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Expression of *recA* in *Deinococcus radiodurans*

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***Deinococcus* (formerly *Micrococcus*) *radiodurans* is remarkable for its extraordinary resistance to ionizing and UV irradiation and many other agents that damage DNA. This organism can repair >100 double-strand breaks per chromosome induced by ionizing radiation without lethality or mutagenesis. We have previously observed that expression of *D. radiodurans recA* in *Escherichia coli* appears lethal. We now find that the RecA protein of *D. radiodurans* is not detectable in *D. radiodurans* except in the setting of DNA damage and that termination of its synthesis is associated with the onset of deinococcal growth. The synthesis of *Shigella flexneri* RecA (protein sequence identical to that of *E. coli* RecA) in *recA*-defective *D. radiodurans* is described. Despite a large accumulation of the *S. flexneri* RecA in *D. radiodurans*, there is no complementation of any *D. radiodurans recA* phenotype, including DNA damage sensitivity, inhibition of natural transformation, or inability to support a plasmid that requires RecA for replication. To ensure that the cloned *S. flexneri recA* gene was not inactivated, it was rescued from *D. radiodurans* and was shown to function normally in *E. coli*. We conclude that neither *D. radiodurans* nor *S. flexneri* RecA is functional in the other species, nor are the kinetics of induction and suppression similar to each other, indicating a difference between these two proteins in their modes of action.**

The eubacterial family *Deinococcaceae* contains five gram-positive species that are extraordinarily resistant to both ionizing and UV irradiation and many other agents that damage DNA (for reviews, see references 17, 18, and 21). *Deinococcus radiodurans*, the first species to be discovered (1), differs from the others in that it is naturally transformable and therefore is amenable to genetic manipulation (29). While it is well-established that *D. radiodurans* DNA damage resistance is due to exceedingly efficient DNA repair (17, 18), very little is known about the repair pathways employed by this organism. Previous studies in this laboratory and others have demonstrated that extremely efficient homologous recombination occurs following DNA damage and that this organism is able to repair fully >100 double-strand breaks per chromosome (introduced by ionizing irradiation) without lethality or mutagenesis. Deinococcal RecA is required for this remarkably efficient double-strand-break repair (6, 7, 10, 13).

To date, three deinococcal DNA repair genes, *recA*, *pol*, and *uvrA*, have been cloned and sequenced (10, 12, 17, 17a). At the level of the deduced amino acid sequence, none of these differ greatly from their *Escherichia coli* homologs. Mutagenization of any of these three *D. radiodurans* loci results in marked sensitivity to a range of different kinds of DNA damage. *D. radiodurans pol* and *uvrA* mutant strains can be restored to wild-type levels of DNA damage resistance by expression of their respective cloned *E. coli* homologs in the *D. radiodurans* mutant strain, indicating functional equivalence and suggesting that neither *pol* nor *uvrA* is uniquely responsible for the high levels of DNA damage resistance exhibited by *D. radiodurans* (11, 17a).

This functional equivalence does not appear to extend to the *recA* gene of *D. radiodurans*, as addressed in the present work. We previously reported results suggesting that expression of the deinobacterial *recA* gene in *E. coli* results in cellular death (10). This is unusual; cross-species functional interchangeability of *recA* genes is routinely achieved (24). Furthermore, as demonstrated in the present study, expression of the *Shigella flexneri recA* gene in *D. radiodurans* does not complement the *D. radiodurans recA* phenotype. The studies presented here extend our earlier work on the isolation and characterization of the deinobacterial *recA* gene (10).

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MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used are described in Table 1. *D. radiodurans* strains were grown in TGY broth (0.8% Bacto Tryptone, 0.1% glucose, 0.4% Bacto Yeast Extract) at 32°C with aeration or on TGY plates solidified with 1.5% agar. *E. coli* strains were grown in Luria-Bertani broth at 37°C with aeration, or on Luria-Bertani plates containing 1.5% agar. For *E. coli*, selective drug concentrations were 100 μg of ampicillin and 20 μg of chloramphenicol per ml. For *D. radiodurans*, selective concentrations of drugs were 3 μg of chloramphenicol and 8 μg of kanamycin per ml.

Transformation. *E. coli* strains were transformed with plasmid DNA by electroporation. A Bio-Rad Gene Pulser apparatus was used at the following settings: 1.8 kV, 25 mF, and 200 Ω. *D. radiodurans* strains were rendered competent with $CaCl_2$ and were transformed with plasmid DNA and high-molecular-weight genomic DNA as previously described (14, 16).

Protein extraction. A 500-ml *D. radiodurans* culture was harvested by centrifugation, and the cells were washed twice in 30 ml of saline, once in prelysis buffer (10 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 0.2 mM EDTA, 5 mM β-mercaptoethanol, 5% [vol/vol] glycerol), and once in butanol-saturated prelysis buffer and were resuspended in 1.5 ml of lysis buffer (0.25% Triton X-100 in prelysis buffer). The cell suspension was passed twice through a French pressure cell at 20,000 lb/in^2, and the lysate was centrifuged at 17,000 × *g* at 20°C for 20 min. The supernatant was aliquoted and stored at −70°C.

