# Transcriptional profile in response to ionizing radiation at low dose in *Deinococcus radiodurans* *

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**Abstract** The genome-wide transcription profile of *Deinococcus radiodurans* cells was investigated after treatment with low dose irradiation (2 kGy). From the expression profile, we found that the process of DNA repair was induced in order, *i.e.* genes involved in base excision repair, nucleotide excision repair and single-strand annealing were induced immediately after ionizing radiation, and genes for recombination repair, including *recA*, *recD* and *recQ* were then activated. Especially, *recD* and *recQ* were specifically induced at low dose irradiation, and this phenomenon informed us that these two genes would play a certain role in anti-oxidation. Some genes such as *ddrA* and *ssb* were activated during the whole repair phase. Furthermore, the response of oxidative stress-related genes under low dose irradiation showed a different pattern from that of the acute high-level irradiation, many anti-oxidative genes were induced to scavenge reactive oxygen species directly, other associated systems also changed their expression patterns during the recovery time, such as iron metabolism systems, intracellular mutagenic precursors sanitize systems. These characteristics indicate that there is a powerful and orderly recovery process in *Deinococcus radiodurans*.

**Keywords:** ***Deinococcus radiodurans*, microarry, oxidative stress, ionizing radiation.**

*Deinococcus radiodurans* (*D. radiodurans*) is a gram-positive, red-pigmented, nonmotile bacterium[1] well known for its extreme resistance to ionizing radiation[2]. The exponentially growing culture of *D. radiodurans* has an extraordinary ability to tolerate 5000—12000 Gy acute gamma radiation without loss of viability[3], and can grow continuously in the presence of chronic radiation (60 Gy/h). It also shows remarkable resistance to the damage caused by desiccation[4] and other DNA-damaging conditions (e.g., hydrogen peroxide, UV light). The ring-like structure of *D. radiodurans* chromosomes was proposed to be an important factor of radioresistance[5], but there exist some controversial phenomena[6]. It has been suggested that multiple copies of the *D. radiodurans* genome may be organized to facilitate recombinational repair processes[7]. However, most of the annotated DNA repair enzymes do not reveal its extreme radiation resistance. Although it is the common knowledge that these multiple resistance ability is owing to highly efficient DNA repair processes, the underlying mechanisms of its preeminent ionizing radiation resistance remain unclear.

Microarray technology has allowed the parallel study of the expression of every gene in an organism. This approach has already been successfully used in studying *D. radiodurans* gene expression under a number of different growth conditions[8—11]. Here, we describe the whole genomic expression profile of *D. radiodurans* during their recovery from ionizing radiation at a low dose (2 kGy).

## 1 Materials and methods

### 1.1 Growth, radiation treatment of bacteria

All *D. radiodurans* strains used in this work were grown at 30℃ in TGY (0.5% Tryptone, 0.3% yeast extract, 0.1% glucose) broth or on TGY plates supplemented with 1.5% Bacto-agar. For irradiation exposure, 400 mL of an exponential growth phase *D. radiodurans* culture was divided in half. One

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half was irradiated on ice at a total dose of 2 kGy, and the other half, used as the nonirradiated control culture. After exposure, the irradiated cell culture was incubated at 30℃ in an orbital shaker and harvested at five recovery time points (0, 0.25, 0.5, 1, 2, and 5 hour), 30 mL of the harvest cells was pipetted into a 50 mL conical tube containing 4.5 mL of ice-cold EtOH/Phenol stop solution (5%, pH< 7.0 water-saturated phenol in ethanol). Then the cells were spun down at 4000 r/min for 2 min at 4℃, frozen in liquid nitrogen and stored at −80℃ until used.

### 1.2 Microarray design and constructions

All genes are identified as described in the published genome sequence (ftp://ftp.ncbi.nih.gov/genomes/Bacteria/Deinococcus_radiodurans/). PCR primers were designed by PRIMEGENS software[12] and 2996 pairs of gene-specific primers were obtained. The rest specific primers were designed by Primer3 and screened through Blastn. In total, 3096 pairs of primers were synthesized.

PCR products were generated and purified as published[7,8,12], yielding a collection of 3084 distinct open reading frames (ORFs) (single band and >100 ng/μL). Each ORF was printed twice on each array (Fig. 1). Microarray fabrication was carried out as described previously[8,13].

