ATP-type DNA ligase requires other proteins for its activity in vitro and its operon components for radiation resistance in *Deinococcus radiodurans* in vivo

Swathi Kota, Vidya A. Kamble, Yogendra S. Rajpurohit, and Hari S. Misra

Abstract: A multiprotein DNA processing complex isolated from *Deinococcus radiodurans* contains the DNA repair protein PprA, an ATP-type DNA repair ligase (LigB) encoded by the *drB0100* gene, and protein kinase activity. An ATP-dependent DNA end-joining activity was detected in the complex. To elucidate the function of the *drB0100* gene, we generated the deletion mutant for the DR_B0100 ORF. The mutant exhibited a nearly 2-log cycle reduction in growth rate when exposed to a 10 000 Gray dose of γ-radiation, and a significant loss in mitomycin C and methylmethane sulphonate tolerance as compared with wild type. Functional complementation of these phenotypes required the wild-type copy of *drB0100* along with other genes such as *drb0099* and *drb0098*, organized downstream in the operon. The in vitro DNA ligase activity of LigB was stimulated severalfold by PprA in the presence of the recombinant DRB0098 protein. However, this activity did not improve when PprA was substituted with purified DRB0099 protein or when DRB0098 protein was substituted with the DRB0099 protein in the presence of PprA in solution. These results suggest that PprA and DRB0098 protein are required for LigB function. Furthermore, they also suggest that the LigB operon components contribute to radiation resistance and double-strand break (DSB) repair in *D. radiodurans*.

Key words: Deinococcus, DNA repair ligase, protein–protein interaction, radioresistance.

Résumé : Un complexe de maturation de l'ADN isolé de *Deinococcus radiodurans* comporte la protéine de réparation de l'ADN PprA, une ligase de réparation de l'ADN de type ATP (LigB) codée par le gène *drB0100*, ainsi qu'une activité protéine kinase. Une activité d'accolement des extrémités d'ADN dépendante de l'ATP a été détectée dans ce complexe. Afin d'élucider la fonction du gène *drB0100*, nous avons généré un mutant de délétion du cadre de lecture ouvert DR_B0100. Comparativement au type sauvage, le mutant montrait une réduction de presque 2 log du taux de croissance à la suite d'une exposition à une dose de radiations γ de 10 000 Gray, et une perte significative de tolérance à la mitocycine C et au méthylméthanesulfonate. La complémentation fonctionnelle de ces phénotypes nécessitait une copie sauvage du gène *drB0100* parallèlement à d'autres gènes, notamment *drb0099* et *drb0098* organisés en aval dans l'opéron. L'activité ADN ligase de LigB in vitro était stimulée de plusieurs fois par PprA en présence de DRB0098 recombinante. Cependant, cette activité n'était pas améliorée lorsque PprA était substitué par DRB0099 purifiée ou lors que DRB0098 était substituée par DRB0099 en présence de PprA en solution. Ces résultats suggèrent que PprA et DRB0098 sont requises à la fonction de LigB. De plus, ils suggèrent aussi que les composantes de l'opéron LigB contribuent à la résistance aux radiations et à la réparation des bris doubles brins chez *D. radiodurans*.

Mots-clés : Deinococcus, ligase de réparation d'ADN, interaction protéine–protéine, radiorésistance.

[Traduit par la Rédaction]

Introduction

Deinococcus radiodurans R1 survives nearly 200 double-strand breaks and 3000 single-strand breaks per genome without detectable loss of viability (Battista 2000). This phenotype is supported by an efficient biphasic double-strand break (DSB) repair (Daly and Minton 1996) and a strong oxidative stress tolerance (Markillie et al. 1999) mechanism along with other processes, including DNA protection and the removal of potential mutagenic nucleotides generated by oxidative damage of DNA, etc. (Makarova et al. 2001; Blasius et al. 2008). DSB repair is biphasic and is supported by extended synthesis dependent strand annealing (ESDSA), a major pathway in phase I (Zahradka et al. 2006). Phase II involves the slow crossover events in homologous recombination by the RecFOR pathway, leading to the circularization and maturation of individual chromosomes (Slade et al. 2009). Gamma-irradiated cells witness a very high rate of DNA synthesis as compared with non-irradiated cells. The

