Extracellular dGMP Enhances *Deinococcus radiodurans* Tolerance to Oxidative Stress

Mingfeng Li[1,9], Hongxing Sun[1,2,3,9], Qiong Feng[1], Huiming Lu[1], Ye Zhao[1], Hui Zhang[1], Xin Xu[1], Jiandong Jiao[1], Liangyan Wang[1]*, Yuejin Hua[1]*

1 Key Laboratory for Nuclear-Agricultural Sciences of Chinese Ministry of Agriculture and Zhejiang Province, Institute of Nuclear-Agricultural Sciences, Zhejiang University, Hangzhou, China, 2 Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiao Tong University School of Medicine, Shanghai, China, 3 Shanghai Institute of Immunology, Institutes of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Abstract

Free extracellular DNA provides nutrition to bacteria and promotes bacterial evolution by inducing excessive mutagenesis of the genome. To understand the influence of extracellular DNA fragments on *D. radiodurans*, we investigated cell growth and survival after extracellular DNA or dNMPs treatment. The results showed that the extracellular DNA fragments inhibited the growth of *D. radiodurans*. Interestingly, dGMP, a DNA component, enhanced *D. radiodurans* tolerance to H_2O_2 and gamma-radiation significantly. Further experiments indicated that extracellular dGMP stimulated the activity of one catalase (KatA, DR1998), and induced gene transcription including *the extracellular nuclease* (*drb0067*). When this only extracellular nuclease gene (*drb0067*) in *D. radiodurans* was deleted, the mutant strain showed more sensitive to H_2O_2 and gamma-radiation than the wild type strain. These results suggest that DRB0067 plays an important role in oxidative stress resistance. Taken together, we proposed a new anti-oxidation mechanism in *D. radiodurans*. This mechanism acts to increase expression levels of DRB0067 which then secretes active nuclease to degrade extracellular DNA fragments. The extracellular nuclease has a two-fold benefit, creating more free dNTPs for further cell protection and the removal of extracellular DNA fragments.

Introduction

Bacteria cell death releases cytoplasmic contents, including DNA components into the microenvironment [1]. In addition, many living bacteria such as *Acinetobacter*, *Azotobacter*, *Bacillus*, *Deinococcus*, *Neisseria* and *Pseudomonas* release DNA into the surrounding environment during cell growth [1–6]. These bacteria benefit in several ways from free extracellular DNA and its degradation product [7–11]. For instance, the uptake of extracellular DNA, from the same or different organisms, promotes the evolution of bacteria. This occurs via horizontal gene transfer, such as transformation, transduction, or conjugation between bacteria [7,8]. Extracellular DNA is also required for the initial establishment of bacterial biofilms, such as in *Pseudomonas aeruginosa*. The degradation of extracellular DNA by DNase I can strongly inhibit biofilm formation [9,10]. Extracellular DNA, both homospecific and heterospecific, is known as an important nutrient source for organisms [11]. However, if extracellular DNA is not degraded immediately, it can threaten the survival of organisms by reincorporating damaged bases into the genome [12]. In most case, extracellular DNA components are degraded by extracellular nucleases secreted by many kinds of bacteria [13–

16]. As a result, a threat to the organism is removed, and its by-products, dNMPs, are a nutrient source for bacteria [11,12,16].

Deinococcus radiodurans are extremely resistant to ionizing radiation, UV radiation, hydrogen peroxide and desiccation [12,17–21]. The high resistance of this bacterium to reactive oxygen species (ROS) results from the strong ability of oxidative resistance [22] and an efficient DNA repair mechanism [23,24]. Ionizing radiation [17] or UV [25] radiation attacks intracellular DNA producing large amounts of damaged oligonucleotides within the nucleotide pool [26]. These damaged oligos are exported into the surrounding medium and finally degraded [27]. However, it is not known if *D. radiodurans*, the most ionizing radiation resistant bacteria, has the ability to reuse these damaged extracellular DNA fragments.

Recently, Daly *et al.* demonstrated that *D. radiodurans* ultrafil-trate, which was enriched in Mn, phosphate, peptides, nucleosides and bases, could protect proteins from ionizing radiation-induced ROS damage [28,29]. These findings implied that degradation and re-absorption of damaged DNA components might contribute to this organism's extreme ROS resistance. Here, we investigated the effects of extracellular DNA fragments and dNMPs on cell

growth, H_2O_2 resistance, as well as UV and gamma-radiation in both *D. radiodurans* and *E. coli*. Our results indicated that the uptake of extracellular DNA fragments represented a new mechanism of protection from oxidative damage.

Results

Extracellular DNA fragments inhibit the growth of *D. radiodurans* but not *E. coli*

Free extracellular DNA is abundant in the environment, and its existence may have an effect on the growth of bacteria. To understand this effect, we grew *D. radiodurans* and *E. coli* cells in the presence or absence of 3.6 mg/ml DNA fragments or dNMPs. We observed large amounts of DNA fragments resulted in a distinct growth inhibition of *D. radiodurans*. Absorption reading (OD_{600}) was 0.26 after 10 hours in the presence of DNA fragments, while control cells OD_{600} reading was 2.38 and that with dNMPs treatment was 2.19 (Fig. 1A). In *E. coli*, the OD_{600} values of control, DNA fragments and dNMPs treated groups were almost the same ($OD_{600} \approx 2.3$) after 4 hours, which indicates neither DNA fragments nor dNMPs affect the growth of *E. coli* (Fig. 1B). These data suggested that DNA fragments and dNMPs have different effects on *D. radiodurans* and *E. coli* growth rates. Extracellular DNA fragments instead of dNMPs were harmful to *D. radiodurans* cells growth.

Extracellular dGMP greatly enhances *D. radiodurans* tolerance to H_2O_2

The influence of extracellular DNA fragments or dNMPs on *D. radiodurans* and *E. coli* cell survival under oxidative stress was evaluated. The presence of DNA fragments caused a modest decrease in H_2O_2 resistance in *D. radiodurans* (Fig. 2A). Similarly, the presence of DNA fragments did not have an obvious effect on *E. coli* resistance to H_2O_2 as well (Fig. 2B). However, the survival rate of *D. radiodurans* was dramatically increased when 10 mM dNMPs was present (Fig. 2A). There was a 33-fold increase in survival when compared to samples without dNMPs treatment. In *E. coli*, no distinct difference was observed between either the DNA fragments or dNMPs treatment groups (Fig. 2B). To understand which dNMPs accounted for this effect, dAMP, dTMP, dCMP and dGMP were separately added to *D. radiodurans* growths. Here

we observed only dGMP had an effect, which dramatically increased H_2O_2 resistance by approximately 57-fold (Fig. 3). In addition, dGMP enhanced the resistance to gamma-radiation, but not UV (Fig. S1A). In sum, extracellular dGMP has an important role in *D. radiodurans* anti-oxidation, but not in *E. coli*.

