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Involvement of a Protein Kinase Activity Inducer in DNA Double Strand Break Repair and Radioresistance of *Deinococcus radiodurans*▽†

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Transgenic bacteria producing pyrroloquinoline quinone, a known cofactor for dehydrogenases and an inducer of a periplasmic protein kinase activity, show resistance to both oxidative stress and protection from nonoxidative effects of radiation and DNA-damaging agents. *Deinococcus radiodurans* R1 encodes an active pyrroloquinoline quinone synthase, and constitutive synthesis of pyrroloquinoline quinone occurred in wild-type bacteria. Disruption of a genomic copy of *pqqE* resulted in cells that lacked this cofactor. The mutant showed a nearly 3-log decrease in γ radiation resistance and a 2-log decrease in mitomycin C tolerance compared to wild-type cells. The mutant cells did not show sensitivity to UVC radiation. Expression of pyrroloquinoline quinone synthase in *trans* showed that there was functional complementation of γ resistance and mitomycin C tolerance in the *pqqE* mutant. The sensitivity to γ radiation was due to impairment or slow kinetics of DNA double strand break repair. Low levels of ^{32}P incorporation were observed in total soluble proteins of mutant cells compared to the wild type. The results suggest that pyrroloquinoline quinone has a regulatory role as a cofactor for dehydrogenases and an inducer of selected protein kinase activity in radiation resistance and DNA strand break repair in a radioresistant bacterium.

Pyrroloquinoline quinone (PQQ) has been shown to be a redox cofactor for periplasmic as well as cytosolic dehydrogenases, contributing to the mineral phosphate solubilization phenotype in bacteria (11). This compound has been reported to act as an antioxidant in vitro (33), in animal systems (13), and in bacterial systems (18) in vivo and as a member of the B group vitamins (16). He and coworkers (13) have shown that the antioxidant nature of PQQ is concentration dependent. Higher concentrations of PQQ induce oxidative stress for mitochondrial activity in rats, which leads to both apoptotic and necrotic cell death. Further studies indicated that the necrotic cell death could be selectively inhibited in the presence of antioxidants, while apoptotic cell death continued by a still-unknown mechanism. Further, a possible role for PQQ as an inducer for proteins kinases involved in distinctly different metabolic and physiological processes has been suggested (20).

Deinococcus radiodurans R1, a gram-positive bacterium, exhibits extraordinary tolerance to various abiotic stresses, including radiation, desiccation, and other DNA-damaging factors (3). DNA double strand break repair in *D. radiodurans* R1 follows biphasic kinetics (8). Phase I is RecA independent and involves an extended synthesis-dependent strand annealing mechanism for reassembly of the fragmented genome (42), while phase II involves RecA-dependent slow crossover events (9). The extreme phenotypes of this bacterium are believed to be due to the presence of an efficient DNA strand break repair mechanism (1, 31) and strong oxidative stress tolerance (27). A comparison of the genome sequence of *D. radiodurans* R1 (41) with the genome sequence of a radiation-sensitive and extensively studied *Escherichia coli* strain (5) showed the presence of very similar DNA recombination and repair complements in the two organisms, except for the absence of the canonical RecBC recombination pathway in *D. radiodurans* R1. However, unlike other bacteria, this organism contains a large number of uncharacterized proteins and several annotated open reading frame (ORFs), whose products do not match any of the typical classes of proteins known and listed in databases (25).

The *D. radiodurans* R1 genome contains the *pqqE* gene, which encodes a functional PQQ synthase enzyme in transgenic *E. coli* cells (18). These cells showed greater resistance to the photodynamic effect of rose bengal and improved resistance to UVC and γ radiation compared to wild-type *E. coli* (20). Hence, the significance of the presence of PQQ without the mineral phosphate solubilization function (18) in *D. radiodurans* R1 would be worth investigating. This study reports the role of PQQ in the double strand break repair and radiation resistance phenotypes of this organism. Synthesis of PQQ was detected in *D. radiodurans* R1 cells harboring wild-type *pqqE*. Disruption of the genomic copy of *pqqE* with *nptII* made these cells PQQ deficient. These cells showed a nearly 3-log decrease in sensitivity to γ radiation and a 2-log decrease in mitomycin C (MMC) tolerance compared to the wild type. The γ radiation sensitivity of the *pqqE* mutant has been attributed to the defect in DNA double strand break repair and to changes in total protein phosphorylation profiles. The expression of wild-type PQQ synthase in *trans* showed nearly complete complementation of impaired phenotypes in mutant cells. These re-

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sults suggest that PQQ has a regulatory role in the double strand break repair and radiation resistance of *D. radiodurans*.

