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Short communication

Involvement of *recQ* in the ultraviolet damage repair pathway in *Deinococcus radiodurans*

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Abstract

Deinococcus radiodurans is a bacterium which can survive extremely DNA damage. To investigate the relationship between *recQ* and the ultraviolet radiation (UV) damage repair pathway, we created a four mutant strain by constructing *recQ* knockout mutants in *uvrA1*, *uvrA2*, and *uvsE* backgrounds. Using the *rpoB*/Rif^r system, we measured the mutation frequencies and rates in wild type, *recQ* (MQ), *uvsE uvrA1 uvrA2* (TNK006), and *uvsE uvrA1 uvrA2 recQ* (TQ). We then isolated Rif^r mutants of these strains and sequenced the *rpoB* gene. The mutation frequency of TQ was 6.4, 10.1, and 2.43 times that of wild type, MQ, and TNK006, respectively, and resulted in rates of 4.7, 6.71, and 2.15 folds higher than that of wild type, MQ, and TNK006, respectively. All the strains demonstrated specific mutational hotspots. Furthermore, the TQ strain showed a transversion bias that was different from the other three strains. The results indicate that *recQ* is involved in the ultraviolet damage repair pathway via the interaction between *recQ* and *uvrA1*, *uvrA2*, and *uvsE* in *D. radiodurans*.

Keywords: Mutators; DNA repair; Mutation; RecQ; UV pathway

1. Introduction

The bacterium *Deinococcus radiodurans* can survive extremely DNA damage whether caused by ionizing radiation, ultraviolet (UV) radiation, oxidative stress, or the action of alkylating agents [1]. For instance, *D. radiodurans* is 33-fold more resistant to UV than *Escherichia coli*. However, the mechanism of its extremely resistance to UV damage is poorly understood [2].

The UV damage repair pathways are composed of UvsE-dependent excision repair (UVER) and UvrA1-dependent pathways. Earl et al. demonstrated that interruption of *uvrA1* greatly sensitizes *D. radiodurans* to UV light [3]. Furthermore, Tanaka et al. determined the biological significance of *uvsE*, *uvrA1* and *uvrA2* to UV resistance in *D. radiodurans*, by showing that the loss of *uvsE*, *uvrA1* and *uvrA2* reduced but not completely abolish the ability to recover from UV damage. They also showed that homologous recombination (HR) played a more significant role than UVER or nucleotide excision repair (NER) in the organism's resistance to UV [4].

As one of four pathways for the initiation of homologous recombination repair, the RecF pathway contributes to the repair of UV-damaged DNA [5–9]. RecQ also takes part in the RecF recombination pathway [10]. As a member of the SF1 helicase family, the RecQ helicase family was first identified in an *E. coli* mutant sensitive to thymine starvation [11]. Loss of RecQ helicase causes genomic instability in all organisms studied [12]. For example, mutations in human RecQ-related genes *BLM*, *WRN* and *RECQ4* cause Bloom's, Werner's and Rothmund-Thomson syndromes in humans [12].

Most RecQ helicases have three conserved domains: helicase, RecQ-C-terminal (RQC), and helicase-and-RNaseD-like-C-terminal (HRDC) [13]. The helicase domain is involved in coupling the energy of NTP hydrolysis to the separation of

Abbreviations: UV, ultraviolet; MQ, *recQ*; TNK006, *uvrA1 uvrA2 uvsE*; TQ, *uvrA1 uvrA2 uvsE recQ*; HR, homologous recombination; NER, nucleotide excision repair; UVER, UvsE-dependent excision repair; RQC, RecQ-C-terminal; HRDC, helicase-and-RNaseD-like-C-terminal; PB, phosphate buffer; 5AZ, 5-azacytidine; NTG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

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Table 1
Strains used in this study

