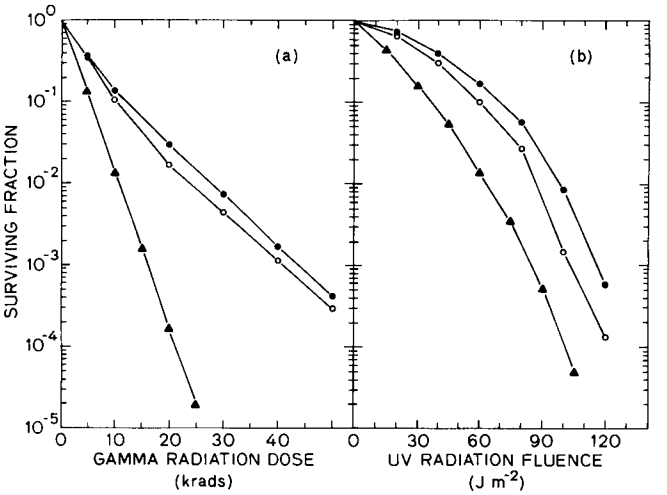

FIG. 1. Radiation survival of a wild-type, *xthA*, and a mutagenized *xthA* strain of *E. coli*. Cells grown in SMM were treated with γ (a) or uv (b) radiation before plating on SMM. Symbols: wild type (SR749) (○), *xthA14* (SR750) (●), radiation-sensitive mutant of strain SR750 (SR993) (▲). All points are the mean of data from three experiments. Note that strain SR749 is the wild-type parent from which strain SR750 was derived by mutagenesis and transduction (*14*).

strain SR750 divided by the D_1 for strain SR993 was equal to 2.7 (27.9 krad/10.5 krad), while a similarly calculated D_1 ratio for uv radiation was only 1.6 (98 J m^{-2}/63 J m^{-2}).

To locate the mutation(s) causing the radiation sensitivity of strain SR993, a *trp thyA* derivative (SR1156) was mated with an Hfr KL16 derivative (SR841). Of the exconjugants selected for nonrequirement of thymine, 5 of 29 (17%) became γ-radiation resistant (data not shown). These and other data obtained from the conjugation experiment allowed the production of a "gradient of transmission" (*9*) that indicated that the location of the radiation-sensitizing mutation(s) in strain SR1156 was at ~51 min on the *E. coli* K-12 linkage map (*3*) (data not shown). Several bacteriophage P1-mediated crosses were performed using strain SR993 as the DNA donor to transduce markers in the vicinity of 51 min on the linkage map. These yielded the result that a radiation-sensitizing mutation was closely linked with the *pheA* locus; i.e., 10 of 20 (50%) transductants of strain SR263 selected for phenylalanine prototrophy (Phe^+) were also γ-radiation sensitive (data not shown). The mutation in strain SR993 was purified by transducing it (with Phe^+) three times sequentially into a wild-type strain (SR991) to yield strains SR1015, SR1026, and SR1060 (Table I), all of which showed similar γ-radiation survival (data not shown). This procedure seemed necessary because out of the first transduction for purification, we obtained two classes of γ-radiation-sensitive transductants. However, the class with the lesser γ-radiation sensitivity was later found to result from P1::Tn*9*cts lysogeny (data not shown). Strains SR1015, SR1026, and SR1060 all were tested for lysogeny (see Materials and Methods) and were found to be nonlysogens (data not shown) and to carry the same radiation-sensitizing mutation, which shall be called

TABLE II

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

Selected phenotype in the transduction[a]	*Nonselected phenotypes detected*[b]				*Cotransduction frequency of radB gene with selected marker*[c] *(%)*	*Calculated distance between radB gene and selected marker*[d] *(min)*	*Map position of selected marker*[e] *(min)*	*Calculated position of the radB gene*[f] (min)
	Phe$^+$	*Nad*$^+$	*Pur*$^+$	*Rad*$^-$				
Tyr$^+$ (99)	NT	NT	NT	49	49	0.42	56.2	56.6
Phe$^+$ (89)	—	32	33	49	55	0.36	56.1	56.5
Nad$^+$ (63)	27	—	29	19	30	0.66	55.2	55.9
PurI$^+$ (100)	4	14	—	2	2	1.46	54.7	56.2

[a] Strains SR857 (*tyrA2*) and SR1016 [*pheA*::Tn*10*(Tc^r) *nadB4 purI66*] were transduced [P1vir · SR1026 (*radB101*)] to tyrosine prototrophy (SR857), or phenylalanine prototrophy, nicotinic acid nonrequirement, or purine nonrequirement (SR1016). The parenthetical value is the number of transductants used.