MATERIALS AND METHODS

Bacterial strains and materials. *D. radiodurans* R1 was a generous gift from M. Schafer (39). The wild type and derivatives of the wild type were grown aerobically in TGY broth (0.5 Bacto tryptone, 0.3% Bacto yeast extract, 0.1% glucose) or on TGY agar plates as required at 32°C. Antibiotics, including chloramphenicol (3 μg/ml) and kanamycin (8 μg/ml), were added when required. The TGY agar plates containing *D. radiodurans* R1 and its derivatives were incubated at 32°C for 48 h before the colonies were counted. The shuttle expression vector pRADgro and derivatives of this vector were maintained in *E. coli* strain HB101 as described previously (34). Other recombinant techniques used have been described previously (37).

Construction of PQQ synthase expression plasmid. Genomic DNA of *Deinococcus* was prepared as described previously (2). A 1,128-bp DNA fragment was PCR amplified from the genomic DNA of *D. radiodurans* R1 using *pqqE* gene-specific primers (forward primer 5′CCGGGGCCCATGGTGGCATTTCTCCG TGGC3′ and reverse primer 5′GCTCTAGATCATGCGTGACTTACCAATG GA3′). The identity and correctness of the *pqqE* gene were ascertained by restriction analysis and partial nucleotide sequencing. The PCR product was ligated at ApaI and XbaI sites in pRADgro (34) to obtain pGro*pqqE*. The recombinant plasmid was transformed into *D. radiodurans* as described previously (29), and chloramphenicol-resistant clones were isolated on TGY agar plates containing chloramphenicol (5 μg/ml). Plasmid DNA was prepared from these clones, and the presence of an insert in the plasmid samples was confirmed by restriction analysis.

Generation of *pqqE* disruption mutant derivative of *D. radiodurans* R1. A 1-kb upstream fragment which contained 500 bp of the 5′ region of *pqqE* coding sequences along with 500 bp upstream of this ORF was PCR amplified using forward primer 5′CTAGGGCCCCAGTGGGAGTACCTC3′ and reverse primer 5′GGAATTCCTACTGTTAGACTGTTG3′. The PCR product was cloned at ApaI and EcoRI sites in pNOKOUT (20) to obtain pNok*pqqE*1. The 1,455-bp downstream fragment, which contained 500 bp of the 3′ half of the *pqqE* coding sequence along with 955 bp downstream of this sequence, was PCR amplified using forward primer 5′CGGGATCCATGTCCAAATTTAAGCA TC3′ and reverse primer 5′CTCTAGACTGCGACTGGGAATGAAG3′ and cloned at BamHI and XbaI sites in pNok*pqqE*1 to obtain pNok*pqqE*. Recombinant plasmid pNok*pqqE* was linearized with ScaI, gel purified, and transferred into *D. radiodurans* R1. The transformants were grown for several generations in TGY medium supplemented with kanamycin (8 μg/ml) to obtain a homozygous *pqqE* disruption mutant. Homozygosity was ascertained by scoring amplification of a 2.2-kb PCR product, which included both *pqqE* (1.1 kb) and the *nptII* cassette (1.1 kb), using *pqqE* coding sequence-specific forward primer 5′ATGG TGGCATTTCTCCGTGGC3′ and reverse primer 5′TCATGCGTGACTTACC AATGGA3′. Clones showing the complete absence of the normal *pqqE* gene (1.1 kb) were considered homozygous *pqqE* disruption mutants and were used for further studies.

Detection of PQQ in *D. radiodurans*. PQQ was extracted from stationary-phase cells of *D. radiodurans* R1 using a modified protocol described previously (40). In brief, the cells were sonicated, and the cell extract was digested with 50% acetonitrile at 65°C for 2 h. The mixture was centrifuged at 15,000 × *g* for 10 min; the clear supernatant was collected and dried with a concentrator under a vacuum. The residues were dissolved in 50% *n*-butanol at 1 mg/ml, and PQQ was extracted at 50°C overnight. The clear supernatant was dried under a vacuum and dissolved in 100% methanol (high-performance liquid chromatography [HPLC] grade). HPLC analysis was carried out using a C_{18} μBondapak column and elution with a mobile phase that comprised 45% methanol in HPLC-grade water at a flow rate 1 ml/min. Each fraction was scanned with a UV detector set at 289 nm. The identity of the PQQ was ascertained by comparing the retention time of a peak with the retention time of a standard sample and by its possible absence in *pqqE* mutant cells.

