Molecular Microbiology (2006) **59**(4), 1308–1316
doi:10.1111/j.1365-2958.2005.05005.x
First published online 12 December 2005

An exonuclease I-sensitive DNA repair pathway in *Deinococcus radiodurans*: a major determinant of radiation resistance

Hari S. Misra,* Nivedita P. Khairnar, Swathi Kota, Smriti Shrivastava, Vasudha P. Joshi and Shree K. Apte
Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai- 400 085, India.

Summary

Deinococcus radiodurans **R1 recovering from acute dose of γ radiation shows a biphasic mechanism of DNA double-strand break repair. The possible involvement of microsequence homology-dependent, or non-homologous end joining type mechanisms during initial period followed by RecA-dependent homologous recombination pathways has been suggested for the reconstruction of complete genomes in this microbe. We have exploited the known roles of exonuclease I in DNA recombination to elucidate the nature of recombination involved in DNA double-strand break repair during post-irradiation recovery of *D. radiodurans*. Transgenic *Deinococcus* cells expressing exonuclease I functions of *Escherichia coli* showed significant reduction in γ radiation radioresistance, while the resistance to far-UV and hydrogen peroxide remained unaffected. The overexpression of *E. coli* exonuclease I in *Deinococcus* inhibited DNA double-strand break repair. Such cells exhibited normal post-irradiation expression kinetics of RecA, PprA and single-stranded DNA-binding proteins but lacked the divalent cation manganese [(Mn(II)]-dependent protection from γ radiation. The results strongly suggest that 3′ (ρ) 5′ single-stranded DNA ends constitute an important component in recombination pathway involved in DNA double-strand break repair and that absence of *sbcB* from deinococcal genome may significantly aid its extreme radioresistance phenotype.**

Introduction

Deinococcus radiodurans exhibits extraordinary tolerance to several abiotic stresses including high doses of ionizing and non-ionizing radiations (Minton, 1994). Radioresistance phenotype of *D. radiodurans* is largely contributed by: (i) the unique responses of this organism to oxidative stress (Markillie *et al*., 1999), (ii) removal of modified or oxidized nucleotide bases by NUDIX hydrolases (Bessman *et al*., 1996) and (iii) the presence of a highly efficient DNA double-strand break (DSB) repair (Daly *et al*., 1994) in addition to several other supportive mechanisms (reviewed in Makarova *et al*., 2001). Novel antioxidants and protein recycling phenomena have recently been implicated in deinococcal radioresistance (Khairnar *et al*., 2003; Joshi *et al*., 2004; Misra *et al*., 2004).

Cells recovering from the effect of high doses of ionizing radiations show biphasic kinetics of DSB repair. The phase I, which is RecA-independent, precedes subsequent to RecA-dependent mechanisms of DSB repair (Daly and Minton, 1996). Loss of ionizing radiation resistance in *D. radiodurans* R1 was seen when certain important housekeeping genes such as *recA* (Gutman *et al*., 1994) and *polA* (Gutman *et al*., 1993) and a few unique genes like *pprI* (*irrE*) (Hua *et al*., 2003), *pprA* (Narumi *et al*., 2004) and polymerase X (*polX*) (Lecointe *et al*., 2004) were mutated. Narumi *et al*. (2004) have suggested that *pprA*, a pleiotropic DNA damage repair protein, might be important in early phase of DSB repair. Disruption of *polX* gene in *D. radiodurans* R1 showed a delayed kinetics of DSB repair and its role in RecA-independent phase has been indicated (Lecointe *et al*., 2004). However, the DNA repair pathway(s) through which these proteins contribute to DSB repair have remained largely unknown.

Different components and their activities in DNA recombination have been studied in *Escherichia coli* and reviewed (Mahajan and Datta, 1979; Clark, 1991; Kowalczykowski *et al*., 1994). Experimental evidence on the survival of *recBC* mutants of *E. coli* showed that these mutants were deficient in DNA recombination (RecF recombination in the absence of RecBC recombination pathways) and hence failed to form colonies, unless they acquired mutations in three loci namely *sbcA* or a combination of *sbcB* and *sbcC/sbcD* (Lloyd and Buckman, 1988; Clark and Sandler, 1994). The effect of *sbcB*, encoding SbcB protein, was attributed to the inhibition of RecF pathways of recombination (Clark, 1991) which was restored by the overexpression of bacteriophage λ exonuclease as shown through independent studies

Accepted 14 November, 2005. *For correspondence. E-mail hsmisra@barc.ernet.in; Tel. (+91) 22 2559 2806; Fax (+91) 22 2550 5151.

(Armengod, 1981). Additionally, in *E. coli*, overexpression of SbcB enzyme (hereafter referred to as ExoI) has been shown to suppress both illegitimate recombination (both RecE-dependent and RecE-independent) and RecF recombination involving either microsequence homologous or non-homologous single-stranded DNA molecules (Clark, 1991; Shimizu *et al*., 1997; Yamaguchi *et al*., 2000).

Both the *in vivo* (Wang and Smith, 1983) and *in vitro* (Kowalczykowski *et al*., 1994; Anderson and Kowalczykowski, 1997) studies have shown that RecBC pathway repairs DNA DSBs while RecF pathway repairs DNA by assimilation of single-stranded gapped DNA molecules in *E. coli*. The absence of *E. coli* RecBCD-like recombinase (ExoV), RecE homologue and ExoI in *D. radiodurans* R1 genome (White *et al*., 1999) may permit RecE-independent illegitimate and/or RecF recombination pathways in DSB repair to a greater extent. Under such genetic background, it would be worth investigating the effect of ExoI of *E. coli* on radiation resistance phenotype of this bacterium. In the present study we have cloned the *E. coli sbcB* gene in a shuttle expression vector under the control of deinococcal *groESL* promoter. Transgenic *D. radiodurans* R1 cells expressing ExoI showed inhibition of DSB repair and loss of γ radiation resistance while continuing to exhibit tolerance to hydrogen peroxide and far-UV (FUV), similar to wild type. These cells also lacked the divalent cation manganese [(Mn(II)]-dependent protection from γ radiation effects. Our data suggest that repair of ionizing radiation induced DNA strand breaks is regulated by a recombination pathway that is sensitive to 3′ → 5′ single-stranded DNA exonuclease activity of ExoI. Such ExoI sensitivity of DSB repair is unaffected by the presence of normal levels of RecA, PprA and single-stranded DNA-binding (SSB) proteins.