[b] Transductants were picked and isolated on the same type of medium on which they were selected. Then they were transferred to other appropriate media for identifying Phe$^+$, Nad$^+$, or Pur$^+$. The Rad phenotype was determined by growing isolated transductants in YENB to OD_{650} = 0.5, diluting them in PB, γ-irradiating them with 20 krad, and plating diluted cells on YENB. Surviving fractions were $\sim 3 \times 10^{-1}$ for Rad$^+$ and $\sim 2 \times 10^{-3}$ for Rad$^-$. NT = not tested. — = All of the transductants grew on these control plates.

[c] Calculated by dividing the Rad$^-$ value by the parenthetical value in the first column and multiplying by 100%.

[d] The distances listed were calculated according to Wu (*15*) as equal to $L(1\text{-}F^{1/3})$, where L is the length of the DNA carried by the transducing bacteriophage, i.e., 2.0 min (*16*), and F is the frequency of cotransduction divided by 100%.

[e] Map positions are from Bachmann and Low (*3*).

[f] Map positions were calculated by adding the distance between the *radB* locus and the selected marker (e.g., 0.42 min) to the map position of the selected marker (e.g., 56.1 min). This calculation depends on the *radB* locus being separated from the *nadB* locus by the *pheA* locus. That this is true was strongly suggested by finding that of 59 transductants that acquired both the Phe$^+$ and Nad$^+$ phenotypes, 24 did not become Rad$^-$ (data not shown).

radB101. Strain SR1059, a wild-type cotransductant with SR1060, is also a nonlysogen (data not shown).

Fine mapping of the *radB101* mutation was attempted by transducing it from strain SR1026 (*radB101*) into strains SR857 (*tyrA2*) and SR1016 [*pheA*::Tn*10*(Tc^r) *nadB2 purI66*] (Table II). The cotransduction data obtained with the markers (*pheA* and *tyrA*) closest to the putative map position were consistent with the *radB* locus being at 56.5 min on the linkage map, and very close to the *grpE* locus. Since the *radB* strain (SR1060) showed the same plating efficiency for bacteriophage λ as the wild-type strain (SR1059) (data not shown), it was assumed that the *radB* gene is different than the *grpE* gene, which is involved in the initiation of λ DNA replication (*17*). When the *radB101* mutation was transduced from strain SR1026 (*radB101*) into strain SR1107 (*pheA grpE280*), 92% (46/50) of the transductants that became Grp$^+$ also became Rad$^-$ and 96% (46/48) of the transductants that became Rad$^-$ also became Grp$^+$ (Table III). These data are consistent with a tight linkage between the *radB* and *grpE* loci, and tentatively suggest that the *radB* locus is just to the right of the *grpE* locus as shown in Fig. 2.

Relative to the wild-type strain (SR1059), the *radB101* mutant (SR1060) was sensitive to γ and uv radiation, and MMS (Fig. 3). Gamma-radiation D_1 values (Figs. 1a and b) were used to calculate the oxygen enhancement ratio, i.e., D_1 (oxic irradiation)/D_1 (anoxic irradiation), which was 3.4 for the wild-type strain (57 krad/17 krad) and for the *radB* strain (32 krad/9.4 krad). The D_1 ratio (wild type/mutant) was 1.8 for γ radiation [oxic (17 krad/9.4 krad) or anoxic (57 krad/32 krad)] and 1.7 (88 J m^{-2}/53 J m^{-2}) for uv radiation, indicating a slight specificity of putative *radB* gene-dependent repair processes for γ-radiation-induced cellular damage. In order to compare these data with those for other radiation-sensitizing mutations, survival curves were produced for isogenic strains carrying those mutations (data not shown) and the D_1 ratios were calculated as above (Table IV). The result was that the *radB* mutant was second only to the *recB* mutant in showing a relatively higher sensitization to γ radiation than to uv radiation.

