

## Genetic Evidence that the *uvrA* Gene Product of *Deinococcus radiodurans* R1 Is a UV Damage Endonuclease

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**An in vitro transposition system, developed to facilitate gene disruption in *Deinococcus radiodurans* R1, has been used to inactivate the gene designated *dr1819* in *uvrA-1*<sup>+</sup> and *uvrA-1* backgrounds. *dr1819* encodes a protein with homology to a UV DNA damage endonuclease expressed by *Schizosaccharomyces pombe*. Interruption of *dr1819* greatly sensitizes the *uvrA-1* strain but not the *uvrA-1*<sup>+</sup> strain to UV light, indicating that the *dr1819* gene product is a component in a DNA repair pathway that can compensate for the loss of nucleotide excision repair in this species. Clones of *dr1819* will restore UV resistance to UVS78, a *uvrA-1 uvrA* strain, indicating that *dr1819* and *uvrA* are the same locus.**

The *Deinococcus radiodurans* R1 genome encodes homologues of all components of the nucleotide excision repair pathway of *Escherichia coli*, including two UvrA-like proteins, UvrA-1 and UvrA-2 (34). The *uvrA-1* gene product has 52% identity with the UvrA proteins of *E. coli* and *Micrococcus luteus* and appears to be the protein that functions in nucleotide excision repair (1, 26). Inactivation of *uvrA-1* sensitizes an otherwise wild-type strain of *D. radiodurans* to mitomycin C.

There are two well-characterized null mutations of the *uvrA-1* gene of *D. radiodurans* R1. Strain 262 carries a 1,300-bp insertion sequence (IS) element, designated IS2621, that inserted 986 bp from the 3' end of the *uvrA-1* gene (26). Strain 302 has a 144-bp deletion that removes the first 34 bp of the *uvrA-1* coding sequence and 110 bp of upstream sequence (26). Each mutation completely eliminates *D. radiodurans*' ability to grow in the presence of 60 ng of mitomycin C per ml (21, 32). The *E. coli uvrA* gene will complement the *D. radiodurans uvrA-1* alleles, restoring mitomycin C resistance (1) to strains 262 and 302.

Unlike the *uvrA* strains of *E. coli*, the *uvrA-1* strains of *D. radiodurans* exhibit nearly wild-type levels of resistance to UV light. A second locus, *uvrB*, must be inactivated before *uvrA-1* strains become UV sensitive (9, 10, 22). The *uvrB* gene product, also called endonuclease  $\beta$ , appears to be part of an alternative excision repair pathway that completely compensates for the loss of nucleotide excision repair. Three mutations have been described that inactivate endonuclease  $\beta$  activity in *D. radiodurans* strain 302 (10), and it has been reported that each mutation affects a separate coding sequence, designated *uvrB*, *uvrC*, and *uvrD*. Evans and Moseley (9) have argued that endonuclease  $\beta$  is a multisubunit protein, but there is no definitive biochemical evidence to support this claim. A cell that is *uvrA-1* and that carries any one of the three *uvrB* mutations is unable to incise its DNA following UV irradiation and has lost the capacity to remove pyrimidine dimers from its chromosomal DNA (22).

Although this alternative excision repair pathway has not been thoroughly characterized, Gutman et al. (14) have proposed that endonuclease  $\beta$  may be a pyrimidine dimer DNA glycosylase (PD glycosylase), because expression of the *denV* gene of bacteriophage T4 partially restores UV resistance to a *uvrA-1 uvrA* strain of *D. radiodurans*. Two lines of evidence, however, argue against this suggestion. (i) The annotation of the *D. radiodurans* R1 genome failed to identify a coding sequence similar to known PD glycosylases (19, 34). (ii) Endonuclease  $\beta$  does not cleave the *N*-glycosyl bond of the 5' base in a pyrimidine dimer as do known PD glycosylases. Instead, this enzyme appears to recognize pyrimidine dimers and cleave a phosphodiester bond immediately 5' to the lesion (8). In this respect, endonuclease  $\beta$  mimics the behavior of the UV damage endonucleases (UVDE proteins) of the eukaryotes *Neurospora crassa* (35) and *Schizosaccharomyces pombe* (6, 30). The eukaryotic proteins recognize a wide range of UV-induced DNA damage and introduce an incision into the DNA backbone 5' to the lesion, initiating a sequence of events that remove the damage (7, 15, 30, 36). The nick in the DNA results in exonucleolytic digestion of the strand carrying the damage, followed by DNA synthesis to fill in the resulting gap (2, 16, 17).

The annotation of the *D. radiodurans* genome did reveal the existence of a gene, *dr1819*, that encodes a protein that has approximately 30% amino acid sequence identity with the UVDE protein of *S. pombe*, suggesting that *dr1819* encodes endonuclease  $\beta$ . (The gene designations used throughout this paper are based on those used in The Institute for Genomic Research [TIGR] Comprehensive Microbial Resource [http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gdr]. The prefix is given in lowercase letters, and loci are italicized.) In addition, a DNA sequence (accession number BAA85759), annotated as the *uvrB* coding sequence, was deposited in the National Center for Biotechnology Information (NCBI) database in 1999 without evidence supporting the annotation. This sequence is identical to *dr1819*.