### 1.3 RNA isolation, probe preparation and microarray hybridization

Total RNA was extracted from cultures of irradiated and unirradiated *D. radiodurans* using TRI Reagent (Invitrogen), following liquid nitrogen grinding. Then the RNA sample from each condition was treated with 10 units of RNase free DNase I (Promega) and purified using phenol-chloroform extraction. RNA quality and quantity were evaluated by UV absorbance at 260 and 280 nm. Hybridization probes and microarray hybridisation were prepared as described by Thompson et al.[13].

### 1.4 Measurement of spot intensity and normalization

Microarrays were scanned using a GenePix 4000B confocal laser microscope. GenePix pro 5.1 was used to quantify hybridization signals. Prior to channel normalization, microarray outputs were filtered to remove spots of poor signal quality by excluding those data points with a mean intensity less than two standard deviations above background in both channels[14].

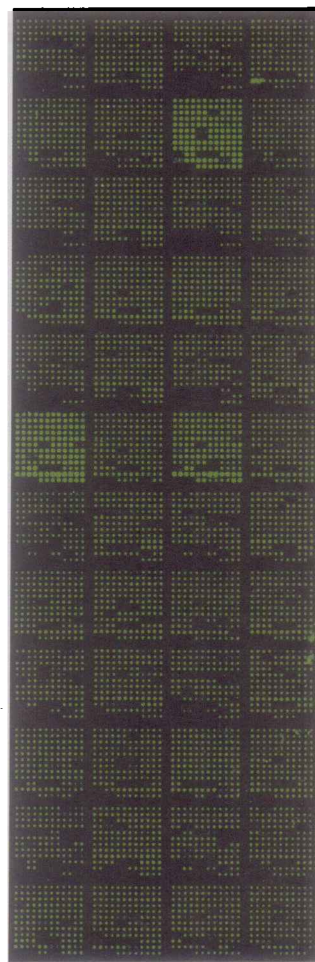

Fig. 1. Cy3-channel scanned image. Hybridization was done using 9-mer random Cy3-probes. Array is composed of 48 blocks (4 × 12), each with 144 features (12 × 12), negative control spots are included in each block randomly. Diameter of features is 100 μm and space between features is 340 μm.

Normalization and statistical analysis were carried out in the R computing environment (2.11, Raqua on the Windows) using the linear models for microarray data package (Limma)[15]. Within Limma, global LOESS normalization was carried out for each microarray[16,17]. The 2-replicate spots per gene in each array were used to maximize the robustness of differential expression measurement of each gene via the "lmFit" function within Limma. This step uses a pooled correlation estimation to generate a more robust estimation of the gene expression across replicate spots, compared to a straight average of replicate spots[18]. All the normalized data can be found in supplemental Table A at www.cab.zju.edu.cn/

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INAS/personal% 20web/Hyj/ProgressinNaturalScience/TableA. xls; supplemental Table B and supplemental Fig. 1 also are supporting information at www. cab. zju. edu. cn/INAS/personal% 20web/Hyj/ProgressinNaturalScience. Genes that showed a statistically significant difference in expression ($P < 0.05$) and increased 2-fold (or decreased 1/2-fold) in magnitude as up-regulated (or down-regulated) were used for further analysis[19].

1.5 Quality control of microarray data

Due to a relatively high frequency of error, at each time point, triplicate biological replicate samples were obtained, and RNA obtained from each sample was hybridized with two microarrays using dye-swap. Each microarray slide contained duplicate sets of gene fragments. Therefore, 12 data points were obtained for each time point. Real-time PCR and operons analysis were additional methods to test the robustness of the microarray hybridization data.

1.6 Quantitative real-time PCR

Quantitative real-time PCR was performed as previously described[9]. In short, first-strand cDNA synthesis was carried out in 20 μL of reactions containing 1 μg of each DNase I-treated, purified total RNA sample combined with 3 μg of random hexamers. The real-time PCR amplification used Quant SYBR Green PCR (TIANGEN) following the manufacturer's instructions. Seven interested genes were used to design specific primers (supplemental Table B). Of these genes, one housekeeping gene DR1343, whose expression was putatively unaffected through the whole process, was used as a normalization factor.