Western blotting (immunoblotting). Protein extracts were analyzed by Western immunoblotting by standard techniques (30). Polyclonal anti-RecA antisera were prepared from rabbits by Berkeley Antibody Co. (Berkeley, Calif.) with commercially supplied *E. coli* RecA (U.S. Biochemical, Inc.). Signal detection utilized goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase (Bio-Rad), which was used at a dilution of 1:2,000. Visualization was chromogenic (4-chloro-1-naphthol) or by the enhanced chemiluminescence system (ECL; Amersham), which was used according to the manufacturer's instructions.

RNA purification and Northern (RNA) hybridization. Total nucleic acid was isolated by a protocol which uses hexadecyltrimethyl ammonium bromide (CTAB) as described previously (10). DNA was removed by degradation with RNase-free DNase I (Sigma). RNA electrophoresis, transfer, and hybridization were as described elsewhere (25).

Densitometry. Densitometric analysis of autoradiograms was performed with a Hewlett Packard Scan Jet IIc and the program NIH 1.5.7. By repeated scannings, it was found that the error range for any given peak was no more than 9% of the peak size.

TABLE 1. Strains and plasmids used[a]

Strain or plasmid	Relevant genotype and phenotype[b]	Source or reference
D. radiodurans		
R1 wild type	gamma^r UV^r	1
R1-R	Spontaneous Rif^r mutant of R1	16
S77	pS11 transformant of R1; Km^r gamma^r UV^r	This work
rec30	MNNG-mutagenized strain of R1; gamma^s UV^s *recA*	20
1R1A	*recA* derivative of R1 acquired by site-specific insertional mutagenesis of the *recA*$^+$ gene	10
rec30$^+$	*recA*$^+$ derivative of rec30 acquired by transformation of strain rec30 with the cloned *D. radiodurans* *recA* gene from pJDC100; gamma^r UV^r	This work
S78	pS11 transformant of rec30; Km^r gamma^s UV^s	This work
S79	pP31 transformant of rec30; Km^r gamma^s UV^s	This work
S208	pP113 transformant of rec30; Cm^r gamma^s UV^s	This work
S209	pEL2 transformant of rec30; Cm^r gamma^s UV^s	This work
E. coli		
DH5α	*recA1 E. coli* cloning strain	Life Technologies
AB1157	*recA*$^+$ *E. coli* strain	8
JC10289	AB1157 derivative; Δ(*recA-srl*)	5
Plasmids		
pI3	*E. coli-D. radiodurans* shuttle vector; replicates as plasmid in both hosts; Ap^r in *E. coli* and Cm^r in *D. radiodurans*	15
pEL2	*E. coli-D. radiodurans* shuttle vector; expresses Ap^r in *E. coli* and Cm^r in *D. radiodurans*; integrative in *D. radiodurans*	28
pS11	*E. coli-D. radiodurans* shuttle vector; expresses Km^r in *E. coli* and *D. radiodurans*; integrative in *D. radiodurans*	28
pMK20	*E. coli* plasmid; 4 kb; *aphA* (Km^r) marker and *E. coli* replicon used in constructing pS19	27
pS19	*E. coli-D. radiodurans* shuttle vector derived from pUE11 and pMK20; replicates as plasmid in both hosts; Ap^r in *E. coli* and Km^r in *D. radiodurans*	27
p*Trc*99A	*E. coli* expression vector	Pharmacia
pSP72	*E. coli* expression vector	Promega
pBluescript SK$^+$	*E. coli* expression vector	Stratagene
pBluescript II SK$^+$	*E. coli* expression vector	Stratagene
pPROK-*recA*	1.2-kb *S. flexneri recA*-containing an *Eco*RI-*Hin*dIII fragment ligated into the *E. coli* expression vector pPROK-1	Kevin McEntee
pJDC100	1.4-kb *Alw*I-*Mae*III *D. radiodurans recA*-containing fragment from *D. radiodurans* R1, inserted into the *Xba*I site of pBluescript II SK$^+$	10
pP24	3.1-kb *Stu*I-*Fsp*I Cm^r-conferring fragment from pI3 ligated with pBluescript SK$^+$ cut with *Eco*RV	This work
pP23	2.0-kb *Nru*I-*Cla*I Cm^r-conferring fragment from pP24 ligated with pSP72 cut with *Eco*RV and *Cla*I	This work
pP26	1.2-kb *S. flexneri recA*-containing *Eco*RI-*Hin*dIII fragment from pPROK-*recA* ligated with pP23 cut with *Eco*RI and *Hin*dIII	This work
pP31	2.6-kb deinococcal promoter plus *S. flexneri recA Bgl*II blunt-end fragment from pP26 ligated with pS11 cut with *Bgl*II and *Dra*I	This work
pP84	1.2-kb *S. flexneri recA*-containing fragment from pPROK-*recA* ligated with p*Trc*99A	This work
pP89	5.0-kb *Eco*RI recircularized fragment from S79, containing pMK20 sequences and *S. flexneri recA*; replicates and expresses Km^r in *E. coli*	This work
pP90	2.5-kb *S. flexneri recA*-containing *Eco*RI-*Xho*I fragment from pP89, ligated with p*Trc*99A, cut with *Eco*RI and *Sal*I	This work
pP113	2.6-kb deinococcal promoter plus *S. flexneri recA Bgl*II blunt-end fragment from pP26, converted to a *Bam*HI-*Bgl*II fragment and ligated with pEL2 cleaved with *Bcl*I	This work