Effect of DNA-damaging agents on cell survival. *Deinococcus* cells were treated with different doses of UV and γ radiation as described previously (19). In brief, mutant and wild-type *D. radiodurans* cells and *pqqE* mutant cells harboring pGro*pqqE* were grown in TGY medium to the late log phase at 32°C. The cells were suspended in sterile phosphate-buffered saline (PBS) and exposed to different doses of γ radiation at a rate of 7.2 kGy per h (Gamma 500; ^{60}CO; Board of Radiation and Isotopes Technology, Department of Atomic Energy, India). Appropriate dilutions were plated on TGY agar plates and incubated at 32°C. To determine UV effects, cells were prepared as described above, and different dilutions were plated. Cells were exposed to different doses of UV radiation at 254 nm and incubated at 32°C. MMC treatment was performed as described by Harris et al. (12). In brief, late-log-phase cells were treated with MMC (20 μg/ml), and aliquots were removed at regular intervals. The appropriate dilutions were plated on TGY agar plates supplemented with kanamycin (8 μg/ml) when required, and the plates were incubated at 32°C for colony formation. Hydrogen peroxide treatment was performed as described previously (34). In brief, late-log-phase cells were treated with different concentrations of hydrogen peroxide for 30 min with vigorous aeration. Cells were diluted with PBS, and different dilutions were plated on TGY agar plates. The numbers of CFU were recorded after 48 h of incubation at 32°C.

Protein phosphorylation. In vivo phosphorylation of proteins was studied using cells grown in the presence of [^{32}P]phosphoric acid overnight as described previously (26). For determination of ^{32}P incorporation, the labeled cells were treated with lysozyme (10 mg/ml) for 1 h at 37°C, followed by 1% NP-40 in cell lysis buffer (20 mM Tris-HCl [pH 8.0], 50 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol). Treated cells were disrupted by repeated cycles of freezing at the temperature of liquid nitrogen and thawing at room temperature. Cell extracts were obtained by centrifugation at 20,000 × *g* and were treated with DNase I (50 μg/ml) and RNase I (50 μg/ml) for 2 h at 37°C. Treated samples were passed through a Sephadex G-25 (GE Healthcare, United States) spin column in 50-μl batches. The incorporation of ^{32}P was monitored by trichloroacetic acid precipitation as described previously (32). Equal amounts of total proteins from both types of cells were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and ^{32}P-labeled phosphoproteins were detected by autoradiography.

DNA strand break repair studies. For DNA strand break repair studies, the cells were irradiated with 6 kGy γ radiation (7.2 kGy/h) at different times during postirradiation recovery. Cell lysis and restriction digestion were carried out in gels. The DNA fragments were separated by pulsed-field gel electrophoresis using the modified protocol described for a previous method (24). In brief, the cells were washed with 70% ethanol in PBS (pH 7.5) for 5 min. Agarose plugs containing the cells were incubated with lysis buffer I (5 mg/ml lysozyme in 5 mM EDTA, pH 8.0) for 2 h at 37°C, followed by overnight incubation at 55°C in lysis buffer II (0.5 M EDTA [pH 8.0], 1% sodium sarcosine, 2 mg/ml proteinase K). The plugs were washed four times with TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8.0]) and then with restriction enzyme buffer for 1 h each time at 55°C. For restriction digestion, the plugs were transferred to fresh tubes containing enzyme buffer and 100 U of XbaI and incubated overnight at 37°C. DNA fragments were analyzed on 0.8% low EEO agarose (Bangalore Genei, India) for 25 h as described previously (24).

