Quantitation of the Involvement of the *recA, recB, recC, recF, recJ, recN, lexA, radA, radB, uvrD,* and *umuC* Genes in the Repair of X-Ray-Induced DNA Double-Strand Breaks in *Escherichia coli*[1]

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SARGENTINI, N. J., AND SMITH, K. C. Quantitation of the Involvement of the *recA, recB, recC, recF, recJ, recN, lexA, radA, radB, uvrD,* and *umuC* Genes in the Repair of X-Ray-Induced DNA Double-Strand Breaks in *Escherichia coli. Radiat. Res.* **107,** 58–72 (1986).

Isogenic *Escherichia coli* strains carrying single DNA-repair mutations were compared for their capacity for (i) the repair of X-ray-induced DNA double-strand breaks (DSB) as measured using neutral sucrose gradients; (ii) medium-dependent resistance, i.e., a *recA*-dependent X-ray survival phenomenon that correlates closely with the capacity for repairing DSB; and (iii) the growth medium-dependent, *recA*-dependent repair of X-ray-induced DNA single-strand breaks (SSB) as measured using alkaline sucrose gradients (about 80% of these SSB are actually parts of DSB). These three capacities were measured to quantitate more accurately the involvement of the various genes in the repair of DSB over a wide dose range. The mutations tested were grouped into five classes according to their effect on the repair of X-ray-induced DSB: (I) the *recA, recB, recC,* and *lexA* mutants were completely deficient; (II) the *radB* and *recN* mutants were about 90% deficient; (III) the *recF* and *recJ* mutants were about 70% deficient; (IV) the *radA* and *uvrD* mutants were about 30% deficient; and (V) the *umuC* mutant resembled the wild-type strains in its capacity for the repair of DSB.

INTRODUCTION

DNA double-strand breaks (DSB) are considered to play a key role in the ionizing radiation-induced killing of *Escherichia coli* and other species (*1–3*). DSB can be induced by several mechanisms. They can be induced directly by the deposition of energy in both strands of the duplex or indirectly by the formation of hydroxyl radicals and their subsequent attack on the DNA (e.g., (*4*)). DSB can also result from enzymatic activity during the repair of ionizing radiation-induced DNA base damage and/or single-strand breaks (SSB) (*5*).

Early studies on whether DSB could be repaired yielded conflicting results (discussed in (*4, 6*)), but overwhelming evidence now indicates that the repair of DSB is a general phenomenon occurring in bacteria, yeast, and mammalian cells (reviewed in (*7*)). In *E. coli* the repair of DSB requires a functional *recA* gene (a gene required for genetic

[1] This paper is dedicated to the memory of Dr. Henry S. Kaplan (deceased: February 4, 1984), who pioneered the study of the repair of DNA double-strand breaks and who made major contributions to the study of the repair of DNA single-strand breaks.

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0033-7587/86 $3.00

recombination as well as for the regulation of cellular responses to DNA damage; reviewed in (*8*)) and duplicate genomes (*6*). Similarly, the repair of DSB in *Saccharomyces cerevisiae* requires the *RAD52* gene (a gene required for meiotic and induced mitotic recombination) and duplicate genomes (reviewed in (*9*)). The repair of DSB has also been demonstrated *in vitro* in a RecA-mediated reaction that requires homology between the participating DNA duplexes (*10*). These results strongly suggest that the repair of DSB is a recombinational process. This model is most clear in *S. cerevisiae* where the repair of DSB is proposed as a required step in meiotic recombination (*11*). However, there is also some evidence in *E. coli* and *S. cerevisiae* for fast, recombination-independent repair of DSB, perhaps requiring only DNA ligase (*12, 13*). Similarly, fast and slow components for the repair of DSB have also been demonstrated in mammalian cells (*12, 14*).

The repair of ionizing radiation-induced DSB is enhanced by a pretreatment with uv radiation (*15, 16*), and this enhancement is prevented by treating the cells between the pretreatment and challenge doses with the inhibitors of protein synthesis, rifampicin and chloramphenicol (*16, 17*). These characteristics, along with the *recA* requirement, suggest that the *in vivo* repair of ionizing radiation-induced DSB requires induction and is an "SOS" function (reviewed in (*8*)).

Since the process for the repair of DSB *in vivo* appears to (i) deal with DSB formed by different mechanisms (i.e., DSB with potentially different terminal groups), (ii) show both recombination-dependent and -independent features, (iii) show both fast and slow components, and (iv) require both regulatory and enzymatic elements, it can be predicted that the repair of DSB *in vivo* is an exceedingly complex process and involves many more gene products than might be suggested by the *in vitro* demonstration of the repair of DSB noted above.

To gain some insight into the complexity of the repair of DSB in *E. coli,* we have selected mutations (*recA, recB, recC, recF, recJ, recN, lexA, radA, radB, uvrD,* and *umuC*) that are known to block or diminish *recA*-dependent processes and have tested them for their effect on the repair of X-ray-induced DSB. Because of the difficulty in quantitating the repair of DSB over a wide range of X-ray doses, we have used three criteria to measure the repair of DSB. We have measured (i) the repair of DSB using neutral sucrose gradients, (ii) medium-dependent resistance (MDR, a phenomenon closely correlated with the capacity for the repair of DSB (*18*)), and (iii) growth medium-dependent (Type III) repair of DNA SSB [about 80% of these SSB are actually parts of DSB (*19*)].

MATERIALS AND METHODS

Bacterial strains. The strains used in this work are listed in Table I. Transductions were accomplished generally as described by Miller (*23*).

