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RecBC enzyme overproduction affects UV and gamma radiation survival of *Deinococcus radiodurans*

*Nivedita P. Khairnar, Vidya A. Kamble, Hari S. Misra**

Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai 400085, India

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ABSTRACT

Deinococcus radiodurans recovering from the effect of acute dose of gamma (γ) radiation shows a biphasic mechanism of DNA double strands breaks repair that involves an efficient homologous recombination. However, it shows higher sensitivity to near-UV (NUV) than *Escherichia coli* and lacks RecBC, a DNA strand break (DSB) repair enzyme in some bacteria. Recombinant *Deinococcus* expressing the *recBC* genes of *E. coli* showed nearly three-fold improvements in near-UV tolerance and nearly 2 log cycle reductions in wild type γ radiation resistance. RecBC over expression effect on radiation response of *D. radiodurans* was independent of indigenous RecD. Loss of γ radiation tolerance was attributed to the enhanced rate of *in vivo* degradation of radiation damaged DNA and delayed kinetics of DSB repair during post-irradiation recovery. RecBC expressing cells of *Deinococcus* showed wild type response to Far-UV. These results suggest that the overproduction of RecBC competes with the indigenous mechanism of γ radiation damaged DNA repair while it supports near-UV tolerance in *D. radiodurans*.

1. Introduction

Mechanism of recombinational repair has been extensively studied in *Escherichia coli*, exposed to various DNA damaging agents [1,2]. Both genetic and biochemical studies suggested that the initiation of homologous recombination follows RecBCD or the RecFOR pathways. Both *in vivo* [3] and *in vitro* [4] studies have shown that RecBCD promotes the repair of double stranded DNA (dsDNA) breaks, where as RecFOR is involved in the repair of single stranded DNA (ssDNA) gaps [5]. Both the pathways act to provide ssDNA molecules coated with RecA to allow the invasion of a homologous molecule. Through the helicase activity of RecB and RecD, the RecBCD complex moves along the DNA duplex [6,7] by unwinding the DNA strands while concurrently cleaving DNA strand in 3′ → 5′ direction at its entry site [8]. This activity gets attenuated when it encounters an eight nucleotides long repeat motif (5′-GCTGGTGG-3′), a canonical CHI sequence of *E. coli* (CHI_{EC}) [9]. Mutation in the *recBC* genes confers low viability to *E. coli* cells unless they acquire mutation in the *sbcA*, *sbcB* and *sbcC*/*sbcD* genes and become proficient in the RecF pathway of homologous recombination and/or illegitimate recombination [10,11]. Thus, RecBCD helps in generating ssDNA for the loading of ssDNA with RecA (*E. coli*-RecA) [12] to initiate homologous recombination. *Deinococcus radiodurans* lacks RecBC enzyme [13] and encodes RecA which prefers dsDNA as a substrate over ssDNA for an efficient DSB repair [14].

D. radiodurans strain R1 exhibits extraordinary tolerance to several abiotic stresses including high doses of ionizing and non-ionizing radiations [15], except near-UV (NUV) [16]. The radiation resistance phenotype of *D. radiodurans* is largely contributed by (i) strong oxidative stress tolerance mechanism [17] (ii) removal of modified/oxidized nucleotide bases by NUDIX hydrolases [18] and (iii) a highly efficient DNA strand breaks (DSB) repair [19,20]. DSB repair in gamma (γ)-irradiated cells occurs by RecA independent but DNA polymerase dependent process of extended synthesis-dependent strand annealing (ESDSA) [21] which proceeds to a very slow reciprocal

* *Corresponding author*. Tel.: +91 22 25592806; fax: +91 22 25505151.
E-mail address: hsmisra@barc.gov.in (H.S. Misra).
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cross-over events involving RecA dependent homologous recombination [19]. It has been shown that RecA from *D. radiodurans* (Dr-RecA) shows preference to double stranded DNA substrate *in solution* [22] and does not complements for *E. coli*-RecA functions [23]. *Deinococcus* lacks RecBC and SbcB proteins while it has the other genetic elements of RecBC recombination pathway, like the canonical CHI_{EC} sequence (739 times in all four genomes against 1008 times in *E. coli*) and an aberrant RecD protein (classified as solo-RecD type helicase) [5]. *D. radiodurans* cells expressing exonuclease I, an inhibitor of RecF/illegitimate recombination, becomes sensitive to gamma radiation and DSB repair was found to be impaired [24]. Genetic interaction of RecBC and RecFOR pathways has been demonstrated in *E. coli* [25]. Absence of the classical RecBC pathway and an extraordinary radiation tolerance dependent on RecFOR pathway in *D. radiodurans*, makes it an interesting system to study the role of RecBC overproduction on the radiation damaged DNA repair phenotype of this organism. In present study, the *recBC* genes of *E. coli* were expressed in *D. radiodurans* R1 and response of recombinant cells to γ and UV radiations were investigated. Recombinant *D. radiodurans* expressing RecBC showed nearly 100-fold improved tolerance to NUV radiation and nearly 2 log cycle reduction in γ radiation resistance as compared to wild type. They exhibited the delayed kinetics of DSB repair and higher rate of post-irradiated genome degradation *in vivo*. These results show the differential effect of RecBC on γ and UV resistance of *Deinococcus*. Increased *in vivo* DNA degradation upon gamma irradiation and delayed kinetics of DSB repair contribute to the γ radiation sensitivity of RecBC expressing *Deinococcus* cells.