## 2 Results and discussion

2.1 General patterns of gene expression and quality of array data

After a dose of 2 kGy, a total of 477 genes were activated and 317 genes were repressed at least at one time point during *D. radiodurans* recovery (Fig. 2). These genes were classified into 19 categories according to their functional assignments in the COG database (http: //www. ncbi. nlm. nih. gov/COG/old/palox. cgi? fun = all) (Supplemental Fig. 1). Genes encoding proteins in the largest category are the genes with unknown functions, suggesting that the capability of the extreme resistance of *D. radiodurans* may be partly attributed to these unknown proteins and unknown repair pathways. Compared our transcriptional profiles data with Liu et al.'s results[8], we found a lot of genes involved in metabolism were repressed in our data, as they were induced in Liu's work, such as ribosomal proteins. In their study, stationary-phase cultures of *D. radiodurans* were irradiated and this culture was transferred to fresh media to recover, therefore, the cells recovered from stationary-phase into exponential-phase, while the control cells were still at stationary-phase[8,9]. Therefore, We believe that this could be a result of that the irradiated bacteria were at different growth phases.

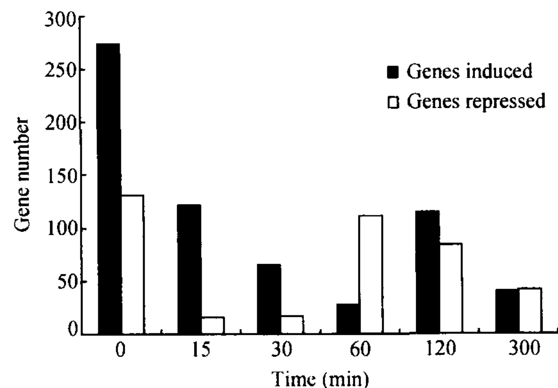


Fig. 2. Magnitude of gene responses at each of the 6 time points. The number of differential expression of genes at each time point is showed, and differential express genes are defined by statistically significant expression ratios showing larger than 2-fold change.

It was found that cell growth was inhibited for about one hour and a lot of stress response-related genes, replication, repair, and recombination genes were highly expressed in this phase. This suggests that the recovery phase is one hour after ionizing. To distinguish potentially damage-induced genes from cell-growth-related genes, we mainly focused on the variation of gene expression level that occurred during the recovery period.

To validate the reliability of the data, correlation of gene expression with the predicted operon organization[21] and multi-subunit complex are shown in Fig. 3. Seven genes were selected and analyzed by real-time quantitative PCR, and the results were highly correlated with those of array data (Fig. 3). Besides these three methods, an uncharacterized induced gene in postirradiation recovery was knocked out to verify its contribution in recovery phase. Although MT1127 (DR1127 mutant) did not show deficiencies in metabolism or growth, it lost 40% survival rate ver-

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sus the wild type after gamma radiation (data not shown).

