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RadA: A protein involved in DNA damage repair processes of *Deinococcus radiodurans* R1

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Abstract RadA is highly conserved in bacteria and belongs to the RecA/RadA/Rad51 protein superfamily found in bacteria, archaea and eukarya. In *Archaea*, it plays a critical role in homologous recombination process due to its RecA-like function. In *Escherichia coli*, it takes part in conjugational recombination and DNA repair but is not as important as that of archaea. Using PSI-BLAST searches, we found that *Deinococcus radiodurans* RadA had a higher similarity to that of bacteria than archaea and eukarya. Disruption of *radA* gene in *D. radiodurans* resulted in a modestly decreased resistance to gamma radiation and ultraviolet, but had no effect on the resistance to hydrogen peroxide. Complementation of the *radA* disruptant by both *E. coli radA* and *D. radiodurans radA* could fully restore its resistance to gamma radiation and ultraviolet irradiation. Further domain function analyses of *D. radiodurans* RadA showed that the absence of the zinc finger domain resulted in a slightly more sensitive phenotype to gamma and UV radiation than that of the *radA* mutant, while the absence of the Lon protease domain exhibited a slightly increased resistance to gamma and UV radiation. These data suggest that *D. radiodurans* RadA does play an important role in the DNA damage repair processes and its three different domains have different functions.

Keywords: ***Deinococcus radiodurans*, RadA, DNA damage repair, domain, complementation function.**

RadA protein belongs to RecA/RadA/Rad51 protein superfamily that exists extensively in bacteria, archaea and eukaryota[1−4]. The RecA proteins of eubacteria and the Rad51 proteins of eukaryotes have been found to play a central role in homologous DNA recombination by searching for sequence homology and exchanging the DNA strands[5−8]. The amino acid sequences of archaeal RadA are much more similar to those of eukaryotic Rad51 homologs than to those of bacterial RecA homologs[9,10]. The archaeal RadA proteins have functionally similar properties to the RecA family proteins from eubacteria and they also play a critical role in recombination and DNA repair[2,11−13]. The N-terminal regions of eukaryotic Rad51-like proteins are highly conserved, but the corresponding region is absent in bacterial RecA proteins. Instead, bacterial RecA has an extra C-terminal region. The C-terminal region of bacterial RecA possibly works by catching the double-stranded DNA with the sequence homologous to the ssDNA, which is complicated with RecA protein[14]. Eukaryotic Rad51 can interact with other proteins, such as Rad52, Rad54, Rad55/57, p53, Brca1, and Brca2[15]. The N-terminal domain of the human Rad51 protein constitutes a DNA binding domain[1] and probably has the same function as the C-terminal domain of RecA, although they have no structural homology to each other[15].

The RadA of *Escherichia coli* was initially identified by virtue of the radiation-sensitive phenotype of its mutant[16]. The *radA* mutant showed a modestly decreased resistance to UV or gamma radiation exposure and exhibited a slight decrease in repair of DNA breaks[17]. Further studies have shown that it plays an important role in postsynaptic DNA processing, especially those lacking the RecG branch migration helicase[18]. These results suggest that *E. coli* RadA also participates in the homologous recombination processes.

Deinococcus radiodurans is characterized firstly due to its extraordinary resistance to gamma radiation[19,20]. It also exhibites extreme resistance to other DNA-damaging agents such as ultraviolet, hydrogen peroxide and desiccation[21,22]. Available evidence indicates that the radioresistance may be attributed to both an unusual chromosomal structure and extremely efficient DNA repair processes[23−25]. Among the types of DNA damage, double-stranded DNA breaks (DSBs) are considered to be most lethal to the cell's survival. In *E. coli*, the processes of DSBs repair are RecA-dependent and need the assistance of other pathways, such as RecBCD and RecFOR pathways. However, *D. radiodurans* does not have an intact RecBCD pathway and have an intact

RecFOR pathway[23]. *D. radiodurans* RecA has the three functions of that of *E. coli* RecA, but promotes DNA strand exchange via an inverse pathway compared with *E. coli* RecA[26]. Using the PSI-BLAST searches, we found that the amino acid sequences of *D. radiodurans* RadA are more similar to those of bacteria than those of achraea and eukarya. To date, we still do not know whether *D. radiodurans* RadA also takes part in the process of homologous recombination and DNA damage repair.

