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ORIGINAL PAPER

# Three *nth* homologs are all required for efficient repair of spontaneous DNA damage in *Deinococcus radiodurans*

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**Abstract** *Deinococcus radiodurans* is a bacterium that can survive extreme DNA damage. To understand the role of endonuclease III (Nth) in oxidative repair and mutagenesis, we constructed *nth* single, double and triple mutants. The *nth* mutants showed no significant difference with wild type in both IR resistance and $H_2O_2$ resistance. We characterized these strains with regard to mutation rates and mutation spectrum using the *rpoB*/Rif$^r$ system. The Rif$^r$ frequency of mutant MK1 (△*dr0289*) was twofold higher than that of wild type. The triple mutant of *nth* (ME3)generated a mutation frequency 34.4-fold, and a mutation rate 13.8-fold higher than the wild type. All strains demonstrated specific mutational hotspots. Each single mutant had higher spontaneous mutation frequency than wild type at base substitution (G:C → A:T). The mutational response was further increased in the double and triple mutants. The higher mutation rate and mutational response in ME3 suggested that the three *nth* homologs had non-overlapped and overlapped substrate spectrum in endogenous oxidative DNA repair.

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**Keywords** Mutators · DNA repair · Mutation · Endonuclease III

**Abbreviations**

| | |
|---|---|
| Nth | Endonuclease III |
| Rif | Rifampicin |
| UV | Ultraviolet |
| PCR | Polymerase chain reaction |
| CFU | Colony-forming units |
| PBS | Phosphate buffer saline |

## Introduction

*Deinococcus radiodurans* is a robust bacterium that can survive extreme DNA damage whether caused by ionizing radiation (IR), ultra-violet (UV), desiccation and mitomycin C (Slade and Radman 2011). For instance, *D. radiodurans* is 33-fold more resistant to UV than *E. coli*. It has been suggested that this elevated resistance of *D. radiodurans* is due to a combination of various DNA repair and recombination proteins and mechanisms, but understanding the molecular mechanisms responsible for radiation resistance needed further work (Blasius et al. 2008).

To recover the genome from oxidative DNA damage caused by IR, *D. radiodurans* repairs its genome with an efficient and accurate DNA damage repair system (Slade and Radman 2011). Of many type of oxidative DNA damage produced by reactive oxygen species (ROS) induced by IR, 8-hydroxy-guanine is a well-investigated purine lesion which induces G:C → T:A transversion mutations via mispairing with adenine during DNA replication (Boiteux and Radicella 1999). In addition, various pyrimidine modifications were generated via ROS attack, e.g., the formamide remnant

derived from pyrimidine bases and the thymine glycol modification of thymine base (Boiteux et al. 1992; Iijima et al. 2009). Oxidative damage in DNA is repaired primarily via base excision repair (Demple and Harrison 1994; David and Williams 1998; Barzilai and Yamamoto 2004). In this pathway, DNA glycosylases recognize damaged or altered bases and cleave the glycosidic bond. Then, apurinic–apyrimidinic (AP) endonucleases incise the backbone of the DNA before the repair of the DNA by DNA polymerase and DNA ligase (Krokan et al. 1997; Daley et al. 2010).

As a bifunctional enzyme that contains both glycosylase and AP lyase activities, the homologs of endonuclease III (Nth) appear in all three phylogenetic domains (Eisen and Hanawalt 1999). Archaeal homologs from *Pyrobaculum aerophilum* (PaNth) and *Archaeoglobus fulgidus* (AfNth) had been characterized as similar to eukaryotic homologs from yeast (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*), bovine, mouse, and human (Watanabe et al. 2005). Structurally, the HhH DNA *N*-glycosylase superfamily, to which Nth belong, contain a helix–hairpin–helix (HhH) motif for DNA binding, a proline/glycine-rich loop (GPD motif), and an iron–sulfur cluster loop (FCL motif) which contains four cysteine residues for binding of a [4Fe–4S] cluster (Yang et al. 2001).