[a] *D. radiodurans* R1 was the first deinococcal strain to be isolated; it was discovered in X-ray-sterilized canned meat that had spoiled in Oregon in 1956 (1). It is the only deinococcal strain to be studied in detail with respect to its radiobiology and photobiology (17, 18). All *D. radiodurans* strains used here are R1 derivatives. *D. radiodurans* SARK is another *D. radiodurans* wild-type strain, which was discovered as a hospital contaminant in Ontario, Canada (22). SARK has only 33% DNA homology with the R1 strain (3). SARK has two natural plasmids, pUE10 and pUE11 (27), neither of which is present in R1. These plasmids have been modified for use as *D. radiodurans* R1-*E. coli* shuttle vectors (15, 27). The following shuttle vectors were used here: pI3 (from pUE10) and pS19 (from pUE11).

[b] gamma, gamma irradiation; UV, ultraviolet irradiation at 254 nm; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

Construction of pP31 and pP113. To determine whether a *recA* gene from another bacterial species could function in *D. radiodurans*, two *S. flexneri recA*-containing tandem duplication integrative expression vectors (pP31 [Km^r] and pS113 [Cm^r]) were constructed such that deinococcal promoters were immediately upstream of the *S. flexneri recA* gene (Fig. 1). The *S. flexneri* RecA protein is identical to the *E. coli* RecA protein; however, the gene of the former has more convenient restriction sites. Construction of pP31 uses the tandem duplication vector pS11 (11, 28). In *E. coli*, pS11 replicates as a plasmid because of the portion derived from the *E. coli* plasmid pMK20 (Table 1), which contains the *aphA* gene (Km^r) (11, 28). The *D. radiodurans* chromosomal DNA fragment in pS11 allows tandem duplication insertion into the chromosomal DNA in *D. radiodurans* (11, 28). The *aphA* gene permits Km drug selection of *D. radiodurans* transformants with the flanking *S. flexneri recA* gene. The construction of pP31 (Fig. 1) was as follows. The 3.1 kb Cm^r-conferring *Stu*I-*Fsp*I fragment from the shuttle plasmid pI3 (Table 1) was ligated with pBluescript SK$^+$ (Table 1) cut with *Eco*RV, generating the plasmid pP24. The pBluescript SK$^+$ Cm^r determinant in the fragment taken from pI3 to generate pP24 was excised from pP24 as a 2.0-kb *Nru*I-*Cla*I fragment, which was then ligated with pSP72 (Table 1) that had been cleaved with *Eco*RV and *Cla*I, generating the plasmid pP23. The *cat* gene was excised from pP23 by cleaving pP23 with *Eco*RI and *Hin*dIII, and it was replaced with a 1.2-kb *Eco*RI-*Hin*dIII fragment, containing the cloned *S. flexneri recA* gene from the plasmid pPROK-*recA* (10). The resulting plasmid, which contained *S. flexneri recA* downstream of a deinococcal promoter, was termed pP26. The promoter-*recA* combination was prepared as a 2.6-kb *Bgl*II blunt-end fragment.

