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Genetic Mapping of Mutations in a Highly Radiation-Resistant Mutant of *Salmonella typhimurium* LT2

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The genes involved in the high radiation resistance of mutant R68 of *Salmonella typhimurium* LT2 were mapped by conjugation. It was observed that the high radiation resistance involved genes localized in two regions of the chromosome, which have been designated as *garA* and *garB* for high gamma resistance. The *garA* gene mapped near *gal* and *uvrB* at about 18 map units, and the *garB* gene mapped near *purC* at about 49 map units. The resistance of R68 was reduced to the wild-type level by the acquisition of the two wild-type alleles, $garA^+$ and $garB^+$. Recombinants carrying the *garA* or *garB* gene repaired single-strand breaks in their DNA faster than did the wild-type strain. However, only those with the *garA* mutation showed a marked increase in UV irradiation resistance above the wild-type level, whereas those with *garB* mutation exhibited an increased rate of spontaneous degradation of DNA beyond the level observed in *recA* cells.

Radiation-resistant cultures of *Salmonella typhimurium* LT2 strain DB21 were developed through a series of repeated exposures to gamma irradiation followed by outgrowth of survivors (5). The mutants showed stepwise increases in resistance to both gamma and UV irradiation. The most resistant strain was designated D21R6008 (R68 in this paper). Resistant cells were often swollen, oval to spherical, and larger than the parent cells (DB21) and contained about two times more RNA and protein, but they had the same amount of DNA.

A study of the response of R68 to irradiation showed that it degraded less DNA after exposure to gamma irradiation and repaired single-strand breaks in its DNA much faster than did the wild type (6). This mutant also showed a high rate of spontaneous DNA degradation in buffer, increased host cell reactivation of gamma-irradiated phage P22, and increased activities of DNA polymerase I and polynucleotide-joining enzyme. These results suggested that these factors are partly responsible for the high increase in radiation resistance.

These mutants were developed to provide a model for studying the genetics and mechanisms of gamma resistance in *S. typhimurium*, using gamma-resistant mutants rather than sensitive ones.

The question of which types of mutation would make one cell more resistant to radiation than the other has led to very extensive studies, especially with mutants of *Escherichia coli*. More than twice as many loci affecting radiation response have been mapped on the chromosomal linkage map of *E. coli* K-12 (4) as on that of *S. typhimurium* (16). A few of the *E. coli* K-12 morphological genes have been associated with elevated radiation resistance, namely, *rodA* at 14 min (11), *envA* at 2 min (13), and *envB* at 70 min (12). The *rodA* gene induces rounded morphology and increase in resistance to UV irradiation. The *envA* mutation mediates chain formation, *envB* confers anomalous spheroid cell formation, and both mutations lead to increased UV resistance. In *E. coli* B, a *lar-1* mutation at 68 min (9) was found to be responsible for the formation of large cells and the phenotypic changes in the P6 camphor mutant of Ogg and Zelle (14). The mutant cells had three times more DNA and RNA and were more resistant to X-ray irradiation than were the parent cells. However, this mutation has not been found in *E. coli* K-12; hence, it has been removed from the map drawing (4). Ahmad et al. (3) recently reported the isolation of a mutant of *E. coli* K-12, highly resistant to UV radiation. The mutant cells were not changed in morphology but could not grow on minimal media. They synthesized DNA polymerase I and endonuclease I constitutively, and the activities of these enzymes together with that of exonuclease III were increased. The activities of exonuclease I,

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exonuclease II, and DNA ligase, however, were not changed. The mutation(s) have not been mapped. Of the above-mentioned mutations associated with radiation resistance, only *envB* has been mapped in *S. typhimurium* at 70 map units (16).

Two approaches were employed in mapping the genes involved in radiation resistance of R68. The first approach was the transfer of gamma sensitivity alleles from wild-type Hfr strains to R68 auxotrophs to reduce resistance. The second approach involved the use of resistant Hfr strains, isolated from crosses of R68 auxotrophs with Hfr strains, to transfer resistance alleles to sensitive and wild-type female strains.

Selected recombinants from the crosses were characterized in terms of their resistance to gamma and UV irradiation and their ability to repair single-strand breaks in their DNA and to degrade their DNA spontaneously. From the series of intermediate-type recombinants observed, it was concluded that the high radiation resistance of R68 is due to mutations in two genes designated as *garA* and *garB*.

MATERIALS AND METHODS

Organisms. The bacterial strains used and their characteristics are listed in Table 1.

Media. Luria broth (LB) plus agar was used as the complete medium for the cultivation of cells and enumeration of viable counts. The LB medium consisted of 1% tryptone (Difco Laboratories), 0.5% yeast extract, and 0.5% NaCl. The basic minimal salts (M9) medium and the EM9 medium were prepared as reported by Davies and Sinskey (5).

Selective media consisted of M9 medium supplemented with the appropriate amino acids, sugars, purines, and pyrimidines at 20 μg/ml. Streptomycin was used, when indicated, at 1,200 μg/ml.