In this study, the possible functions of *D. radiodurans* RadA were investigated. Disruption of *D. radiodurans radA* gene resulted in a modest decrease in the resistance to gamma radiation and ultraviolet irradiation, but exhibited no effect on the resistance to hydrogen peroxide. These phenotypes of *radA* disruptant could be complemented by the exogenous expression of *E. coli* RadA in *D. radiodurans*, suggesting that *D. radiodurans* RadA may have similar functions to that of *E. coli* RadA. Further domain-functional analyses showed that the absence of the zinc finger domain resulted in a slightly more sensitive phenotypes to gamma and UV radiation than that of the *radA* mutant, while the absence of the Lon protease domain exhibited a slightly increased resistance to gamma and UV radiation, indicating that its three different domains have different functions.

1 Materials and methods

1.1 Strains, plasmids and growth conditions

All strains and plasmids used in this study are listed in Table 1. All *D. radiodurans* strains were grown at 30℃ in TGY broth or on TGY plates supplemented with 1.5% agar. *E. coli* strain DH5α was purchased from Invitrogen (La Jolla, CA) and was grown at 37℃ in LB broth or on LB plates solidified with 1.5% agar. Antibiotics were added as appropriate concentrations when required: ampicillin 50 μg/mL, kanamycin 20 μg/mL, chloramphenicol 3 μg/mL.

1.2 Construction of the radA disruptant in D. radiodurans

Disruption of *radA* gene (dr1105) was carried out as described previously[27]. A fragment containing the *radA* gene was amplified by PCR using LA Taq polymerase (Takara, Japan) with the primers: 5′-GAGTTGTTGGAGGGCGAT-3′ and 5′-AACGGCTTTCACGGCTTCTT-3′. The PCR product was ligated into pMD18-T vector (Takara, Japan). The resulting plasmid was propagated in *E. coli* DH5α and digested with *Bss*H II and was ligated with about 1045 bp *Bss*H II fragment containing the kanamycin-resistant gene and *groEL* promoter from pRADK (stock in lab). The re-

Table 1 Strains and plasmids

	Description	Ref.
Strains		
D. radiodurans R1	ATCC 13939	[19]
DH5α	host strain for plasmid construction	Invitrogen
MR	R1 *radA* null mutant	this study
MR-C1	MR carrying pRADK*radA*-D	this study
MR-C2	MR carrying pRADK*radA*-E	this study
MR-C3	MR carrying pRADK*radA*△N	this study
MR-C4	MR carrying pRADK*radA*△C	this study
MR-C5	MR carrying pRADK*radA*△NC	this study
Plasmids		
pMD18	TA-cloning vector	Takara
pRADK	*E.coli-D.radiodurans* shuttle vector carrying *D. radiodurans groEL* promoter	[29]
pRADK*radA*-D	pRADK derivative expressing *D. radiodurans* RadA	this study
pRADK*radA*-E	pRADK derivative expressing *E. coli* RadA	this study
pRADK*radA*△N	pRADK derivative expressing the *D. radiodurans* RadA without N-terminal zinc finger domain (1－150 aa)	this study
pRADK*radA*△C	pRADK derivative expressing the *D. radiodurans* RadA without C-terminal Lon protease domain (351－513 aa)	this study
pRADK*radA*△NC	pRADK derivative expressing the RecA-like domain (151－350 aa) of *D. radiodurans* RadA	this study

sulting plasmid was linearized and was transformed into an exponential-phase R1. Putative kanamycin-resistant recombinants were selected on TGY plates supplemented with 20 μg/mL kanamycin. To confirm gene replacement, primers (5′-ATGGAGCGGGTGGCGG-3′/5′-TCAGGCCAAACGGCTTTC-3′), which anneal outside the coding sequence of *radA* gene, were used to generate PCR fragments from genomic DNA from the disruptant candidates and R1. The resulting *D. radiodurans radA* disruptant was named as MR.