Media. MM is a 0.4% glucose–salts medium (*24*) supplemented with *L*-arginine, *L*-histidine, *L*-leucine, *L*-proline, and *L*-threonine (all at 1 m*M*) and thiamine · HCl at 0.5 μg/ml. YENB is yeast extract (Difco) at 0.75% and nutrient broth (Difco) at 0.8%. Media were solidified by adding Noble agar (Difco) at 1.6%. ^{3}H-YENB is YENB containing [methyl-^{3}H]thymidine (New England Nuclear, 80 Ci/mmol) at 20 μCi/ml. PB is Na_2HPO_4 at 5.83 g/liter and KH_2PO_4 at 3.53 g/liter, pH 7.0.

Cell preparation and X irradiation. Cells were grown overnight and diluted into homologous medium (YENB or ^{3}H-YENB, 500-fold; MM, 100-fold). Cultures were shaken at 37°C until their optical density at 650 nm (OD_{650}; Zeiss PMQ II spectrophotometer) reached 0.4, which for wild-type cells corresponds to 5

TABLE I

Strains of *Escherichia coli* K-12 Used[a]

Stanford radiology number	*Genotype*	*Source, reference, or derivation*[b]
SR48	As SR749 but *trp* Lac$^+$ *recC22*	JC5410, A. J. Clark
SR192	F$^+$? *metE thyA36 deo(C2?) lacZ53 rpsL151* λ$^-$ *lexA101*	(*21*)
SR255	As SR749 but *deoB16 recB21*	(*21*)
SR655	As SR749 but *uvrA6 recN262*	SR1474 × P1 · SR749, Tyr$^+$
SR669	HfrPO45 *ilv-318 thr-300 srlA300*::Tn*10 rpsE300* λ$^-$ *recA56*	JC10240, A. J. Clark
SR749	F$^-$ *argE3 hisG4 leuB6 Δ(gpt-proA)62 thr-1 thi-1 ara-14 galK2 lacY1 mtl-1 xyl-5 tsx-33 rpsL31 supE44* λ$^-$	AB1157, B. J. Bachmann
SR776	As SR749 but Thr$^+$ *radA100*	(*22*)
SR777	As SR749 but Thr$^+$	(*22*)
SR884	As SR749 but *tnaA300*::Tn*10 recF143*	JC12334, A. J. Clark
SR894	As SR749 but *srlA300*::Tn*10*	SR749 × P1 · SR669, Tc^r
SR895	As SR749 but *srlA300*::Tn*10 recA56*	As SR894
SR990	Hfr Hayes V *pheA*::Tn*10* pro*XIII thi lac*	NK6024, N. Kleckner
SR1018	As SR749 but *umuC122*::Tn*5*	GW2100, G. C. Walker
SR1026	F$^-$ *leuB19 metE70 thyA36 deo(C2?) bioA2 lacZ53 malB45 rha-5 rpsL151* λ$^-$ *radB101*	(*21*)
SR1075	As SR749 but *pheA*::Tn*10*	SR749 × P1 · SR990, Tc^r
SR1086	As SR749	SR1075 × P1 · SR1026, Phe$^+$
SR1087	As SR749 but *radB101*	As SR1086
SR1119	F$^-$ *deoC araD139 Δ(lac)U169 malE*::Tn*5 f16B relA rpsL*	T5M7, T. Silhavy
SR1120	As SR749 but *malE*::Tn*5*	SR749 × P1 · SR1119, Kn^r
SR1157	As SR749 but *thyA*	SR749, Tmp^r
SR1158	As SR749	SR1157 × P1 · SR255, Thy$^+$

TABLE I—*Continued*

Stanford radiology number	*Genotype*	*Source, reference, or derivation*[b]
SR1159	As SR749 but *recB21*	As SR1158
SR1165	As SR749 but *umuC122*::Tn*5*	SR749 × P1 · SR1018, Kn^r
SR1214	As SR749 but *tna300*::Tn*10*	SR749 × P1 · SR884, Tc^r
SR1215	As SR749 but *tna300*::Tn*10* *recF143*	As SR1214
SR1259	F$^-$ *argH1 his-4 met lacMS286* ϕ80dII *lacBK1 malΔ1 mtl-1 xyl-7 supE44 rpsL* λ^- *uvrD254*::Tn*5*	SK3451, S. R. Kushner
SR1277	As SR749 but *uvrD254*::Tn*5*	SR749 × P1 · SR1259, Kn^r
SR1278	As SR749	SR1120 × P1 · SR192, Mal$^+$
SR1279	As SR749 but *lexA101*	As SR1278
SR1474	As SR749 but *tyrA16*::Tn*10* *uvrA6 recN262*	SP264, S. M. Picksley
SR1552	As SR749	SR1075 × P1 · SR655, Phe$^+$
SR1553	As SR749 but *recN262*	As SR1552
SR1642	As SR749	SR1157 × P1 · SR48, Thy$^+$
SR1643	As SR749 but *recC22*	As SR1642
SR1660	As SR749 but Tsx$^+$ Sup$^+$ *recB21 recC22 sbcA23 recJ284*::Tn*10*	JC12105, A. J. Clark
SR1663	As SR749 but *recJ284*::Tn*10*	SR749 × P1 · SR1660, Tc^r

[a] Genotype nomenclature is that of Bachmann (*20*).

[b] Tc^r, Kn^r, and Tmp^r indicate that isolates were resistant to tetracycline, kanamycin, and trimethoprim (i.e., a *thyA* mutant), respectively. P1 indicates the use of P1vir, P1vira, or P1::Tn*9cts* as transducing bacteriophage.