Irradiation procedures. The experimental techniques used for gamma and UV irradiation were as described by Davies and Sinskey (5). Exponential-phase cells were irradiated in 0.067 M phosphate buffer at 0°C. Gamma irradiation was provided by a 30-kc, pool-type, U.S. Atomic Energy Commission, ^{60}Co, Mark I Food Irradiator. Samples received 4.90 ± 0.07 krads/min at the beginning of the study. A General Electric germicidal 15-W lamp, type G8T5, operated in a lightproof chamber at ambient temperature, was used to provide UV irradiation. The incidental UV dose rate at a distance of 30 cm was 1.7 J/m^2

TABLE 1. *S. typhimurium* LT2 strains

Strain	Sex type	Radiation resistance	Markers	Source
DB63	HfrK4	Wild type	*serA13*	Botstein
DB46	HfrB2	Wild type	*hisD gal*	Botstein
PV42	HfrA	Wild type	*purC7 strA*	Magasanik
PV77	HfrB3	Wild type	*hisD23 gal-50*	Magasanik
SI6	HfrA	Resistant	*his garA*	Ibe
SI7	HfrK4	Resistant	*garA*	Ibe
SI8	HfrB2	Resistant	*metE garB*	Ibe
DB21	F$^-$	Wild type	Prototroph	Botstein
DB25	F$^-$	Wild type	*thyA*	
DB251	F$^-$	Wild type	*thyA$^+$ garB*	Ibe
DB5055	F$^-$	Wild type	*leuD ara his-1099 trp*	Botstein
SI1	F$^-$	Wild type	*leuD proB ara strA*	Ibe
SI2	F$^-$	Wild type	*leuD ara trp his-1099 strA*	Ibe
SI3	F$^-$	Sensitive	*recA thyA*	Ibe
DB47	F$^-$	Sensitive	*recA*	Botstein
SI31	F$^-$	Resistant	*thy$^+$ rec$^+$ garB*	Ibe
SI4	F$^-$	Wild type	*gal50 hisD23 purC213 metC30 strA*	Ibe
SI41	F$^-$	Resistant	*gal50 hisD$^+$ purC metC garB strA*	Ibe
TA1531	F$^-$	Sensitive	*hisC207 Δ(gal-bio-uvrB-chl)*	ATCC
SI5	F$^-$	Sensitive	*hisC207 strA Δ(gal-bio-uvrB-chl)*	Ibe
SI51	F$^-$	Resistant	*hisC$^+$ strA (gal-bio-uvrB)$^+$ garA*	Ibe
D21R6008 (R68)	F$^-$	Highly resistant	Prototroph *garA garB*	Davies
SI9	F$^-$	Highly resistant	R68 *araB garA garB*	Ibe
SI91	F$^-$	Resistant	R68 *araB$^+$ garB*	Ibe
SI10	F$^-$	Highly resistant	R68 *metE garA garB*	Ibe
SI101	F$^-$	Resistant	R68 *metE$^+$ his garB*	Ibe
SI12	F$^-$	Highly resistant	R68 *metE trp garA garB*	Ibe
SI13	F$^-$	Highly resistant	R68 *serA garA garB*	Ibe
SI131	F$^-$	Resistant	R68 *serA$^+$ garA*	Ibe
SI14	F$^-$	Highly resistant	R68 *his strA garA garB*	Ibe
SI15	F$^-$	Highly resistant	R68 *his serA garA garB*	Ibe
SI18	F$^-$	Wild type	R68 *garA$^+$ garB$^+$*	Ibe

per s as measured with a Blak-Ray dosimeter (Fisher Scientific Co.).

Isolation of auxotrophic mutants. (i) UV mutagenesis. A modified method of Gorini and Kaufman (7) was used. Cells were grown in M9 medium at 37°C. When the culture reached a density of about 5×10^8 to 5×10^9 cells per ml, a 5-ml suspension was exposed in a sterile petri dish to a dose of UV irradiation which reduced the viable count by a factor of 10^3 to 10^4. The irradiated sample was inoculated into 100 ml of M9 medium supplemented with a single nutrient, or multiple nutrients in the case of multiple auxotrophs. The cells were grown overnight with shaking at 37°C.

(ii) Chemical mutagenesis. A 0.1-ml volume of ethyl methane sulfonate was added to 5 ml of an exponential-phase culture at 10^9 cells per ml in LB at 37°C. The tube was allowed to stand at 37°C for 30 min, and 0.2 ml of the mutagenized culture was added to 20 ml of fresh LB and grown overnight with shaking at 37°C.

(iii) Penicillin selection. The desired auxotrophs were enriched by penicillin treatment of the mutagenized culture by the method of Gorini and Kaufman (7).

(iv) Mapping of mutations in auxotrophs. Mutations in methionine mutants were mapped by the auxanographic procedure of Smith (17). Mutants which showed growth only in the presence of both vitamin B12 and methionine were characterized as *metE* mutants. Mutations of the other mutants were mapped by interrupted mating experiments with appropriate Hfr strains according to Sanderson and Demerec (15).