Results

ExoI expression does not affect growth of D. radiodurans *R1*

The role of the *sbcB* gene, which encodes the inhibitor of illegitimate and/or RecF recombination processes in *E. coli* (Clark, 1991), in DSB repair in *Deinococcus* was investigated. A 1434 bp DNA fragment carrying *sbcB* gene was polymerase chain reaction (PCR) amplified from wild-type strain of *E. coli* and PCR product was sequenced to ascertain the correctness of the *sbcB* gene and absence of any mutation incorporated during PCR cycles. The *sbcB* gene was cloned at ApaI and XbaI in a shuttle expression vector, pRADgro (Fig. 1A), which was constructed by introducing a 264 bp fragment (Fig. 1B) of *Deinococcus* genomic DNA having constitutively expressing *groESL* promoter and translation signals of *groE* gene, into pRAD1. The recombinant plasmid, called pGrosbcB (Fig. 1C), when restriction digested with *ApaI* and XbaI, released the ~1.4 kb ApaI–XbaI fragment (Fig. 1D). The pGrosbcB was used to transform *D. radiodurans* strains R1 and the expression of recombinant protein under the control of *PgroESL* was ascertained on SDS-PAGE. Figure 2 shows the expression of an expected size protein of ~55 kDa. *Deinococcus* cells expressing recombinant SbcB protein showed normal growth characteristics similar to wild type (Fig. 3). This showed that expression of recombinant ExoI in RecBC minus and SbcC/SbcD plus background in *D. radiodurans* did not affect its viability, as has earlier been reported in the case of *E. coli*.

Effect of ExoI on response to radiation stress

Deinococcus radiodurans cells transformed with pRADgro and pGrosbcB were grown in rich medium containing chloramphenicol (3 μg ml^{-1}). The late-log phase cells were exposed to different doses of γ and FUV radiations and cell survival was monitored on rich medium containing chloramphenicol (3 μg ml^{-1}). The recombinant cells showed differential responses to ionizing (Fig. 4A) and non-ionizing radiations (Fig. 4B). Cells expressing ExoI were marginally sensitive to high doses (> 1.0 kJ m^{-2}) of non-ionizing radiation (Fig. 4B) but showed major (> 2 log cycles) loss of resistance to ionizing radiation (Fig. 4A). Compared with wild type (D_{10} = 12 kGy ^{60}CO γ-rays), the D_{10} value for transgenic cells decreased to 2 kGy for γ-rays. *D. radiodurans* strain R1 expressing ExoI showed H_2O_2 tolerance similar to wild-type *Deinococcus* cells (Fig. 4C).

Mn(II) protection of ExoI-expressing Deinococcus *cells from γ radiation damage*

Recently, Mn(II) has been shown to protect *D. radiodurans* R1 from γ radiation damage (Daly *et al*., 2004). Mn(II)-grown *D. radiodurans* cells expressing ExoI were as sensitive to γ radiation as the untreated cells (Fig. 5). However, wild-type cells showed a Mn(II)-dependent protection at higher doses of γ irradiation. The effect of Mn(II) on characteristics such as the frequency of single cells, diplococci and tetrad population were similar to that of wild type (H.S. Misra, unpublished). This indicates that the mechanism(s) by which manganese enhances the radiation tolerance in wild-type cells are not sufficient to protect ExoI-expressing *Deinococcus* cells from γ radiation damage.

Role of RecA, PprA and SSB protein in radioresistance of ExoI-expressing D. radiodurans

Loss of radiation resistance in *D. radiodurans* R1 has been shown when important single genes, such as *recA*