The *nth* mutants in *E. coli* were shown to about as resistant as wild-type cells to IR and $H_2O_2$, and had a weak mutator effect (Cunningham and Weiss 1985). Three homologs of putative Nth are present in *D. radiodurans*: DR0928 and DR2438 are of archaeal type, and DR0289 is close to yeast protein (Makarova et al. 2001). The three homologs of Nth raised the question of whether their functions are essential for repair endogenous oxidative DNA damage or if these genes serve as mutual backup functions. To understand the role of Nth in oxidative repair and mutagenesis, we constructed *nth* single, double and triple mutations, respectively measured the cell survival rate of *nth* mutants, and characterized these strains with regard to mutation rates and mutation spectrum.

## Materials and methods

### Bacterial strains, media and antibiotics

Restriction enzymes, T4 ligase, and Taq DNA polymerase were purchased from Takara Bio Inc. (Otsu, Shiga, Japan). All *D. radiodurans* cultures were grown at 30 °C in TGY media (0.5 % bacto tryptone, 0.1 % glucose, and 0.3 % bacto yeast extract) with aeration or on TGY plates supplemented with 1.3 % agar. Rifampicin (Rif; Xiamen Sanland Chemicals Co. Ltd., China) was dissolved in dimethyl sulfoxide (Sigma Chemical Co, St Louis, MO, USA). Kanamycin, chloramphenicol and streptomycin were purchased from Sangon Biocompany (Shanghai, China).

### Construction of *D. radiodurans* mutants

The mutants were generated in a three-step gene splicing by overlap extension technique (Horton et al. 1989). Briefly, to generate MK1, we constructed a DNA fragment based on wild type, in which the entire coding region of the *dr0289* gene was replaced with a chloramphenicol-resistance cassette under the control of a constitutively expressed *D. radiodurans kat* promoter. The DNA fragment was transformed into *D. radiodurans* R1. MK1 was selected on TGY agar supplemented with 3 μg/mL chloramphenicol. The mutant was confirmed by PCR and DNA sequencing.

To generate MK2, we generated a fragment of genomic DNA in which a kanamycin-resistance cassette was fused upstream and downstream of *dr0928*. The DNA fragment was transformed into *D. radiodurans* R1. MK2 was selected on TGY agar supplemented with 20 μg/mL kanamycin. The mutant was confirmed by PCR and DNA sequencing.

MA3 was generated as a fragment of genomic DNA in which a streptomycin-resistance cassette was fused upstream and downstream of *dr2438*. The DNA fragment was transformed into *D. radiodurans* R1. MA3 was selected on TGY agar supplemented with 8 μg/mL streptomycin. The mutant was confirmed by PCR and DNA sequencing. Strain ME2 was constructed by inactivating *dr0928* in MK1. Strain MT2 was constructed by inactivating *dr2438* in MK2. Strains ME3 was constructed by inactivating *dr2438* in ME2.

### Phenotypic characterization of *nth* mutants

For assays with $\gamma$-rays, cell suspensions (200 μL) of various *nth* strains were irradiated at room temperature for 24 h with $^{137}$Cs $\gamma$-rays at various distances from the source, which correspond to doses from 0 to 8 kGy. After irradiation, the various *nth* strains were plated on TGY plates and incubated at 30 °C for 3 days prior to colony enumeration. Various concentrations of $H_2O_2$ were added to the growing cultures. Samples were immediate diluted and plated on TGY agar as previously described (Huang et al. 2007). Survival rates in the assays are calculated as a percentage of the number of colonies obtained with untreated cells.

### Measurement of mutation frequency and rate

Mutation frequency assays were as previously described (Kim et al. 2004; Hua et al. 2008). The calculation of mutation rate followed the method of Drake (1991). The differences in mutation frequencies and rates were tested by Student's two-tailed *t* test. Colonies in the Rif$^r$ TGY

**Table 1** Oligonucleotides used for PCR and sequencing

| Name of the primes | Sequence (5′–>3′) |
|---|---|
| rpoB1S1 | AAACTGTGCCGATGGTGGAC |
| rpoB1A1 | TAGCTCACGCGGCCATTCAC |
| rpoB1seq | CATGCTGCTCGGCAACCC |
| rpoB2S1 | TCTTTCCCATCGACGAGTCC |
| rpoB2A1 | CACGATGGGGCGGTTGTT |

**Table 2** Strains used in this study

| Strain | Description | Reference(s) or source |
|---|---|---|
| *Deinococcus radiodurans* | | |
| R1 | Wild type | ATCC |
| MK1 | As R1 but △*dr0289::chlo* | This study |
| MK2 | As R1 but △*dr0928::kana* | This study |
| MA3 | As R1 but △*dr2438::aad* | This study |
| ME2 | As MK1 but △*dr0928::kana* | This study |
| MT2 | As MK2 but △*dr2438::aad* | This study |
| ME3 | As ME2 but △*dr2438::aad* | This study |
| *Escherichia coli* TG1 | *supE hsd*△*5 thi* △*(lac-proAB)*/F' [*traD36 proAB*$^{+}$*lac I*$^{q}$ *lacZ*△*M15*] | Takara |

plates were used for isolating genomic DNA and PCR sequencing.

