Chapter

An Antioxidant Defense System in Radiation-Resistant Bacterium *Deinococcus geothermalis* against Oxidative Stress

Chanjae Lee, Min K. Bae and Sung-Jae Lee

Abstract

A radiation-resistant bacterium, *Deinococcus geothermalis* has various stress response mechanisms, including antioxidation. Features that maintain vitality at high radiation doses include the following: enzymatic scavengers of ROS such as catalase, SOD, and peroxidase; strain-specific DNA repair systems such as Deinococcal unique proteins; non-enzymatic responses such as manganese complexes, carotenoids, and DNA-binding proteins. This chapter summarizes the primary response mechanism by redox balance centered on the cystine transporter. It also reviews action characteristics of DNA-binding protein Dps and a putative LysR family protein, and effects on loss of function of the carotenoid biosynthesis genes by transposition of insertion sequences. Environmental adaptation and molecular evolution of radiation-resistant bacterium are also considered to explain the potentials of molecular behavior induced by oxidative stress.

Keywords: cystine ABC transporter, Dps, LysR regulator, oxidative stress, redox-potential, transposition

1. Introduction

The radiation-resistant bacterium of genus *Deinococcus* is an essential resource for research to understand responses to oxidative stress and mechanisms for recovering direct double-strand break damage to DNA caused by gamma-radiation [1–3]. High gamma-ray resistance is caused by the unique DNA-damage repair proteins and various protective mechanisms in these radiation-resistant *Deinococcus* bacteria [4–6]. Many researchers have studied the properties of their unique proteins. These studies have expanded our scientific understanding [7–9]. Technological advances have recently been made to understand life phenomena through genomics, metabolomics, and proteomics studies [10–12]. Despite the remarkable progress in recent omics studies, it is still difficult to fully understand these cell recovery characteristics from various cellular stress damages. The accumulation of various creative research results will eventually lead to a complete understanding of such characteristics.

Only 20% of DNA damage is directly caused by radiation. In comparison, the remaining 80% is indirectly caused by reactive oxygen species (ROS) such as

superoxide and hydroxyl radicals which are chemically reactive molecules that can damage cell structures such as cell membrane, proteins, and nucleic acids (DNA and RNA) [1, 10]. Bacteria have a natural ROS scavenging system composed of enzymatic antioxidants (e.g. catalase, peroxidase, superoxide reductase, and superoxide dismutase (SOD)), and non-enzymatic antioxidants (e.g. intracellular manganese, pyrroloquinoline quinone, carotenoids), small antioxidant thiols (e.g. cystine, bacillithiol, or mycothiol), and DNA-protecting proteins [2, 13–15].

Specific regulators tightly control many stress response defense systems. Enzymatic ROS scavengers are regulated by the global transcriptional regulator OxyR, a LysR family regulator [16–20]. OxyR of *Deinococcus radiodurans* is a 1-Cys-type that can activate the transcription of genes encoding catalase (*katE*), ferrous iron transporter (*feoB*), and iron(III) dicitrate transporter (drb0125). It is also a repressor of *dps* and *mntH* transcription to control antioxidant functions and Mn/Fe ion homeostasis [21].

These gene regulation systems are also susceptible to intracellular redox balance through specific ABC transporters and chemical modification of low-molecular-weight (LMW) thiol compounds using unique enzyme reactions. The cystine importer is one of the redox controlling ABC transporters [22–25]. It could sense the redox balance and affect gene regulation for enzymatic defense through the OxyR activation [15, 20]. There are also some exceptional OxyR regulons in bacteria [26–28].

This redox balance affects various enzymatic and chemical modification processes through a progressive transformation. For example, acetylation is a conserved modification used to regulate various cellular pathways such as gene expression, protein synthesis, detoxification, and virulence. Acetyltransferase enzymes can transfer an acetyl moiety, usually from acetyl coenzyme A (AcCoA), onto a target substrate, thereby modulating the activity or stability [29]. Gcn5-related N-acetyltransferase (GNAT) members can acetylate the amino group of an extensive range of substrates. They are classified into three groups: (1) small molecule acetyltransferases such as aminoglycosides and mycothiol; (2) peptide acetyltrandferases such as the peptidoglycan that is part of the cell wall; and (3) protein acetyltransferases such as the histone family [30]. In Gram-positive *Actinomycetes* and *Firmicutes*, alternative LMW thiols such as mycothiol (MSH) and bacillithiol (BSH) play related as glutathione surrogates of Gram-negative bacteria, respectively [31].

As antioxidant substances, carotenoid compounds also act as scavengers of ROS. *Deinococcales* species generally have a reddish color phenotype due to carotenoid biosynthesis. The metabolic pathway in *Deinococcus* is well conserved and industrially applicable [32].

As one of bacterial nucleoid proteins in gene expression specificity of growth phase-dependent manner, Dps (DNA-binding protein from starved cells) is initially suppressed at the exponential cell growth phase. It is then expressed in large quantities in the stationary growth phase to become the major protein [33, 34]. These sequential nucleoid protein transitions and overexpression of a particular protein demonstrate the function of a defense mechanism that can protect against cell damage during stress due to increased ROS and reduced nutrients that cells can consume. Dps proteins are found almost ubiquitously in bacterial genomes. Each bacterial genome contains species-specific Dps genes. Dps has multifaceted roles such as DNA binding, iron sequestration, and ferroxidase activity in various stress responses [35–37]. Dps was described initially in *Escherichia coli* as a protein that could protect the bacteria in a malnourished environment by DNA-binding [38]. Dps has a shell-like structure with a spherical hollow cavity in the center. This hollow cavity of Dps acts as an iron storage compartment and iron sequestration that

is important in iron detoxification and homeostasis. Dps is a small DNA-binding protein having approximately 150 to 300 amino acid residues with a positive charge along the entire chain. While Dps is a significant protein when it is over-expressed, Dps plays another essential role in condensing and protecting the stationary-phase nucleoid from severe environmental stress such as oxidative and radioactive damages [34, 39, 40]. Therefore, many bacteria express more than one Dps depending on environmental factors around them. How the condensed DNA is untangled when proteins are expressed remains unclear. It merits further studies.