(v) Spontaneous mutants. Streptomycin-resistant mutants were obtained spontaneously by plating a sample of the culture on LB agar with streptomycin sulfate and incubating it at 37°C for 48 h.

(vi) Thymine-requiring mutants. Thymine-requiring mutants were isolated by the procedure of Stacey and Simson (18), which employed trimethoprim (a gift of George Hitchings of Burroughs Wellcome Co.).

Conjugation experiments. Standard mating experiments were performed by the method of Sanderson and Demerec (15).

Transfer of gamma sensitivity. Interrupted mating experiments were performed between wild-type donor Hfr strains and resistant recipient strains. The strains used are listed in Table 2. Selective minimal agar plates supplemented appropriately were used to obtain recombinants. The time of transfer of the gamma-sensitive marker was obtained by screening 100 single-colony isolates of the recombinants at each mating time interval. The colonies were transferred onto LB plates and incubated overnight. Replicas of the colonies were made on LB plates and were immediately exposed to 150 krads of gamma irradiation. The plates were scored for number of gamma-sensitive recombinants. The gamma- and UV-irradiation survival curves of the sensitive recombinants were obtained to determine the level of resistance remaining.

Further transfer of gamma sensitivity was carried out in a cross between PV77 (HfrB3) and SI101, a *his* mutant of the SI10 *met*$^+$ recombinant, which had acquired the *garA*$^+$ locus. The *his*$^+$ recombinants were screened for sensitivity to 125 krads, and survival curves of the sensitive recombinants were obtained.

Isolation of gamma-resistant Hfr strains. Gamma-resistant Hfr strains were isolated by carrying out crosses between wild-type Hfr strains and resistant auxotrophs carrying late markers (Table 3). The late marker recombinants from 5.5 h of uninterrupted mating were screened for those which were still resistant to 150 krads and had acquired the Hfr terminus together with the late marker. The Hfr ability was assayed by plate mating, employing the female strains and media listed in Table 3. The Hfr recombinants were also screened for a given auxotrophic marker which would be used for counter-selection of the Hfr in crosses with female strains.

Transfer of gamma resistance. Interrupted mating experiments were performed between gamma-resistant Hfr strains and wild-type or sensitive female strains (Table 4). The appropriate selective media were used for mapping the time of transfer of the auxotrophic markers. To check for transfer of gamma resistance genes directly, the mating mixture was plated on LB agar with streptomycin and incubated at 37°C for 3 h to allow for phenotypic expression, and the plates were exposed to 100 krads of irradiation. Transfer of gene *uvrB*$^+$ was selected for directly by exposure of LB plates to 81 J of UV irradiations per m^2. Single-colony isolates of recombinants were also checked for acquisition of resistance to 100 krads by replica plating. Gamma- and UV-irradiation survival curves of resistant recombinants were obtained to determine the level of resistance transferred.

Spontaneous degradation of DNA. Cells were labeled by growing the cells in 20 ml of EM9 medium with 2.5 μCi of [^{3}H]thymidine (specific activity, 6.7 Ci/mmol) and 500 μg of deoxyadenosine per ml for 16 h at 37°C with aeration. The cultures were chilled, centrifuged, and washed twice with cold M9 medium. The cells were resuspended in cold medium and incubated for 30 min. Spontaneous degradation of DNA was measured as the percentage of total radioactivity of acid-insoluble material, using a method similar to that of Davies et al. (6).

Repair of single-strand breaks. The repair of single-strand breaks in DNA was studied by zone sedimentation on alkaline sucrose gradients, using a modified method of Davies et al. (6). Sedimentation was done in a Spinco SW50.1 rotor spun for 40 min at 40,000 rpm.

Post-irradiation DNA degradation during repair.

TABLE 2. Transfer of gamma sensitivity from wild-type Hfr strains to R68

Donor D10 (krads)	Recipient D10 (krads)	Recombinant D10 (krads)	Map position of sensitivity marker (units)	Map position avg (units)
PV42 (HfrA) (13)	SI10 (163)	SI101 *metE*$^+$ *garA*$^+$ (38 ± 11)	13.6	17.5
DB63 (HfrK4) (13)	SI9 (188)	SI91 *araB*$^+$ *garA*$^+$ (39 ± 11)	21.4	
PV77 (HfrB3) (15)	SI13 (200)	SI131 *serA*$^+$ *garB*$^+$ (88 ± 18)	49.5	49.5
PV77 (HfrB3) (15)	SI101 *garA*$^+$ (38)	SI18 *his*$^+$ *garA*$^+$ *garB*$^+$ (13 ± 1)	>48	

TABLE 3. Isolation of gamma-resistant Hfr strains

Donor D10 (krads)	Markers	Recipient D10 (krads)	Markers	Selective medium	F^-, (markers), and medium for plate mating	Markers of the selected recombinants	Symbol D10 (krads)
PV42 (HfrA) (13)	*purC7 strA*	SI15 (200)	*serA his*	M9 + his	SI12 (*metE trp*) on M9 + trp	HfrA *his garA*	SI6 (35)
DB46 (HfrB2) (13)	*hisD gal*	SI12 (163)	*metE trp*	M9 + met	SI14 (*his strA*) on M9 + str	HfrB2 *metE garB*	SI8 (43)
DB63 (HfrK4) (10)	*serA13*	SI10 (163)	*metE*	M9	SI1 (*leuD proB ara strA*) on M9 + pro + str	HfrK4 *garA*	SI7 (35)

When cells were lysed for DNA single-strand breakage studies, 0.1-ml samples of irradiated cells were also used to obtain the percentage of degradation of DNA at various time intervals during repair. The procedure for spontaneous DNA degradation was followed. Unirradiated cells served as controls.