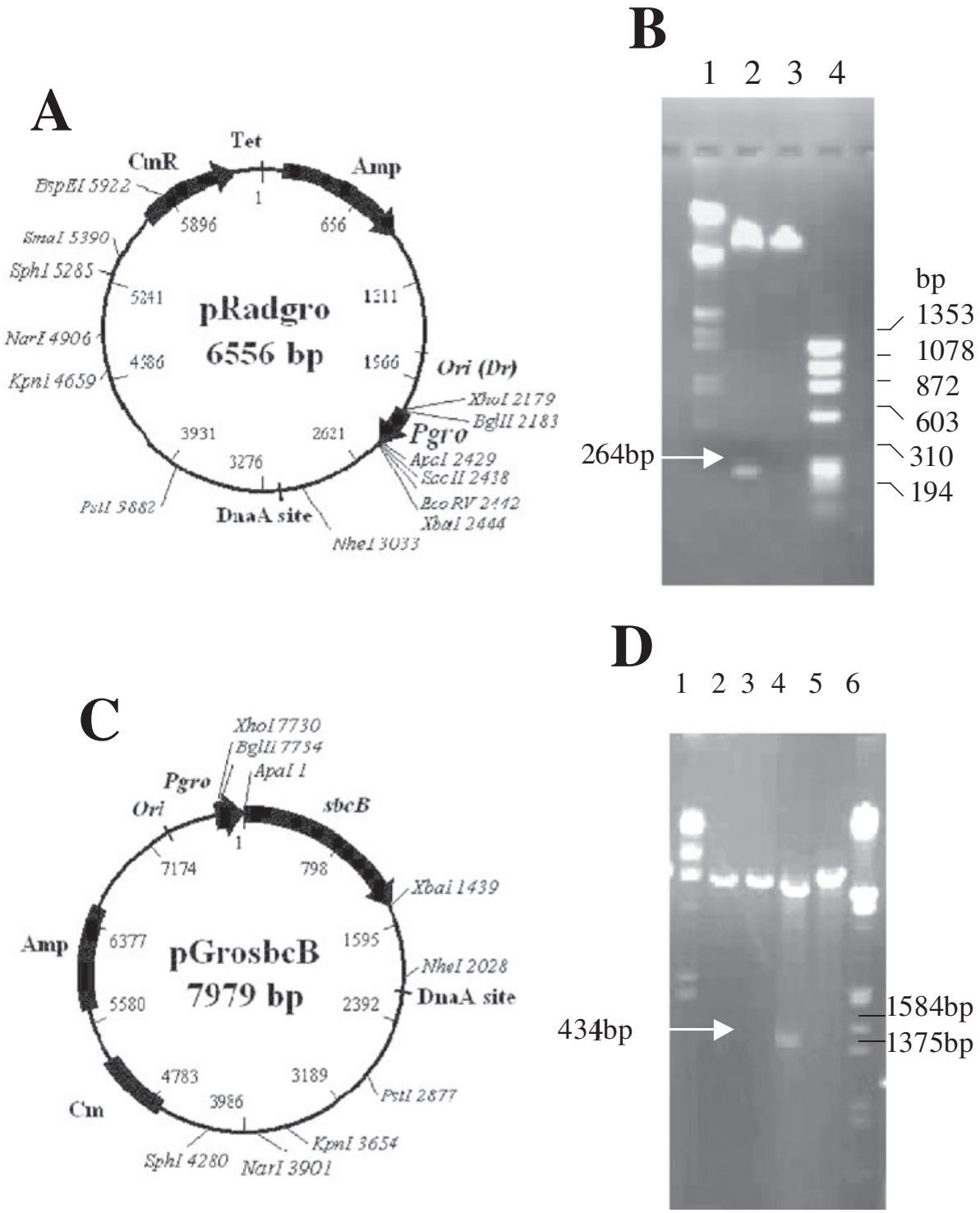

Fig. 1. Construction of expression plasmid having *sbcB* gene from *Escherichia coli* under the control of deinococcal *groESL* promoter.
A. Partial restriction map of pRADgro.
B. Agarose gel electrophoretic analysis of pRADgro plasmid DNA digested with BglII and XbaI. An expected size 264 bp restriction fragment (lane 2) containing necessary signal for the expression of transgene(s) in *Deinococcus radiodurans* was estimated by comparing with DNA size marker ϕX174 digest of HaeIII (lane 4) and λ DNA digest of HindIII/EcoRI (lane 1). Lane 3 shows pRAD1 linearized with BglII.
C. Partial restriction map of recombinant plasmid pGrosbcB containing *E. coli sbcB* gene under the control of deinococcal *PgroESL*. The 1434 bp coding sequence of *sbcB* was PCR amplified and cloned at ApaI and XbaI sites in pRADgro.
D. Restriction digestion analysis of pGrosbcB with ApaI and XbaI showing the release of 1.434 kb insert from the recombinant plasmid. Lanes 1: λ DNA digested with HindIII; 2: pRADgro linearized with BglII; 3: pGrosbcB digested with ApaI; 4: pGrosbcB digested with ApaI and XbaI; 5: pGrosbcB digested with XbaI; and 6: λ DNA digest of HindIII/EcoRI.

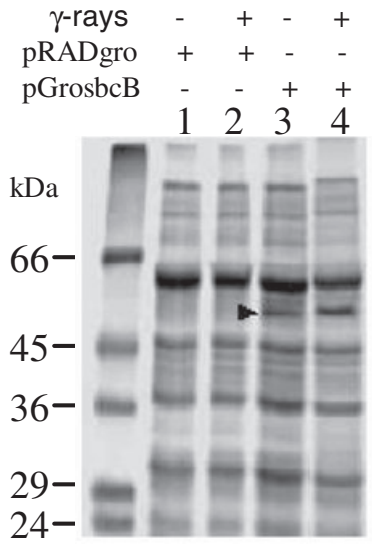

Fig. 2. Expression of recombinant SbcB protein in *Deinococcus radiodurans*. *D. radiodurans* R1 cells (1–4) harbouring pRADgro or pGrosbcB, as indicated, were irradiated with 2 kGy (^{60}CO γ-rays) radiation and allowed to recover for 4 h. Cells were lysed by boiling in Laemmli's sample buffer and soluble proteins in clear cell-free extract were separated on 5–14% gradient SDS-PAGE. Approximately 55 kDa polypeptide appeared only in clones harbouring pGrosbcB, which gets induced in response to irradiation.

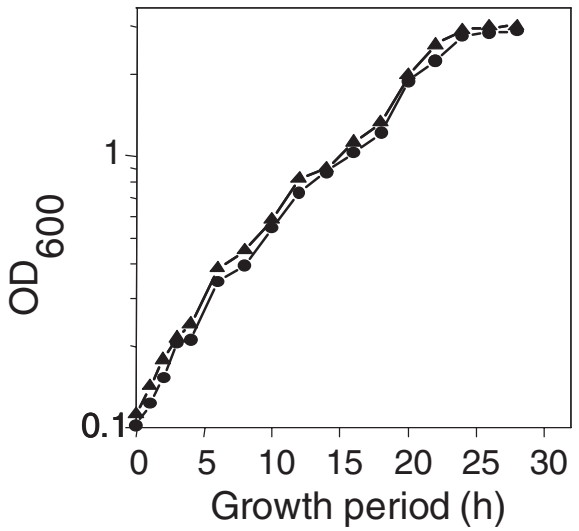

Fig. 3. Growth characteristics of *Deinococcus* expressing exonuclease I from *E. coli*. *Deinococcus radiodurans* R1 harbouring pRADgro (●) or pGrosbcB (▲) were grown in the presence of chloramphenicol (3 μg ml^{-1}) overnight and then subcultured in fresh TYG broth containing chloramphenicol. The turbidity was measured at 600 nm at regular interval.