2. Materials and methods

2.1. Bacterial strains

D. radiodurans strain R1 was generous gift from Dr. M. Schafer [26]. Wild type and their respective derivatives were grown aerobically in TGY (0.5 Bacto Tryptone, 0.3% Bacto Yeast Extract, 0.1% Glucose) in presence of chloramphenicol (3 μg/ml), when appropriate at 32 °C. Construction of shuttle expression vector was carried out and maintained in *E. coli* strain HB101 as described earlier [24]. Other recombinant techniques used in this study are as described earlier [27].

2.2. Construction of expression plasmids

Genomic DNA of *D. radiodurans* was prepared as published previously [28]. The 3560 bp DNA fragment was PCR amplified from the genomic DNA of *E. coli* strain W3110, using *recB* specific primers (DR2, 5′-GTGGGCCCATGAGTGATGTCGCCGA-3′ and DR3, 5′-TCCCCGCGGTTACGCCTCCTCCAGGGT-3′). The identity and correctness of *recB* gene, was ascertained by restriction analysis and partial nucleotide sequencing. PCR product was ligated at ApaI and SacII sites in pRADgro [24] to yield pGrorecB. Subsequently, a 3388 bp genomic DNA fragment was PCR amplified using *recC* specific primers (DR4, 5′-AGGAGGGGATATCATGTTAAGGGTCTACCAT-3′ and DR5, 5′-GCTCTAGAT CATGACTGATTAAAGCG-3′) and identity of the gene was ascertained by partial nucleotide sequencing. The probable deinococcal ribosome binding site [29] was introduced upstream of *recC* through upstream (DR4) primer. The PCR product was end filled with T4 DNA polymerase and digested with XbaI to generate blunt end-XbaI insert. The SacII digested pGrorecB was end filled with T4 DNA polymerase and then cut with XbaI to generate blunt-XbaI vector and ligated with blunt-XbaI *recC* to yield pGroBC. Both plasmids, pGrorecB and pGroBC, were transformed into *D. radiodurans* as described earlier [29] and chloramphenicol resistant clones were isolated on TGY agar plates containing chloramphenicol (5 μg/ml). Plasmid DNA was prepared from these clones and presence of plasmid was confirmed by restriction analysis.

2.3. Construction of pNOKOUT and generation of recD deletion mutant of D. radiodurans R1

The 1.1 kb nptII cassette expressing under deinococcal promoter was cloned at SmaI site of pBluescript SK+ to make pNOKOUT vector. The 1.0 kb genomic DNA fragment upstream to *recD* coding sequences were PCR amplified using forward primer (5′-GGGGTACCAGACCGTC CGGGGGTCGC-3′) and reverse primer (5′-AAAACTGCAGGACGCCTTAAGAGTTCAGA-3′). PCR product was cloned at KpnI and PstI site in pNOKOUT to yield pRecD1. The 960 bp genomic fragment downstream to *recD* coding sequences was PCR amplified using forward (CGGGATCCGCCAGGCAGGATAGCGCGGA) and reverse (GCTC-TAGACCTGTAAACGG CCTGAAGCT) primers and cloned at BamHI and XbaI site in pRecD1 to yield pNOKrecD. Recombinant plasmid pNOKrecD was linearized with ScaI and used to transform *D. radiodurans* R1. Transformants were grown several generations to obtain homozygous *recD* deletion which was checked by looking the amplification of 450 bp internal *recD* fragment. The clones showed the complete absence *recD* was considered as homozygous *recD* deletion mutant and used for further studies.

2.4. Expression studies of RecB and RecC in D. radiodurans

Recombinant *D. radiodurans* cells harbouring pRadgro, pGrorecB and pGroBC were grown till late logarithmic phase and divided in two sets. One set was irradiated with 6 kGy γ radiation as described earlier [24] and other set was maintained as control. Both sets were incubated for 2 h at 32 °C at 200 rpm and cells were collected by centrifugation. Cells were treated with butanol saturated PBS and suspended in 25 mM Tris–HCl, pH 7.6, 50 mM NaCl, 1 mM DTT, 1 mM EDTA and 0.5 mM PMSF. Cells were broken by sonication at 50% duty cycle using 10 s pulse and 1 min cooling cycle. Lysate was centrifuged at 30,000 × *g* and supernatant was used as cell free extract. Approximately 10 μg total proteins from each sample were separated on SDS-PAGE and transferred on Immobilon P (Millipore). Blots were independently probed with monoclonal antibodies against RecB and RecC as described earlier [30].

2.5. Radiation stress studies

D. radiodurans cells were treated with different doses of UV and γ radiations as described earlier [24]. In brief, *D. radio-*