RESULTS

Transfer of gamma sensitivity. The results of a typical interrupted mating experiment between wild-type HfrPV77 and R68 auxotroph SI13 are shown in Fig. 1a, and the typical gamma-irradiation survival curves of the parent and recombinant strains are shown in Fig. 1b. Data on other crosses are shown in Table 2. In a cross between PV42 (HfrA) and SI10, $metE^+$ was transferred early. When 100 recombinants were scored for gamma sensitivity to 150 krads, a gamma sensitivity factor designated $garA^+$ was transferred at about 45 min of mating. Since HfrA has its origin at 81 map units, the $garA^+$ marker thus extrapolates to a map position of $(45/138 \times 100) - 10 = 13.6$ map units. The gamma-irradiation survival curves of the double recombinants indicated that the resistance of R68 *metE* was not reduced to the wild-type level by the acquisition of this factor. The D10 value of SI10 was reduced from 163 to 38 ± 11 (Table 2).

The $garA^+$ locus was confirmed in another cross between DB63 (HfrK4) and SI9. The $garA^+$ factor was transferred to R68 $araB^+$ recombinants at about 35 min of mating. Since HfrK4 starts transferring its DNA at 96 map units, $garA^+$ thus extrapolates to a map position of $(35/138 \times 100) - 4 = 21.4$ map units. The double recombinants also had a mean D10 value of 39 ± 11, indicating that gamma resistance of R68 *araB* was not reduced to the wild-type level.

From both crosses, the average map position for the $garA^+$ marker was therefore 17.5 map units (Table 2).

Another gamma sensitivity marker, designated $garB^+$, was acquired by R68 $serA^+$ recombinants from PV77 (HfrB3) in a cross between this Hfr and SI13 (Fig. 1a). This factor was transferred at about 27 min of mating. HfrB3 has its origin at 69 map units; therefore, $garB^+$ extrapolates to a map position of $69 - (27/138 \times 100) = 49.5$ map units. The gamma-irradiation survival curves are shown in Fig. 1b. The D10 value of the R68 parent was reduced from 200 to 88 ± 18, also not to the wild-type level.

Further transfer of gamma sensitivity. In a cross between SI101, a *his* mutant of the R68 $metE^+$ $garA^+$ double recombinant, and PV77 (HfrB3), the his^+ recombinants were scored for sensitivity to 125 krads of irradiation. The recombinants were found to have acquired the $garB^+$ factor before his^+. his^+ was transferred at 29 min of mating, extrapolating to a map position of $69 - (29/138 \times 100) = 48$ map units. The $garB^+$ factor therefore mapped at a position greater than 48 map units. The recombinants had the wild-type level of resistance. Therefore, the resistance of R68 *metE* was reduced to the wild-type level with a D10 value of 13 ± 1 by the acquisition of both *ga* A^+ and $garB^+$ markers from HfrA and HfrB3, respectively (Table 2).

TABLE 4. Transfer of gamma resistance to wild-type and sensitive female strains

Donor D10 (krads)	Recipient D10 (krads)	Recombinant D10 (krads)	Map position of resistance marker (units)	Map position avg (units)
SI6 (HfrA) (35)	SI5 (8)	SI51 (*gal-bio-uvrB*)$^+$ his^+ *garA* (23 ± 4)	16.5	18.5
SI7 (HfrK4) (35)	SI2 (13)	SI2 *garA* ND[a]	20.6	
SI8 (HfrB2) (43)	DB25 (13)	DB251 $thyA^+$ *garB* (34 ± 8)	<64.9	
SI8 (HfrB2) (43)	SI3 (4)	SI31 $thyA^+$ $recA^+$ *garB* (34 ± 8)	<70.8	
SI8 (HfrB2) (43)	SI4 (13)	SI41 $hisD^+$ *garB* (34 ± 8)	49	49

[a] ND, Not determined.