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involvement of both DNA polymerases, i.e., DNA polymerase A (Gutman et al. 1993) and X-family DNA repair polymerase (Lecointe et al. 2004; Khairnar and Misra 2009) in radioresistance and DSB repair has been demonstrated in *D. radiodurans*. This bacterium contains two types of DNA ligase (White et al. 1999): NAD-type bacterial ligase (LigA) and ATP-type DNA repair ligase (LigB), encoded by the *dr2069* and *drb0100* genes (Le et al. 2008), respectively; *ligB* is the first gene of the *drb0100–drb0099–drb0098* operon (i.e., the LigB operon) (Makarova et al. 2001). This operon is inducible by γ radiation and expressed as polycistronic mRNAs under the *ligB* promoter (Liu et al. 2003). Although the functional redundancy of these ligases in normal growth of this bacterium has not been studied, the indispensability of NAD-type ligase in other bacterium having multiple DNA ligases has been demonstrated (Petit and Ehrlich 2000). The ATP-type DNA ligase is an ortholog of mammalian DNA repair ligase III/IV (Aravind and Koonin 1999; Ellenberger and Tomkinson 2008). A multiprotein complex isolated from *D. radiodurans* exhibits various DNA repair functions (Kota and Misra 2008). It comprises 24 proteins, including LigB and a pleiotropic protein promoting DNA repair (PprA), and exhibits protein kinase and ATP-sensitive nuclease activity that is presumably required for DNA end-joining activity in vitro. Requirement of ATP for DNA ligase activity in a multiprotein complex containing LigB polypeptide and absence of ligase activity in purified recombinant LigB (Blasius et al. 2007) might indicate that this protein possibly interacts with other proteins similar to eukaryotic DNA repair ligases and thus expresses the DNA end-joining activity in protein complex.

Here, we have demonstrated that DNA end-joining activity of recombinant LigB requires DRB0098, another protein of the LigB operon, and PprA in solution. Unlike individual deletions of *drb0098* and *drb0099*, the LigB deletion (*ΔligB*) makes this operon non-functional and resulted in the loss of DNA damage tolerance in wild-type cells. The *ΔligB* mutant phenotype was complemented by expression of the entire LigB operon in *trans*, under the *groESL* promoter, but not by LigB alone. These results suggested that the expression of LigB activity in DNA damage tolerance requires the other host proteins, specifically PprA and DRB0098, as is shown in this study.

Materials and methods

Bacterial strains and materials

Deinococcus radiodurans ATCC13939 was a generous gift from Dr. M. Schaefer, Germany (Schäfer et al. 2000). Wild type and their respective derivatives were grown aerobically in TGY (0.5% Bacto tryptone, 0.3% Bacto yeast extract, 0.1% glucose) broth or on agar plates as required at 32 °C. Antibiotics such as chloramphenicol (5 μg·mL^{-1}) and kanamycin (8 μg·mL^{-1}) were supplemented as 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. Shuttle expression vector pRADgro and its derivatives were maintained in *Escherichia coli* HB101 as described previously by Misra et al. (2006). Other recombinant techniques used were as described in Sambrook and Russell (2002). All of the molecular biology grade chemicals including restriction enzymes and DNA modifying enzymes were purchased from the Sigma Chemical Company Roche Biochemicals, New England Biolabs, and Bangalore Genei.

Generation of *drb0100* deletion mutant derivative of *D. radiodurans*

The DNA sequences located 1 kb upstream and 1 kb downstream of the coding region of *drb0100* were amplified using 100upF (5′-GGAATTCGACGGCACGATTCAGCTGGA-3′) and 100upR (5′-AACTGCAGCAGGATCACTGCTGCGTA-3′) primers for upstream fragment, and 100dnF (5′-CGGGATCCGCAAAAACCGTACCGAACA-3′) and 100dnR (5′-GCTCTAGATCAAAG GGGTCGAGGCCT-3′) primers for downstream fragment. The upstream fragment was first cloned at *Eco*RI and *Pst*I sites in pNOKOUT (Khairnar et al. 2008) to yield pNOK100up. Subsequently, the downstream fragment was cloned at *Bam*HI–*Xba*I sites in pNOK100up to yield pNOK100 (Fig. S1).[2] The recombinant plasmid was linearized with *Sca*I and transformed into *D. radiodurans*. The transformants were scored on TGY plates containing kanamycin (Kan) (10 μg·mL^{-1}) and grown for several generations in TGY broth supplemented with Kan (8 μg·mL^{-1}) to obtain the homozygous *drb0100* deletion mutant. Homozygous replacement of *drb0100* with *nptII* in the bacterial genome was ascertained by PCR amplification using *nptII*-specific and DR81 and DR82 primers for the *drb0100* gene. Clones showing the complete absence of target gene (0.632 kb) were considered homozygous deletion mutants of *drb0100* and named *ΔligB*.

Construction of expression plasmids

Genomic DNA of *Deinococcus* was prepared as published previously in Battista et al. (2001). The 633 bp fragment of *ligB* was PCR amplified using gene-specific primers DR79 (5′-GGAATTCCATATGCGAGTCAAATACCCTTCCA-3′) and DR80 (5′-CGGGATCCTCATGACTGCTCCTGGCGCT-3′). Similarly, the 1.242 kb *drb0098* was PCR amplified using 98F (5′-GGAATTCATATGTCTGCCCTCATCTACCTC-3′) and 98R (5′-CGGATCCTTAGCCGCTCAGCTCCCCCTCCA-3′) primers. The 858 bp *drb0099* was PCR amplified using 99F (5′-GGAATTCCATATGAACCGCAAAAACCGTACCGA-3′) and 99R (5′-CGGGATCCTCAGGAGGTAGATGAGGGCAGA-3′) primers. PCR products of *drb0098*, *drb0099*, and *ligB* were cloned at *Nde*I and *Bam*HI sites in pET28a+ and the recombinant plasmids were named pETB100, pET99, and pETB98, respectively. These plasmids were individually transformed into *E. coli* BL21 (DE3) pLysS for the expression of recombinant protein.

