JOURNAL OF BACTERIOLOGY, June 1994, p. 3508–3517
0021-9193/94/$04.00+0

Vol. 176, No. 12

In Vivo Damage and *recA*-Dependent Repair of Plasmid and Chromosomal DNA in the Radiation-Resistant Bacterium *Deinococcus radiodurans*

MICHAEL J. DALY, LING OUYANG, PINHAS FUCHS,† AND KENNETH W. MINTON*

Department of Pathology, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799

Received 8 February 1994/Accepted 15 April 1994

Deinococcus radiodurans **R1 and other members of this genus share extraordinary resistance to the lethal and mutagenic effects of ionizing radiation. We have recently identified a RecA homolog in strain R1 and have shown that mutation of the corresponding gene causes marked radiosensitivity. We show here that following high-level exposure to gamma irradiation (1.75 megarads, the dose required to yield 37% of CFU for plateau-phase wild-type R1), the wild-type strain repairs >150 double-strand breaks per chromosome, whereas a *recA*-defective mutant (rec30) repairs very few or none. A heterologous *Escherichia coli-D. radiodurans* shuttle plasmid (pMD68) was constructed and found to be retained in surviving *D. radiodurans* R1 and rec30 following any radiation exposure up to the highest dose tested, 3 megarads. Plasmid repair was monitored in vivo following irradiation with 1.75 megarads in both R1/pMD68 and rec30/pMD68. Immediately after irradiation, plasmids from both strains contained numerous breaks and failed to transform *E. coli*. While irradiation with 1.75 megarads was lethal to rec30 cultures, a small amount of supercoiled plasmid was regenerated, but it lacked the ability to transform *E. coli*. In contrast, wild-type cultures showed a cell division arrest of about 10 h, followed by exponential growth. Supercoiled plasmid was regenerated at normal levels, and it readily transformed *E. coli*. These studies show that *D. radiodurans* retains a heterologous plasmid following irradiation and repairs it with the same high efficiency as its chromosomal DNA, while the repair defect in rec30 prevents repair of the plasmid. Taken together, the results of this study suggest that plasmid DNA damaged in vivo in *D. radiodurans* is repaired by *recA*-dependent mechanisms similar to those employed in the repair of chromosomal DNA.**

Deinococcus (formerly *Micrococcus*) *radiodurans* R1 was discovered by Anderson and coworkers (1) in X-ray-sterilized canned meat that was found to have undergone spoilage. Culture yielded a red-pigmented nonsporulating gram-positive bacterium that was extremely resistant to ionizing radiation and many other agents that damage DNA. Subsequently, four similar radioresistant species have been identified and grouped with *D. radiodurans* in the family *Deinococcaceae*, one of the 10 known eubacterial families (27, 30, 32). The deinobacteria are the most ionizing and UV-radiation-resistant organisms known, with full survival reported at exposures of 0.5 to 3 megarads and 600 to 1,000 J/m^2, respectively (3, 19, 27, 32).

Since its discovery, studies on *D. radiodurans* have shown that its extreme resistance is attained via extraordinarily efficient DNA repair (for a review, see reference 27). For example, ionizing radiation produces double-strand breaks (dsb) in the DNA of *D. radiodurans* with the same efficiency as in the DNA of other organisms; however, wild-type *D. radiodurans* can mend >100 dsb per chromosome without lethality or mutagenesis (5, 7, 11, 20), whereas most other organisms can repair no more that 2 or 3 (18).

Using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) mutagenesis, Moseley and Copeland (28) obtained a *D. radiodurans* strain (rec30) that was markedly sensitive to DNA damage. They used "rec" in the strain designation because the strain was also defective in natural transformation, a process that requires recombination. We have recently identified the defective locus in rec30 and found that it encodes a RecA homolog, as judged by the predicted protein sequence of the gene and by immunological cross-reactivity of this deinococcal RecA with RecA of *Escherichia coli* (12). Both rec30 and a strain we generated that contains a 3.1-kb insertional mutation within the deinococcal *recA* gene (strain 1R1A) have a markedly radiosensitive phenotype, with a 70-fold reduction in the dose required to yield survival of 37% of CFU (D_{37}) relative to that of the wild-type (12). The RecA protein from *D. radiodurans* appears unusual in at least one respect: *recA* genes have been cloned from a wide variety of bacterial species, and the vast majority of such genes, when expressed in *E. coli*, complement the *E. coli recA* phenotype with respect to recombinase deficiency and/or DNA damage sensitivity (35). In contrast, synthesis of *D. radiodurans* RecA in *E. coli* is highly toxic, suggesting that its mode of interaction with other proteins and/or nucleic acids is different from that of *E. coli* RecA (12). While the unusually efficient mechanism(s) of repair in *D. radiodurans* are not understood, the requirement for a RecA-type protein suggests that homologous recombination is central.

In the present work, we report studies on repair of chromosomal and plasmid DNA in wild-type *D. radiodurans* and in strain rec30. Analysis of repair and mutagenesis of plasmid DNA has proven valuable for the study of a variety of repair pathways in both prokaryotes and eukaryotes. Such studies employ damaged plasmid DNA that is introduced by transfec-

* Corresponding author. Mailing address: Department of Pathology, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., Bethesda, MD 20814-4799. Phone: (301) 295-3476. Fax: (301) 295-1640. Electronic mail address: minton@usuhsb.usuhs.mil.

† Permanent address: Department of Virology, Israel Institute for Biological Research, Ness-Ziona, Israel.

tion, injection, or transformation into the cell type of interest, followed by a range of assay techniques for plasmid repair or mutagenesis (8, 14, 22, 29, 33, 36–38, 40). These approaches all require inducing plasmid damage in vitro, prior to introduction of the plasmid into a cell. This is necessary because damaging the plasmid in vivo would require supralethal damage of the chromosomal DNA, since the plasmid is a very small target compared with the chromosome(s). This constraint, however, does not apply to *D. radiodurans*, since extreme plasmid damage can be inflicted in vivo at exposures commensurate with cellular survival. This provides the potential opportunity to use specially designed reporter plasmids as indicators of cellular repair processes occurring simultaneously in the chromosomal DNA. In the current work, we have constructed pMD68, an autonomously replicating *D. radiodurans-E. coli* shuttle vector, and report the results of experiments on its damage, repair, and survival following ionizing irradiation delivered in vivo as a plasmid in *D. radiodurans*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *D. radiodurans* R1 is the wild-type strain (1) and the *D. radiodurans* R1 derivative rec30 was obtained via MNNG mutagenesis (28). rec30 is defective in a gene that is homologous to *recA* of *E. coli*, resulting in reduced natural transformation efficiency of homologous chromosomal DNA, marked sensitivity to DNA damage, and slow growth (12). We have previously compared the phenotype of rec30 to a strain we had generated (strain 1R1A) that is isogenic with the wild-type strain except for a large 3.1-kb Cm^r-conferring insertion centrally placed within the chromosomal 1.1-kb *recA* protein-coding sequence. Strains rec30 and 1R1A are phenotypically indistinguishable, manifesting the same deficiencies to exactly the same extent (12), leading us to suggest that both strains are probably globally defective in deinococcal RecA function. Strain rec30, rather than 1R1A, was used for the current studies, because it lacks the heterologous chromosomal 3.1-kb segment present in 1R1A that contains *E. coli* plasmid sequences shared with pMD66 and pMD68. *D. radiodurans* readily recombines homologous linear and circular DNA with its chromosome (12, 21, 25, 42, 43) if homology is provided. Consequently, use of rec30 (instead of 1R1A) precludes homologous plasmid-chromosome recombination, which is undesirable for this study, which addresses *recA*-mediated DNA repair among plasmids, rather than repair occurring by plasmid-chromosome recombination. *D. radiodurans* was grown in TGY broth (0.8% Bacto Tryptone, 0.1% glucose, 0.4% Bacto Yeast Extract; Difco Laboratories) at 32°C with aeration or on TGY plates solidified with 1.5% agar. Selective drug concentrations for *D. radiodurans* were 3 μg of chloramphenicol per ml, 8 μg of kanamycin (KM) per ml, or 2.5 μg of tetracycline (TC) per ml. The *E. coli recA1* cloning strain DH10B (Life Technologies) was grown at 37°C in Luria-Bertani (LB) broth with aeration or on LB plates solidified with 1.5% agar. Selective drug concentrations for *E. coli* were 50 μg of ampicillin per ml, 30 μg of TC per ml, or 30 μg of KM per ml.