1.3 Construction of complementation plasmids

The *radA* coding sequence was amplified from the genomic DNA of *D. radiodurans* using primers as follows: 5′-GGAATTCCATATGGAGCGGGTGGCGG-3′ and 5′-CGCGGATCCTCAGCGCCAAACGGCTTTC-3′. The PCR product was sequenced to confirm the identity and absence of mutation. The 1512-bp fragment was digested by *Nde* I and *Bam*H I and cloned into the pRADK vector pre-digested by the same enzymes. The resulting plasmid was named pRADK*radA*-D. The complementation plasmid pRADK*radA*-E expressing *E. coli* RadA was constructed in a similar manner to pRADK*radA*-D using the primers: 5′-GGAATTCCATATGGTGGCAAAAGCTCCAAAAC-3′ and 5′-CGCGGATCCTTATAAGTCGTCGAACAC-GCTAAG-3′. To further investigate the functions of *D. radiodurans* RadA, other three complementation plasmids were constructed, which expressed three different truncated *D. radiodurans* RadA proteins (Fig. 1). These plasmids were transformed into the *radA* disruptant.

1.4 Cells survival assays under irradiation and hydrogen peroxide treatment

D. radiodurans cultures were grown to A_{600}=0.8, and were suspended in phosphate buffer. These cells were treated at room temperature with different doses of gamma radiation. After irradiation, the cells were diluted to an appropriate concentration and plated on TGY plates. For UV treatment, the exponential-staged cultures were serially diluted with phosphate buffer and plated on TGY plates. Then, the cells were exposed to different doses of UV radiation. The effect of hydrogen peroxide on cell survival was measured as described earlier[28]. Cells in exponential growth were treated with different concentrations (0—40 mmol/L) of hydrogen peroxide at 4℃ for 30 min and serially diluted to an appropriate concentration, then plated on TGY agar plates. For the above three treatments, colony-forming units were determined after incubation for 3 d.

2 Results

2.1 RadA is highly conserved in eubacteria

With the *D. radiodurans* RadA as the seed, PSI-BLAST searches results showed that the RecA-like domain of *D. radiodurans* RadA is much more similar to those of bacteria than to those of archaea and eukarya (Table 2). *D. radiodurans* RadA has 47% identity and 61% similarity with *E. coli* RadA, and many critical amino acids are highly conserved in both of them (Fig. 2(b)). From the comparison between *D. radiodurans* RadA and *E. coli* RadA using the Clustal W