Strain	Description	Reference(s) or source
Deinococcus radiodurans R1	Wild type	ATCC
TNK006	As R1 but Δ*uvrA1* Δ*uvrA2* Δ*uvsE*	[4]
MQ	As R1 but Δ*recQ*	[13]
TQ	As TNK006 but Δ*recQ*	This study
Escherichia coli TG1	*supE*, *hsd*Δ*5*, *thi*, Δ(*lac-proAB*)/F' [*traD36*, *proAB*$^+$, *lac I*^{q}, *lacZ*Δ*M15*]	Takara

nucleic acid duplexes [12]. The RQC domain is suggested to play a role in regulating specific protein–protein interactions and modulating DNA binding affinity. Finally, the HRDC domain is thought to be involved in nucleic acid substrate binding [13]. The *recQ*-deficient *D. radiodurans* is sensitive to γ-irradiation, UV, H_2O_2, and mitomycin C. Further biochemical experiments have shown that the helicase and all three HRDC domains are indispensable for complete DNA damage resistance. Furthermore, the three tandem HRDC domains increase the efficiency of the unwinding and ATPase activity [13].

Hanada et al. investigated the relationship between RecQ helicase and UvrAB in *E. coli* and demonstrated that the UvrAB complex suppressed illegitimate recombination in a pathway shared with RecQ helicase [14]. In our present study, we determined the mutation spectrum of wild type *D. radiodurans* and mutants *uvsE uvrA1 uvrA2* (TNK006, referred to in Table 1), *recQ* (MQ), and *uvsE uvrA1 uvrA2 recQ* (TQ) by means of spontaneous mutation in the *rpoB*/Rif^r mutational analysis system. We found that *recQ* is involved in the ultraviolet damage repair pathway via the interaction between *recQ* and *uvrA1*, *uvrA2*, and *uvsE* in *D. radiodurans*.

2. Materials and methods

2.1. Bacterial strains, plasmids, media and oligonucleotides

Restriction enzymes, T4 ligase, and Taq DNA polymerase were purchased from TaKaRa (Otsu, Shiga, Japan). All *E. coli* cultures [TG1] were grown at 37 °C or as otherwise indicated in LB broth or on LB plates supplemented with the appropriate antibiotics (ampicillin, 50 μg/mL; kanamycin, 30 μg/mL). All *D. radiodurans* cultures were grown at 30 °C in TGY media (0.5% bacto tryptone, 0.1% glucose, 0.3% bacto yeast extract) with aeration or on TGY plates supplemented with 1.3% agar. Oligonucleotides were synthesized by Invitrogen (Rockville, MD).

2.2. Construction of recQ disruption strain

A *recQ* disruption strain (designated TQ) was generated using a three-step gene splicing by overlap extension technique [15]. Briefly, TQ was constructed based on TNK006, in which the entire coding region of the *recQ* gene was replaced with a kanamycin resistance cassette under the control of a constitutively expressed *D. radiodurans GroEL* promoter (Fig. 1A). TQ was selected on TGY agar supplemented with 20 μg/mL kanamycin. The mutant was confirmed by PCR and DNA sequencing using primers AF1 (5′-GCCGTTGTCTTCCAGTTCCCAGTGC-3′) and AR3 (5′-TTATGAAGCTTGGGCCGCCGGGCCA-3′) (Fig. 1B).

2.3. Measurement of mutation frequency

For mutation frequency assays, cells were harvested in early stationary phase, washed twice, with the phosphate buffer (20 mM, pH 7.4) (PB) and re-suspended in PB [13]. Serial dilutions with PB were plated onto TGY plates containing