Transformation. *E. coli* was transformed by the $CaCl_2$ technique employing 10 to 100 ng of plasmid per 10^8 recipients. Transformation of *D. radiodurans* employed exponentially growing cultures that were resuspended in fresh TGY broth that contained 25 mM $CaCl_2$, as previously detailed (25). Transformation of *D. radiodurans* typically employed 0.5 to 1 μg of DNA per 2.5×10^7 recipients. Recipients were grown for 2 generations prior to selective plating to allow expression of transformed markers to occur (25). Therefore, scoring of transformants is expressed per 10^8 recipients.

DNA isolation and manipulation. Isolation of plasmid DNA from *E. coli*, use of enzymatic reagents, gel electrophoresis, blotting, hybridization, washing of blots, and autoradiography were as previously described (21, 42). Minipreps of *D. radiodurans* genomic DNA were performed by a protocol that employs hexadecyltrimethyl ammonium bromide (CTAB) (2). Purification of *D. radiodurans* plasmid DNA was accomplished by a scale-up of the miniprep technique, starting with 1 liter of stationary-phase *D. radiodurans*, and completed by CsCl-ethidium bromide density gradient separation of plasmid DNA from linear DNA. This approach provided greater plasmid yields than our prior method (41).

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed with a transverse alternating field electrophoresis (TAFE) apparatus (Geneline; Beckman Instruments, Inc.), using 0.9% Beckman Low Endo-osmosis (LE) agarose gels (10 cm long, 7.6 cm wide, and 0.64 cm thick) and 0.5× TBE running buffer (50 mM Tris, 1 mM EDTA, 50 mM boric acid, pH 8.3; 0.1 μg of ethidium bromide per ml). *D. radiodurans* R1 (wild type) and rec30 genomic DNA was isolated as described above, and aliquots were electrophoresed in the LE agarose gel with a conventional horizontal gel electrophoresis unit at 100 V for 15 min, at which time essentially all DNA in the wells had migrated into the gel, as determined by ethidium bromide staining. The gel was then removed from the conventional electrophoresis unit, mounted vertically in the TAFE unit, and electrophoresed at 13°C with ramped pulse times ranging from 5 to 40 s over 15 h.

Construction of pMD66 and pMD68. As discussed above, *D. radiodurans* readily recombines homologous linear and plasmid DNA with its chromosome. Thus, to study plasmid repair, it was necessary to generate a useful vector with minimal homology to *D. radiodurans* R1 (wild type) or rec30 genomic DNA. For this reason, sequences from *D. radiodurans* SARK, which has only 33% homology with strain R1 (4) were employed. There are two additional constraints in the construction of the *D. radiodurans* R1-*E. coli* shuttle plasmid pMD66. One constraint is the requirement for separate promoters for *E. coli* and *D. radiodurans*, since each of these organisms poorly recognizes promoters from the other (43). Consequently, to express the same plasmid-borne drug marker in low-copy number in both *E. coli* and *D. radiodurans*, two upstream promoters are required, one for *E. coli* and one for *D. radiodurans* (41). The other constraint is two origins of replication, one for *D. radiodurans* and one for *E. coli*. We used the following SARK-derived plasmids as starting materials for construction of pMD66: pS19, a *D. radiodurans* plasmid derived from SARK plasmid pUE11 that replicates and confers Km^r in *D. radiodurans* SARK and R1 (41); pI3, a *D. radiodurans-E. coli* shuttle vector derived from SARK plasmid pUE10 that replicates in SARK and R1, conferring Cm^r, and replicates in *E. coli* where it confers Ap^r (24); and pS18, a derivative of SARK plasmid pUE11 that contains a portion of *E. coli* plasmid pMK20 (41). A 10.7-kb *Eco*RI-*Eco*47III fragment of pS18 that contains the *aphA* gene (from pMK20; Km^r) with its *E. coli* promoter and a deinococcal promoter immediately upstream was ligated to the 4.2-kb *Eco*RI-*Ssp*I fragment of pBR322 to make pMD65. The 12-kb *Xba*I-*Eco*RI fragment of pI3 that contains the pI3 (pUE10) deinococcal origin of replication and a strong deinococcal promoter was ligated to the 14.9-kb *Eco*RI-*Xba*I fragment of pMD65 to make pMD66 (Fig. 1). pMD66 replicates in *D. radiodurans* because of the origin of replication in the pI3-derived segment and replicates in *E. coli* because of the pBR322 origin of replication. In *D.*

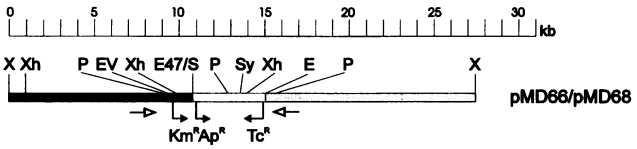

FIG. 1. Restriction map of pMD66 and pMD68. pMD66 and pMD68 are identical, except that pMD66 is purified from *E. coli* while pMD68 is purified from *D. radiodurans* (see Materials and Methods). The sequences derived from pS18 (black segment), pBR322 (white segment), and pI3 (grey segment). Restriction sites abbreviations: E, *Eco*RI; E47/S, fusion of *Eco*47III-cleaved site with *Ssp*I-cleaved site (both enzymes leave blunt ends); EV, *Eco*RV; P, *Pvu*II; Sy, *Sty*I; X, *Xba*I; Xh, *Xho*I. The *Eco*RI and *Xba*I sites are unique. The small black arrows indicate the locations of the *aphA* gene (Km^r) from pMK20 and of the *bla* (Ap^r) and *tet* (Tc^r) genes from pBR322. All are preceded by the respective *E. coli* promoting sequence. The open arrows indicate deinococcal promoting sequences from pS18 and pI3.

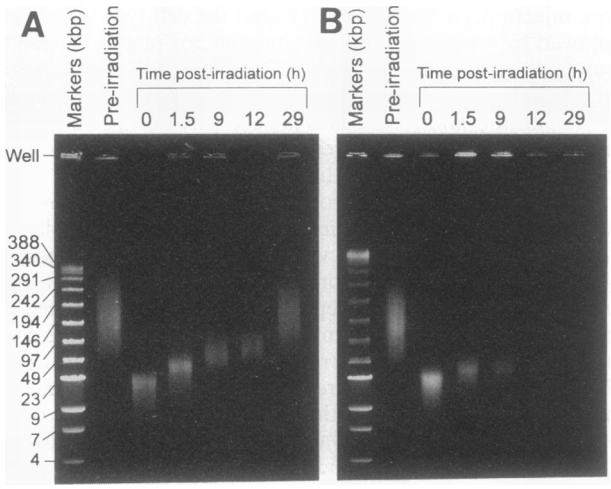

FIG. 2. PFGE of total DNA from *D. radiodurans* R1 (wild type) (A) and rec30 (B). Cultures of R1 and rec30 were grown to early plateau phase and gamma irradiated on ice to 1.75 megarads. Total genomic DNA was purified by the miniprep technique immediately thereafter, or the cells were diluted 1/50 in fresh TGY broth and incubated at 32°C with aeration for the times indicated, and total DNA was purified by the same technique. Each lane contains the DNA from 3×10^6 cells, as determined by counting with a hemocytometer. The DNA is visualized by ethidium bromide staining. The markers are composed of a λ ladder plus λ phage DNA digested with *Hin*dIII. The average molecular size of DNA in the unirradiated-cell samples was determined with a Shimadzu Chromato Scanner CS-930 and a Shimadzu DR-2 data recorder and analyzer using the gel standards.

radiodurans, pMD66 confers Km^r because of pS18 (pUE11) deinococcal promoting sequences and Tc^r because of pI3 (pUE10) promoting sequences. In *E. coli*, pMD66 confers Ap^r because of the *bla* gene from pBR322, confers Km^r because of the *E. coli* promoting sequences from the pMK20 portion of pS18, and confers Tc^r because of the *E. coli* promoting sequences from pBR322. Plasmid pMD68 is identical to pMD66, except for an apparent restriction methylation acquired by passage in *D. radiodurans* (see Results).

