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Identification and Characterization of *uvrA*, a DNA Repair Gene of *Deinococcus radiodurans*

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***Deinococcus radiodurans* is extraordinarily resistant to DNA damage, because of its unusually efficient DNA repair processes. The *mtcA*$^+$ and *mtcB*$^+$ genes of *D. radiodurans*, both implicated in excision repair, have been cloned and sequenced, showing that they are a single gene, highly homologous to the *uvrA*$^+$ genes of other bacteria. The *Escherichia coli uvrA*$^+$ gene was expressed in *mtcA* and *mtcB* strains, and it produced a high degree of complementation of the repair defect in these strains, suggesting that the UvrA protein of *D. radiodurans* is necessary but not sufficient to produce extreme DNA damage resistance. Upstream of the *uvrA*$^+$ gene are two large open reading frames, both of which are directionally divergent from the *uvrA*$^+$ gene. Evidence is presented that the proximal of these open reading frames may be *irrB*$^+$.**

Deinococcus (formerly *Micrococcus*) *radiodurans* and other members of the same genus share extraordinary resistance to the lethal and mutagenic effects of UV- and ionizing radiation and chemical agents that form bulky adducts and cross-links in DNA (27, 47–49). While it is known that this extreme resistance to DNA damage is due to exceedingly efficient DNA repair, the precise repair mechanisms involved are poorly understood (33). Two mitomycin (MM)-sensitive strains of *D. radiodurans*, 302 and 262, carrying mutations that were in genes designated *mtcA* and *mtcB*, respectively, were obtained by chemical mutagenesis using *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (34). These strains, however, were not sensitive to UV (254 nm) but were sensitive to bulky alkylating agents. The *mtcA* strain was further mutagenized, yielding UV-sensitive isolates designated as being doubly defective in *mtcA* and one of the following loci: *uvsC*, *uvsD*, and *uvsE* (35). It was proposed that in *D. radiodurans* there are two endonucleases that recognize pyrimidine dimers in DNA, one encoded by *mtcA* and *mtcB* (UV endonuclease-α) and the other encoded by *uvsC*, *uvsD*, and *uvsE* (UV endonuclease-β) (2, 17, 35); that either of these two endonucleases is independently sufficient to endow *D. radiodurans* with wild-type resistance to UV; and that both had to be inactivated to obtain a UV-sensitive, excisionless phenotype. While UV endonuclease-α has not been detected in vitro, UV endonuclease-β has been identified as a 36,000-Da nuclease absent in *uvsC*-, *uvsD*-, or *uvsE*-mutant extracts. It has no known substrate other than pyrimidine dimers and incises as an endonuclease rather than as a glycosylase (15, 17). On the other hand, it is believed that UV endonuclease-α (*mtcA*$^+$ *mtcB*$^+$) not only recognizes UV-induced bipyrimidine damage but also recognizes bulky chemical adducts and interstrand cross-links (2, 17, 32, 35).

In this communication, we report that the *mtcA*$^+$ and *mtcB*$^+$ genes are a single gene that is highly homologous to the *uvrA*$^+$ genes of other bacteria and that its gene product is functionally similar to the UvrA protein of *Escherichia coli*.

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(A preliminary account of this work was given in a review article [32].)

MATERIALS AND METHODS

Bacterial strains, plasmids, growth conditions, and transformation. Bacterial strains and plasmids are given in Table 1. *D. radiodurans* was grown in TGY broth (0.8% Bacto-tryptone, 0.1% glucose, 0.4% Bacto-yeast; Difco Laboratories) at 32°C with agitation or on TGY plates solidified with 1.5% agar. *E. coli* was grown in Luria-Bertani broth at 37°C with agitation or on Luria-Bertani plates containing 1.5% agar. Selective concentrations of antibiotics were as follows: for *D. radiodurans*, 10 μg of kanamycin (KM) per ml; for *E. coli*, 50 μg of ampicillin per ml, 30 μg of chloramphenicol per ml, and 30 μg of KM per ml. Plasmid transformation of *E. coli* was by the $CaCl_2$ technique, and natural transformation of *D. radiodurans* also employed $CaCl_2$ as previously described (30).

DNA isolation and manipulation. Isolation of genomic DNA from *D. radiodurans*, isolation of plasmid DNA from *E. coli* DH5α, use of enzymatic reagents, gel electrophoresis, blotting, hybridization, washing of blots, and autoradiography were as previously described (9, 29). DNA fragments cut from agarose gels were purified using the Qiaex DNA extraction kit (Qiagen, Inc.). Small DNA restriction fragments (50 to 200 bp) were separated by sodium dodecyl sulfate–8% polyacrylamide gel electrophoresis (SDS-PAGE).

DNA sequencing and data analysis. DNA sequencing was carried out by the dideoxy technique using α-^{35}S-dCTP on double-stranded plasmids (Fig. 1). Both strands were sequenced. Forward and reverse universal primers and 30 sequence-specific primers (each primer, 18 to 20 nucleotides long) were employed. Databases were searched using the IRX network service at the National Center for Biotechnology Institute, Bethesda, Md.

Immunoblotting of *E. coli* UvrA. Cells were grown in 500 ml of Luria-Bertani broth (*E. coli*, 37°C) or TGY broth (*D. radiodurans*, 32°C) overnight with vigorous shaking. The cells were pelleted and resuspended in 5 ml of Tris-HCl (15 mM, pH 8.0) containing 2-betamercaptoethanol (2 mM) and passed three times through a French pressure cell at 10,000 lb/in^2 at 4°C. Protein content was measured with the Bio-Rad Protein Assay kit. Proteins in the crude extract were separated by one-dimensional 0.1% SDS–29.2% PAGE in minigels run at a constant 200 V. The gels were blotted onto an Immobilon polyvinylidene difluoride transfer membrane (Millipore, Inc.) and incubated overnight in blocking buffer (1% bovine serum albumin, 50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.05% NaN_3) at 4°C. The blots were probed by incubation with a 3:1 dilution of primary monoclonal antibody A2A3 in blocking buffer lacking NaN_3 for 18 h at 4°C with rocking, followed by washing three times for 20 min each at 23°C in 150 mM NaCl–50 mM Tris-HCl (pH 7.4)–0.05% Tween 20. The blots were then incubated with secondary antibody horseradish-peroxidase goat anti-mouse immunoglobulin G (Bio-Rad Laboratories) and imaged by enhanced chemiluminescence (Amersham, Inc.) according to the manufacturer's instructions.