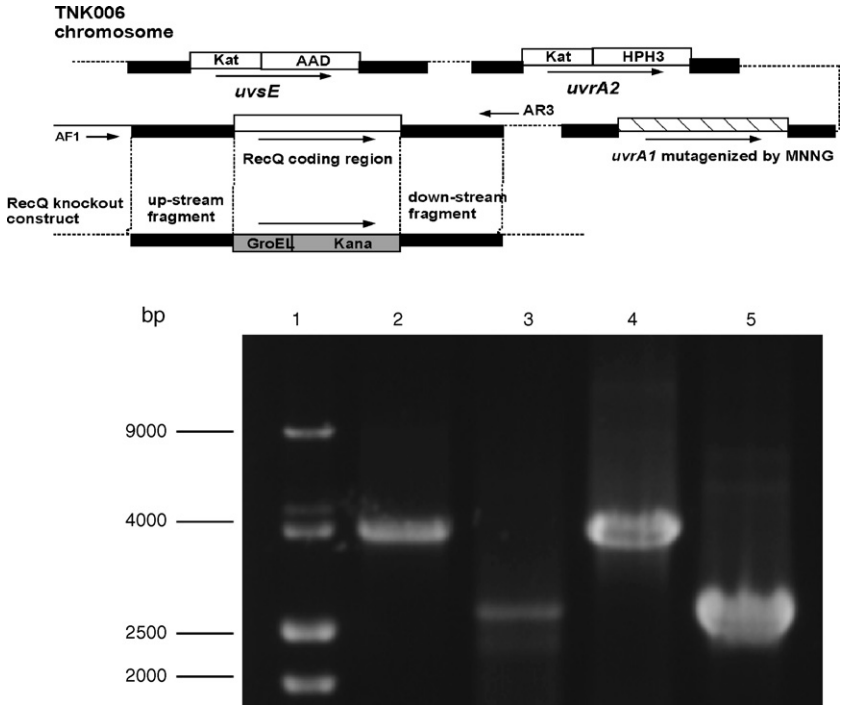

Fig. 1. Construction and verification of *Deinococcus radiodurans uvrA1 uvrA2 uvsE recQ* four-knockout mutant. (A) Schematic representation of the targeting strategy for generating the four-knockout mutant. (B) Verification of the *D. radiodurans uvrA1 uvrA2 uvsE recQ* four-knockout mutant strain. Lane 1: DNA size marker; lanes 2–4: PCR products using primers AF1 and AR3; lane 2: PCR products of wild type; lane 3: PCR products of MQ; lane 4: PCR products of TNK006; lane 5: PCR products of TQ.

Table 2
D. radiodurans rpoB mutation frequencies (f) and rates[a] (μ, $\times 10^8$)

Strains	f	μ
R1	5.6 (5.0–6.1)[b]	1.42 (1.34–1.50)
MQ	3.5 (0.7–6.4)	1.00 (0.54–1.47)
TNK006	14.6 (13.7–15.5)	3.12 (3.00–3.24)
TQ	35.6 (21.4–49.6)	6.71 (4.47–8.96)

[a] Rates are calculated by the method of Drake [16].
[b] 95% confidence limits.

50 μg/mL rifampicin (Rif) and incubated for 4 days before scoring. The total number of colony-forming units (CFU) was determined by plating serial dilutions on TGY plates. The frequency of mutations conferring resistance to Rif was determined as the ratio of mutant bacteria on Rif plates to total viable bacteria on TGY plates [4]. The mutation rate calculation followed the method of Drake [16]. Colonies in the Rif^r TGY plates were used for isolating genomic DNA and PCR sequencing.

2.4. DNA isolation and sequencing

Saturated 1.5 mL cell cultures were centrifuged at 13,000 × *g* for 1 min and re-suspended in 200 μL of PB buffer. The stripped cells were mixed with 200 μL of phenol, 200 μL of chloroform/isoamyl alcohol (ratio: 24/1) and 100 μL of silica. The cells were then ground by Mixer Mill type MM 301 (Retsch GMbh & Co. KG, Germany) followed by centrifugation at 13,000 × *g* for 30 min. The supernatant was transferred to a new tube with 200 μL of isopropyl alcohol. The samples were mixed by inversion and kept at −20 °C for 30 min, followed by a centrifugation at 13,000 × *g* for 10 min. The DNA pellet was washed twice with ethanol, and 30 μL of MilliQ water was added to the tube.