Bacterial insertion sequence (IS) elements consist of a gene encoding transposase (one or two), terminal inverted repeats (TIR), and direct repeats (DR). Their lengths are less than 3 kb [41, 42]. According to the database of IS finding platform such as ISfinder, IS types vary [43]. IS densities are significantly less in chromosomes than in plasmids in bacteria. Perhaps plasmids are the primary IS carrier [44]. These IS elements can be transferred by high temperature, γ-irradiation, oxidative stress, and substances that could damage DNA and result in gene breakdown [45–47]. Regulation of IS transposition is also affected by various factors such as transcriptional repressors and inhibitors, ribosome frameshifting, methylation, mRNA stability, and target sequences [48–51]. Gene breakdown by transposition of IS elements does not just have deleterious aspects. IS-mediated gene inhibition offers various advantages such as virulence, antibiotic/xenobiotic resistance, metabolism, and small promoter obtained by IS migration in the genome of a strain [52].

A comprehensive paper on *D. radiodurans*'s antioxidant mechanism and control systems of specific regulator proteins in response to oxidation stress has been published during the preparation of this chapter [53]. Here, we will focus on antioxidation mechanisms in *Deinococcus geothermalis*, a radiation-resistant bacterium used within the research destination's scope [54]. We characterized the cystine importer and used transcriptomics analysis to detect the critical players in intracellular antioxidant responses. The putative Dps protein and LysR family regulator's functional role was then determined and IS transposition events were selected on carotenoid biosynthesis path defect (**Figure 1**). Understanding the

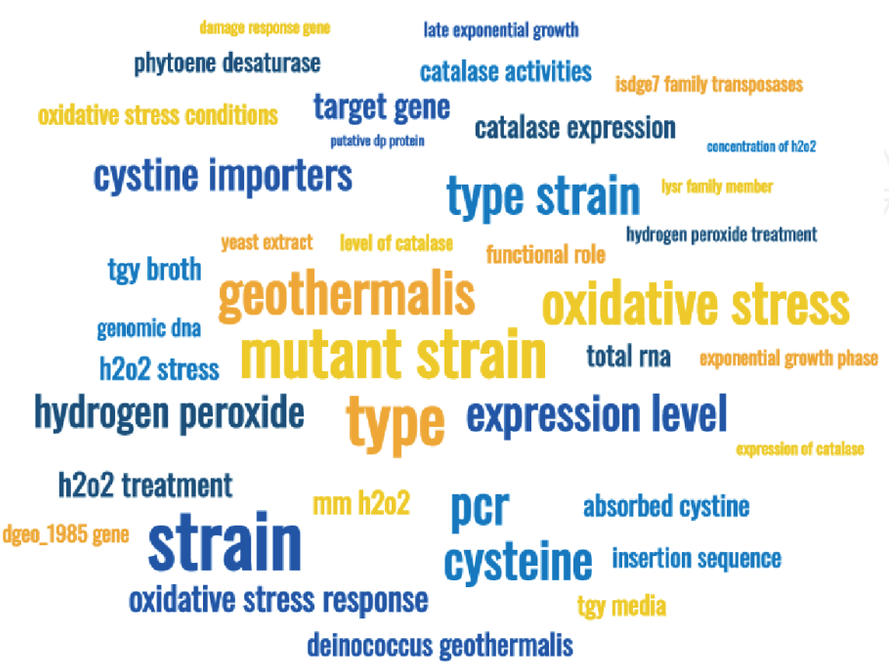

Figure 1.
WordCloud analysis of our owns four published papers [25, 55, 61, 62] by the WordCloud generator 40 quantity of MonkeyLearn.

sophisticated antioxidant system of living organisms is a problematic and challenging study like a puzzle game.

2. An antioxidant defense system in a radiation-resistant bacterium

2.1 A cystine importer, redox balance and control of gene expression

D. geothermalis contains a cystine importer as a substrate-binding protein and a membrane permease Dgeo_1986-87 which is highly expressed at the late exponential growth phase [25, 55]. Its intracellular total thiol level is affected by the expression level of this cystine importer. *Deinococcus* has specific genes that can repair when DNA is damaged. Using *dgeo*_1986-87 a cystine importer disrupted mutant strain, we have detected the expression levels of unique DNA repair proteins, *ppr*A, *ddr*A, and *ddr*B. These DNA repair proteins were highly up-regulated under oxidative stress conditions induced by 50 mM H_2O_2 [55]. However, when cystine importer expression is enhanced in a mutant Dgeo_1985R strain, DNA repair proteins are entirely down-regulated [25]. The increased intracellular thiol concentration strongly repressed the expression level of these DNA repair-related unique genes, excluding *rec*A gene in *Deinococcus* through overexpressed cystine transporter. Therefore, unique DNA repair proteins in *Deinococcus* are controlled by redox potential levels. If there is a direct controlling system for unique DNA repair genes, maybe it is repressed by the reduced redox potential through the cystine transporter's overexpression.

In general, the primary antioxidant enzyme, e.g. catalase, is highly induced by an oxidative stress condition. It is positively controlled by a global transcriptional regulator OxyR [16]. In *D. radiodurans*, the redox sensor OxyR has a single cysteine residue in the active site. It controls the expression of catalase and iron/manganese uptake proteins positively [21]. However, in *D. geothermalis* wild-type and Δ*dgeo*_1986-87 cystine importer disrupted strain, expression level of *oxyR* is strongly induced. OxyR is not proportionally affected on catalase expression level. Thus, OxyR is not a positive regulator of catalase.

