Biochemical and Biophysical Research Communications 450 (2014) 575–580

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A PerR-like protein involved in response to oxidative stress in the extreme bacterium *Deinococcus radiodurans*

Chengzhi Liu [1], Liangyan Wang [1], Tao Li, Lin Lin, Shang Dai, Bing Tian *, Yuejin Hua *

Key Laboratory for Nuclear-Agricultural Sciences of Chinese Ministry of Agriculture and Zhejiang Province, Institute of Nuclear-Agricultural Sciences, Zhejiang University, 310029 Hangzhou, China

ARTICLE INFO

Article history:
Received 20 May 2014
Available online 10 June 2014

Keywords:
PerR
Fur homolog
ROS
Deinococcus radiodurans

ABSTRACT

Response and defense systems against reactive oxygen species (ROS) contribute to the remarkable resistance of *Deinococcus radiodurans* to oxidative stress induced by oxidants or radiation. However, mechanisms involved in ROS response and defense systems of *D. radiodurans* are not well understood. Fur family proteins are important in ROS response. Only a single Fur homolog is predicted by sequence similarity in the current *D. radiodurans* genome database. Our bioinformatics analysis demonstrated an additional guanine nucleotide in the genome of *D. radiodurans* that is not in the database, leading to the discovery of another Fur homolog DrPerR. Gene disruption mutant of DrPerR showed enhanced resistance to hydrogen peroxide (H_2O_2) and increased catalase activity in cell extracts. Real-time PCR results indicated that DrPerR functions as a repressor of the catalase gene *katE*. Meanwhile, derepression of *dps* (DNA-binding proteins from starved cells) gene under H_2O_2 stress by DrPerR point to its regulatory role in metal ions hemostasis. Thus, DrPerR might function as a Fur homolog protein which is involved in ROS response and defense. These results help clarify the complicated regulatory network that responds to ROS stress in *D. radiodurans*.

1. Introduction

Aerobic metabolism is necessary for sustain cell energy, but its overproduced byproducts—reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hydroxyl radical, have toxic effects on cells by damaging membrane and macromolecules. The relatively mild oxidant H_2O_2 possess a longer cellular half-life (~1 ms) than other ROS [1], but its unlimited diffusion across cell membrane and promotion of the production of high toxic hydroxygen radical (·OH) via Fenton Chemistry make H_2O_2 highly toxic [2]. Aerobic microorganisms evolve various strategies including systems to respond to and defend against ROS such as antioxidant enzymes and their regulators.

ROS defense system is coordinated by thiol-base responsive regulator through either activating or inactivating the expression of genes [3]. Site-specific, covalent and reversible modification on the thiol of cysteine made it a potential sensor for transforming oxidative signal into a response against ROS. OxyR, a sensor and regulator, is generally found in gram-negative bacteria and functions as a global positive regulator of peroxide stress response [4]. Ferric uptake regulator (Fur) family protein was first known to response to the exceeding level of intracellular Fe (II) [2], which catalyzes the Fenton chemistry. Therefore, Fur proteins are involved in fighting against intracellular ROS. Fur family members are generally repressors in the response to several metal ions including Fe (II), Mn (II), Zn(II), Ni (II), and H_2O_2, referred as Fur, Mur, Zur, Nur [5–7] and PerR, respectively. PerR integrates submicromolar levels of H_2O_2 and the cytosolic concentration of metal Mn (II) and Fe (II) as signals [8], and is considered as an important protein for responding to ROS stress.

Deinococcus radiodurans is an aerobic bacterium well known for its extreme resistance to ionizing radiation, UV radiation, oxidants [9,10] which generate lethal ROS to cells. Many studies have investigated the extreme resistance mechanism of *D. radiodurans* to ROS stress [11], but the details remain unclear. Recent proposals state that under lethal ROS, the integrity of proteome is prior to genome for cell survival [12,13]. Cell survival depends on the functions of proteins involved in DNA repair, replication and metabolisms. ROS responding and defense systems are indispensable for protecting proteins and maintaining proteome integrity. *D. radiodurans* has several strategies to respond to ROS including maintaining a high level of Mn/Fe concentration ratio, increasing the supply of

* Corresponding authors. Address: Institute of Nuclear-Agricultural Sciences, Zhejiang University, No. 268, Kaixuan Road, 310029 Hangzhou, China. Fax: +86 571 86971703.

E-mail addresses: tianbing@zju.edu.cn (B. Tian), yjhua@zju.edu.cn (Y. Hua).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.06.015
0006-291X/

small-molecular antioxidant and enhancement of ROS-scavenging enzyme activity. Gene mutations for ROS-scavenging enzymes such as catalases, superoxide dismutase resulted in significant decline of *D. radiodurans* survival under oxidative stress [14]. The efficient non-enzymatic small-molecule antioxidants in *D. radiodurans* comprise pyrroloquinoline-quinone and carotenoids [15–17].