Survival measurements. *D. radiodurans* cells were grown to stationary phase with vigorous shaking. All challenges were administered to stationary-phase cells. Following a challenge, the cells were incubated on TGY plates at 32°C, and colonies were counted after 3 to 5 days. For treatment with 4,5′,8-trimethylpsoralen (Me_3psoralen) plus near-UV (360-nm) exposure, 10 ml of cells was pelleted and resuspended in 1 ml of double-distilled H_2O. A 10-μl volume of a saturated ethanol solution of Me_3psoralen was added, resulting in a final con-

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description; relevant genotype[a]	Source or reference[b]
D. radiodurans		
R1	Wild type	5
302	*mtcA*; MNNG-mutagenized strain R1 γ ray^r UV^r Mm^s	34
321	302ΩpS11; *mtcA*	This work; 302 × pS11
322	302ΩpHA101; *mtcA*, *E. coli uvrA*$^+$	This work; 302 × pHA101
262	*mtcB*; MNNG-mutagenized strain R1 γ ray^r UV^r Mm^s	34
270	262ΩpS11; *mtcB*	This work; 262 × pS11
271	262ΩpHA101; *mtcB*, *E. coli uvrA*$^+$	This work; 262 × pHA101
E. coli		
DH5α	*recAuvrA*$^+$	Life Technologies, Inc.
N3055	*recA*$^+$ *uvrA277*::Tn*10*	Barbara Bachman, Yale University
Plasmid		
pUE502	*D. radiodurans* chromosomal DNA in cosmid pJBFH; 26 kb; *mtcA*$^+$ *mtcB*$^+$	1
pBluescriptIISK(+)	2.96-kb phagemid cloning vector; Ap^r	Stratagene, Inc.
pBCSK(+)	3.4-kb phagemid cloning vector; Cm^r	Stratagene, Inc.
pHA15	pBluescriptIISK(+) *Eco*RI::5.6-kb *D. radiodurans Eco*RI fragment from pUE502; *mtcA*$^+$	This work; In vitro ligation
pHA15b	pBluescriptIISK(+) *Acc*I-*Eco*RI-A::3.1-kb *D. radiodurans* DNA *Acc*I-*Eco*RI fragment from pHA15; *mtcA*$^+$	This work; In vitro ligation
pHA15b.1	pBCSK(+) *Xho*I::1.1-kb *Xho*I fragment from pHA15; *mtcA*$^+$	This work; In vitro ligation
pHA15b.2	pBCSK(+) *Acc*I-*Xho*I-A::1.8-kb *D. radiodurans* DNA *Acc*I-*Xho*I fragment from pHA15b	This work; In vitro ligation
pHA16	pBluescriptIISK(+) *Eco*RI::2.6-kb *Eco*RI fragment of *D. radiodurans* DNA from pUE502; *mtcB*$^+$	This work; In vitro ligation
pHA17	pBluescriptIISK(+) *Eco*RV::1.3-kb *Sty*I fragment from pHA17	This work; fill in of *Sty*I fragment with T4 DNA polymerase, followed by blunt-end ligation; *mtcB*$^+$
pHA18	pBluescriptIISK(+) *Eco*RI::316-bp fragment of *D. radiodurans* DNA from pUE502	This work; In vitro ligation
pS11	16.5-kb *E. coli-D. radiodurans* shuttle vector; replicates as plasmid in *E. coli*, integrative in *D. radiodurans*; Km^r	45
pSST10	8.0-kb plasmid with *uvrA* gene under regulation of λ phage p_L promoter	O. Kovalsky, L. Grossman 50
pHA101	pS11 *Bgl*II-*Dra*I-A::3.9-kb *Bam*HI-*Hin*dIII-fragment from pSST10 containing the *E. coli uvrA* gene; 19.5 kb	This work (Fig. 4)

[a] Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; ::, linkage; Ω, chromosomal insertion. Plasmids are described in terms of parental restriction fragments as previously suggested (37).

[b] Transformations are designated recipient strain × donor DNA.

centration of 0.6 μg of Me_3psoralen per ml (43). The cells were incubated for variable time periods at 32°C and then irradiated with agitation to 8 kJ/m^2. The cells were then washed, resuspended, and diluted in fresh TGY, and aliquots were plated. For treatment with far-UV (254-nm) exposure, cells were serially diluted and plated on TGY agar. The lids of the agar plates were removed, and the plates were exposed to UV at a dose rate of 1.0 J/m^2/s for up to 1,500 J/m^2. Following irradiation, the petri dishes were covered and incubated at 32°C. For treatment with ionizing radiation, 1.0 ml of stationary-phase culture without change of broth was irradiated with ^{60}Co at 1.3 Mrads (13 kGy)/h at 0°C for durations of up to 1.5 h. Following irradiation, the cells were diluted and spread on TGY plates. For treatment with MM, it was added to stationary-phase cells without a change of broth to 1 μg of MM per ml. At 30-min intervals, samples were taken, washed, diluted in fresh TGY, and spread on TGY plates. Treatment with 4-nitroquinoline-1-oxide (4NQO) was achieved by adding 0.33 ml of an

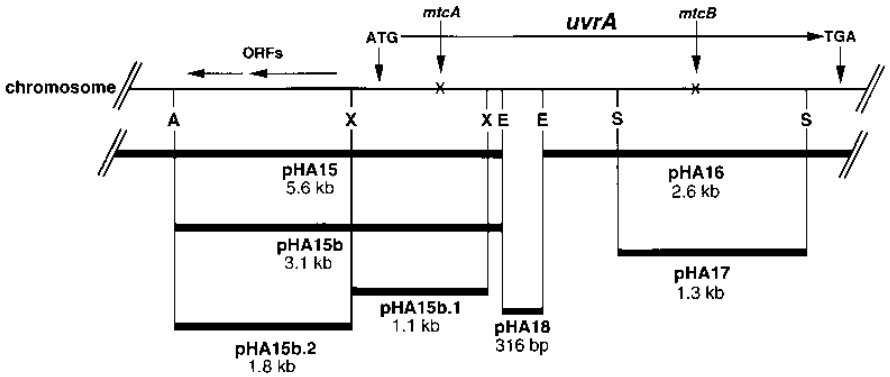

FIG. 1. Map of the *uvrA* gene of *D. radiodurans*. The amino acid coding sequence is indicated by the initiating ATG and terminating TGA, with an intervening arrow indicating the direction of transcription. The *D. radiodurans* DNA inserts in the various clones used for DNA sequencing are shown below, and their sizes are indicated. The locations shown for the defects in *mtcA* (*uvrA1*) and *mtcB* (*uvrA2*) in strains 302 and 262 are approximate, indicating that these strains can be transformed to Mm^r by pHA15b.1 and pHA17, respectively. Restriction enzyme recognition sites: A, *Acc*I; E, *Eco*RI; S, *Sty*I; X, *Xho*I. ORFs, open reading frames.