RESULTS AND DISCUSSION

Disruption of *pqqE* results in deinococcal cells that lack PQQ. The genomic copy of *pqqE* (DRC0034) was disrupted with the *nptII* cassette and used to generate a *pqqE* disruption mutant. Homozygosity was confirmed by PCR amplification with gene-specific primers (Fig. 1). PQQ was extracted from cell extract of both wild-type and *pqqE* mutant stationary-phase cells, and its presence was detected by HPLC. The extract from wild-type cells showed elution of several species, including a species with a retention time of 3.29 min, which matches the retention time of standard PQQ (3.27 min) under similar chromatographic conditions (Fig. 2). The extract from mutant cells showed a metabolite profile similar to that of the wild type except that there was a peak with a retention time of 3.29 min in the wild-type extract and a retention time of 3.27 min in the standard PQQ profiles (Fig. 2C). This strongly indicated that only wild-type cells make extractable PQQ. Also, transgenic *E coli* cells expressing deinococcal *pqqE* were bioassayed for synthesis of PQQ (18). The results indicate that there is synthesis of PQQ in *D. radiodurans* R1.

The *pqqE* mutants showed higher sensitivity to DNA-damaging agents. The *pqqE* disruption mutant cells were checked for their response to γ radiation, UVC, MMC, and hydrogen peroxide. Mutant cells showed a 3-log decrease in the level of γ radiation resistance compared to wild-type cells (Fig. 3A)

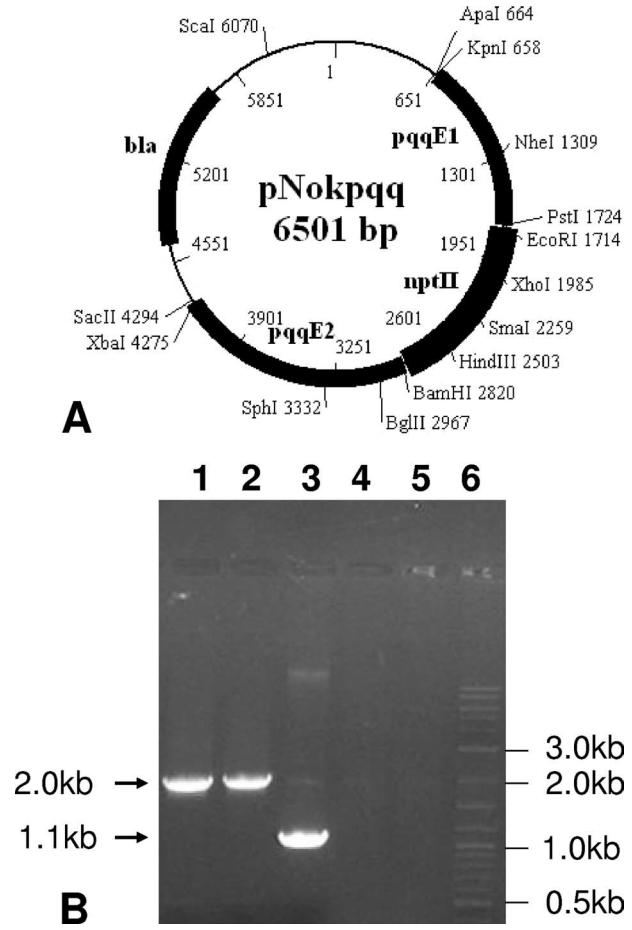

FIG. 1. Construction of a recombinant suicide plasmid and generation of a *pqqE* disruption mutant derivative of *D. radiodurans* R1. The *pqqE* gene sequence was divided at the middle of the gene into two equal halves. Both fragments were PCR amplified along with upstream (*pqqE1*) and downstream (*pqqE2*) sequences and cloned in pNOKOUT (19) to obtain pNOK*pqqE* (A). The recombinant plasmid was linearized with ScaI and transformed into *D. radiodurans* R1. Recombinant clones were subcultured for several generations in order to completely replace normal copies with a disrupted copy on the genome. Genomic DNA from two homozygous *pqqE* mutant clones, clones 1 (lane 1) and 2 (lane 2), and the wild type (lane 3) was PCR amplified using *pqqE* coding sequence-flanking primers (B). Clone 1 was subsequently used for further studies.