According to present annotated genome at the National Center for Biotechnology Information (NCBI), there is only one Fur homolog (DR0865) encoded in the genome of *D. radiodurans*. However, the homolog does not seem to function as the regulator in iron uptake pathway. The genome of *D. radiodurans* was sequenced in 1999 [18], so inaccuracy and missing data might be attributed to the limitation of sequencing techniques at that time [19]. Using RNA sequencing (RNA-seq) data, we found transcripts matching non-coding regions according to previous genome annotation. We reanalyzed the *D. radiodurans* genome data and identified a "missing" functional gene.

In the present study, we report a novel PerR-like protein of Fur family in *D. radiodurans* that is not annotated in the current database and has a different function from DR0865. *In vivo* experiments revealed that this gene was responsible for responding to H_2O_2 and regulating a serial of genes related to oxidative stress.

2. Material and methods

2.1. Bacterial strains and growth conditions

All strains and plasmids used in this study are listed in Supporting Information Supplementary Table.1. The wild type and mutant strains of *D. radiodurans* R1 (ATCC 13939) were grown in TGY medium (0.5% (w/v) Bactotryptone, 0.1% (w/v) glucose, 0.3% (w/v) Bacto yeast extract) at 30 °C or on TGY plates supplemented with 1.5% (w/v) agar. Ampicillin (100 $\mu g ml^{-1}$), chloramphenicol (3 $\mu g ml^{-1}$), and kanamycin (20 $\mu g ml^{-1}$) were added to the medium if required.

2.2. Identification of novel transcripts in D. radiodurans by RNA sequencing

RNA sequencing of *D. radiodurans* was performed by applying SOLiD system. A Perl script was written to search novel transcripts located at the non-coding region or the antisense of coding region base on the RNA sequencing data. The results were compared with Glimmer3 and Prodigal annotation [20,21]. The candidates were predicted by Blastp program.

2.3. Construction of D. radiodurans knockout mutant and complement strains

Mutants were constructed as previously described [22,23]. Primers are listed in Supporting Information Supplementary Table.2. Primers were used to amplify the upstream and downstream of target gene with the *Bam*HI and *Hind*III cutting sties respectively. After digestion, these segments were ligated to a kanamycin resistance cassette and transformed into the exponential phase cells with $CaCl_2$ method. The mutant strain (*ΔdrperR*) was screened with kanamycin, and confirmed by PCR products analysis and sequencing. *drperR* gene sequence has been submitted to NCBI with a No. KJ817356.

2.4. Survival assay under oxidative stress

Solutions (1 M) of manganese chloride, ferric trichloride, hydrogen peroxide (Sigma) were prepared in milli-Q water and filter-sterilized. Cells grown to OD_{600} about 1.0 were plated on TGY plates and overlaid with 5-mm sterile filter discs containing 10 μl of various cations solutions. The plates were incubated for three days, and the inhibition zone of each disc was measured.

For the concentration-dependent H_2O_2 treatment, the cell cultures were treated with different concentrations (ranged from 0 to 50 mM) of H_2O_2 for 30 min after a series of dilution of H_2O_2. For the time-dependent H_2O_2 treatment, cell cultures were treated with 40 mM H_2O_2, and sampled at intervals of 20 min. After treatment, 5 μl of sample was dropped onto the TGY plate and cells grown were measured.

2.5. Assays of cellular antioxidant capacity and activities of catalase and superoxide dismutase

Cellular antioxidant capacity was measured by ABTS assay. The activity was expressed as μmol of Trolox equivalent antioxidant capacity per gram whole cell extract protein (μmol TEAC/g). Total catalase activity was further determined as described previously [24]. Activities of specific catalases were further assayed by the native PAGE gel stained with horseradish peroxidase-diaminobenzidine [25]. Superoxide dismutase activity was analyzed by SOD activity assay and protein stain method on gel visualized with diaminobenzidine and hydrogen peroxide [26].

2.6. QRT-PCR analysis of related genes

QRT-PCR wad performed according to the previous method [26]. Total RNA was extracted using Trizol Reagent (Invitrogen) followed by treatment with RNase free Dnase I (Promega). The first-strand cDNA synthesis was carried out in 20 μl of reaction mixture containing 1 μg of RNA sample combined with 3 μg of random hexamers using SuperScript III Reverse Transcriptase kit (Invitrogen). SYBR Premix Ex Taq™ (TaKaRa) was used to amplify with STRATAGENE Mx3005P™ Real-time detection system.