Measurements of viable and visible counts and the time course of repair. Overnight cultures were irradiated without change of broth on ice using ^{60}Co at 1.33 megarads/h. For survival studies (viable counts), aliquots were taken at various time points during irradiation, diluted and plated on TGY agar or TGY agar supplemented with KM and/or TC, and the plates were incubated for at least 3 days prior to counting colonies. The dilutions prior to plating were aimed at yielding about 250 colonies per agar plate. Quantification of visible cells was done with a Neubauer hemocytometer. Dilutions were made, and the hemocytometer chamber was gently filled with a micropipet. Sixty-four squares (a volume of 16 nl) from four different areas of the chamber were counted. The dilutions prior to hemocytometer counting were aimed at yielding about 500 cells present within 64 squares of the Neubauer hemocytometer. In the measurements of the time course of repair, the cells were irradiated with 1.75 megarads and immediately thereafter diluted 1/50 into fresh TGY broth without antibiotics, followed by incubation at 32°C with aeration for up to 50 h, with samples taken at intervals. The dilution of cells following irradiation is necessary to observe strand-break rejoining (as well as cell growth kinetics), since *D. radiodurans* has been shown to mend dsb only at cell densities suitable for growth (7), an observation we have confirmed (unpublished results).

RESULTS

dsb mending of chromosomal DNA in *D. radiodurans* is *recA* dependent. In experiments in which a single radiation dose was used, the dose was usually the D_{37} for wild-type *D. radiodurans* R1, which is 1.75 megarads under our standard conditions employing irradiation of cultures that have been grown overnight without change of broth prior to irradiation (12). The D_{37} (statistically, an average of one lethal hit per CFU) is therefore closely related to the maximum repair capacity of the cell, while at the same time resulting in minimal detrimental effects on growth curves, since the initial population (100%) is regenerated in only 1.4 cell divisions. We have previously measured dsb mending of wild-type *D. radiodurans* chromosomal DNA by PFGE (11) by the conventional approach that calls for embedding of whole cells in an agarose plug, followed by in situ preparation and restriction cleavage of the cellular DNA in the plug. The purpose of embedding cells in agarose is to avoid the shearing of chromosomal DNA that typically occurs if prepared in liquid. However, agarose embedding of whole cells prior to DNA preparation often results in the retention or trapping of a variable, and often substantial, fraction of the DNA in the agarose plug, as is frequently seen following electrophoresis (in DNA from all organisms and all electrode conformations; e.g., see the gels in reference 34). While this is not a problem when determining genome size or mapping, variable retention of DNA in the plug was problematic in interpretation of *D. radiodurans* dsb mending (11). We therefore modified the procedure to eliminate the agarose plug by carrying out DNA purification in liquid, then running all DNA into the gel by conventional electrophoresis for 15 min, followed by field alternation for 15 h. We previously determined the size of the *D. radiodurans* chromosome to be 3.58×10^6 bp by summation of chromosomal *Not*I restriction fragments (by PFGE using the agarose plug technique [11]), which is essentially the same as a value previously obtained by Hansen (16), who calculated genome size by renaturation kinetics. By using the modified method in which the DNA is prepared in liquid rather than agarose, no restriction cleavage was necessary, since the average DNA fragment size was about 1.6×10^5 bp (Fig. 2), indicating that shearing during purification of DNA from unirradiated cells introduced 22 dsb per chromosome. However, following irradiation with 1.75 megarads, the average-size fragment was equal to or less than 2.3×10^4 bp (Fig. 2), indicating the presence of >150 dsb per chromosome. Since the maximum-size fragment after irradia-

TABLE 1. Transformation restriction of pMD66 and pMD68 in *D. radiodurans*

Plasmid	No. of transformants[a]	
	D. radiodurans	*E. coli*
None	0	0
pMD66 (constructed in *E. coli*)	5×10^1	4.0×10^5
pMD68[b] (i.e., pMD66 purified from a *D. radiodurans* transformant)	1.0×10^6	1.5×10^5
pMD66[c] (i.e., pMD68 purified from an *E. coli* transformant)	1.0×10^2	5.0×10^5

[a] Tc^r transformants per microgram of plasmid per 1×10^8 *D. radiodurans* R1 or *E. coli* recipients.
[b] A diagnostic restriction map of pMD68 from a *D. radiodurans* transformant was identical to the original pMD66 constructed in *E. coli*.
[c] A diagnostic restriction map of pMD66 (pMD68 passaged through *E. coli*) was identical to pMD66 as originally constructed in *E. coli*.

tion is smaller than the minimum-size fragment (~50 kb) generated by shearing of DNA from unirradiated cells, it is likely that little or no dsb caused by shearing are generated during sample preparation in the postirradiation zero-time samples, because the starting material is already shorter than what is generated by shear forces in the unirradiated samples. Hence, essentially all DNA breaks (>150 dsb per chromosome) at the postirradiation zero-time point are introduced by irradiation. Following irradiation, these dsb were rejoined by the wild-type strain (Fig. 2A), while rec30 cells show some dsb mending at the 1.5-h point but no further mending at later times (Fig. 2B). *D. radiodurans* contains 4 to 10 chromosomal copies per cell (27), and RecA-mediated homologous recombination among these chromosome copies may be required for dsb mending.

Transformation restriction of pMD66 DNA in *D. radiodurans*. After construction in *E. coli*, pMD66 was used to transform *D. radiodurans* to Tc^r; however, the transformation efficiency of pMD66 into *D. radiodurans* recipients was very low (Table 1), presumably in part because of unmethylated restriction cleavage sites within the plasmid, as suggested by the following observations. After purification of this plasmid from *D. radiodurans*, the plasmid transformed *D. radiodurans* at very high efficiency (Table 1); consequently, pMD66 purified from *D. radiodurans* was given a new designation, pMD68, to indicate the high transforming efficiency conferred by passage through *D. radiodurans*. pMD66 and pMD68 were identical, as assessed by detailed restriction mapping, and both transformed *E. coli* recipients with equally high efficiency (Table 1). However, if pMD68 was used to transform *E. coli* and plasmid purified from such a transformant, then the *E. coli*-derived plasmid no longer transformed *D. radiodurans* with high efficiency but instead had the same low transformation efficiency for *D. radiodurans* characteristic of the original *E. coli*-purified pMD66 (Table 1). These observations indicate that the modification (e.g., methylation) performed in *D. radiodurans* that renders this plasmid highly transformable in *D. radiodurans* does not occur in *E. coli*. Restriction of transformed plasmids is not a general property of *D. radiodurans-E. coli* shuttle vectors. For instance, pI3 (24) and similar plasmids (43) are readily transferred between *E. coli* and *D. radiodurans* without transformation restriction. Consequently, we suggest that the restriction of transformation of pMD66 in *D. radiodurans* is related to the several kilobases of