(Gutman *et al*., 1994), *pprA* (Narumi *et al*., 2004), *pprI* (Hua *et al*., 2003) or *polA* (Gutman *et al*., 1993) were mutated. SSB protein is an essential protein in all organisms and is involved in DNA replication, recombination and repair leading to the survival of the cells. Possible involvement of RecA, PprA and SSB proteins in the observed radiosensitivity of ExoI-expressing cells was investigated in term of the levels of these proteins in cells recovering from radiation effect. Both ExoI-expressing transgenic cells and wild-type cells showed nearly similar

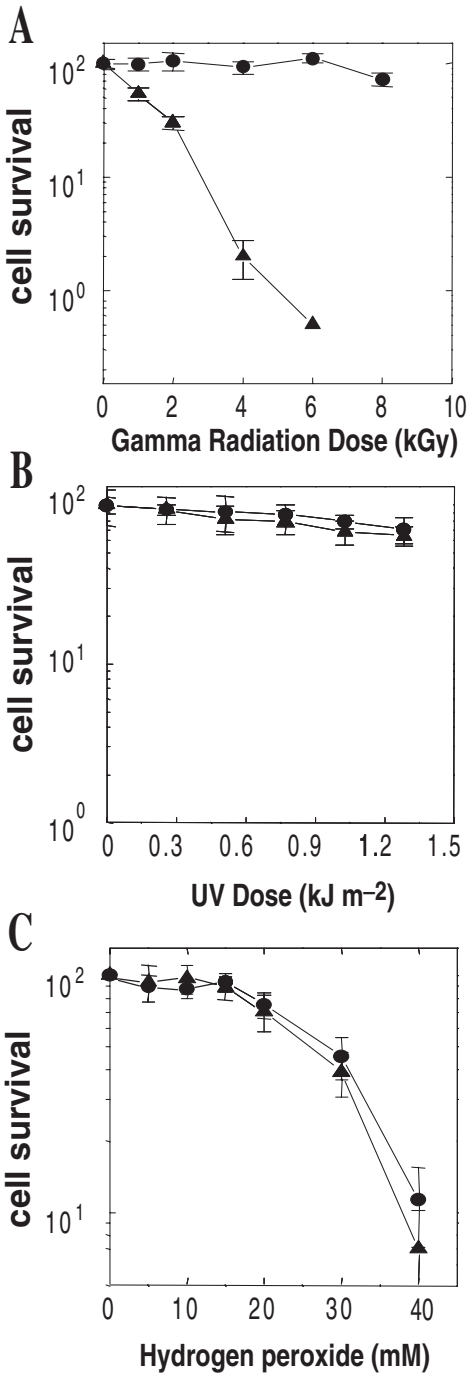

Fig. 4. Differential response of *Deinococcus radiodurans* expressing exonuclease I to radiations and oxidative stress. Cells containing shuttle vector, pRADgro, were used as control (●) and compared with exonuclease I-expressing *Deinococcus* cells (▲). Exonuclease I-expressing cells showed loss of resistance to γ radiation when compared with control (A) while no effect was seen against far-UV (B) or hydrogen peroxide (C).

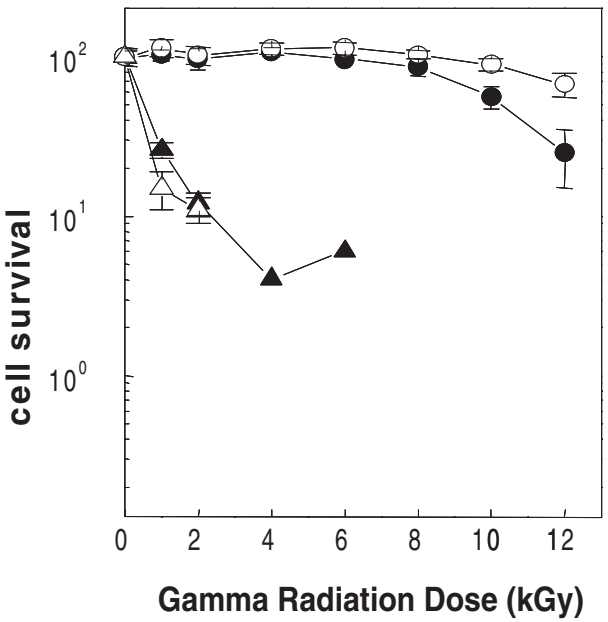

Fig. 5. Effect of Mn(II) on the γ radiation response of *Deinococcus radiodurans* expressing ExoI from *E. coli*. *D. radiodurans* R1 harbouring pRADgro (●) or pGrosbcB (▲) were grown in the presence (○ and △) of 2.5 μM $MnCl_2$ and exposed to different doses of γ radiation. Irradiated cells were diluted appropriately and plated on TYG agar containing chloramphenicol (3 μg ml^{-1}) and colony-forming units were estimated after 36 h of incubation at 32°C.

typical post-irradiation expression kinetics of RecA, PprA and SSB proteins (Fig. 6). This suggests that the ExoI-dependent loss of γ radiation tolerance in *Deinococcus* relates to a pathway where ExoI does not involve expression of these proteins.

Effect of ExoI on the kinetics of DNA DSB repair

To understand the effect of ExoI during rejoining of DSBs produced from γ irradiation of the transgenic *Deinococcus* cells, the kinetics of DSB repair was monitored using pulsed field gel electrophoresis. Wild-type cells showed a typical pattern (Fig. 7) of DSB repair and full-length genome was reassembled in 36 h after irradiation. In contrast, in the ExoI clone the damaged DNA was not repaired and DNA fragments persisted for several hours after irradiation. This suggested that ExoI activity that removes 3′ → 5′ single-stranded DNA ends, either generated from radiation effect or tailored by 5′ → 3′ exonuclease functions of recombination proteins, inhibited the rejoining of DNA fragments (DSBs) by homologous recombination. It is also noteworthy that γ-ray-triggered DNA strand breaks were otherwise fairly protected from nucleolytic degradation till 24 h after irradiation.