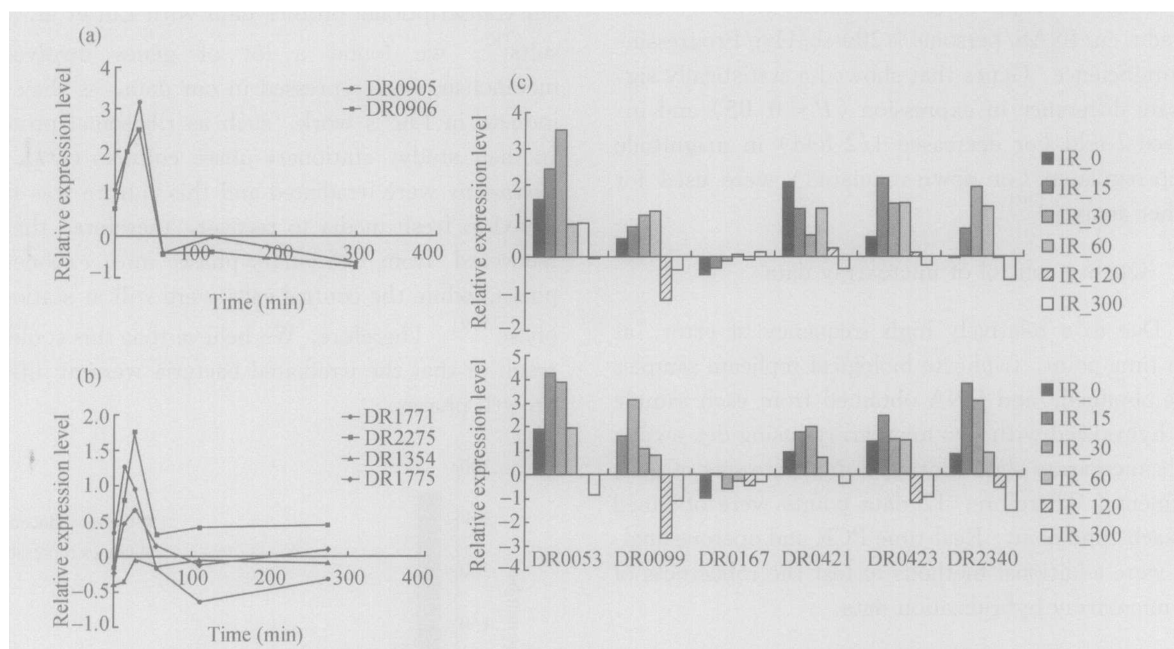


Fig. 3. Three methods to test microarray data quality. (a) Predicted operon consisting of DR0905 and DR0906. (b) Subunits of the UvrABCD excinuclease. These two figures show that the expression profiles of genes belonging to the same operon or multi-subunit complex are highly correlated. (c) Comparative studies for six genes between microarray data (upper) and real-time quantitative PCR data (lower). Note the high concordance of the results when two distinct methods are used.

## 2.2 Induction of DNA repair system

Ionizing radiation leads to a lot of double-strand breaks (DSBs), single-strand gaps and sites of DNA base damage on chromosome[22]. It is known that *D. radiodurans* has repair pathways including excision repair, mismatch repair, single-strand annealing (SSA), non-homologous end joining (NHEJ) and recombinational repair[23,24]. The repair processes could be separated into two phases according to our time-coursing microarray data. The first repair phase (0—0.25 h) was activated immediately after ionizing radiation. Excision repair, mismatch repair, and SSA are considered to be involved in this phase (Table 1). In this repair phase, the physical length of many chromosomal DNA fragments could be increased, thereby reducing DNA damage caused by exonucleases. Another benefit of increasing the length of substrate DNA might be that larger fragments are more easily engaged in *recA*-dependent DNA repair. There is some supporting evidence that about one-third of the DSBs were repaired in the first phase[25]. The second repair phase was induced between 0.25 and 1 hour after ionizing radiation. RecA-dependent and RecA-independent recombination repairs belong to this phase (Table 2). Besides *recA*, genes such as *recQ* and *recD* were significantly induced in this phase. Although the RecBCD recombinase is missing, *D. radiodurans* does encode an apparent ortholog of one of the helicase-related subunits of the complex RecD. This protein is unusual for it contains an N-terminal region of about 200 amino acid residues that consist of three tandem predicted HhH DNA-binding domains. This suggests that there is a different recombination repair based on RecD from *E. coli*. RecQ helicase is another component of the recombination repair system in *D. radiodurans*, it has an unusual domain architecture, three tandem copies of the C-terminal helicase-Rnase D (HRD) domain. This three-HRD domain in *D. radiodurans* could contribute to the repair phenotype given the interactions of RecQ with RecA in recombination. RecQ can suppress illegitimate recombination and improve the efficiency of repair. Furthermore, we found both of them were specifically up-regulated at low dose irradiation and their mutants were very sensitive to $H_2O_2$ (unpublished data), it informed us that they may have function in against oxidative stress.

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Table 1. The first repair phase (0—0.25 h) which was activated immediately after ionizing radiation

| Gene ID | Gene name | Repair pathway[a)] | Protein description |
|---|---|---|---|
| DR1771 | *uvrA* | NER | Excinuclease ABC, subunit A, ATPase, DNA binding |
| DR1775 | *uvrD* | NER | Helicase II; initiates unwinding from a nick |
| DR1572 | *uvrD* | NER | Helicase II; initiates unwinding from a nick |
| DR2275 | *uvrB* | NER | Excinuclease ABC, subunit B, Helicase |
| DR2438 | *nth* | BER | Endonuclease III and thymine glycol DNA glycosylase |
| DR0928 | *nth* | BER | Endonuclease III and thymine glycol DNA glycosylase |
| DR1707 | *polA* | BER | DNA polymerase I |
| DR1663 | *ung* | BER | Uracil DNA glycosylase |
| DR0689 | *ung* | BER | Uracil DNA glycosylase |
| DR0715 | *mug* | BER | G/T mismatch-specific thymine DNA glycosylase |

a) Abbrevistion of DNA repair pathway: NER, nucleotide excision repair; BER, base excision repair.