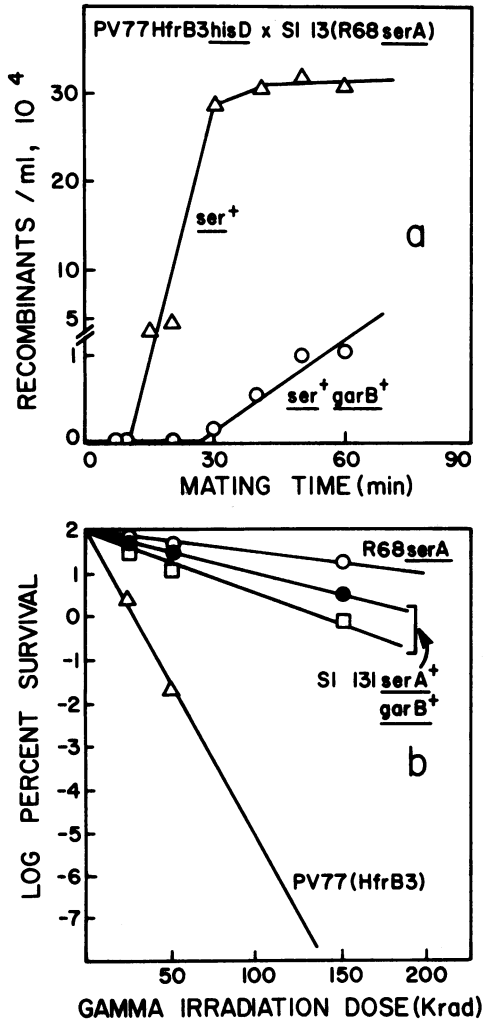

FIG. 1. (a) Kinetics of transfer of *ser*$^+$ and gamma sensitivity locus *garB*$^+$ from PV77 (HfrB3) to SI13 (R68 *serA*). One hundred *ser*$^+$ recombinants at each mating time interval were transferred onto LB plates and incubated overnight at 37°C. Replicas of the colonies were made on LB plates and immediately exposed to 150 krads of gamma irradiation. The plates were scored for gamma-sensitive recombinants after incubation at 37°C for 24 h. The numbers of *ser*$^+$ *garB*$^+$ double recombinants were then calculated. (b) Gamma-irradiation survival curves for SI13, PV77, and SI131, a *serA*$^+$ *garB*$^+$ double recombinant of SI13. Exponential-phase cells were irradiated in 0.067 M phosphate buffer at 0°C for various time intervals. Samples were diluted and plated on LB agar.

Transfer of gamma resistance. The results of a typical interrupted mating experiment between gamma-resistant HfrSI8 and the wild-type female multiple auxotroph SI4 are shown in Fig. 2a, and the typical gamma-irradiation survival curves of the parents and recombinant strains are shown in Fig. 2b. Data on other crosses are listed in Table 4. SI7 (gamma-resistant HfrK4) was isolated from a cross of DB63 (HfrK4) with SI10 (R68 *metE*) (Table 3). In a cross of SI7 with SI2 *ara leuD trp his strA*, transfer of gamma resistance factor *garA* was selected for directly by exposure of the mating mixture on LB-streptomycin plates to 100 krads of irradiation. *ara*$^+$ was transferred early before 10 min = < 2 map units; *garA* at 34 min = (34/138 × 100) − 4 = 20.6 map units; *trp*$^+$ at 52 min = 33.7 map units; and *his*$^+$ at 68 min = 45.3 map units. SI7 turned

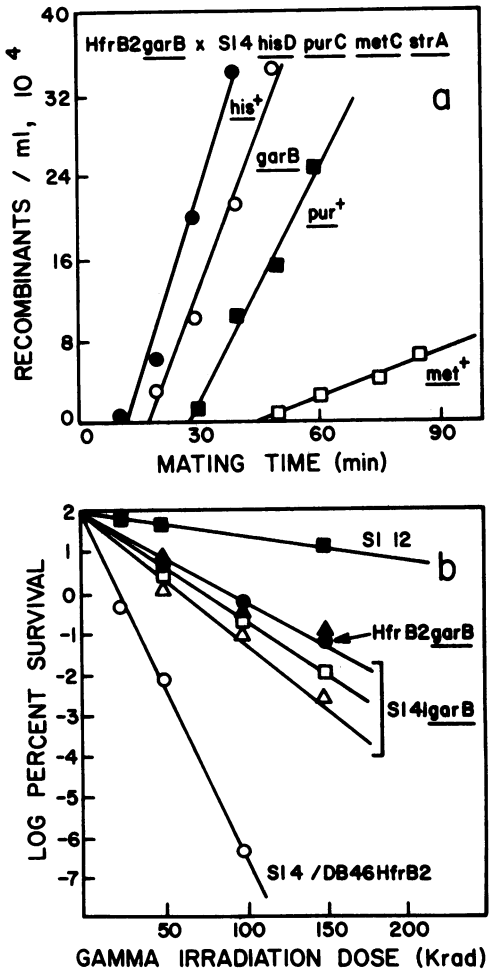

FIG. 2. (a) Kinetics of transfer of *hisD*$^+$, *garB*, *purC*$^+$, and *metC*$^+$ markers from SI8 (HfrB2 *garB*) to SI4, a streptomycin-resistant multiple auxotroph. Recombinants were selected directly on the appropriate selective medium. To check for transfer of the *garB* marker, the mating mixture was plated on LB agar with streptomycin and incubated at 37°C for 3 h before the plates were exposed to 100 krads of irradiation. (b) Gamma-irradiation survival curves for SI12, DB46, SI8, SI4, and SI41, a *his*$^+$*garB* recombinant. SI8 was isolated from a cross of DB46 with SI12.

out to be unstable; hence, further studies could not be carried out after this preliminary cross.