3. Results

3.1. Identification of a novel Fur homolog protein embedded in the sequenced genome data of D. radiodurans

By RNA-seq and analysis, we found transcripts from noncoding regions of the *D. radiodurans* NCBI genome. One of the transcripts originated at the downstream of RecA, which follows the DR2341 (Fig. 1A and B). This founding is in consistence with the prediction by Glimmer3 and Prodigal (Prokaryotic Dynamic Programming Genefinding Algorithm), which are bacterial and archaeal genefinding programs [20,21]. The ORF appeared to encode a Fur homolog that was 80 amino acids, which was shorter than other Fur family members. We designed primers to clone an extended region containing this putative homolog. Sequencing showed that omission of a guanine at Chromosome I 2,340,150 led to a frameshift (Fig. 1C). Blastp analysis of the corrected ORF identified conservation sites of Fur family including a putative N-terminal DNA-binding helix, a C-terminal dimerization domain, a structural Zn (II)-binding site with two CX_2C coordinated motifs, and a regulatory metal-binding site. Further alignment analysis demonstrated that the new Fur homolog, designated *DrPerR* (data submitted to NCBI), shared 32% identity with DR0865, 32% identity with the PerR of *Bacillus subtilis* and 23% identity with the *E. coli* Fur (Fig. S2). Moreover, the *drperR* genes flanking *birA* in a divergent direction and located near RecA were observed in *D. radiodurans*, *Deinococcus gobiensis*, *Deinococcus geothermalis*, and *Deinococcus proteolyticus* (Fig. 1B).