Table 2. The second repair phase (0.25—1 h). Recombination repairs belong to this phase

| Gene ID | Gene name | Repair pathway[a)] | Protein description |
|---|---|---|---|
| DR0596 | *ruvB* | RER | Helicase subunit of the RuvABC resolvasome |
| DR1289 | *recQ* | RER | Helicase; suppressor of illegitimate recombination |
| DR1902 | *recD* | RER | Helicase/exonuclease |
| DR2340 | *recA* | RER | Recombinase; sigle-stranded DNA-dependent ATPase |
| DRA0346 | *pprA* | RER | RecA-independent recombination |

a) Abbrevistion of DNA repair pathway: RER, recombinational repair.

Interestingly, a group of genes were activated through the repair phase (Table 3), including *ddrA* (DR0423), *ssb* (DR0099), DR0936 and DR0663. First, DdrA protein seems to function as a DNA-end-protection protein, which binds to the exposed DNA ends and prevents nuclease digestion of the chromosomal DNA[26]. Ssb protein could bind single strand DNA and stabilize single-stranded regions, this gene is also a DNA replication protein. Both of them are thought to play an important role in the whole repair process. Further, DR0936 is similar to ELP3, which is part of the six-subunit elongator complex in eukaryote, and its histone acetyltransferase (HAT) domain indicates that it is responsible for transcription, and it has an iron ion-binding domain. A phospholipase D/nuclease domain has been detected in DR0663. This superfamily includes enzymes involved in signal transduction, lipid biosynthesis, endonuclease and open reading frames in pathogenic viruses and bacteria. Furthermore, it shows 32% identity to helicase. Taken together, these data suggested that this protein might have the function to hydrolyze damaged DNA and protect the organism from elevated levels of mutagenesis by preventing the reincorporation of damaged bases during DNA synthesis and recombination. Based on the characteristics of these proteins, it could be implied that *D. radiodurans* keeps genetic integrity by mechanisms that limit degradation of damaged DNA and the diffusion of DNA fragments produced following irradiation.

Table 3. Genes activated throughout the whole repair phase

| Gene ID | Gene name | Protein description and comments |
|---|---|---|
| DR0099 | *ssb* | Single-stranded DNA-binding protein |
| DR0423 | *ddrA* | Rad52/22 double-strand break repair protein |
| DR2574 | DR2574 | Helix-turn-helix motif, DNA binding |
| DR0053 | DR0053 | DNA damage-inducible (dinB) |
| DR2479 | DR2479 | Function unknown |
| DR0663 | DR0663 | Phospholipase D/nuclease |
| DR2441 | DR2441 | GCN5-related N-acetyltransferase |
| DR0936 | DR0936 | Elongator protein 3/MiaB/NifB (eukaryote) |

Previous research confirmed that *D. radiodurans* did not possess a functional error-prone DNA repair (SOS) response system. But in our microarray data, we found that orthologs of several genes involved in the SOS response of *E. coli* and *Bacillus subtilis* were activated, including *recA*, *ssb*, *uvrABCD*, *ruvB*, *dinB* and *lexA* (DRA0074). Furthermore, *uvrA* was induced to express when two *lexA* genes were knocked out (data not shown). These data suggest that *D. radiodurans* may have a specific SOS response system that is distinct from the classical *E. coli*-type SOS response. In agreement with a recent report[27], our expression data showed that DrHU (DRA0065) was not significantly induced after irradiation. In contrast to *E. coli* HU, DrHU does not exhibit a marked preference for DNA with nicks or gaps compared to perfect duplex DNA, suggesting that this protein is not involved in DSBs recombination repair and may serve to stabilize four-

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way junction structures in preparation for subsequent repair events. PprI protein is considered as a key protein in the repair process, and *D. radiodurans* deficient in PprI is very sensitive to ionizing radiation[28]. In consistence with our previous result of Western blot[29], PprI was found to be not activating at the protein level. We also compared this result with Liu's microarray data and found that PprI was activated at only one time point in their data[8]. Bioinformatics analysis showed that PprI has regulatory functions and does not possess an enzymatic function in the direct repair of DNA DSBs[28]. It might be a reason why this protein is not induced significantly in the repair phase.

