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DNA Repair Mutants of *Rhodobacter sphaeroides*

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The genome of the photosynthetic eubacterium *Rhodobacter sphaeroides* 2.4.1 comprises two chromosomes and five endogenous plasmids and has a 65% G+C base composition. Because of these characteristics of genome architecture, as well as the physiological advantages that allow this organism to live in sunlight when in an anaerobic environment, the sensitivity of *R. sphaeroides* to UV radiation was compared with that of the more extensively studied bacterium *Escherichia coli*. *R. sphaeroides* was found to be more resistant, being killed at about 60% of the rate of *E. coli*. To begin to analyze the basis for this increased resistance, a derivative of *R. sphaeroides*, strain 2.4.1ΔS, which lacks the 42-kb plasmid, was mutagenized with a derivative of Tn*5*, and the transposon insertion mutants were screened for increased UV sensitivity (UV^s). Eight UV^s strains were isolated, and the insertion sites were determined by contour-clamped homogeneous electric field pulsed-field gel electrophoresis. These mapped to at least five different locations in chromosome I. Preliminary analysis suggested that these mutants were deficient in the repair of DNA damage. This was confirmed for three loci by DNA sequence analysis, which showed the insertions to be within genes homologous to *uvrA*, *uvrB*, and *uvrC*, the subunits of the nuclease responsible for excising UV damage.

The photosynthetic bacterium *Rhodobacter sphaeroides* was the first prokaryote shown to have multiple chromosomes (48). Wild-type strain 2.4.1 comprises a genome of two circular chromosomes, as well as five additional plasmids with unknown functions. This genome architecture raises significant questions pertaining to DNA metabolism, an area about which virtually nothing is known in this multichromosomal prokaryote. Furthermore, the nature of DNA replication, repair, and recombination in *R. sphaeroides* is of interest because of other aspects of the lifestyle of this organism. Being photosynthetic, it is subjected to potentially lethal UV irradiation, making its mode of DNA repair of special interest. The high G+C content (65%) of the *R. sphaeroides* genome makes this of further interest because of the expected higher content of Z DNA, the greater need for uracil repair following cytosine deamination, and the role of the latter process in UV mutagenesis when it occurs in pyrimidine dimers (49). In addition, *R. sphaeroides* can use a range of compounds as terminal electron acceptors, including toxic metal oxides and organic compounds with a potential for free radical formation (34). Thus, it is not only likely but probable that there are specific mechanisms that protect the genome against damage from these compounds.

The current paradigm for prokaryotic DNA replication, repair, and recombination is based on DNA metabolism in *Escherichia coli*. Since this is an enteric organism with exposure to a limited environment and has a single chromosome with a base composition of about 50% G+C, there is reason to expect that the *E. coli* model is incomplete or not entirely appropriate for free-living bacteria such as *R. sphaeroides*. Comparisons of enteric species (or animal pathogens) with nonenteric counterparts of the same genera indicate that enteric microorganisms in a genus are usually more sensitive to UV irradiation (2–4, 8, 10, 13, 19, 30, 45). Thus, further studies of nonenteric microbes should be rewarded by expanding our knowledge of DNA repair mechanisms.

Although much of the *E. coli* paradigm of DNA repair processes is applicable to other systems, numerous differences have been noted. Many bacteria lack photoreactivation systems (e.g., *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *Deinococcus radiodurans*, and *Neisseria gonorrhoeae*) (10, 24) or show absent or altered inducible mutagenic repair responses (20, 41, 43), even enteric organisms closely related to *E. coli* (41). Although RecA functions are widely distributed, their role may vary from that in *E. coli*, as in the cyanobacteria, where RecA is apparently essential (35), or in *D. radiodurans*, where considerably more recombinational repair occurs than in *E. coli* (32). Elaborations of the UvrABC excision repair system of *E. coli* have also been noted, such as in *D. radiodurans*, where there is also a second excision repair system, contributing to the extreme UV resistance of this organism (32). At the other extreme, *Pasteurella haemolytica* has been reported to be naturally deficient in excision repair (29). The methyl-directed mismatch repair system (33) and the very short patch repair system (26, 28) of *E. coli* may also be different, since neither adenine nor cytosine methylation is universal (21, 44). Thus, it is already clear that there are considerable variations on the *E. coli* DNA repair model.

Because of the multichromosomal genome, high G+C content, and environmental lifestyle of *R. sphaeroides*, we believe it is important to examine the DNA metabolism of this organism. Fortunately, a number of genetic tools have been developed for use with *R. sphaeroides* to facilitate this analysis (15). In this report, we present an initial study of DNA repair-deficient mutants and identify the genes for the subunits of the UvrABC excision repair nuclease.

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MATERIALS AND METHODS

Bacteria, plasmids, phage, and cell culture. The bacterial strains, plasmids, and phage used in this work are described in Table 1. All bacterial strains were grown in Luria-Bertani medium (LB). *R. sphaeroides* 2.4.1ΔS and *E. coli* MG1655

TABLE 1. Bacterial strains, bacteriophage, and plasmids used in this study

Strain, phage, or plasmid	Relevant genotype (phenotype)	Source
R. sphaeroides		
2.4.1ΔS	2.4.1 with 42-kb plasmid deleted	A. Suwanto
GR0117	2.4.1ΔS *leuC*::Tn*5*TpMCS (Tp^r Leu^-)	This study
GR0221	2.4.1ΔS *rprA*::Tn*5*TpMCS (Tp^r $UvrA^-$)	This study
GR0228	2.4.1ΔS *rprE*::Tn*5*TpMCS (Tp^r Uv^s)	This study
GR0302	See GR0333	This study
GR0304	2.4.1ΔS *rprD*::Tn*5*TpMCS (Tp^r Uv^s)	This study
GR0310	2.4.1ΔS *rprA*::Tn*5*TpMCS (Tp^r Uv^s)	This study
GR0311	2.4.1ΔS *rprC*::Tn*5*TpMCS (Tp^r $UvrC^-$)	This study
GR0328	2.4.1ΔS *rprA*::Tn*5*TpMCS (Tp^r $UvrA^-$)	This study
GR0333	2.4.1ΔS *rprB*::Tn*5*TpMCS (Tp^r $UvrB^-$)	This study
Phage RS1	*R. sphaeroides* bacteriophage	
E. coli		
MG1655	F^- λ^-	
S17-1	*pro hsdR hsdM*$^+$ *recA* with plasmid RP4 integrated in host chromosome Tc::Mu-Km::Tn*7*	A. Puhler
XL1-Blue MRF′	Δ*mcrA* Δ(*mcrCB hsdSMR mrr*) *recA lac* carrying episome F′ *proAB*::Tn*10* (Tc^r)	Stratagene
GR0479	XL1-Blue MRF′/pBS479	This study
GR0491	XL1-Blue MRF′/pBS491	This study
GR0492	XL1-Blue MRF′/pBS492	This study
GR0493	XL1-Blue MRF′/pBS493	This study
GR0494	XL1-Blue MRF′/pBS494	This study
Plasmids		
pSUP5Tp	Tn*5*-derived transposon carrying Tp^r	C. Sasakawa
pSUPTn5TpMCS	Polylinker insertion at unique *Spe*I site within pSUP5Tp	This study
pBluescript SK (−)	Ap^r with T3 and T7 promoters	Stratagene
pBS479	pBluescript::6-kb *Eco*RI fragment with Tp^r from strain GR0221	This study
pBS491	pBluescript::3.3-kb *Eco*RI fragment with Tp^r from strain GR0304	This study
pBS492	pBluescript::3.9-kb *Eco*RI fragment with Tp^r from strain GR0311	This study
pBS493	pBluescript::3.6-kb *Eco*RI fragment with Tp^r from strain GR0328	This study
pBS494	pBluescript::9.5-kb *Eco*RI fragment with Tp^r from strain GR0333	This study