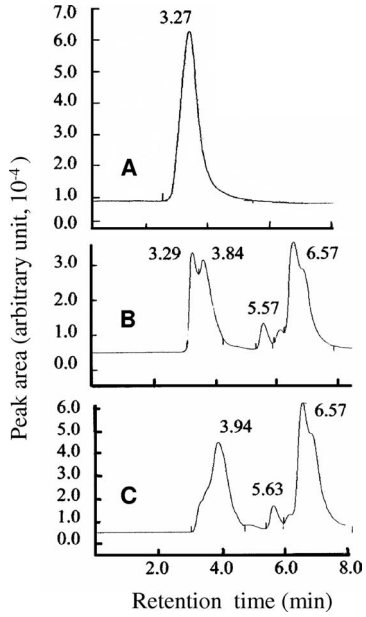

FIG. 2. Detection of PQQ in *D. radiodurans* R1: elution profiles of commercially available PQQ (A) and PQQ extracted from extracts of wild-type (B) and *pqqE* disruption mutant (C) cells.

and a 2-log decrease in MMC tolerance (Fig. 3B). These cells were more sensitive to UVC at 0.8 kJ m^{-2} than wild-type cells, while at a higher dose the UVC responses of wild-type and mutant cells were similar (Fig. 3C). Both γ radiation and MMC treatment produce a high density of double strand breaks in the genome (17), while UVC produces the maximum number of single strand breaks and less than 1% double strand breaks in the genome (6). The unique effect of PQQ in response to γ radiation and MMC clearly indicated the role of PQQ in the regulation of DNA double strand break repair. Our previous studies indicated that PQQ-expressing transgenic *E. coli* shows improved resistance to both single strand and double strand breaks caused by UVC, γ radiation, and MMC (20). In this study PQQ disruption did not affect the UVC phenotype of wild-type cells. This suggested that UVC tolerance in this bacterium may be supported by a mechanism that does not require PQQ. Previous findings have also shown that γ-radiation-sensitive derivatives of *D. radiodurans* R1 are not always sensitive to UVC radiation (19, 34). *E. coli* does not harbor the strong indigenous mechanism for DNA double strand break repair; therefore, strengthening of the DNA double strand break repair mechanism with PQQ would have a significant effect on UVC tolerance in *E. coli*.

PQQ-synthesizing *E. coli* cells show improved tolerance to oxidative stress caused by the photodynamic effect of rose bengal (18). The antioxidant role of PQQ was further supported by the greater reactivity of this compound with artificially produced reactive oxygen species in solution (33). Mutant cells showed a nearly fivefold decrease in hydrogen peroxide tolerance compared to wild-type cells (Fig. 4), suggesting that PQQ has a role in the oxidative stress tolerance of *D. radiodurans* R1 as well. However, the contributions of PQQ to oxidative stress tolerance in *Deinococcus* were not as pronounced as the effect of PQQ on DNA double strand break repair. The higher sensitivity of PQQ-deficient *D. radiodurans* R1 cells to γ radiation and MMC and the response of PQQ-synthesizing transgenic *E. coli* to DNA double strand break-producing agents (20) clearly suggest that PQQ contributes to DNA double strand break repair in bacteria. Thus, functions of PQQ in both oxidative stress tolerance and DNA double strand break repair could be strongly suggested. Although the molecular mechanism of PQQ action in DNA double strand break repair is not clear, the role of PQQ as a cofactor for periplasmic protein kinase, which is involved in DNA strand break repair and homologous recombination, has recently been demonstrated (20).

PQQ synthase expressed on a low-copy-number plasmid complements the *pqqE* mutant phenotype. To ascertain that *pqqE* mutant phenotypes are due to a lack of functional PQQ