Extracellular dGMP induces KatA activity

In *D. radiodurans*, catalases and SODs protect proteins from ROS-mediated damage *in vivo* [30]. PAGE activity-staining assay reveals that *D. radiodurans* stain possesses activity corresponding to two catalases and one SOD, and stains that carry mutations in these genes (*katA and sodA*) are more sensitive to ionizing radiation than wild type [30]. In order to understand how dGMP increases *D. radiodurans* tolerance to H_2O_2, the activity change in catalases and SODs was measured after 2.5 mM dGMP was added to growth medium by PAGE activity-staining assay. The additional dGMP enhanced whole cell KatA activity by ~50%, but had no effect on KatB or any of the SODs assayed (Fig. 4A/4B). It is possible that extracellular dGMP increases *D. radiodurans* tolerance to H_2O_2 by inducing KatA catalase activity *in vivo*.

Extracellular dGMP stimulates transcription of anti-oxidation related genes

To better understand extracellular dGMP's involvement in anti-oxidation, the expression patterns of ROS response genes were investigated using Real-time quantitative PCR (Table 1). The addition of dGMP increased the transcriptional level of *katA* gene (*dr1998*) about 2.8-fold, but not any other catalase or SOD genes, which agrees with the results from the PAGE activity-staining assay. In other words, extracellular dGMP induces the activity of KatA by increasing the expression level of KatA *in vivo*.

The high intracellular Mn/Fe ratio in *D. radiodurans* could contribute to its remarkable resistance to environmental stresses [28,29]. Here, the extracellular dGMP also increased the transcription levels of genes regulating the intracellular Mn/Fe ratio. DR2244, a sensory transduction histidine kinase, was induced by extracellular dGMP, which might be a signaling response to oxidative stress. Considering the role of extracellular nuclease in degrading extracellular DNA, the transcription of *drb0067*, the extracellular nuclease gene in *D. radiodurans*, was also investigated. When extracellular dGMP was present, transcription

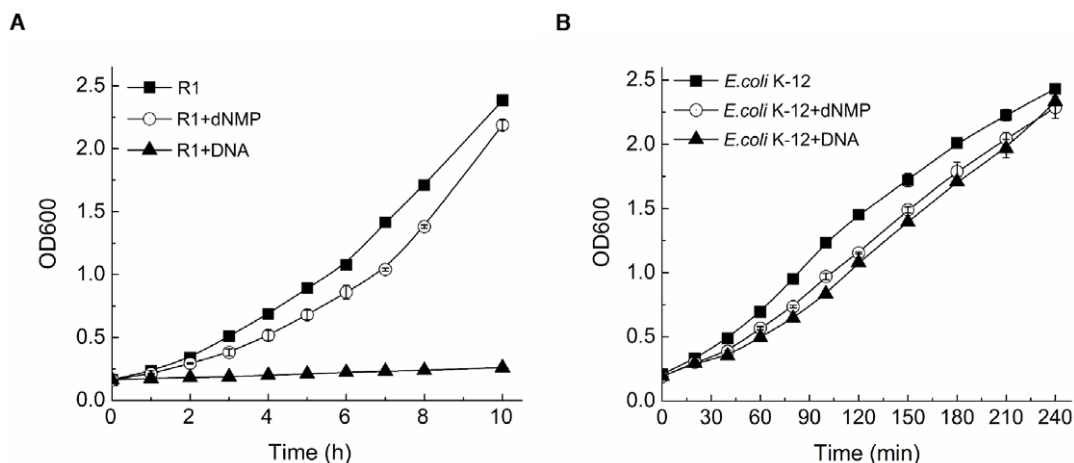**Figure 1. *D. radiodurans* and *E. coli* cell growth after DNA fragments or dNMPs treatment.** (A) Growth of *D. radiodurans* after the addition of 3.6 mg/ml DNA fragments or dNMPs. (B) Growth of *E. coli* K-12 after the addition of 3.6 mg/ml DNA fragments or dNMPs. Values are the mean ± standard deviation of three independent experiments. R1, *D. radiodurans* wild type strain.
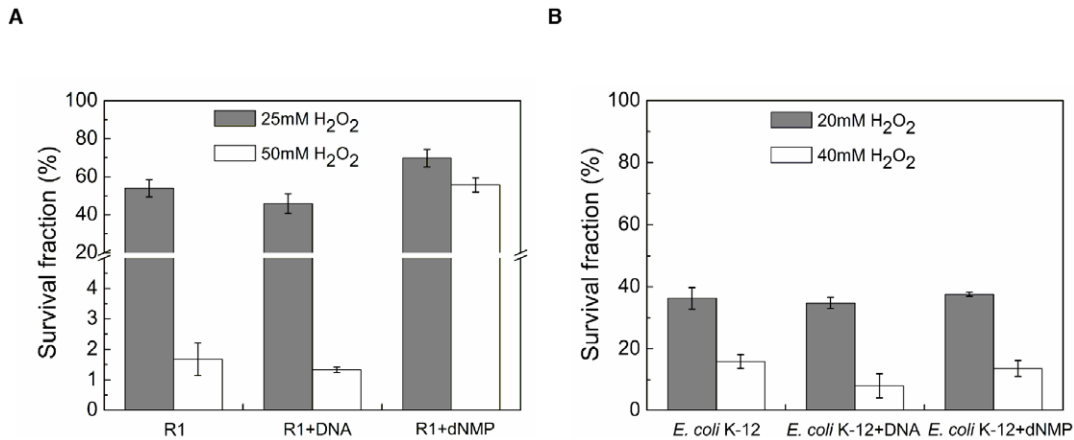doi:10.1371/journal.pone.0054420.g001

Figure 2. H$_2$O$_2$ sensitivity in *D. radiodurans* and *E. coli* treated with DNA fragments or dNMPs. (A) Sensitivity of *D. radiodurans* to H$_2$O$_2$ after the addition of 3.6 mg/ml DNA fragments or dNMPs. (B) Sensitivity of *E. coli* K-12 to H$_2$O$_2$ after the addition of 3.6 mg/ml DNA fragments or dNMPs. Each data point represents the mean±SD of three replicates. R1, *D. radiodurans* wild type strain.
doi:10.1371/journal.pone.0054420.g002

of *drb0067* was stimulated (2-fold), thus enhancing the degradation of extracellular DNA fragments and increasing the pool of dNMPs.