$\times 10^7$ (YENB) or 2×10^8 (MM) colony-forming units per milliliter. Cells were filter-harvested, washed, resuspended in PB at an OD_{650} of 1.0, and X-irradiated (oxic, 50 kVp, room temperature) as described earlier (*25*) except that only the 50 mA X-ray tube was used and the dose rate was 2.8 krad min^{-1}. [For comparison, note that 50 kVp X rays produce 1.93 more DSB/krad/genome and 1.57 more SSB/krad/genome than ^{137}Cs γ rays (*26*).]

Cell survival. Cells were diluted in PB, plated in duplicate on homologous medium, and incubated 1 (YENB) or 2 (MM) days at 37°C.

Assay for DNA single-strand breaks. Irradiated ^{3}H-YENB-grown cells were diluted 20-fold into PB and YENB and shaken at 37°C for 20 and 120 min, respectively. The OD_{650} of the cultures was maintained at 0.05–0.4 by dilution. After incubation, cells were converted to spheroplasts by adding 0.2 ml of cells (OD_{650} = 0.05) to 0.2 ml of ice-cold 0.07 *M* Tris, 0.017 *M* EDTA, pH 7.6, containing lysozyme (Worthington Biochemical Corp.) at 400 μg/ml. After 10 min on ice, 0.1 ml of spheroplast suspension was layered onto

4.8-ml linear alkaline sucrose gradients (sucrose at 5–20%, NaOH at 0.1 *N*, Triton X-100 at 0.1%), each possessing a 0.1-ml cap of 0.5 *N* NaOH. After standing for more than 60 min at room temperature, the gradients were centrifuged (Beckman SW 50.1 rotors) at 10,000 rpm for 16 h at 20°C. Methods for gradient fractionation, the determination of trichloroacetic acid-insoluble radioactivity, and the calculation of DNA strand breaks have been described (*18, 25*).

Assay for DNA double-strand breaks. Unless otherwise noted, the procedures were as described above. Irradiated cells were diluted into YENB and repair-incubation times were either <15 s (i.e., "0 min") or 120 min. Spheroplasts were layered onto neutral sucrose gradients (as in (*5*), but containing Triton X-100 at 0.1%). After standing for more than 90 min at room temperature, gradients were centrifuged at 3700 rpm for 40 h at 20°C.

RESULTS

One complication in the quantitation of DSB using neutral sucrose gradients is that, for a time during the repair process after higher doses of ionizing radiation, the DNA from irradiated cells sediments faster than the DNA from nonirradiated cells (*6, 18, 27, 28*). This fast-sedimenting DNA makes the quantitation of nonrepaired DSB in *E. coli* increasingly difficult after 50 kVp X-ray doses greater than 4 krad. In this work, we have quantitated the repair of DSB in an essentially isogenic set of *E. coli* mutants, both for the lower dose range and for the higher dose range, where the fast-sedimenting DNA is observed. In Fig. 1, we show the putative initial and final yields of DSB induced by 1–4 krad. The *recA, recB, recC,* and *lexA* mutants were completely deficient in the repair of DSB; in fact, as best exemplified by the *recA* strain, some strains accumulated more DSB during the repair-incubation period than they initially possessed. Over this dose range, the *recF, recJ, recN, radA, radB, uvrD,* and *umuC* mutants were as proficient in the repair of DSB as the parental wild-type strain. These observations regarding the repair of DSB after 1–4 krad are summarized in Table II.

After 20 krad, a dose that ultimately induces fast-sedimenting DNA in wild-type cells, kinetic studies of DNA sedimentation on neutral sucrose gradients were used to assess the repair of DSB in our set of mutants. The DNA sedimentation profiles in Fig. 2 are for isogenic pairs of strains (i.e., mutant and wild type) and show the DNA sedimentation characteristics both immediately after X irradiation and after 120 min of repair incubation. The DNA sedimentation rate for irradiated DNA after minimal repair incubation (i.e., 0 min) was the same for each mutant and its cotransductant wild type (data not shown), and this sedimentation rate, which reflects the presence of DSB, was slow compared to the rate for nonirradiated DNA (cf., 0-min DNA for mutants and for nonirradiated wild-type cells, Fig. 2). The *recA, recB, recC,* and *lexA* strains were unable to convert the slow-sedimenting DNA to faster-sedimenting DNA during 120 min of repair incubation. The *recF, recJ, recN,* and *radB* strains were able to convert, to different degrees, slow-sedimenting DNA to faster-sedimenting DNA, while the *radA, uvrD,* and *umuC* strains were able to perform this conversion in a fashion similar to the wild-type strains. Of these last strains, the *radA* and *uvrD* mutants showed more fast-sedimenting DNA at the 120-min time point than did their cotransductant wild-type strains. The disappearance of slow-sedimenting DNA observed in Fig. 2 is quantitated in Table II.

MDR is defined as the enhanced ionizing radiation survival that is seen for logarithmic-phase cells grown in and plated on rich medium (e.g., yeast extract + nutrient