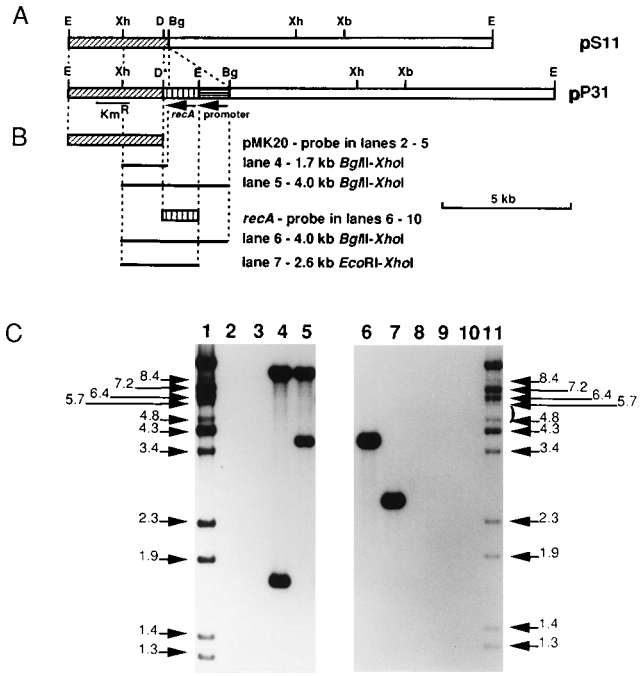

FIG. 1. Construction of pP31. (A) Restriction maps of the integrative vector pS11 (28) and the *recA*-containing derivative pP31. pMK20-derived DNA is indicated by diagonal hatching. Cloned *S. flexneri recA* and deinococcal promoter sequences derived from the shuttle vector pI3 (15) are indicated by vertical and horizontal hatching, respectively. The position of the *aphA* gene (Km^r) is represented by a horizontal line, and the relative locations and orientations of *recA* and the pI3-derived promoter are indicated by arrows. Abbreviations: Bg, *Bgl*II; D, *Dra*I; E, *Eco*RI; Xb, *Xba*I; Xh, *Xho*I; D*, a *Dra*I site that was destroyed by ligation with a Klenow-blunted *Hin*dIII end. (B) The respective positions of the pMK20 and the *S. flexneri recA* probes, shown as boxed segments. Restriction fragments that hybridize with the probes are indicated by horizontal black lines. Indicated in each case are (i) the size of the fragment to which each probe hybridizes, (ii) the restriction endonucleases that generate the complementary fragments, and (iii) the lane that contains it (see panel C). (C) Southern hybridization of chromosomal DNA from strains R1, rec30, S78 (rec30 containing a pS11 tandem duplication), and S79 (rec30 containing a pP31 chromosomal tandem duplication). Lanes 2 to 5 were probed with pMK20 DNA. The lanes contain *Bgl*II-*Xho*I double digests of total DNA from wild-type R1 (lane 2), rec30 (lane 3), S78 (lane 4), and S79 (lane 5). Lanes 6 to 10 were probed with the *S. flexneri recA* gene. These lanes contain *Bgl*II-*Xho*I double digests of total DNA, except for lane 7, which contains an *Eco*RI-*Xho*I double digest. Lanes: 6 and 7, S79; 8, S78; 9, wild-type R1; and 10, rec30 (lane 10). Lanes 1 and 11 contain DNA size standards (λ phage DNA cleaved with *Bst*EII). Sizes are expressed in kilobase pairs.

This was accomplished as follows: pP26 was cleaved with *Hin*dIII, and then the *Hin*dIII end was filled in with Klenow fragment and the linear DNA was cut with *Bgl*II. pP31 was generated by ligating this *Bgl*II blunt-end fragment with pS11 (Table 1) DNA cleaved with *Bgl*II and *Dra*I (Fig. 1). Thus, pP31 is the same as pS11, except that the *S. flexneri recA* gene and an adjacent deinococcal promoter are present (Fig. 1).

pEL2 and pP113 (not shown) are tandem duplication constructions similar to pS11 and pP31. pEL2, a tandem duplication vector in *D. radiodurans*, replicates in *E. coli* as a plasmid because of the *E. coli* plasmid pEL1 that contains *cat* (Cm^r) (28). pEL2 contains a segment of *D. radiodurans* chromosomal DNA that permits tandem duplication in *D. radiodurans*. The *cat* gene allows chloramphenicol drug selection for *D. radiodurans* transformants that contain the tandem duplications (28). pP113 is derived from pEL2, introducing a deinococcal promoter plus the *S. flexneri recA* gene. To construct pP113, the *Bgl*II blunt-end fragment described above was ligated with pSP72 which had been cut in the multiple cloning site with *Bgl*II and *Eco*RV. The resulting plasmid, pP100, was then cut with *Bgl*II and *Bam*HI, so that the promoter-*recA*-containing fragment was excised as a *Bgl*II-*Bam*HI fragment. This was ligated with pEL2 (Table 1) which had been cut with *Bcl*I, giving pP113.

The plasmids pS11 and pP31 (both Km^r) and pEL2 and pP113 (both Cm^r) were transformed into the *D. radiodurans recA* mutant strain rec30 (10, 20), and Cm^r or Km^r transformants were recovered in each case. Genomic DNA was purified from a representative number of these, and the placements and respective structures of the integrated plasmids were confirmed by Southern hybridization, with pMK20, pEL2, and the 1.2-kb *S. flexneri recA*-containing fragment from pPROK-*recA* (shown for pS11 and pP31; Fig. 1) used as probes.

RESULTS

Expression of *recA* in *D. radiodurans*. Detection of the RecA protein of *D. radiodurans* by Western immunoblotting used anti-RecA antiserum raised against *E. coli* RecA, since the deduced amino acid sequences of *D. radiodurans* and *E. coli* RecA are highly homologous (10). Immunoblots performed on one-dimensional sodium dodecyl sulfate-polyacrylamide gels of *D. radiodurans* protein extracts did not show the presence of a RecA protein with the use of chromogenic or chemiluminescence visualization. However, it was found that in extracts made after gamma irradiation, the deinococcal RecA was clearly visible (Fig. 2). In the *recA*-defective strain, rec30, deinococcal RecA protein was not detected by antiserum under any circumstances (not shown).

Northern blot analysis of *recA* expression in *D. radiodurans*. The failure to demonstrate RecA in unirradiated cultures by anti-RecA antiserum could have been due to a number of possibilities, including the following: (i) the anti-RecA antibodies in the antiserum did not have sufficient affinity to *D. radiodurans* RecA to allow detection, since the *D. radiodurans* protein may have been present in small amounts; or (ii) the *D. radiodurans recA* gene is novel in that it is not expressed in undamaged cells, or its products are not normally processed in cultures that are not DNA damaged. To differentiate between these possibilities, the presence of RecA-encoding mRNA was assessed by Northern blot analysis with the radiolabelled cloned *D. radiodurans recA* gene as a probe (Fig. 3). Northern blots showed no evidence for *recA* mRNA in preirradiated total RNA samples and the sample prepared from cells immediately postirradiation. A homologous band was observed in the 1.5-, 3-, 5-, 9-, and 12-h lanes. However, no homology was detected from time samples at 24 or 48 h postirradiation (Fig.