The following primers were used to amplify the DNA for sequencing: 5′-AAACTGTGCCCATGGTGGAC-3′ (5′ position 1058) and 5′-TAGCTCACGCGGCCATTCAC-3′ (5′ position 173). The PCR products were purified and sequenced by Invitrogen (Shanghai, China), using sequencing primer 5′-CATGCTGCTCGGCAACCC-3′ (5′ position 1221).

3. Results

3.1. Mutation frequencies and rates

We measured the mutation frequencies of the colonies grown on TGY plates and Rif^r TGY plates, and calculated the mutation rates using Drake's method [16]. Table 2 lists the mutation frequencies and rates of these strains. The MQ strain did not show a higher rate of spontaneous mutations compared to wild type, while the Rif^r frequency of TNK006 was 2.6-fold higher than that of wild type. Strikingly, the combination of mutations from the MQ (*recQ*), and TNK006 (*uvrA1 uvrA2 uvsE*) strains which were combined to generate the TQ strain, generated a mutation frequency 6.4, and a mutation rate 4.7-fold higher than that of wild type.

3.2. Distribution of mutation sites leading to Rif^r expression in the four strains

We isolated Rif^r mutants from *D. radiodurans* wild type, TNK006, MQ, and TQ and sequenced the *rpoB* regions of their genomes. Table 3 shows the results of 71 mutations that activate the Rif^r phenotype, including 19 mutations occurring in wild type, 18 mutations in TNK006, 15 mutations in MQ, and 19 mutations in TQ.

3.3. Spontaneous mutation in wild type D. radiodurans

We detected one type of 9-bp deletion and four types of base substitution hotspots in the wild type background, all of which correspond to the findings of Kim et al. [17]. The percentage of deletion (1258–1266 9-bp deletion) reached 42% (8 of 19). In addition, there exists one base substitution hotspot at position 1273 (G:C → A:T), with an incidence of 32% (6 of 19).

3.4. Spontaneous mutation hotspots in mutator strains of D. radiodurans

TNK006 conserves the three mutation hotspots at positions 1273 and 1303 (G:C → A:T), and 1259 (A:T → C:G) of the wild type. Together, these three hotspots accounted for 13 of the 17 base substitutions (76%). We also determined a previously unreported [17] *rpoB* mutation at position 1505 (A:T → G:C) in the MQ strain. Most other mutations were transition mutations (66.6%), with G:C → A:T (1273) mutations predominating over A:T → G:C mutations. Additionally, in the TQ strain, most mutations were transversions (89.4%) at posi-

Table 3
Distribution of mutations leading to Rif^r in *D. radiodurans*

D. radiodurans site (bp)	Amino acid change	Base-pair change	Wild type	TNK006	MQ	TQ	*E. coli* site (bp)
1505	K502R	AT ⇒ GC[a]	0	0	1	0	1838
1273	D425N	GC ⇒ AT	0	3	10	1	1546
1303	H435Y	GC ⇒ AT	2	7	2	0	1576
1319	S440F	GC ⇒ AT	0	0	0	1	1592
1418	P473L	GC ⇒ AT	0	2	0	0	1691
1265	Q422L	AT ⇒ TA	0	2	0	0	1538
1325	L442Q	AT ⇒ TA	1	0	0	0	1598
1259	L420R	AT ⇒ CG	2	3	0	17	1532
1273	D425Y	GC ⇒ TA	6	0	0	0	1546
1305	H435Q	GC ⇒ CG	0	0	1	0	1578
1258-66		9-bp deletion	8	1	1	0	1531-39
Total			19	18	15	19	

The DNA sequence change in *rpoB* was determined in each case.
[a] The corresponding base and base change is different in *E. coli*.

Table 4
Mutation rates at sites in *rpoB*

rpoB site (location no. in bp)	Mutation rate μ ($\times 10^9$)			
	Wild type	TNK006	MQ	TQ
I (1259)	1.495	5.2	–[a]	60.037
II (1265)	–[a]	3.467	–[a]	–[a]
III (1273)	4.484	5.2	6.667	3.532
IV (1303)	1.495	12.133	1.333	–[a]
V (1325)	0.747	–[a]	–[a]	–[a]
Average/site (total no. of sites)	2.84 (5)	5.2 (6)	2 (5)	22.367 (3)
Average/transition site (total no. of transition sites)	14.2 (1)	6.93 (3)	2.89 (3)	3.53 (2)

[a] Mutation rates cannot be determined at these sites.

tion 1259 (A:T → C:G), while the remaining two mutations were both transitions (G:C → A:T).