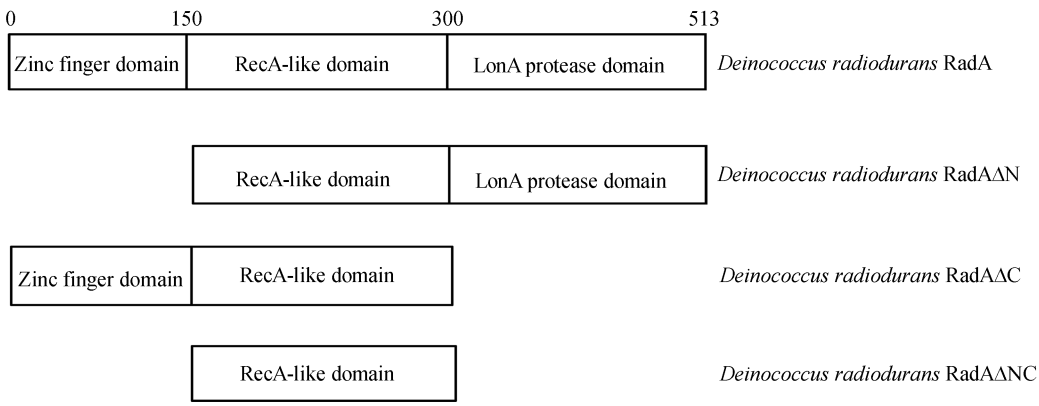

Fig. 1. Domain analysis and three truncated fragments of *D. radiodurans* RadA. *D. radiodurans* RadA is composed of N-terminal zinc finger domain, RecA-like domain and C-termianl Lon protease domain, which is consistent with *E. coli* RadA. Three truncated RadA proteins of *D. radiodurans* are used for the functional analyses of the three domains.

Table 2 Comparison of the identity and similarity of the RadA proteins or orthologs from other organisms to the *D. radiodurans* RadA

Genus	Species	Name	Identity (similarity) to DR1105
Bacteria			
Bacillus	*B. cereus*	RadA	45% (64%)
	B. licheniformis	RadA	45% (64%)
	B. weihenstephanensis	RadA	45% (64%)
Deinococcus	*D. geothermalis*	RadA	87% (93%)
	D. radiodurans	RadA	100%
Escherichia	*E. coli*	RadA	*47% (61%)*
Thermus	*T. thermophilus*	RadA	58% (69%)
Archaea			
Ferroplasma	*F. acidarmanus*	Rad51-related protein	35% (55%)
Methanothermobacter	*M. thermautotrophilcum*	RadA	26% (43%)
Methanosarcina	*M. mazei*	RadB	25% (39%)
Thermoplasma	*T. acidophilum*	Lon	25% (49%)
	T. volcanium	RadB	35% (52%)
Eukarya			
Arabidopsis	*A. thaliara*	DNA repair protein	37% (54%)
Bombyx	*B. mori*	DMC homolog	27% (44%)
Drosophila	*D. melanogaster*	Rad51	28% (40%)
Homo	*H. sapiens*	Rad51	27% (41%)
Saccharomyces	*S. cerevisiae*	Rad51	48% (66%)

program, the zinc finger motif, walker box A and B responsible for ATP binding and conserved sequence (KNRFG), which are existing in *E. coli* RadA, are also found in *D. radiodurans* RadA (Fig. 2(a)). Like *E. coli* RadA, *D. radiodurans* RadA has three domains: the N-terminal zinc finger domain, RecA-like domain and the C-terminal Lon protease domain (Fig. 1).

2.2 Disruption of radA gene of D. radiodurans has a modest effect on the resistance to gamma and UV radiation

The *D. radiodurans radA* disruptant was constructed using the direct insertional mutagenesis technique. The results of PCR confirmation showed that the size of PCR fragment from disruptant candidate is 2560 bp and the size of PCR product from R1 is 1512 bp, which are consistent with the expected sizes (Fig. 3). This mutant exhibited a modest increase in sensitivity to gamma and UV radiation; however, disruption of *D. radiodurans radA* had no apparent effect on the resistance to hydrogen peroxide (Fig. 4).

2.3 E. coli and D. radiodurans RadA proteins can compensate fully the resistance phenotypes of the radA mutant to gamma and UV irradiation

To examine whether both *E. coli* RadA and *D. radiodurans* RadA have the same function, we constructed the complementation plasmids: pRADK*radA*-E expressing *E. coli* RadA and pRADK*radA*-D expressing *D. radiodurans* RadA. They were transformed into the *radA* mutant of *D. radiodurans*. The complementation of this *radA* mutant by *D. radiodurans* RadA could recover completely the resistance to gamma and UV radiation, which also proves that the modestly sensitive phenotypes of this mutant do is due to the diruption of *radA* gene. Furthermore, the *E. coli* RadA could restore the resistance of the *radA* mutant to that of wild type R1 (Fig. 5). It suggests that *D. radiodurans* RadA has a similar function in the DNA damge repair process as that of *E. coli*.