Drb0067 encodes the only extracellular nuclease in *D. radiodurans*

Upon investigation of the *D. radiodurans* genome, we found that *drb0067* is the only extracellular nuclease gene. To further explore the role of DRB0067 in *D. radiodurans*, a *drb0067* null mutant (Δ*drb0067*) was constructed and verified by PCR (Fig. 5A). To test the mutant and wild type strains ability to degrade extracellular DNA, each strain was inoculated onto DNase test agars plates. A distinct clear zone was observed surrounding the wild type cells, but not Δ*drb0067* (Fig. 5B). Moreover, there was no nuclease activity detected in the culture medium of Δ*drb0067* (Fig. 5C).
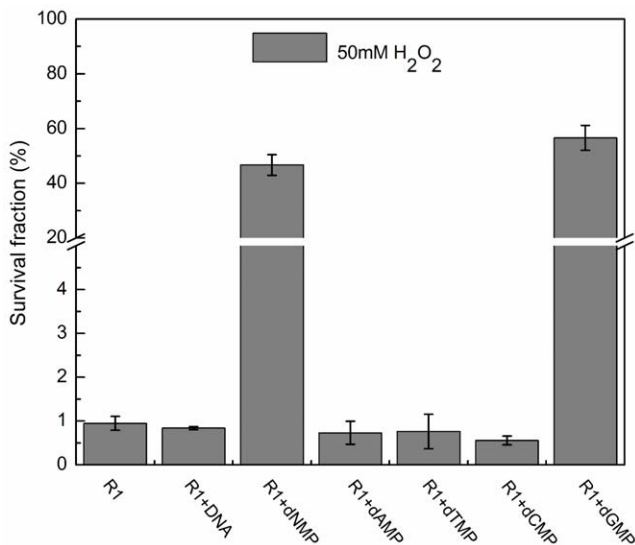

Figure 3. H$_2$O$_2$ sensitivity in *D. radiodurans* treated with dAMP, dTMP, dCMP or dGMP. Sensitivity of *D. radiodurans* to 50 mM H$_2$O$_2$ with the addition of DNA fragments (3.6 mg/ml), dNMPs, dAMP, dTMP, dCMP or dGMP (10 mM for each). Each data point represents the mean±SD of three replicates. R1, *D. radiodurans* wild type strain.
doi:10.1371/journal.pone.0054420.g003

Bases on these two assays, we demonstrated DRB0067 is the only extracellular nuclease in *D. radiodurans*. And this extracellular nuclease is secreted through the secretory pathway since the deletion of *secD/secF* gene (*dr1822*) inhibited the secretion of DRB0067 (data not shown).

The extracellular nucleases act as a modulator for natural transformation in some bacteria, such as *Vibrio cholerae* [16]. Next, we investigated the transformation frequency for the Δ*drb0067* strain and found the transformation efficiency was ~4.5-fold higher than the wild type strain (Fig. 5D). These results suggest that the DRB0067 protein is also an important modulator for natural transformation in *D. radiodurans*.

We next investigated the effect of DNA fragments on growth in the wild type and Δ*drb0067* strains. In the absence of extracellular DNA fragments, wild type and Δ*drb0067* strains had similar growth patterns except that the mutant strain (OD$_{600}$≈4.61) exhibited slightly lower OD readings than wild type (OD$_{600}$≈5.75) at the stationary phase. However, in the presence of DNA fragments, Δ*drb0067* grwoths were more sensitive than the wild type growths (Fig. 5E). Under these conditions, the wild type strain entered stationary phase after 29 hours (OD$_{600}$≈4.60), whereas Δ*drb0067* was still in logarithmic phase (OD$_{600}$≈2.45) at that time. Therefore, the presence of DNA fragments resulted in a severe cell growth decline in the Δ*drb0067* strain when compared to wild type.

Deletion of *drb0067* impairs H$_2$O$_2$ resistance of *D. radiodurans*

Our experiments indicated that the extracellular dGMP, not extracellular DNA, enhanced the resistance of *D. radiodurans* to H$_2$O$_2$ and gamma-radiation. To understand the role of DRB0067 in this process, the H$_2$O$_2$ resistance of wild type and Δ*drb0067* strains were measured. These results, which were expected, revealed the *drb0067* mutation to have a decreased resistance to H$_2$O$_2$. The survival rate of the Δ*drb0067* strain was 6 times lower than that of the wild type strain under 30 mM H$_2$O$_2$ treatment (Fig. 6A/6B). Furthermore, the Δ*drb0067* was more sensitive to gamma-radiation, though not UV radiation (Fig. S1B). Next, the effects of oligo(dG)50 and dGMP on Δ*drb0067* resistance were investigated with varying concentrations of H$_2$O$_2$. Here, we found that the addition of dGMP (2.5 mM) restored the mutant strain's resistance to H$_2$O$_2$. The survival fraction with dGMP treatment
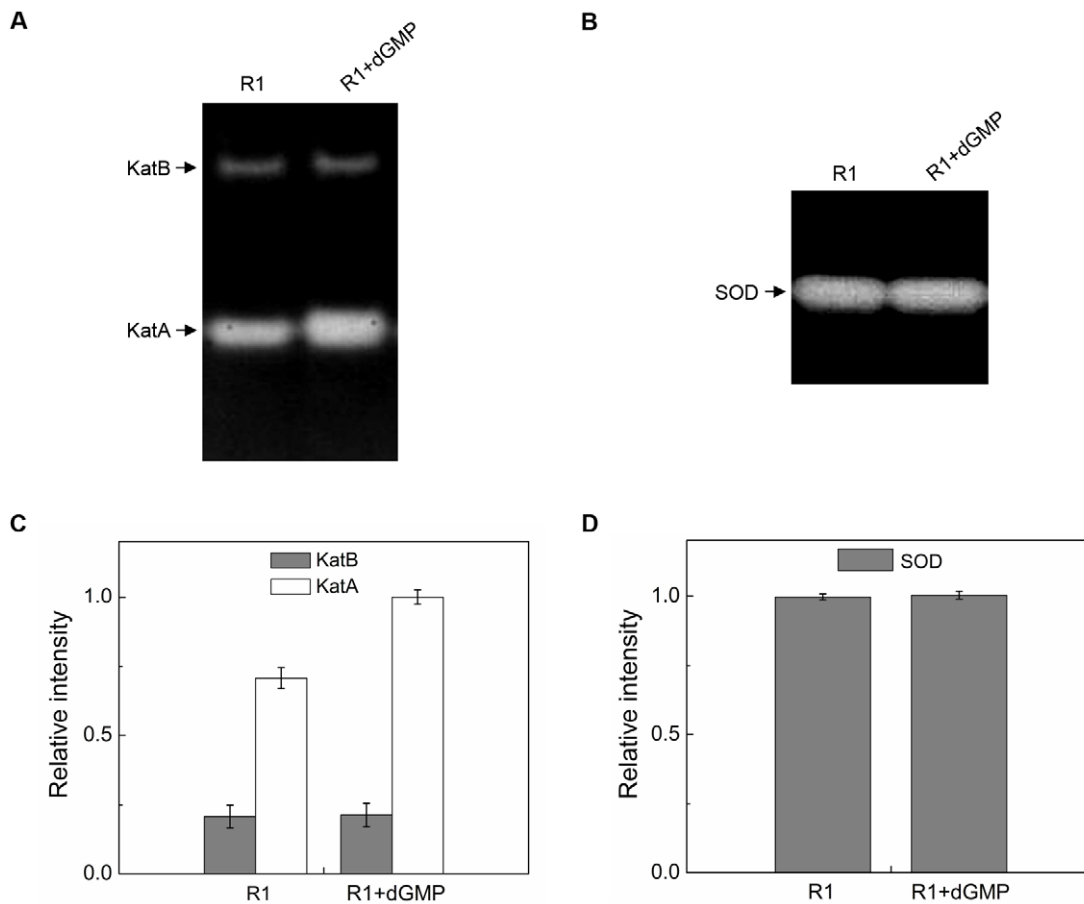
Figure 4. Addition of extracellular dGMP increases the activity of KatA in *D. radiodurans*. (A) Extracellular dGMP (2.5 mM) increased the activity of KatA, but not KatB. (B) Extracellular dGMP (2.5 mM) had no effect on the activity of SOD. (C) and (D) Quantification of the intensity of bands was performed using ImageJ. Each sample contains 80 μg of total protein. Values are the mean ± standard deviation of three independent measurements. R1, *D. radiodurans* wild type strain; KatA, catalase A; KatB, catalase B.
doi:10.1371/journal.pone.0054420.g004