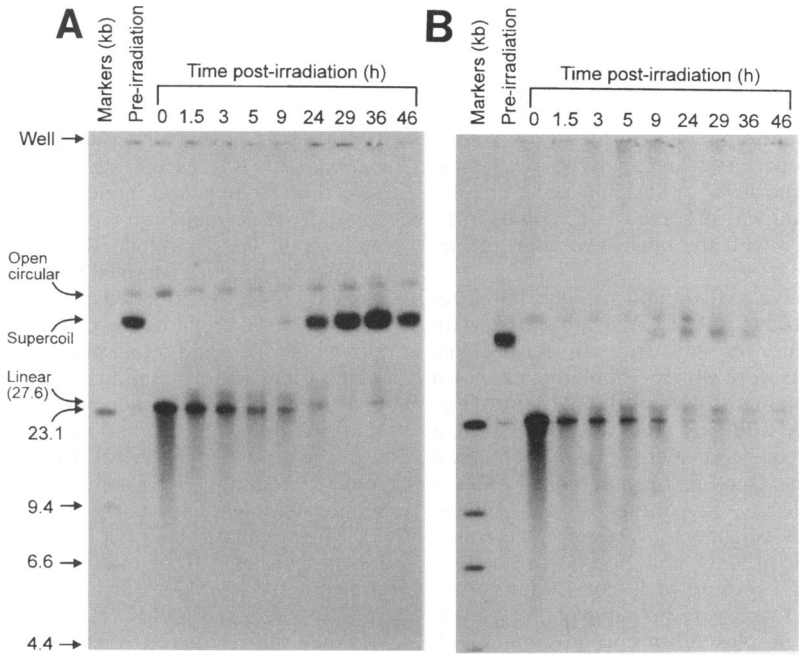

FIG. 3. Breakage and regeneration of plasmid in *D. radiodurans* R1/pMD68 (A) and rec30/pMD68 (B). Early-plateau-phase cultures were gamma irradiated with 1.75 megarads while on ice, and total DNA was purified immediately by the miniprep technique, or the cells were diluted 1/50 in fresh TGY broth and allowed to incubate at 32°C with aeration for the indicated time prior to purification of DNA by the same technique. Each lane contains DNA from 3×10^6 cells, as determined by hemocytometer count. Electrophoresis was in a 0.55% agarose gel for 12 h at 75 V prior to blotting and probing of the blot with a 1.2-kb fragment of the pBR322 *tet* gene that had been radiolabeled with [^{32}P]dCTP by the random priming method. The markers are λ phage DNA digested with *Hin*dIII.

E. coli plasmid present in pMD66 that are lacking in pI3 and similar shuttle vectors (24, 43).

Plasmid repair following in vivo irradiation of *D. radiodurans* is *recA* dependent. As shown above, extensive damage and repair of chromosomal DNA can be observed in wild-type *D. radiodurans* following exposure to extreme levels of ionizing radiation compatible with a high degree (37%) of cellular survival. Because of the ability of *D. radiodurans* to survive high doses of irradiation, it seemed possible that damage and repair could be detected in a resident plasmid, since the high-radiation exposure might be sufficient to damage a small target. The *E. coli-D. radiodurans* shuttle plasmid, pMD68, that confers Km^r and Tc^r in *D. radiodurans* and Km^r, Tc^r, and Ap^r in *E. coli* was constructed (Fig. 1) as described in Materials and Methods and purified from *D. radiodurans*. Before and immediately following 1.75 megarad irradiation of R1/pMD68 and rec30/pMD68, total cellular DNA was prepared, and electrophoresed in agarose gels under conventional (not PFGE) conditions. Following irradiation, supercoiled plasmid was no longer detectable by Southern blotting, while a small amount of open circular plasmid and a large amount of linearized plasmid were detected in either the wild type (R1) (Fig. 3A, lanes Pre-irradiation and 0 h) or rec30 (Fig. 3B, lanes Pre-irradiation and 0 h). To determine whether the small amount of residual open circular DNA was the result of repair during irradiation (78 min on wet ice; ~0°C), the cells were frozen on dry ice during irradiation (−70°C) and then rapidly melted and fixed by immersion in 100% ethanol. At the D_{37} under these frozen irradiation conditions (D_{37} = 3.0 megarads at −70°C; freezing of any bacteria, including *D. radiodurans*, increases radioresistance because of diminished DNA oxygen effects [26, 31]), the extent of both chromosomal and plasmid breakage was identical to that seen at the D_{37} under the usual (~0°C) irradiation conditions (data not shown). Since no repair is likely to occur while the cells were frozen, we concluded that the presence of the same amount of residual open circular plasmid form seen following irradiation at ~0°C is not the result of repair. To ascertain that the degree of plasmid breakage seen in *D. radiodurans* is not modulated by any effects peculiar to *D. radiodurans*, *E. coli*/pMD66 was irradiated at ~0°C with 1.75 megarads and immediately following irradiation total cellular DNA was prepared and analyzed for plasmid breakage (Fig. 4). The same level of residual open circular plasmid following irradiation was observed in *E. coli* as in *D. radiodurans* (compare Fig. 3 with the uncleaved lanes of Fig. 4).

Following irradiation of R1/pMD68 and rec30/pMD68, recovery was allowed to proceed in fresh TGY broth at 32°C with aeration and in the absence of any drug. In R1/pMD68, degraded plasmid fragments were slowly eliminated, as shown by the diminution of fragmented DNA in the gel lanes (Fig. 3A). Before recovery of the wild type (R1) from lag phase, a small amount of new supercoil was evident (Fig. 3A, 9 h lane); after recovery from lag phase (see Fig. 6), supercoiled pMD68 had returned to normal levels. In contrast to R1/pMD68, rec30/pMD68 showed only slight recovery of supercoiled pMD68 (Fig. 3B) detectable at 9, 24, and 29 h postirradiation of rec30/pMD68. Following exposure of *E. coli* (either $recA^+$ or *recA*) to 1.75 megarads, there were no survivors, there was no evidence of repair, and no plasmid or chromosomal DNA was detectable by 12 h postirradiation (data not shown).

All *D. radiodurans* survivors following irradiation retain pMD68. Because of radiation-induced damage to pMD68, it is possible that the plasmid might not be repaired or retained by each *D. radiodurans* bacterium following irradiation and that the results shown in Fig. 3 for repair of plasmid represent an occurrence in a subset of the *D. radiodurans* population. pMD68 was specifically constructed not to be essential to *D. radiodurans* R1, except when required for drug resistance selection. Deinococcal sequences in pMD68 are derived exclusively from *D. radiodurans* SARK, which has little homology with strain R1 (4). While R1 recognizes the replication and promoter sequences derived from SARK (41, 43), the plasmid can be cured by extended propagation on nonselective medium. Thus, it might be the case that only some *D. radiodurans* survivors repair and retain pMD68, specifically that fraction in which there is no plasmid damage or minor damage. As one test to determine whether pMD68 is uniformly repaired and retained following irradiation, R1/pMD68 and rec30/pMD68 were assessed for survival following various exposures to ionizing radiation by plating on TGY or on TGY supplemented with KM, as a marker for the presence of pMD68 (Fig. 5). The survival rates of R1/pMD68 and rec30/pMD68 were indistinguishable from that found for wild-type and rec30 in previous studies (12). Furthermore, the survival rates of R1/pMD68 and rec30/pMD68 were unaffected, regardless of whether the irradiated cells were plated on nonselective or selective medium containing KM (Fig. 5), indicating that the Km^r determinant was still present. Identical results were also obtained when plated either on selective medium containing TC or on agar that contained selective concentrations of both TC and KM (not shown). Diagnostic restriction cleavage analysis of plasmid from R1/pMD68 and rec30/pMD68 survi-

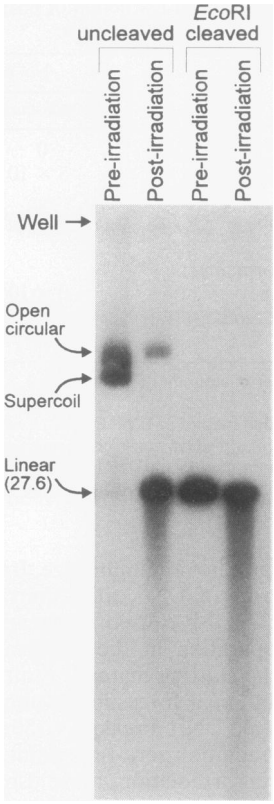

FIG. 4. Breakage of plasmid in *E. coli* DH10B/pMD66 following gamma irradiation. Cells were exposed to 1.75 megarads on ice, and total DNA was purified immediately thereafter. Each lane contains DNA from 3×10^5 cells, as determined by hemocytometer count. Electrophoresis, blotting, and probing were done as described in the legend to Fig. 3.