A second cross was carried out between SI6 (HfrA *garA*) and SI5, a radiation-sensitive *his strA* mutant with a *gal-bio-uvrB-chl* deletion. The *garA* marker was transferred simultaneously with gal^+ bio^+ $uvrB^+$ at about 49 min of mating and his^+ at about 80 min. Therefore, *garA* mapped at about (49/138 × 100) − 19 = 16.5 map units. The recombinants had an average D10 value of 23 ± 4, compared with a D10 of 8 for the radiation-sensitive parent. The average map position of *garA* gene, using HfrK4 and HfrA, was therefore estimated at 18.5 map units (Table 4).

In conjugation experiments between SI8 (HfrB2 *garB*) and DB25 (*thyA*) and SI3 (*thyA recA*), thy^+ was transferred at about 40 and 48 min of mating, respectively. The thy^+ recombinants were found to have acquired the *garB* marker before *thyA*; hence, *garB* mapped before 36 + (40/138 × 100) = 64.9 map units. The presence of *recA* in SI3 slowed down recombination. The thy^+ *garB* recombinants exhibited a mean D10 value of 34 ± 8 krads (Table 4).

A more precise mapping of the *garB* mutation was carried out in a cross of SI8 with SI4, a multiple auxotroph. *garB*, selected for directly, was transferred after his^+ and before $purC^+$ (Fig. 2a). his^+ was transferred at 11 min, (36 + 8) = 44 map units; *garB* at 18 min, [36 + (18/138 × 100)] = 49 map units; $purC^+$ at 27 min, (36 + 19.5) = 54.5 map units; and $metC^+$ at 45 min, (36 + 32.6) = 68.6 map units. The his^+ *garB* recombinants studied had an average D10 value of 34 ± 8 krads (Table 4). The gamma-irradiation survival curves are shown in Fig. 2b.

Average map position of gamma resistance markers. The average map position of the *garA*/$garA^+$ marker was therefore 18 map units with HfrA and HfrK4 and 49.3 map units for the *garB*/$garB^+$ marker with HfrB2 and HfrB3 (Tables 2 and 4).

UV resistance of recombinants. In Fig. 3, survival curves of representative recombinants and parent strains are shown. The recombinants segregated into two sets. Those carrying the *garA* mutation showed a level of UV resistance much higher than wild-type level, whereas those carrying the *garB* mutation expressed a level of UV resistance only slightly higher than wild-type level. None of the recombinants exhibited a level of UV resistance as high as that of R68.

Spontaneous degradation of DNA. The relative amounts of spontaneous DNA degradation in selected recombinants and parents are shown in Fig. 4. The recombinants also fell into two groups with respect to this phenotypic expression. Those carrying the *garB* mutation exhibited a high level of DNA degradation, equal to or higher than that of DB47, a *recA* mutant. Recombinants with the *garA* mutation did not degrade their DNA spontaneously. It was significant to observe that SI18 (R68 wild-type recombinant) showed no degradation of its DNA; nor did SI131 (R68 $serA^+$ *garA* recombinant), which acquired the $garB^+$ locus and still retained high gamma resistance. The only exception to this observation was that made with SI6 (HfrA), which was assumed to be carrying only the *garA* mutation. This strain showed a high rate of spontaneous DNA degradation and so may be carrying a *garB* mutation. This strain had a D10 value of 35 krads, and none of the SI51 recombinants which acquired *garA* from it attained its level of gamma resistance. This suggests the presence of other factors in SI6 not transferred to the sensitive female strain SI5. Again, none of the recombinants showed a level of spontaneous DNA degradation as high as that observed in R68.