were grown without selection at 30 and 37°C, respectively. Matings were carried out on LB plates at 30°C. Selective agents were added to the medium for the following strains: *E. coli* S17-1/pSUPTn*5*TpMCS, 50 μg of trimethoprim (TP) per ml; 34 μg of chloramphenicol per ml, and 60 μg of ampicillin (AP) per ml; *E. coli* XL1-Blue MRF′/pBluescriptSK(−)::UV^s insert, 100 μg of AP per ml and 50 μg of TP per ml. Immediately after mating, strains of *R. sphaeroides* containing transposon insertions were selected on medium supplemented with 50 μg of TP per ml and 10 μg of potassium tellurite (Te); the latter acted as a selective agent against *E. coli* S17-1. Subsequently, these strains were grown on plates or in liquid containing 50 μg of TP per ml alone.

Transposon mutagenesis and isolation of UV-sensitive mutants. The mobilizable suicide plasmid pSUPTn*5*TpMCS was introduced into *R. sphaeroides* 2.4.1ΔS by mating from *E. coli* S17-1. The plasmid carries a Tn*5* derivative (Tn*5*TpMCS) encoding TP resistance (40). Within the Tn*5* lies a cluster of restriction sites (*Ase*I, *Dra*I, *Sna*BI, *Ssp*I, and *Spe*I) which cut infrequently in the *R. sphaeroides* genome (47). These sites enable positioning of transposon insertions in the *R. sphaeroides* genome by digestion of DNA in agarose plugs, followed by contour-clamped homogeneous electric field (CHEF) pulsed-field gel electrophoresis.

R. sphaeroides 2.4.1ΔS was mutagenized with Tn*5*TpMCS as described previously (11) and spread onto LB-TP-Te plates. After 3 days of incubation, the TP-resistant colonies were replica plated onto fresh LB-TP-Te plates and the replicas were exposed for 45 s to 0.8 J of UV light per m^2 per s generated by a Sylvania germicidal lamp (GTE). UV light intensity was measured with a Spectronics DM-254N UV meter. Both sets of plates were then incubated for 2 days. Colonies which failed to grow on the replica plates were picked from the master as putative UV^s mutants.

Determination of UV sensitivity curves. For each strain examined, 10-ml cultures were grown to 200 Klett units (KU) and harvested by centrifugation at 4,000 × *g*. The pellets were resuspended in 10 ml of M63 salts (31) to 100 KU and then placed on ice. A 5-ml volume of the suspension was transferred to a 90-mm-diameter glass petri dish. The dish was placed on a rotary shaker (100 rpm), and the cells were exposed to 0.25 J of UV light per m^2 per s. Aliquots (0.5 ml) were removed after 0, 10, 20, 30, 40, 60, 90, and 180 s of exposure. The aliquots were serially diluted in LB and plated, and after 3 days the viable cell titer was determined. All aliquots were kept on ice and under foil between exposure, dilution, and plating.

Ten percent survival assay. Cultures were grown to 200 KU, and 0.1 ml of a 10^{-5} dilution was plated onto each of two plates. One of the plates was then exposed to 25 J of UV light per m^2. After 3 days of incubation at 30°C, the colonies were counted and the surviving fraction was determined. This UV dose gave 10% survival of the wild type (about 100 colonies) while allowing no growth of putative UV^s mutants.

MMC sensitivity. A 0.2-ml volume of a 100-KU suspension of cells in M63 salts (see above) was plated onto an LB plate containing mitomycin C (MMC) at a concentration of 0, 0.0125, 0.025, or 0.05 μg/ml. After 3 days of incubation, the number of colonies per plate was determined.

Phage viability assay. Aliquots of a high-titer (≈10^{10} PFU/ml) lysate of *R. sphaeroides*-specific phage RS1 (1, 14) were exposed for 60 and 90 s to 0.8 J of UV light per m^2 per s. The titers of the exposed and unexposed lysates were then determined on various *R. sphaeroides* strains. The UV-irradiated phage assay was performed by growing *R. sphaeroides* 2.4.1ΔS, GR0117, and UV^s mutants to 100 KU. A 10-μl volume of diluted phage lysate was added to a 0.2-ml aliquot of the bacterial culture and mixed briefly, and the phage was allowed to adsorb for 20 min at room temperature. A 3-ml volume of 0.6% LB agar was added, mixed, and overlaid on an LB plate. After 3 days of incubation, phage viability was determined.

Mapping the position of transposon insertion. A modification of the previously described protocol (37) for pulsed-field gel electrophoresis of *R. sphaeroides* DNA was used for CHEF agarose gel electrophoresis analysis as follows. Five-milliliter cultures were grown to 300 KU, 1.5 ml was removed, and the cells were pelleted in a microcentrifuge. The cells were washed by resuspension in 1 ml of PIV (10 mM Tris-HCl [pH 7.6], 1 M NaCl) and repelleted as before. The pellet was resuspended in 350 μl of PIV, 425 μl of molten (45°C) 1% InCert agarose (FMC BioProducts, Rockland, Maine) in TE (10 mM Tris, 1 mM EDTA, pH 8.0) was added, and the mixture was briefly vortexed. A repeater pipette was used to aliquot 20 10-μl drops onto a disposable plastic weighing dish. Once set (about 15 min but no longer than 30 min to prevent drying), the plugs were scooped off the boat with a plastic spatula (cut from a piece of X-ray film) into 15-ml polypropylene tubes containing 10 ml of EC solution (6 mM

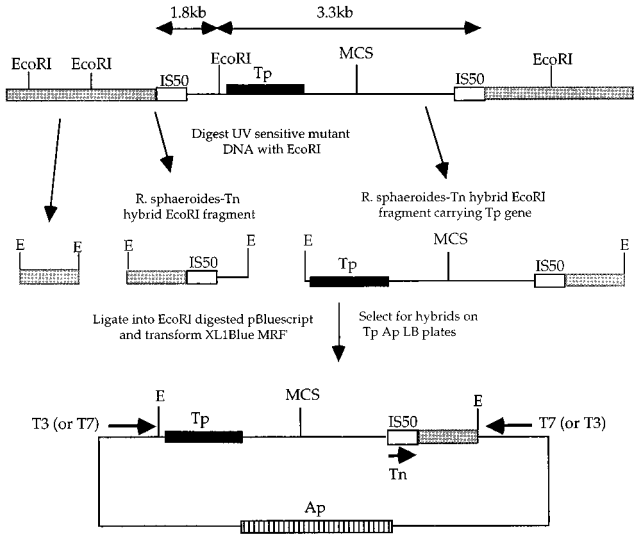

FIG. 1. Strategy used to subclone and sequence *R. sphaeroides* genomic DNA flanking transposon insertions. *Eco*RI libraries were made from each UV^s mutant in pBluescript SK (−) as described in Materials and Methods. For each clone, the DNA sequence was obtained by using the T3, T7, and Tn primers (bold arrows). The Tn primer (which anneals to IS*50* of the transposon) gave a DNA sequence corresponding to genomic DNA adjacent to the insertion (stippled area). The T3 and T7 primers gave DNA sequences of the transposon's *dhr* gene (black rectangle) and a segment of genomic DNA distal to the point of insertion (stippled area). The particular primer (T3 or T7) that gave these sequences (*dhr* or genomic) depended on the insert's orientation. MCS, cluster of rarely cutting restriction endonuclease sites used in CHEF pulsed-field gel electrophoretic analysis; Ap, β-lactamase resistance gene; Tp, TP resistance (*dhr*) gene.