In this study, we have disrupted the coding sequence of *dr1819* using an in vitro transposition system designed specifically for *D. radiodurans* and demonstrate that the resulting

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Reference or source
<i>D. radiodurans</i>		
R1	ATCC 13939	Anderson et al. (3)
302	As R1 but <i>uvrA-1</i>	Moseley and Copland (21), Narumi et al. (26)
UVS78	As 302 but <i>uvrE</i>	Moseley and Evans (22)
LSU1000	As 302 but <i>dr1819::TnDrCat</i>	This study
LSU2000	As R1 but <i>dr1819::TnDrCat</i>	This study
<i>E. coli</i> DH5 $\alpha$ MCR	K-12 F <sup>-</sup> <i>mcrA</i> $\Delta$ ( <i>mrr-hsdRMS-mcrBC</i> ) $\phi$ 80d <i>lacZ</i> $\Delta$ 15 $\Delta$ ( <i>lacZYA-argF</i> )U169 <i>endA1 recA1 deoR thi-1 phoA supE44</i> $\lambda^-$ <i>gyrA96 relA1</i>	Invitrogen Inc.
Plasmids		
pGEM-T	Amp <sup>r</sup>	Promega, Madison, Wis.
pBC	Amp <sup>r</sup>	Stratagene, La Jolla, Calif.
pGPS3	Amp <sup>r</sup> Kan <sup>r</sup>	New England Biolabs, Beverly, Mass.
pTTC101	pGEM-T derivative with a <i>TnDrCat</i> (2,623-bp) insert; Cat <sup>r</sup> Amp <sup>r</sup>	This study
pGTC101	pGPS3 derivative with a <i>TnDrCat</i> insert; Cat <sup>r</sup> Kan <sup>r</sup> Amp <sup>r</sup>	This study
<i>pdr1771</i>	pGEM-T derivative with 3,441 bp of <i>dr1771</i> ( <i>uvrA-1</i> ) and adjacent sequence, Amp <sup>r</sup>	This study
<i>pdr1819</i>	pGEM-T derivative with 3,441 bp of <i>dr1819</i> (putative <i>uvrE</i> ) and adjacent sequence, Amp <sup>r</sup>	This study
<i>pdr1819-54</i>	<i>pdr1819</i> derivative with a <i>TnDrCat</i> insertion within the <i>dr1819</i> coding sequence	This study
<i>pdr1819-65</i>	<i>pdr1819</i> derivative with a <i>TnDrCat</i> insertion within the <i>dr1819</i> coding sequence	This study
<i>pdr1819-77</i>	<i>pdr1819</i> derivative with a <i>TnDrCat</i> insertion within the <i>dr1819</i> coding sequence	This study
<i>pdr1819-80</i>	<i>pdr1819</i> derivative with a <i>TnDrCat</i> insertion within the <i>dr1819</i> coding sequence	This study

strain is sensitive to UV light in a *uvrA-1* background. We also provide evidence that *dr1819* is the *uvrE* gene described in earlier literature.

#### MATERIALS AND METHODS

**Bacterial strains and plasmids.** Bacterial strains and plasmids used in this study are listed in Table 1. All *D. radiodurans* strains were grown at 30°C in TGY broth (0.5% tryptone, 0.3% yeast extract, 0.1% glucose) or on TGY agar (1.5% agar). *E. coli* strains were grown in Luria-Bertani broth (28) or on Luria-Bertani plates at 37°C. Plasmids were propagated in *E. coli* strain DH5 $\alpha$  MCR.

**Transformation in liquid culture.** Calcium chloride from a 1 M stock solution was added to *D. radiodurans* cultures in exponential growth until a final concentration of 30 mM was achieved. This mixture was incubated at 30°C for 80 min (32). Either 1  $\mu$ g of plasmid DNA or 10  $\mu$ g of chromosomal DNA was added to 1 ml of TGY containing  $2 \times 10^7$  cells and incubated on ice for 30 min. The transformation mixture was diluted 10-fold with TGY broth and incubated for another 18 h at 30°C. When this transformation protocol was used to identify *uvrA*<sup>+</sup> cells, transformants were selected on TGY plates containing 60 ng of mitomycin C per ml (32).

**Chromosomal DNA isolation.** TGY broth (200 ml) was inoculated with a 2-ml overnight culture (approximately  $2 \times 10^8$  CFU/ml) of *D. radiodurans* R1. After 48 h, the 200-ml culture was harvested by centrifugation at 4°C at  $3,000 \times g$  for 15 min. Pellets were resuspended in 20 ml of 95% ethanol and held at room temperature for 10 min to remove the *D. radiodurans* outer membrane. The ethanol-stripped cells were collected by centrifugation at 4°C at  $3,000 \times g$  for 15 min, and the resulting pellet was resuspended in 9 ml of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA [pH 8.0]). Two milligrams of lysozyme (Sigma Chemical, St. Louis, Mo.) was added to the stripped cells, and this mixture was incubated at 37°C for 30 min. Then 0.5 ml of 10% sodium dodecyl sulfate (SDS) and 50  $\mu$ l of 20-mg/ml proteinase K (Sigma Chemical) were added to lysozyme-treated cells and incubated for 3 h at 56°C.