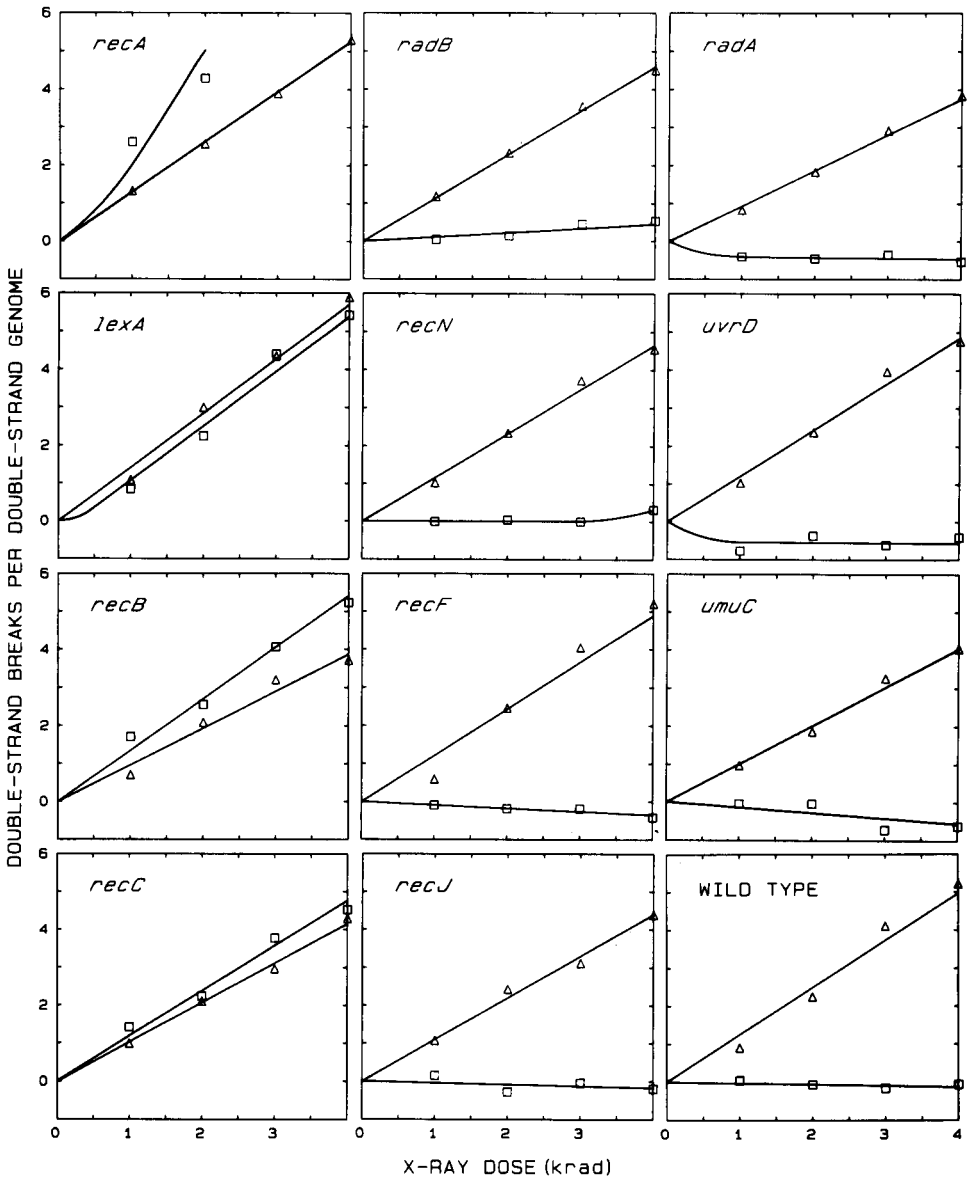

FIG. 1. Effect of DNA repair mutations on the repair of DNA double-strand breaks in X-irradiated (1–4 krad) *E. coli*. Cells were grown in YENB (yeast extract + nutrient broth), irradiated in buffer, then incubated at 37°C in YENB for 0 min (△) or for 120 min (□) before assaying for double-strand breaks per double-strand genome using neutral sucrose gradients. All strains were derived by transduction from SR749 (see Table I). Strains used are *recA* (SR895), *recB* (SR1159), *recC* (SR1643), *recF* (SR1215), *recJ* (SR1663), *recN* (SR1553), *radA* (SR776), *radB* (SR1087), *lexA* (SR1279), *uvrD* (SR1277), *umuC* (SR1165), and wild type (SR749). Points are the means from duplicate or more experiments.

TABLE II

Effect of Mutations on Phenomena Relevant to the Repair of X-Ray-Induced DNA Double-Strand Breaks

Class	*Mutation*[a]	*Relative repair of DNA double-strand breaks after 1–4 krad*[b]	*Relative disappearance of slow-sedimenting double-stranded DNA after 20 krad and incubation*[c]	*Relative medium-dependent resistance*[d]	*Relative type III repair of DNA single-strand breaks*[e]
I	*recA*	⩽0	−0.05, 0.15 (0.05)	0.01	−0.07
	lexA	0	0.07, 0.06 (0.06)	0.01	0.01
	recB	<0	−0.03, 0.09 (0.03)	0.00	0.09
	recC	<0	0.06, −0.03 (0.02)	−0.01	0.07
II	*radB*	1	0.21, 0.18 (0.20)	0.12	0.09
	recN	1	0.31, 0.39 (0.35)	0.13	0.31
III	*recF*	1	0.55, 0.56 (0.56)	0.26	0.31
	recJ	1	0.67, 0.60 (0.64)	0.31	0.46
IV	*radA*	1	0.93, 0.92 (0.92)	0.45	0.69
	uvrD	1	1.13, 1.18 (1.16)	0.87	0.62
V	*umuC*	1	0.97, 1.02 (1.00)	0.98	>1.00
	Wild type	1	1.00, 1.00 (1.00)	1.00	1.00

[a] Mutant strains are those listed for Fig. 1.

[b] Data shown in Fig. 1 were used to estimate the relative repair of DNA double-strand breaks after 1–4 krad. A value of 1 indicates complete repair; a value of zero or less, no repair.

[c] Data shown in Fig. 2 were used to quantitate the relative disappearance of "0-min" slow-sedimenting DNA. This quantitation yielded the first values listed. The second values listed were derived from a duplicate experiment (as in Fig. 2, but data not shown). The mean values are given in parentheses. These values are the fraction of 0-min DNA, between the relative distances sedimented of 0.0 and 0.5, that disappeared from that half of the gradient during 120 min of repair incubation in the mutants as compared to their wild-type strain.