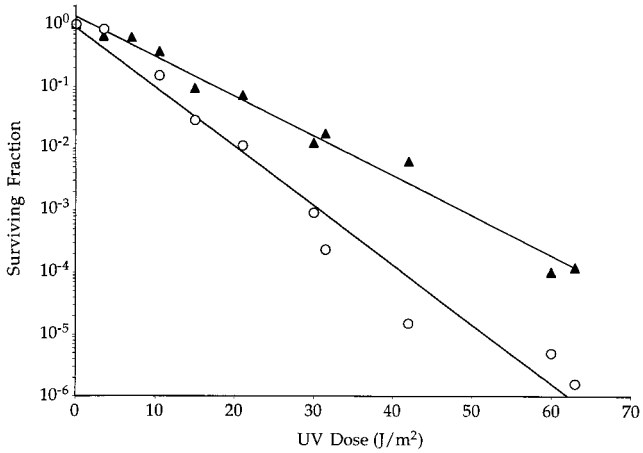

FIG. 2. UV sensitivity of wild-type strains of *E. coli* and *R. sphaeroides*. *R. sphaeroides* 2.4.1ΔS (▲) and *E. coli* MG1655 (○) were grown, irradiated, and plated as described in Materials and Methods. The data shown are the means of two experiments performed on different days.

Tris-HCl [pH 7.6], 1 M NaCl, 0.1 M EDTA [pH 7.5], 0.5% Brij 35, 0.2% deoxycholate, 0.5% Sarkosyl, 20 μg of DNase-free RNase [Sigma] per ml, 1 mg of egg white lysozyme [Sigma] per ml). The plugs were incubated with gentle shaking at 37°C for 24 h. The mouth of each tube was then covered with a single layer of sterile gauze, and the EC solution was decanted. Plugs sticking to the gauze were returned to the tube by tapping the base of the tube on the bench. A 10-ml volume of ESP solution (0.5 M EDTA [pH 9.3], 1% Sarkosyl, 200 μg of nuclease-free proteinase K [Boehringer] per ml) was then added, and the mixture was incubated at 55°C for 56 h. The solution was decanted and replaced with 10 ml of TE and incubated at 4°C for 3 h with gentle shaking. The TE was changed three more times, and the final TE solution was removed from the tube. Plugs were transferred to microcentrifuge tubes for digestion by sucking them onto the end of a 1-ml micropipette tip.

An advantage of this method is high throughput; 50 different samples can be prepared at a time by one person. Also, the DNA is aliquoted at a fixed concentration, giving greater reproducibility between lanes and between gels. We have found that this method gives DNA of as high a quality as that prepared by conventional methods and does not show signs of degradation after 1 year.

For each UV^s strain, DNA plugs were digested in single-enzyme reactions with the rarely cutting enzymes *Ase*I, *Sna*BI, *Dra*I, and *Spe*I. After digestion, the plugs were dialyzed against TE for 20 min and melted at 75°C for 10 min. The molten agarose-DNA mixture was loaded into the wells of a 1× TBE (38)–1.2% SeaPlaque GTG agarose (FMC) gel. The gels were run in 1× TBE with a CHEF DRII tank (Bio-Rad, Hercules, Calif.) at 10°C and 200 V. Pulse times were increased linearly from 5 to 75 s over 24 h to separate fragments in the 50- to 400-kb range, and the gel was stained and photographed. The run was then continued for a further 18 h but with pulse times ranging from 90 to 160 s to separate 0.9- to 1.6-Mb fragments. The positions of the transposon insertions were then deduced from the previously published map of the *R. sphaeroides* 2.4.1 genome (47, 48).

Subcloning of *R. sphaeroides* DNA flanking the site of transposon insertion. A unique *Eco*RI site within Tn*5*TpMCS divides the transposon into two fragments, one of which contains the intact dihydrofolate reductase (*dhr*) gene, which confers trimethoprim resistance (Fig. 1). Digestion of UV^s mutant genomic DNA with *Eco*RI generates a fragment containing the *dhr* gene of the transposon and flanking genomic DNA. To clone this fragment, we constructed *Eco*RI libraries in pBluescript SK (−) and selected for transformants on AP-TP plates.

The UV^s mutants were grown to 300 KU, and 1.5 ml of the culture was used to prepare genomic DNA (5), yielding approximately 5 μg of genomic DNA that was greater than 50 kb in size. A 2-μg sample of this DNA was digested with *Eco*RI and ligated to 0.5 to 1 μg of *Eco*RI-digested pBluescript SK (−) for 4 h at room temperature. A 0.1- to 0.2-μg sample of DNA was then used to transform 0.2 ml of TSS (12)-prepared XL1-Blue MRF′ cells. After allowing 20 min for transformation, 1 ml of LB was added to the cells; this was followed by an outgrowth for 1 to 2 h at 37°C. The cells were then briefly centrifuged, resuspended in 0.2 ml of LB, and spread onto LB-TP-AP plates. After 24 h, Tp^r colonies were picked and used to prepare plasmid DNA with Wizard minipreps (Promega). To ensure that Tp^r was carried by the plasmid, as opposed to spontaneous Tp^r of the host, the plasmids were used to retransform XL1-Blue MRF′.

DNA sequencing. Plasmid DNA was purified by the alkali lysis-cesium chloride method (38) and resuspended in sterile deionized water at a concentration of 800 μg/ml. A primer (5′-TTCAGGACGCTACTTGTGTA-3′) complementary to the end of the IS*50* segment of the transposon was used to sequence across the transposon-*R. sphaeroides* DNA junction. T3 and T7 primers were used to obtain sequences from opposite ends of the *Eco*RI DNA inserts. Sequencing reactions were performed by the *Taq* Dye-deoxy Terminator method (Applied Biosystems) and run on an Applied Biosystems Sequenator. This generated sequences from either the region at the *Eco*RI site of the transposon or the region around the *Eco*RI site of the flanking *R. sphaeroides* DNA (Fig. 1). Each template was sequenced twice with each primer.