Lysed cells were transferred to a centrifuge tube and extracted once with an equal volume of phenol-chloroform (1:1) and twice with equal volumes of chloroform-isoamyl alcohol (24:1). The DNA was precipitated from the extracted material with 1 ml of 3 M sodium acetate (pH 7.0) and 20 ml of ice-cold 100% ethanol. The DNA was spooled out with a curved glass rod and washed twice with 70% ethanol. The DNA was air dried, dissolved in 5 ml of TE buffer (pH 8.0), and stored at 4°C.

**Survival curves.** Only *D. radiodurans* cultures in exponential growth ( $2 \times 10^6$  to  $4.5 \times 10^7$  CFU/ml) were evaluated for their ability to survive UV or ionizing radiation. All *D. radiodurans* cultures were treated at 25°C. UV irradiation was conducted with a germicidal lamp with a calibrated dose rate of 25 J/m<sup>2</sup>/s generated UV light. Gamma irradiation was conducted with a model 484R <sup>60</sup>Co irradiator (J. L. Shepherd & Associates, San Fernando, Calif.) at a rate of 30.8

Gy/min. Irradiated cultures were diluted, plated in triplicate on TGY agar plates, and incubated for 3 days at 30°C before scoring for survivors.

**Construction of *pdr1819* and *pdr1771*.** A PCR fragment encoding the putative *uvrE* gene (*dr1819*) of *D. radiodurans* R1 was amplified directly from purified chromosomal DNA using an appropriate pair of primers derived from the published sequence of the R1 genome. The primers, designated *uvsup* and *uvsdwn* (Table 2), generate a 1,348-bp fragment that includes the coding sequence plus 180 bp of upstream and 190 bp of downstream sequence. PCRs were carried out using Ready-To-Go PCR Beads (Amersham Pharmacia, Piscataway, N.J.) and were supplemented with betaine and dimethyl sulfoxide at final concentrations of 1.3 M and 1.3%, respectively. The PCR products were isolated using the Prep-A-Gene DNA purification systems (Bio-Rad, Hercules, Calif.). The *dr1819*-containing PCR fragments were inserted directly into the vector pGEM-T (Promega, Madison, Wis.) to generate the construct *pdr1819*.

Plasmid *pdr1771* was constructed in a similar manner. A PCR fragment encoding the *uvrA-1* gene of *D. radiodurans* R1 was amplified using a pair of primers derived from the published sequence of the R1 genome (Table 2). The primers, designated *uvrAFP* and *uvrADP*, generate a 3,320-bp fragment that includes the *uvrA-1* coding sequence plus 306 bp of upstream and 87 bp of downstream sequence. The PCR product was ligated in pGEM-T as described above. The inserts were sequenced to confirm that the clones were the *D. radiodurans* R1 genes designated *dr1819* and *dr1771*. The sequences obtained were identical to that reported at the TIGR web site (<http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.pl?database=gdr>).

TABLE 2. Primers used for construction of pGTC101, *pdr1819*, and *pdr1771*

Primer	Sequence
<i>uvsup</i>	5'-CGAATCCATCGGAACCTCCTCAGAGTAAGC
<i>uvsdwn</i>	5'-GTACTTCTGGCAAACCGCCGACTGCGTGAC
<i>uvrAFP</i>	5'-TCGAAGACCGGCAGCTTATCG A
<i>uvrADP</i>	5'-TCCATCTCCCGCAGGACGTAT T
Tu1	5'-AGCTTTGTTTAAACACGTGTACAACGCCTCC AAGGAC
Tu2-1	5'-TGATTTTTTCTCCATTGTCTTACTCCCTCCAA GCGGTG
Cat1-1	5'-GGAGGGAGTAAGACAATGGAGAAAAAATC ACTGGATATACCAC
Cat2-2	5'-ACTTATTTCAGGCATGCAACCAGGC
N	5'-ACTTTATTGTTCATAGTTTATGATCTATTTTG
S	5'-ATAATCCTTAAAAACTCCATTTCACCCCT