The strain's cystine transport has been found to be dependent on the growth phase. In other words, some features are often expressed in the latter half of the exponential phase. In a mutant with the importer gene removed, it reacts relatively sensitive to oxidative stress. However, if the importer is overexpressed, its resistance to hydrogen peroxide is increased. A mutant that artificially overexpression the importer shows increased resistance to hydrogen peroxide without being affected by catalase expression, which results from an increase in the content of total thiol entering the cell through the cystine importer [15]. Therefore, the intracellular reduction state through enhancing thiol contents is a primary defense system of *D. geothermalis* against oxidative stress without induction of enzymatic ROS defense factors.

2.2 Hints from transcriptomic analysis

We performed transcriptomic analysis using RNA-Seq technology to define functional roles of bacterial TrmB (Dgeo_1985), Dps (Dgeo_0257), a cystine importer (Dgeo_1986-87), and LysR family regulator (Dgeo_2840). We constructed target gene disrupted mutants. Expression levels of all genes at OD_{600} 4.0 as a late exponential growth phase in mutants were then compared to those in

wild-type *D. geothermalis* [55]. Data have been deposited in NCBI's Gene Expression Omnibus. They are accessible through GEO series accession number GSE151903.

First, a transcriptomic study was done to compare gene expression levels between wild-type and cystine importer deleted mutant of *D. geothermalis*. Genes up-regulated more than 3.0-fold of log value are listed in **Table 1**. Both CRISPR-Cas system gene clusters, Dgeo_0233-38 gene cluster and Dgeo_0956-65 gene cluster, were up-regulated 35.1-105.5-fold and 10.3-65.2-fold, respectively. Iron transporter Dgeo_2443 and 2444 genes were up-regulated 4.69 and 12.42-fold, respectively. Three gene clusters for GCN5, Dgeo_0369-70, 2125, and 2313, were up-regulated 12.53, 3.73, and 11.2-fold, respectively. Four MFS transporters, Dgeo_0249, 0530, 1968, and 2330, were up-regulated 3.75, 6.41, 5.57, and 3.22-fold, respectively. Four ABC transporters, Dgeo_0543, 0647, 1805, and 2581, were up-regulated 3.34, 6.96, 8.03, and 3.6-fold, respectively.

Δ*dgeo*_0257 and Δ*dgeo*_2840 mutant strains were revealed many no effect and several fluctuated patterns. The CRISPR-Cas system's slightly upregulated expression was also found in the LysR family regulator Dgeo_2840 disrupted mutant, but not in a putative Dps gene Dgeo_0257 disrupted mutant. In the case of Δ*dgeo*_2840 mutant, a different iron transporter *dgeo*_1370 was up-regulated 3.35-fold. However, gene expression levels of GCN5 and MFS transporter gene clusters were not affected in Δ*dgeo*_0257 or Δ*dgeo*_2840 mutant strain. When the intracellular redox potential was reduced through disruption of a cystine importer, why these gene clusters with several distinct physiological functions showed dramatic overexpression? Do they somehow have a relationship with antioxidant responses? These questions are interesting. Future studies in this field of antioxidation research are needed. We focused on two antioxidant biosynthesis pathways for bacillithiol and mycothiol because these pathways are related to up-regulated GCN5 gene clusters.

Gene clusters	Genes	△*dgeo*_1986-87	△*dgeo*_0257	△*dgeo*_2840
CRISPR-Cas	Dgeo_0233-38	35.1–105.6	—	3.36
	Dgeo_0956-65	10.3–65.2	—	4.59–5.46
Iron transporter	Dgeo_1370	—	—	3.35
	Dgeo_2443-44	4.69–12.42	—	—
GCN5	Dgeo_0369-70	12.53	—	—
	Dgeo_2125	3.73	—	—
	Dgeo_2313	11.2	—	—
MFS transporter	Dgeo_0249	3.75	—	—
	Dgeo_0530	6.41	—	—
	Dgeo_1968	5.57	—	—
	Dgeo_2330	3.22	—	—
ABC transporter	Dgeo_0543	3.34	0.35	1.62
	Dgeo_0647	6.96	1.54	—
	Dgeo_1805	8.03	—	—
	Dgeo_2581	3.60	0.69	—
RpiR family	Dgeo_2822	—	—	3.20
	Dgeo_2619	0.20	0.29	0.28

Table 1.
Transcriptomics analysis for some target genes among wild-type and mutants.

2.3 Mycothiol as a major under oxidation state

D. geothermalis genome contains 28 GNAT proteins [56]. Four GNAT genes, *dgeo*_0369-0370, 2125, and 2313, contribute to its redox-balancing regulation. In Δ*dgeo*_1986-87 mutant, these four GNAT genes were up-regulated over 3.0-fold (**Table 1**). Dgeo_2125 is an acetyltrans_3 family member. However, its function has not been characterized yet. Dgeo_0370 is a putative phosphinothricin acetyltransferase, a broad-spectrum herbicide that acts as a competitive inhibitor of glutamine synthetase. Dgeo_0369 is a putative RimI which is a S18 ribosomal protein acetylation enzyme. Dgeo_2313 is a putative mycothiol synthase MshD (**Table 2**). Thus, Dgeo_2313 is a gene direct-related to redox potential because mycothiol acts as a total thiol balance. Mycothiol is the main LMW thiol in most *Actinomycetes*, including *Mycobacterium tuberculosis* [31]. MshD and MshC were strongly induced in Δ*dgeo*_1986-87 mutant with the absence of hydrogen peroxide. However, when hydrogen peroxide was present, MshD expression was strongly down-regulated to be under 0.3-fold.