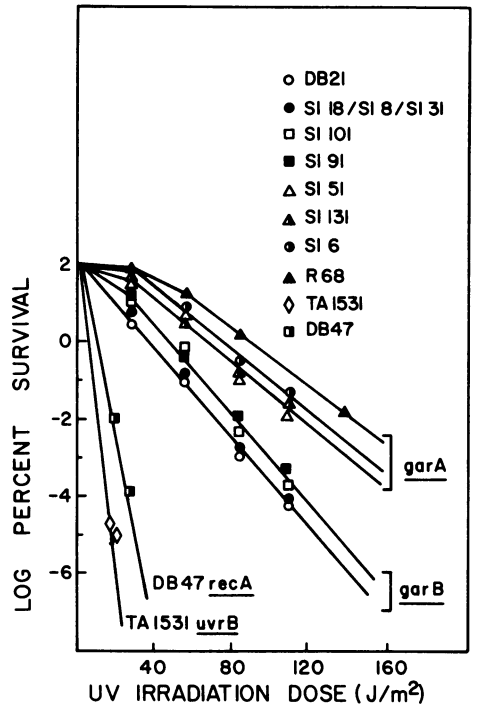

FIG. 3. UV-irradiation survival curves of recombinant and parent strains. The D10 value of each strain (in kilorads) is shown in parentheses. Symbols: ▲——▲, R68 *garA garB* (163); ◑——◑, SI6 (HfrA *garA*) (35); Δ——Δ, SI131 R68 $serA^+$ *garA* (88); △——△, SI51 his^+ (*gal bio uvrB chl*)$^+$ *garA* (23); □——□, SI101 R68 $metE^+$ *garB* (38); ■——■, SI91 R68 $araB^+$ *garB* (39); ●——●, SI18 R68 $garA^+$ $garB^+$ (wild type) (13), SI8 (HfrB2 *garB*) (43), and SI31 thy^+ rec^+ *garB* (34); ○——○, DB21 wild type (13); ◨——◨, DB47 *recA* (4); ◇——◇, TA1531 *uvrB* (8).

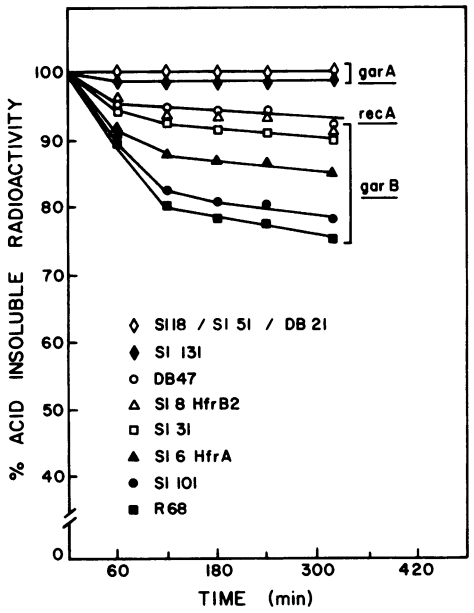

FIG. 4. Spontaneous degradation of DNA of recombinant and parent strains during incubation in 0.067 M phosphate buffer at 37°C. Stationary-phase cells labeled with [^{3}H]thymidine were suspended in nonradioactive EM9 medium for 30 min at 37°C. The cells were centrifuged and transferred into 20 ml of 0.067 M phosphate buffer. DNA degradation was measured by transferring 0.1-ml samples onto filter disks at various time intervals. The disks were dried, washed in bulk twice with cold 5% trichloroacetic acid and once in acetone, dried, added to vials with 10 ml of scintillation fluor, and counted. The percentage of total radioactivity which was acid insoluble was plotted against incubation time at 37°C.

Repair of single-strand breaks. The relative abilities of the recombinants to repair single-strand breaks induced by different doses of gamma irradiation were studied by using three strains: (i) SI18 (R68 wild-type recombinant), (ii) the parent R68, and (iii) SI51 (*gal*$^+$ *garA* recombinant with intermediate resistance). The observations are shown in Table 5. Both SI18 and SI51 repaired breaks induced by 25 krads within 40 min and those induced by 50 krads within 120 min and 75 min, respectively. However, SI18 could not repair breaks induced by 75 krads, whereas SI51 could repair these breaks after 120 min. When a dose of 100 krads was used, only R68 could repair the single-strand breaks induced, and repair was achieved within 120 min.

It appeared, therefore, that the *garA* marker inherited by SI51 controls activities which result in higher ability to repair single-strand breaks, although the *garB* marker is essential for the realization of the high repair ability of R68. Loss of the *garA* and *garB* markers in SI18 reduced repair ability and, hence, the gamma resistance of R68 to the wild-type level.

Post-irradiation DNA degradation. DNA breakdown in the three strains used for single-strand breakage studies was determined by measuring the radioactivity of acid-insoluble material during incubation of the exponential-phase cells after gamma irradiation. The results of the percent DNA degradation at 120 min (Table 5) show that as the dose was increased, the wild-type recombinant, SI18, degraded more of its DNA, followed by SI51, and the least degradation was observed in R68. There was complete degradation of the DNA of SI18 and SI51 after exposure to 100 krads at 120 min, compared with 48% degradation in R68.

DISCUSSION

The observations from interrupted mating experiments show that there are only two genes involved in the high radiation resistance of R68, and they map at about 18 and 49 map units on the linkage map of *S. typhimurium*.

This was indicated by the production of multiple classes of recombinants with different levels of resistance when R68 was crossed with wild-type Hfr strains and acquired wild-type alleles from two map positions. The formation of Hfr strains which acquired part of the resistance factors of R68 in the two locations and transferred these factors to females confirmed the existence of mutations at two loci. The reduction of the resistance of R68 to wild-type level by the acquisition of wild-type alleles from the two genes provided the final evidence for mutations in two genes. Results from phenotypic studies seemed to show that the actions of the two genes were additive towards attaining the high radiation resistance of R68.