3.5. Strain-specific mutation hotspots in rpoB

The relative frequencies of *rpoB* mutations among the four strains of *D. radiodurans* at five selected sites (sites I–V in Table 4) can be seen in Fig. 2. The four strains differed significantly with respect to relative mutation frequencies at sites I and IV. Table 4 converts these relative frequencies to mutation rates per site. Site I may best represent the different mutation rates of the four strains. The TQ strain, which combines the TNK006 and MQ strains, produced the highest mutation rate at this site. Site II showed the difference between TNK006 and the others, as well as site IV. Only the wild type strain exhibited mutations at site V.

Table 4 shows the average mutation rate per site in *rpoB*, based on five sites in wild type, six in TNK006, five in MQ and three in TQ. In wild type, the percentage of transition mutations was similar to the percentage of transversion mutations (Fig. 3). The vast majority of the spontaneous base substitutions in *rpoB* that lead to Rif^r in TNK006 and MQ were transitions. Transversions, however, were the major source of mutations in TQ.

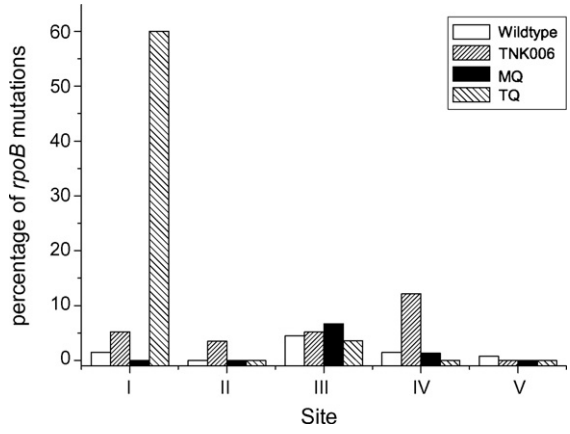

Fig. 2. Comparison of relative mutation frequencies observed at specific sites in *rpoB*. The values for five sites in *rpoB* in four different strains are shown as the percentage of all detected *rpoB* mutations that lead to Rif^r mutants.

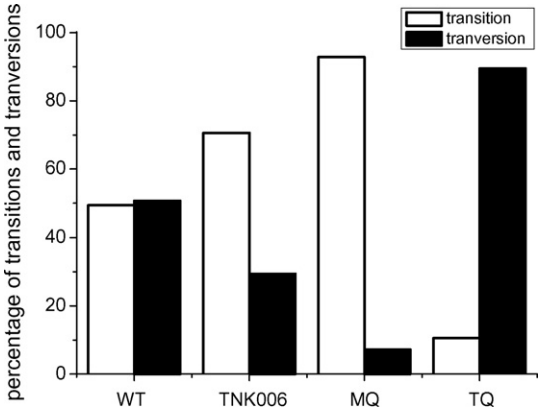

Fig. 3. Comparison of the percentage of transitions and transversions in *rpoB*. White square: the percentage of transition mutations; black square: the percentage of transversion mutations.