TABLE III

Phenotype of Phe$^+$ Transductants from SR1107 × P1vir·SR1026[a]

Phe$^+$ transductant phenotype[b]			*Fraction of total Phe$^+$ transductants*
Phe	*GrpE*	*RadB*	
+(D)	−(R)	+(R)	33/85
+(D)	+(D)	+(R)	4/85
+(D)	+(D)	−(D)	46/85
+(D)	−(R)	−(D)	2/85

[a] Recipient: SR1107 (*pheA grpE280*). Donor: SR1026 (*radB101*). Phe$^+$ means prototrophic for phenylalanine.

[b] GrpE: ability (+/−) to show normal plating efficiency of bacteriophage λ (see Materials and Methods). RadB: ability (+/−) to survive γ irradiation (see Table II). D = donor phenotype, R = recipient phenotype.

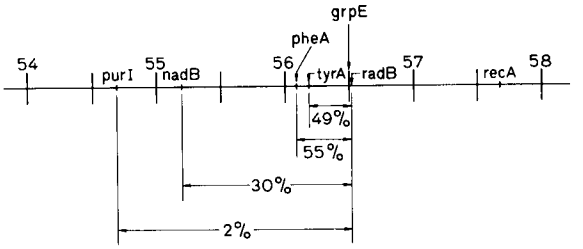

FIG. 2. Order of genetic loci in the *purI–recA* region of the *E. coli* K-12 chromosome. The average frequency of cotransduction between markers is shown (taken from Table II). The position of each of the genes (in terms of min) except *radB* (which we calculate to be at about 56.5 min) is from Bachmann and Low (*3*).

A series of experiments were designed to determine in what repair processes the *radB* gene might play a role. The *radB* strain was found to be completely proficient in the reactivation of γ- or uv-irradiated bacteriophage λvir (Fig. 4). The *radB* mutation sensitized a *polA1* strain, but not a *recA* strain, to γ radiation (Fig. 5); it sensitized *uvrB5* and *polA1* strains, but not the *recA* strain, to uv radiation (Fig. 6). Since repair processes can exhibit both error-free and error-prone DNA repair modes (*18, 19*), the *radB* mutant was tested for its sensitivity to γ- and uv-radiation mutagenesis. The *radB* mutant was not significantly different from the wild-type strain for either kind of radiation mutagenesis (Table V).

Several radiation-sensitive mutants also exhibit a deficiency in recombination ability [reviewed in Ref. (*20*)] and show abnormal radiation-induced DNA degradation (*21, 22*). The *radB* mutant was found to be ~60% deficient in recombination ability (Table VI). This is only a minor deficiency if one compares it to that for *recA* or

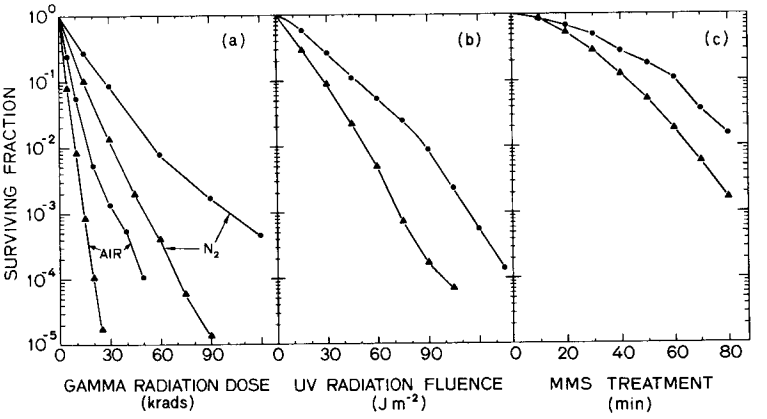

FIG. 3. Radiation and methyl methanesulfonate (MMS) survival of wild-type and *radB* cotransductant strains of *E. coli*. Cells grown in SMM were treated with γ (a) or uv (b) radiation, or MMS (c) before plating on SMM. Gamma irradiation was either oxic (AIR) or anoxic (N_2). Symbols: wild type (SR1059) (●), *radB101* (SR1060) (▲). All points are the mean of data from three experiments.