[d] Data shown in Fig. 3 were used to determine the doses necessary to produce surviving fractions of 10^{-1}, 10^{-2}, and 10^{-3} for both YENB-grown (Y) and MM-grown (M) cells. Then at each survival level, the difference in dose survived by the Y and M mutant cells was divided by the difference in dose survived by the Y and M parental wild-type cells, SR749, to determine the fraction of medium-dependent resistance; the mean fraction for the three survival levels is listed.

[e] Data shown in Fig. 4 were used to determine the doses necessary to yield 15 single-strand breaks per single-strand genome in YENB-grown cells after maximal repair in buffer and in YENB. The difference in the doses obtained for each mutant was divided by the analogous value for the parental wild-type strain, SR749, to yield the fractional value listed.

broth) when compared to cells grown in and plated on minimal medium (e.g., glucose + mineral salts). When compared at equal-killing doses, rich medium-grown cells survive a three- to fourfold greater X-ray dose than do minimal medium-grown cells, and this survival is *recA*- and *lexA*-dependent (*25*). Because MDR is correlated with the repair of DSB (*18*), we tested the mutants for their ability to show MDR (Fig. 3). The *recA, recB, recC,* and *lexA* mutants did not show significant MDR. The *recF, recJ, recN, radA, radB,* and *uvrD* mutants showed partial deficiencies in MDR, while

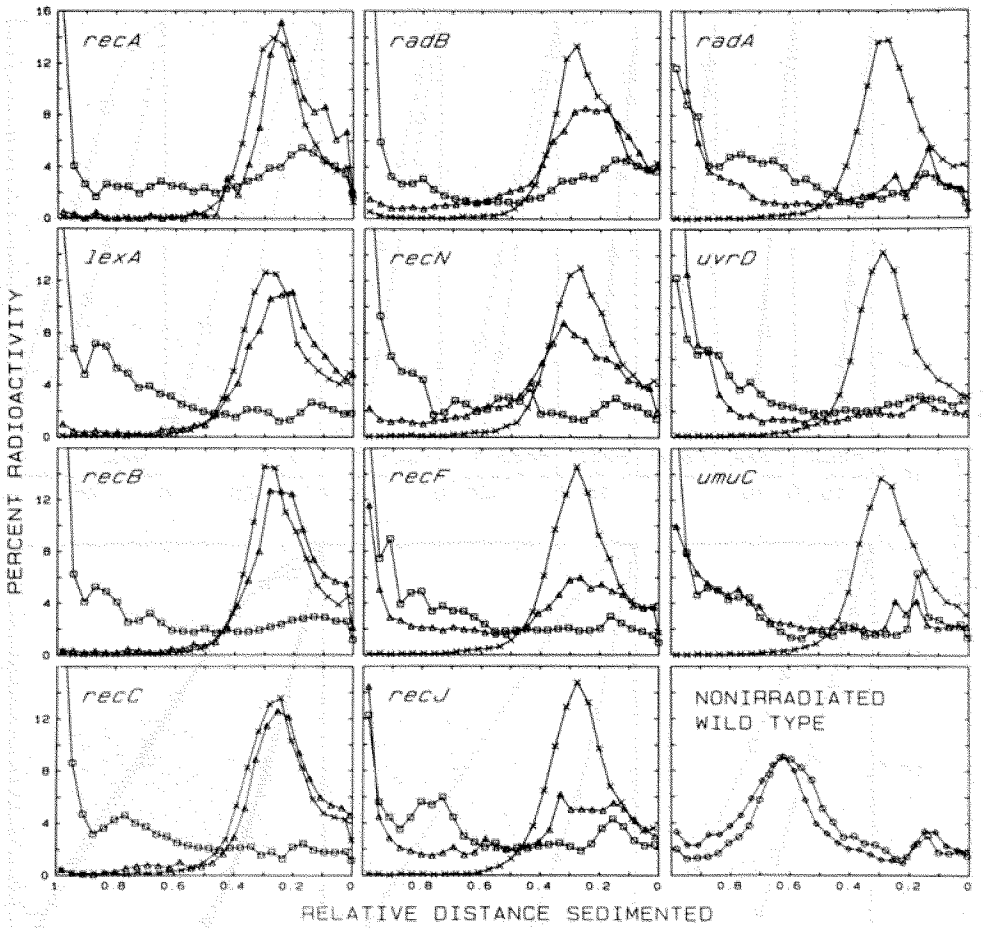

FIG. 2. Effect of DNA repair mutations on the repair of DNA double-strand breaks in X-irradiated (20 krad) *E. coli*. Cells were prepared as for Fig. 1; however, in this case the DNA profiles from the neutral sucrose gradients are shown. The DNA profiles for irradiated wild-type cells are for 120 min (□) of incubation; for irradiated mutant cells, the profiles are for 0 min (×) and 120 min (△) of incubation. The DNA profiles for nonirradiated wild-type cells (SR749) are for 0 min (◇) and 120 min (○) of incubation. Mutant strains used are those listed for Fig. 1; wild-type strains used are the isogenic cotransductants paired in Table I with those mutant strains, except for SR749, which was used for comparison with the three insertion mutants (*recN*, *uvrD*, and *umuC*). Duplicate experiments (not shown) gave similar results.

the *umuC* strain showed essentially a wild-type level of MDR. The relative amounts of MDR shown by these mutants have been quantitated in Table II.