### DNA isolation and sequencing

DNA isolation was as previously described (Hua et al. 2008). The primers (Table 1) were used to amplify the DNA for sequencing. Most mutations were in the PCR product of rpoB1S1 and rpoB1A1, and the rest in the region obtained with rpoB2S1 and rpoB2A1. The PCR products were purified and sequenced by Jinsite Biotechnology (Nanjing, China), using sequencing primer rpoB1-seq (5′ position 1221) and rpoB2seq (5′ position 323).

## Results

### Phenotypic characterization of *nth* mutants

To identify the role of Endonuclease III in *D. radiodurans*, we constructed six *nth* mutants, including three single knockout, two double knockout and one triple knockout (Table 2). We measured the cell survival rate of *nth* mutants under the stress of $\gamma$-rays and $H_2O_2$. As shown in Fig. 1, the survival rate of *nth* mutants were not significantly altered compared to wild-type when exposed to

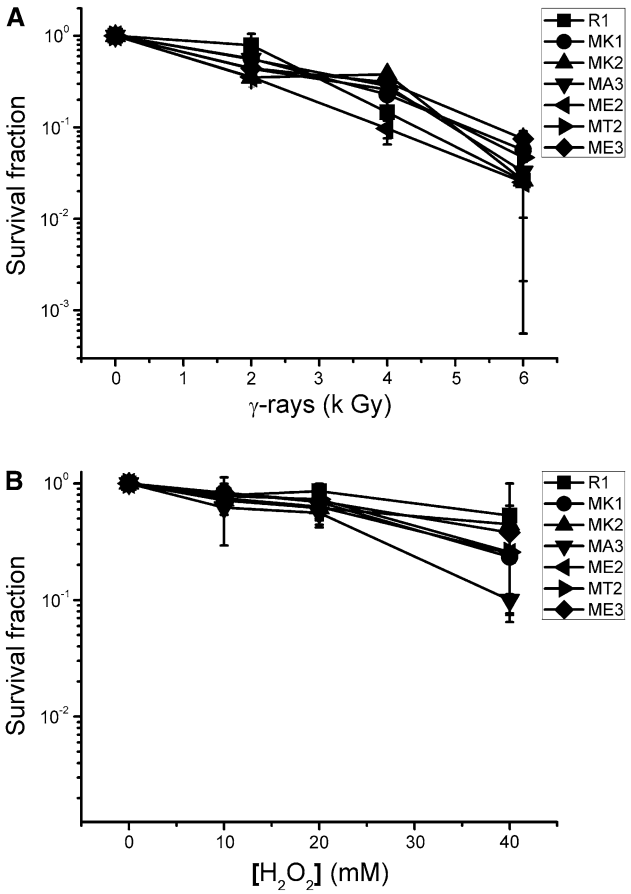


**Fig. 1** Phenotypic characterization of *nth* mutants with ionizing radiation and DNA damaging agent. **a** $\gamma$-rays and **b** $H_2O_2$

$\gamma$-radiation. We observed the same results in $H_2O_2$ survival assay.

### 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 (Table 3) by Drake's method (Drake 1991). Of three single mutants, MK2 and MA3 strains had 1.75-fold and 1.26 higher mutations rate than wild type, respectively, while the Rif$^{r}$ frequency of MK1 was 3.3-fold higher than wild type. ME2, the combination of MK1 and MK2, was 2.35-fold higher than wild type in mutation rate. The combination of MK2 and MA3, MT2, by contrast, did not show any mutator phenotype. Strikingly, the triple mutant of *nth* (ME3) generated a mutation frequency 34.3-fold, and a mutation rate 13.8-fold higher than the wild type.