TABLE IV

Gamma-Radiation Sensitivity of DNA Repair-Deficient Mutants relative to Their Ultraviolet-Radiation Sensitivity (G^s/uv^s)

Strain	*Genotype relevant to DNA repair*	*D_1 for γ radiation*[a] *(krad)*	*Relative γ-radiation sensitivity (G^s)*[b]	*D_1 for uv radiation*[a] *($J\ m^{-2}$)*	*Relative uv-radiation sensitivity (uv^s)*[b]	*(G^s/uv^s)*[c]
SR1163	+	19.7	—	87.2	—	—
SR1164	*recB21*	7.9	2.5	37.6	2.3	1.09
SR1059	+	17.2	—	88.4	—	—
SR1060	*radB101*	9.4	1.8	52.8	1.7	1.06
SR1073	+	17.6	—	80.0	—	—
SR1074	*polA1*	7.8	2.3	21.2	3.8	0.61
SR1104	+	17.1	—	79.6	—	—
SR1105	*recF143*	14.4	1.2	33.2	2.4	0.50
SR1108	+	17.3	—	84.0	—	—
SR1109	*lexA101*	6.8	2.5	9.6	8.8	0.28
SR922	+	18.5	—	81.2	—	—
SR923	*recA56*	6.5	2.8	4.8	16.9	0.17
SR392	+	19.0	—	77.6	—	—
SR393	*uvrD3*	20.8	0.91	9.6	8.1	0.11
SR629	+	16.5	—	74.0	—	—
SR349	*uvrA6*	20.5	0.80	5.8	12.8	0.06

[a] D_1 is the dose required to kill 99% of the irradiated cells. Data were taken from the survival curves (Figs. 3a, b, or not shown).

[b] The G^s and uv^s values were calculated by dividing the D_1 value for the wild-type strain by the D_1 value for its cotransductant mutant strain, i.e., the strain listed directly below it in the first column of the table. Note that G^s and uv^s are set at 1.0 for the wild-type strains.

[c] G^s/uv^s is the γ-radiation sensitivity of a mutant strain relative to its uv-radiation sensitivity. Note that G^s/uv^s is 1.0 for the wild-type strains.

recB mutants (*20*). Also, γ- and uv-radiation-induced DNA degradation was only slightly enhanced relative to that in the wild-type strain (Fig. 7).

DISCUSSION

The *radB* gene was shown to be located at 56.5 min on the linkage map for *E. coli* K-12 (Fig. 2). The *recA* (*lexB, recH, tif, umuB, zab*) gene mapping at 57.7 min is the closest gene to the *radB* locus that is also known to be involved in the repair of radiation-induced damage (*3*). However, the *recA* locus theoretically cannot be linked by bacteriophage P1 transduction to the *purI* locus [as is the *radB* gene (Table II)], since they are separated by ~3.0 map units (*16*). These data are consistent with *radB* being a heretofore undescribed gene for radiation resistance. Moreover, the *radB* gene promises to be of special interest to workers interested in repair processes for ionizing-radiation-induced DNA damage, because most other DNA repair mutations (except *recB21*) can be characterized as specifically affecting the repair of uv-radiation-induced damage more than they affect the repair of γ-radiation-induced

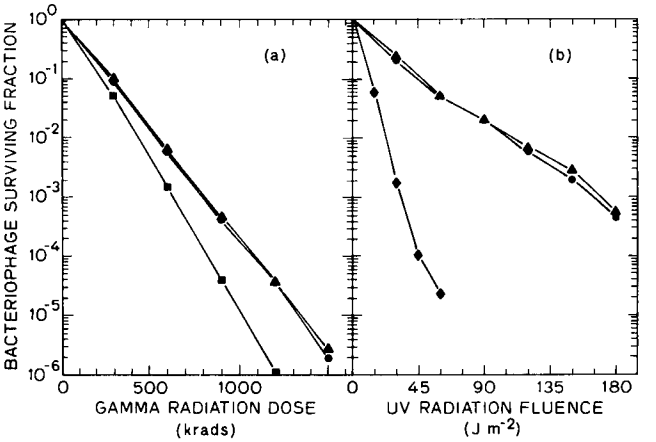

FIG. 4. Reactivation of irradiated bacteriophage λ in DNA repair-deficient host cells. *E. coli* strains were tested for their ability to host-cell reactivate bacteriophage λvir (see Materials and Methods) that had been treated with γ (a) or uv (b) radiation. Symbols: wild type (SR1059) (●), *radB101* (SR1060) (▲), *polA1* (SR1074) (■), *uvrB5* (SR250) (◆). All points are the mean of data from two experiments.

damage (Table IV). Note that the only known ionizing-radiation-sensitizing, uv-radiation-normal mutation, *rorA* (*23*), is a *recB* allele (*24*).