Type III repair is defined as the additional repair that is detected for X-ray-induced SSB when one compares cells allowed to repair their SSB in growth medium with cells incubated in buffer (*29*) and is mostly the repair of DSB (*18, 19*). The measurement of Type III repair provides a quantitation of the repair of DSB after higher X-ray doses than can be studied with the neutral sucrose gradient technique. Therefore, we have quantitated the Type III repair of X-ray-induced SSB in rich growth medium for the

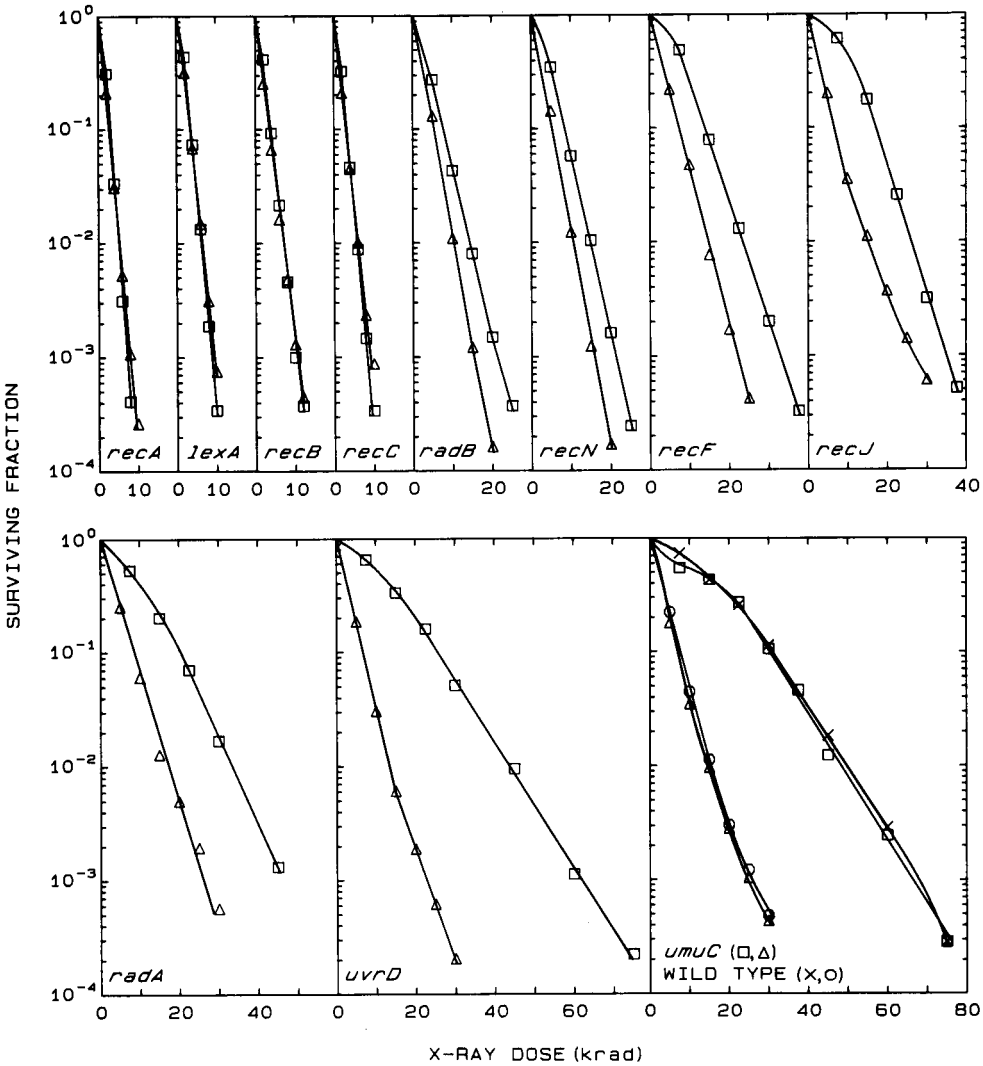

FIG. 3. Effect of DNA repair mutations on medium-dependent resistance in X-irradiated *E. coli.* Cells were grown in YENB (yeast extract + nutrient broth) (□, ×) or MM (minimal medium) (△, ○) irradiated in buffer, and plated on homologous media. Strains used are those listed for Fig. 1. Points are the means from triplicate experiments.

mutants listed above. The incubation times selected were more than sufficient to allow the completion of repair in the parental wild-type strain, SR749 (data not shown). Data in Fig. 4 indicate that the *recA* and *lexA* mutants do not perform Type III repair, while the *recB, recC, recF, recJ, recN, radA, radB,* and *uvrD* strains showed partial proficiencies, and the *umuC* strain showed the wild-type level of Type III repair. The relative amounts of Type III repair shown by these mutants have been quantitated in Table II.