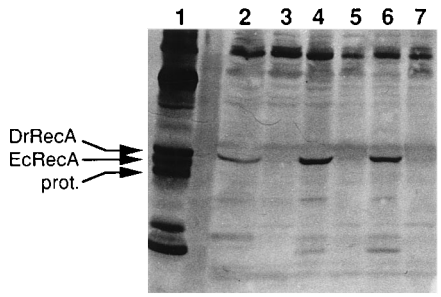

FIG. 2. RecA protein induction in *D. radiodurans*; Western immunoblot probed with rabbit polyclonal anti-*E. coli* RecA antiserum (1:100 dilution), showing induction of deinococcal RecA protein. *D. radiodurans* R1 was grown overnight to early plateau phase (10^8 cells per ml). Aliquots (50 ml) of culture without change of broth were gamma irradiated with ^{60}Co to 5×10^5 rads (5 kGy) on ice at a dose rate of 1 megarad/h. This radiation exposure under our culture and irradiation conditions (above) does not produce any lethality. Each 50-ml aliquot was separately pelleted in a clinical centrifuge and resuspended in 50, 250, or 600 ml of fresh TGY broth, and the suspensions were incubated for 2 h at 32°C with aeration. Controls were treated identically, except that they were mock irradiated. Protein extraction, electrophoresis, and chromogenic visualization are as described in Materials and Methods. Enhanced chemiluminescence visualization yielded identical results (not shown). All lanes contain 10 μg of protein. Lane 1 contains markers for *D. radiodurans* and *E. coli* RecA obtained as previously described (10). DrRecA, *D. radiodurans* RecA, which migrates more slowly than *E. coli* RecA because deinococcal RecA is 10 amino acids longer (10); EcRecA, *E. coli* RecA; prot., a major proteolytic fragment of *E. coli* RecA (10). Lanes 2, 4, and 6 contain protein derived from cells that had been irradiated and subsequently incubated in 50, 250, or 600 ml of fresh medium, respectively. Lanes 3, 5, and 7 are identical to lanes 2, 4, and 6, except that the cells were mock irradiated.

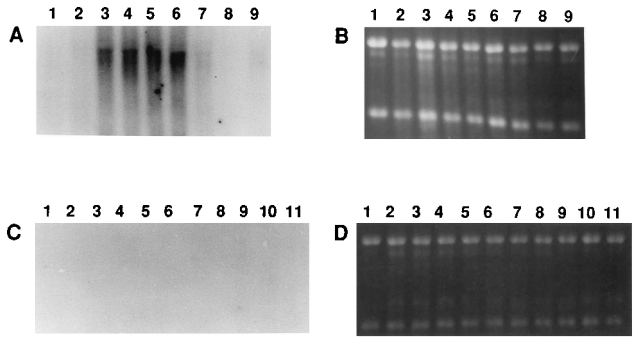

FIG. 3. *recA* mRNA induction in *D. radiodurans*. Total RNA was purified from *D. radiodurans* R1 at various time points following gamma irradiation and was probed with the *D. radiodurans recA* gene. A culture of 20 ml of *D. radiodurans* R1 was grown overnight to stationary phase. A 10-ml aliquot was removed and placed on ice. The remaining 10 ml was exposed to 1.75 megarads of ^{60}Co gamma radiation on ice at 1 megarad/h. The 1.75-megarad exposure is the D_{37} for *D. radiodurans* under the irradiation and culture conditions used in this study, where D_{37} is the dose required to obtain 37% viability of the cells. The cells were harvested, resuspended in 500 ml of fresh TGY broth, and incubated at 32°C with aeration. At the indicated times, 50-ml aliquots of culture were removed. RNA was purified as noted in Materials and Methods, and 500 ng of RNA from each time point was electrophoresed, transferred, and hybridized with radiolabelled *D. radiodurans recA* DNA consisting of the 1.4-kb fragment from pJDC100 (Table 1) for 18 h at 10^6 cpm/ml. (A) RNA purified from irradiated strain R1. Lanes 1 and 2, RNA from R1 immediately before and immediately after irradiation, respectively; lanes 3 to 9, RNA purified 1.5, 3, 5, 9, 12, 24, and 48 h postirradiation, respectively. (B) Ethidium bromide-stained agarose gel from which the Northern blot in panel A was generated, illustrating the rRNA in each lane. (C) RNA from a mock-irradiated control culture. Lanes 1 to 11, RNA purified at 0, 1, 2, 3, 4, 5, 6, 7, 10, 12, and 24 h, respectively. (D) Ethidium bromide-stained agarose gel from which the Northern blot in panel C was prepared.

3). In unirradiated cells diluted and incubated identically for the same duration, there was no evidence for *recA* mRNA (Fig. 3). rec30 total RNA samples failed to show detectable mRNA in this assay (not shown). This is consistent with the finding that RecA was undetectable on rec30 Western blots. These observations suggest that *D. radiodurans* RecA is not synthesized in *D. radiodurans* except after DNA damage. In addition, these findings suggest that the mutation in strain rec30 is regulatory in nature, since mRNA, protein, or fragments of either were not detected.