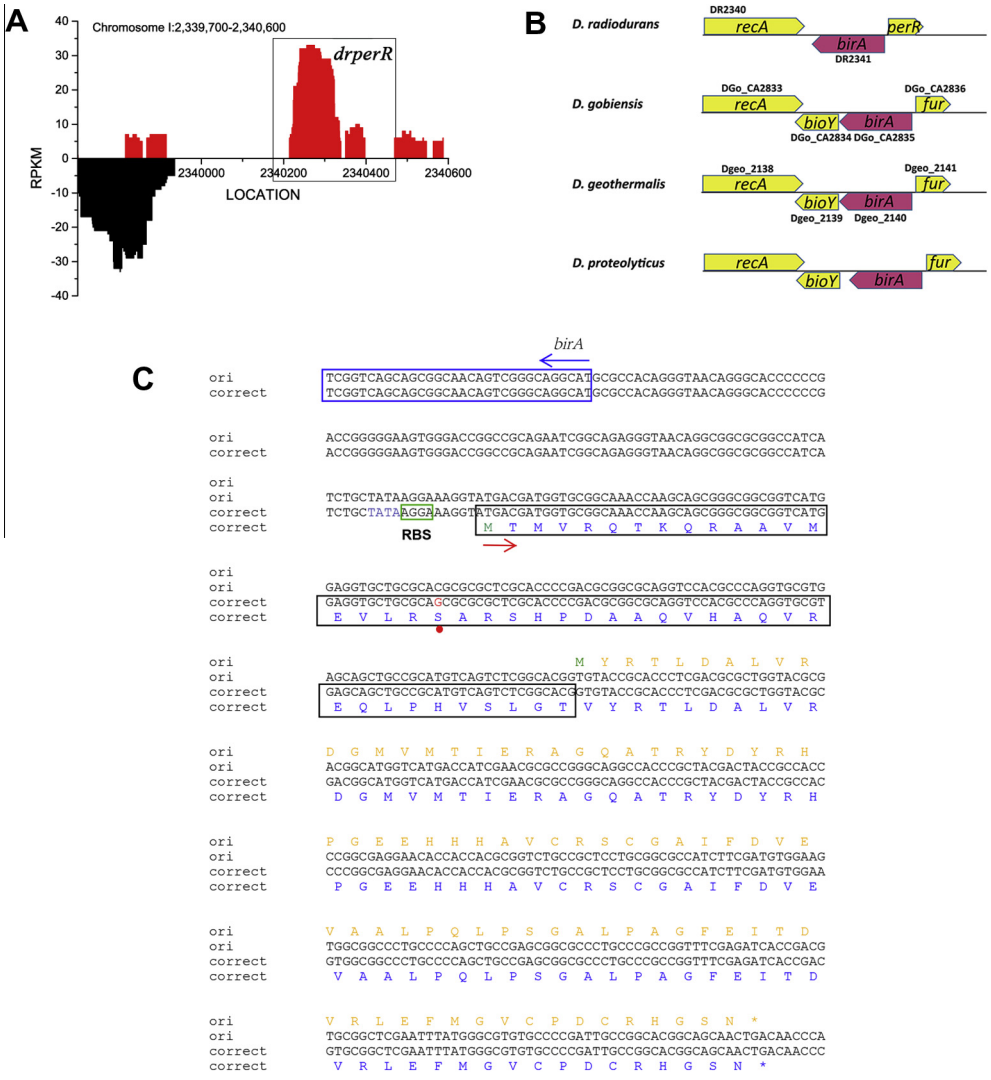

Fig. 1. Identification of a novel ORF embedded in the sequenced genome data of *D. radiodurans*. (A) RNA-seq data suggested a novel ORF at the positive strand. (B) Relative location of the ORF (*fur*) in *D. radiodurans, D. gobiensis, D. geothermalis,* and *D. proteolyticus.* (C) Nucleotide sequence of the *D. radiodurans drperR* gene. The predicted amino acid sequence (orange) was presented above the nucleotide sequence according to previous database (frame +3), and the re-sequenced segment was below (blue, frame +1), with a green M (Met) indicated the first code, respectively. TATA-box (blue) and ribosomal binding site (green box) was indicated at the corrected sequence. And the omission of G was indicated by a red circular symbol. The amino acid sequence affected by frame shift was shown in a black box. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Disruption of drperR gene increase the resistance of D. radiodurans to H_2O_2

A mutant *ΔdrperR* strain was less sensitive to H_2O_2 than wild type R1 (Fig. 2A), indicating that the *drperR* gene might be involved in cellular resistance to oxidative stress. However, the size of the inhibition zone was not substantially different in the present of metal ions, suggesting that the *drperR* gene might not contribute directly to Mn or iron uptake. The H_2O_2 sensitivity of *ΔdrperR* was further determined at different treatment concentration and time. In concentration-dependence assays, *ΔdrperR* survival was not affected at 10–50 mM H_2O_2, while the wild type strain was dramatically inhibited at 30 mM (Fig. 2B). In time-dependence assays, a decrease in *ΔdrperR* survival was not observed until 80 min (Fig. 2C).

3.3. drperR mutation reduced the cellular antioxidant capacity, activities of catalase and superoxide dismutase

Antioxidants play important roles in preventing the damages of ROS and thus contribute to cell survival under oxidative stress. Cellular antioxidant assay showed a 43% increase of antioxidant capacity in the *ΔdrperR* strain (0.6394 mM trolox/g) compared to the wild type (0.4469 mM trolox/g) (Fig. 3A).

Catalase and superoxide dismutase (SOD) are important antioxidant enzymes for ROS scavenging. Disruption of *drperR* leads to an increase of total catalases activity to 4.45-fold under normal condition. *D. radiodurans* genome has three catalase genes (*dr1998*, *dra0146* and *dra0259*). PAGE activity-staining assay demonstrated that disruption of *drperR* led to an increase of the activity of two catalases: KatE (DR1998) and KatB (DRA0146) (Fig. 3B). However, the activity of DRA0259 is not significantly different in the wild type and mutant strains. On the other hand, total SOD activity was not affected by the disruption of *drperR,* consistent with the PAGE activity-staining assay (Fig. 3C). Therefore, the increased cellular tolerance to H_2O_2 of the *ΔdrperR* strain might be at least partly attributed to the enhanced catalase activity.

3.4. Transcriptional levels of a catalase and dps are under the control of DrPerR

Quantitative real time PCR analysis (qRT-PCR) was performed to compare gene expression in the *ΔdrperR* and wild type R1 strain.

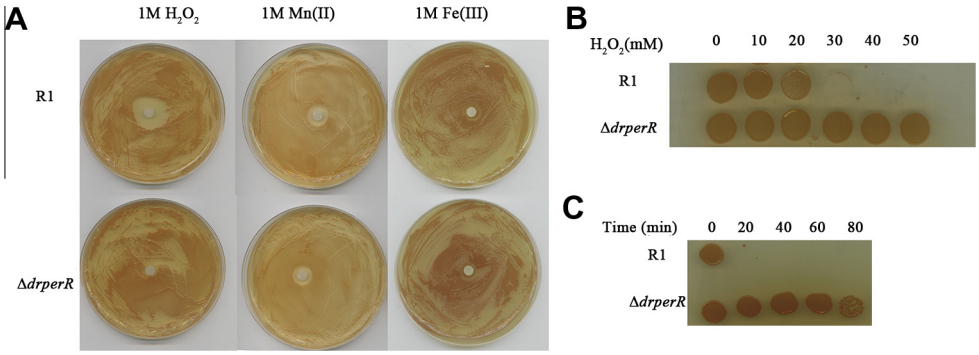

Fig. 2. Metal ions and hydrogen peroxide sensitivity of the wild type strain and the mutant *ΔdrperR*. (A) Inhibition zone of 1 mol/L hydrogen peroxide (left) 1 mol/L manganese ion (middle), and 1 mol/L ferric ion (right). (B) Growth of wild-type R1 and *ΔdrperR* under 0 ~ 50 mM H_2O_2 treatment. (C) Growth of wild-type R1 and *ΔdrperR* under 0–80 min treatment with 40 mM H_2O_2.