The coding sequences of *ligB* and the LigB operon (containing all three genes) were cloned under the deinococcal–*groESL* promoter in pRADgro (Misra et al. 2006). In brief, the ~632 bp *drb0100* was PCR amplified using DR81 (5′-GTGGGCCCATGCGAGTCAAATACCCTTCCA-3′) and DR82 (5′-GCTCTAGATCATGACTGCTCCTGGCGCT-3′) primers and cloned at *Apa*I–*Xba*I sites in pRADgro to yield pGroB100 (Fig. S1).[2] Similarly, the 2704 bp LigB operon

[2] Supplementary data for this article are available on the journal Web site (http://bcb.nrc.ca).

was PCR amplified using OpeF (5′-GGAATTCCATATGCGAGTCAAATACCCTTCCA-3′) and OpeR (5′-CGGATCCTTAGCCGCTCAGCTCCCCCTCCA-3′) primers and cloned at *Nde*I–*Bam*HI sites in pRADgro to yield pGroOpe (Fig. S1).[2] Recombinant plasmids were transformed into *D. radiodurans* and *ΔligB* cells as described earlier (Meima et al. 2001). The chloramphenicol-resistant clones were isolated on TGY agar plates containing chloramphenicol (5 μg·mL^{-1}) and plasmid DNA was prepared using a modified protocol as published in Meima et al. (2001). In brief, the cell pellet was suspended in GTE (50 mmol·L^{-1} glucose, 10 mmol·L^{-1} Tris–HCl (pH 8.0), 2 mmol·L^{-1} EDTA (pH 8.0)) buffer and incubated at 37 °C for 30 min. The treated cells were subsequently treated similar to *E. coli*, for plasmid isolation using High Pure Plasmid Isolation Kit (Roche Applied Sciences, Mannheim, Germany). Plasmid DNA was digested with appropriate restriction enzymes and release of insert was ascertained on 1% agarose. The insert was sequenced to confirm the correctness of the gene.

The effect of DNA-damaging agents on cell survival

Deinococcus cells were treated with different doses of UV and γ radiations as described in Misra et al. (2006). In brief, the *ΔligB* and wild-type *D. radiodurans* cells, as well as those harboring pGroB100 and pGroOpe, were grown in TGY until the late log phase at 32 °C. These cells were suspended in sterile phosphate-buffered saline (PBS) and exposed to different doses of γ radiation at 7.2 kGy·h^{-1} (Gamma cell 5000, ^{60}Co, Board of Radiation and Isotopes Technology, DAE, India). Appropriate dilutions were plated on TGY agar plates and incubated at 32 °C. For UV effects, the cells were prepared as described above and different dilutions were plated. Cells were exposed to different doses of UV radiation at 254 nm (FUV) and incubated at 32 °C. Cells were treated with mitomycin C (MMC) as described in Keller et al. (2001) and with methylmethane sulphonate (MMS) as described in Zhang et al. (2003). In brief, the late log phase cells were treated with MMC (20 μg·mL^{-1}) and MMS (10 μg·mL^{-1}) for different time intervals. The appropriate dilutions of treated cultures were plated on TGY agar plates supplemented with Kan (8 μg·mL^{-1}) when required, and the plates were incubated at 32 °C. The colony forming units were recorded after 48 h of incubation.

Expression and purification of recombinant proteins

The recombinant proteins LigB (B100), DRB0099 (B99), DRB0098 (B98), and PprA were purified from transgenic *E. coli* BL21 (DE3) pLysS harbouring pETB100, pETB98, pET99, and pETpprA (Kota and Misra 2006) by nickel-affinity chromatography as described previously (Misra et al. 1998; Fig. 1). In brief, the clear supernatant was passed through an immobilized metal-affinity chromatography (IMAC) column and washed with 20 volumes of washing buffer (50 mmol·L^{-1} NaH_2PO_4, 300 mmol·L^{-1} NaCl, 50 mmol·L^{-1} imidazole (pH 8.0)). The proteins were eluted with a gradient of 50–500 mmol·L^{-1} imidazole in buffer containing 50 mmol·L^{-1} NaH_2PO_4 and 300 mmol·L^{-1} NaCl (pH 8.0). Fractions were analysed on 10% SDS–PAGE and the fractions containing more than 95% pure protein were pooled and dialyzed against 50 mmol·L^{-1} NaH_2PO_4, 300 mmol·L^{-1} NaCl (pH 8.0), 1 mmol·L^{-1} DTT, 1 mmol·L^{-1} PMSF, and 50% glycerol. Protein concentration was determined using Bradford's dye-binding method.