Somehow, intracellular redox potential affects these GNAT regulations. If two artificial conditions such as oxidation and reduction are provided, the expression levels of redox potential-dependent GNAT genes would be detected. These variable expression levels of GNAT genes will provide stress response control. At the moment, the physiological roles of these four GNAT proteins remain unclear. In general, proteins in the GNAT superfamily have broad-spectrum physiological functions. Their amino acid sequence identities are very low. Thus, predicting their functional roles through protein sequence similarities is difficult.

How about expression levels of bacillithiol (BSH) biosynthesis-related genes in the transcriptome of *D. geothermalis*? The genome of *D. geothermalis* contains BSH biosynthesis enzymes BshA (Dgeo_1099; BSH biosynthesis glycosyltransferase), BshB1 (Dgeo_2305; BSH biosynthesis deacetylase), BshC (Dgeo_1276; BSH biosynthesis cysteine-adding enzyme), and BstA (Dgeo_1829; BSH transferase) as Drad BSH-related genes. It also contains BSH reductase (Dgeo_2331; YpdA) and bacilliredoxin (Dgeo_1464; YtxJ). Despite all genes involved in BSH biosynthesis and degradation pathway, expression levels of these genes were not affected in the intracellular oxidation state of Δ*dgeo*_1986-87 strain. However, they might be affected by other stressors such as heat shock and hydrogen peroxide treatment.

2.4 Dps and its mysterious roles

D. radiodurans have two paralogous Dps proteins, each known to play a different role. DrDps1 (DR2263) binds to both linear and coiled DNA. However, DrDps2 (DRB0092) preferentially binds to coiled DNA, forming different conformation of protein-DNA complexes to protect DNA against ROS, although its protection occurs at different iron to protein ratios. The difference between two DrDps could result from the fact that DrDps1 has higher iron oxidation rate in the presence of hydrogen

Genes	△*dgeo*_1986-87	△*dgeo*_0257	△*dgeo*_2840
MshA (Dgeo_2307)	0.79	0.98	0.69
MshB (Dgeo_1021)	1.14	1.00	1.10
MshC (Dgeo_1714)	10.78	0.88	1.36
MshD (Dgeo_2313)	11.2	1.21	0.99

Table 2.
Expression levels of MSH biosynthesis-related genes.

peroxide and higher affinity to bind DNA than DrDps2 [37]. In summary, DrDps1 may function in DNA metabolism, while DrDps2 may protect against exogenously derived ROS [57].

D. geothermalis has two Dps proteins homologous to Dps proteins of *D. radiodurans*. Dgeo_0281 is homologous to DrDps1 (DR_2263). The novel Dgeo_0257 has been proposed to be one of Dps DNA-binding proteins in *D. geothermalis*. It probably has different roles from DrDps1 homologous protein, Dgeo_0281. Dgeo_0257 shares 72% amino acid sequence identity to DR_0528 of *D. radiodurans*, suggesting the need for research as another candidate protein of DrDps. The DrDps2 (DR_B0092) did not share any significant sequence identities with proteins of *D. geothermalis*. Dgeo_0281 and Dgeo_0257 share only 11.5% amino acid sequence identity, lower than 16% amino acid sequence identity shared between DrDps1 and DrDps2.

We prepared both *dps* genes disrupted mutant strains, Δ*dgeo*_0257 and Δ*dgeo*_0281, and they were more susceptible to H_2O_2 than the wild-type strain. The novel putative Dps Dgeo_0257 might play a role in DNA protection and antioxidant reactions such as Dgeo_0281. DNA-binding capacities of purified Dgeo_0257 and Dgeo_0281 proteins were then determined by electrophoretic mobility shift assay (EMSA). Gel filtration assay was also performed for conformational determination [58]. Dgeo_0257 protein has a 5-fold higher DNA-binding affinity than Dgeo_0281. Interestingly, both Dps proteins were found to have similar metal-sensing behavior (**Figure 2**). When ferrous ion was present, Dps proteins could not bind to DNA. Their DNA-binding activity was found to be non-specific for DNA sequence. To determine the physiological functions of these two Dps proteins, we performed quantitative real-time (qRT)-PCR analysis for both *dgeo*_0257 and *dgeo*_0281 genes in wild-type, Δ*dgeo*_0257, and Δ*dgeo*_0281 mutant strains at different growth phase in a time-course study. Surprisingly, the *dgeo*_0281 gene was early expressed at OD_{600} 2.0. Its expression then gradually reduced at OD_{600} 4.0 and 8.0. However, *dgeo*_0257 was dramatically induced in a stationary phase at OD_{600} 8.0. Thus, we predicted that both Dps proteins of *D. geothermalis* had growth phase-dependent specificity.

2.5 Active transposition of insertion sequences under oxidative stress condition

Various selectable approaches have detected transposition events of ISs. For example, IS*Dra2* was induced by irradiation, causing the *thyA* (thymidylate synthase) gene to be destroyed in *D. radiodurans*. As a result, *thyA* mutant became