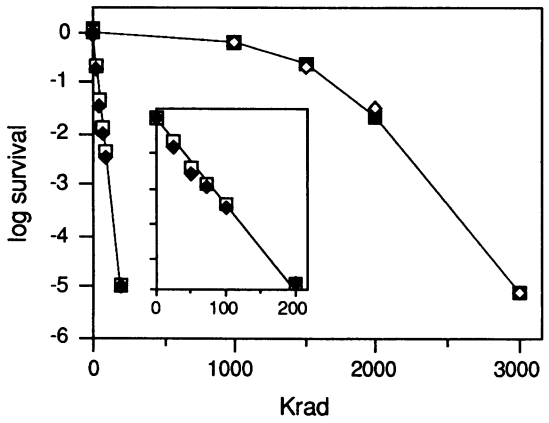

FIG. 5. Survival of *D. radiodurans* R1/pMD68 and rec30/pMD68 following gamma irradiation. Cells were grown to early plateau phase and irradiated on ice at 1.33 megarads/h. Aliquots were withdrawn at intervals, diluted, and plated on TGY agar or TGY agar that was supplemented with KM. Symbols: ■, R1/pMD68 plated on TGY agar; ◇, R1/pMD68 plated on selective TGY agar supplemented with KM; □, rec30/pMD68 plated on TGY agar; ◆, rec30/pMD68 plated on selective TGY agar supplemented with KM. The inset box shows an expanded view of the rec30/pMD68 survival results.

vors always showed the original plasmid, without deletions or rearrangements, regardless of the plating conditions. These results indicate that the plasmid is retained in R1 and rec30 following irradiation and is repaired with an efficiency similar to the chromosome. Furthermore, to our limits of detection (about 0.5% in these experiments), isolates selected on TC are also Km^r and isolates selected on KM are also Tc^r, suggesting that point mutagenesis is a rare occurrence in pMD68 following irradiation.

Biological activity of pMD68 following irradiation of *D. radiodurans* is *recA* dependent. Biological activity of pMD68 following ionizing radiation of R1/pMD68 and rec30/pMD68 was assessed after irradiation by determining the ability of plasmid present in total DNA preparations to transform *E. coli* DH10B to Km^r (Fig. 6). Following exposure of plateau-phase cells to 1.75 megarads, cells were diluted to about 10^6 per ml into fresh medium and incubated at 32°C with aeration, and samples were taken at intervals. Immediately following irradiation, the ability of a total DNA preparation from R1/pMD68 to transform *E. coli* DH10B to Km^r was reduced from about 1,000 transformants per μg of total deinococcal DNA to 2 or 3 transformants per μg of total DNA as a result of plasmid damage (Fig. 6). Following irradiation of the wild type, there was a division arrest for about 10 h. This profound growth lag following any form of DNA damage (nonlethal or partially lethal) in *D. radiodurans* has been well described (for a review, see reference 27), during which a very limited degree of genomic DNA degradation occurs, intrinsic to the repair processes. Furthermore, it appears that *recA* cells do not exhibit rapid "reckless" degradation of their genomic DNA, as evidenced by rec30's slow loss of DNA following high-dose irradiation (Fig. 2), which has been noted by others (28). The growth arrest in R1/pMD68 was followed by seven divisions over 15 h, which is the normal exponential growth rate for *D. radiodurans*, and an additional eighth division over the subsequent 4 h to achieve a plateau phase of about 10^8 cells/ml (Fig. 6). Recovery of plasmid transfer efficiency (as measured by *E. coli* Km^r transformants per μg of total R1/pMD68 or total rec30/pMD68 DNA) began to return with recovery from lag phase in R1/pMD68 (Fig. 6), while no recovery of plasmid transfer was seen in rec30/pMD68 (Fig. 6). The plasmid in *E. coli* DH10B Km^r transformants was always found to be bona fide pMD66, as determined by diagnostic restriction digestions of plasmid.

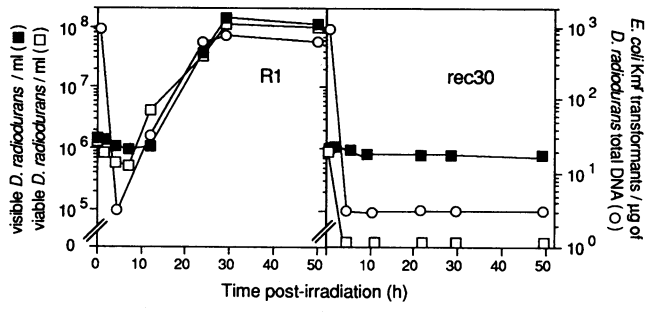

FIG. 6. Viable cells, visible cells, and pMD68 transformation activity for R1/pMD68 and rec30/pMD68. Early-plateau-phase cells (7.3×10^7 cells per ml) were irradiated with 1.75 megarads on ice, then diluted 1/50 in fresh TGY medium, and allowed to incubate at 32°C for the indicated times. Viable cell counts were determined by plating for colony formation at the times indicated. Visible cell counts were determined by hemocytometer count at the times indicated. Plasmid pMD68 transforming activity was determined by purification of total DNA by the miniprep technique at the times indicated, 1 μg of which was used to transform *E. coli* DH10B to Km^r as determined by plating on selective agar containing KM.

Following exposure of rec30/pMD68 to 1.75 megarads, there was no detectable survival of rec30, as determined both by colony formation assay and the inability of liquid cultures to resume growth at any time postirradiation (Fig. 6), consistent with the known radiosensitivity of rec30. After irradiation, the ability of rec30/pMD68 total DNA to transform *E. coli* DH10B decreased from about 1,000 Km^r transformants per μg of rec30/pMD68 DNA (the same efficiency as unirradiated R1/pMD68) to 2 or 3 transformants per μg of rec30/pMD68 total DNA. As in the case with *E. coli* Km^r transformants deriving from R1/pMD68 total DNA, the rare *E. coli* Km^r isolates resulting from transformation of DNA from postirradiation rec30/pMD68 cells also contained bona fide pMD66, as determined by diagnostic restriction digestions. Unlike R1/pMD68, rec30/pMD68 total DNA never recovered its ability to transform *E. coli* DH10B to Km^r at preirradiation levels of efficiency.

At postirradiation times of 9, 24, and 29 h, the presence of a small amount of supercoiled plasmid can be detected in rec30 by autoradiography (Fig. 3B). In Fig. 3, each lane contained total DNA from 3×10^6 cells, as determined by hemocytometer counts. In the wild type, the amount of DNA per lane (i.e., per 3×10^6 cells) steadily diminished until 5 to 9 h postirradiation to a minimum of about half of the original amount, as estimated by ethidium bromide staining of the gel (not shown). Thereafter, the amount of DNA per lane increased to control levels. In rec30, the amount of DNA per lane diminished slightly more rapidly than in the wild type and was no longer visible by ethidium bromide staining long after irradiation (not shown). The transformation of *E. coli* DH10B to Km^r by deinococcal total DNA is expressed in Fig. 6 as transformants per microgram of total DNA. That is, minipreps from a much larger number of rec30 experiments and many more transformations were required to obtain meaningful numbers of transformants than in the case of the wild-type. Because transformation per microgram of cellular DNA was reduced in rec30, it must also be concluded that residual supercoil in rec30