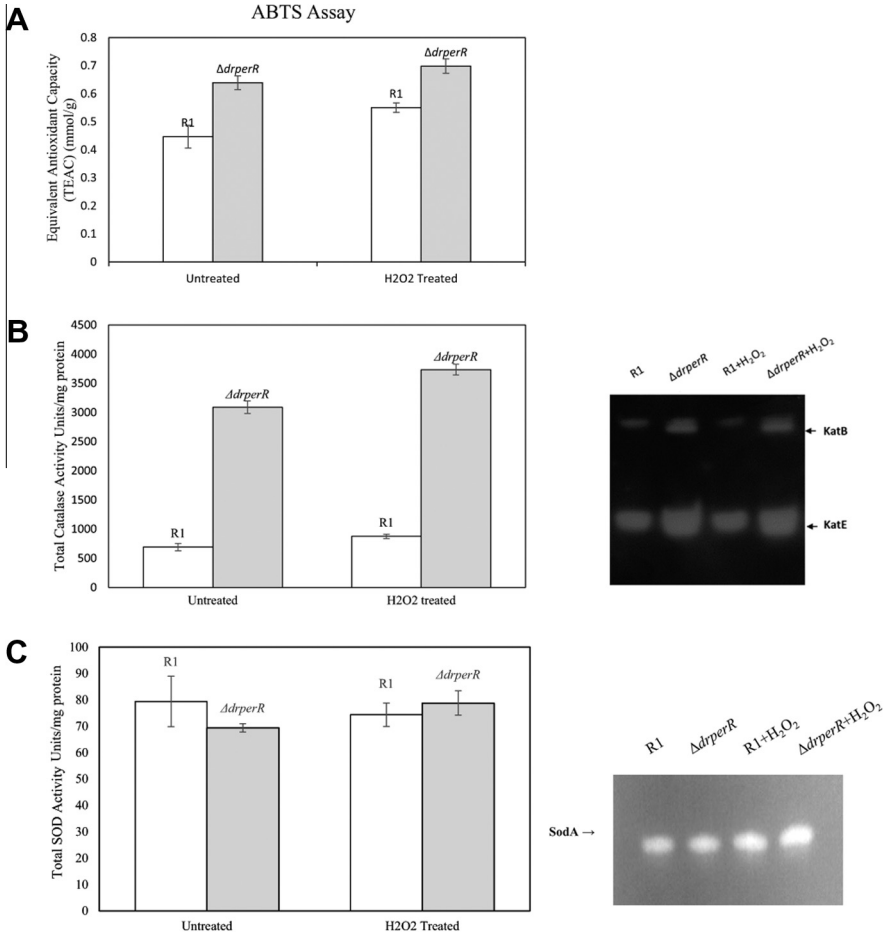

Fig. 3. Disruption of *drperR* increased the antioxidant activity in *D. radiodurans*. (A) Cellular antioxidant activity assay by ABTS method. (B) Catalase activity assay. (C) Superoxide dismutase activity. Assays were performed three times and values were presented as mean ± standard deviation. For PAGE activity-staining assay, each lane contained 20 μg of protein.

Under normal condition and H_2O_2 stress, *katE* transcription was 4.2-fold increased in *ΔdrperR* stain compared to wild type (Fig. 4). That was in consistence with the results of catalases activity measurement. However, the transcription of *dra0146* and *dra0259* did not show a substantially increase. In *B. subtilis*, PerR is proposed to bind to the promoter of *mrgA*, which was induced under oxidative stress [27,28]. *D. radiodurans* has two MrgA homologs: Dps-1 (DR2263) and Dps-2 (DRB0092), which function as iron chelating proteins to protect against oxidative stress. Their induction under oxidative stress implies negative control [29]. Therefore, we checked whether the transcription of *dps* was affected, and found a 1.4-fold increase in transcription of *dps-1*. The significant change of the *dps-2* transcription was not observed.

4. Discussion

ROS-response proteins modify the expression of defense and repair proteins to contribute to cell survival under oxidative stress [30,31]. In this work, we identified a novel Fur homolog (PerR)

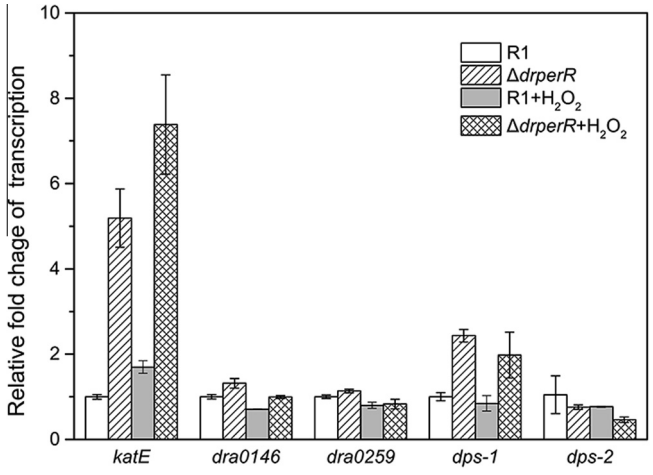

Fig. 4. Analysis of DrPerR-dependent genes. Real-time PCR analysis of the DrPerR-dependent genes expression with *dr0089* as internal control.