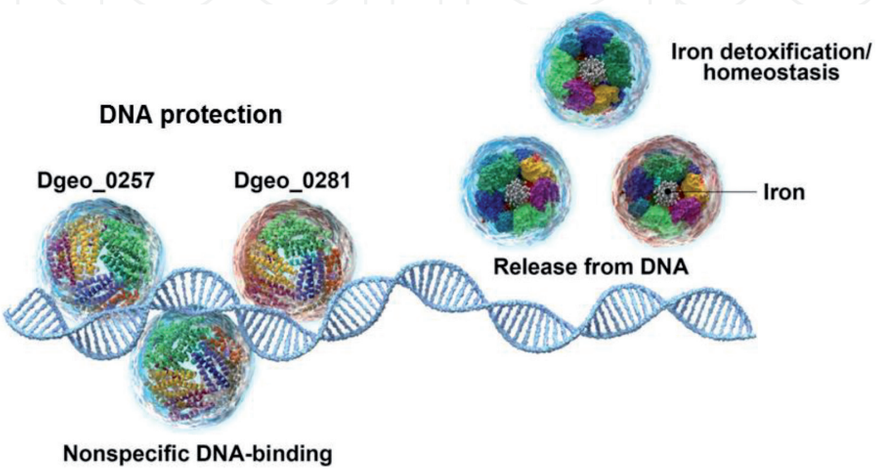

Figure 2.
Illustration of DNA protection and iron detoxification roles of two Dps proteins in D. geothermalis.

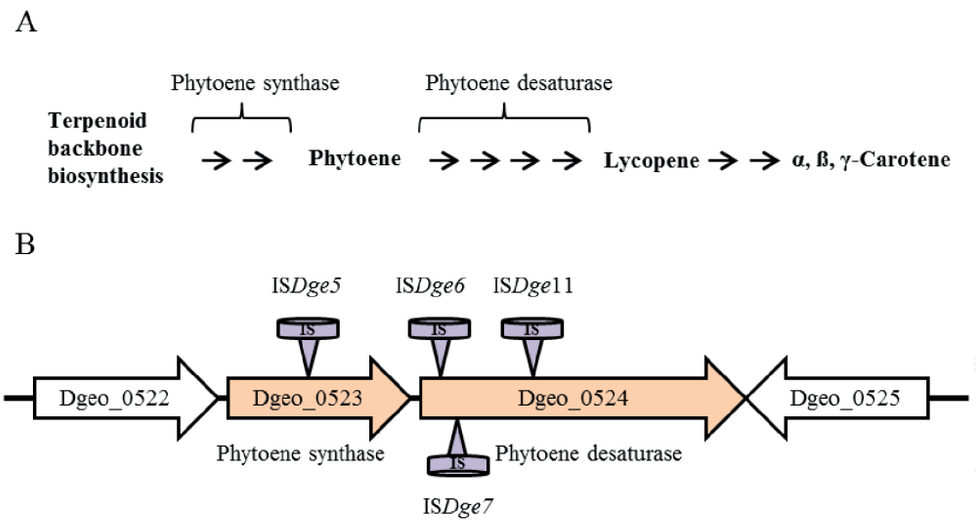

Figure 3.
Brief scheme of metabolic pathway (A) and the ISs integrated loci in the gene cluster for carotenoid biosynthesis (B).

resistant to trimethoprim [47, 59]. In the case of antibiotic-resistant phenotype, a certain IS element was integrated into *rsmG* gene disrupted by IS*Tth7* of IS*5* family in *Thermus thermophilus*, resulting in streptomycin-resistance [60].

The genome of *D. geothermalis* contains a total of 73 ISs. *Deinococcus* species were found to have pink or reddish colored colonies. However, *D. geothermalis* wild-type, Dps-like gene disrupted mutant (Δ*dgeo*_0257 mutant), and LysR gene disrupted mutant (Δ*dgeo*_2840 mutant) were found to have white-colored colonies under an oxidative stress condition. The reason was that phytoene desaturase function of Dgeo_0524 as a carotenoid pathway-related gene was interrupted by the transposition of each IS element: IS*Dge6* for Δ*dgeo*_2840 mutant, IS*Dge7* for Δ*dgeo*_0257 mutant, and IS*Dge11* for wild-type (**Figure 3**) [32, 61, 62]. Among down-regulated genes in RNA-seq, two genes (*dgeo*_0928 and *dgeo*_1785) were disturbed by IS*Dge5* in the Δ*dgeo*_0257 mutant strain. A new biomarker for finding transposition loci with antibiotic streptomycin-resistance was also used easily to selecting colonies on streptomycin contained media. When the IS*Dge6* element was inserted into the *rsmG* gene (*dgeo*_2335) encoding ribosomal RNA small subunit methyltransferase and a point mutation or frameshift mutation on *rsmG* gene occurred, mutant strains were resistant to 50 μg/ml streptomycin (prepared manuscript). In the current discovery, IS*Dge5*, IS*Dge6*, IS*Dge7*, and IS*Dge11* were all replicating transposition modes through PCR detection of target genes. We found that each IS element was transposed, explicitly depending on DNA-binding proteins from these active transposition events. There is an open question. Although the genome of *D. geothermalis* contains a high copy number of IS elements such as IS*Dge2*, IS*Dge3*, IS*Dge4*, and IS*Dge13*, transposition events have not been found yet. Nevertheless, in the case of IS*Dge2*, its transposase gene expression was strongly induced by oxidative stress. Thus, it is a big challenge to detect DNA-binding protein-dependent IS transposition occurrence. We can imagine that when the environmental factor is changed from oxidative stress to others such as other source radiations, gravity, pressure, and certain chemicals, specialized IS elements might be transposed into other loci in the genome.