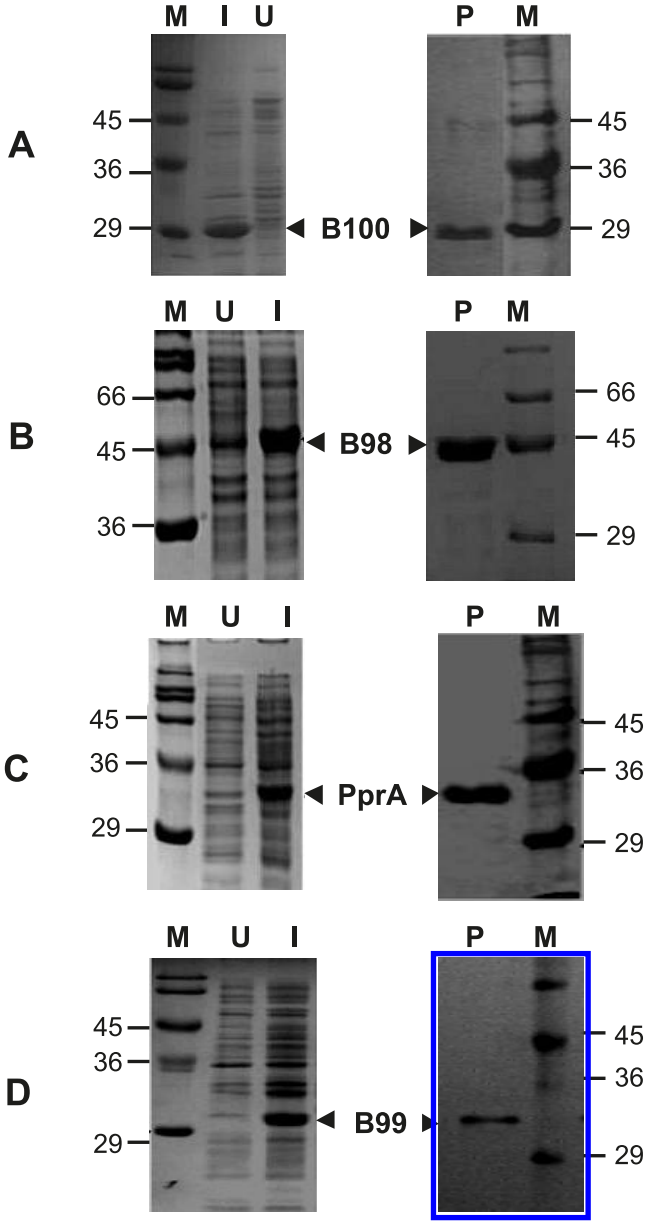


Fig. 1. Inducible expression and purification of recombinant proteins from transgenic *Escherichia coli*. The *E. coli* BL21 harboring pETB100 (A), pETB98 (B), pETPprA (C), and pETB99 (D) were induced with 200 μmol·L^{-1} IPTG (I) and total proteins were compared with uninduced control (U). The LigB (B100), DRB0099 (B99), DRB0098 (B98), and PprA (PprA) recombinant proteins were purified (P) in different fractions and sizes were estimated using molecular size markers (M) as standard.

DNA end-joining activity assay

The plasmid pET28a+ DNA was linearized with *Nde*I and pBluescript SK+ was linearized with *Eco*RI to generate cohesive ends in dsDNA substrate and DNA ligation was carried out using standard protocol (Sambrook and Russell 2002). In brief, approximately 500 ng linearized DNA was mixed with 2 μmol·L^{-1} LigB in a 10 μL reaction volume

containing 50 mmol·L^{-1} Tris–HCl, 1 mmol·L^{-1} $MgCl_2$, 5 mmol·L^{-1} ATP, 20 mmol·L^{-1} DTT, and 1% PEG. Approximately 2 µmol·L^{-1} each of DRB0098, DRB0099, and PprA were added to the ligated mixture in different combinations, as required. The reaction mixture was incubated at 17 °C overnight and subsequently heated to 65 °C in the presence of 25 mmol·L^{-1} EDTA for 15 min to dissociate the nucleoprotein complex, and products were analyzed on 1.0% agarose. The ligase activity was also assayed by monitoring the *E. coli* transformation efficiency of linear DNA incubated in the presence of these proteins and heated as described before transformation, using standard protocols (Sambrook and Russell 2002).