Sequence analysis. Sequence files were analyzed by using the BLAST server at the National Center for Biotechnology Information (Bethesda, Md.). This involved translating each sequence in all six reading frames and using these probes to search the nonredundant protein sequence database with the BLASTX program. All other analyses, such as removing vector sequences and sequence assembly projects, were carried out with the Genetics Computer Group software package.

Nucleotide sequence accession numbers. The sequences described in Results were entered into GenBank. Their accession numbers are as follows: *uvrA* from GR0328 (T3 or Tn primer), U23398; *uvrA* from GR0221 (Tn primer), U23399; *mmsA* from GR0221 (T3 primer), U23400; *uvrB* from GR0333 (Tn primer), U23401; *uvrC* from GR0311 (Tn primer), U23402; *pgsA* from GR0311 (T7 primer), U23403.

RESULTS

UV radiation resistance of *R. sphaeroides*. We first examined the relative UV sensitivities of *R. sphaeroides* 2.4.1ΔS and *E. coli* MG1655 (Fig. 2). *R. sphaeroides* was 10-fold more resistant than *E. coli* at a dose of 30 J/m^2 and 100-fold more resistant at 60 J/m^2. The rate of killing of *R. sphaeroides* was about 60% of that of *E. coli*. Given that their genome sizes are similar, this indicates that *R. sphaeroides* is more UV resistant than *E. coli*.

Isolation and mapping of DNA repair-deficient mutants. Approximately 10^4 transposon insertion mutants from seven independent mutagenesis experiments were screened for increased sensitivity to UV light by replica plating. A four-point UV sensitivity curve (data not shown) was then obtained from a liquid culture of each of 50 UV^s candidates. Seven strains,

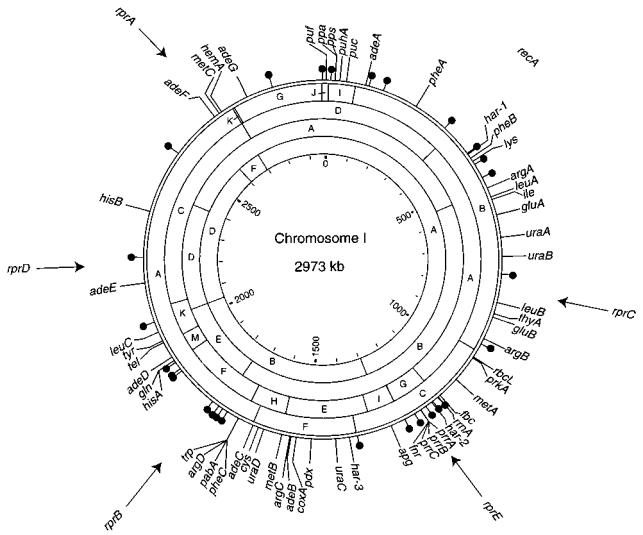

FIG. 3. Physical map of chromosome I of *R. sphaeroides* 2.4.1. The four concentric circles show cleavage maps for (outer to inner) *Ase*I, *Dra*I, *Sna*BI, and *Spe*I. Map position zero is the *Ase*I J-I junction (12 o'clock on the outer circle). The locations of transposon insertions giving a UV^s phenotype and other insertions and markers are shown. Moving clockwise from zero, insertions at the following loci gave a UV^s phenotype: *rprC*, GR0311; *rprE*, GR0228; *rprB*, GR0302 and GR0333; *rprD*, GR0304; *rprA*, GR0221, GR0310, and GR0328. Insertions shown by black lollipops were isolated as candidate UV^s mutants that were wild type in retests. The position of *recA* was determined by hybridization with a *recA* gene (unpublished results). Most auxotrophic markers were localized by using the transposon mutagenesis and mapping techniques described in this report (unpublished results).

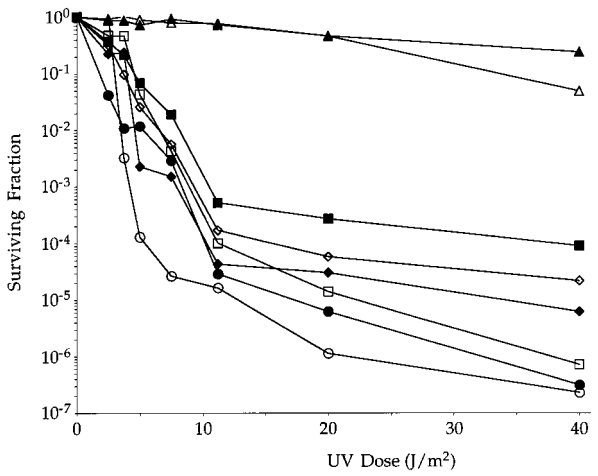

FIG. 4. UV sensitivity of wild-type and UV^s mutants of *R. sphaeroides* 2.4.1ΔS. Survival was measured as described in the legend to Fig. 2. The points shown are the means of duplicate experiments performed on different days. Symbols: ▲, wild-type *R. sphaeroides* 2.4.1ΔS △, auxotroph GR0117 (*leuC* Leu$^-$); ◆, UV^s GR0221 (*rprA* UvrA$^-$); ■, UV^s GR0304 (*rprD*); ●, UV^s GR0310 (*rprA*); ◇, UV^s GR0311 (*rprC* UvrC$^-$); □, UV^s GR0328 (*rprA* UvrA$^-$); ○, UV^s GR0333 (*rprB* UvrB$^-$).

designated GR0221, GR0302, GR0304, GR0310, GR0311, GR0328, and GR0333, showed significant UV sensitivity.

The 50 UV^s mutant candidates were also spread on plates and irradiated with a UV dose which gave 10% survival of the wild-type parent. The seven strains identified above were UV^s in this assay, and in addition, an eighth strain, GR0228, was found to be UV sensitive. Further analysis of GR0228 is in progress, and the results are not presented here.

The position of the transposon insertion in each of the eight UV^s strains was determined by CHEF pulsed-field gel electrophoresis (Fig. 3). By using the J-I *Ase*I junction on the physical map as the zero position for chromosome I and moving clockwise around the chromosome, the insertions were located at the following positions: GR0311, 791 kb; GR0228, 1,212 kb; GR0302 and GR0333, 1,768 kb; GR0304, 2,203 kb; GR0221, GR0310, and GR0328, 2,663 kb. GR0302 and GR0333 mapped to the same position and were derived from the same mutagenesis experiment. Since they could be siblings, GR0302 was not examined further. GR0221, GR0310, and GR0328 contained insertions which also mapped to the same position. Because these strains were from independent experiments and their restriction patterns differed slightly, all three were analyzed further.

Transposon insertion mutants that were indistinguishable from the wild type in their UV^s (as judged by survival curves) were also located within the genome. These mapped to 27 locations on chromosome I (Fig. 3) and 6 locations on chromosome II (data not shown; see reference 11). Insertions also mapped to the endogenous plasmids in three strains. The insertions were localized to the 110-kb plasmid in two of these and to the 95-kb plasmid in the third strain.

During screening for increased UV^s, we also screened for auxotrophy (unpublished data). The positions of the insertions in these auxotrophic strains, their phenotypes, and other mapped genes are also presented in Fig. 3.