Expression of *S. flexneri recA* in *D. radiodurans*. To determine whether a *recA* gene from another bacterial species could function in *D. radiodurans*, *S. flexneri recA* integrative tandem duplication expression vectors, p31 and p113, were constructed (Fig. 1; Materials and Methods). The *S. flexneri* RecA protein is identical to the *E. coli* RecA protein; however, the gene of the former has more convenient restriction sites. pP31 confers Km^r and pP113 confers Cm^r, and the two vectors contain different segments of *D. radiodurans* chromosomal DNA, thereby allowing duplication insertion at two different *D. radiodurans* chromosome sites. After confirmation of duplication insertion by Southern blotting (Materials and Methods), protein extracts were prepared from strains S78 (containing pS11 alone [Table 1]), S79 (containing pP31), S209 (containing pEL2 alone), and S208 (containing pP113). The presence of *S. flexneri* RecA was demonstrated by Western immunoblotting with rabbit anti-*E. coli* RecA antiserum (Fig. 4). Protein bands characteristic of *E. coli* RecA were demonstrated in the *D. radiodurans* S79 and S208 extracts but were not present in those of S78 or S209 (Fig. 4).

There is no detectable activity of *S. flexneri* RecA in *D. radiodurans*. (i) Ionizing and UV radiation resistance. Strains S77, S78, S79, S208, and S209 were tested for resistance to gamma

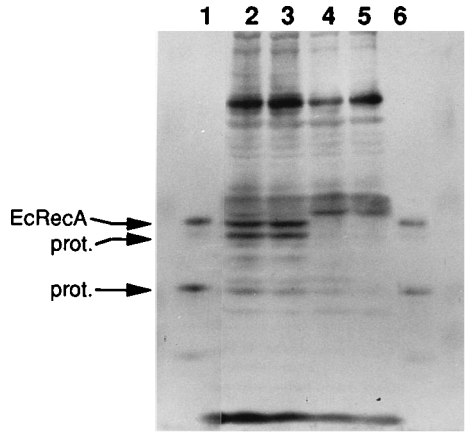

FIG. 4. *S. flexneri* RecA protein synthesis in *D. radiodurans*; Western immunoblot probed with rabbit anti-*E. coli* RecA antiserum (1:100 dilution), showing the presence of *S. flexneri* RecA in *D. radiodurans* S79 (rec30 that contains *S. flexneri recA* linked to Km^r) and S208 (rec30 that contains *S. flexneri recA* linked to Cm^r). All lanes contain 10 μg of protein. Lane 2, protein from strain S79; lane 3, protein from S208; lane 4, protein from S78 (rec30 plus pS11); and lane 5, protein from S209 (rec30 plus pEL2). Lanes 1 and 6 contain commercially obtained *E. coli* RecA protein (U.S. Biochemical). EcRecA, *E. coli* RecA; prot., proteolytic fragments of *E. coli* RecA.

ray and UV radiation (Fig. 5). The expression of *S. flexneri recA* had no detectable effect on the ionizing or UV radiation resistance of strain S79, which expresses *S. flexneri recA*, compared with strain S78, the pS11 (vector) transformant (Fig. 5). Similar results were obtained with S208 (which expresses *S. flexneri recA*) compared with the S209 control that contains only the vector pEL2 (not shown).

(ii) Transformability. Strains R1, rec30, S77, S78, and S79 were tested for transformability to rifampin resistance (Rif^r) with high-molecular-weight linear genomic DNA from the Rif^r strain R1-R (Table 1) and to Cm^r with the tandem duplication

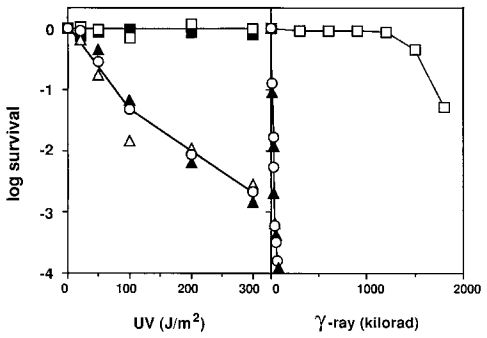

FIG. 5. Survival of *D. radiodurans* strains. For UV irradiation, overnight cultures in early plateau phase (10^8 cells per ml) were suitably diluted, and 0.5 ml in TGY was placed on agar plates. Instead of stroking of the dilution into the plates, they were allowed to dry by permitting sufficient time for the liquid medium to be absorbed by the plate (approximately 30 min) at 23°C. The plates were then exposed to UV radiation with the use of a 254-nm-wavelength germicidal lamp, which was calibrated with a UVX digital radiometer (Ultra-Violet Products, Inc.) at a dose rate of 0.15 J/m^2 s^{-1}. For gamma irradiation, overnight cultures in early plateau phase in TGY broth in aliquots of 10 to 50 ml were irradiated with ^{60}Co at a dose rate of 1 megarad (10 kGy) per h as determined with an ionization chamber. Aliquots were irradiated at 23°C. After irradiation, suitable dilutions were made and plated on TGY agar. Colonies were scored after 3 days. Symbols: solid squares, wild-type R1; open squares, strain S77 (R1 that contains the pS11 tandem duplication); open triangles, strain rec30; solid triangles, strain S78 (rec30 that contains the pS11 tandem duplication); and open circles, strain S79 (rec30 that contains pP31 tandem duplication and expresses *S. flexneri recA*).

vector pEL2. When transformed with Rif^r DNA, wild-type R1 and S77 both yielded approximately 5×10^5 Rif^r CFU per μg of transforming DNA, whereas the *recA* strains rec30, S78 (strain rec30 containing vector pS11), and S79 (rec30 containing pP31) yielded only 2×10^2 CFU per μg. These results show that *S. flexneri* RecA does not complement the deinococcal *recA* phenotype with respect to high-molecular-weight DNA transformation.