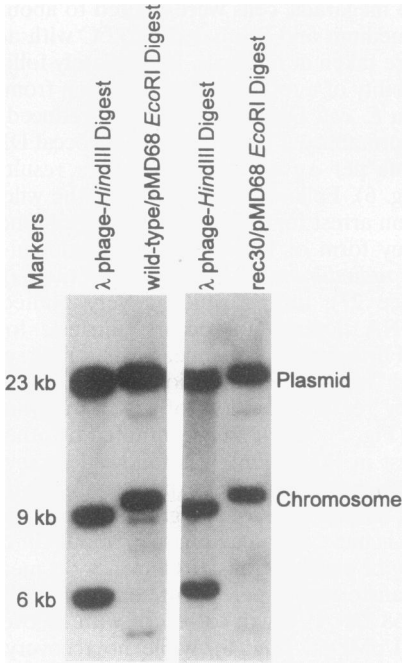

FIG. 7. *D. radiodurans* R1 (wild type) and rec30 contain approximately six pMD68 copies per cell. R1/pMD68 (left gel) and rec30/pMD68 (right gel) were grown to plateau phase in TGY broth in the presence of 2.5 μg of TC per ml. Total DNA was prepared and cleaved with *Eco*RI, and 500 ng of DNA per lane electrophoresed. *Eco*RI has only one cleavage site in pMD68, thereby linearizing the plasmid. The gel was blotted and hybridized with purified probes, each of 10 kb, one specific for pMD68 and one specific for a 10-kb *Eco*RI fragment of the chromosome. The plasmid probe was a *Sac*II-*Sac*II 10-kb fragment of pMD66, while the genomic probe was the 10-kb *Eco*RI deinococcal chromosomal fragment from the plasmid pS14 (42). Both 10-kb fragments were labeled by random priming, hybridized at 10^6 cpm per probe for ~18 h, and subjected to stringent washing and then autoradiography for ~18 h. The upper band, just above the 23-kb marker, is the *Eco*RI-linearized pMD68, while the lower band is a 10-kb *Eco*RI fragment of chromosomal DNA. Following exposure, the autoradiogram was scanned and peaks were integrated using the densitomer described in the legend to Fig. 3. The chromosome copy number per cell in plateau phase (the present case) has previously been determined to be 4 (16, 27). By comparison to the chromosomal fragment, the numbers of plasmids per cell were 5.90 for pMD68 in R1 and 6.47 for pMD68 in rec30.

is qualitatively defective (in addition to quantitatively defective).

***D. radiodurans* contains six copies of pMD68 per cell, but plasmid transfer is accomplished by a single monomeric plasmid.** *D. radiodurans* at all times contains a minimum of four chromosomal copies (6, 16, 17), which is sufficient for recombinational repair. We would expect that recombinational repair of plasmids would also require several copies of the plasmid. We have observed that cell lines that contain pMD68 (or other plasmids), maintain about six copies of plasmid per cell (Fig. 7), compatible with the potential occurrence of interplasmidic recombinational repair among them.

Different bacteria achieve plasmid transformation by different means. It is believed that in *E. coli* and *Salmonella typhimurium*, transformation requires no more than a single monomeric double-stranded DNA open circular or supercoiled plasmid; plasmid is not degraded during uptake and appears to enter the cell via channels at zones of adhesion (for a review, see reference 15). In contrast, plasmid transfer in *Bacillus subtilis* is thought to require uptake of multimeric plasmids or numerous copies of monomeric plasmid, since during uptake the plasmid DNA is degraded by double-stranded endonucleolytic cleavage, rendered single stranded by exonucleolytic cleavage, and is subsequently reassembled intracellularly by recombinational processes to form functional plasmid (for a recent review, see reference 9).

TABLE 2. Plasmid transfer in *D. radiodurans*

Plasmid[a]	No. of transformants[b]		
	Cm^r	Km^r	Cm^r Km^r
pS19	0	4.0×10^5	0
pI3	2.9×10^5	0	0
None	0	0	0
pS19 + pI3	2.7×10^{5c}	4.9×10^{5d}	1.4×10^3

[a] 500 ng of pS19 or pI3, separately or in combination (1 μg of total plasmid for the two), was used.
[b] Drug-resistant transformants per 1×10^8 *D. radiodurans* recipients.
[c] 100 Cm^r colony isolates were streaked on selective medium containing KM. All were Km^s.
[d] 100 Km^r colony isolates were streaked on selective medium containing chloramphenicol. All were Cm^s.

It is unknown whether the mechanism of plasmid transfer in *D. radiodurans* resembles *E. coli*, *B. subtilis*, or any other organism. In transformation of *D. radiodurans* we customarily employ plasmid in 100-fold excess of recipient bacteria. Thus, it should be possible for *D. radiodurans* to take up numerous copies of plasmid under these conditions. To determine how many plasmids *D. radiodurans* R1 recipients actually take up under these conditions, cotransformation experiments were carried out with two compatible plasmids derived from *D. radiodurans* SARK, pUE11 (pS19; Km^r) and pUE10 (pI3; Cm^r). R1 is readily transformed by either of these plasmids. When one plasmid is already resident in R1, transformation for the second plasmid occurs with the same efficiency as that seen with recipients that contain no plasmid. pS19 at 100 copies per recipient and pI3 at 250 copies per recipient *D. radiodurans* bacterium were used. A higher copy number of pI3 than of pS19 was used, because pS19 is slightly more transformable (Table 2). When the plasmids were mixed prior to transformation, it was found that cotransformation of the two occurred with a frequency of 1.4×10^{-5}, which is equal to the frequency of transformation for each of the individual markers (3×10^{-3}) squared (Table 2). This indicates that transformation for each marker was independent. Additionally, we have previously shown that transformation of *D. radiodurans* is a linear function of plasmid concentration employed in the transformation mixture, also indicating that only one plasmid is required to accomplish transformation (25). To determine whether the monomeric plasmid fraction was responsible for transformation, we employed sucrose gradient separation of monomeric plasmid from small quantities of multimeric plasmid and found that monomeric plasmid is fully active in plasmid transfer (not shown). This indicates that the plasmid dimer fraction (which carries its own redundant sequences) is not responsible for the vast majority of plasmid transfer in R1. From the above evidence, it may be concluded that plasmid transfer under the current conditions is almost entirely accomplished by a single monomeric plasmid.

Repair of plasmid in *recA*$^+$ *D. radiodurans* requires more than one copy of pMD68. As shown above, *recA D. radiodurans* appears to be both quantitatively and qualitatively defective in

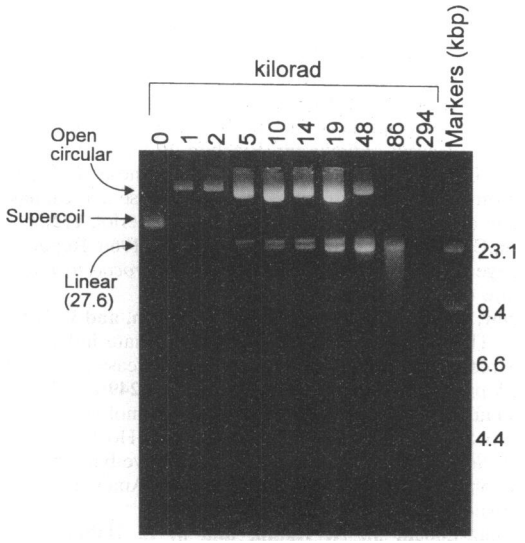

FIG. 8. Breakage of pMD68 by gamma irradiation in water. Purified pMD68 was gamma irradiated under aerobic conditions in water at ~0°C (on ice) to the doses indicated and electrophoresed in a 0.8% agarose gel. Each lane contains 200 ng of pMD68. The markers are λ phage DNA digested by *Hin*dIII.

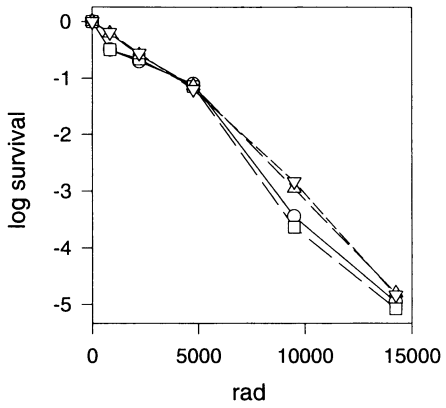

FIG. 9. Transformation of *D. radiodurans* R1 (wild type) and rec30 by pMD68 that had been gamma irradiated in water prior to transformation. The horizontal axis indicates the dose of gamma irradiation to which the plasmid had been exposed prior to use for transformation. The vertical axis indicates the transformation frequency. The transformation frequency of unirradiated pMD68 was 2×10^5 Km^r transformants per μg of pMD68 per 1×10^8 wild-type or rec30 recipients. Symbols: ○, wild type plated on selective agar containing TC; □, wild type plated on selective agar containing KM; △, rec30 plated on selective agar containing TC; ▽, rec30 plated on selective agar containing KM.