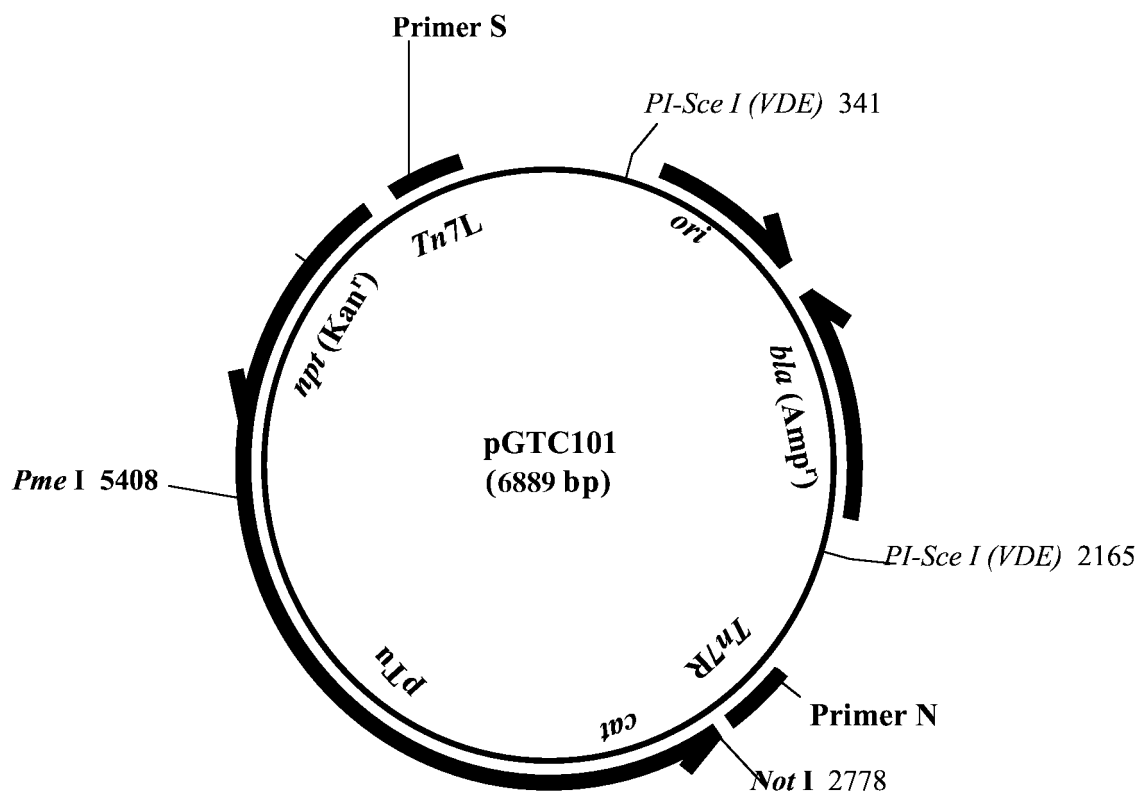


FIG. 1. Plasmid pGTC101. A *Pme*I- and *Not*I-digested TuCat fragment from pTTC101 was cloned into pGPS3. *PI-Sce* I(VDE), *PI-Sce* I (intein from VDE [*VMAI*-derived endonuclease of *S. cerevisiae*]).

**Construction of pGTC101.** The *tuf1* (*dr0309*) gene product, elongation factor Tu, is the only protein known that is constitutively expressed at high level in *D. radiodurans* (31). For this reason, we chose to fuse the 1,907-bp sequence immediately upstream of *tuf1* (here designated pTu) to the *cat* gene of Tn9 in a three-step process. (i) Primers were designed (Table 2) that permitted efficient amplification of pTu. Prior to amplification, *D. radiodurans* R1 chromosomal DNA (200 ng) was denatured in the presence of the primers (150 ng of each) by heating to 95°C for 5 min. Four units of *Pfu* polymerase (Stratagene, La Jolla, Calif.) were added to the denatured template to initiate PCR. The conditions for the first 15 cycles of amplification were denaturation at 95°C for 1 min, annealing at 64°C for 1 min, and extension at 72°C for 4 min. For the next 10 cycles, the length of the extension step was increased by 10 s per cycle. After 25 cycles, the reaction was held at 72°C for 10 min. The resulting PCR product duplicated pTu, beginning 1,907 bp upstream of the initiation codon of the *tuf1* gene and ending with the base pair adjacent to the initiation codon. Primer Tu1-1 included a tail that, when amplified, resulted in formation of a *Pme*I restriction site. Primer Tu2-1 included a tail that overlapped the *cat* gene found on the vector pBC (Stratagene, La Jolla, Calif.).

(ii) The 716 bp of the *cat* gene from pBC was also amplified as described using the primers Cat1-1 and Cat1-2 (Table 2). The Cat1-1 primer included a tail that overlapped the pTu promoter region. The Cat1-2 primer included a tail that, when amplified, resulted in formation of a *Not*I restriction site.

(iii) The purified PCR products from steps i and ii were combined with primers Tu1-1 and Cat1-2 in a final PCR. The overlap between the amplified pTu fragment and the amplified *cat* gene resulted in the formation of a fusion product with a *Pme*I site upstream of pTu and a *Not*I site downstream of *cat*. For undetermined reasons, it was not possible to efficiently cut the spliced fragment with either *Pme*I or *Not*I. The spliced PCR fragment was therefore inserted into pGEM-T (Promega), a vector that provides a 3'-compatible overhang for PCR products. The resulting construct, designated pTTC101, was propagated in the *E. coli* strain DH5 $\alpha$  MCR (Invitrogen, Inc., Gaithersburg, Md.).

Purified pTTC101 was digested with *Pme*I and *Not*I restriction enzymes, and the pTu-*cat* fusion was isolated. This fragment was inserted into *Pme*I- and *Not*I-digested pGPS3 (New England Biolabs, Beverly, Mass.), creating a construct that could be used in an in vitro transposition reaction. The resulting construct, designated pGTC101 (Fig. 1), was propagated in DH5 $\alpha$  MCR.