In vitro studies suggest that exonuclease III-deficient (*xthA*) strains ought to be

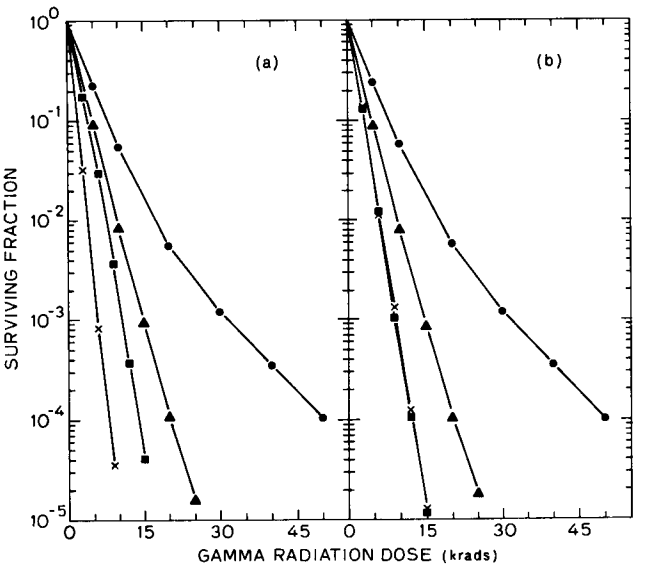

FIG. 5. Gamma-radiation survival of wild-type, *radB*, *polA*, *recA*, *radB polA*, and *radB recA* strains of *E. coli*. Cells were grown and plated on SMM. Symbols for (a): wild type (SR1069) (●), *polA1* (SR1070) (■), *radB101* (SR1071) (▲), *radB101 polA1* (SR1072) (×). Symbols for (b): wild type (SR1065) (●), *recA56* (SR1066) (■), *radB101* (SR1067) (▲), *radB101 recA56* (SR1068) (×). All points are the mean of data from three experiments.

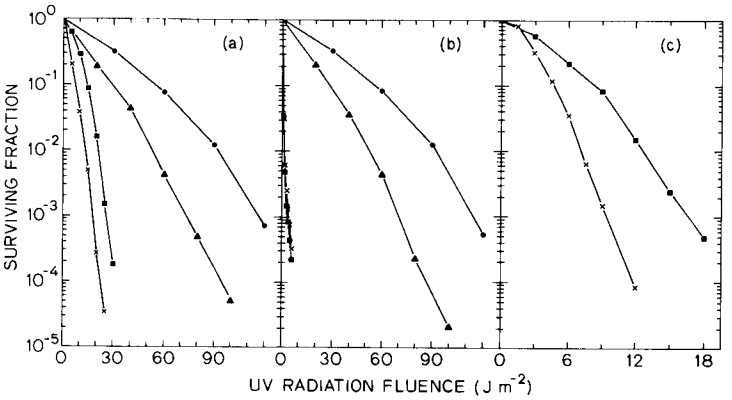

FIG. 6. Ultraviolet-radiation survival of wild-type, *radB*, *polA*, *recA*, *uvrB*, *radB polA*, *radB recA*, and *uvrB radB* strains of *E. coli*. Symbols for (a) and (b) are the same as those for Fig. 5. Symbols for (c): *uvrB5 (SR1110)* (■), *uvrB5 radB101* (SR1111) (×). All points are the mean of data from three experiments.

deficient in the repair of ionizing-radiation-induced DNA damage (*25, 26*). Since such mutants are not very sensitive to ionizing radiation (*27, 28*), it was suggested that the *in vivo* function of exonuclease III might be fulfilled by alternate enzymes (*25*). It was for this reason that we chose to search for new γ-radiation-sensitive