regeneration of pMD68. Both *D. radiodurans* R1/pMD68 and rec30/pMD68 contain six copies of pMD68 per cell, and it might be expected that *recA*-dependent repair requires the homology provided by multiple copies of pMD68. Consequently, we sought to determine whether *recA*-dependent repair occurred when there was only a single copy of pMD68 per cell. On the basis of studies detailed in the preceding section, it appears likely that plasmid transformation of *D. radiodurans* under the current conditions is the result of the entry of a single monomeric circular plasmid into the recipient. Therefore, we irradiated plasmid in vitro and used the damaged plasmid to transform R1 (wild type) and rec30 to assess whether the $recA^+$ wild-type cells had enhanced transformation efficiency of damaged plasmid. Irradiation of DNA in a nonprotective aqueous solution resulted in far more DNA damage per rad than irradiation of plasmid in vivo in *D. radiodurans* or *E. coli* (Fig. 8; compare to Figs. 3 and 4). This effect occurs in intracellular DNA in all organisms, because the DNA is protected by conformational restraints, small molecule radical scavengers, and most importantly binding proteins (reference 23 and citations therein). Relatively low doses of irradiation resulted in plasmid DNA strand breakage in vitro, converting supercoil to open circular and linear forms, and at the highest dose tested, 3×10^5 rads, the plasmid was converted entirely to low-molecular-weight DNA (Fig. 8). Transformation of plasmid DNA irradiated in vitro showed no difference in efficiency between *D. radiodurans* wild-type and rec30 recipients (Fig. 9), suggesting that RecA does not participate in repair of exogenously damaged plasmid upon transformation, presumably because of a lack of homologous sequences.

DISCUSSION

D. radiodurans R1 has the extraordinary ability to rejoin in excess of 100 dsb per chromosome without lethality or mutagenesis (20, 27). Here, it is shown that this remarkably efficient chromosomal dsb mending and survival is *recA* dependent (Fig. 2 and 5), and these observations have been extended to include a heterologous plasmid, pMD68 (Fig. 3). pMD68 was constructed from pBR322 and SARK sequences and shares very little or no homology with R1 sequences to ensure that recombinational repair was not occurring via homology between the reporter plasmid, pMD68, and the R1 chromosomal sequences. We found that pMD68 was present in six copies per cell (Fig. 7) and could therefore recombine among identical copies. In addition, in an attempt to mimic chromosomal repair, which occurs without drug-related selection, these studies employed no drug selection pressure for plasmid maintenance during repair. At the outset, therefore, it was not known (i) whether plasmid DNA would be maintained following irradiation, (ii) whether it would be subjected to DNA repair, and (iii) if repaired, whether such repair would be efficient. In answer to these questions, we found that all viable cells did indeed retain plasmid without drug selection (Fig. 5), that plasmid was repaired (Fig. 3 and 6), and that such repair was efficient in $recA^+$ cells, but not *recA*-mutant cells.

It is possible that plasmid repair is not actually mediated by RecA itself but instead is a secondary effect resulting from the loss of cellular growth and the presence of DNA degradation. In our view, however, it is much more likely that RecA participates directly in plasmid repair. In rec30, there is a quantitative defect in the repair of pMD68 compared with that of the wild type, but limited regeneration of supercoil is evident in rec30 at 9, 24, and 29 h following irradiation (Fig. 3B). This supercoil may have arisen by non-RecA-dependent processes from the residual open circular plasmid present after irradiation. However, in addition to the quantitative defect, there is a qualitative defect in the repair of pMD68 in rec30, since the restored supercoil did not regain the capacity to transform *E. coli* to Km^r, unlike R1/pMD68 (Fig. 6). This suggests that the regeneration of supercoil in rec30 by non-RecA-dependent processes alone results in the introduction of lethal defects in the plasmid. Perhaps lacking RecA, the plasmids are unable to compare themselves by a process involving Holliday junction strand migration and are conse-

quently unable to detect deletions, insertions, or rearrangements.

With respect to chromosomal DNA, the wild type was able to regenerate full-length chomosomes (Fig. 2A), while rec30 cells could not (Fig. 2B). However, at the 1.5-h postirradiation time point in rec30, there is a small but highly reproducible increase in DNA fragment size (Fig. 2B). Unlike the wild type, no further increase in fragment size was seen in rec30 at later times. Perhaps the initial increase in fragment size in rec30 cells is due to rejoining by the *recA*-independent single-strand annealing pathway similar to that in *E. coli* (37, 39) and *Saccharomyces cerevisiae* (10). The single-strand annealing pathway employs exonucleolytic degradation of one strand of duplex DNA fragments, followed by annealing of the resulting single-stranded regions (if complementary) by a renaturase. Renaturases, such as the *E. coli* RecT protein, facilitate the hybridization of complementary single strands but do not mediate RecA-like strand invasion of duplex DNA (13). Given the extraordinary DNA damage imposed by 1.75 megarads to the multiple chromosomes of *D. radiodurans*, some annealing of homologous single-stranded regions generated by exonucleolytic action is expected.

By a different approach, we suggest another argument that plasmid repair is *recA* dependent, based on the observation that transformation of pMD68 damaged by irradiation in vitro yields identical transfer efficiencies of damaged plasmid into the wild type and rec30 (Fig. 8 and 9). We show that under the conditions employed, *D. radiodurans* transformation proceded essentially by the entry of a single monomeric plasmid (Table 2). Consequently, the failure of the wild type to have enhanced transfer of damaged plasmid over that of rec30 suggests that the wild type cannot repair damaged plasmid upon transformation because the plasmid is monomeric and present in only a single copy, and consequently there is inadequate homology available for a *recA*-dependent repair pathway to occur.

The current work is to our knowledge the first instance in which repair of plasmid damaged in vivo has been studied, made possible by the remarkable radioresistance of *D. radiodurans*. The requirement for RecA for repair of plasmid damaged in vivo suggests that the study of plasmid repair strategies in vivo will aid understanding of the unusually efficient *recA*-dependent repair of large numbers of dsb in the chromosome. While we have shown that both chromosomal and plasmid DNA require *recA*-dependent functions for the extraordinary repair capabilities of this organism, physical evidence of homologous or nonhomologous recombination or gene conversion is still lacking. We are currently developing a two-plasmid system in which each plasmid contains different mutant alleles of a drug marker gene as well as physical polymorphisms that will facilitate the characterization of DNA damage-induced recombination.

ACKNOWLEDGMENT

This work was supported by USPHS grant GM39933.