Table 1. Influence of dGMP (2.5 mM) on *D. radiodurans* transcription levels.

ORF	Annotation	Fold	*p* value
catalase and sod genes			
DR1998	catalase	2.80	5.16E-06
DRA0146	catalase	1.01	0.85
DRA0259	catalase	0.65	2.12E-05
DR1279	Mn family superoxide dismutase	0.88	0.18
DR1546	Cu/Zn superoxide dismutase	0.95	0.29
DRA0202	Cu/Zn superoxide dismutase	0.73	0.78
other genes			
DR2283	manganese ABC transporter permease	2.98	1.79E-05
DR2523	Manganese/iron transport system substrate-binding protein	1.36	0.0003
DR2539	Mn-dependent transcriptional regulator	2.25	0.00014
DRB0016	iron complex transport system ATP-binding protein	2.79	0.0047
DRB0092	starvation-inducible DNA-binding protein	2.49	8.73E-05
DRB0121	iron ABC transporter, ATP-binding protein	1.62	1.70E-05
DRB0124	iron-chelator utilization protein, putative	9.71	2.43E-06
DRB0067	extracellular nuclease	2.00	0.0005
DR2244	sensory transduction histidine kinase	2.01	0.0032

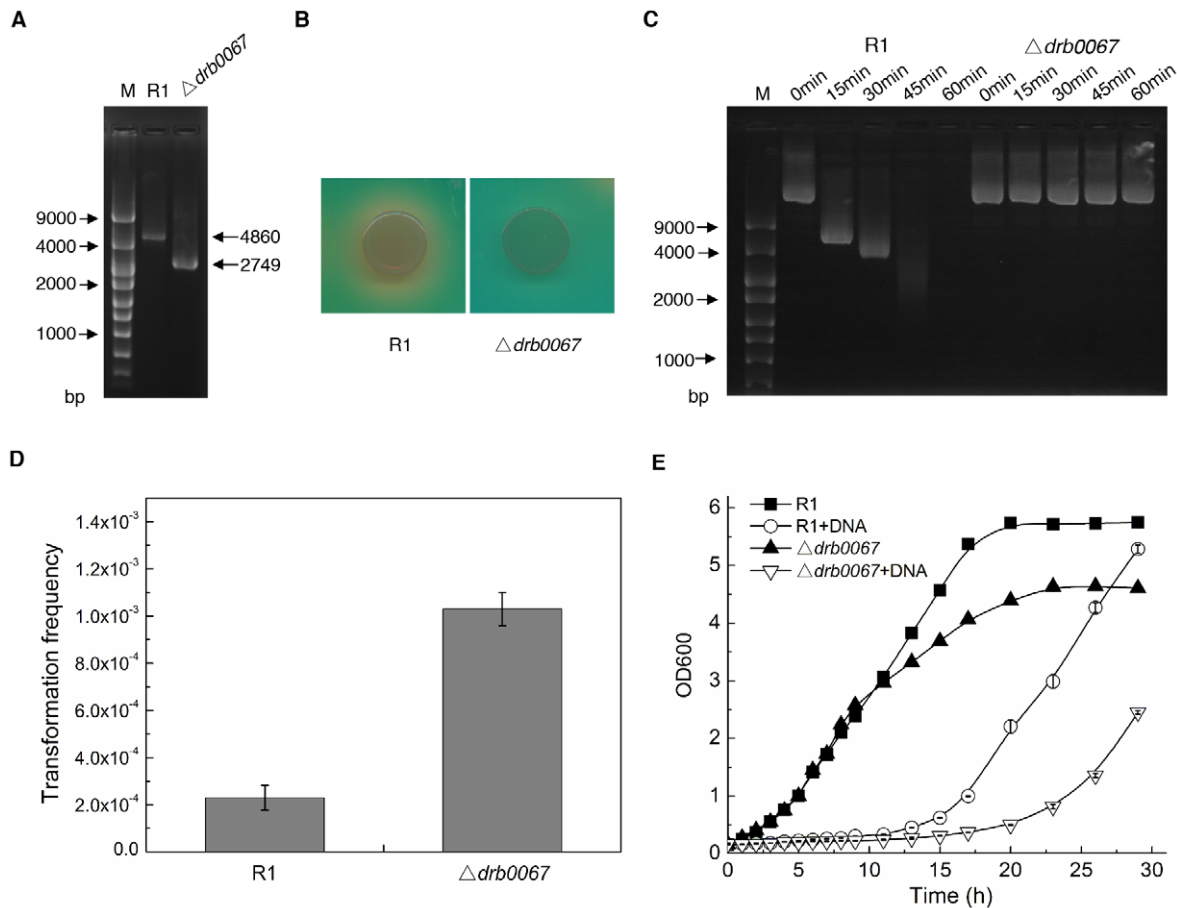doi:10.1371/journal.pone.0054420.t001

Figure 5. Disruption of *drb0067* gene and its phenotypes test. (A) PCR analysis of the mutant with primers 0067upF and 0067downR. (B) DNase Test Agar with Methyl Green to analyse R1 and Δ*drb0067*. 20 μl of cells (OD$_{600}$≈1.0) were dripped onto DNase test agars plates. (C) Enzymatic activity to analyse R1 and Δ*drb0067*. The cells were cultured in TGY until the OD$_{600}$≈2.5–3.0, and then centrifuged to collect the supernatant for enzyme reaction. (D) Transformation analysis of R1 and Δ*drb0067*. 1 μg pRADK plasmid was used for each transformation. (E) Growth of R1 and Δ*drb0067* with the addition of 3.6 mg/ml DNA fragments or dNMPs. M denotes molecular standards. All the experiments are performed three times and values are mean ± standard deviation. R1, *D. radiodurans* wild type strain; Δ*drb0067*, the *drb0067* null mutant.
doi:10.1371/journal.pone.0054420.g005

was about 4 times higher than that with oligo(dG)50 treatment, and 5 times higher than control under the stress of 30 mM H$_2$O$_2$ (Fig. 6C/6D). In addition, *D. radiodurans* secreted more active extracellular nuclease after gamma-radiation treatment (Fig. S2). These results suggested that DRB0067 might be involved in ROS resistance through degradation of extracellular DNA to dNMPs, which increases the pool of dGMP. This pool then aids in enhancing the *D. radiodurans* tolerance to oxidative stress.

Discussion