TABLE V

Gamma- and Ultraviolet-Radiation Mutagenesis in Wild-Type (SR1059) and *radB101* (SR1060) Strains[a]

Mutagen and mutation assay	*Dose*	*Surviving fraction*		*Mutants per 10^8 survivors*	
		SR1059(+)	*SR1060(radB)*	*SR1059(+)*	*SR1060(radB)*
Gamma radiation (oxic)	0.3 krad	0.87	0.85	5.0 ± 0.7	5.1 ± 1.3
(*lacZ53*→Lac$^+$)	0.6	0.85	0.78	9.1 ± 0.5	7.6 ± 0.6
	1.0	0.77	0.51	12.2 ± 0.2	14.3 ± 3.3
	2.0	0.58	0.39	24.3 ± 2.4	20.4 ± 6.1
	4.0	0.34	0.14	49.0 ± 6.6	49.2 ± 6.0
Gamma radiation (anoxic)	0.9 krad	0.93	0.85	8.4 ± 0.3	7.9 ± 1.5
(*lacZ53*→Lac$^+$)	1.8	0.86	0.68	16.7 ± 2.6	17.2 ± 4.2
	3.0	0.84	0.65	26.4 ± 1.4	21.3 ± 3.2
	6.0	0.65	0.34	48.4 ± 8.0	52.0 ± 9.8
	12.0	0.43	0.18	95.2 ± 16.4	87.3 ± 4.0
Ultraviolet radiation	1.0 J m^{-2}	0.94	0.87	2.1 ± 0.2	1.3 ± 1.0
(*lacZ53*→Lac$^+$)	2.5	0.94	0.77	5.2 ± 2.0	4.9 ± 2.5
	5.0	0.90	0.71	21.1 ± 1.2	16.8 ± 3.0
Ultraviolet radiation	1.0 J m^{-2}	0.94	0.87	0.2 ± 0.1	0.4 ± 0.1
(*leuB19*—Leu$^+$)	2.5	0.94	0.77	1.0 ± 0.2	1.0 ± 0.5
	5.0	0.90	0.71	4.4 ± 0.2	3.3 ± 0.8
	7.5	0.90	0.64	8.6 ± 0.2	9.6 ± 3.0
	10.0	0.78	0.58	14.9 ± 0.2	14.7 ± 3.4

[a] Data are the mean of those from three experiments per protocol. The second value listed in the mutant frequency data is the standard deviation.

TABLE VI

Effect of the *radB101* Mutation on Recombination Ability (Conjugation Test)[a]

Recipient strain	Expt. No.	Lac^+ F'-ductants per ml[b]	Leu^+ recombinants per ml[c]		Recombination deficiency[d] (%)	Lac^+ recombinants per ml[c]		Recombination deficiency[d] (%)
			Actual	Normalized		Actual	Normalized	
SR1059	1	1.32×10^7	2.36×10^4	1.79×10^{-3}	—	6.35×10^3	4.81×10^{-4}	—
(+)	2	1.61×10^7	3.33×10^4	2.07×10^{-3}	—	9.57×10^3	5.94×10^{-4}	—
	3	1.71×10^7	1.36×10^4	7.95×10^{-4}	—	6.43×10^3	3.76×10^{-4}	—
SR1060	1	1.24×10^7	9.65×10^3	7.78×10^{-4}	57	3.50×10^3	2.82×10^{-4}	41
(*radB101*)	2	1.73×10^7	1.22×10^4	7.05×10^{-4}	66	5.40×10^3	3.12×10^{-4}	47
	3	1.71×10^7	2.77×10^3	1.62×10^{-4}	80	2.83×10^3	1.65×10^{-4}	56
					Mean: 68			Mean: 48

[a] Both recipient strains were mated simultaneously in each experiment, with the same donor-strain cultures. Techniques are described under Materials and Methods.

[b] Recipient strains were mated with an F′ donor strain (SR865) in order to determine each recipient's ability to take up donor DNA irrespective of recombination ability.