2.4 The absence of any of three domains of D. radiodurans RadA results in the sensitive phenotypes of the radA mutant

We constructed three complentation plasmids expressing different truncated *D. radiodurans* RadA proteins in order to study the function of different domains of *D. radiodurans* RadA (Fig. 1). The absence of the zinc finger domain resulted in a slightly more sensitive phenotypes to gamma and UV radiation than that of the *radA* mutant, while the absence of the Lon protease domain exhibited a slightly increased resistance to gamma and UV radiation. The phenotypes of the *radA* mutant by the complementation of only RecA-like do-

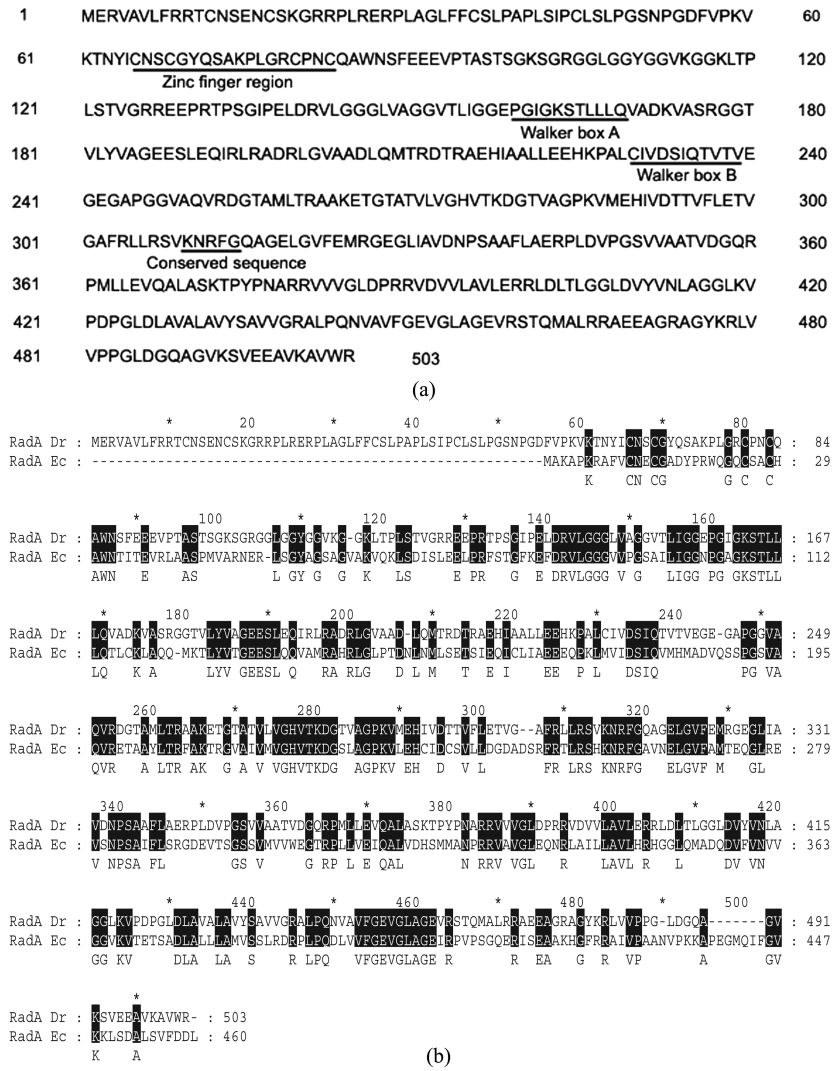

Fig. 2. *Deinococcus radiodurans* RadA has a high similarity to *E. coli* RadA. (a) Locations of known predicted motifs within the primary amino acid sequence of *D. radiodurans* RadA. Zinc finger motif, walker boxes A and B, conserved sequence are indicated. (b) Amino acid sequence comparison of *D. radiodurans* RadA with *E. coli* RadA using Clustal W program. Black boxes denote identity.