Discussion

Role of exonuclease I in DNA recombination has been studied in *E. coli* where it was observed that the cells lacking active RecBC must undergo mutation in *sbcB*, *sbcC* and *sbcD* genes to survive under DNA-damaging conditions. *D. radiodurans* R1 naturally lacks the *recBC* but contains as yet uncharacterized *sbcC*/*sbcD* genes and the expression of SbcB protein in this background did not influence the normal growth characteristics of the bacterium (Fig. 3). Through mutational studies in *E. coli*, the genetic dependence of *sbcB* and *sbcC/sbcD* genes and

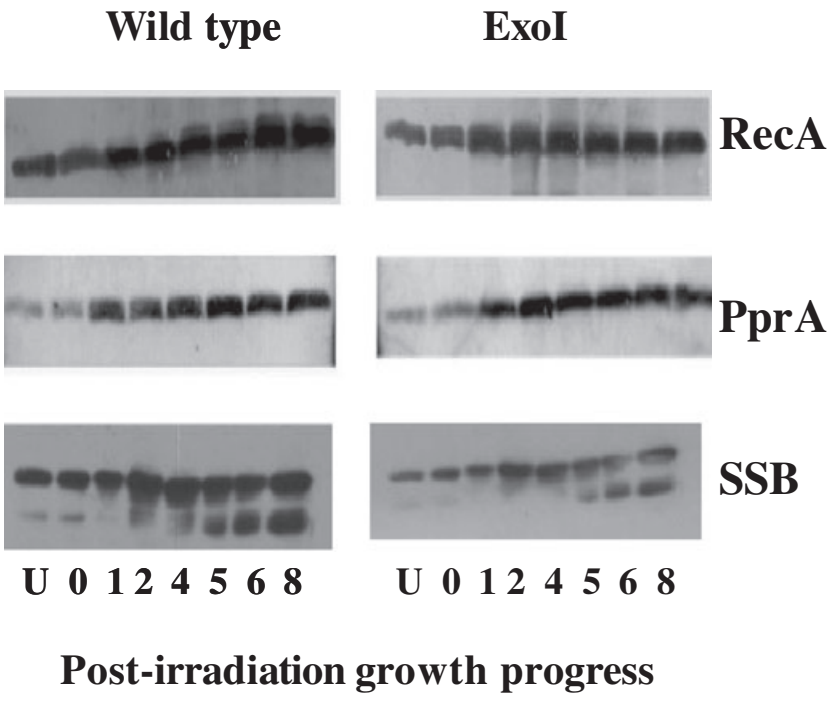

Fig. 6. Expression kinetics of RecA, PprA and single-stranded DNA-binding (SSB) proteins in the wild type and ExoI-expressing *Deinococcus radiodurans* R1 cells. Logarithmically growing transgenic cells (U) were irradiated with 3 kGy of γ-rays and allowed to grow at 32°C. Aliquots were drawn at different time intervals (0, 1, 2, 4, 5, 6, 8 h) after irradiation and total proteins were immunoblotted and cross-reacted with antibodies against RecA, PprA or SSB protein. All lanes contain equal amount of protein (10 μg).

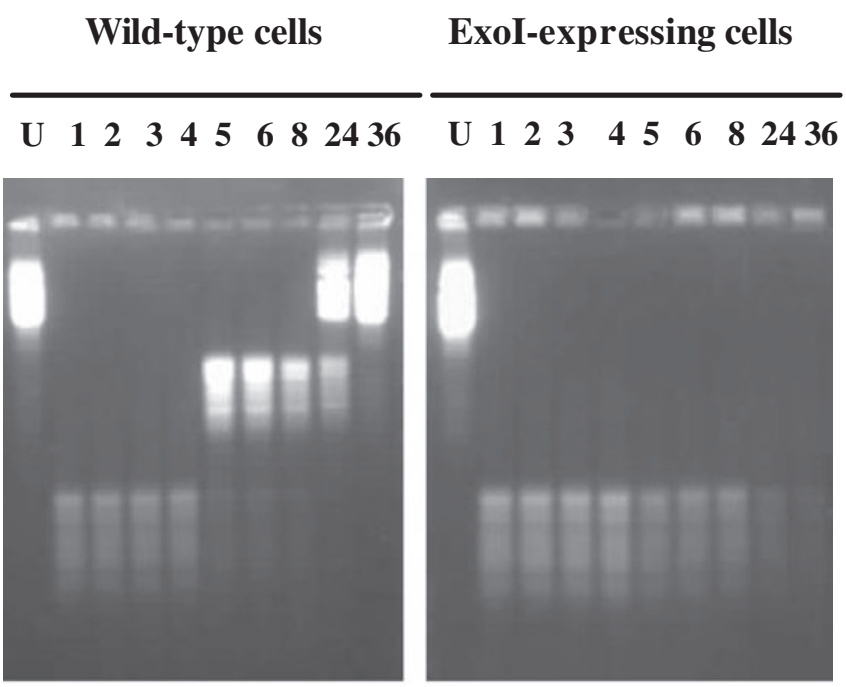

Fig. 7. Differential kinetics of DNA double-strand break repair in the wild type and ExoI-expressing cells of *Deinococcus radiodurans* R1. Logarithmically growing *Deinococcus* cells (U) were irradiated with 3 kGy of γ-rays and allowed to recover from radiation effect. Aliquots were drawn at different time intervals (0, 1, 2, 3, 4, 5, 6, 8, 24, 36 h) and extent of DNA strand breaks or their repair were monitored by pulsed field gel electrophoresis.

their interaction in the survival of *recBC* minus cells have been demonstrated (Clark, 1991).