Characterization of repair-deficient mutants. To test for the ability to repair chemically induced DNA damage, the six UV^s mutant strains (the eight mutants described above, except for GR0302 and GR0228) were spread on plates containing various concentrations of MMC. *R. sphaeroides* 2.4.1ΔS and an *R. sphaeroides* 2.4.1ΔS leucine auxotroph containing a Tn*5*TpMCS insertion (*leuC* mutant GR0117) were used as controls. The eight strains tested each formed several hundred colonies on plates without MMC or containing 0.0125 μg of MMC per ml. On plates containing 0.025 μg of MMC per ml, the wild-type controls gave approximately 100 colonies while the six UV^s strains failed to grow. This suggested that the UV sensitivity of the six mutants was due to altered DNA repair rather than a defect in UV shielding or some other protection mechanism.

Survival curves (Fig. 4) were generated to determine the relative sensitivity of each of the UV^s mutants to UV light. *R. sphaeroides* 2.4.1ΔS and GR0117 were again used as controls. GR0304 was the least UV sensitive, GR0333 was the most UV sensitive, and the rest were intermediately sensitive. Reduction of the surviving fraction of the mutants to 10^{-1} required a UV dose of 2.5 to 7.5 J/m^2, and in contrast, a dose of at least 35 J/m^2 was required to reduce the surviving fraction of the controls by a similar amount. Another distinctive feature of the mutant survival curves was their biphasic pattern. There was a rapid decline in the surviving fraction of cells until a UV dose of 15 J/m^2 was reached, at which point there was a decreased rate of killing. This appeared to be independent of the level of survival attained at this dose.

We also compared the survival curves of the UV^s mutants to that reported (9) for two *recA* mutants of *R. sphaeroides* 2.4.1 (Fig. 5). A UV dose of 15 J/m^2 was reported to reduce the surviving fraction of a *recA* mutant to 10^{-1}, whereas the same dose reduced the survival of the UV^s mutants to 10^{-3} to 10^{-5}. This observation is unlike that for *E. coli*, a *recA* mutant of which is slightly more sensitive to UV light than other classes of DNA repair mutants (27).

We next tested whether the UV^s mutants were defective in

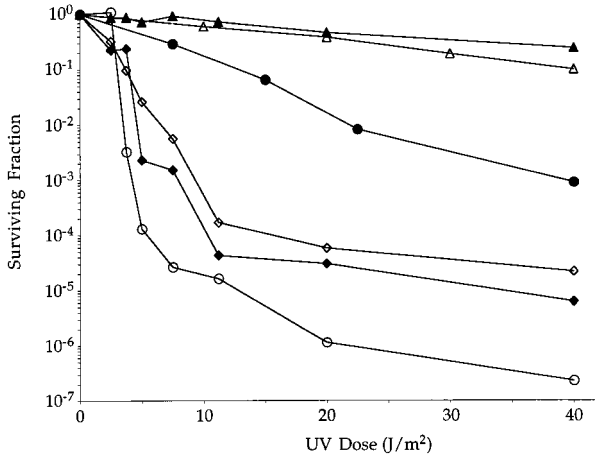

FIG. 5. Comparison of UV sensitivity of RecA$^-$ and UV^s mutants of *R. sphaeroides* 2.4.1ΔS. Data presented by Calero et al. (9) on the sensitivity of wild-type *R. sphaeroides* 2.4.1 (△) or *recA* mutants of this strain are compared with those on the sensitivity of the mutants described in this report. Only one *recA* mutant is shown, that with insertion mutation *recA*::ΩKm (●), since the two mutants were similar. The data for *R. sphaeroides* 2.4.1ΔS (▲) and UV^s mutants GR0221 (*rprA* UvrA$^-$) (◆), GR0311 (*rprC* UvrC$^-$) (◇), and GR0333 (*rprB* UvrB$^-$) (○) were taken from Fig. 4.

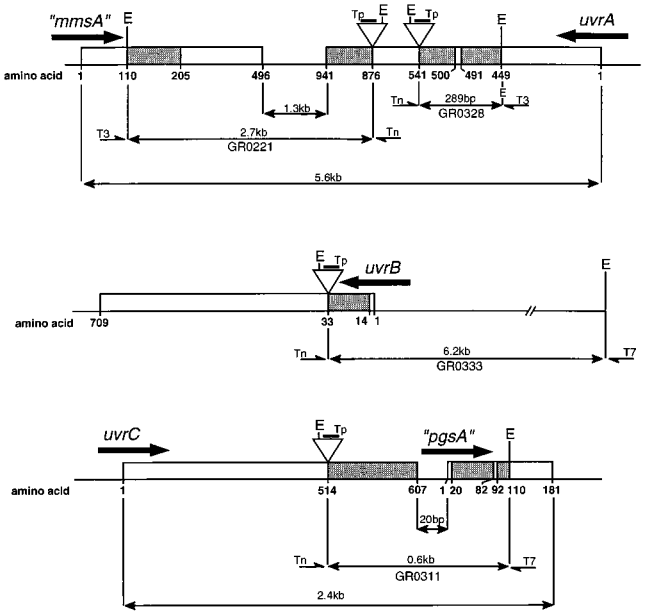

FIG. 6. Schematics of the *R. sphaeroides uvrA*, *uvrB*, and *uvrC* regions. The name of each gene is given above a black arrow that indicates the direction of transcription. *Eco*RI sites (E) in the transposon and in flanking *R. sphaeroides* DNA are shown. Transposon Tn*5*TpMCS is shown as a triangle with the internal *Eco*RI site (E) and the *dhr* gene (Tp). The numbers beneath each gene indicate amino acid positions within the polypeptide. The sizes and extents of *Eco*RI fragment inserts in clones from UV^s strains are also indicated, as are the estimated sizes of other regions. Half arrows indicate the directions and locations of primers (Tn, T3, and T7) used for sequencing. Areas of genes having high scores of similarity to *R. sphaeroides* sequences in BLAST searches are shaded. The matches of amino acid sequence are shown in more detail in Table 3. Genes "*mmsA*" and "*pgsA*" are designated as such only on the basis of BLAST matches and not on the basis of functional analysis.

the ability to repair UV-irradiated phage DNA. To do this, we used *R. sphaeroides* phage RS1 (1, 14). As shown in Table 2, an unirradiated phage lysate gave approximately the same titer (2.4×10^{10} to 3.0×10^{10} PFU/ml) on the UV^s mutants and control strains, showing that these strains had the same ability to propagate the phage. However, when irradiated with a UV dose of 50 or 75 J/m^2, the phage titer on the UV^s mutants was reduced by 100-fold or more compared with that on the wild-type strains. These results confirmed that the UV^s mutants were defective in the ability to repair damaged DNA.

Cloning of transposon insertions. We successfully obtained Tp^r clones from five of the UV^s strains (GR0221, GR0304, GR0311, GR0328, and GR0333) as described in Materials and Methods. The pBluescript SK (−) clones contained *Eco*RI inserts of 6.0, 3.3, 3.9, 3.6, and 9.5 kb, respectively. The transposon component was calculated to comprise 3.3 kb of each hybrid fragment (40), indicating that the flanking *R. sphaeroides* genomic DNA comprised 2.7, 0, 0.6, 0.3, and 6.2 kb of the insert DNA, respectively. It was later found by DNA sequencing that only 35 bp of *R. sphaeroides* flanking DNA existed in the subclone generated from GR0304, which proved to be insufficient sequence to produce meaningful results by database searching. Therefore, the analysis of this strain is not described further. We were unsuccessful in subcloning the fragment from GR0310.