Of particular interest was the finding that several genes directly associated with DNA replication were induced. Specifically, genes such as DR0002, DR1913 and DR0906 are involved in replication complex that could initiate DNA replication. DR0507, DR0001, DR0467 and DR1707 encode DNA polymerase III alpha subunit, DNA polymerase III beta subunit, hypothetical DNA polymerase, DNA-directed DNA polymerase I, respectively. Further, DR0899 encodes an RNase H domain, it can hydrolyse the RNA portion of RNA and DNA hybrids, and then DNA polymerase I filled Okazaki fragments. This finding implied that DNA replication was necessary during the repair phase.

### 2.3 Induction of genes defence against oxidative stress

Many oxidative stress-related genes had been detected during the recovery time (Table 4). Genes that are directly involved in scavenging reactive oxygen species (ROS) were significantly up-regulated at least one time point during the recovery, including *sodA* (1.59-fold) and *katE* (2.11-fold). In addition, *msrA* (DR1849) was induced to repair oxidized proteins and *arginase* (DRA0149) was activated, which can inhibit the toxic effects of oxygen[30]. Few genes associated with oxidative stress response were activated in Liu's data, and in fact, many genes were significantly repressed. However, many of them were induced at a low dose. In addition, DR1127 is proposed to function as a toxic anion resistant protein and its mutant was more sensitive to hydrogen peroxide than irradiation (data not shown), suggesting this protein should belong to ROS defence system. Previous studies had shown that about 80% of DNA damage is caused indirectly by irradiation-induced ROS, the remaining about 20% by direct interaction between $\gamma$-photons and DNA during irradiation processes[31]. We presumed that proteins might be totally degraded but not partially damaged in response to acute irradiation (15 kGy), and repair of damaged molecules may be not necessary at a high dose of irradiation. At a low dose, a cell would activate its antioxidant defenses to repair partially damaged molecules.

Table 4. Induction of oxidative stress-related genes in *D. radiodurans* following ionizing irradiation

| Gene ID | Name | Gene annotation | Relative gene expression and $P$ value | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | T0 | P0 | T15 | P15 | T30 | P30 | T60 | P60 |
| DR0412 | *ahpF* | Thioredoxin fold reductase | 1.866 | 0.002 | 1.434 | 0.117 | 0.963 | 0.819 | 0.835 | 0.139 |
| DR0846 | *bcp* | Antioxidant type thioredoxin fold protein | 1.623 | 0.001 | 1.111 | 0.572 | 0.545 | 0.457 | 0.775 | 0.459 |
| DR1208 | *bcp* | Antioxidant type thioredoxin fold protein | 0.707 | 0.016 | 0.891 | 0.447 | 0.929 | 0.594 | 1.854 | 0.806 |
| DR1279 | *sodA* | Superoxide dismutase, Mn or Fe dependent | 1.189 | 0.276 | 1.593 | 0.031 | 1.147 | 0.103 | 1.486 | 0.006 |
| DR1538 | *osmC* | Alkylperoxide and oxidative stress response | 2.497 | 0.004 | 1.935 | 0.000 | 1.008 | 0.951 | 0.939 | 0.844 |
| DR1849 | *msrA* | Peptide methionine sulfoxide reductase PMSR | 1.485 | 0.020 | 1.177 | 0.101 | 1.148 | 0.158 | 1.021 | 0.811 |
| DR1857 | *osmC* | Alkylperoxide and oxidative stress response | 0.804 | 0.470 | 2.005 | 0.001 | 1.363 | 0.009 | 1.587 | 0.005 |
| DR1998 | *katE* | Catalase | 0.429 | 0.000 | 0.948 | 0.579 | 2.109 | 0.00 | 0.268 | 0.002 |
| DR2085 | *grxA* | Glutaredoxin | 6.543 | 0.000 | 3.728 | 0.006 | 2.618 | 0.00 | 1.545 | 0.129 |
| DRA0202 | *sodC* | Superoxide dismutase, Cu/Zn dependent | 2.428 | 0.019 | 0.925 | −0.222 | 0.693 | 0.015 | 0.978 | 0.714 |
| DRA0259 | *katE* | Catalase | 2.128 | 0.007 | 1.499 | 0.037 | 1.256 | 0.096 | 0.711 | 0.593 |