ethanolic solution of 4NQO to 10 ml of stationary-phase cells, giving a final concentration of 50 μg of 4NQO per ml. Following incubation, the cells were washed and diluted in fresh TGY and spread on TGY plates.

Nucleotide sequence accession number. The sequence of the *D. radiodurans uvrA* gene has been deposited in GenBank under accession number U52145.

RESULTS

DNA and deduced protein sequence of *D. radiodurans uvrA*. Identification of the defective loci in strains 302 (*mtcA*) and 262 (*mtcB*) proceeded by determining which DNA fragments from wild-type *D. radiodurans* R1 could transform these strains to mitomycin resistance (Mm^r) as determined by the ability of transformants to grow on TGY plates containing 0.04 μg of MM per ml (20). It had previously been determined that cosmid pUE502 (26 kb) from wild-type *D. radiodurans* could transform both strains 302 and 262 to Mm^r (1) and that two separate *Eco*RI subclones of pUE502, pUE58 (*D. radiodurans* DNA insert which is the same as in pHA15 in the present work) and pUE59 (insert which is the same as in pHA16), could transform strains 302 and 262, respectively, but they could not transform both loci (2). These clones were unmapped with respect to the parental cosmid pUE502. We further subcloned these regions to give pHA15b.1 and pHA17 (Fig. 1), which were able to transform strains 302 and 262 to Mm^r, respectively (Fig. 1). After full sequencing of these two clones and additional sequencing of their parental clones pHA15 and pHA16, as well as pHA18 and pUE502, the *uvrA* sequence was completed (Fig. 2).

A computer-assisted search showed that the DNA sequence homology of the *D. radiodurans* open reading frame with *E. coli uvrA* is 59% and the deduced amino acid sequence identity is 57% (Fig. 3). *D. radiodurans* shows greater homology at the DNA level with *Micrococcus luteus* (72%) than with *E. coli uvrA*, presumably because both *D. radiodurans* and *M. luteus* DNA are G+C rich (approximately 70%). At the amino acid sequence level, the deduced identity between *D. radiodurans* and *M. luteus* is 54%. A multiple protein sequence alignment of these three genes is shown in Fig. 3. Among all three amino acid sequences, there is a 40.2% identity and a 68% homology. The length of the *D. radiodurans* gene (3,045 bp, 1,015 amino acids [aa]) is similar to those of *E. coli* (2,820 bp, 940 aa) and *M. luteus* (2976 bp, 992 aa) (24, 42).

Chromosomal insertion of the *E. coli uvrA*$^+$ gene in *uvrA*-defective *D. radiodurans*. Heterologous genes are expressed poorly in *D. radiodurans* because of the inability of this organism to recognize promoters from other bacteria, including *E. coli* and *Staphylococcus* and *Bacillus* spp. (44, 46). One means of circumventing this expression problem in *D. radiodurans* is to introduce a heterologous gene at high copy number, using the duplication insertion vector pS11 that we have previously described (7, 21, 22, 45). This vector is composed of a *D. radiodurans* chromosomal fragment covalently linked to the *E. coli* plasmid pMK20; pMK20 contains a kanamycin resistance (Km^r) determinant (*aphA*; aminoglycoside-3′-phosphotransferase type I) which is weakly expressed in *D. radiodurans*. While pS11 replicates as a plasmid in *E. coli* because of the pMK20 portion, in *D. radiodurans* it does not replicate autonomously and instead confers Km^r by integrating into the chromosome via recombination between the pS11 vector deinococcal sequence and the recipient homologous chromosomal sequence (45). The integration event yields an insertion of the heterologous DNA (the pMK20 portion) flanked by a direct repeat of the host sequence. The presence of flanking direct repeats fosters gene amplification (presumably by uneven homologous recombination of daughter chromosomes [25, 39]) and KM selection produces up to 50 tandem copies per chromosome of the pS11 amplification unit, each unit consisting of the heterologous DNA plus flanking host sequence (45). *Dra*I and *Bgl*II cleavage sites adjacent to the *aphA* gene in pS11 (Fig. 4) may be used to introduce a gene to be expressed in *D. radiodurans*. Transformation of *D. radiodurans* followed by KM selection produces amplification of the gene inserted at