TABLE 2. Reactivation of UV-irradiated bacteriophage RS1

Strain	Relative RS1 phage titer[a] after exposure to UV dose (J/m^2) of:		
	0	50	75
ΔS	1	1.3×10^{-2}	5.4×10^{-4}
GR0117	1	1.2×10^{-2}	7.0×10^{-4}
GR0221	1	4.7×10^{-4}	3.1×10^{-7}
GR0304	1	3.9×10^{-4}	1.1×10^{-6}
GR0310	1	5.2×10^{-4}	3.0×10^{-7}
GR0311	1	7.2×10^{-4}	9.4×10^{-7}
GR0328	1	5.0×10^{-4}	3.3×10^{-7}
GR0333	1	1.7×10^{-4}	3.3×10^{-7}

[a] The titers of lysates of *R. sphaeroides* phage RS1 after irradiation with UV light at 50 and 75 J/m^2 are presented as fractions of the titer of the unirradiated lysate (0 J/m^2). The titers of unirradiated lysates varied from 2.4×10^{10} to 3.3×10^{10} PFU/ml for the various strains.

For each pBluescript clone, either the T3 or the T7 primer gave the DNA sequence of the transposon at its internal *Eco*RI site while the other primer gave the sequence of genomic DNA flanking the *Eco*RI site in the chromosome (Fig. 1). This showed that clones had been isolated as predicted. By using automated sequencing with CsCl-purified template DNA, we obtained an approximately 450-bp sequence with a few percent unknown bases for each run. This accuracy was sufficient for identification of similarities to existing sequences in database searches. The matches described below and illustrated in Table 3 and Fig. 6 were the highest-scoring segment pairs in the database searches.

GR0328. The cloned *R. sphaeroides* DNA flanking the insertion was 284 bp long. Both DNA strands were sequenced twice, with the Tn and T3 primers, and a consensus sequence was generated. A BLAST search with this consensus translated in the +1 frame showed high-scoring segment pairs with the *uvrA* gene product from *Micrococcus luteus*, *Salmonella typhimurium*, *E. coli*, *Mycoplasma capricolum*, and *Vitreoscilla* sp. Depending on the species, the deduced *R. sphaeroides* amino acid sequence showed 52 to 64% amino acid identity and 74 to 83% amino acid similarity over an approximately 90-amino-acid region of subunit A of the UvrABC excinuclease. In *M. luteus*, this polypeptide comprises 992 amino acids, with the site of insertion in the mutant corresponding to amino acid

TABLE 3. Results of database searches with sequences flanking UV[s] insertions[a]

UV[s] strain	Best match	Organism showing best match	% Amino acid identity[b]	% Amino acid similarity[c]	Length of region[d]	Length of polypeptide[e]	Sequence alignments found by BLAST[f]
GR0328	*uvrA*	*M. luteus*	57	78	42	992	GR0328: 163 LTAQKNEIARAILKEIRERLGFLVNVGLDYLSMSRAAGTLSG 288 LT +++IA +LKEI RL FL++VGL+YL+ + R AGTLSG *uvrA*: 500 LTDRERQIADQVLKEILARLAFLLDVGLEYLNLERPAGTLSG 541
	uvrA	*M. luteus*	37	53	43	992	GR0328: 4 FERYQNNRPCHVCGGYRLKPEALAVKIGGLHIGQVVQMSIKEA 132 +E + PC C G RL P L V +GGL I ++ ++EA *uvrA*: 449 YESFMREIPCPACHGARLNPTVLNVLVGGLSIADATRLPMREA 491
GR0221	*uvrA*	*S. typhimurium*	62	80	66	941	GR0221: 5 HELVEQSNTVVVIEHNLDVVKTADWIIDIGPEGGDGGGKIVAEGTPEEVXKVEVSHXGRYLRDIXK 202 H+L +Q NT+VVIEHNLDV+KTADWI+D+GPEGG GGG+I+ GTPE V + E SH R+L+ + K *uvrA*: 876 HQLRDQGNTIVVIEHNLDVIKTADWIVDLGPEGGSGGGEILVAGTPETVAECEASHTARFLKPMLK 941
GR0221	"*mmsA*"	*P. aeruginosa*	55	62	96	496	GR0221: 1 EFCIGAPHLLKGEFTDSAGPGIDXYSMRQPLGVGAGITPFNFPGMIPLWKMGPALGAGNA 180 E P LL GE ++ ID YS+ QPLGV GITPFNFP MIPLW A+ GNA "*mmsA*": 110 EHACNVPSLLMGETVENVARNIDTYSITQPLGVCVGITPFNFPAMIPLWMFPLAIACGNA 169 GR0221: 181 FILKPSERDPSVPLXLXEIFQEGGLPDGVLQVVERG 288 FILKPSE+ P + L E+F E G P GVLQVV G "*mmsA*": 170 FILKPSEQVPLTSVRLAELFLEAGAPKGVLQVVHGG 205
GR0333	*uvrB*	*M. luteus*	70	90	20	709	GR0333: 68 FEMKTPFQPAGDQPTAIAEL 9 FE+ +P+QP+GDQP AIAEL *uvrB*: 14 FEVISPYQPSGDQPKAIAEL 33
GR0311	*uvrC*	*P. fluorescens*	40	65	92	607	GR0311: 5 FALRMNDPVLYFVQRLRDEAHRWAIGAHRAKRXKAVSATPLDEIPGVGXARKRALLAHFG 184 F L+ + P L+ +Q++RDEAHR+AI HRA+R K + L+++ GVG R+R LL HFG *uvrC*: 514 FTLKGDSPALHLIQQIRDEAHRFAITGHRARRGKTRRTSTLEDVAGVGPKRRRDLLKHFG 573 GR0311: 185 SDKAVARAGVPDLXAVEGISETMAQSIHDYFH 280 + ++RA + ++ GIS+ +A+ I+ H *uvrC*: 574 GLQELSRASIEEIAKAPGISKKLAELIYANLH 605
	"*pgsA*"	*E. coli*	50	73	63	181	GR0311: 276 VMFLYFXRPWADWFALTLFILAAVTDFFDGYLARLWKQESKFGAMLDPIADKAMVVIALVIIT 88 V+ Y W+ + A +F +AAVTD+FDG+LAR W Q ++FGA LDP+ADK +V IA+V++T "*pgsA*": 20 VLVFYLPVTWSPFAAALIFCVAAVTDWFDGFLARRWNQSTRFGAFLDPVADKVLVAIAMVLVT 82
			42	68	19	181	GR0311: 57 LPVTLILFREVFVSGLREF 1 LP ++ RE+ +S LRE+ "*pgsA*": 92 LPAATMIAREIIISALREW 110

[a] The highest-scoring segment pairs found when BLAST was used to search the nonredundant protein databases with the *R. sphaeroides* UV[s] mutant sequences are presented.
[b] Percentage of *R. sphaeroides* amino acids which are identical to those within the region of homology of the protein.
[c] Sum of *R. sphaeroides* amino acids which are identical and similar, expressed as a percentage of the total number of amino acids found within the region of homology.
[d] Length of the region (expressed as number of amino acids) of the polypeptide which gave a match with the translated *R. sphaeroides* nucleotide sequence.
[e] The length of the complete subject polypeptide, expressed as amino acids, found in the BLAST search.
[f] Two amino acid sequences are presented for each alignment. The upper sequence is the amino acid sequence deduced by BLAST that corresponds to the *R. sphaeroides* nucleic acid sequence. The numbers at the ends of the upper lines are nucleotide positions in the *R. sphaeroides* DNA sequences. The lower sequence is the polypeptide match found in the database by BLAST. The numbers at the ends of the lower sequences are amino acid positions in the full-length polypeptides.