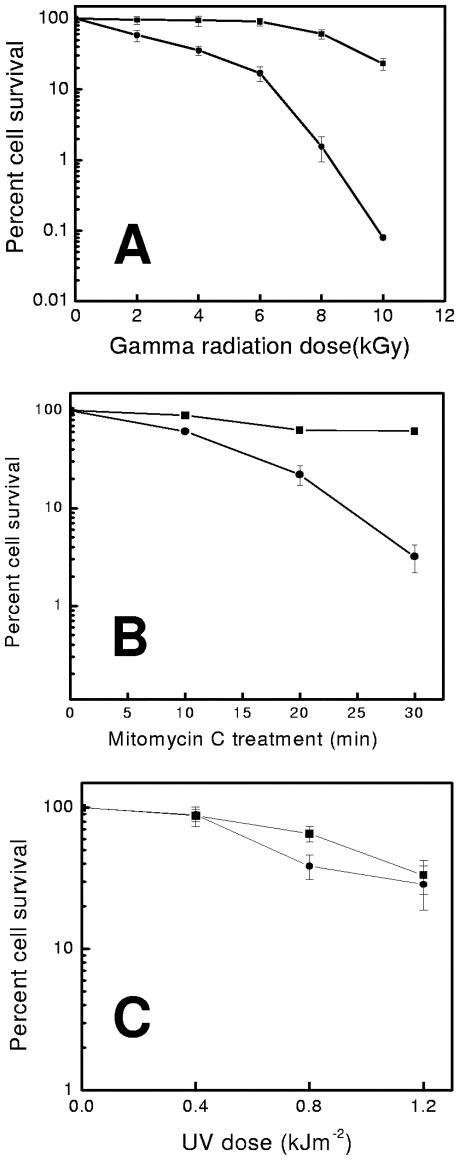

FIG. 3. Cell survival response of *pqqE* disruption mutant to DNA-damaging agents. *D. radiodurans* cells harboring the wild-type *pqqE* gene (■) and a disrupted copy (●) of this gene were treated with different doses of γ radiation (A) and UVC radiation (B) and exposed to MMC (20 μg/ml) for different times (C), and cell survival was monitored. The initial cell density of cultures used in all experiments was approximately 10^7 cells/ml.

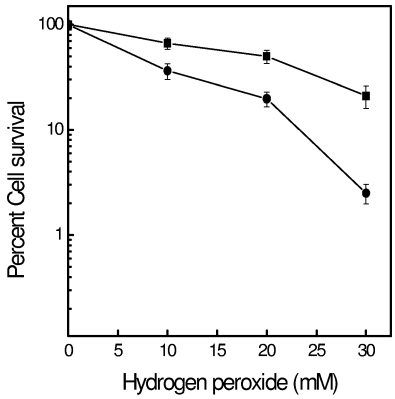

FIG. 4. Effect of *pqqE* disruption on the hydrogen peroxide response of *D. radiodurans*. *D. radiodurans* wild type (■) and *pqqE* disruption mutant (●) cells were exposed to different concentrations of hydrogen peroxide, and cell survival was monitored.

synthase per se, the enzyme was expressed in *trans*, and the responses of the cells to DNA double strand break-producing agents were monitored. The *pqqE* mutant cells harboring pGro*pqqE* (see Fig. S1 in the supplemental material) showed recovery of lost γ radiation and MMC resistance phenotypes to nearly wild-type levels (Fig. 5). The levels of complementation were nearly 90% of the wild-type levels. This suggested that *pqqE* mutant phenotypes were not due to an absence of any proteins downstream of *pqqE* but to the absence of PQQ synthase per se. The expression of a transgene under control of the P_{groESL} promoter in pRADgro has been demonstrated in previous studies (19, 22). Mutant cells showing the functional complementation of lost phenotypes also confirmed the expression of the *pqqE* gene on the pGro*pqqE* plasmid. These results strongly suggest that PQQ has a role in the DNA double strand break repair and radiation resistance of *D. radiodurans* R1. The mechanism of PQQ action in double strand break repair is most intriguing. PQQ acts as an antioxidant in bacterial systems (18), as an essential nutrient in mammalian systems (13), as an inducer of apoptotic and necrotic cell death in tumor cell lines (R. Pandey, B. Sankar, K. B. Sainis, and Hari S. Misra, unpublished data), and as a member of the B-group vitamins (16). These diverse roles suggest that PQQ might act as a signaling molecule, and its role in DNA damage-induced signal transduction could be hypothesized.

Further, PQQ is known as a redox cofactor for bacterial dehydrogenases (28). PQQ interacts with these enzymes through the conserved amino acid motifs distributed in the primary structure of the dehydrogenases. A database search of these motif-containing proteins showed that the motif is present in proteins kinases in all the organisms examined (http://www.sanger.ac.uk; http://smart.embl-heidelberg.de). Using this information, the interaction of one such protein, YfgL, with PQQ has been characterized (20), and it has been shown that interaction with PQQ stimulates the autophosphorylation activity of this protein in solution. The *D. radiodurans* R1 genome contains five ORFs for putative Ser/Thr kinases, which have PQQ binding motifs in their primary structure. None of these kinases have been characterized yet. However, the possibility that PQQ is required for stimulation of the activity of these enzymes and in radiation stress tolerance of *D. radiodurans* R1 cannot be ruled out and should be investigated independently.