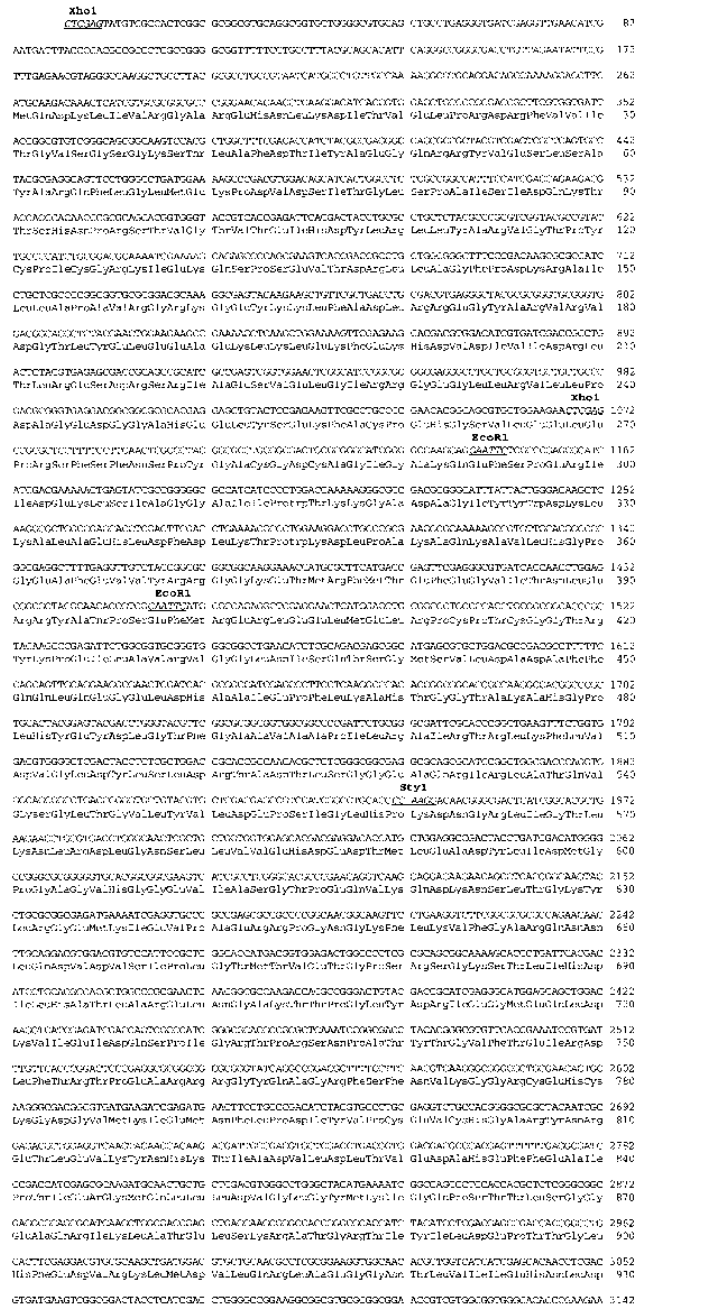

FIG. 2. The nucleotide and deduced amino acid sequences of the *D. radiodurans uvrA* gene. Restriction enzyme recognition sites corresponding to those indicated in Fig. 1 are italicized, underlined, and labeled with the appropriate enzyme above the sequence.

FIG. 3. Alignment of protein sequences of *uvrA* genes of *M. luteus*, *E. coli*, and *D. radiodurans*. Identical amino acids are indicated by an asterisk, and similar amino acids are indicated by a dot below the alignment. The dashed lines above the alignment corresponding to aa 32 to 46 and 678 to 692 of the *D. radiodurans* sequence indicate Walker A-type ATP recognition sequences. The pairs of short solid lines above the alignment corresponding to aa 259 to 286 and 277 to 803 of the *D. radiodurans* sequence indicate zinc finger motifs. The single, long line above the alignment corresponding to aa 534 to 552 of the *D. radiodurans* sequence is a helix-turn-helix motif.

these sites as a nonselected passenger within the pS11 amplification unit, resulting in substantial expression of the passenger gene by virtue of high copy number (21–23). When grown in drug-free culture, amplification units are lost from the chromosome by intrachromosomal recombination between repeats producing monomeric and multimeric circular forms of the pS11 amplification unit (30). This process results in cured Km^s isolates that have lost the chromosomal pS11 insertion and are identical to the original recipients prior to transformation.

A duplication insertion transformation construction designed to express the *E. coli uvrA*$^+$ gene was generated by inserting this gene into pS11, giving pHA101 (Fig. 4). Plasmids pS11 and pHA101 were introduced to the *uvrA*-defective strains 302 and 262, and Km^r transformants were selected. Gel electrophoresis of restriction-cleaved plasmids pS11 and pHA101 (pS11::*uvrA*) (plasmids were grown in *E. coli*, since pS11 is integrative in *D. radiodurans*) and chromosomal DNA from pS11- and pHA101-transformed cell lines shows substantial amplification of these insertions (Fig. 5). This is recognized as prominent ethidium bromide-stained bands at the proper locations in the chromosomal digestions. Southern blotting and probing with an internal fragment of the *E. coli uvrA* gene show the presence of this gene in the pHA101-transformed strains (Fig. 5).

Measurement of *E. coli* UvrA by immunoblotting in *uvrA*-defective *D. radiodurans*. To determine if the inserted *E. coli uvrA*$^+$ sequence was expressed in *D. radiodurans* hosts, cellular extracts were assessed for the presence of UvrA by affinity to the monoclonal antibody A2A3. This antibody recognizes the region of *E. coli* UvrA between amino acids 680 and 940, as determined by affinity to an ordered set of UvrA deletion mutants (27a, 28). Both strains 302 (Fig. 6) and 262 (data not shown) synthesized *E. coli* UvrA if the cells contained the pS11::*uvrA*$^+$ insertion.

Survival of *D. radiodurans uvrA*-defective strains that contain the *E. coli uvrA*$^+$ gene. Survival of strains 302 and 262 was enhanced to *D. radiodurans* wild-type or near-wild-type resistance by the presence of the pS11::*uvrA* duplication insertion

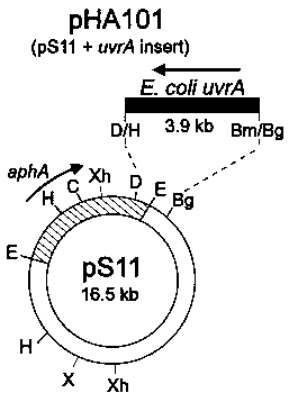

FIG. 4. Construction of integration vector pHA101 from pS11 and pSST10. pSST10 (not shown) was first cleaved with *Hin*dIII, and the resulting 5′ overhang was filled in with T4 DNA polymerase. The plasmid was then cleaved with *Bam*HI. This gives a 3.9-kb fragment from pSST10 that contains the complete 2,820-bp *E. coli uvrA*$^+$ gene (black segment). The fragment was inserted into pS11 that had been doubly cleaved with *Dra*I and *Bgl*II. Ligation resulted in a *Bam*HI-*Bgl*II fusion at one end of the *uvrA* insert and a fusion between the blunt-ended *Hin*dIII site and the naturally blunt-ended *Dra*I cleavage site in pS11 at the other end. The hatched segment of pS11 is the pMK20 portion, while the white segment is the *D. radiodurans* chromosomal DNA portion. Restriction endonuclease sites: Bg, *Bgl*II; Bm, *Bam*HI; Bm/Bgl, *Bam*HI-*Bgl*II fusion; C, *Cla*I; D, *Dra*I; D/H, *Dra*I–blunt-ended *Hin*dIII fusion; E, *Eco*RI; H, *Hin*dIII; X, *Xba*I; Xh, *Xho*I.