The *garA* gene mapped near *gal* and *uvrB*, and the recombinants with *garA* mutation showed a much higher UV resistance level than the wild type. This observation is significant for two reasons. Firstly, the *rodA* mutation at 14 min near *gal* in *E. coli* K-12 confers rounded morphology and elevated UV resistance (11); therefore, it is possible that *garA* is the counterpart of *rodA* in *S. typhimurium*. Secondly, it has confirmed that the radiation resistance of R68 is not due to mutation to filamentous forms, because if the *envA* mutation at 2 min in *E. coli* were involved, it would have been transferred early by HfrA and HfrK4 used in mapping. R68 cells were indeed not found to be filamentous.

The *rodA* cells should be examined for resistance to gamma irradiation, ability to repair single-strand breaks, and lack of spontaneous degradation of their DNA, which are characteristics of *garA* cells. Cell envelope studies on a *rod*

TABLE 5. Effect of gamma irradiation dose on repair of single-strand breaks in DNA and DNA degradation during repair

Strain	Dose (krads)	Repair ability[a]	Time taken (min)	DNA degradation at 120 min (%)[b]
SI18	25	+	40	35
	50	+	120	58
	75	−	∞	60
	100	−	∞	95
SI51	25	+	40	ND[c]
	50	+	75	20
	75	+	120	35
	100	−	∞	95
R68	25	+	40	10
	50	+	75	15
	75	+	75	20
	100	+	120	48

[a] Two-milliliter samples of exponential-phase cells of SI18, SI51, and R68 prelabeled with [^{3}H]thymidine were suspended at 10^8 cells per ml in 0.067 M phosphate buffer and exposed to 0-, 25-, 50-, 75-, and 100-krad doses of ^{60}Co gamma rays. The cell suspensions were centrifuged, suspended in 5 ml of LB broth, and incubated at 37°C for repair periods of 40, 75, and 120 min. The cells were chilled, centrifuged, and suspended in 15% chilled sucrose in 0.01 M Tris-hydrochloride, pH 8.1. The cells were converted into protoplasts, and 0.1-ml samples were lysed on top of 5 to 20% alkaline sucrose gradients. The gradients were centrifuged at 40,000 rpm for 40 min in a Spinco SW50.1 rotor at 20°C, collected, and counted as by Davies et al. (6). The times within which repair of the single-strand breaks occurred were calculated from the sedimentation profiles.

[b] [^{3}H]thymidine radioactivity counts in trichloroacetic acid-insoluble fraction of 0.1-ml cell samples were obtained at various time intervals during incubation of irradiated cells in Luria broth. The counts were used to calculate the percentage of DNA degradation. Unirradiated cells served as controls.

[c] ND, Not determined.

mutant have been carried out (8). However, there has as yet been no relationship established between rounded morphology of *rodA* cells and their high UV resistance. Therefore, if the *garA* mutation is found to be responsible for the spheroid from of R68, the recombinants would provide useful tools for further studies on the relationship between radiation resistance and morphology of *S. typhimurium* cells.

The *garB* gene mapped near *purC* after *his* at about 49 map units, a map position which appears distant from known markers that affect radiation resistance. The *lar-1* mutation in *E. coli* B at 68 min was not present in R68 because this marker would have been transferred very early by HfrB3, which has its origin at 69 map units and transfers in a counterclockwise direction. This observation is significant because it confirms the difference in the phenotypes of the two mutants. The *lar* mutant contained three times more DNA and RNA than did the parent cell (9), whereas the R68 cell had the same DNA content as the wild type and was not polyploid, though it was large (5). The *envB* locus at 70 map units was also not involved because it was not transferred by any of the Hfr strains used in this study, either by virtue of their origins and orientation or by the mating times used. It should be mentioned that the giant *E. coli* K-12 cells of Adler et al. (2) were reconstructed by Long et al. (10) by combining *envB* and *lon* mutations. The combination, however, yielded radiation-sensitive large cells because of the *lon* mutation which confers filamentous cell formation and radiation sensitivity (1). This study therefore shows that neither *envB* nor *lon* at 10 min was involved in the large cell shape of R68 because of their map positions and the radiation sensitivity induced by *lon* mutation. R68 cells were large but not giant cells.

The stationary-phase cells of the *garB* recombinants showed a high rate of spontaneous DNA degradation, equal to or higher than that observed in *recA* cells, and their UV resistance was only slightly elevated above wild-type level, unlike the *garA* recombinants. The *garB* mutants could be used to study the effect of the mutation on DNA nucleases and cellular activities responsible for this phenotype and its relationship to gamma resistance since these activities do not control highly elevated UV resistance. The UV-resistant mutant strain SA236 of Ahmad et al. (3) could not grow on minimal media, a fact that makes it unlikely that the unmapped mutation(s) involved are *garA* or *garB* since *garA* and *garB* recombinants and R68 grew on minimal media. Besides, the level of DNA polymerase activity in R68 was increased by a maximum of sevenfold (6) and not 35-fold, and DNA ligase activity was increased in R68 but not in SA236. Finally, it is concluded that a study of the activities of DNA polymerase I, endonuclease I, exonucleases I, II, and III, and DNA ligase in the *garA* and *garB* recombinants will throw more light on the phenotypic differences observed and may even help unravel the mysteries of biological damage by various kinds of radiation.

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