Recent research showed that the high intracellular Mn/Fe concentration ratio of *D. radiodurans* is associated with the efficiency of its repair pathways by protecting cells from ROS generated during recovery[30,32]. As we know, iron metabolism is co-ordinately regulated with oxidative stress defences because iron can produce hydroxyl radicals through Fenton reaction, which indiscriminately damage all cellular

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components. Although we did not find significant changes of these predicted Mn transporters (DR1709, DR2283, DR2284, DR2523), iron metabolism systems did significantly change their expression levels. We observed that *D. radiodurans* evoked five mechanisms to decrease free intracellular iron concentration during the repair phase. Firstly, Fe-dependent transporter systems were repressed, including hemin transport system (DRB0014—DRB0016), one iron transport system substrate-binding protein (DRB0125) and one iron-chelator utilization protein (DRB0124). Secondly, the DNA-binding ferritin-like proteins (DR2263 and DRB0092), which play a central role in protecting DNA from oxidative damage by directly binding to DNA were stimulated and to bind free iron ions[33]. Thirdly, *D. radiodurans* strongly suppressed ROS producing genes expression during the recovery phase, such as DR0952 (*sdhA*), a gene involved in TCA pathway's superoxide ($O_2^-$) radical-generating step. Superoxide could oxidize and inactivate the protein containing iron-sulfur cluster, then resulting in improved free iron concentration. Fourthly, some proteins (DR2438, DR0928 and DR0951) containing iron-sulfur cluster loop were expressed, which could bind free iron and decrease the intracellular iron concentration. Thereby this may cause the ratio of Mn and Fe ions increased remarkably and inhibited the intracellular ROS generation[32]. Lastly, in relation to iron metabolism, we also found genes that responsible for heme biosynthesis (DR1131) and heme binding (DR2095) were activated. Heme is biosynthesis by protoporphyrin and free ferrous iron and its compounds are cofactors for cytochromes, catalases and peroxidase.[34]. Moreover, we found the cells would prefer to use MnSOD (DR1279) rather than iron-dependent peroxidase (DRA0145) to remove ROS.

### 2.4 Other associated systems contributing to DNA repair

During the period of stasis, it can be expected that the cells would induce some other mechanisms to assist DNA repair. Firstly, the cells exported the DNA degradation products, and protected the organism from elevated levels of mutagenesis by preventing the reincorporation of damaged bases during DNA synthesis[22]. We found that some poorly characterized ABC transporters (DR0511 and DR2316) were highly induced at 0—0.25 h, which might be involved in the export of damaged products before DNA repair. Secondly, DR0004, a member of MutT/Nudix family, was induced to sanitize the remaining intracellular mutagenic precursors via pyrophosphohydrolases.

## 3 Conclusions

The present study provided a comprehensive description of the global transcriptional responses evoked by *D. radiodurans* cells when challenged with sublethal irradiation. The research revealed how the transcriptome profile of *D. radiodurans* was shifted during its cellular response to irradiation stress, which involved growth inhibition and resumption. Notably, we found that the process of DNA repair was induced in order. Base damage, nucleotide damage and single strand damage were repaired in the first phase, and then DSBs repair occurred in the second phase. Most strikingly, *recD* and *recQ* were specifically induced at low dose irradiation; some evidence showed that they play a certain role in against oxidative stress (our unpublished data). We also found that the response of oxidative stress-related genes under low dose irradiation showed a different pattern from that of the acute high-level irradiation. Furthermore, we showed the repression of iron uptake and induction of storage-related genes, which could decrease the concentration of free iron significantly. In addition, genes responsible for heme biosynthesis (DR1131) and heme binding (DR2095) were activated. It is generally known that heme compounds consist of ferrous ion and protoporphyrin IX. They are also cofactors for anti-oxidative genes, such as peroxidase, cytochromes and catalase.

Overall, this microarray study identified sets of putative coregulated genes and enabled the generation of testable hypotheses of possible functions for their encoded products. Hence, we are currently exploring whether the induction of the responsible genes helps repair damaged DNA and protects against oxidants in *D. radiodurans*.

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