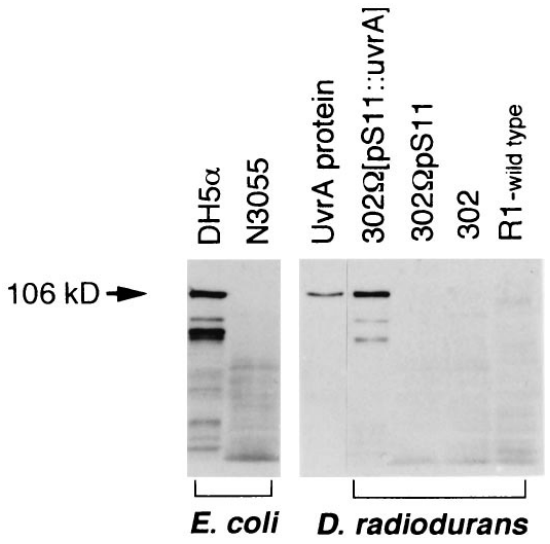

FIG. 6. Western blot of UvrA protein using monoclonal antibody A2A3. The procedure used is described in Materials and Methods. All lanes contain 100 μg of protein, except for the lane labeled "UvrA protein," which contains 200 ng of purified UvrA. The two *E. coli* lanes are controls with *uvrA*$^+$ strain DH5α and *uvrA* strain N3055, which contains a Tn*10* insertion in the *uvrA* coding sequence. *D. radiodurans* strain 302 shows evidence of UvrA protein only if transformed with pHA101 ([pS11::*E. coli uvrA*$^+$]; strain 322) and not if transformed with pS11 (strain 321).

with respect to treatment with MM, 4NQO, and Me_3psoralen plus near-UV exposure (Fig. 7). The presence of the pS11 duplication insertion (lacking *E. coli uvrA*$^+$) did not enhance survival of strain 302 or 262. Survival following UV (254-nm) exposure of strains 302 and 262 was wild type and displayed no change as a result of the duplication insertions (data not shown), since 302 and 262 contain the *uvsCDE* gene product, UV endonuclease-β (see above). Survival following ionizing-radiation exposure of strains 302 and 262 was also wild type and displayed no change as the result of the duplication insertions (data not shown), consistent with the fact that cells defective in either or both UV endonuclease-α and UV endonuclease-β are already wild type with respect to ionizing radiation (35). Strains 302 and 262 that had contained the pS11::*uvrA*$^+$ duplication insertion but were cured of the insertion by passage on nonselective agar (Fig. 5) displayed survival to MM, 4NQO, and Me_3psoralen identical with that of the parental *uvrA*-defective strains 302 and 262 (data not shown).

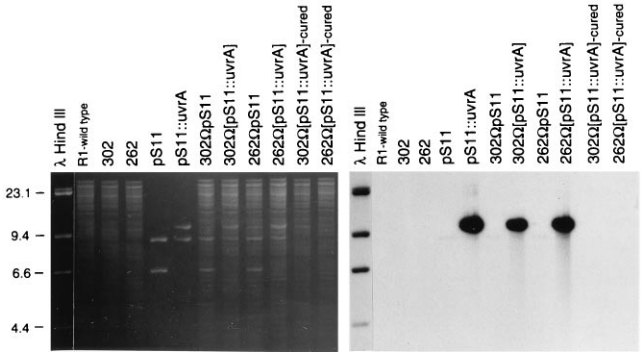

FIG. 5. Agarose gel electrophoresis and Southern blotting of transformed strains. Each lane contains 5 μg of DNA. Chromosomal DNA from the various strains and DNA from purified plasmid preparations of pS11 and pS11::*E. coli uvrA*$^+$ (pHA101) were digested with *Xho*I and *Xba*I and electrophoresed in 1% agarose at 50 V. The agarose gel was then stained with ethidium bromide (left) followed by blotting onto nitrocellulose. The gene amplification in transformed strains is evident in the ethidium bromide-stained gel from the brightness of the pS11 or pS11::*uvrA* characteristic bands. Strains 322 [302Ω(pS11::*uvrA*)] and 271 [262Ω(pS11::*uvrA*)] were propagated on KM-free agar yielding Km^s isolates that had lost the duplication insertion [302Ω(pS11::*uvrA*) cured and 262Ω(pS11::*uvrA*) cured]. The blots were probed with an internal 0.3-kb *Eco*RI fragment of the *E. coli uvrA*$^+$ gene (24), end labeled with [γ-^{32}P]ATP.

DNA sequence upstream of *uvrA*. In most bacteria examined, the single-stranded DNA binding protein gene (*ssb*) is normally located 200 to 900 bases upstream of *uvrA* and transcribed divergently, as seen in *E. coli* (41), *Proteus mirabilis* (12), *Serratia marcescens* (10, 11), *Salmonella typhimurium* (3), *Haemophilus influenzae* (26), and *Brucella abortus* (56). An exception to this rule is *Bacillus subtilis*, wherein *ssb* is located at approximately 4° on the chromosome (38) and *uvrA* is located at 305° (4). The 2,000 bp that lay upstream of the *D. radiodurans uvrA* gene does not show any appreciable homology to *ssb* or any other sequences in other organisms. The two largest open reading frames are 837 and 504 bp, both in the same reading frame and divergent from *uvrA*. Because the novel DNA repair gene *irrB* has a 22% cotransformation frequency with *uvrA1* (51), we suggest that the first of these reading frames, the 837-bp open reading frame, may be the *irrB*$^+$ gene (see Discussion on cotransformation frequency).