## RESULTS

**In vitro transposition using pGTC101.** In vitro transposition was performed using the protocol developed by New England Biolabs for the GPS-M mutagenesis system. Twenty nanograms of purified, circular pGTC101 was combined with the TnsABC\* transposase supplied with the system (4) and target DNA (*pdr1819*). The target-to-donor molar ratio was maintained at 4:1. Following the transposition reaction, unreacted pGTC101 molecules were destroyed by digestion with 8 U of *PI-Sce*I (a rare-cutting site-specific DNA endonuclease derived from an intein in *Saccharomyces cerevisiae*; New England Biolabs) for 4 h at 37°C. The parent of pGTC101, pGPS3, was constructed to contain a pair of *PI-Sce*I restriction sites. The transposon generated by the action of the TnsABC\* transposase on pGTC101 was designated TnDrCat.

The transposition reaction mixture was transformed by electroporation into approximately  $5 \times 10^5$  CFU of electrocompetent DH5 $\alpha$  MCR. Successful transposon insertions into the target were selected by plating the electroporated cells onto LB medium containing 25  $\mu$ g of chloramphenicol per ml. Approximately 200 Cat<sup>+</sup> colonies were recovered following in vitro transposition and electroporation. Thirty of the Cat<sup>+</sup> colonies were picked, and the plasmids they carried were isolated. These plasmids were digested with a combination of *Apa*I and *Pst*I to release the gene of interest from the vector. Digests were separated on 1% agarose and stained with ethidium bromide to determine whether the transposon had inserted into the putative *uvrE* gene. Four of the first 80 *pdr1819*::TnDrCat plasmids examined appeared to have inserts in the putative *uvrE*

TABLE 3. Position of insertions within the *dr1819* coding sequence after in vitro transposition with TnDrCat

Plasmid	Transposon inserted after base:
<i>pdr1819-54</i>	2
<i>pdr1819-65</i>	1030
<i>pdr1819-77</i>	1203
<i>pdr1819-80</i>	726

sequence. DNA sequencing using primer-binding sites (primer N and primer S; Table 2) within TnDrCat confirmed the position of insertion. The position of each insertion is identified in Table 3.

A *Pst*I-*Apa*I fragment was isolated and purified from *pdr1819-80*::TnDrCat. One microgram of this fragment was added to competent cultures of *D. radiodurans* strain 302 (approximately  $10^7$  CFU/ml). After an 8-h incubation, 300  $\mu$ l of the transformation mixture was plated onto TGY agar plates containing 5  $\mu$ g of chloramphenicol per ml. Following this protocol, 20 to 50 Cat<sup>r</sup> colonies were recovered from each transformation reaction. Individual colonies were used to inoculate TGY broth containing 5  $\mu$ g of chloramphenicol per ml, and cultures were grown to stationary phase. One hundred microliters of this broth culture was used to inoculate TGY broth containing 10  $\mu$ g of chloramphenicol per ml, and cultures were grown to stationary phase. This culture was diluted (1:10<sup>6</sup>) and plated on TGY agar containing 10  $\mu$ g of chloramphenicol per ml.

Since *D. radiodurans* is multigenomic, individual colonies had to be screened to determine if they were homozygous for the disruption. TnDrCat insertions into *dr1819* of strain 302 were verified by PCR. The set of primers designed to amplify *dr1819*, *uvsup* and *uvsdwn*, were combined with primer S, which anneals within the transposon (Table 2). A 1,300-bp fragment corresponding to intact *dr1819* was the only product produced when the three primers were combined with strain 302 genomic DNA in the reaction (Fig. 2, lane 3). In contrast, an approximately 750-bp product was obtained using genomic DNA isolated from the strain carrying the transposon (Fig. 2, lane 2). The 750-bp fragment was sequenced, and the sequence obtained was that of the 5' end of *dr1819*, signifying that the 750-bp product is the result of an amplification involving *uvsup* and primer S. We did not observe the 1,340-bp fragment corresponding to intact *dr1819* in the strain carrying TnDrCat, indicating that the strain was homozygous for the transposon insertion. The strain containing the disruption was designated LSU1000.

Genomic DNA from LSU1000 was isolated and used to transform *D. radiodurans* R1 to chloramphenicol resistance. Resulting colonies were screened as described above to establish that they were homozygous for the disruption. This strain, LSU2000, was *uvrA-1*<sup>+</sup> (mitomycin C resistant) and *dr1819*::TnDrCat.

**Disruption of *dr1819* in *D. radiodurans* 302 sensitizes this strain to the lethal effects of UV light.** LSU1000 (*uvrA-1 dr1819*::TnDrCat) is very sensitive to UV light (Fig. 3), and the enhanced sensitivity was observed at every UV dose examined. For example, there is an approximately 100-fold increase in sensitivity at the 250-J/m<sup>2</sup> dose relative to the parent strain. The shoulder that typifies the UV resistance of strains R1 and

302 disappears in LSU1000, indicating that the gene product of *dr1819* is necessary for the UV resistance observed in a *uvrA-1* background. Disruption of *dr1819* in a *uvrA-1*<sup>+</sup> background (LSU2000) had no effect on survival following UV treatment, demonstrating a complete overlap of function in the repair pathways involving DR1819 and nucleotide excision repair.