[c] Recipient strains were mated with Hfr strains, SR96 (for Leu^+ selection) and SR359 (for Lac^+ selection), in order to determine the ability of the recipient strains to produce genetic recombinants. "Normalized" data are the "actual" recombinants per ml values corrected for DNA uptake by dividing the "actual" values by the "Lac^+ F'-ductants per ml" value.

[d] Recombination deficiency is determined by dividing the "normalized" values for the *radB101* strain (SR1060) by the "normalized" values for the control strain (SR1059) determined in the same experiment, subtracting the quotient from 1.00, and multiplying by 100%.

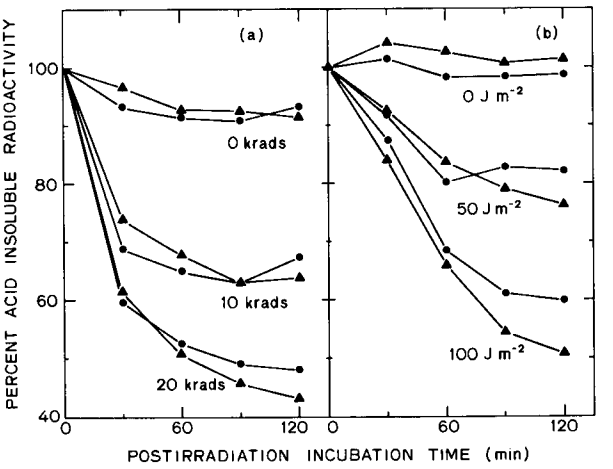

FIG. 7. Radiation-induced DNA degradation in wild-type and *radB* strains of *E. coli*. Cells labeled with [*methyl*-^{3}H]thymine were tested for their ability to degrade their DNA (see Materials and Methods) after treatment with 0, 10, or 20 krad of γ radiation (a), or after 0, 50, or 100 J m^{-2} of uv radiation (b) and incubation in SMM at 37°C. After graded times of incubation, duplicate samples (quadruplicate for nonirradiated, nonincubated samples) were taken for the assay of their nondegraded DNA (trichloroacetic acid-insoluble radioactivity). All points are the percentage of counts in the samples, relative to the nonirradiated, nonincubated samples, and are the mean of data from three experiments. Symbols: wild type (SR1059) (●), *radB101* (SR1060) (▲).

mutants in an *xthA* background. This approach seems to be validated by isolating the *radB* mutation, which appeared to sensitize the *xthA* strain to γ radiation more (D_1 ratio was 2.7) (Fig. 1a) than it sensitized the wild-type strain (D_1 ratio was 1.8) (Fig. 3a), while it sensitized both strains similarly to uv radiation [the D_1 ratio using *xthA* strains was 1.6 (Fig. 1b), while it was 1.7 (Fig. 3b) using *xthA*$^+$ strains]. Since these data could also be explained by an unknown, third mutation in the *xthA radB* strain, in the future we plan to combine the *xthA14* and *radB101* mutations in a "clean" wild-type strain for analysis.

On the basis of interactions of the *radB* mutation with known DNA repair mutations, we can tentatively assign the *radB* gene to certain genetic pathways of DNA repair. (1) Since the *radB* mutation sensitized the *polA* strain but not the *recA* strain to γ radiation (Fig. 5), it should inhibit the *recA* gene-dependent (Type III) repair of γ-radiation-induced DNA single-strand breaks (*29*). (2) Since the *radB* mutant is Hcr$^+$ (Fig. 4), the *radB* gene probably plays no role in the *polA* gene-dependent short-patch excision repair pathway for the repair of uv-radiation-induced DNA damage [note that *polA* strains are partially Hcr$^-$, Ref. (*30*)], but could play a role in the *recA* gene-dependent long-patch excision repair pathway for the repair of uv-radiation-induced DNA damage [reviewed in Ref. (*1*)] [Note that *recA* strains are Hcr$^+$, Ref. (*31*)]. (3) Since the *radB* mutation sensitizes the *uvrB* strain, but not the *recA* strain, to uv radiation (Fig. 6), the *radB* gene ought to play a role in postreplication repair of uv-radiation-induced DNA damage [reviewed in Ref. (*1*)]. The addition of new DNA repair genes to already-proposed genetic pathways of DNA repair serves the valuable function of increasing both the definition of these genetic pathways and the chances of resolving their underlying biochemical mechanisms.

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