DISCUSSION

The DNA damage sensitivity of strains 302 (*mtcA*) and 262 (*mtcB*) was found to be due to defects in a single gene that was highly homologous with the *uvrA* genes of other bacteria. Thus, the mutations in strains 302 and 262 can now be designated *uvrA1* and *uvrA2*, respectively. Historically, it has been believed that independent transformation of two mutational markers in *D. radiodurans* indicated that the mutations were in two separate genes (33), in the present case the *mtcA* and the *mtcB* genes (2, 34). This notion is consistent with observations from work with other organisms: the average size of a DNA

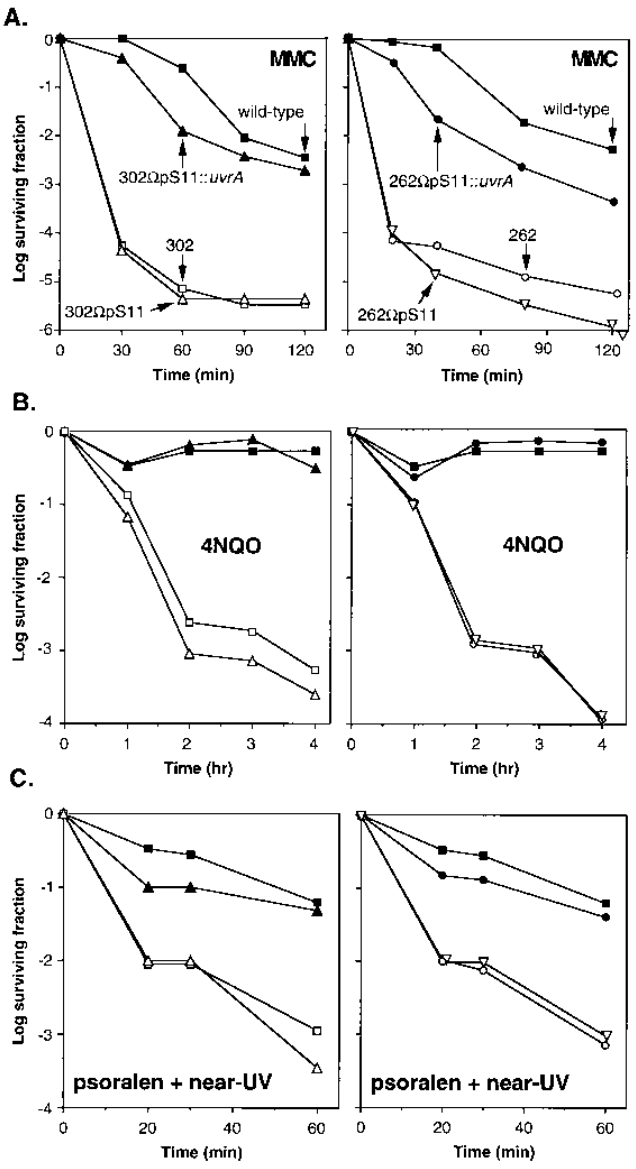

FIG. 7. Survival of *D. radiodurans* strains following exposures to DNA-damaging agents. Treatments were as described in Materials and Methods. (A) MM (MMC), 1 μg/ml; (B) 4NQO, 50 μg/ml; (C) Me_3psoralen, 0.6 μg/ml for variable durations followed by a fixed near-UV exposure. Symbols: black square, wild-type R1; white square, strain 302 (*mtcA*); white circle, strain 262 (*mtcB*); white triangle, strain 311 (302ΩpS11; *mtcA*); inverted white triangle, strain 270 (262ΩpS11; *mtcB*); black triangle, strain 322 [302ΩpHA101(pS11::*E. coli uvrA*$^+$); *mtcA*]; black circle, strain 271 [262ΩpHA101(pS11::*E. coli uvrA*$^+$); *mtcB*].

fragment incorporated into *B. subtilis*, *Streptococcus pneumoniae*, and *H. influenzae* during natural transformation has been estimated to be 4.3, 5, and 18 kb, respectively (13, 14, 19). However, it has recently been deduced that in *D. radiodurans* the size of the piece of DNA incorporated into the recipient strain during transformation is less than 1 kb (31). Thus, two mutations in the same gene could have very low-level or absent cotransformation efficiency. Transformation data obtained in this study indicate that the mutations in *mtcA* and *mtcB* are at least 0.9 kb and as much as 3.1 kb distant, as indicated by the ability to transform *mtcA* with pHA15b.1 and *mtcB* with pHA17 (Fig. 1).

Structural features of the *D. radiodurans* UvrA protein. In addition to the high homology of *D. radiodurans* UvrA protein and *E. coli* UvrA, there are striking similarities at particular small functional domains of critical importance that have been defined by mutagenesis studies of the *E. coli* UvrA protein. The two Walker A-type ATP binding consensus sequences, G-X_3-GKT(S)-X_6-I(L) (24, 53) are completely conserved (Fig. 3), consonant with findings that both ATPase sites in *E. coli* UvrA have functional roles in nucleotide excision repair (50). The 20-aa helix-turn-helix motif in the midportion of the protein is very highly conserved (Fig. 3), also consistent with the observation that mutations in this motif in *E. coli* UvrA protein eliminate its specificity for UV-damaged DNA (54). *E. coli* UvrA contains two zinc finger consensus sequences ($CXXCX_{18-20}CXXC$ [6]), each of which coordinates a zinc atom by bonding to the four cysteines (36). Zinc fingers are implicated in DNA binding (6). The C-terminal zinc finger in UvrA is fully conserved in *D. radiodurans*, consistent with site-specific mutagenesis studies of *E. coli* UvrA that indicate that this zinc finger is necessary for UvrA protein to bind DNA (52, 55). The N-terminal zinc finger is not fully conserved in *D. radiodurans*, lacking a cysteine residue at aa 262. However, the function of the N-terminal zinc finger in *E. coli* UvrA is unknown, since site-specific mutagenesis studies of this zinc finger show modest or no changes in UvrA function or cellular survival (36, 52, 55). Thus, the meaning of this variation at the N-terminal zinc finger motif in *D. radiodurans* is uncertain.