LSU1000 and LSU2000 are fully resistant to ionizing radiation. The disruption of *dr1819* did not alter the resistance of strain 302 or R1 to ionizing radiation at any of the doses examined (data not shown).

***pdr1819* restores UV resistance to a *uvrA-1 uvs* strain of *D. radiodurans*.** The pattern of UV sensitivity observed in LSU1000 duplicates that reported for UVS78, a *uvrA-1 uvsE* strain of *D. radiodurans*, suggesting that *dr1819* encodes the *uvsE* gene product. To test this possibility, an attempt was made to transform UVS78 to UV resistance with *pdr1819*. UVS78 cultures were grown to a density of approximately  $10^6$  CFU/ml before 1  $\mu$ g of transforming DNA was added to the culture. Following the transformation protocol, cells were diluted 1:100 in TGY broth and allowed to grow for 14 h at 30°C. The resulting cultures grew to a density of  $4.8 \times 10^7 \pm 0.6 \times 10^7$  CFU/ml.

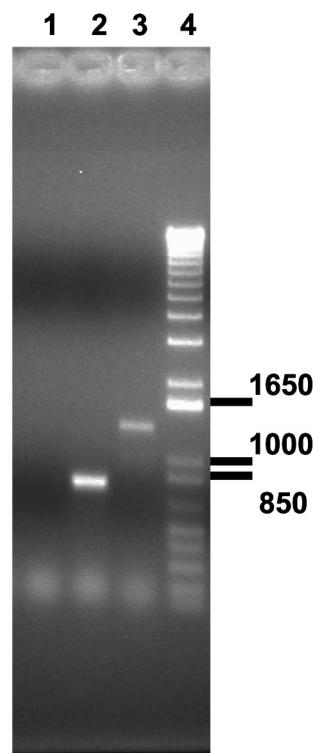


FIG. 2. Ethidium bromide-stained agarose gel illustrating that LSU1000 carries a homozygous insertion of *dr1819-80*::TnDrCat. Chloramphenicol-resistant colonies were isolated, and their genomic DNA was screened using appropriate primers and PCR to establish whether intact *dr1819* remained in LSU1000. Lane 1, attempt to amplify *dr1819* from LSU1000 genomic DNA with primers *uvsup* and *uvsdwn*. Lane 2, amplification of a 750-bp product when primers *uvsup*, *uvsdwn*, and S are combined with LSU1000 genomic DNA. Lane 3, amplification of a 1,300-bp product when primers *uvsup*, *uvsdwn*, and S are combined with R1 genomic DNA. Lane 4, 1-kbp ladder (Invitrogen, Gaithersburg, Md.). Sizes are shown in base pairs.