Effect of SbcB protein on radioresistance phenotype of transgenic *Deinococcus* was examined to evaluate the possible existence of an ExoI-regulated recombination pathway as reported in *E. coli* and to ascertain whether it has a role in DSB repair and radiation tolerance phenotype of this bacterium. ExoI-expressing *Deinococcus* cells showed loss of resistance to γ radiation (Fig. 4) while no effect was observed with FUV radiation or H_2O_2. Loss of γ radiation resistance appeared to be correlated with the inability of such cells to mend DNA DSBs (Fig. 6) during post-irradiation survival. This suggests that ExoI expression in *Deinococcus* inhibits the repair of γ-ray-induced DNA damage but has no effect on the repair of FUV-damaged DNA.

Inhibition of DSB repair was earlier reported in many radiosensitive mutants of *Deinococcus* having mutation in genes such as *recA*, *pprA*, etc. ExoI-expressing *Deinococcus* cells having normal levels of such proteins (Fig. 5) still showed loss of radiation tolerance and inhibition in DSB repair. Although these results are consistent with the action of ExoI before the actions of RecA and SSB, they are also consistent with a subsequent action. That is not the case with PprA, however, as this protein stimulates DNA ligase activity, which is thought to be the final step in recombination. Differential effect of ExoI on normal and ionizing radiation-stressed growth conditions could be accounted for the contribution of ExoI sensitive recombination pathway in restructuring of its genome from DSBs. Such effect might have been less pronounced under normal conditions and have escaped the lethality due to the presence of multiple copies of housekeeping genes on multiple genomes.

Molecular mechanism of ExoI action has not been demonstrated *in vitro*. However, Yamaguchi *et al*. (2000) have demonstrated that 3′ → 5′ exonuclease activity of wild-type ExoI inhibits illegitimate recombination activated by 5′ → 3′ exonuclease function of RecE/RecT. Mythili and Muniyappa (1993) have shown that overexpression of lambda phage exonuclease (*red*-system) reverts the inhibitory effect of ExoI on plasmid multimerization, a characteristic of RecF recombination. The lambda integration in host genome was sensitive to the mutation in RecF recombination gene and to the presence of wild-type copy of *sbcB* (Clark, 1991) in *recBC* strain of *E. coli.* Furthermore, it has been demonstrated that null mutations in *sbcB* and *sbcC* genes allow RecF-mediated repair of arrested replication fork in *rep recBC* mutant of *E. coli* (Bidnenko *et al*., 1999). Thus, ExoI activity appears to regulate the recombination pathways that involve the assimilation of either short region or long gapped region of single-stranded DNA molecules. *Deinococcus* genome does not encode the *E. coli* homologues for RecE and RecBC recombination pathways. Therefore, the radiosensitivity caused by overexpression of ExoI in *Deinococcus* strongly suggest that RecF-like pathway may be a major component in DSB repair that controls radiation tolerance of this bacterium. *D. radiodurans* R1 genome does contain majority of the components of RecF recombination pathway (White *et al*., 1999).

DNA recombination repair mechanisms appear to be

critical for DSB repair to restore genome integrity during post-irradiation recovery in *Deinococcus*. The present study has brought forth some interesting facts and provides evidence, although indirect, for the first time for the involvement of 3′ → 5′ single-stranded DNA ends in efficient DSB repair and radiation tolerance in *Deinococcus*. It further emphasizes the need to identify other candidate recombination genes involved in DSB repair which function either by interacting with SbcB protein or by working in tandem independently. This study also shows that the recombination repair of DNA damaged from ionizing radiation follows a different mechanism from UV-damaged DNA repair, which would be interesting to study in an organism that lacks photoreactivation and SOS recombination repair. Furthermore, the persistence of DNA fragments in ExoI cells without their assimilation to higher size even considerable time after irradiation and their mechanism of protection would be of great interest to investigate subsequently. Last, but not least, in the loss of ExoI function, *Deinococcus* appears to have gained immensely in its radioresistance.

Experimental procedures

Bacterial strains

Deinococcus radiodurans strain R1 was a generous gift from Dr M. Schafer (Schafer *et al*., 2000). The strain R1 and its derivatives were grown aerobically in TYG (0.5 Bacto Tryptone, 0.3% Bacto Yeast Extract, 0.1% Glucose) in the presence of chloramphenicol (3 μg ml^{-1}) at 32°C. Construction of shuttle expression vector was carried out in *E. coli* strain HB101, which was maintained in our laboratory under standard laboratory conditions.

Construction of expression plasmid

Genomic DNA of *D. radiodurans* was prepared as described previously (Battista *et al*., 2001). DNA fragment from chromosome 1 (Accession No. NC001263) of *D. radiodurans* R1 from base pair position 617641–617878 containing a functional P*groESL* promoter sequence along with ribosome binding site (RBS) and first three codons of *groE* gene of *D. radiodurans* R1 were PCR amplified using 'Bgl' primer (5′-GAAGATCTGTATTGTCGCCCTAC-3′) and 'Apa' primer (5′-CGCCCATGGGCCCTTTCAGCATGTGGGGT-3′). ApaI site was incorporated in 'Apa' primer at the 5′ end followed by RBS and first three codons of *groESL* operon. PCR amplification was carried out at optimum conditions and PCR product obtained was used as template for second PCR for the incorporation of other restriction enzyme sites SacII, EcoRV and XbaI through 'Xba' primer 5′-GCTCTAGATATCCGCG GCCATGGGCCCTTT-3′) and 'Bgl' primer. The 264 bp PCR-amplified product was sequenced to ascertain the modifications, if any, and cloned at the compatible sites in pRAD1 (Meima and Lidstrom, 2000), yielding pRADgro (Fig. 1). The 1434 bp coding sequence of *sbcB* was PCR amplified from wild-type *E. coli* strain W3110, using gene-specific primers (DR6F, 5′-GTGGGCCCATGATGAATGACGGT-3′ and DR6R, 5′-GCTCTAGATTAGAGAATCTCTTCCGCGTA-3′) and cloned at ApaI and XbaI sites in pRADgro. The recombinant plasmid was named as pGrosbcB (Fig. 2) and transformed into *D. radiodurans* as described earlier (Udupa *et al*., 1994) and chloramphenicol-resistant clones were isolated on TYG agar plates containing chloramphenicol (5 μg ml^{-1}). These clones were characterized for the presence of pGrosbcB and used for monitoring the expression of the SbcB in *D. radiodurans* strains R1.