3. Conclusion

As a model for oxidative stress response, *Deinococcus* species is a beneficial model organism to understand its survival strategies in the presence of harsh

environmental stressors such as ionizing radiation, desiccation, and ultraviolet light. It is also a useful model organism to understand DNA damage repair mechanisms and industrial application such as bioremediation of toxic substances. For these reasons, many researchers are interested in applying extreme conditions, including microgravity and universe exposure outside the international space station, to a type strain of *D. radiodurans* recently. Here, we focused on several aspects of oxidative stress defense systems dependent on our research destination, for example, intracellular redox balance through a cystine importer, antioxidant substance carotenoid biosynthesis, DNA protecting and iron detoxification protein Dps, and transposition of IS elements under oxidative stress. We hope this chapter will provide an opportunity to open up a new horizon in traditional research as we learn about the phenomena linked differently to known antioxidant response mechanisms in radiation-resistant strains.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Chanjae Lee, Min K. Bae and Sung-Jae Lee*
Department of Biology, Kyung Hee University, Seoul, Korea-South

*Address all correspondence to: sungjaelee@khu.ac.kr

References

[1] Battista JR, Earl AM, Park MJ. Why is *Deinococcus radiodurans* so resistant to ionizing radiation? Trends in Microbiology. 1999;7:362-365. DOI:10.1016/s0966-842x(99)01566-8.

[2] Blasius M, Hubscher U, Sommer S. *Deinococcus radiodurans*: what belongs to the survival kit? Crit Rev Biochem Mol Biol. 2008;43:221-238. DOI: 10.1080/10409230802122274.

[3] Agapov AA, Kulbachinskiy AV. Mechanisms of stress resistance and gene regulation in the radioresistant bacterium *Deinococcus radiodurans*. Biochem. (Mosc.) 2015;80:1201-1216. DOI: 10.1134/S0006297915100016.

[4] Cox MM, Battista JR. *Deinococcus radiodurans* - the consummate survivor. Nat Rev Microbiol. 2005; 3:882-892. DOI: 10.1038/nrmicro1264.

[5] Daly MJ. A new perspective on radiation resistance based on *Deinococcus radiodurans*. Nat Rev Microbiol. 2009;7:237-245. DOI: 10.1038/nrmicro2073.

[6] Slade D, Radman M. Oxidative stress resistance in *Deinococcus radiodurans*. Microbiol. Mol Biol Rev. 2011;75:133-191. DOI: 10.1128/MMBR.00015-10.

[7] Hua Y, Narumi I, Gao G, Tian B, Satoh K, Kitayama S, Shen S. PprI: a general switch responsible for extreme radioresistance of *Deinococcus radiodurans*. Biochem Biophys Res Comm. 2003;306:354-360. DOI: 10.1016/s0006-291x(03)00965-3.

[8] Tanaka M, Earl AM, Howell HA, Park MJ, Eisen JA, Peterson SN, Battista JR. Analysis of *Deinococcus radiodurans*'s transcriptional response to ionizing radiation and desiccation reveals novel proteins that contribute to extreme radioresistance. Genetics. 2004;168:21-33. DOI: 10.1534/genetics.104.029249.

[9] Selvam K, Duncan JR, Tanaka M, Battista JR. DdrA, DdrD, and PprA: components of UV and mitomycin C resistance in *Deinococcus radiodurans* R1. PLoS One. 2013;8:e69007. DOI: 10.1371/journal.pone.0069007.

[10] Luan H, Meng N, Fu J, Chen X, Xu X, Feng Q, other authors. Genome-wide transcriptome and antioxidant analysis on gamma-irradiated phases of *Deinococcus radiodurans* R1. PLoS One. 2014;9:e85649. DOI: 10.1371/journal.pone.0085649.

[11] Lim S, Jung J-H, Blanchard L, de Groot A. Conservation and diversity of radiation and oxidative stress resistance mechanisms in *Deinococcus* species. FEMS Microbiol Rev. 2019;43:19-52. DOI:10.1093.femsre/fuy037.

[12] Ott E, Kawaguchi Y, Ozgen N, Yamagishi A, Rabbow E, Rettberg P, Weckwerth W, Milojevic T. Proteomic and metabolomics profiling of *Deinococcus radiodurans* recovering after exposure to stimulated low earth orbit vaccum conditions. Front Microbiol. 2019;10:909. DOI: 10.3389/fmicb.2019.00909.

[13] Chiancone E, Ceci P. The multifaceted capacity of Dps proteins to combat bacterial stress conditions: detoxification of iron and hydrogen peroxide and DNA binding. Biochim Biophys Acta. 2010;1800:798-805. DOI: 10.1016/j.bbagen.2010.01.013.

[14] Helmann JD. Bacillithiol, a new player in bacterial redox homeostasis. Antioxid Redox Signal. 2011;15:123-133. DOI: 10.1089/ars.2010.3562.

[15] Hillion M, Antelmann H. Thiol-based redox switches in prokaryotes.

Biol Chem. 2015;396:415-444. DOI: 10.1515/hsz-2015-0102.

[16] Christman MF, Storz G, Ames BN. OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli* and Salmonella typhimurium, is homologous to a family of bacterial regulatory proteins. Proc Nat Acad Sci. USA. 1989;86:3484-3488. DOI: 10.1073/pnas.86.10.3484.

[17] Storz G, Tartaglia LA, Ames BN. The OxyR regulon. Antonie van Leeuwenhoek. 1990;58:157-161. doi: 10.1007/bf00548927. DOI: 10.1007/BF00548927.

[18] Schell MA. Molecular biology of the LysR family of transcriptional regulators. Annu Rev Microbiol. 1993;47:597-626. DOI: 10.1146/annurev.mi.47.100193.003121.

[19] Åslund F, Zheng M, Beckwith J, Storz G. Regulation of the OxyR transcription factor by hydrogen peroxide and the cellular thiol disulfide status. Proc Natl Acad Sci USA. 1999;96:6161-6165. DOI: 10.1073/pnas.96.11.6161.

[20] Kim SO, Merchant K, Nudekman R, Beyer Jr WF, Keng T, DeAngelo J, Hausladen A, Stamler JS. OxyR: a molecular code for redox-related signaling. Cell. 2002;109:383-396. DOI: 10.1016/s0092-8674(02)00723-7.