Sensitivity to γ radiation correlates with defects in double strand break repair and less incorporation of ^{32}P in mutant cells. *D. radiodurans* R1 cells exposed to 6 kGy γ radiation were allowed to recover under normal growth conditions. Aliquots were removed at different time intervals, and the kinetics of DNA double strand break repair was monitored by pulsed-field gel electrophoresis. The results showed that there was strong impairment of the double strand break repair mechanism in mutant cells, while wild-type cells showed a normal

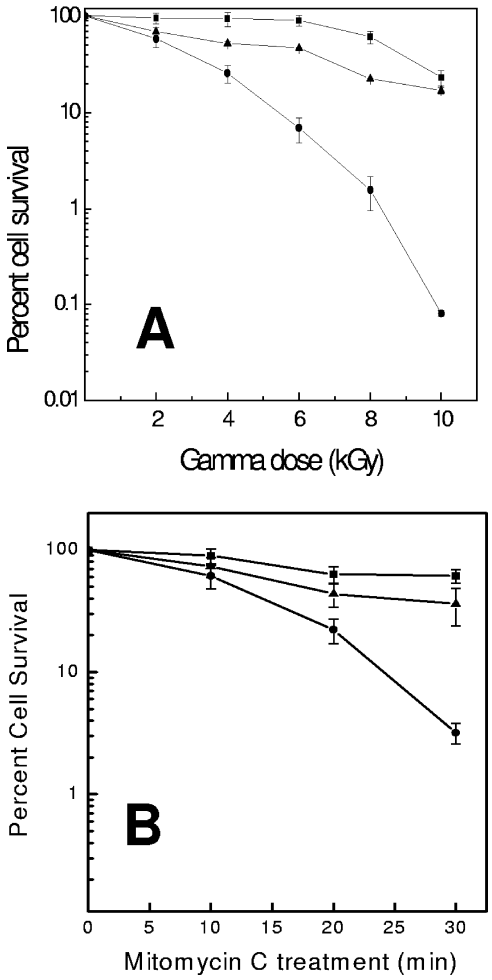

FIG. 5. Functional complementation of *pqqE* mutant phenotypes with wild-type PQQ synthase. The wild type (■), the *pqqE* mutant (●), and mutant cells expressing wild-type PQQ synthase (▲) on plasmid pGropqq (see Fig S1 in the supplemental material) were treated with different doses of γ radiation (A) and MMC (B), and cell survival was compared with that of controls. The initial cell density of cultures used in this experiment was approximately 2×10^8 cells/ml.

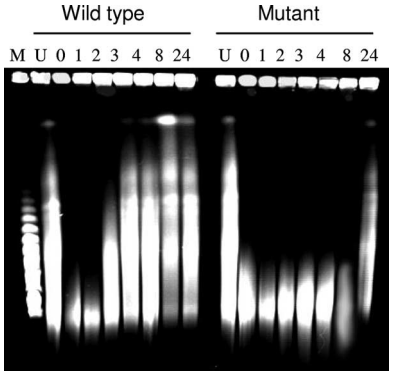

FIG. 6. DNA double strand break repair kinetics of the wild type and *pqqE* disruption mutant derivatives of *D. radiodurans* during postirradiation recovery. Logarithmically growing *Deinococcus* cells (lane U) were irradiated with 6 kGy γ radiation (7.2 kGy/h) and allowed to recover after irradiation. Aliquots were removed at different times (0, 1, 2, 3, 4, 8, and 24 h [lanes 0, 1, 2, 3, 4, 8, and 24, respectively]), and genomic DNA was digested with XbaI in agarose plugs. The extent of DNA strand breaks and the repair kinetics were monitored by pulsed-field gel electrophoresis by comparing the increase in the size of genomic DNA with the DNA size ladder for pulsed-field gel electrophoresis (lane M).

pattern of double strand break repair (Fig. 6). Wild-type cells showed recovery of the normal-size genome at 4 h after irradiation. The mutant cells showed no sign of recovery up to 8 h after irradiation. This result indicated that DNA double strand break repair occurred at a much lower rate in mutant cells than in wild-type cells. Delayed double strand break repair kinetics leading to γ radiation sensitivity has also been reported by other groups of workers (4, 12). Thus, the role PQQ in DNA double strand break repair was strongly supported.