Results and discussion

Recombinant LigB was not active in isolated form

The recombinant DRB0099 (B99), DRB0098 (B98), PprA, and LigB (B100) of *Deinococcus radiodurans* were purified from transgenic *E. coli* expressing these proteins on multicopy plasmids. All of the proteins were purified to near homogeneity (Fig. 1) and used for subsequent studies. The recombinant LigB was checked for cohesive end DNA ligation of *Eco*RI-digested pBluescriptSK+ DNA in the presence of ATP and NAD$^+$ cofactors. The reaction mixture was heated at 65 °C for 15 min to break the DNA–protein interaction and products were analysed on 1% agarose gel. The purified recombinant protein did not show detectable DNA ligase activity with either ATP or NAD$^+$ (Fig. 2A). However, the T4 DNA ligase control showed DNA end-joining activity and the DNA products migrating both slower and faster than substrate were observed (Fig. 2A). This indicated that the recombinant LigB either lacks ligase activity or requires other components for its efficient function. This agreed with earlier results from Blasius and colleagues (2008). However, the presence of LigB as one of the components of a multiprotein complex exhibiting ATP-type DNA ligase activity indicates the possibility that LigB is active along with other proteins in the complex (Kota and Misra 2008). The complex contains PprA, which has been reported for its role in stimulation of DNA ligase (Narumi et al. 2004) activity in solution. The complex also shows a yet unidentified kinase activity in vitro. Hence, the possible requirement of PprA for DNA ligase activity of LigB is hypothesized.

Recombinant LigB required PprA and DRB0098 for its activity in solution

The ligase activity of LigB was checked in the presence of purified recombinant DRB0098, DRB0099, and PprA in different combinations. Individually, these proteins did not show a detectable end-joining activity as detected on agarose gel. When LigB was incubated with PprA, the DNA end-joining activity was detected, which further improved in the presence of both PprA and DRB0098 (Fig. 2B). However, the stimulation of DNA end-joining activity of LigB was not observed when DRB0098 was substituted with DRB0099 in presence the of PprA or when DRB0098 was incubated with or without DRB0099 in the absence of PprA (Fig. 2C). This indicated that the LigB activity was stimulated by PprA and DRB0098 together, but not by DRB0099.

Fig. 2. DNA end-joining activity assay of recombinant DRB0100 (LigB). The purified recombinant protein was assayed with linear plasmid substrate (A, C) and with 1 kb PCR amplified DNA substrate (B). Purified LigB (DB) was incubated with cohesive-end linear plasmid DNA substrate in the presence of ATP (1 and 5 mmol·L^{-1}) and NAD$^+$ (1 mmol·L^{-1}) at 16 °C overnight, and products were analyzed on 1% agarose gel with T4 DNA ligase (T4) as a positive control (A). The effect of PprA (B) and DRB0099 (C) on DRB0098-supported ligation efficiency of LigB was assayed on agarose gel. The effect of different combinations of these proteins on ligase activity of LigB was also monitored by *E. coli* transformation assay (D). The ligation mixtures obtained from incubation of DNA substrate with LigB alone (1) and LigB with DRB0098 (2), PprA (3), and PprA with DRB0098 (4), respectively, were transformed into *E. coli.*

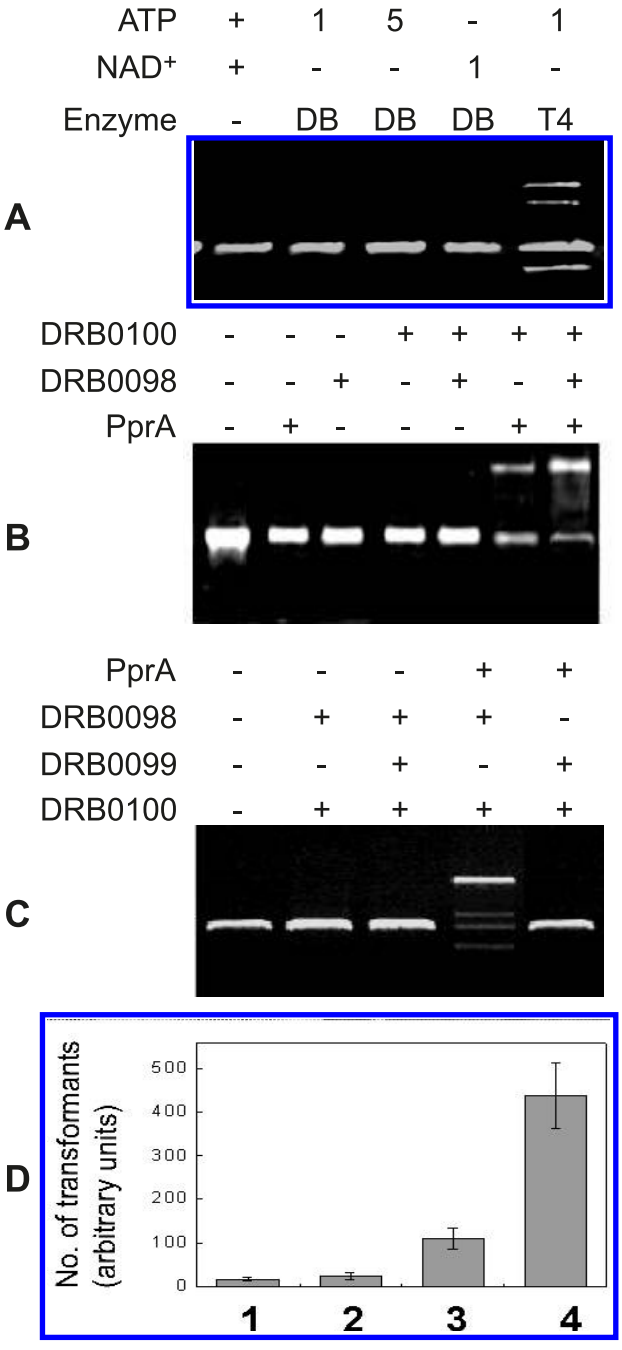
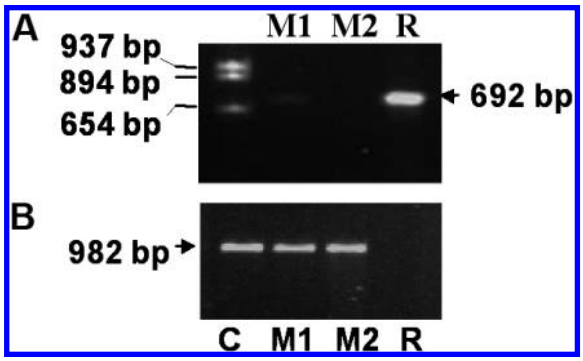