The fact that the *E. coli* UvrA protein can fully or partially complement the repair defect in *uvrA D. radiodurans* (Fig. 7) is remarkable, in that it implies that *E. coli* UvrA is interacting with a hypothetical *D. radiodurans* UvrB to form the $UvrA_2B$ complex integral to the UvrABC pathway (8, 18, 40). On the basis of studies of serial deletion mutants, it is believed that the first 230 aa of *E. coli* UvrA contains the minimal region necessary for interactions with UvrB (8). Within this region, we find that extremely high identity and similarity exist for the first 120 aa of *E. coli* and *D. radiodurans* UvrA, as well as high identity with the corresponding region of the *M. luteus* UvrA. Similarity among these sequences on the same order does not occur again until approximately aa 500. This leads us to suggest that the first 120 aa may form the interface between $UvrA_2$ and UvrB and that the failure of *E. coli* UvrA to fully complement *uvrA D. radiodurans* with respect to survival to MM and Me_3psoralen plus near UV (Fig. 7) may be due to suboptimal conformation for the requisite interface between $UvrA_2$ and UvrB.

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REFERENCES

1. **Al-Bakri, G. H.** 1985. Ph.D. thesis. University of Edinburgh, Edinburgh.
2. **Al-Bakri, G. H., M. W. Mackay, P. A. Whittaker, and B. E. B. Moseley.** 1985. Cloning of the DNA repair genes *mtcA*, *mtcB*, *uvsC*, *uvsD*, *uvsE* and the *leuB* gene from *Deinococcus radiodurans*. Gene **33:**305–311.
3. **Alberti, M., Y. Li, A. Sancar, and J. E. Hearst.** 1992. *Salmonella typhimurium* single stranded DNA binding protein (ssb) gene, 5′ end; UvrA gene, complete coding sequence. GenBank accession no. M93014. Unpublished data.
4. **Anagnostopoulos, C., P. J. Piggot, and J. A. Hoch.** 1993. The genetic map of *Bacillus subtilis*, p. 425–461. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria. American Society for Microbiology, Washington, D.C.
5. **Anderson, A., H. Nordan, R. Cain, G. Parrish, and D. Duggan.** 1956. Studies on a radioresistant micrococcus. I. Isolation, morphology, cultural characteristics, and resistance to gamma radiation. Food Technol. **10:**575–578.
6. **Berg, J. M.** 1986. Potential metal-binding domains in nucleic acid binding proteins. Science **232:**485–487.

7. **Carroll, J. D., M. J. Daly, and K. W. Minton.** 1996. Expression of *recA* in *Deinococcus radiodurans*. J. Bacteriol. **178:**130–135.
8. **Claassen, L. A., and L. Grossman.** 1991. Deletion mutagenesis of the *Escherichia coli* UvrA protein localizes domains for DNA binding, damage recognition, and protein-protein interactions. J. Biol. Chem. **266:**11388–11394.
9. **Daly, M. J., L. Ouyang, P. Fuchs, and K. W. Minton.** 1994. In vivo damage and *recA*-dependent repair of plasmid and chromosomal DNA in the radiation-resistant bacterium *Deinococcus radiodurans*. J. Bacteriol. **176:**3508–3517.
10. **de Vries, J., J. Genschel, C. Urbanke, H. Thole, and W. Wackernagel.** 1994. The single-stranded-DNA-binding proteins (SSB) of *Proteus mirabilis* and *Serratia marcescens*. Eur. J. Biochem. **224:**613–622.
11. **de Vries, J., and W. Wackernagel.** 1993. Cloning and sequencing of the *Serratia marcescens* gene encoding a single-stranded DNA-binding protein (SSB) and its promoter region. Gene **127:**39–45.
12. **de Vries, J., and W. Wackernagel.** 1994. Cloning and sequencing of the *Proteus mirabilis* gene for a single-stranded DNA-binding protein (SSB) and complementation of *Escherichia coli ssb* point and deletion mutations. Microbiology **140:**889–895.
13. **Dubnau, D.** 1982. Genetic transformation in *Bacillus subtilis*, p. 147–178. *In* D. Dubnau (ed.), The molecular biology of the bacilli, vol. 1. Academic Press, Inc., New York.
14. **Dubnau, D., and C. Cirigliano.** 1972. Fate of transforming deoxyribonucleic acid after uptake by competent *Bacillus subtilis*: size and distribution of integrated donor segments. J. Bacteriol. **111:**488–494.
15. **Evans, D. M., and B. E. B. Moseley.** 1983. Roles of the *uvsC*, *uvsD*, *uvsE*, and *mtcA* genes in the two pyrimidine dimer excision repair pathways of *Deinococcus radiodurans*. J. Bacteriol. **156:**576–583.
16. **Evans, D. M., and B. E. B. Moseley.** 1985. Identification and initial characterization of a pyrimidine dimer UV endonuclease (UV endonuclease-beta) from *Deinococcus radiodurans*: a DNA-repair enzyme that requires manganese ions. Mutat. Res. **145:**119–128.
17. **Evans, D. M., and B. E. B. Moseley.** 1988. *Deinococcus radiodurans* UV endonuclease-beta DNA incisions do not generate photoreversible thymine residues. Mutat. Res. **207:**117–119.
18. **Grossman, L., and S. Thiagalingam.** 1993. Nucleotide excision repair, a tracking mechanism in search of damage. J. Biol. Chem. **268:**16871–16874.
19. **Gurney, T. J., and M. S. Fox.** 1968. Physical and genetic hybrids formed in bacterial transformation. J. Mol. Biol. **32:**83–100.
20. **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.
21. **Gutman, P. D., P. Fuchs, and K. W. Minton.** 1994. Restoration of the DNA damage resistance of *Deinococcus radiodurans* DNA polymerase mutants by *Escherichia coli* DNA polymerase I and Klenow fragment. Mutat. Res. DNA Repair **314:**87–97.
22. **Gutman, P. D., H. Yao, and K. W. Minton.** 1991. Partial complementation of the UV sensitivity of *Deinococcus radiodurans* excision repair mutants by the cloned *denV* gene of bacteriophage T4. Mutat. Res. DNA Repair **254:**207–215.
23. **Hansen, M. T.** 1980. Four proteins synthesized in response to deoxyribonucleic acid damage in *Micrococcus radiodurans*. J. Bacteriol. **141:**81–86.
24. **Husain, I., B. van Houten, D. C. Thomas, and A. Sancar.** 1986. Sequences of *Escherichia coli uvrA* gene and protein reveal two potential ATP binding sites. J. Biol. Chem. **261:**4895–4901.
25. **Janniere, L., B. Niaudet, E. Pierre, and S. Ehrlich.** 1985. Stable gene amplification in the chromosome of *Bacillus subtilis*. Gene **40:**47–55.
26. **Jarosik, G. P., and E. J. Hansen.** 1994. Cloning and sequencing of the *Haemophilus influenzae ssb* gene encoding single-strand DNA-binding protein. Gene **146:**101–103.
27. **Kitayama, S., S. Asaka, and K. Totsuka.** 1983. DNA double-strand breakage and removal of cross-links in *Deinococcus radiodurans*. J. Bacteriol. **155:** 1200–1207.