involved in the ROS response by regulating expression of antioxidant enzymes in the extreme bacterium *D. radiodurans*. With the emergence of next generation sequencing techniques from 454, Illumina and SOLiD, high-throughput and high-accuracy sequence analysis is possible. RNA sequencing using SOLiD technique identified a transcript from a noncoding region of the annotated *D. radiodurans* genome. The ORF showed high homology to PerR protein from the Fur family, but was different from the predicted Fur homolog DR0865 in *D. radiodurans*. The novel PerR from *D. radiodurans* had homologs with an identity above 50% in other Deinococcus-Thermus (Deide_19480, Dgeo_2141, Deipr_1246, Deima_0520, Deipe_1848, DGo_CA2836, and TTC1639) (Supplementary Fig. 1). In addition, the amino acid compositions had high identity to each other in the metal binding sites.

Further sequence analysis (Supplementary Fig. 2) showed that DrPerR contained two $CX_{2-4}C$ motifs (Cys 84, Cys 87 and Cys 123, Cys 126). Corresponding sites were found in most of Fur homologues including EcFur (*E. coli*), HpFur (*Helicobacter pylori*), especially in PerR-like metalloregulators such as BsPerR (*B. subtilis*). The second metal-binding site is thought to be a regulatory site that varies in different bacteria. In HpFur, the second metal binding site is co-coordinated by His 42, Glu 90, His 97, His 99 and Glu110, while in BsPerR the five residues were His 37, His 91, His 93, Asp 85 and Asp 104 [32]. The substitution of Glu with Asp was common among the PerR-like family members [32]. DrPerR shares a similar amino acid composition (His 23, His 79, His 81, Asp 61, and Asp 92) to BsPerR at the site. DR0865 possess a slightly different composition (Glu 70, His 77 and His 79, lacking Glu or Asp) from BsPerR and DrPerR. DrPerR seem to lack a third metal binding site seen in HpFur and DR0865.

DrPerR contributed to cellular resistance of *D. radiodurans* to oxidative stress, because gene disruption resulted in increased resistance to H_2O_2 and increased catalases activity in cell extraction. The *katE* (*dr1998*) gene encodes the major periplasmic catalase in *D. radiodurans* [14]. Increasing activity of KatE enhances H_2O_2 scavenging. We had verified that *katE* (*dr1998*) was also controlled by a ROS responding protein, OxyR [29]. Thus, *katE* transcription might be co-regulated by OxyR and DrPerR in *D. radiodurans*. In normal conditions, cells do not require excess catalase so its expression might be tightly repressed by DrPerR. When ROS signals reach certain level, *katE* expression was derepressed by inactivation of DrPerR via unknown pathway. Increase of the ROS level also activates the OxyR and thus promotes the expression of *katE*. This strategy would facilitate *D. radiodurans* adaption to fluctuant environment stresses. Moreover, DtxR (DR2539) and the Fur homologue (DR0865) are suggested to regulate KatE activity [33]. Therefore, the regulation of *katE* appears to be a complicated mechanism which might contribute to the precise adaption mechanism of *D. radiodurans* under oxidative stress.

Under oxidative stress, free Fe (II) is toxic to cells by Fenton Chemistry and should be tightly controlled. Dps is suggested to be responsible for free Fe (II) chelating and protecting DNA. A Dps homolog of *D. radiourans* is regulated by OxyR [29]. QRT-PCR results show that DrPerR repressed the expression of *dps*-1 (*dr2263*) under normal condition, while no significant changes was observed in the transcription of *dps-2* (*drb0092*). The regulation of Dps-1 by DrPerR links DrPerR to metal homeostasis, similarly to the regulation of *mrgA* by PerR in *B. subtilis* [28].

D. radiodurans possesses a powerful antioxidant system, and several vital regulators including OxyR have been investigated in *D. radiodurans*. However, the whole picture is still to be clarified. Here we discovered a novel, unpredicted Fur homologue in the *D. radiodurans* genome and characterized it as a PerR-like transcription regulator that responses to H_2O_2 and functions as a negative regulator of *katE* and *dps*, which are responsible for ROS scavenging and metal homeostasis, respectively. These results add to our understanding of the complex *D. radiodurans* regulatory network of antioxidants. Further research is needed to elucidate the interactions between the OxyR, DtxR, DR0865 and PerR in ROS response and metal ion homeostasis in *D. radiodurans*. This study also provided implications on how to utilize sequenced genome data and the importance of genome data mining. Resequencing of *D. radiodurans* genome by next generation sequencing would resolve the problems in genome data.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (31170079, 31210103904, 31370119, 31370102), a major project for genetically modified organisms breeding from the Ministry of Agriculture of China (2013ZX08009003-002), a special Fund for Agroscientific Research in the Public Interest (201103007), a Program for New Century Excellent Talents in University (NCET-10-0739), the Natural Science Foundation of Zhejiang Province (LY13C010001), the Fundamental Research Funds for the Central Universities and the Project for Zhejiang Provincial Construction of Technology Innovation Team (2010R50033).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.06.015.