### Distribution of mutation sites leading to Rif$^{r}$ expression in the six strains

We isolated Rif$^{r}$ mutants from *D. radiodurans* wild type, MK1, MK2, MA3, ME2, and ME3 and sequenced the *rpoB*

regions of their genomes. Table 4 shows the results of 197 mutations which activate the Rif$^r$ phenotype, included 50 mutations in wild type, 22 mutations in MK1, 26 in MK2, 26 in MA3, 28 in ME2, and 43 in ME3.

**Table 3** *D. radiodurans* rpoB mutation frequencies ($f$) and rates ($\mu$) $\times 10^{-8}$

| Strains | $f$ | $\mu$ |
|---|---|---|
| R1 | 2.46 (0.06–4.86)[a] | 1.12 (0.49–1.75) |
| MK1 | 6.77 (3.16–10.38) | 3.73 (1.98–5.48) |
| MK2 | 4.45 (1.03–7.86) | 1.96 (0.46–3.46) |
| MA3 | 3.55 (1.37–5.71) | 1.42 (0.80–2.05) |
| ME2 | 6.02 (2.27–9.77) | 2.64 (1.28–4.00) |
| MT2 | 2.3 (1.02–3.57) | 0.85 (0.56–1.15) |
| ME3 | 84.47 (24.2–144.92) | 15.50 (6.43–24.58) |

Rates are calculated by the method of Drake (1991)

[a] 95 % confidence limits

## Spontaneous mutation hotspots in mutator strains and wild type of *D. radiodurans*

We detected two types of deletions of 9 bp and three types of base substitution hotspots in the wild-type background. Most mutation types were mentioned in Kim et al. (2004). One of the base-substitution hotspots was (G:C → A:T) at position 1273 (13 of 50, 26 %). There was also another base-substitution hotspot at 1259 (A:T → C:G), with an incidence of 20 % (10 of 50). In addition, deletion reached 16 % (8 of 50).

MK1 showed three hotspots at positions 1259 (A:T → C:G), 1273 (G:C → T:A) and 1303 (G:C → A:T); these three hotspots accounted for 19 of 22 base substitutions (77 %). We determined a previously unreported *rpoB* mutation at position 1313 (G:C → A:T) in MK1 strain. MK2 conserved one hotspot at position 1259 (A:T → C:G) of the wild type, and there was another hotspot at position 1273 (G:C → A:T); these two were 50 % of hotspots (13 of

**Table 4** Distribution of mutations leading to Rif$^r$ in *D. radiodurans*

| *D. radiodurans* site (bp) | Amino acid change | Base-pair change | Wild type | MK1 | MK2 | MA3 | ME2 | MT2 | ME3 | *E. Coli* site |
|---|---|---|---|---|---|---|---|---|---|---|
| 1252 | S418P | AT → GC[a] | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1525 |
| 1273 | D425N | GC → AT | 13 | 0 | 6 | 6 | 1 | 0 | 4 | 1546 |
| 1303 | H435Y | GC → AT | 0 | 5 | 3 | 4 | 7 | 0 | 25 | 1576 |
| 1313 | R438H | GC → AT | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1586 |
| 1319 | S440F | GC → AT | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 1592 |
| 1327 | G443R | GC → AT | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1600 |
| 1411 | E471K | GC → AT | 8 | 0 | 2 | 4 | 5 | 0 | 1 | 1684 |
| 1418 | P473L | GC → AT | 0 | 0 | 0 | 0 | 1 | 1 | 9 | 1691 |
| 1435 | G479S | GC → AT | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1708 |
| 457 | I153F | AT → TA[a] | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 436 |
| 1265 | Q422L | AT → TA | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1538 |
| 1274 | D425V | AT → TA | 1 | 0 | 0 | 3 | 2 | 0 | 0 | 1547 |
| 1304 | H435L | AT → TA | 3 | 0 | 1 | 0 | 0 | 1 | 0 | 1577 |
| 1325 | L439Q | AT → TA | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1598 |
| 1441 | I481F | AT → TA | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1714 |
| 1259 | L420R | AT → CG | 10 | 8 | 7 | 1 | 6 | 5 | 3 | 1532 |
| 1304 | H435P | AT → CG | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1577 |
| 1441 | I481L | AT → CG | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1714 |
| 1443 | I481M | AT → CG[a] | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1716 |
| 1273 | D425Y | GC → TA | 0 | 4 | 1 | 0 | 0 | 4 | 0 | 1546 |
| 1303 | H435Q | GC → TA | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1576 |
| 1250–1258 | | 9-bp deletion | 7 | 1 | 1 | 3 | 2 | 3 | 0 | 1523–1531 |
| 1258–1266 | | 9-bp deletion | 1 | 1 | 1 | 3 | 1 | 3 | 0 | 1531–1539 |
| 1287–1292 | CAACCC | 6-bp insertion | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1259–1265 |
| 1274–1288 | TCTCGCAGT TCAAGG | 15-bp insertion | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1246–1260 |
| Total | | | 50 | 22 | 26 | 26 | 28 | 23 | 43 | |