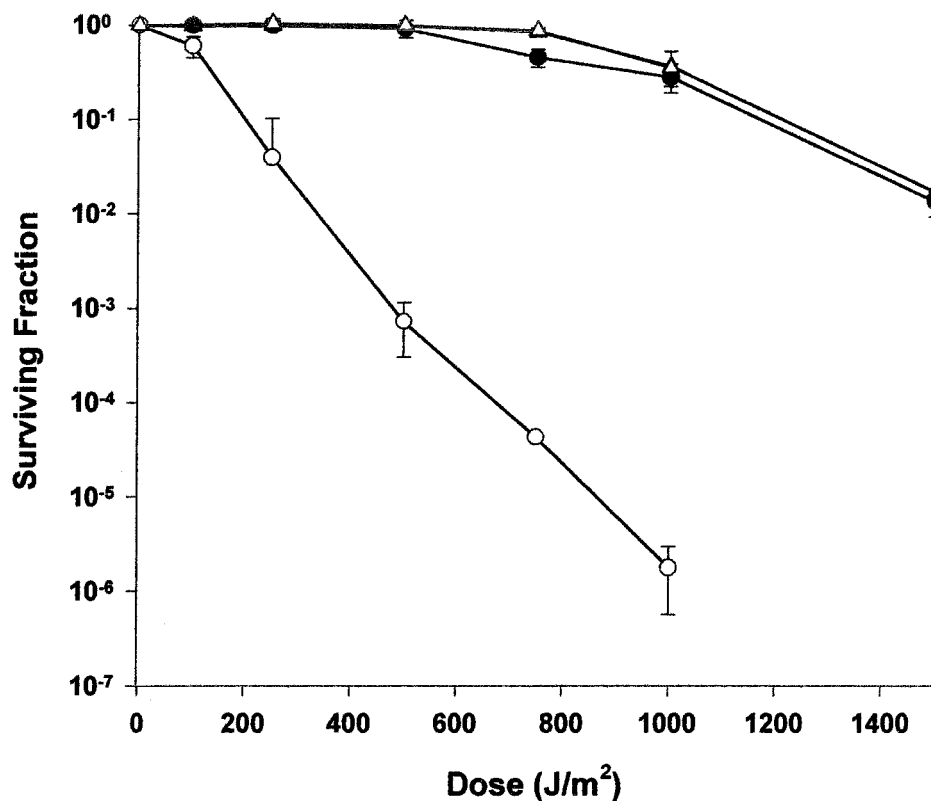


FIG. 3. Representative survival curves for *D. radiodurans* strain LSU1000 *uvrA-1 dr1819::TnDrCat* (open circles) following exposure to UV radiation. Survival of strains R1 (triangles), 302 *uvrA-1* (circles), and LSU2000 *dr1819::TnDrCat* (open triangles) is also shown. Values are the means  $\pm$  standard deviations of triplicate experiments ( $n = 9$ ).

Aliquots of these cultures were exposed to 500 J of UV light  $m^{-2}$ , and the surviving fraction was determined (Table 4). Genomic DNA isolated from UVS78 could not transform UVS78 to UV resistance, as evidenced by the failure to increase survival of the irradiated transformed culture versus the culture that did not receive transforming DNA. In contrast, UVS78 cultures transformed with genomic DNA isolated from strains R1 and 302 exhibited a 20-fold increase in survival after exposure to 500  $J m^{-2}$  relative to the nontransformed control. Plasmids *pdr1771* and *pdr1819* were equally effective in restoring UV resistance to UVS78, yielding a 50-fold-higher number of survivors following UV irradiation. Since incorporation of the wild-type sequence of *dr1819* is sufficient to restore UV resistance to UVS78, we assume that *dr1819* is the *uvrE* gene described earlier (9, 10, 22).

## DISCUSSION

Although perhaps better known for its resistance to ionizing radiation, *D. radiodurans* also displays a startling ability to tolerate UV light (22–24). The UV radiation survival curves of log-phase cultures of *D. radiodurans* R1 exhibit a characteristic shoulder of resistance in which there is no loss of viability to approximately 500  $J m^{-2}$ . The  $D_{37}$  dose for *D. radiodurans* R1 is typically reported to be between 600 and 700  $J m^{-2}$ , at least 20-fold higher than the  $D_{37}$  dose of *E. coli* cultures UV irradiated under the same conditions (29). The energy deposited by a 500- $J m^{-2}$  dose of UV is sufficient to convert approxi-

mately 1% of the thymine in the irradiated cell's DNA to pyrimidine dimers (5, 33). Since the *D. radiodurans* genome is 3.26 Mbp with a 67% GC content (34), as many as 5,000 thymine-containing pyrimidine dimers could form per genome following exposure to UV at 500  $J m^{-2}$ .

When *D. radiodurans* cultures are UV irradiated, there is an immediate and characteristic burst of chromosomal DNA degradation (25, 33), and it has been assumed that this phenomenon is a manifestation of necessary DNA repair processes, since strains that do not exhibit this DNA degradation are highly sensitive to UV light. Moseley and Evans (22) established that the process of UV-induced DNA degradation in *D.*

TABLE 4. Transformation of UVS78 to UV resistance with isolated DNA encoding the *dr1771* (*uvrA-1*) and the *dr1819* (putative *uvrE*) gene products<sup>a</sup>

Transforming DNA	Mean no. of cells surviving <sup>b</sup> (10 <sup>4</sup> ) $\pm$ SD	Fold increase <sup>c</sup>
None	3.8 $\pm$ 0.01	
Genomic DNA from UVS78	3.2 $\pm$ 0.01	0.84
Genomic DNA from R1	100 $\pm$ 8	26
Genomic DNA from 302	75 $\pm$ 4.7	20
<i>pdr1771</i>	190 $\pm$ 12	50
<i>pdr1819</i>	200 $\pm$ 46	53

<sup>a</sup> Cultures of UVS78 were exposed to UV light (500  $J m^{-2}$ ) after transformation (see description in text).

<sup>b</sup> Mean  $\pm$  standard deviation ( $n = 54$ ).

<sup>c</sup> Fold increase relative to the nontransformed UVS78 culture.

*radiodurans* was initiated by either of two nucleases, which they named endonuclease  $\alpha$  and endonuclease  $\beta$ . Each of these enzymes appears to be capable of recognizing UV-induced DNA damage and incising the genome in response to that damage. Furthermore, both endonucleases appear to be equally adept at protecting the cell from UV-induced lethality. Mutations inactivating each endonuclease were identified, and it was established that loss of either nuclease alone failed to affect the UV resistance of this species (10).

Although endonuclease  $\alpha$  was subsequently identified as a homologue of the UvrABC endonuclease of *E. coli*, (1, 26) little is known about the function of endonuclease  $\beta$ . Like the characterized PD glycosylases of *M. luteus* (13) and bacteriophage T4 (12, 27), endonuclease  $\beta$  catalyzes an incision adjacent to a pyrimidine dimer, facilitating its removal, but the *D. radiodurans* protein appears to incise the DNA by a different mechanism (8). The PD glycosylases cleave the N-glycosyl bond of the 5' base in the dimer, generating a structure that will release free thymine on photoreversal. The action of endonuclease  $\beta$  does not produce this structure. Free thymine is not released when photoreversal is attempted on UV-irradiated DNA exposed to partially purified endonuclease  $\beta$ , and from this result it has been inferred that endonuclease  $\beta$  cleaves a phosphodiester bond adjacent to the lesion (8).