Fig. 3. Confirmation of *drb0100* deletion in bacterial genome. *Deinococcus radiodurans* (R) was transformed with pNOK100 (C) and recombinant cells were grown for several generations. The genomic DNA from prospective mutants (M1 and M2) was prepared and used as template for PCR amplification of 692 bp of *drb0100* (A) and 982 bp of *nptII* (B) using sequence-specific primers. Subsequent studies were carried out with the M2 mutant designated Δ*ligB*.

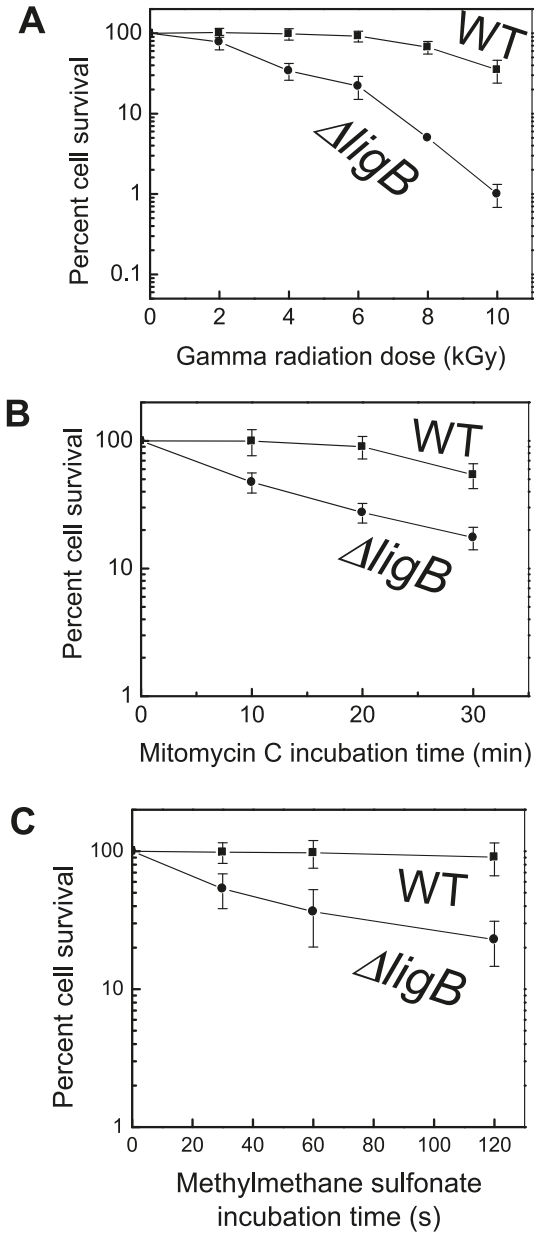


It was possible that LigB confers low DNA end-joining activity, which could not be assayed on agarose gel; thus, a more sensitive bioassay of *E. coli* transformation was employed. The linear plasmid DNA substrates were incubated with LigB in the presence of PprA and (or) DRB0098 proteins in different combinations at 16 °C and transformed into *E. coli*. LigB alone showed low activity, which did not increase upon incubation with DRB0098. However, the incubation of LigB with PprA showed nearly 6-fold higher DNA end-joining activity, which increased by another 4 fold in the presence of DRB0098 (Fig. 2D). LigB activity detected in the presence of DRB0098 and PprA was dependent on ATP, as no activity was observed in the presence of NAD^+ (data not shown). This suggested that LigB was an ATP-type DNA ligase and required DRB0098 and PprA for its efficient DNA end-joining activity. The presence of DRB0099, the second component of the operon, did not improve LigB activity in vitro. This suggested that the efficient functioning of LigB requires at least PprA and DRB0098 in vitro.