References

[1] C.E. Paulsen, K.S. Carroll, Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery, Chem. Rev. 113 (2013) 4633–4679.
[2] K. Hantke, Iron and metal regulation in bacteria, Curr. Opin. Microbiol. 4 (2001) 172–177.
[3] H. Antelmann, J.D. Helmann, Thiol-based redox switches and gene regulation, Antioxid. Redox Signal. 14 (2011) 1049–1063.
[4] Q. Wei, P.N. Minh, A. Dotsch, F. Hildebrand, W. Panmanee, A. Elfarash, S. Schulz, S. Plaisance, D. Charlier, D. Hassett, S. Haussler, P. Cornelis, Global regulation of gene expression by OxyR in an important human opportunistic pathogen, Nucleic Acids Res. 40 (2012) 4320–4333.
[5] B.E. Ahn, J. Cha, E.J. Lee, A.R. Han, C.J. Thompson, J.H. Roe, Nur, a nickel-responsive regulator of the Fur family, regulates superoxide dismutases and nickel transport in *Streptomyces coelicolor*, Mol. Microbiol. 59 (2006) 1848–1858.
[6] E. Diaz-Mireles, M. Wexler, G. Sawers, D. Bellini, J.D. Todd, A.W. Johnston, The Fur-like protein Mur of *Rhizobium leguminosarum* is a Mn(2+)-responsive transcriptional regulator, Microbiology 150 (2004) 1447–1456.
[7] M. Fuangthong, J.D. Helmann, Recognition of DNA by three ferric uptake regulator (Fur) homologs in *Bacillus subtilis*, J. Bacteriol. 185 (2003) 6348–6357.