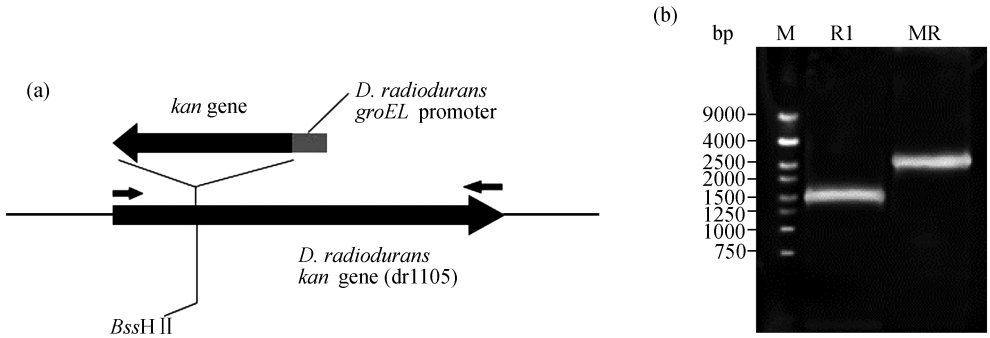

Fig. 3. Construction and verification of the disruptant of *D. radiodurans radA*. (a) Schematic representation of *D. radiodurans radA* disruption. The two large arrows indicate the position and orientation of *radA* and kanamycin-resistant genes, respectively. The small arrows indicate the positions of specific primers used for PCR. (b) Verification of the *radA* disruption by PCR analysis. Purified PCR fragments were amplified from the genomic DNA of strain R1 and MR using primers that flank the coding sequences for the *radA* gene. The PCR products of R1 revealed a band lenghth of ~1512 bp, whereas those of MR resulted in a ~2560 bp band. M denotes molecular standards.

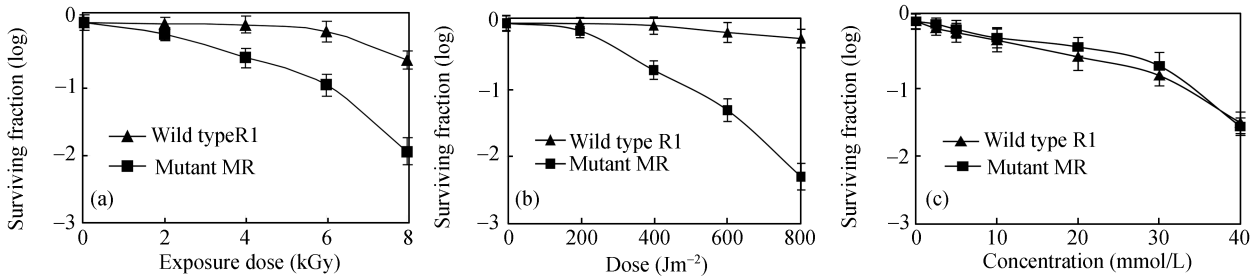

Fig. 4. Response of *radA* disruptant of *D. radiodurans* to gamma radiation, UV radiation and hydrogen peroxide. (a) Representative survival curves for *D. radiodurans* R1 (closed triangles) and mutant MR (closed squares) following exposure to gamma radiation. Values are the mean ± standard deviation of four independent experiments. (b) Representative survival curves for *D. radiodurans* R1 (closed triangles) and mutant MR (closed squres) following exposure to UV radiation. Values are the mean±SD of four independent experiments. (c) Representative survival curves for *D. radiodurans* R1 (closed triangles) and mutant MR (closed squares) following exposure to different concentrations of hydrogen peroxide for 30 min at room temperature. Values are the mean±SD of three independent experiments.