Here, we report extracellular dGMP enhanced the resistance of *D. radiodurans* to H$_2$O$_2$ and gamma-radiation. These findings suggest extracellular dGMP plays an important role in the organism's anti-oxidation pathway. Interestingly, we observed extracellular dGMP enhance the expression levels of KatA (DR1998) in *D. radiodurans*. These findings have yielded clues that may reveal the underlying mechanism of extracellular dGMP in anti-oxidation. Moreover, we found extracellular dGMP modulated expression of other genes, including one sensory transduction histidine kinase gene (*dr2244*), and genes involved in the regulation of manganese/iron. The up-regulation of these genes may enhance the tolerance of H$_2$O$_2$ and gamma-radiation as well.

Both cAMP and cGMP, as second messengers, have been widely studied in eukaryotes. It has been reported that cGMP can protect eukaryotic cells from oxidative stress, as in endothelial progenitors [31]. In bacteria, the production of cGMP has also been demonstrated. However, the physiological role of cGMP is still not well defined [32]. Recently Misra *et al.* reported that a DNA damage-induced signaling mechanism including secondary messengers and signaling enzymes exist in *D. radiodurans* [33]. Considering that the GC content (66.6%) in *D. radiodurans* is higher than most of other bacteria [27], we hypothesize that guanine base, obtained from the breakdown of extracellular dGMP [1], could be converted into cGMP after absorbed, and protect the cells from oxidative damage. However, further experiments are required to full characterize how dGMP enhances *D. radiodurans* tolerance to oxidative stress.

In *D. radiodurans*, DRB0067, encoded on the mega-plasmid, is an extracellular nuclease [27]. We demonstrated the absence of DRB0067 completely abolished nuclease activity from medium (Fig. 5B/5C). These results indicate DRB0067 is the only extracellular nuclease in this bacterium. Further experiments suggested this extracellular nuclease is secreted through the secretory pathway. Under normal growth conditions, DRB0067 is bound to the carotenoid-containing hexagonal layer [27].
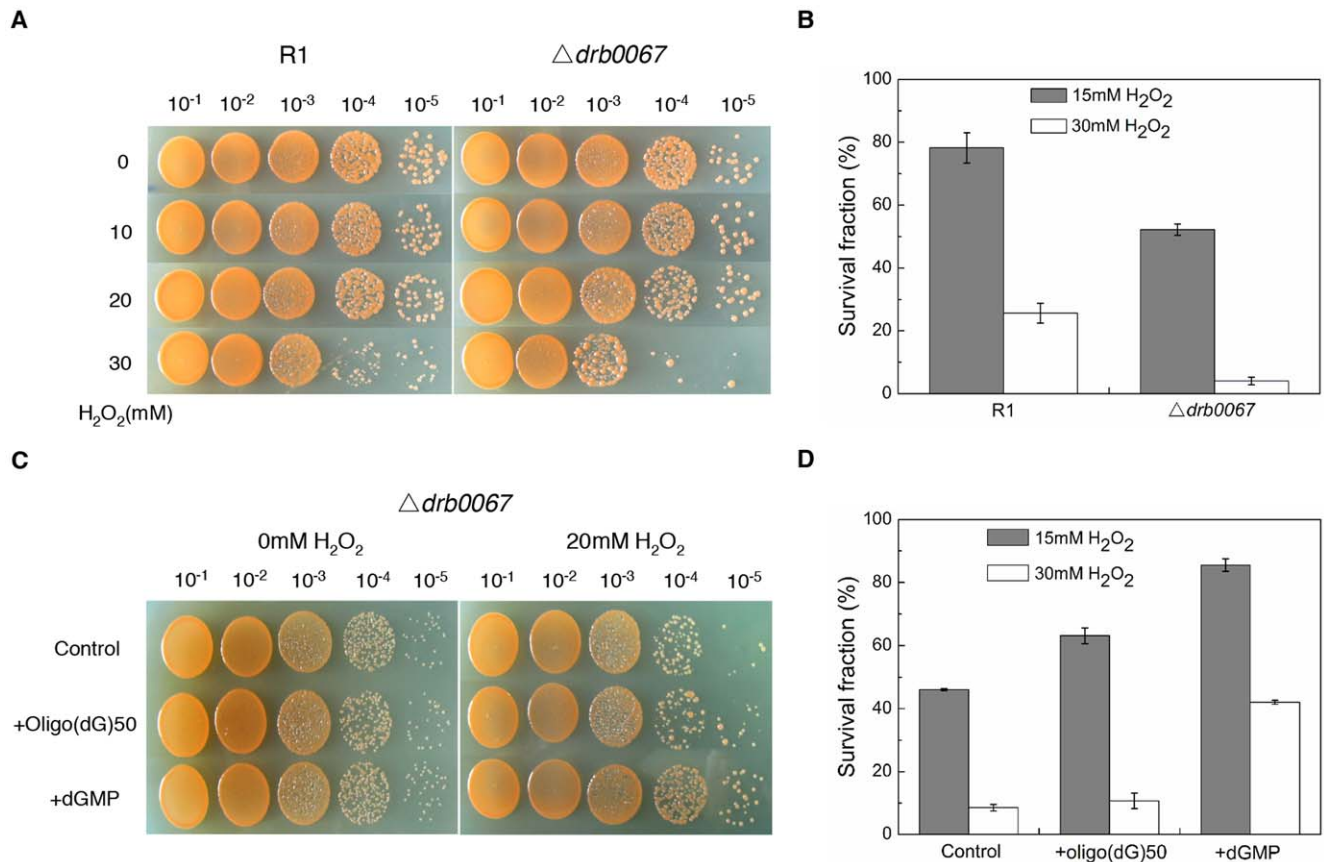
Figure 6. Sensitivity of Δ*drb0067* strain subjected to H₂O₂. (A) and (B) Sensitivity of R1 and Δ*drb0067* to different concentration of H₂O₂. (C) and (D) Sensitivity of Δ*drb0067* to different concentration of H₂O₂ with the addition of 0.05 mM oligo(dG)50 or 2.5 mM dGMP. Data represent the means ± deviations of three independent experiments. R1, *D. radiodurans* wild type strain; Δ*drb0067*, the *drb0067* null mutant.
doi:10.1371/journal.pone.0054420.g006