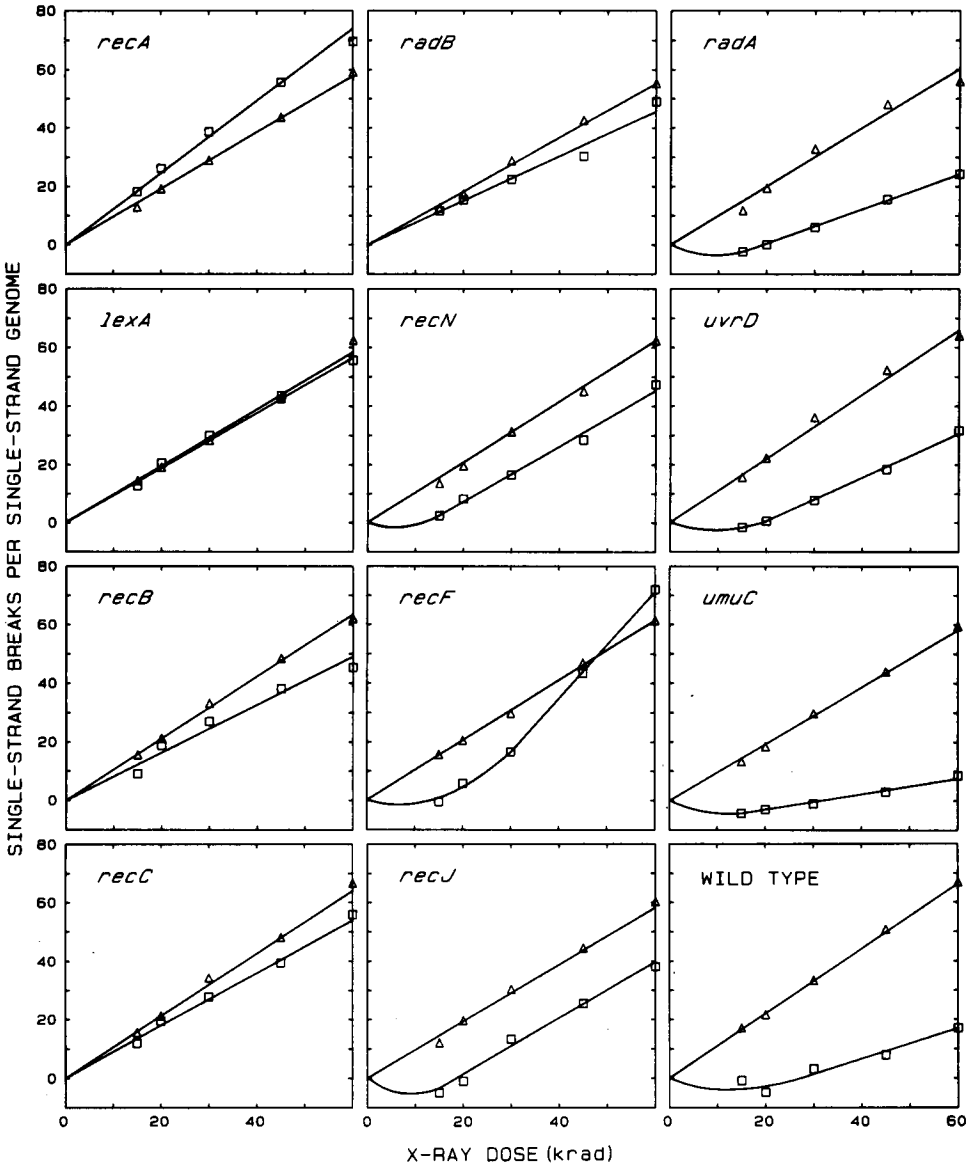

FIG. 4. Effect of DNA repair mutations on Type III repair of DNA single-strand breaks in X-irradiated *E. coli*. Cells were grown in YENB (yeast extract + nutrient broth), irradiated in buffer, then incubated at 37°C in buffer for 20 min (△) or in YENB for 120 min (□) before being assayed on alkaline sucrose gradients for single-strand breaks per single-strand genome. Strains used are those listed for Fig. 1. Points are the means from triplicate experiments.

DISCUSSION

To study the involvement of different gene products in the repair of X-ray-induced DSB, we tested a set of *E. coli* mutants using both direct and indirect assays for the

repair of DSB. For *E. coli* cells X-irradiated with 1–4 krad (Fig. 1), one can conclude that the *recA, recB, recC,* and *lexA* genes are essential for the repair of DSB and that the *recF, recJ, recN, radA, radB, uvrD,* and *umuC* genes are not involved. However, kinetic data for cells irradiated with 20 krad indicate that the *recF, recJ, recN, radA, radB,* and *uvrD* genes do play a role in the repair of DSB (Fig. 2). To confirm this and to better quantitate the involvement of these genes in the repair of X-ray-induced DSB, we used two indirect assays: the effect of mutations in these genes on MDR and on Type III repair. MDR is relevant because, compared to cells grown in minimal medium, cells grown in rich medium show a large enhancement in their capacity to survive X irradiation (i.e., they show MDR; (*25*)), and they show a large enhancement in their capacity to repair X-ray-induced DSB (*18*). Similarly, Type III repair is relevant because about 80% of the SSB dealt with by Type III repair are actually parts of DSB (*19*).

Based on these direct and indirect assays whose results are summarized in Table II, one can divide the mutations into five classes according to their effect on the repair of X-ray-induced DSB.

Class I mutations, i.e., *recA, recB, recC,* and *lexA,* are absolutely required for the repair of DSB induced by 1–4 or 20 krad of X rays. In support of this conclusion, these mutations completely blocked MDR and blocked 90–100% of Type III repair. Since about 20% of the SSB that require growth medium for their repair are not parts of DSB (*19*) and the *recB* and *recC* mutants appear to repair a similar fraction of SSB (compared vertically, Fig. 4), one can surmise that while the *recA* and *lexA* genes are essential for the Type III repair of SSB, the *recB* and *recC* genes are required only for the repair of those SSB that are actually parts of DSB. The conclusion that the *recA* gene is essential for the repair of X-ray-induced DSB has been stated earlier (*6, 30*). Our finding that the *lexA* gene is also required is consistent with the knowledge that it regulates most *recA*-dependent processes (reviewed in (*8*)). The absolute requirement of the *recB* gene is in agreement with its requirement for the repair of DSB occurring at nonrepaired daughter-strand gaps in uv-irradiated *E. coli* cells (*31, 32*). The requirement for the *recC* gene is consistent with the fact that both the *recB* and *recC* genes are required to code for a functional exonuclease V (e.g., (*33*)).