The transforming efficiency of tandem duplication vector pEL2 was found to be 2.4×10^3 CFU per μg in strain S77 (R1 plus pS11), whereas those for strains S78 (rec30 plus pS11) and S79 (rec30 plus pP31) were 7 and 7.5 CFU per μg, respectively. These results demonstrate that *S. flexneri* RecA does not substitute for *D. radiodurans* RecA with respect to tandem duplication insertion in *D. radiodurans.*

(iii) *S. flexneri* RecA does not support pS19 replication in *recA D. radiodurans*. pS19 is a 40-kb *E. coli-D. radiodurans* shuttle vector (Table 1). This vector consists of the full-length sequence of the naturally occurring plasmid pUE11 from *D. radiodurans* SARK (Table 1, footnote *a*) joined to sequences from the *E. coli* plasmid pMK20 (Table 1). pS19 confers Km^r and replicates autonomously in *E. coli* and in *D. radiodurans* R1. In the present study, it was found that pS19 requires RecA for replication in *D. radiodurans* R1, since strains without a *recA* mutation were readily susceptible to plasmid transformation with pS19, while *recA* strains rec30 and 1R1A (Table 1) were not transformable by pS19. *D. radiodurans* R1 contains no plasmids homologous to pS19. pI3 (Table 1), which is a shuttle vector derived from a different SARK plasmid (pUE10 rather than pUE11; Table 1, footnote *a*), transforms both *recA*$^+$ and *recA D. radiodurans* R1 with high efficiency.

Strains rec30, rec30$^+$, S208 (Cm^r; rec30 which contains pP113 and expresses *S. flexneri recA* [Fig. 4]), S209 (Cm^r; a strain S208 control that contains only the vector portion of pP113, i.e., just pEL2), and wild-type R1 were transformed with pS19 DNA. When strains R1 and rec30$^+$ were transformed with pS19 DNA, Km^r transformants were recovered with an efficiency of 3.7×10^4 and 1.23×10^5 CFU per μg, respectively. No pS19 transformants were recovered when strain rec30, S208, or S209 was transformed, despite the fact that all three strains were readily transformed with pI3. These results show that *S. flexneri* RecA, unlike *D. radiodurans* RecA, cannot support the replication of pS19.

Rescue of *S. flexneri recA* from *D. radiodurans* and expression in *E. coli*. Because the cloned and expressed *S. flexneri recA* gene in *D. radiodurans* failed to complement any feature of the deinococcal *recA* phenotype, it was necessary to verify that this gene in *D. radiodurans* was still functional. This was accomplished by rescuing the gene from strain S79 (rec30 plus pP31) and testing whether it complemented *E. coli recA* mutants. Genomic DNA from this strain was cut with *Eco*RI (see restriction map in Fig. 1), self-ligated, and transformed into *E. coli* DH5α. Km^r transformants were recovered and were found to harbor a plasmid, which was termed pP89. This plasmid consisted of pMK20 sequences ligated with the *S. flexneri recA* gene. A 2.5-kb *Eco*RI-*Xho*I fragment, containing the *S. flexneri recA* gene and a portion of pMK20, was excised and ligated with the expression vector p*Trc*99A which had been cleaved with *Eco*RI and *Sal*I in the multiple cloning site. The resulting plasmid, pP90, contained *recA* immediately adjacent to the *trc* promoter. pP90 was transformed into the *recA*-deleted *E. coli* strain JC10289 (5). In addition, pP84 (the 1.2-kb *S. flexneri recA*-containing *Eco*RI-*Hin*dIII fragment from pPROK-*recA* ligated with p*Trc*99A; Table 1) and p*Trc*99A without an insert were transformed into JC10289. Transformants containing pP90, pP84, and p*Trc*99A and the wild-type *E. coli* AB1157 (7) were tested for resistance to gamma radiation at doses of as much as 60 kilorads. pP84 and pP90 conferred wild-type levels of gamma ray resistance on JC10289. In contrast, JC10289 and the JC10289 transformant containing p*Trc*99A (no insert) were highly sensitive to gamma radiation (not shown).

The *recA* activity of the *recA* gene rescued from *D. radiodurans* was also determined by complementation of bacteriophage λ *red gam* mutants. Such mutants require a functional *recA* gene for propagation and cannot be propagated on *recA E. coli* strains (26). On AB1157, the titer of λ *red gam* was found to be 1.5×10^8 phage per ml. JC10289 transformants containing p*Trc*99A, pP84, or pP90 were infected with a λ *red gam* mutant. No plaques were detected when the recipient contained p*Trc*99A. However, when JC10289 cells containing either pP84 or pP90 (Table 1) were infected with the λ *red gam* mutant, the titer was 1.7×10^8 phage per ml. These results show that the *S. flexneri recA* gene rescued from *D. radiodurans* was functional, because it complemented an *E. coli* Δ*recA* mutant in conferring radioresistance and in supporting replication of λ *red gam* mutants.