541. Matches were to two regions of the UvrA polypeptide on either side of the region from amino acids 491 to 500.

GR0221. The sequence generated from the Tn primer also gave high-scoring segment pairs with the *uvrA* gene product from *S. typhimurium*, *M. luteus*, *E. coli*, *Vitreoscilla* sp., and *Paracoccus denitrificans*. Depending on the species, amino acid identity to the *R. sphaeroides* deduced amino acid sequence varied between 62 and 72% and amino acid similarity was 74 to 93% over a 62- to 73-amino-acid region of the UvrA polypeptide. This region was near the C terminus of the protein, and the site of the insertion corresponded to codon 874 within the 941-amino-acid UvrA polypeptide from *S. typhimurium*. The region immediately downstream of the gene gave no relevant matches when used in a separate BLAST search.

When the *R. sphaeroides* flanking DNA was sequenced with the T3 primer (translated in the +2 frame), BLAST gave the highest-scoring segment pair to *mmsA*, the gene encoding methylmalonate-semialdehyde dehydrogenase from *Pseudomonas aeruginosa* (46). Matches to other members of the aldehyde dehydrogenase gene family from *Saccharomyces cerevisiae*, plants, and humans, as well as other species of bacteria, were also found. Depending on the species found in the search, the deduced *R. sphaeroides* amino acid sequence showed between 40 and 55% amino acid identity and between 56 and 66% similarity to polypeptides within this family over a 70- to 90-amino-acid region. The *Eco*RI site in the *R. sphaeroides* DNA coincided with amino acid 110 of the 496-amino-acid *mmsA* gene product of *P. aeruginosa*. Matches were found by BLAST over the next 96 amino acids towards the C terminus of the polypeptide. Beyond this region, matches were not found by BLAST. However, translation of the *R. sphaeroides* nucleotide sequence within the nonmatching region showed the presence of the strict consensus residues P, P, G, and K at amino acid positions 207, 209, 236, and 242, respectively, which occur within the aldehyde dehydrogenase family (25). This suggested that although amino acid sequence differences occur, the putative aldehyde dehydrogenase amino acid sequence from *R. sphaeroides* is in keeping with the family motif.

The sequence information from GR0221 and GR0328 taken together suggests that these strains have insertions in *uvrA* at different locations. The insertions are in opposite orientations and are separated by approximately 1 kb. At 1.3 kb downstream and on the opposite strand lies the 3′ end of a second gene which encodes a putative aldehyde dehydrogenase. Between these genes is an unsequenced 1.3-kb region which has sufficient coding capacity for a third gene. In *P. aeruginosa*, the *mmsA* gene is followed in an operon by the 897-bp *mmsB* gene (46).

GR0333. A BLAST search with the sequence generated from the Tn primer, translated in the −3 frame, gave high-scoring segment pairs with the *uvrB* gene products from *M. luteus*, *E. coli*, and *Mycoplasma genitalium*; the *uvr*/*dinA* gene product of *B. subtilis*; and the Uvr-402 protein of *S. pneumoniae*. The match to the *uvrB* gene product of *M. luteus* was 70% amino acid identity and 90% similarity over a 20-amino-acid region. The orientation of the transposon was such that the sequence generated with the Tn primer gave only the region at the 5′ end of the gene, with a match corresponding to amino acid 33 of the 709-amino-acid polypeptide and accounting for the short length of the homologous region. A GTG codon that could be used as the initiator was found in the *R. sphaeroides* sequence at the predicted position of the methionine start codon. A methionine codon (ATG) was also found 5 codons upstream. Among the other organisms, the *R. sphaeroides* amino acid sequence gave a range of matches, the highest to the *uvr*/*dinA* gene product of *B. subtilis* (57% amino acid identity, 76% similarity) and the lowest to the *S. pneumoniae* Uvr-402 protein (47% amino acid identity, 80% similarity). In each case, the match was to a 20-amino-acid region lying at the amino terminus of the protein found in the search.

The genomic DNA sequence flanking the *Eco*RI site was generated by using the T7 primer. However, a BLAST search using this sequence did not give matches that were considered significant.

GR0311. A BLAST search with the sequence generated from the Tn primer, translated in the +2 frame, gave high-scoring segment pairs with the *uvrC* gene product from *Pseudomonas fluorescens*, *E. coli*, and *B. subtilis*. The matches ranged, depending on the species, from 39 to 45% amino acid identity and 60 to 68% amino acid similarity over a 71- to 92-amino-acid region. In *P. fluorescens*, the *uvrC* gene product is a 607-amino-acid polypeptide. The site of the insertion was at amino acid 512. The orientation of the insertion within the gene generated a sequence from the end of the insertion to beyond the 3′ end of the gene.

In this subclone, the T7 primer generated a sequence from the *Eco*RI site of the *R. sphaeroides* flanking DNA. A BLAST search with this sequence, translated in the −2 frame, gave high-scoring segment pairs with the product of the *pgsA* gene from *E. coli*, *P. fluorescens*, and *Mycobacterium leprae*. The *pgsA* gene encodes phosphatidylglycerophosphate synthase, which catalyzes the committed step to the synthesis of acid phospholipids in *E. coli*. The polypeptide is composed of 181 to 193 amino acids, depending on the organism. The deduced *R. sphaeroides* sequence gave matches to two regions of the *pgsA* gene product in each organism. This is exemplified by the *E. coli* matches to amino acid regions 20 to 82 and 92 to 110, which gave 50 and 42% amino acid identity, respectively. The sequence generated from the T7 primer started at amino acid 110 within PgsA and continued beyond the 5′ end of the gene. In *E. coli*, the start codon of *pgsA* is 55 bp downstream of the termination codon of the *uvrC* gene (22). In addition, the *pgsA* promoter begins 9 bp upstream of the *uvrC* stop codon, i.e., within the *uvrC* gene itself. In *R. sphaeroides*, we estimated that the distance between *uvrC* and *pgsA* is 20 bp, and as in *E. coli*, the genes are tandemly arranged. However, we have not found a σ^{70} promoter-like sequence for *pgsA* within *uvrC*.