The total phosphoprotein profiles of wild-type and *pqqE* mutant cells of *D. radiodurans* R1 were analyzed in vivo. The results showed that there was nearly 10-fold less incorporation of ^{32}P in mutant cells than in wild-type cells. The levels of ^{32}P incorporation in proteins from mutant cells were 4.23×10^4 cpm per μg total proteins, compared to 3.12×10^5 cpm per μg total proteins in wild-type cells. SDS-PAGE analysis of equal amounts of total soluble proteins from wild-type and mutant cells showed that there was less incorporation of ^{32}P in *pqqE* mutant proteins; this led to the absence of certain phosphoproteins with molecular masses of 28, 37, 44, 67, and 93 kDa (Fig. 7). The low levels of protein phosphorylation observed in the *pqqE* mutant could have been due to an absolute requirement for PQQ for certain protein kinase activities and/or to PQQ stimulation of the residual activities of the protein kinases in the cells. Our assumption concerning the involvement of protein kinases in double strand break repair may explain the possibility of low kinase activity in the absence of PQQ that might explain the delayed double strand break repair observed in mutant cells. These cells could reassemble a shattered-genome in 24 h of postirradiation recovery at low efficiency (Fig. 6).

Previous studies have emphasized the contribution of efficient DNA double strand break repair (7, 31) and strong oxi-

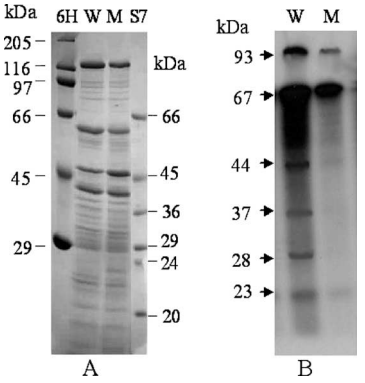

FIG. 7. Effect of *pqqE* mutation on total protein phosphorylation in *D. radiodurans* R1. Both wild-type (lane W) and *pqqE* disruption mutant (lane M) cells were labeled with ^{32}P in vivo. Total proteins from both types of cells were separated by SDS-PAGE (A), and the levels of ^{32}P incorporation in phosphoproteins were determined by autoradiography (B).

dative stress tolerance (10, 27) in the radioresistance phenotype of *D. radiodurans* R1. The DNA recombination and repair complements of *D. radiodurans* R1 (41) are very similar to those of *E. coli* (5). The extraordinary performance of *D. radiodurans* R1 in DNA repair raises the strong possibility that there is an undiscovered DNA damage response mechanism leading to efficient DNA strand break repair, and the possible contribution by uncharacterized proteins (25) could be emphasized. The present study resulted in a previously unreported concept for prokaryotes, the regulation of DNA strand break repair by an inducer of a DNA double strand break repair protein kinase activity, as shown in *E. coli* (20). DNA damage-induced signal transduction and the involvement of protein phosphorylation in DNA damage repair and cell cycle regulation have been reported in eukaryotes (21, 23, 38). The requirement for CDK1 during DNA end resection, homologous recombination, and DNA damage checkpoint activation has been demonstrated in budding yeast (15). Although the phenomenon of signal transduction has been extensively studied in bacteria (14, 36), the involvement of signal transduction and protein phosphorylation in DNA strand break repair and post-repair resumption of bacterial growth has not been demonstrated. Recently, Cox and Battista discussed the linkage between the DNA damage checkpoint, double strand break repair, and cell division in *Deinococcus* (7). They argued that *Deinococcus* cells do not divide until the cells have repaired the DNA double strand breaks. How cells sense the signal for the onset of cell division has not been determined. However, the phosphorylation of DNA recombination/repair protein has been reported in *Bacillus subtilis* (30). Workers have shown that the phosphorylation of a single-stranded DNA binding protein increases its affinity for single-stranded DNA and its ability to discriminate between double-stranded DNA and single-stranded DNA substrates. Our findings provide clear evidence concerning the role of PQQ, an inducer of a DNA repair and homologous recombination protein kinase, in radiation resistance and double strand break repair in *D. radiodurans* R1. We therefore propose the strong possibility that there is a DNA damage-induced signaling mechanism and that it is significant for DNA recombination/repair and cell growth of this bacterium. The identification of a protein kinase(s) and the mechanism of protein phosphorylation contributing to double strand break repair and radiation resistance in bacteria would be interesting to investigate separately.

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