Interestingly, the nuclease is released into the medium after ionizing radiation [34–36], indicating that this nuclease participates in *D. radiodurans* post-irradiation recovery. This hypothesis was indirectly supported by a transcriptome study reporting that the expression level of DRB0067 was induced after ionizing radiation [37]. Here, we have found direct evidence that deletion of *drb0067* decreases the survival ability of *D. radiodurans* after H₂O₂ or gamma-radiation treatment. Moreover, we report gamma-radiation enhances the secretion of DRB0067, indicating an important role for this protein in anti-oxidation. It is quite possible DRB0067 is required to degrade damaged DNA fragments that are exported after radiation damage to avoid genome mutagenesis. This mechanism also provides essential nutrition for cells' recovery. Interestingly, dGMP, one product from DNA degradation, dramatically stimulates *D. radiodurans* resistance to oxidative stress, which may indicate another purpose for DRB0067 induction after DNA damage stress.

Free extracellular DNA is a source of natural transformations. By degrading extracellular DNA, nucleases act as a modulator for natural transformation, such as the Dns protein in *V. cholerrae* [16]. In our experiments, an absence of DRB0067 increased the natural transformation rate of *D. radiodurans* (Fig. 5D), suggesting that DRB0067 acts as a natural transformation modulator in this bacterium. Free extracellular DNA is also a source of nutrients for organisms. However, the presence of large amounts of extracellular DNA inhibits *D. radiodurans* cell growth. Furthermore, the disruption of the *drb0067* gene amplified this effect. While in *E. coli*

extracellular DNA had no effect on its growth. These findings support the importance of extracellular nuclease in this organism. Taken together, the degradation of extracellular DNA into dNMPs by extracellular nuclease DRB0067 serves many purposes in *D. radiodurans*. First by converting extracellular DNA into nutrients, thus reversing cell growth inhibition, and finally by enhancing *D. radiodurans* tolerance to oxidative stress.

Materials and Methods

Strains, media, and growth conditions

The bacterial strains and plasmids used in this study are listed in Table S1. *D. radiodurans* (ATCC 13939) was used as the wild-type strain and for construction of mutants. All cells were cultured at 30°C in TGY medium (0.5% Bacto tryptone, 0.3% Bacto yeast extract, 0.1% glucose) or on TGY plates containing 1.5% Bacto agar powder. *E. coli* strain DH5α was used for propagation of plasmids and was grown at 37°C on LB media with appropriate antibiotics.

Disruption of the *drb0067* gene in *D. radiodurans*

Disruption of *D. radiodurans drb0067* gene was performed using the double crossover recombination method [38]. In brief, the 0067upF and 0067upR primers (Table S1) were used for the upstream fragment and 0067downF and 0067downR primers (Table S1) for the downstream fragment. The upstream and downstream were digested by *Hin*dIII and *Bam*HI respectively,

and ligated to the *Bam*HI-*Hin*dIII fragment of the kanamycin resistance cassette containing the *gro*EL promoter. The kanamycin resistance cassette was obtained from pRADK, a shuttle plasmid modified from pRADZ3 [39]. The fragment was then transformed into *D. radiodurans* R1 with CaCl$_2$ as described previously [40]. The mutant strain was obtained on TGY agar with 30 μg/ml kanamycin, and was confirmed by PCR with the primers 0067upF and 0067downR primers.

Growth curve and survival fraction tests

Bacteria growth was determined using optical density data (OD) at 600 nm. The strains were cultured in 20 ml liquid TGY or LB medium until an OD$_{600}$≈0.15 was reached, and DNA fragments (Herring sperm DNA from Sigma-Aldrich Company) or dNMPs, at a final concentration of 3.6 mg/ml, were added. The cultures were incubated with 250 rpm at 30°C or 37°C and samples were taken to measure the OD$_{600}$ value at different time. All experiments were repeated in triplicate.

For the sensitivity assay, the strains were cultured in 5 ml liquid TGY medium until an OD of OD$_{600}$≈0.75 was reached. Then DNA fragments, dNMPs, dGMP, or oligo(dG)50 were added to the growths (Table S1). Cultures were grown for another 3 hours. As a negative control autoclaved distilled water was added to a culture of each strain. After washed and diluted to an appropriate concentration with PBS solution, the cultures were treated with different concentrations of H$_2$O$_2$ (25 mM, 50 mM or 15 mM, 30 mM) for 30 min at 4°C. After treatment, the cells were plated on TGY plates and incubated at 30°C for 3 days before colonies were enumerated. The H$_2$O$_2$ survival assays on *E. coli* were performed as described above, except the cells were treated with 20 mM or 40 mM H$_2$O$_2$, plated on LB agar, and incubated at 37°C for 15 hours. Survival fraction (%) was calculated using the following equation: Survival fraction (%) = $N_{sample}/N_{control} \times 100\%$, where $N_{control}$ is the number of control colonies and N_{sample} is the number of H$_2$O$_2$ treated colonies. For the dripping test, the cultures were washed and serially diluted 1:10 with PBS solution, and then treated with H$_2$O$_2$ (10 mM, 20 mM or 30 mM), gamma-radiation (2.5 h for 2 kGy) or UV (408 J/m^2) separately [41]. 20 μl of cells were dripped onto TGY plates.