The DNA sequence change in *rpoB* was determined in each case

[a] The corresponding base and base change is different between *D. radiodurans* and *E. coli*

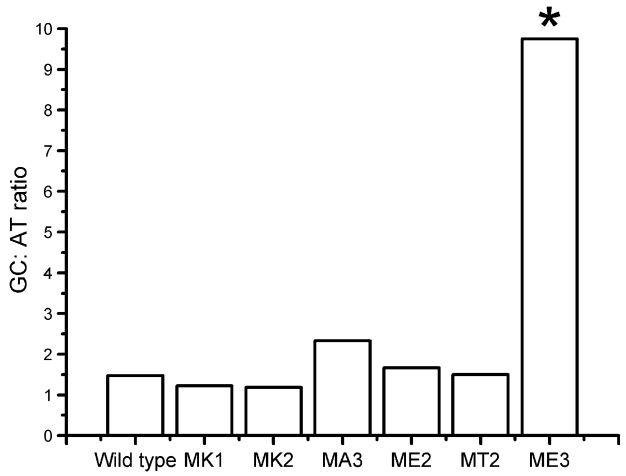


**Fig. 2** Histogram showing the ratio of GC:AT per mutated *rpoB* sequences determined from wild-type and mutant strains of *D. radiodurans*. The *black asterisks* are placed on histograms where the ratio was significantly different ($P < 0.05$) from wild type by Fisher's exact test

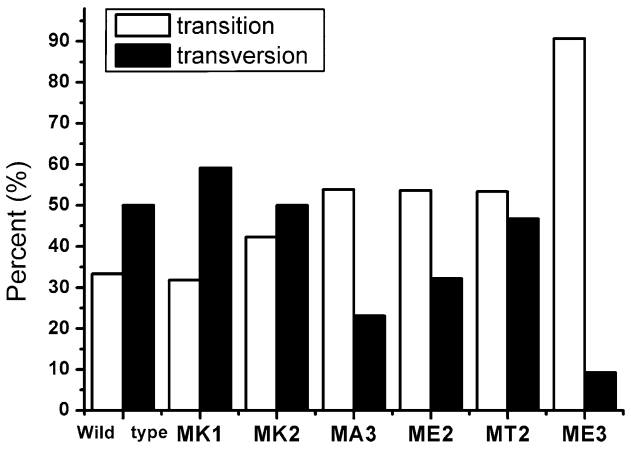


**Fig. 3** Comparison of percentage of transitions and transversions in *rpoB* in *D. radiodurans* mutants and wild type. *Open squares* transition mutations; *filled squares* transversion mutations

26). In the MA3 strain, there were three hotspots that were all transitions (53.8 %) at position 1273 (G:C → A:T), 1303 (G:C → A:T) and 1411 (G:C → A:T); at the same time, deletion reached 23% (6 of 26). As the combination of MK1 and MK2, ME2 showed three hotspots at positions 1303 (G:C → A:T), 1411 (G:C → A:T) and 1259 (A:T → C:G), representing 64.3 % (18 of 28). In addition, we found a previously unreported *rpoB* insertion mutation at positions 1286–1292 (CAACCC). As the combination of MK2 and MA3, MT2 showed two hotspots at positions 1259 (A:T → C:G) and 1273 (G:C → T:A). We also found a *rpoB* insertion mutation at position 1274–1288 (TCTC GCAGTTCAAGG). For ME3 strains, most mutations were transitions (90 %, 39 of 43), while the remaining four mutations were both transversions (A:T → C:G).