The drb0100 deletion mutant was sensitive to DNA damage

The complete replacement of the chromosomal copy of *ligB* from the *Deinococcus* genome with selection marker gene *nptII* was confirmed by PCR amplification (Fig. 3). The mutant (Fig. 3, lane M2) that showed complete replacement of *ligB* with *nptII* was named *ligB* deletion mutant and used in subsequent studies. The effect of DNA damage on the survival of Δ*ligB* was monitored. Mutant cells showed a nearly 10-fold decrease in γ radiation tolerance at 10 kGy (Fig. 4A) as compared with wild type. These cells were ~4-fold less tolerant to MMC when treated for 20 min, and (Fig. 4B) ~5-fold less tolerant to MMS treatment lasting 2 min (Fig. 4C), as compared with wild type. Mutant response to UV (254 nm) was similar to wild type (data not shown). Earlier, Makarova and colleagues had reported insignificant effect of *drb0100* deletion on γ radiation tolerance of *D. radiodurans* (Makarova et al. 2007), but the effect of UV (254 nm), MMS, and MMC on *drb0100* deletion has not previously been reported. The differences in γ radiation response of Δ*ligB* isolated in these studies could be attributed to the different conditions and dose rates used for γ radiation treatment. Alternatively, it is possible that the Δ*ligB* mutant isolated in this study has acquired additional spontaneous mutation(s) during its isolation and should be investigated separately. These results, however, indicated that the *ligB* mutant of *D. radiodurans* was sensitive to γ

Fig. 4. Cellular response of Δ*ligB* mutant to various DNA damaging agents. The Δ*ligB* (-●-) and wild-type (-■-) cells were grown until late logarithmic phase and exposed to different doses of γ radiation (A) and treated for different time intervals with 20 $\mu g \cdot mL^{-1}$ mitomycin C (B) and 10 $\mu g \cdot mL^{-1}$ methylmethane sulfonate (C). Treated cells were plated on TGY agar and surviving fraction was counted. One hundred percent for wild-type and Δ*ligB* cells correspond to ~2.4×10^8 and ~4.1×10^7 cells·mL^{-1}, respectively, for panels A and B, and 1.9×10^8 and 7.8×10^7 cells·mL^{-1}, respectively, for panel C.



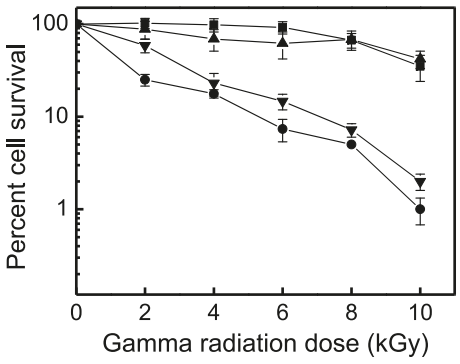
radiation, thereby elucidating the role of LigB operon components in DSB repair. Both MMC and MMS are DNA cross-linking agents that mimic the effect of γ radiation on DNA. These agents also methylate nitrogenous bases, which subsequently fix mutations if not corrected faithfully. Normally, these modifications are corrected by their direct reversals involving both specific and non-specific DNA base methylases and by the base excision repair mechanism (Sedgwick 2004). In absence of the error-free repair processes, MMS-induced base modifications are repaired by SOS repair mechanism, which fixes mutations as shown in *E. coli* (Sikora et al. 2010).

Functional complementation of *ΔligB* cells required other proteins of the LigB operon

To ascertain that the *ΔligB* mutant response to γ radiation was due to the absence of LigB and not the polar effect of this deletion, the wild-type allele of *ligB* was expressed in *trans* in *ΔligB* cells and the γ radiation effect was monitored. The expression of wild-type LigB alone showed partial functional complementation in *ΔligB* (Fig. 5) cells. Surprisingly, *ΔligB* cells become non-transformable with other plasmids like the pRADgro vector and its derivatives containing *drb0099* and *drb0098*, separately. This is intriguing and prohibited us from understanding the individual effect of other genes of the operon on *ΔligB* phenotypes. Since *drb0100* deletion would have disrupted the LigB operon and LigB expressing on plasmid alone did not complement fully, the requirement of other proteins of this operon for functional complementation of *ligB* deletion was checked. Transgenic *ΔligB* cells expressing a complete LigB operon on a plasmid were constructed. These cells were treated with γ radiation and cell survival was monitored. The results showed the complete recovery of γ radiation resistance in mutant cells (Fig. 5). Higher levels of transgene expression under the *groESL* promoter have been previously demonstrated (Misra et al. 2006; Khairnar et al. 2008). These results suggested that all the proteins of the LigB operon were possibly required for radiation resistance in this bacterium. The DRB0098 polypeptide contains a putative nucleotide kinase domain similar to T4 polynucleotide kinase and has been shown to be a polynucleotide kinase (Blasius et al. 2008). This suggests the possible interaction of LigB, a DNA ligase, with other components of LigB operon, at least DRB0098, in radiation resistance in this bacterium. The DRB0098 stimulation of DNA end-joining activity of LigB on *Eco*RI-digested DNA substrate is also intriguing, as this substrate was already in phosphorylated form and DRB0098 is reported to be a polynucleotide kinase type enzyme. This, however, indicated that DRB0098 might have some other roles in addition to its polynucleotide kinase activity.