Transformation test

The plasmid pRADK was used to test the effect of the extracellular nuclease DRB0067 on the natural transformation. Here, 1 μg plasmid was used for each transformation. The pRADK was then transformed into *D. radiodurans* R1 with CaCl$_2$ as described previously [40]. Natural transformation frequencies were determined using the following equation: Natural transformation frequencies = N_{TK}/N_{TGY}, where N_{TK} is the number of clones on the TGY plates with 30 μg/ml kanamycin and N_{TGY} is the number of clones on the TGY plates.

Extracellular nuclease activity assay

DNase test agars were used to test the extracellular nuclease activity. The cells were cultured in TGY until an OD of OD≈1.0 was reached, then 20 μl of cells were dripped onto DNase test agars plates (plates contain 42.0 g of DNase Agar Base (Qingdao Hope Bio-Technogy Co., Ltd, China), 0.05 g of methyl green and 2 g of glucose per liter of distilled water). Cultures were incubated at 30°C for 3 days. We used pRADK plasmid to test the activity of nuclease outside of the cells. The cells were cultured in TGY, and then centrifuged to collect the supernatant for enzyme reaction, during which 10 mM MgCl$_2$ was added.

Activity measurement of Catalase and SOD

Cells were treated with dGMP (2.5 mM) when they reached OD≈0.8, incubated for 3 hours and then disrupted with an ultrasonicator. The protein concentration of the supernatant was measured by the Bradford's method [42]. The catalase activity was assayed by the horseradish peroxidase-diaminobenzidine method [43]. In detail, samples were separated, using electrophoresis, in an 8% non-denaturing polyacrylamide gel matrix at 4°C for 4–5 hours (15 mA). The gel matrix was then washed with distilled water for 3 times. Next, it was incubated with a 0.06% H$_2$O$_2$ solution for 20 min under slow shaking. The gel was washed again and then incubated with FeCl$_3$ (2%)/K$_3$Fe(CN)$_6$ (2%) (V/V = 1:2) until bright strips appeared on the gel. For the assay of SOD activities, a 10% non-denaturing polyacrylamide gel was used according to the nitroblue tetrazolium-riboflavin method [44]. This method was nearly the same as the one described above except for the gel-running time (2–2.5 h) and the staining solution (2.45 mM nitroblue tetrazolium chloride (NBT), 28 mM TEMED, 28 μM riboflavin, and 100 mM EDTA, pH = 7.8). The gel was stained for 30 min and then exposed under lamplight until the bright strips appeared. For the activity measurement experiments 80 μg proteins were used per lane.

Real-time quantitative PCR

Real-time quantitative PCR was used to determine the influence of the extracellular dGMP on the expression levels of Catalases, SODs and other genes of interest in *D. radiodurans*. In short, cells were grown to an OD$_{600}$≈0.2 and then 2.5 mM dGMP was added. Cells were harvested by centrifugation at 4000 rpm for 5 min at 4°C when an OD$_{600}$ of 0.4~0.45 was reached. The extraction of total RNA and cDNA synthesis were performed as described previously [45]. SYBR *Premix Ex Taq*™ (TaKaRa Biotechnology (Dalian) Co, Ltd, China) was used for amplification, and all assays were performed using the STRATA-GENE Mx3005P™ Real-time detection system.

Supporting Information

Figure S1 UV and gamma-ray sensitivity in R1 and Δ*drb0067* strains. (A) Sensitivity of R1 to UV (408 J/m^2) and gamma-radiation (2 kGy) with the addition of 10 mM dNMPs or 10 mM dGMP. (B) Sensitivity of R1 and Δ*drb0067* to UV (408 J/m^2) and gamma-radiation (2 kGy). R1, *D. radiodurans* wild type strain; Δ*drb0067*, the *drb0067* null mutant.
(TIF)

Figure S2 Gamma-radiation enhances the secretion of active extracellular nuclease. The cells were cultured in TGY until the OD$_{600}$≈1.0, treated with 2 kGy or 4 kGy gamma-radiation, and then centrifuged to collect the supernatant for enzyme reaction. The extracellular nuclease is secreted more from *D. radiodurans* after treatment. But no obvious extracellular nuclease activity change is observed from Δ*drb0067* after gamma-radiation treatment. M denotes molecular standards. R1, *D. radiodurans* wild type strain; Δ*drb0067*, the *drb0067* null mutant.
(TIF)

Table S1 Strains, plasmids and primers used in this study.
(DOC)

Author Contributions

reagents/materials/analysis tools: QF YZ HZ XX JJ. Wrote the paper: ML HS HL.