### Strain-specific mutation hotspots in *rpoB*

The strains differed significantly in respect to relative mutation frequencies of five different base substitutions. Base substitution (G:C → A:T) may be the best example of the different mutation rates in the six strains. The knockout of three *nth* homologs significantly increased the mutation at G:C base pairs (Fig. 2).

For substitutions at base pairs, there were clear differences between wild-type and mutant strains (Table 4). Mutation in wild type was predominantly in AT base pairs. In contrast, mutant strains generated mutations at GC base pairs much more frequently than at AT base pairs. The rate of mutation in GC base pairs in mutant strains correlated with the increase of knock-out genes. In wild type and the two single mutants, MK1 and MK2, transversion was predominant (Fig. 3). However, the majority of mutations in single mutant MA3 were transition. It was puzzling that ME2, the combination of MK1 and MK2, had a contrasting result to MK1 and MK2. The combination of MK2 and MA3, MT2 showed a mixed result of MK2 and MA3. The vast majority of spontaneous base substitutions in *rpoB* that lead to $Rif^r$ in the triple mutant ME3 were transitions.

## Discussion

In this work, we created *nth* mutator strains by constructing the following knockout mutants: *dr0289* (MK1), *dr0928* (MK2), *dr2438* (MA3), and the double knockout *dr0289 dr0928* (ME2), *dr0928 dr2438* (MT2) and the triple mutant *dr0289 dr0928 dr2438* (ME3). We measured the cell survival rate of *nth* mutants. The *nth* mutants showed no significant difference with wild type in both IR resistance and $H_2O_2$ resistance. *Nth* mutant in *E. coli* was shown as resistant as wild type (Cunningham and Weiss 1985). The result of survival assay in *D. radiodurans* indicated that there was a backup system to BER for DNA repair in *D. radiodurans*. NER may serve as a backup system when the major BER pathways are inactivated (Dianov et al. 1998).

Kim et al. (2004) developed an *rpoB*/$Rif^r$ mutation analysis system for *D. radiodurans* based on assays measuring the frequency of forward mutations to resistance to rifampicin. The $\beta$ 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 (Garibyan et al. 2003). This mutation analysis system had been widely used (Garibyan et al. 2003; Wolff et al. 2004; Miller et al. 2002; Davidsen et al. 2005; Meier and Wackernagel 2005; Morlock et al. 2000; Anthony et al. 2005; Wang et al. 2001; Nicholson and Maughan 2002; Maughan et al. 2004;

O'Sullivan et al. 2008). Furthermore, Kim et al. (2004) analyzed 185 spontaneous, 33 NTG-induced, 195 AZ-induced, and 17 *uvrD* mutations, and defined 33 base substitutions at 22 different mutational sites (base pairs) in *D. radiodurans*. We extended this system by increasing two base-substitution sites and two insertion sites. The insertion site could be explained by an Okazaki fragment forming a hairpin structure, and allowing one region of the template to be replicated twice, leading to expansion (Gordenin et al. 1997).

A constructed *E. coli nth* mutant showed a mutator effect (4- to 22-fold enhancement) via Arg + revertant assay (Cunningham and Weiss 1985). Another study reported that a *nth* mutant had a 7.7-fold higher mutation frequency than wild type in *E. coli* via Arg + His + revertant assay (Jiang et al. 1997). The Nth homologs in yeast, *ntg1* and *ntg2* single mutants, had considerably increased spontaneous mutation frequency, which was further increased in the double mutant (Gellon et al. 2001). Downregulation of the Nth homolog in mammals, NEIL1, enhanced spontaneous mutation in the Hprt locus by about three-fold in both Chinese hamster V79 and human bronchial A549 cell lines. The mutant frequency was further enhanced (7- to 8-fold) under oxidative stress (Maiti et al. 2008). We measured mutation rates of *nth* mutators in *D. radiodurans*. MK1, MK2 and MA3 were weak mutators, although they enhanced the effect when they were part of the ME3 triple mutant. The elevated levels of spontaneous mutation for MK1, MK2 and MA3 demonstrated that the three *nth* homologs were required to repair endogenous oxidative DNA damage. The higher mutation rate in the triple mutant ME3 suggested that the three enzymes had non-overlapped substrates in oxidative DNA repair.