[8] P. Zuber, Management of oxidative stress in Bacillus, Annu. Rev. Microbiol. 63 (2009) 575–597.
[9] Y. Hua, I. Narumi, G. Gao, B. Tian, K. Satoh, S. Kitayama, B. Shen, PprI: a general switch responsible for extreme radioresistance of *Deinococcus radiodurans*, Biochem. Biophys. Res. Commun. 306 (2003) 354–360.
[10] K.S. Makarova, L. Aravind, Y.I. Wolf, R.L. Tatusov, K.W. Minton, E.V. Koonin, M.J. Daly, Genome of the extremely radiation-resistant bacterium *Deinococcus radiodurans* viewed from the perspective of comparative genomics, Microbiol. Mol. Biol. Rev. 65 (2001) 44–79.
[11] D. Slade, M. Radman, Oxidative stress resistance in *Deinococcus radiodurans*, Microbiol. Mol. Biol. Rev. 75 (2011) 133–191.
[12] A. Krisko, M. Radman, Biology of extreme radiation resistance. the way of *Deinococcus radiodurans*, Cold Spring Harb. Perspect. Biol. 5 (2013).
[13] M.J. Daly, E.K. Gaidamakova, V.Y. Matrosova, J.G. Kiang, R. Fukumoto, D.Y. Lee, N.B. Wehr, G.A. Viteri, B.S. Berlett, R.L. Levine, Small-molecule antioxidant proteome-shields in *Deinococcus radiodurans*, PLoS One 5 (2010) e12570.
[14] L.M. Markillie, S.M. Varnum, P. Hradecky, K.K. Wong, Targeted mutagenesis by duplication insertion in the radioresistant bacterium *Deinococcus radiodurans*: radiation sensitivities of catalase (katA) and superoxide dismutase (sodA) mutants, J. Bacteriol. 181 (1999) 666–669.
[15] J.R. Battista, A.M. Earl, M.J. Park, Why is *Deinococcus radiodurans* so resistant to ionizing radiation?, Trends Microbiol 7 (1999) 362–365.
[16] B. Tian, Y. Hua, Carotenoid biosynthesis in extremophilic Deinococcus-Thermus bacteria, Trends Microbiol. 18 (2010) 512–520.
[17] D. Ghosal, M.V. Omelchenko, E.K. Gaidamakova, V.Y. Matrosova, A. Vasilenko, A. Venkateswaran, M. Zhai, H.M. Kostandarithes, H. Brim, K.S. Makarova, L.P. Wackett, J.K. Fredrickson, M.J. Daly, How radiation kills cells: survival of *Deinococcus radiodurans* and *Shewanella oneidensis* under oxidative stress, FEMS Microbiol. Rev. 29 (2005) 361–375.
[18] O. White, J.A. Eisen, J.F. Heidelberg, E.K. Hickey, J.D. Peterson, R.J. Dodson, D.H. Haft, M.L. Gwinn, W.C. Nelson, D.L. Richardson, K.S. Moffat, H. Qin, L. Jiang, W. Pamphile, M. Crosby, M. Shen, J.J. Vamathevan, P. Lam, L. McDonald, T. Utterback, C. Zalewski, K.S. Makarova, L. Aravind, M.J. Daly, K.W. Minton, R.D. Fleischmann, K.A. Ketchum, K.E. Nelson, S. Salzberg, H.O. Smith, J.C. Venter, C.M. Fraser, Genome sequence of the radioresistant bacterium *Deinococcus radiodurans* R1, Science 286 (1999) 1571–1577.
[19] J.M. Eggington, N. Haruta, E.A. Wood, M.M. Cox, The single-stranded DNA-binding protein of *Deinococcus radiodurans*, BMC Microbiol. 4 (2004) 2.
[20] D. Hyatt, G.L. Chen, P.F. Locascio, M.L. Land, F.W. Larimer, L.J. Hauser, Prodigal: prokaryotic gene recognition and translation initiation site identification, BMC Bioinformatics 11 (2010) 119.
[21] A.L. Delcher, K.A. Bratke, E.C. Powers, S.L. Salzberg, Identifying bacterial genes and endosymbiont DNA with Glimmer, Bioinformatics 23 (2007) 673–679.
[22] B. Tian, Z. Xu, Z. Sun, J. Lin, Y. Hua, Evaluation of the antioxidant effects of carotenoids from *Deinococcus radiodurans* through targeted mutagenesis, chemiluminescence, and DNA damage analyses, Biochim. Biophys. Acta 1770 (2007) 902–911.
[23] Z. Xu, B. Tian, Z. Sun, J. Lin, Y. Hua, Identification and functional analysis of a phytoene desaturase gene from the extremely radioresistant bacterium *Deinococcus radiodurans*, Microbiology 153 (2007) 1642–1652.
[24] P. Esposito, G. Varvara, S. Caputi, G. Perinetti, Catalase activity in human healthy and inflamed dental pulps, Int. Endod. J. 36 (2003) 599–603.
[25] M. Li, H. Sun, Q. Feng, H. Lu, Y. Zhao, H. Zhang, X. Xu, J. Jiao, L. Wang, Y. Hua, Extracellular dGMP enhances *Deinococcus radiodurans* tolerance to oxidative stress, PLoS One 8 (2013) e54420.
[26] L. Wang, G. Xu, H. Chen, Y. Zhao, N. Xu, B. Tian, Y. Hua, DrRRA: a novel response regulator essential for the extreme radioresistance of *Deinococcus radiodurans*, Mol. Microbiol. 67 (2008) 1211–1222.
[27] M.J. Faulkner, Z. Ma, M. Fuangthong, J.D. Helmann, Derepression of the *Bacillus subtilis* PerR peroxide stress response leads to iron deficiency, J. Bacteriol. 194 (2012) 1226–1235.
[28] M. Fuangthong, A.F. Herbig, N. Bsat, J.D. Helmann, Regulation of the *Bacillus subtilis* fur and perR genes by PerR: not all members of the PerR regulon are peroxide inducible, J. Bacteriol. 184 (2002) 3276–3286.
[29] H. Chen, G. Xu, Y. Zhao, B. Tian, H. Lu, X. Yu, Z. Xu, N. Ying, S. Hu, Y. Hua, A novel OxyR sensor and regulator of hydrogen peroxide stress with one cysteine residue in *Deinococcus radiodurans*, PLoS One 3 (2008) e1602.
[30] J.M. Dubbs, S. Mongkolsuk, Peroxide-sensing transcriptional regulators in bacteria, J. Bacteriol. 194 (2012) 5495–5503.
[31] L. Wang, L. Yin, G. Xu, M. Li, H. Zhang, B. Tian, Y. Hua, Cooperation of PprI and DrRRA in response to extreme ionizing radiation in *Deinococcus radiodurans*, Chin. Sci. Bull. 57 (2012) 98–104.
[32] D.A. Traore, A. El Ghazouani, S. Ilango, J. Dupuy, L. Jacquamet, J.L. Ferrer, C. Caux-Thang, V. Duarte, J.M. Latour, Crystal structure of the apo-PerR-Zn protein from *Bacillus subtilis*, Mol. Microbiol. 61 (2006) 1211–1219.
[33] H. Chen, R. Wu, G. Xu, X. Fang, X. Qiu, H. Guo, B. Tian, Y. Hua, DR2539 is a novel DtxR-like regulator of Mn/Fe ion homeostasis and antioxidant enzyme in *Deinococcus radiodurans*, Biochem. Biophys. Res. Commun. 396 (2010) 413–418.