The systemic study of macromolecular complexes has allowed placing the proteins with hitherto unknown roles into a functional context, and functionally these assemblies represent more than the sum of their interacting partners. Recent studies show that macromolecular complexes can act as depots for releasable regulatory proteins (Mazumder et al. 2003; Sampath et al. 2004; Ray et al. 2007). It has been argued that there would be qualitative superiority for these proteins when present in a microenvironment with other components of macromolecular assemblies. Thus, the regulation of these protein functions when they are isolated or in the presence of other proteins of the macromolecular complex seems to be different, presumably because the structural attainability and stability of the proteins assembled in complex are expected to be different from the ones that folded and existed alone. The significance of protein complexes in maintaining structural and functional integrity of DNA has been demonstrated (Cozzarelli et al. 2006). Such concepts become much more relevant in *Deinococcus radiodurans*, where the bacterial genome forms a toroidal structure with proteins and such organization of DNA with proteins was implicated in its efficient DSB repair. Recently, a multiprotein complex identified from *D. radiodurans* (Kota and Misra 2008) shows the presence of DNA repair proteins, phosphoproteins, and protein kinase activity. The DNA ligase activity of the complex containing LigB was ATP dependent, which led to speculation that the ATP-type DNA ligase activity in complex was probably due to the LigB protein.

Fig. 5. Functional complementation of *ΔligB* mutant. Recombinant plasmids containing DRB0100 and LigB operon on multicopy plasmids were transformed into *ΔligB* cells. The wild-type (-■-), *ΔligB* mutant (-●-), and *ΔligB* cells expressing LigB (-▼-) or the LigB operon (-▲-) in *trans* were exposed to different doses of γ radiation and cell survival was monitored. One hundred percent of corresponding unirradiated cells correspond to 3.2×10^8, 8.3×10^7, 6.4×10^7, and 2.1×10^8 cells·mL^{-1}, respectively.



This study has provided evidence to suggest that LigB is as an ATP-type DNA ligase that requires other proteins such as PprA and DRB0098, for its activity in vitro and its role in radioresistance of *Deinococcus* in vivo. These findings provide one of the possible reasons as to why the DNA ligase activity of purified recombinant LigB is low (Le et al. 2008) or absent in other studies (Blasius et al. 2007). The molecular mechanism of this protein's role in radiation resistance is not clear. However, LigB polypeptide shows greater similarities with eukaryotic ATP-dependent DNA ligase 3 (Lig3) and DNA ligase 4 (Lig4) (Aravind and Koonin 1999), and these ligases are classified as DNA repair ligases (Ellenberger and Tomkinson 2008). Their roles in NHEJ and base excision repair (BER) have been demonstrated in eukaryotes. It has been shown that Lig3 interacts with poly (ADP-ribose) polymerase 1 (PARP-1) (Leppard et al. 2003) and XRCC1, which in turn interact with DNA polymerase β and form a BER complex (Kubota et al. 1996). Similarly, Lig4 interaction with the Ku70–Ku80 heterodimer and XRCC4 is facilitated by DNA-PKcs activity during

NHEJ repair functions in eukaryotes (Costantini et al. 2007). Further, the mutual interaction of different proteins including Lig3 and Lig4 and their associated partners of different DNA repair pathways has been shown (Wang et al. 2006). *Deinococcus* contains PprA, a protein that forms a dimeric complex like Ku70–Ku80 (Murakami et al. 2006), can bind with DNA DSBs (Satoh et al. 2006), and stimulates DNA ligase activity in solution (Narumi et al. 2004). NHEJ-type activity has been reported in other bacteria including *Bacillus subtilis* (Weller et al. 2002), which also contains multiple DNA ligases. The functional interaction of LigB with PprA and DRB0098 (Fig. 2), as evidenced by stimulation of DNA ligase activity by these proteins, strongly suggests the possible role of LigB in DNA repair. The BER activity of deinococcal X-family DNA polymerase, a homologue of eukaryotic polymerase β, has been demonstrated in the presence of T4 DNA ligase (Khairnar and Misra 2009). Sensitivity of *ΔligB* cells to MMS (Fig. 4C) strengthened the possibility of LigB's involvement in BER by interacting with other DNA repair proteins including DNA polymerase X, thus contributing to DNA repair of this bacterium. These findings also indicated the significance of protein–protein interaction in regulating LigB activity and the requirement of all three proteins (LigB, DRB0099, and DRB0098) for *ΔligB* complementation, suggesting the role of the LigB operon in radioresistance in this bacterium.

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