Molecular studies

Immunoblotting and immunodetection of total proteins of *Deinococcus* recovering from γ radiation effect was carried out using antibodies against RecA (Karthikeyan *et al*., 1999), deinococcal SSB (A. Alahari and S.K. Apte, unpublished) and PprA (Narumi *et al*., 2004). Equal amount (10 μg) of protein was separated on SDS-PAGE and transferred onto PVDF membrane (Millipore) and incubated with antiserum against RecA (1:15 000), SSBs (1:10 000) and PprA (1:20 000) and allowed to hybridized for 18 h as described earlier (Misra *et al*., 2003). The blots were washed and incubated with horseradish peroxidase-conjugated rabbit IgG antiserum (Bangalore Genie, India). Chemiluminescent signals on the PVDF membrane were visualized using 'Lumi-Light Western Blotting Substrate' (Roche Applied Sciences) following manufacturer's protocols. Pulsed field gel electrophoresis was carried out as described earlier (Mattimore and Battista, 1996).

Radiation and oxidative stress studies

Deinococcus radiodurans cells harbouring pGrosbcB and pRADgro were grown till late-log phase at 30°C in TYG broth supplemented with chloramphenicol (3 μg ml^{-1}) with 2.5 μM $MnCl_2$ when required. These cells were suspended in sterile phosphate-buffered saline (PBS) and treated with different doses of γ radiation on ice using Cobalt 60 (4.87 kGy h^{-1}) irradiator. The cells were appropriately diluted with normal saline and plated on TYG agar plate containing chloramphenicol (5 μg ml^{-1}) and colony-forming units were counted after 48 h of incubation. For UV effects, late-log phase cells of wild type and SbcB clones were serially diluted with normal saline and plated on TYG agar plates containing chloramphenicol (5 μg ml^{-1}). Sets were prepared in triplicates and exposed to different doses of UV radiation at 254 nm. The plates were wrapped with aluminium foil and incubated at 32°C for 36 h before the number of colonies that appeared on plates was recorded. The effect of hydrogen peroxide on cell survival was studied as described earlier (Yun and Lee, 2000).

Acknowledgements

The authors are thankful to Dr Issay Narumi (JAERI, Japan) for providing PprA antibodies, Professor B.J. Rao (TIFR, Mumbai) for providing RecA antibodies, Dr Anuradha Alahari (BARC, Mumbai) for providing SSB antibodies and Dr M. Schafer for a kind gift of *D. radiodurans* R1. We express our

sincere thanks to Dr S.K. Mahajan and Professor B.J. Rao for their comments on data analysis and other discussion in the preparation of this article.

References

Anderson, D.G., and Kowalczykowski, S.C. (1997) The translocating RecBCD enzyme stimulates recombination by directing RecA protein onto ssDNA in a chi-regulated manner. *Cell* **90:** 77–86.

Armengod, M.E. (1981) Role of the *recF* gene of *Escherichia coli* K-12 in lambda recombination. *Mol Gen Genet* **18:** 497–504.

Battista, J.R., Park, M.J., and McLemore, A.E. (2001) Inactivation of two homologous proteins presumed to be involved in the desiccation tolerance of plants sensitizes *Deinococcus radiodurans* R1 to desiccation. *Cryobiology* **43:** 133–139.

Bessman, M.J., Frick, D.N., and O'Handley, S.F. (1996) The MutT proteins or 'Nudix' hydrolases, a family of versatile, widely distributed, 'housecleaning' enzymes. *J Biol Chem* **271:** 25059–25062.

Bidnenko, V., Seigneur, M., Penel-Colin, M., Bouton, M.-F., Ehrlich, S.D., and Michel, B. (1999) *sbcB sbcC* null mutations allow RecF-mediated repair of arrested replication forks in *rep recBC* mutants. *Mol Microbiol* **33:** 846–857.

Clark, A.J. (1991) *rec* genes and homologous recombination proteins in *Escherichia coli*. *Biochimie* **73:** 523–532.

Clark, A.J., and Sandler, S.J. (1994) Homologous genetic recombination: the pieces begin to fall into place. *Crit Rev Microbiol* **20:** 125–142.

Daly, M.J., Gaidamakova, E.K., Matrosova, V.Y., Vasilenko, A., Zhai, M., Venkateswaran, A., *et al.* (2004) Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates γ-radiation resistance. *Science* **306:** 1025–1028.

Daly, M.J., and Minton, K.W. (1996) An alternative pathway of recombination of chromosomal fragments precedes *recA*-dependent recombination in the radioresistant bacterium *Deinococcus radiodurans*. *J Bacteriol* **178:** 4461–4471.

Daly, M.J., Ouyang, L., Fuchs, P., and Minton, K.W. (1994) *In vivo* damage and recA-dependent repair of plasmid and chromosomal DNA in the radiation-resistant bacterium *Deinococcus radiodurans*. *J Bacteriol* **176:** 3508–3517.

Gutman, P.D., Fuchs, P., Ouyang, L., and Minton, K.W. (1993) Identification, sequencing, and targeted mutagenesis of a DNA polymerase gene required for the extreme radioresistance of *Deinococcus radiodurans*. *J Bacteriol* **175:** 3581–3590.

Gutman, P.D., Carroll, J.D., Masters, C.I., and Minton, K.W. (1994) Sequencing, targeted mutagenesis and expression of a *recA* gene required for the extreme radioresistance of *Deinococcus radiodurans*. *Gene* **141:** 31–37.

Hua, Y., Narumi, I., Gao, G., Tian, B., Satoh, K., Kitayama, S., and Shen, B. (2003) Pprl: a general switch responsible for extreme radioresistance of *Deinococcus radiodurans*. *Biochem Biophys Res Commun* **306:** 354–360.