27a. **Kovalsky, O.** Personal communication.

28. **Kovalsky, O. I., and L. Grossman.** 1994. The use of monoclonal antibodies for studying intermediates in DNA repair by the *Escherichia coli* Uvr(A)BC endonuclease. J. Biol. Chem. **269:**27421–27426.
29. **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.
30. **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.
31. **Mattimore, V., K. S. Udupa, G. A. Berne, and J. R. Battista.** 1995. Genetic characterization of forty ionizing-radiation-sensitive strains of *Deinococcus radiodurans*: linkage information from transformation. J. Bacteriol. **177:** 5232–5237.
32. **Minton, K. W.** 1994. DNA repair in the extremely radioresistant bacterium *Deinococcus radiodurans*. Mol. Microbiol. **13:**9–15.
33. **Moseley, B. E. B.** 1983. Photobiology and radiobiology of *Micrococcus* (*Deinococcus*) *radiodurans*. Photochem. Photobiol. Rev. **7:**223–275.
34. **Moseley, B. E. B., and H. J. R. Copland.** 1978. Four mutants of *Micrococcus radiodurans* defective in the ability to repair DNA damaged by mitomycin-C, two of which have wild-type resistance to ultraviolet irradiation. Mol. Gen. Genet. **160:**331–337.
35. **Moseley, B. E. B., and D. M. Evans.** 1983. Isolation and properties of strains of *Micrococcus* (*Deinococcus*) *radiodurans* unable to excise ultraviolet light-induced pyrimidine dimers from DNA: evidence for two excision pathways. J. Gen. Microbiol. **129:**2437–2445.
36. **Navaratnam, S., G. M. Myles, R. W. Strange, and A. Sancar.** 1989. Evidence from extended X-ray absorption fine structure and site-specific mutagenesis for zinc fingers in UvrA protein of *Escherichia coli*. J. Biol. Chem. **264:**16067–16071.
37. **Novick, R. P., R. C. Clowes, S. N. Cohen, R. Curtiss III, N. Datta, and S. Falkow.** 1976. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol. Rev. **40:**168–189.
38. **Ogasawra, N., S. Nakai, and H. Yoshikawa.** 1994. Systematic sequencing of the 180 kilobase region of the *Bacillus subtilis* chromosome containing the replication origin. DNA Res. **1:**1–14.
39. **Peterson, B. C., and R. H. Rownd.** 1983. Homologous sequences other than insertion elements can serve as recombination sites in drug resistance gene amplification. J. Bacteriol. **156:**177–185.
40. **Sancar, A., and M.-S. Tang.** 1993. Nucleotide excision repair. Photochem. Photobiol. **57:**905–921.
41. **Sancar, A., K. R. Williams, J. W. Chase, and W. D. Rupp.** 1981. Sequences of the *ssb* gene and protein. Proc. Natl. Acad. Sci. USA **78:**4274–4278.
42. **Shiota, S., and H. Nakayama.** 1989. *Micrococcus luteus* homolog of the *Escherichia coli uvrA* gene: identification of a mutation in the UV-sensitive mutant DB7. Mol. Gen. Genet. **217:**332–340.
43. **Sinden, R. R., and R. S. Cole.** 1981. Measurement of cross-links formed by treatment with 4,5′,8-trimethylpsoralen and light, p. 69–81. *In* E. C. Friedberg and P. C. Hanawalt (ed.), DNA repair: a laboratory manual of research procedures, vol. 1. Marcel Dekker, Inc., New York.
44. **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.
45. **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.
46. **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.
47. **Sweet, D. M., and B. E. B. Moseley.** 1976. The resistance of *Micrococcus radiodurans* to killing and mutation by agents which damage DNA. Mutat. Res. **34:**175–186.
48. **Tempest, P. R., and B. E. B. Moseley.** 1978. Role of the gene *mtcA* in the resistance of *Micrococcus radiodurans* to the lethal effects of mitomycin C and alkylation mutagenesis, p. 283–286. *In* P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), DNA repair mechanisms. Academic Press, New York.
49. **Tempest, P. R., and B. E. B. Moseley.** 1980. Defective excision repair in a mutant of *Micrococcus radiodurans* hypermutable by some monofunctional alkylating agents. Mol. Gen. Genet. **179:**191–199.
50. **Thiagalingam, S., and L. Grossman.** 1991. Both ATPase sites of *Escherichia coli* UvrA have functional roles in nucleotide excision repair. J. Biol. Chem. **266:**11395–11403.
51. **Udupa, K. S., P. A. O'Cain, V. Mattimore, and J. R. Battista.** 1994. Novel ionizing-radiation-sensitive mutants of *Deinococcus radiodurans*. J. Bacteriol. **176:**7439–7446.
52. **Visse, R., M. de Ruijter, M. Ubbink, J. A. Brandsma, and P. van de Putte.** 1993. The first zinc-binding domain of UvrA is not essential for UvrABC-mediated DNA excision repair. Mutat. Res. DNA Repair **294:**263–274.
53. **Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay.** 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. **1:**945–951.
54. **Wang, J., and L. Grossman.** 1993. Mutations in the helix-turn-helix motif of the *Escherichia coli* UvrA protein eliminate its specificity for UV-damaged DNA. J. Biol. Chem. **268:**5323–5331.
55. **Wang, J., K. L. Mueller, and L. Grossman.** 1994. A mutational study of the C-terminal zinc-finger motif of the *Escherichia coli* UvrA protein. J. Biol. Chem. **269:**10771–10775.
56. **Zhu, Y., S. C. Oliveira, and G. A. Splitter.** 1993. Isolation of *Brucella abortus ssb* and *uvrA* genes from a genomic library by use of lymphocytes as probes. Infect. Immun. **61:**5339–5344.