[21] Chen H, Xu G, Zhao Y, Tian B, Lu H, Yu X, Xu Z, Ying N, Hu S, Hua Y. A novel OxyR sensor and regulator of hydrogen peroxide stress with one cysteine residue in *Deinococcus radiodurans*. PLoS One. 2008;3:e1602. DOI: 10.1371/journal.pone.0001602.

[22] Burguière P, Auger S, Hullo MF, Danchin A, Martin-Verstraete I. Three different systems participate in L-cystine uptake in *Bacillus subtilis*. J Bacteriol. 2004;186:4875-4884. DOI: 10.1128/JB.186.15.4875-4884.

[23] Ohtsu I, Kawano Y, Suzuki M, Morigasaki S, Saiki K, Yamazaki S, Nonaka G, Takagi H. Uptake of L-cystine via an ABC transporter contributes defense of oxidative stress in the L-cystine export-dependent manner in *Escherichia coli*. PLoS One. 2015;10:e0120619. DOI: 10.1371/journal.pone.0120619.

[24] Chonoles Imlay KR, Korshunov S, Imlay JA. Physiological roles and adverse effects of the two cystine importers of *Escherichia coli*. J Bacteriol. 2015;197:3629-3644. DOI: 10.1128/JB.00277-15.

[25] Kim M, Jeong S, Lim S, Sim J, Rhie HG, Lee S-J. Oxidative stress response of *Deinococcus geothermalis* via a cystine importer. J Microbiol. 2017;55:137-146. DOI: 10.1007/s12275-017-6382-y.

[26] Loprasert S, Vattanaviboon P, PratuanW,ChamnongpolS,MongkolsukS. Regulation of the oxidative stress protective enzymes, catalase and superoxide dismutase in *Xanthomonas*-a review. Gene. 1996;179:33-37. DOI: 10.1016/s0378-1119(96)00427-1.

[27] Milse J, Petri K, Rückert C, Kalinowski J. Transcriptional response of *Corynebacterium glutamicum* ATCC 13032 to hydrogen peroxide stress and characterization of the OxyR regulon. J Biotechnol. 2014;190:40-54. DOI: 10.1016/j.jbiotec.2014.07.452.

[28] Liu X, Sun M, Cheng Y, Yang R, Wen Y, Chen Z, Li J. OxyR is a key regulator in response to oxidative stress in *Streptomyces avermitilis*. Microbiol. 2016;162:707-716. DOI: 10.1099/mic.0.000251.

[29] Burckhardt RM, Escalante-Semerena JC. Small-molecule

acetylation by GCN5-related N-acetyltransferases in bacteria. Microbiol Mol Biol Rev. 2020;84:e00090–e00019. DOI: 10.1128/MMBR.00090-19.

[30] Favrot L, Blanchard JS, Vergnolle O. Bacterial GCN5-related N-acetyltransferases: from resistance to regulation. Biochem. 2016;55:989-1002. DOI: 10.1021/acs.biochem.5b01269.

[31] Imber M, Pietrzyk-Brzezinska AJ, Antelmann H. Redox regulation by reversible protein S-thiolation in Gram-positive bacteria. Redox Biol. 2019;20:130-145. DOI: 10.1016/j.redox.2018.08.017.

[32] Tian B, Hua Y. Carotenoid biosynthesis in extremeophilic *Deinococcus-Thermus* bacteria. Trends in Microbiol. 2010;18:512-520. DOI: 10.1016/j.tim.2010.07.007.

[33] Azam TA, Ishihama A. Twelve species of the nucleoid associated protein from *Escherichia coli*: sequence recognition specificity and DNA binding affinity. J Biol Chem. 1999;274:33105-33113. DOI: 10.1074/jbc.274.46.33105.

[34] Ishihama A. The nucleoid: an overview. EcoSal Plus. 2009;3. DOI:10.1128/ecosalplus.2.6.

[35] Zhao G, Ceci P, Ilari A, Giangiacomo L, Laue TM, Chiancone E, Chasteen ND. Iron and hydrogen peroxide detoxification properties of DNA-binding protein from starved cells. A ferritin-like DNA-binding protein of *Escherichia coli*. J Biol Chem. 2002;277:27689-27696. DOI: 10.1074/jbc.M202094200.

[36] Chiancone E, Ceci P. The multifaceted capacity of Dps proteins to combat bacterial stress conditions: detoxification of iron and hydrogen peroxide and DNA binding. Biochim Biophys Acta. 2010;1800:798-805. DOI: 10.1016/j.bbagen.2010.01.013.

[37] Santos SP, Mitchell EP, Franquelim HG, Castanho MARB, Abreu IA, Romao CV. Dps from *Deinococcus radiodurans*: oligomeric forms of Dps1 with distinct cellular functions and Dps2 involved in metal storage. FEBS J. 2015;282:4307-4327. DOI: 10.1111/febs.13420.

[38] Almirón M, Link AJ, Furlong D, Kolter R. A novel DNA binding protein with regulatory and protective roles in starved *Escherichia coli*. Genes Dev. 1992;6:2646-2654. DOI:10.1101/gad.6.12b.2646.

[39] Wolf SG, Frenkiel D, Arad T, Finkel SE, Kolter R, Minsky A. DNA protection by stress-induced biocrystallization. Nature. 1999;400:83-85. DOI: 10.1038/21918.

[40] Ceci P, Cellai S, Falvo E, Rivetti C, Rossi GL, Chiancone E. DNA condensation and self-aggregation of *Escherichia coli* Dps are coupled phenomena related to the properties of the N-terminus. Nucleic Acids Res. 2004;32:5935-5944. DOI: 10.1093/nar/gkh915.