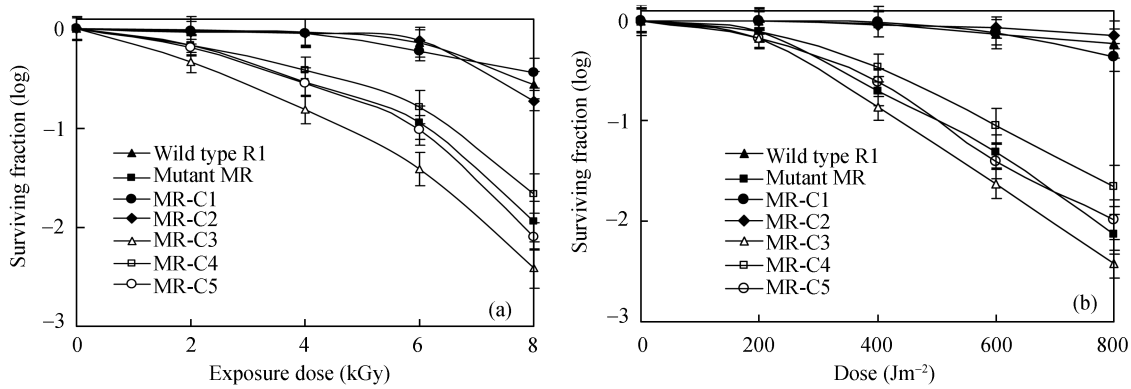

Fig. 5. Representative survival curves of MR-C1 (closed circles), MR-C2 (closed diamonds), MR-C3 (open triangles), MR-C4 (open squares) or MR-C5 (open circles) respectively following exposure to gamma radiation (a) and UV radiation (b) compared with that of R1 (closed triangles) and MR (closed squares). Values are the mean±SD of three independent experiments.

main were the same as those of the *radA* mutant (Fig. 5), indicating that three domains of *D. radiodurans* RadA may have different functions in homologous recombination and DNA repair processes.

3 Discussion

In many organisms, RecA/RadA/Rad51 protein superfamily plays an important role in homologous recombination and DNA repair processes[5,8]. Here, we show that *D. radiodurans* RadA is much more similar to those of bacteria than those of archaea and eukarya, indicating that *D. radiodurans* RadA may have functions similar to that of bacteria. In *D. radiodurans*, gamma irradiation will produce hundreds of double-stranded DNA breaks, thousands of single-stranded DNA damage and many free radicals. These DNA damages are lethal to cell's survival and are repaired by DNA damage repair pathways. Disruption of *radA* gene in *D. radiodurans* resulted in a modest decrease in the resistance to gamma and UV radiation, suggesting that *D. radiodurans* RadA is involved in these processes.

E. coli RadA takes part in the branch-migration process and is partially redundant compared with RecG and RuvABC[18]. We found that *E. coli* RadA could fully compensate for the resistant phenptypes of the *radA* mutant of *D. radiodurans* to gamma and UV radiation. This suggests that *D. radiodurans* RadA may have a same function in the DNA damage repair process as that of *E. coli* and also function in the branch-migration process.

The experiments of complementation of the *radA* mutant by different domains of *D. radiodurans* RadA showed that different domains of *D. radiodurans* RadA have different functions. Only the absence of the N-terminal zinc finger domain resulted in a more sensitive phenotypes to gamma and UV irradiation than those of *radA* mutant. However, only the absence of the Lon protease domain resulted in a more resistant phenotypes to gamma and UV radiation. These suggest that the zinc finger domain may be responsible for promot-

ing DNA binding efficency of *D. radiodurans* RadA and protects DNA from being damaged severely, while Lon protease domain may decompose these proteins involved in DNA damage repair processes and produces these worse phenotypes. The RecA-like domain could not individually work because of the absence of DNA binding activity, but no evidence shows that RadA has the functions of RecA. With regard to the functions of the three domains of *D. radiodurans* RadA, further research and study need to be done in the future.

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