Each single mutant showed higher spontaneous mutation frequency than wild type at base substitution (G:C → A:T). This result is consistent with previous reports (Cunningham and Weiss 1985; Najrana et al. 2000; Saito et al. 1997) that *nth* mutants in both *D. radiodurans* and *E. coli* showed mutator phenotypes of G:C → A:T transitions (Najrana et al. 2000). The mutational response was further increased in the *nth* double and triple mutants. The stimulation in *nth* mutants suggested involvement of *nth* in the G:C → A:T transition (Najrana et al. 2000). The mutational spectrum strongly suggested that oxidized DNA bases, presumably oxidized cytosine, were the major target of *nth* homologs in *D. radiodurans* (Gellon et al. 2001). The G:C → A:T transition mutation was probably caused by a repair defect of uracil glycol, 5-OHC and 5-OHU (Najrana et al. 2000). All the three damaged bases are derived from cytosine, and are known to mispair with adenine (Purmal et al. 1994, 1998). They are expected to be potent mutagenic lesions leading to G:C → A:T transitions (Najrana et al. 2000).

In wild type and the two single mutants, MK1 and MK2, transversion was predominant. ME2, the combination of MK1 and MK2, had a contrasting result to MK1 and MK2. The preference reversal of transversion and transition between MK1/MK2 and ME2 might be explained by followed. It is possible that DR0289 and DR0928 shared a similar substrate spectrum which caused transition mutation, but not the transversion mutation. Then, transversion mutation became dominant when either of DR0289 and DR0928 was knockout. However, the transition mutation was out of control in ME2 cell. ME2 also contributed to 6-bp insertion found in the *rpoB* gene. The 6-bp insertion mutation was a duplication of the 6 nucleotides comprising nucleotides 1287–1292 of *rpoB* that did not disrupt the correct reading frame. A decrease in DNA damage repair capacity would lead to aberrant Okazaki fragment processing in ME2 mutant due to the defect of DR0289 and DR0928. The aberrant Okazaki fragment processing might cause the long-range replication slippage errors and replication error or damage. And this error or damage which was repaired by recombination resulted in the insertion mutation (Tishkoff et al. 1997). The combination of MK2 and MA3, MT2 showed a mixed result of MK2 and MA3. The result indicated that Dr0928 and Dr2438 only had a little overlap in the substrate spectrum of Nth.

Why does *D. radiodurans* possess three homologs of *E. coli* endonuclease III? One possibility is that three nth homologs in *D. radiodurans* may repair oxidative DNA base damage together. The other glycosylase in *D. radiodurans* also has multiple homologs such as alka (DR2074, DR2584), ung (DR0689, DR1663) and mug (DR0715, DR1751, DR0022) (Makarova et al. 2001). DR0689, DR1751 and DR0022 were identified in *D. radiodurans* (Sandigursky et al. 2004). Multiple glycosylase homologs were also found in *Saccharomyces cerevisiae* and mammals (You et al. 1998; van der Kemp et al. 1996; Huffman et al. 2005); they showed differences in their regulation and substrate specificity (You et al. 1998; Huffman et al. 2005). An additional possibility is that the three *nth* homologs in *D. radiodurans* have different transcription modes. NTG1 is DNA damage inducible. In contrast, NTG2 is not induced to any significant extent in yeast (Alseth et al. 1999). Similarly, DR0928 and DR2438 were DNA damage-inducible. In contrast, DR0289 is not induced to any significant extent (Chen et al. 2007). S-phase-specific activation of NEIL1 raises the possibility that it is involved in repair of the damage present in the replication bubble (Dou et al. 2003). In contrast, NEIL2, independent of cell cycle expression, could be involved in transcription-coupled repair (TCR) (Dou et al. 2003). Similarly, Nth homologs in *D. radiodurans* might take different functions in vivo. It is possible that three nth homologs shared a similar substrate spectrum which caused GC to AT

transition mutation, but not the transversion mutation. The shared substrate spectrum explained the increase in mutation rate and the changes in mutation spectrum. The nth homologs had overlapped functions in substrates. The higher mutation rate and mutational response in ME3 suggested that the three *nth* homologs had non-overlapped and overlapped substrate spectrum in endogenous oxidative DNA repair.

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**Conflict of interest** None.

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