DISCUSSION

Previous studies of DNA repair in *R. sphaeroides* have established that this system is amenable to study and sufficiently different from that of *E. coli* to merit further analysis. The related species *R. capsulatus* possesses an inducible SOS repair response and a photoreactivation system (6), and *R. sphaeroides* has a system for repair of alkylation damage (52). However, the alkylation repair response does not induce the cognate *E. coli* repair gene (16) nor does the SOS system induce the *E. coli recA* gene (17). These results indicate some difference from *E. coli*, since many other bacteria were able to induce these heterologous systems.

In the present study, *R. sphaeroides* was mutagenized with a Tn*5* derivative and eight UV^s strains were isolated. Digestion of their genomic DNAs with four restriction enzymes followed by CHEF pulse-field gel electrophoresis permitted the sites of insertion to be located on chromosome I. The mapping resolution was sufficient to show that strains GR0221, GR0328, and GR0310 have nearby but nonidentical insertions. On the basis of sequence analysis of cloned segments from GR0221 and GR0328, we calculate these two insertions to be approximately 1 kb apart. Since the *uvrA* gene is about 3 kb long on the basis of the protein sequence, it is possible that all three insertions

are in this gene, although we cannot rule out the possibility that the insertion in GR0310 is within an upstream regulator of *uvrA* or in a gene within a few kilobases of *uvrA*, which is also involved in DNA repair.

The six strains analyzed in this study showed increased UV sensitivity on solid and in liquid media. In addition, they also showed increased sensitivity to MMC and lower reactivation of UV-irradiated phage than the wild type. That the UV^s phenotype resulted from the inability of the strains to repair damaged DNA was supported by database searches with sequences from the regions flanking the sites of insertion. These led to the conclusion that strains GR0221 and GR0328 had transposon insertions in *uvrA* while strains GR0333 and GR0311 had insertions in *uvrB* and *uvrC*, respectively. These three genes have been shown to encode the UvrABC nuclease in *E. coli* (39, 51). We were unsuccessful in subcloning flanking DNA from strain GR0310, and the sequence we obtained from strain GR0304 was insufficient to provide meaningful matches during the database search. Although we did not perform complementation analysis with cosmid clones covering the sites of insertion (11), it seems clear that the insertions in GR0221, GR0328, GR0333, and GR0311 represent insertions in genes encoding DNA repair functions. The formal possibility remains that in strains GR0304 and GR0310, the UV^s phenotype results from a mutation at a second site. However, we note that in our previous analysis of 25 auxotrophic insertion mutants, in all cases the phenotype was caused by the insertion. Thus, this formal possibility of secondary mutations in *R. sphaeroides* is unlikely. Nevertheless, we are currently using a cosmid library of chromosome I to perform complementation analysis of these strains.

When the relative UV sensitivities of *R. sphaeroides* and *E. coli* were compared, *R. sphaeroides* was approximately 100-fold more resistant to a UV dose of 60 J/m^2. It has been estimated that in bright sunlight, *E. coli* numbers are reduced to 10% in 1 to 2 h (23, 24). Thus, *R. sphaeroides*, in contrast to *E. coli*, can easily survive the exposure of a sunny day, an ability that appears to be necessary for a photosynthetic lifestyle. *E. coli* is, in fact, relatively UV resistant compared with other enteric bacteria (45); thus, the resistance of *R. sphaeroides* is expected to be greater than that of many other prokaryotes. While *R. sphaeroides* is not as resistant as *D. radiodurans* or some subsurface bacteria, its resistance is comparable to that of *M. luteus* (4). However, we do not know how UV resistant *R. sphaeroides* is. We have only examined its UV repair processes under dark conditions and ignored any repair component which may come from photoreactivation or which is inducible under photosynthetic conditions. We also know that *R. sphaeroides* 2.4.1 carries a UV-inducible prophage (unpublished results), and we have not resolved how much UV killing is, in fact, due to prophage induction rather than DNA damage per se.

How does *R. sphaeroides* achieve its greater UV resistance? One area where there are clear differences between *R. sphaeroides* and *E. coli* is the G+C contents of their genomes. To analyze these, we assembled all nonredundant sequences of *R. sphaeroides* from the GenBank-EMBL databases, as well as a representative random sample of *E. coli* sequences (Table 4). In this sequence sample, *R. sphaeroides* has a G+C content of 65% while that of *E. coli* is 51%, comparable to what has been previously measured. From these sequences, it can be seen that the total frequency of pyrimidine dinucleotides (Py_2) is actually slightly higher in *R. sphaeroides*. The pyrimidine trinucleotide (Py_3) frequency is also slightly higher, and this tends to make the number of pyrimidine dimers that can form after irradiation slightly lower than the Py_2 frequency. Thus, there is no significant difference in the frequency of Py_2 between *E. coli* and *R. sphaeroides*. However, the frequency of specific pyrimidine dinucleotide pairs shows considerable differences, with the TT dimer being predominant in *E. coli* and the CC dimer dominating in *R. sphaeroides*. Since the quantum yields for these different dimers are not the same, different numbers of lesions in the two genomes are expected. On the basis of previous measurements of the yield of cyclobutane dimers (36, 42, 50), we estimated that *R. sphaeroides* should have at least 67% of the number of *E. coli* dimers and probably more. Interpretation of these effects must also take into account the other types of lesions derived from pyrimidine dinucleotides following UV irradiation (18). Of particular note is the 6-4 pyrimidine dimer, which is rare among TT dinucleotides and is preferentially formed from TC and CC dinucleotides at the doses used in this study (7). The 6-4 photoproduct is better correlated with mutagenesis than cyclobutane dimers (7) and thus may be biologically more significant. However, beyond this, the relationship of these different types of lesions to lethality is not known; thus, it is not clear that base composition alone can account for the resistance of *R. sphaeroides*, although it may contribute to this phenomenon.

TABLE 4. Genome compositions of *R. sphaeroides* and *E. coli*[a]

Nucleotide(s)	% of total nucleotides in:	
	R. sphaeroides	*E. coli*
A	17.5	24.5
G	32.5	25.5
C	32.5	25.5
T	17.5	24.5
TT	3.2	7.2
TC	7.1	5.8
CT	5.5	5.1
CC	9.4	5.9
Total Py_2	25.2	24.0
Total Py_3	12.2	11.0

[a] Mononucleotide, Py2, and Py3 percentages were calculated from a nonredundant sample of GenBank-EMBL sequences for each organism as follows: *R. sphaeroides*, sample size of 103,009 nucleotides, G+C content of 65%, C+T content of 50%; *E. coli*, sample size of 634,352 nucleotides, G+C content of 51%, C+T content of 50%.

It seems likely that the increased resistance of *R. sphaeroides* compared with *E. coli* is due to its DNA repair systems. We note that although there are differences between the mutants described here, their level of UV sensitivity is approximately the same as for comparable *E. coli* mutants (27). This is not what would be expected if there were significant shielding mechanisms or if the extra resistance were due to base composition effects. Rather, this suggests that the excision repair mechanism and accessory pathways are responsible for the resistance. We also note that the observation that *recA* mutants of *R. sphaeroides* were not as sensitive as the UvrABC mutants implies a different organization of DNA repair. It is possible that recombination does not play a large role in DNA repair in *R. sphaeroides* and that alternative mechanisms exist to fill gaps opposite DNA lesions.

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