Joshi, B., Schmid, R., Altendorf, K., and Apte, S.K. (2004) Protein recycling is a major component of post-irradiation recovery in *Deinococcus radiodurans* strain R1. *Biochem Biophys Res Commun* **320:** 1112–1117.

Karthikeyan, G., Lakshmikant, G.S., Wagle, M.D., Krishnamuthy, G., and Rao, B.J. (1999) RecA interacts with Klenow and enhances fidelity of DNA synthesis *in vitro*. *J Mol Microbiol Biotechnol* **1:** 149–156.

Khairnar, N.P., Misra, H.S., and Apte, S.K. (2003) Pyrroloquinoline-quinone synthesized in *Escherichia coli* by pyrroloquinoline-quinone synthase of *Deinococcus radiodurans* plays a role beyond mineral phosphate solubilization. *Biochem Biophys Res Commun* **312:** 303–308.

Kowalczykowski, S.C., Dixon, D.A., Eggleston, A.K., Lauder, S.D., and Rehrauer, W.M. (1994) Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol Rev* **58:** 401–465.

Lecointe, F., Shevelev, I.V., Bailone, A., Sommer, S., and Hubscher, U. (2004) Involvement of an X family DNA polymerase in double-stranded break repair in the radioresistant organism *Deinococcus radiodurans*. *Mol Microbiol* **53:** 1721–1730.

Lloyd, R.G., and Buckman, C. (1988) Identification and genetic analysis of *sbcC* mutations in commonly used *recBC*, *sbcB* strains of *Escherichia coli* K12. *J Bacteriol* **164:** 836–844.

Mahajan, S.K., and Datta, A.R. (1979) Mechanism of recombination by the RecBC and the RecF pathways following conjugation in *Escherichia coli* K-12. *Mol Gen Genet* **169:** 67–78.

Makarova, K.S., Aravind, L., Wolf, Y.I., Tatusov, R.L., Minton, K.W., Koonin, E.V., and Daly, M.J. (2001) Genome of extremely radiation resistant bacterium, *Deinococcus radiodurans* viewed from the perspectives of comparative genomics. *Microbiol Mol Biol Rev* **65:** 45–79.

Markillie, L.M., Varnum, S.M., Hradechy, P., and Wong, K.K. (1999) Targeted mutagenesis by duplication insertion in the radioresistant bacterium *Deinococcus radiodurans*: radiation sensitivities of catalase (*katA*) and superoxide dismutase (*sodA*) mutants. *J Bacteriol* **181:** 666–669.

Mattimore, V., and Battista, J.R. (1996) Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *J Bacteriol* **178:** 633–637.

Meima, R., and Lidstrom, M.E. (2000) Characterization of the minimal replicon of a cryptic *Deinococcus radiodurans* SARK plasmid and development of versatile *Escherichia coli-D. radiodurans* shuttle vectors. *Appl Environ Microbiol* **66:** 3856–3867.

Minton, K.W. (1994) DNA repair in the extremely radioresistant bacterium *Deinococcus radiodurans*. *Mol Microbiol* **13:** 9–15.

Misra, H.S., Khairnar, N.P., and Mahajan, S.K. (2003) An alternate photosynthetic electron donor system for PSI supports light dependent nitrogen fixation in a non-heterocystous cyanobacterium, *Plectonema boryanum*. *J Plant Physiol* **160:** 33–39.

Misra, H.S., Khairnar, N.P., Barik, A., Indirapriyadarsini, K., Mohan, H., and Apte, S.K. (2004) Pyrroloquinoline-quinone: a reactive oxygen species scavenger in bacteria. *FEBS Lett* **578:** 26–30.

Mythili, E., and Muniyappa, K. (1993) Formation of linear plasmid multimers promoted by the phage lambda *Red-*

system in *lon* mutant of *E. coli*. *J Gen Microbiol* **139:** 2387–2397.

Narumi, I., Satoh, K., Cui, S., Funayama, T., Kitayama, S., and Watanabe, H. (2004) PprA: a novel protein from *Deinococcus radiodurans* that stimulates DNA ligation. *Mol Microbiol* **54:** 278–285.

Schafer, M., Schmitz, C., Facius, R., Horneck, G., Milow, B., Funken, K.-H., and Ortner, J. (2000) Systematic study of parameters influencing the action of Rose Bengal with visible light on bacterial cells: comparison between biological effect and singlet-oxygen production. *Photochem Photobiol* **71:** 514–523.

Shimizu, H., Yamaguchi, H., and Ashizawa, Y. (1997) Short homology-independent illegitimate recombination in *Escherichia coli*: distinct mechanism from short-homology-dependent illegitimate recombination. *J Mol Biol* **266:** 297–305.

Udupa, K.S., O'Cain, P.A., Mattimore, V., and Battista, J.R. (1994) Novel ionizing radiation sensitive mutant of *Deinococcus radiodurans*. *J Bacteriol* **176:** 7439–7446.

Wang, T.C., and Smith, K.C. (1983) Mechanisms for *recF*-dependent and *recB*-dependent pathways of postreplication repair in UV-irradiated *Escherichia coli uvrB*. *J Bacteriol* **156:** 1093–1098.

White, O., Eisen, J.A., Heildelberg, J.F., Hickey, E.K., Peterson, J.D., Dodson, R.J., *et al.* (1999) Genome sequence of the radioresistant bacterium *Deinococcus radiodurans* R1. *Science* **286:** 1571–1577.

Yamaguchi, H., Hanada, K., Asami, Y., Kato, J.-I., and Ikeda, H. (2000) Control of genetic stability in *Escherichia coli*: the SbcB 3′ → 5′ exonuclease suppresses illegitimate recombination promoted by the RecE 5′ → 3′ exonuclease. *Genes Cells* **5:** 101–109.

Yun, E.J., and Lee, Y.N. (2000) Production of two different catalase – peroxidases by *Deinococcus radiophilus*. *FEMS Microbiol Lett* **184:** 155–159.