This study has determined that inactivation of *dr1819* by insertional mutagenesis sensitizes a *uvrA-1* strain of *D. radiodurans* to UV light, even though the same disruption has no effect on the UV resistance of a *uvrA-1*<sup>+</sup> strain (Fig. 3). The *dr1819* gene product apparently catalyzes a process that compensates for the loss of nucleotide excision repair in this species. Since the *dr1819* gene product of *D. radiodurans* has 30% amino acid sequence identity and 40% similarity with the *uve1*<sup>+</sup> gene product (Uve1p) of *S. pombe* (19, 34), we suggest that DR1819 is a UV DNA damage endonuclease that catalyzes repair of UV-induced DNA damage by a mechanism similar to that of Uve1p. However, understanding DR1819's specific role in DNA damage repair will require further biochemical investigation.

Uve1p is an endonuclease that binds to a wide spectrum of DNA lesions, including UV-induced photoproducts (6, 11), apurinic/aprimidinic sites (15), and base-base mismatches (18). This protein introduces a nick immediately 5' to a lesion, leaving a 5' phosphate and a 3'-OH (2). The nicks become the focus of other repair proteins, which first digest the strand containing the damage and then fill in the resulting gapped heteroduplex. Like endonuclease  $\beta$ , the activity of Uve1p overlaps the nucleotide excision repair pathway in *S. pombe*. Strains of *S. pombe* that are nucleotide excision repair deficient remain proficient in the excision of UV photoproducts and are largely UV resistant (7, 15, 30, 36). Double mutants, lacking nucleotide excision repair and Uve1p activities, become hypersensitive to UV light.

UVS78 (10, 22) was used to establish whether the coding sequence *dr1819* was the *uvrE* gene described in earlier literature. The *dr1819* sequence was cloned, and that clone was used as donor DNA in a transformation protocol in which UVS78 was the recipient. As shown in Table 4, the *dr1819* sequence is sufficient but not necessary to restore UVS78 to UV resistance. The coding sequence of *dr1771* (*uvrA-1*) will also restore UV resistance to this strain. We conclude that the

open reading frame *dr1819* is the *uvrE* gene and that *dr1819* encodes endonuclease  $\beta$ .

In addition to UVS78, Evans and Moseley isolated UVS9 and UVS25 while screening mutagenized strain 302 cultures for UV-sensitive strains (10, 22). As with UVS78, the UV sensitivity of these strains was manifest only in *uvrA-1* backgrounds. Genomic DNA isolated from each of these strains could transform UVS78 to UV resistance, indicating that each strain carried a different mutation. Evans and Moseley concluded that the individual mutations were found in separate genes, which they designated *uvrC* and *uvrD*, and that these genes were different from *uvrE*. They reasoned that the mutations found in UVS9, UVS25, and UVS78 could not be located within the same gene because the frequency with which each mutant's genomic DNA restored the others to UV resistance did not differ significantly from that observed when R1 genomic DNA was used in the transformation protocol.

Apparently, Evans and Moseley assumed that if all three mutations were located within the same gene, the efficiency of transformation to UV resistance would be greatly reduced, because one mutation would simply replace the other during recombination. However, this is not a valid assumption when considering natural transformation in *D. radiodurans*. Since the publication of Evans and Moseley's manuscript (10), it has been established that the average size of the DNA fragment inserted during transformation is less than 950 bp, substantially smaller than that reported for other transformable species (20). Genomic DNA from deinococcal strains carrying different mutations within the same gene can transform each other to DNA damage resistance with efficiencies indistinguishable from those observed when R1 genomic DNA is used as the donor. It is therefore possible that *uvrC* and *uvrD* are also alleles of *dr1819*. We could not obtain strains UVS9 and UVS25 to test this possibility.

As part of this study, a method was developed for genetically manipulating *D. radiodurans* R1 that makes use of the commercially available GPS-M in vitro transposition system. A transposon was created that is capable of expressing chloramphenicol resistance in *D. radiodurans* R1 by fusing a 1,900-bp sequence upstream of the *tuf1* gene of R1 to a *cat* gene and inserting that fusion into a vector containing the minimal set of sequences required for transposition. The resulting construct, pGTC101, was used to disrupt the cloned coding sequence of *dr1819*. The vector carrying the disrupted gene was used as transforming DNA, and the chloramphenicol resistance marker was integrated into the R1 genome. The resulting Cat<sup>r</sup> colonies were screened for cells homozygous for the marker. The result was a stable loss-of-function mutation that is easily moved from one strain to another. Since the *D. radiodurans* R1 genome has been sequenced in its entirety and since any segment of that genome can be cloned after amplification in a PCR, it should be possible to rapidly inactivate any nonessential gene in this species using this approach.

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