DISCUSSION

The magnitude of RecA induction after gamma irradiation is very large, since there is no detectable RecA produced in undamaged *D. radiodurans* cells. Densitometry (not shown) of Fig. 2 (Western) and Fig. 3 (Northern) yielded estimates of 50- to 100-fold induction of *recA* expression and RecA synthesis. We have previously shown evidence that the synthesis of deinococcal RecA in *E. coli* is highly toxic or lethal (10). It might be the case that deinococcal RecA in *D. radiodurans* is also inhibitory to growth or is toxic and that only very low basal levels of expression can be tolerated. In this view, unlike other organisms, *D. radiodurans* selectively expresses its own *recA* following DNA damage, when it is central to repair. However, the *recA* locus is not expressed at a detectable level after completion of DNA repair and once growth is possible. This view is supported by the kinetics of cessation of deinococcal *recA* mRNA expression at 10 to 12 h following 1.75 megarads of irradiation (Fig. 3) and by the known time lag of 10 to 12 h following 1.75 megarads of irradiation and the onset of exponential growth (6, 7). In comparison, when RecA synthesis was calibrated by using several nalidixic acid (which induces *recA*) concentrations in *E. coli*, it was found that the optimal concentration for RecA synthesis was intermediate and resulted in an enhancement of RecA synthesis over control RecA synthesis of only sixfold (9).

In the *D. radiodurans recA*-defective strain rec30, *S. flexneri* RecA was expressed at high levels as determined by Western blotting (Fig. 4) but failed to alleviate the *D. radiodurans recA* phenotype; there were no changes in the ionizing and UV resistance of rec30 (Fig. 5), no restoration of natural transformability, and no restoration of the ability of rec30 to support pS19 replication. *S. flexneri recA* encodes the same protein as *E. coli recA*; however, *S. flexneri recA* rather than *E. coli recA* was used because of convenient DNA restriction sites.

The ability of pS19 to replicate in *recA*$^+$ but not *recA D. radiodurans* cells suggests that RecA (either at very low constitutive levels or at induced levels) is associated with plasmid transfer in *D. radiodurans*. While *D. radiodurans* is competent throughout exponential growth (29), it is known that other naturally transformable organisms that develop competence during select phases of growth show enhanced *recA* expression, as in *Streptococcus pneumoniae* and in *Bacillus subtilis* (4, 23). Thus, induction of *recA* may not be only in response to DNA damage in *D. radiodurans* but might also occur during plasmid

transfer. Such an effect would not be seen in Western or Northern blots, because only a tiny fraction of cells undergo plasmid transfer during an experiment (17). Although many hypotheses can be advanced, we suggest that the requirement for the *recA*$^+$ genotype in replication of pS19 may be due to a difference in the origin of replication of pS19 compared with pI3. In *E. coli*, for example, *oriC* is DnaA dependent but RecA independent, while *oriM* is DnaA independent but is dependent on RecA to initiate formation of D-loops (2).

It has been previously shown that the transformation efficiency of high-molecular-weight chromosomal DNA diminishes if the host is irradiated prior to transformation (19), and we have made similar observations (not shown). Thus, the induction of RecA accompanying DNA damage is not in itself sufficient to enhance transformation. We have also recently found that irradiation of wild-type R1 to exposures of as much as 1 megarad (nonlethal exposures, all of which stimulate *recA* induction) has no discernible effect on the high quantitative efficiency of plasmid transfer of pS19 and pI3 to the irradiated cells, which are transformed either immediately following irradiation or at later times (not shown). Thus, the diminished transformation efficiency seen with high-molecular-weight chromosomal DNA noted above may pertain to impared integration of high-molecular-weight DNA into the damaged chromosome. This effect does not apply to plasmid transfer, because no integration step occurs.

The inability of *S. flexneri* RecA to complement *D. radiodurans* rec30 and the apparently toxic effects of *D. radiodurans* RecA in *E. coli* (10) suggest that these proteins are fundamentally different, and this difference may reflect the different roles that these two proteins play in DNA repair. It is possible that the failure of *S. flexneri* RecA to complement the *D. radiodurans recA* phenotype is due to a cryptic mutation in the *S. flexneri recA* gene inflicted during the procedures required for cloning and expression of *S. flexneri recA* in *D. radiodurans* rec30. For this reason, we rescued the *S. flexneri recA* gene from *D. radiodurans* and tested it for functionality in *recA E. coli*, where it complemented both ionizing-radiation survival and the propagation of λ *red gam.*

This latter effort contrasts with our experience with *D. radiodurans recA* expression in *E. coli*. After failed attempts at cloning deinococcal *recA* in a wide variety of *E. coli* systems (10), we finally found that the deinococcal *recA* gene could be cloned in p*Trc*99A. Expression of this clone in *recA E. coli* did not complement any feature of the *E. coli recA* phenotype. Rescue of this deinococcal *recA* gene and introduction into *D. radiodurans* rec30 showed that it also failed to complement any feature of the strain rec30 mutant phenotype, leading to the conclusion that the deinococcal *recA* gene in these experiments had indeed undergone a mutagenic event that permitted its expression in *E. coli*. The nature of that event remains to be determined and may shed light on the differentiating features of these two proteins and their hosts and on cross-complementation endeavors.

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