[41] Mahillon J, Chandler M. Insertion sequences. Microbiol Mol Biol Rev. 1998;62:725-774.

[42] Siguier P, Gourbeyre E, Varani A, Ton-Hoang B, Chandler M. Everyman's guide to bacterial insertion sequences. Microbiol Spectrum. 2015;3:MDNA3-0030-2014. DOI: 10.1128/microbiolspec.MDNA3-0030-2014.

[43] Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res. 2006;34:D32–D36. DOI: 10.1093/nar/gkj014.

[44] Siguier P, Gourbeyre E, Chandler M. Bacterial insertion sequences: their genomic impact and diversity. FEMS Microbiol Rev. 2014;38:865-891. DOI: 10.1111/1574-6976.12067.

[45] Narumi I, Cherdchu K, Kitayama S, Watanabe H. The *Deinococcus radiodurans* uvrA gene: identification of mutation sites in two mitomycin-sensitive strains and the first discovery of insertion sequence element from deinobacteria. Gene. 1997;198:115-126. DOI: 10.1016/s0378-1119(97)00301-6.

[46] Ohtsubo Y, Genka H, Komatsu H, Nagata Y, Tsuda M. High-temperature-induced transposition of insertion elements in *Burkholderia multivorans* ATCC17616. Appl Environ Microbiol. 2005;71:1822-1828. DOI: 10.1128/AEM.71.4.1822-1828.2005.

[47] Pasternak C, Ton-Hoang B, Coste G, Bailone A, Chandler M, Sommer S. Irradiation-induced *Deinococcus radiodurans* genome fragmentation triggers transposition of a single resident insertion sequence. PLoS Genetics. 2010;6:e1000799. DOI: 10.1371/journal.pgen.1000799.

[48] Roberts D, Hoopes BC, McClure WR, Kleckner N. IS10 transposition is regulated by DNA adenine methylation. Cell. 1985;43:117-130. DOI: 10.1016/0092-8674(85)90017-0.

[49] Escoubas JM, Prere MF, Fayet O, Salvignol I, Galas D, Zerbib D, Chandler M. Translational control of transposition activity of the bacterial insertion sequence IS1. EMBO J. 1991;10:705-712.

[50] Kleckner N, Chalmers RM, Kwon D, Sakai J, Bolland S. Tn10 and IS10 transposition and chromosome rearrangements: Mechanism and regulation *in vivo* and *in vitro*. In: Transposable Elements. Curr Top Microbiol Immunol. 1996;204:49-82. DOI: 10.1007/978-3-642-79795-8_3.

[51] Kiss J, Nagy Z, Toth G, Kiss GB, Jakab J, Chandler M, Olasz F. Transposition and target specificity of the typical IS30 family element IS1655 from *Neisseria meningitidis*. Mol Microbiol. 2007;63:1731-1747. DOI: 10.1111/j.1365-2958.2007.05621.x.

[52] Vandecraen J, Chandler M, Aertsen A, Van Houdt R. The impact of insertion sequences on bacterial genome plasticity and adaptability. Crit Rev Microbiol. 2017;43:709-730. DOI: 10.1080/1040841X.2017.1303661.

[53] Qi H, Wang W, He J, Ma Y, Xiao F, He S. Antioxidative system of *Deinococcus radiodurans*. Res Microbiol. 2020;171:45-54. DOI: 10.1016/j.resmic.2019.11.002.

[54] Makarova KS, Omelchenko MV, Gaidamakova EK, Matrosova VY, Vasilenko A, other authors. *Deinococcus geothermalis*: the pool of extreme radiation resistance genes shrinks. PLoS One. 2007;2:e955. DOI: 10.1371/journal.pone.0000955.

[55] Choo K, Kim M, Abdi Nansa S, Bae MK, Lee C, Lee S-J. Redox potential change by the cystine importer affected on enzymatic antioxidant protection in *Deinococcus geothermalis*. Antonie van Leeuwenhoek. 2020;113:779-790. DOI: 10.1007/s10482-020-01388-4.

[56] Ud-Din AIMS, Tikhomirova A, Roujeinikova A. Structure and functional diversity of GCN5-related N-acetyltransferases (GNAT). Int J Mol Sci. 2016;17:1018. DOI: 10.3390/ijms17071018.

[57] Reon BJ, Nguyen KH, Bhattacharyya G, Grove A. Functional comparison of *Deinococcus radiodurans* Dps proteins suggests distinct in vivo roles. Biochem J. 2012;447:381-391. DOI: 10.1042/BJ20120902.

[58] Bae MK. Purification and characterization of DNA binding proteins from starved cell (Dps) of *Deinococcus geothermalis* [thesis]. Seoul: Kyung Hee University; 2020.

[59] Mennecier S, Servant P, Coste G, Bailone A, Sommer S. Mutagenesis via IS transposition in *Deinococcus radiodurans*. Mol Microbiol. 2006;59:317-325. DOI: 10.1111/j.1365-2958.2005.04936.x.

[60] Gregory ST, Dahlberg AE. Transposition of an insertion sequence, ISTth7, in the genome of the extreme thermophile *Thermus thermophiles* HB8. FEMS Microbiol Lett. 2008;289:187-192. DOI: 10.1111/j.1574-6968.2008.01389.x.

[61] Lee C, Choi N, Bae MK, Choo K, Lee S-J. Transposition of insertion sequences was triggered by oxidative stress in radiation-resistant bacterium *Deinococcus geothermalis*. Microorganisms. 2019;7:446. DOI: 10.3390/microorganisms7100446.

[62] Lee C, Choo K, Lee S-J. Active transposition of insertion sequences by oxidative stress in *Deinococcus geothermalis*. Front Microbiol. 2020;11:558747. DOI:10.3389/fmicb.2020.558747.