Class II mutations, i.e., *radB* and *recN,* differ from class I mutations in that the *radB* and *recN* mutants showed the complete repair of DSB after 1–4 krad and showed a small but significant conversion of slow-sedimenting DNA to fast-sedimenting DNA (as measured on neutral sucrose gradients) after 20 krad. This change in DNA sedimentation rate has been correlated with the repair of DSB (*6, 18, 27, 28*). That *radB* and *recN* cells possess a small capacity for the repair of DSB is supported by the fact that they showed a small capacity for MDR and for Type III repair. To a first approximation, we would average the percentage capacities for MDR and Type III repair (Table II) to conclude that these mutants show about a 90% deficiency in the repair of X-ray-induced DSB. For this calculation we have ignored the 31% value for Type III repair in the *recN* strain (Table II). We tend to think that the *radB* and *recN* mutations are alleles because they map to the same locus and have very similar phenotypes ((*21, 34*) and data not shown). It is important to note that while the *recN* gene has been shown to be involved in the repair of ionizing radiation-induced DSB (*34*), our results show that it is required to a lesser degree than are the class I genes.

Class III mutations, i.e., *recF* and *recJ,* allowed more conversion of slow-sedimenting

DNA to fast-sedimenting DNA than did the class I and II mutations. We conclude from our evaluation of the MDR and Type III repair data that these mutants show about a 70% deficiency in the repair of X-ray-induced DSB. The *recF* (*35*) and *recJ* (*36*) mutations are not allelic.

Class IV mutations, i.e., *radA* and *uvrD*, allowed more conversion of slow-sedimenting DNA to fast-sedimenting DNA on neutral sucrose gradients than did classes I–III and, in fact, they did this as well as the wild-type strains. However, class IV strains were deficient or delayed in their conversion of fast-sedimenting DNA to DNA that had an intermediate rate of sedimentation typical of nonirradiated DNA (Fig. 2). The *radC* mutant should also be included as a class IV mutant, based on studies reported earlier (*37, 38*). The *radA, radC,* and *uvrD* mutants show nonrepaired SSB after repair-incubation (Fig. 4 and (*38*)) on alkaline sucrose gradients, where fast-sedimenting DNA is not observed (we presume that it is alkali-labile (*18*)). Therefore, the delayed conversion of fast-sedimenting DNA to intermediate-sedimenting DNA in these mutants is consistent with the presence of nonrepaired DSB. From our evaluation of the MDR and Type III repair data, we conclude that the class IV mutants show about a 30% deficiency in the repair of X-ray-induced DSB. The *radA* (*22*), *radC* (*37*), and *uvrD* (*39*) mutations are not allelic. The deficiency in the repair of DSB in the *radA, radC,* and *uvrD* mutants is significant when compared to the class V mutant (*umuC*) and wild-type strains, which showed no deficiency in the repair of X-ray-induced DSB (Fig. 2, Table II).

Due to the limited knowledge concerning the functions of many of the genes that we have studied, it seems premature to propose an all-inclusive model for their roles in the repair of DSB, but a few points should be made. The *recA, recB,* and *recC* genes play major roles in genetic recombination (reviewed in (*40*)). This function along with the apparent requirement of multiple chromosomes for the repair of DSB (*6*) supports the premise that most of the repair of X-ray-induced DSB in *E. coli* involves a recombination mechanism(s). Models for such mechanisms have been described for the repair of the DSB that arise in uv-irradiated *uvrB* cells (*32*).

The requirement for *de novo* protein synthesis for the repair of DSB (*15, 16*) may relate to the inducible nature of the *recA* (reviewed in (*8*)), *recN* (*41*), and *uvrD* (*42–45*) genes. Like *lexA* (reviewed in (*8*)), the *recF* gene may be involved in the regulation of the *recA* gene (*46*), or it may regulate the protease activity of RecA protein (*47*).

Concomitant with the repair of DSB induced by higher doses of X rays, one can detect the formation of fast-sedimenting DNA on neutral sucrose gradients (*6, 18, 27, 28*). This fast-sedimenting DNA could result from an association with membrane material, since there is evidence for the involvement of DNA–membrane attachments in the repair of DSB in *Micrococcus radiodurans* (*27, 48*) and in *E. coli* (*28*), and since large amounts of RecA protein are associated with membrane fractions in *E. coli* after SOS induction (*49, 50*), as would occur in X-irradiated cells. Alternatively, this fast-sedimenting DNA could be large, unresolved DNA-recombination intermediates formed in the repair process. The delayed disappearance of fast-sedimenting DNA in the *radA, radC,* and *uvrD* mutants and their minor deficiency in the repair of DSB (indicated by the data for Type III repair and MDR) suggest that the repair of DSB is probably still occurring in the fast-sedimenting DNA.

In conclusion, we have shown that the repair of X-ray-induced DSB is completely

dependent on the *recA, recB, recC,* and *lexA* genes; partially dependent on *recF, recJ, recN, radA, radB,* and *uvrD* genes; and independent of the *umuC* gene; we suggest that the repair of DSB is a complex, multistep process.

ACKNOWLEDGMENTS

We are grateful to Dr. Israel Felzenszwalb, Dr. Rakesh C. Sharma, and Dr. Tzu-chien V. Wang for valuable discussions; to Dr. William P. Diver for his contributions to the initial phases of this project; and to Cheryl Cheng for skillful technical assistance. This work was supported by Public Health Service Grant CA-06437 from the National Cancer Institute, DHHS.

RECEIVED: December 16, 1985; REVISED: March 25, 1986

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