REFERENCES

1. **Anderson, A. W., H. C. Nordon, R. F. Cain, G. Parrish, and D. Duggan.** 1956. Studies on a radio-resistant micrococcus. I. Isolation, morphology, cultural characteristics, and resistance to gamma radiation. Food Technol. **10:**575–578.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1987. Current protocols in molecular biology, vol. I, p. 2.4.1–2.4.5. Greene Publishing Associates and John Wiley and Sons, New York.
3. **Bonura, T., and A. K. Bruce.** 1974. The repair of single-strand breaks in a radiosensitive mutant of *Micrococcus radiodurans*. Radiat. Res. **57:**260–275.
4. **Brooks, B. W., R. G. E. Murray, J. L. Johnson, E. Stackebrandt, C. R. Woese, and G. E. Fox.** 1980. Red-pigmented micrococci: a basis for taxonomy. Int. J. Syst. Bacteriol. **30:**627–646.
5. **Burrell, A. D., P. Feldschreiber, and C. J. Dean.** 1971. DNA-membrane association of the repair of double breaks in X-irradiated *Micrococcus radiodurans*. Biochim. Biophys. Acta **247:**38–53.
6. **Chou, F. I., and S. T. Tan.** 1990. Manganese(II) induces cell division and increases in superoxide dismutase and catalase activities in an aging deinococcal culture. J. Bacteriol. **172:**2029–2035.
7. **Dean, C. J., P. Feldschreiber, and J. T. Lett.** 1966. Repair of X-ray damage to the deoxyribonucleic acid in *Micrococcus radiodurans*. Nature (London) **209:**49–52.
8. **Dianov, G. L., M. K. Saparbaev, A. V. Mazin, and R. I. Salganik.** 1991. The chemical mutagen dimethyl sulphate induces homologous recombination of plasmid DNA by increasing the binding of RecA protein to duplex DNA. Mutat. Res. **249:**189–193.
9. **Dubnau, D.** 1993. Genetic exchange and homologous recombination, p. 555–584. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics. American Society for Microbiology, Washington, D.C.
10. **Fishman-Lobell, J., N. Rudin, and J. E. Haber.** 1992. Two alternative pathways of double-strand break repair that are kinetically separable and independently modulated. Mol. Cell. Biol. **12:**1292–1303.
11. **Grimsley, J. K., C. I. Masters, E. P. Clark, and K. W. Minton.** 1991. Analysis by pulsed-field gel electrophoresis of DNA double-strand breakage and repair in *Deinococcus radiodurans* and a radiosensitive mutant. Int. J. Radiat. Biol. **60:**613–626.
12. **Gutman, P. D., J. D. Carroll, C. I. Masters, and K. W. Minton.** 1994. Sequencing, targeted mutagenesis and expression of a *recA* gene required for the extreme radioresistance of *Deinococcus radiodurans*. Gene **141:**31–37.
13. **Hall, S. D., M. F. Kane, and R. D. Kolodner.** 1993. Identification and characterization of the *Escherichia coli* RecT protein, a protein encoded by the *recE* region, that promotes renaturation of homologous single-stranded DNA. J. Bacteriol. **175:**277–287.
14. **Han, E. K., and W. A. Saffran.** 1992. Differential repair and recombination of psoralen damaged plasmid DNA in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **236:**8–16.
15. **Hanahan, D.** 1987. Mechanisms of DNA transformation, p. 1177–1183. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
16. **Hansen, M. T.** 1978. Multiplicity of genome equivalents in the radiation-resistant bacterium *Micrococcus radiodurans*. J. Bacteriol. **134:**71–75.
17. **Harsojo, S. Kitayama, and A. Matsuyama.** 1991. Genome multiplicity and radiation resistance in *Micrococcus radiodurans*. J. Biochem. **90:**877–880.
18. **Hutchinson, F.** 1978. Workshop summary: DNA strand break repair in eukaryotes. ICN-UCLA Symp. Mol. Cell. Biol. **9:**457–463.
19. **Keller, L. C., and R. B. Maxcy.** 1984. Effect of physiological age on radiation resistance of some bacteria that are highly radiation resistant. Appl. Environ. Microbiol. **47:**915–918.
20. **Kitayama, S., and A. Matsuyama.** 1971. Double-strand scissions in DNA of gamma-irradiated *Micrococcus radiodurans* and their repair during postirradiation incubation. Agric. Biol. Chem. **35:** 644–652.
21. **Lennon, E., and K. W. Minton.** 1990. Gene fusions with *lacZ* by duplication insertion in the radioresistant bacterium *Deinococcus radiodurans*. J. Bacteriol. **172:**2955–2961.
22. **Levy, D. D., J. D. Groopman, S. E. Lim, M. M. Seidman, and K. H. Kraemer.** 1992. Sequence specificity of aflatoxin B1-induced mutations in a plasmid replicated in xeroderma pigmentosum and DNA repair proficient cells. Cancer Res. **52:**5668–5673.
23. **Ljungman, M., S. Nyberg, J. Nygren, M. Eriksson, and G. Ahnström.** 1991. DNA-bound proteins contribute much more than soluble intracellular compounds to the intrinsic protection against radiation-induced DNA strand breaks in human cells. Radiat. Res. **127:**171–176.

24. **Masters, C. I., and K. W. Minton.** 1992. Promoter probe and shuttle plasmids for *Deinococcus radiodurans*. Plasmid **28:**258–261.
25. **Masters, C. I., M. D. Smith, P. D. Gutman, and K. W. Minton.** 1991. Heterozygosity and instability of amplified chromosomal insertions in the radioresistant bacterium *Deinococcus radiodurans*. J. Bacteriol. **173:**6110–6117.
26. **Matsuyama, A., M. J. Thornley, and M. Ingram.** 1964. The effect of freezing on the radiation sensitivity of vegetative bacteria. J. Appl. Bacteriol. **27:**110–124.
27. **Moseley, B. E. B.** 1983. Photobiology and radiobiology of *Micrococcus* (*Deinococcus*) *radiodurans*. Photochem. Photobiol. Rev. **7:**223–274.
28. **Moseley, B. E. B., and H. J. R. Copeland.** 1975. Isolation and properties of a recombination-deficient mutant of *Micrococcus radiodurans*. J. Bacteriol. **121:**422–428.
29. **Mudgett, J. S., M. Buckholt, and W. D. Taylor.** 1991. Ultraviolet light-induced plasmid-chromosome recombination in *Escherichia coli*: the role of *recB* and *recF*. Gene **97:**131–136.
30. **Murray, R. G. E., and B. W. Brooks.** 1986. Genus I. *Deinococcus* Brooks and Murray, 1981, 354^{VP}, p. 1035–1043. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. Williams & Wilkins, Baltimore.
31. **Okazawa, Y., and A. Matsuyama.** 1967. A note on radiation resistance of *Micrococcus radiodurans*. Agric. Biol. Chem. **31:** 1505–1508.
32. **Oyaizu, H., E. Stackebrandt, K. H. Schleifer, W. Ludwig, H. Pohla, H. Ito, A. Hirata, Y. Oyaizu, and K. Komagata.** 1987. A radiation-resistant rod-shaped bacterium, *Deinobacter grandis* gen. nov., sp. nov., with peptidoglycan containing ornithine. Int. J. Syst. Bacteriol. **37:**62–67.
33. **Paramio, J. M., C. Bauluz, and R. deVidania.** 1991. Comparative study of the lethal effects of near-UV light (360 nm) and 8-methoxypsoralen plus near-UV on plasmid DNA. Cell. Mol. Biol. **37:**125–137.
34. **Pyle, L. E., L. N. Corcoran, B. G. Cocks, A. D. Bergman, J. C. Whitley, and L. R. Finch.** 1988. Pulsed-field electrophoresis indicates larger-than-expected sizes for mycoplasma genomes. Nucleic Acids Res. **16:**6015–6025.
35. **Roca, A. I., and M. M. Cox.** 1990. The RecA protein: structure and function. Crit. Rev. Biochem. Mol. Biol. **25:**415–456.
36. **Saxena, J. K., J. B. Hays, and E. J. Ackerman.** 1990. Excision repair of UV-damaged plasmid DNA in *Xenopus* oocytes is mediated by DNA polymerase alpha and/or delta. Nucleic Acids Res. **18:**7425–7432.
37. **Seetharam, S., K. H. Kramer, H. L. Waters, and M. M. Seidman.** 1990. Mutational hotspot variability in an ultraviolet-treated shuttle vector plasmid propagated in xeroderma pigmentosum and normal human lymphoblasts and fibroblasts. J. Mol. Biol. **212:**433–436.
38. **Sheibani, N., M. M. Jennerwein, and A. Eastman.** 1989. DNA repair in cells sensitive and resistant to cis-diamminedichloroplatinum(II): host cell reactivation of damaged plasmid DNA. Biochemistry **28:**3120–3124.
39. **Silberstein, Z., M. Shalit, and A. Cohen.** 1993. Heteroduplex strand-specificity in restriction-stimulated recombination by the RecE pathway of *Escherichia coli*. Genetics **133:**439–448.
40. **Smerdon, M. J., J. Bedoyan, and F. Thoma.** 1990. DNA repair in a small yeast plasmid folded into chromatin. Nucleic Acids Res. **18:**2045–2051.
41. **Smith, M. D., R. Abrahamson, and K. W. Minton.** 1989. Shuttle plasmids constructed by the transformation of an *Escherichia coli* cloning vector into two *Deinococcus radiodurans* plasmids. Plasmid **22:**132–142.
42. **Smith, M. D., E. Lennon, L. B. McNeil, and K. W. Minton.** 1988. Duplication insertion of drug resistance determinants in the radioresistant bacterium *Deinococcus radiodurans*. J. Bacteriol. **170:**2126–2135.
43. **Smith, M. D., C. I. Masters, E. Lennon, L. B. McNeil, and K. W. Minton.** 1991. Gene expression in *Deinococcus radiodurans*. Gene **98:**45–52.