4. Discussion

In this work, we created a four mutant strain in *D. radiodurans* by constructing *recQ* knockout mutants in combinations of *uvrA1 uvrA2 uvsE* mutant backgrounds. Each mutant of *D. radiodurans* (Table 1) displayed a specific spontaneous mutation rate in the *rpoB* mutation assay system (Table 2). The β subunit of RNA polymerase, which is involved in rifampicin binding, is highly conserved among prokaryotes, and Rif^r mutants detected in many bacteria are the result of amino acid exchanges [18]. Kim et al. developed an *rpoB*/Rif^r mutation analysis system for *D. radiodurans* based on the assays measuring the frequency of forward mutations to rifampicin, streptomycin, and trimethoprim resistance [17]. This mutation analysis system had been used not only in *E. coli* for the mutators of *mutS*, *mutT*, *mutYM* [18], Pol IV [19], *ndk* and *ndk mutS* [20], but also in other bacteria: *Neisseria* species for *mutY* [21], *Pseudomonas stutzeri* for *mutS* [22], *Mycobacterium tuberculosis* [23,24], *Helicobacter pylori* [25], and *Bacillus subtilis* [26,27]. Furthermore, Kim et al. analyzed 185 spontaneous, 33 NTG induced, 19 5AZ induced, and 17 *uvrD* mutations, as well as defined 33 base change substitutions at 22 different mutational sites (base pairs) in *D. radiodurans* [17]. We extended the analysis by adding a new mutation site at position 1505 of the *rpoB* gene.

We measured the mutation rates of four strains in *D. radiodurans*, and compared their mutation specificities. The mutation

rates of each mutator are listed in Table 2. Although the wild type mutation frequency was higher than the value demonstrated in Kim's previous work [17], there was no significant difference between mutation rates. Rosche and Foster reason that an accurate determination of mutation rate depends on understanding the strengths and limitations of these methods to estimate mutation rates [28]. MQ may not represent a real mutator phenotype since mutator cells typically demonstrate a higher incidence of spontaneous mutations when compared to wild type cells [29]. However, the incorporation of *recQ* (as demonstrated in the TQ strain) enhanced the effect of *uvrA1*, *uvrA2* and *uvsE* seen in MQ, which was quite unexpected. In regards to a similar type of finding, Miller et al. asked whether alleles yielding weaker effects alone have the ability to produce strong effects in the presence of certain other alleles [20]. This may be a result of an interaction between *recQ* and *uvrA1*, *uvrA2*, *uvsE* in our studies. In support of our hypothesis that there may be an interaction between *recQ* and UV damage repair pathways, Hanada et al. have demonstrated that the UvrAB helicase may function as a part of the RecQ pathway in *E. coli* [14].

Additionally, we determined the mutation spectrum of wild type, TNK006, MQ, and TQ in regards to spontaneous mutation by means of the *rpoB*/Rif^r mutational analysis system. The specificity of the various mutators shown here opens the way for an investigation of the relationship between the RecQ and UV repair pathways in *D. radiodurans*.

Next, we compared the average mutation rate per site in *rpoB* in four strains of *D. radiodurans*. As shown in Table 4, the mutation rate at the same sites widely varies. For instance, site I showed a 36-fold higher rate of mutation in TQ than in wild type. Thus, the mutational hotspots were found to be strain-specific. Hotspots are thought to relate to the interactions of polymerase, editing, and repair proteins with the mutational site and surrounding sequences, in which each protein has its own inherent specificity with regard to sequence context [30]. The specificity of hotspots shown here may reveal details of the interaction between *recQ* and *uvrA1*, *uvrA2*, *uvsE*.

In addition, TQ showed a transversion bias that differs from the other strains studied here. Typically, the rates of transitional substitutions are greater than that of transversions, which may be explained by selection on nonsynonymous transversions. It is possible that there exists greater purifying selection against transversions due to the greater biochemical difference in the final protein product as a result of a transversion [31]. Thus, selection is thought to promote DNA repair systems that prevent transversions [32]. The transversion bias of TQ shown here suggests that the interaction between *recQ* and *uvrA1*, *uvrA2*, and *uvsE* might be involved in the prevention of transversions. It is possible that there exists a two-component system in which *recQ* prevents transversions as a first line of defense on specific mutational sites and surrounding sequences, while a second line of defense includes the UV damage repair pathway, or vice versa. In sum, our results suggest that *recQ* is involved in the ultraviolet damage repair pathway via the interaction between *recQ* and *uvrA1*, *uvrA2*, and *uvsE* in *D. radiodurans*.

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