References

1. Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D (2007) Release and persistence of extracellular DNA in the environment. Environ Biosafety Res 6: 37–53.
2. Dillard JP, Seifert HS (2001) A variable genetic island specific for Neisseria gonorrhoeae is involved in providing DNA for natural transformation and is found more often in disseminated infection isolates. Mol Microbiol 41: 263–277.
3. Hamilton HL, Dominguez NM, Schwartz KJ, Hackett KT, Dillard JP (2005) Neisseria gonorrhoeae secretes chromosomal DNA via a novel type IV secretion system. Mol Microbiol 55: 1704–1721.
4. Lorenz MG, Wackernagel W (1994) Bacterial gene transfer by natural genetic transformation in the environment. Microbiol Rev 58: 563–602.
5. Yin X, Stotzky G (1997) Gene transfer among bacteria in natural environments. Adv Appl Microbiol 45: 153–212.
6. Thomas CM, Nielsen KM (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat Rev Microbiol 3: 711–721.
7. Davison J (1999) Genetic exchange between bacteria in the environment. Plasmid 42: 73–91.
8. Dreiseikelmann B (1994) Translocation of DNA across bacterial membranes. Microbiol Rev 58: 293–316.
9. Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS (2002) Extracellular DNA required for bacterial biofilm formation. Science 295: 1487.
10. Steinberger RE, Holden PA (2005) Extracellular DNA in single- and multiple-species unsaturated biofilms. Appl Environ Microbiol 71: 5404–5410.
11. Finkel SE, Kolter R (2001) DNA as a nutrient: novel role for bacterial competence gene homologs. J Bacteriol 183: 6288–6293.
12. Battista JR (1997) Against all odds: the survival strategies of Deinococcus radiodurans. Annu Rev Microbiol 51: 203–224.
13. Ichige A, Oishi K, Mizushima S (1988) Isolation and characterization of mutants of a marine Vibrio strain that are defective in production of extracellular proteins. J Bacteriol 170: 3537–3542.
14. Dodd HN, Pemberton JM (1999) The gene encoding a periplasmic deoxyribonuclease from Aeromonas hydrophila. FEMS Microbiol Lett 173: 41–46.
15. Brnakova Z, Godany A, Timko J (2005) An extracellular endodeoxyribonuclease from Streptomyces aureofaciens. Biochim Biophys Acta 1721: 116–123.
16. Blokesch M, Schoolnik GK (2008) The extracellular nuclease Dns and its role in natural transformation of Vibrio cholerae. J Bacteriol 190: 7232–7240.
17. Vukovic-Nagy B, Fox BW, Fox M (1974) The release of a deoxyribonucleic acid fragment after x-irradiation of Micrococcus radiodurans. Int J Radiat Biol Relat Stud Phys Chem Med 25: 329–337.
18. Minton KW (1994) DNA repair in the extremely radioresistant bacterium Deinococcus radiodurans. Mol Microbiol 13: 9–15.
19. Battista JR, Earl AM, Park MJ (1999) Why is Deinococcus radiodurans so resistant to ionizing radiation? Trends Microbiol 7: 362–365.
20. Makarova KS, Aravind L, Wolf YI, Tatusov RL, Minton KW, et al. (2001) Genome of the extremely radiation-resistant bacterium Deinococcus radiodurans viewed from the perspective of comparative genomics. Microbiol Mol Biol Rev 65: 44–79.
21. Ghosal D, Omelchenko MV, Gaidamakova EK, Matrosova VY, Vasilenko A, et al. (2005) How radiation kills cells: survival of Deinococcus radiodurans and Shewanella oneidensis under oxidative stress. FEMS Microbiol Rev 29: 361–375.
22. Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, et al. (2007) Protein oxidation implicated as the primary determinant of bacterial radioresistance. PLoS Biol 5: e92.
23. Zahradka K, Slade D, Bailone A, Sommer S, Averbeck D, et al. (2006) Reassembly of shattered chromosomes in Deinococcus radiodurans. Nature 443: 569–573.
24. Slade D, Lindner AB, Paul G, Radman M (2009) Recombination and replication in DNA repair of heavily irradiated Deinococcus radiodurans. Cell 136: 1044–1055.
25. Boling ME, Setlow JK (1966) The resistance of Micrococcus radiodurans to ultraviolet radiation. 3. A repair mechanism. Biochim Biophys Acta 123: 26–33.
26. Slade D, Radman M (2011) Oxidative stress resistance in Deinococcus radiodurans. Microbiol Mol Biol Rev 75: 133–191.
27. White O, Eisen JA, Heidelberg JF, Hickey EK, Peterson JD, et al. (1999) Genome sequence of the radioresistant bacterium Deinococcus radiodurans R1. Science 286: 1571–1577.
28. Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, et al. (2004) Accumulation of Mn(II) in Deinococcus radiodurans facilitates gamma-radiation resistance. Science 306: 1025–1028.
29. Daly MJ, Gaidamakova EK, Matrosova VY, Kiang JG, Fukumoto R, et al. (2010) Small-molecule antioxidant proteome-shields in Deinococcus radiodurans. PLoS One 5: e12570.
30. Markillie LM, Varnum SM, Hradecky P, Wong KK (1999) Targeted mutagenesis by duplication insertion in the radioresistant bacterium Deinococcus radiodurans: radiation sensitivities of catalase (katA) and superoxide dismutase (sodA) mutants. J Bacteriol 181: 666–669.
31. Curatola AM, Xu J, Hendricks-Munoz KD (2011) Cyclic GMP protects endothelial progenitors from oxidative stress. Angiogenesis 14: 267–279.
32. Linder JU (2010) cGMP production in bacteria. Mol Cell Biochem 334: 215–219.
33. Kamble VA, Rajpurohit YS, Srivastava AK, Misra HS (2010) Increased synthesis of signaling molecules coincides with reversible inhibition of nucleolytic activity during postirradiation recovery of Deinococcus radiodurans. FEMS Microbiol Lett 303: 18–25.
34. Gentner NE, Mitchel RE (1975) Ionizing radiation-induced release of a cell surface nuclease from Micrococcus radiodurans. Radiat Res 61: 204–215.
35. Mitchel RE (1975) Letter: Origin of cell surface proteins released from Micrococcus radiodurans by ionizing radiation. Radiat Res 64: 380–387.
36. Tanaka A, Hirano H, Kikuchi M, Kitayama S, Watanabe H (1996) Changes in cellular proteins of Deinococcus radiodurans following gamma-irradiation. Radiat Environ Biophys 35: 95–99.
37. Liu Y, Zhou J, Omelchenko MV, Beliaev AS, Venkateswaran A, et al. (2003) Transcriptome dynamics of Deinococcus radiodurans recovering from ionizing radiation. Proc Natl Acad Sci U S A 100: 4191–4196.
38. Funayama T, Narumi I, Kikuchi M, Kitayama S, Watanabe H, et al. (1999) Identification and disruption analysis of the recN gene in the extremely radioresistant bacterium Deinococcus radiodurans. Mutat Res 435: 151–161.
39. Meima R, Lidstrom ME (2000) Characterization of the minimal replicon of a cryptic Deinococcus radiodurans SARK plasmid and development of versatile Escherichia coli-D. radiodurans shuttle vectors. Appl Environ Microbiol 66: 3856–3867.
40. Hua Y, Narumi I, Gao G, Tian B, Satoh K, et al. (2003) PprI: a general switch responsible for extreme radioresistance of Deinococcus radiodurans. Biochem Biophys Res Commun 306: 354–360.
41. Wang L, Xu G, Chen H, Zhao Y, Xu N, et al. (2008) DrRRA: a novel response regulator essential for the extreme radioresistance of Deinococcus radiodurans. Mol Microbiol 67: 1211–1222.
42. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254.
43. Wayne LG, Diaz GA (1986) A double staining method for differentiating between two classes of mycobacterial catalase in polyacrylamide electrophoresis gels. Anal Biochem 157: 89–92.
44. Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44: 276–287.
45. Sun H, Li M, Xu G, Chen H, Jiao J, et al. (2012) Regulation of MntH by a dual Mn(II)- and Fe(II)-dependent transcriptional repressor (DR2539) in Deinococcus radiodurans. PLoS One 7: e35057.