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REVIEW

Mechanisms of Stress Resistance and Gene Regulation in the Radioresistant Bacterium *Deinococcus radiodurans*

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Abstract—The bacterium *Deinococcus radiodurans* reveals extraordinary resistance to ionizing radiation, oxidative stress, desiccation, and other damaging conditions. In this review, we consider the main molecular mechanisms underlying such resistance, including the action of specific DNA repair and antioxidation systems, and transcription regulation during the anti-stress response.

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POSSIBLE MECHANISMS OF STRESS RESISTANCE IN *Deinococcus radiodurans*

Deinococcus radiodurans is a mesophilic, aerobic, nonpathogenic, non-spore-forming Gram-positive bacterium. *Deinococcus radiodurans* cells have coccoid form and often form dyads and tetrads. The colonies have a pink color. A characteristic feature of *D. radiodurans* is its high resistance to radiation. Bacteria of this species were first isolated from canned meat sterilized with γ-irradiation [1]. The culture of *D. radiodurans* survives irradiation doses up to 10,000 Gy without loss in viability, and it is 30 and 1000 times more resistant than *E. coli* and human cell cultures, respectively [2]. Besides γ-irradiation, the *D. radiodurans* cells reveal a high level of resistance to ultraviolet exposure, desiccation, and treatment with various chemical mutagens such as mitomycin C and hydrogen peroxide (see review [3]).

It was long assumed that the main cellular target for the damaging action of various stress factors including ionizing radiation is DNA. Therefore, most studies of stress resistance in *D. radiodurans* were aimed at revealing unique mechanisms of DNA protection and repair that could preserve the genome under stress conditions. However, recent studies demonstrated that the resistance might mainly result from the ability of *D. radiodurans* cells to protect their proteome from oxidative stress resulting from various damaging conditions [3-6]. Based on available data, the following factors were proposed to contribute to the stress resistance in *D. radiodurans*: (1) structural organization of the cell wall; (2) specific structure and packaging of the genome; (3) highly efficient DNA repair systems; (4) proteome protection from oxidative stress; (5) active removal of toxic compounds from the cells; (6) specific features of gene expression and its regulation under stress conditions.

Several excellent reviews on the mechanisms of stress resistance in *D. radiodurans* have been published in recent years [3, 4, 7-9], including a comprehensive review by Slade and Radman [3]. Here, we briefly describe general mechanisms that preserve the integrity of the cell, DNA, and proteins in *D. radiodurans* under stress conditions. The main attention is paid to recent publications in this area and to mechanisms of transcription regulation in *D. radiodurans*.

STRUCTURE OF THE CELL WALL OF *D. radiodurans*

The cell wall of *D. radiodurans* contains two membranes separated by a peptidoglycan layer. Despite the

Abbreviations: BER, base excision repair; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; ROS, reactive oxygen species.

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presence of two membranes, the high content of peptidoglycan and the absence of lipopolysaccharides reveal the relationship of *D. radiodurans* with Gram-positive bacteria. In addition, *D. radiodurans* differs from other bacteria in the phospholipid membrane composition [10, 11]. The outer membrane is covered by the S layer and a polysaccharide layer [12]. The outer membrane, the S layer, and polysaccharides together form a single structure, the pink envelope, which contains carotenoids that determine the pink color of the bacterium [13]. Recent data suggest that the inner membrane is also connected with the outer membrane through protein complexes, a key structural role in which is played by the D-protein [14]. The main protein component of the S layer is Hpi, which forms hexagonal structures [13]. An important bridging role in the pink envelope is also played by the SlpA protein of the S layer [15]. The S layer likely increases the cell wall rigidity, facilitates interactions with various ligands, and increases cell resistance to external stress factors.

The genome of *D. radiodurans* contains 13 genes encoding enzymes of carotenoid synthesis [16]. For comparison, green sulfur bacteria and cyanobacteria contain up to 9 and 10 of such genes, respectively [17, 18]. It was proposed that membrane-bound carotenoids have a protective role by inactivating free radicals and preventing their production. *In vitro* experiments demonstrated that the fraction of carotenoids isolated from *D. radiodurans* has protein-protective properties. Strains lacking carotenoids are characterized by higher levels of protein damage under oxidative stress conditions [19]. At the same time, the *D. radiodurans* cells lacking carotenoids are only slightly more sensitive to radiation than the wild-type bacteria [20]. This can be explained by efficient utilization of free radicals by other protective systems or by the fact that membranes are not the main target for radiation damage (see below) [3].

GENOME ORGANIZATION AND RADIORESISTANCE

The genome of *D. radiodurans* contains $3.28 \cdot 10^6$ bp and consists of two circular chromosomes and two plasmids [21]. During the exponential phase of growth, each cell contains about 10 genome copies [22]. The DNA content depends on the conditions and phase of the growth but never drops below two genomic copies. This allows efficient repair of DNA damages, including double-strand breaks that are induced by ionizing radiation, by homologous recombination.

Comparison of 16S RNA and conserved protein sequences revealed that the *Deinococcus* genus belongs to Gram-positive bacteria and is phylogenetically close to the *Thermus* genus [16, 21, 23]. After the divergence of a common ancestor of the two genera, *Deinococcus* gathered systems of cell resistance to stress conditions from many different bacteria [24]. As a result, the *Deinococcus* genome has a mosaic structure; most genes are homologous to *Thermus*- and *Bacillus*-like genomes, while many individual genes have common origin with genes from other taxons including archaea and eukaryotes. Such extensive horizontal gene transfer was possible due to natural competence of the cells of *D. radiodurans* [25, 26]. Interestingly, *D. radiodurans* is much more efficiently transformed by ultraviolet-irradiated DNA in comparison with other bacteria [25]. This may be the result of a much higher efficiency of DNA repair in *D. radiodurans* (see below). *Deinococcus radiodurans* can incorporate in its genome significant amounts of foreign DNA, up to 500,000 bp, or more than 10% of the genome [27]. The cell wall of *D. radiodurans* contains a DNA processing complex that includes a DNA translocator [14], which could play a role in the high natural transformation efficiency.

The genome of *D. radiodurans* is tightly packaged and forms nucleoid of toroidal architecture. This may serve as a mechanism of DNA protection from mutagenic factors, including ionizing radiation and active radicals produced inside the cell by radiation [28-30]. At the same time, ionizing radiation results in similar levels of DNA damage in cells of *D. radiodurans* and *E. coli* [31]. However, the specific nucleoid structure in *D. radiodurans* may help to keep together the DNA ends that are formed at the sites of double-strand breaks and to facilitate their repair.

MECHANISMS OF DNA REPAIR IN *D. radiodurans*

The DNA repair systems of *D. radiodurans* are highly efficient; this bacterium can successfully repair up to 200 double-strand breaks and 190 interstrand crosslinks per genomic copy without decrease in viability, while *E. coli* cells die in the presence of only a dozen double-strand DNA breaks. *Deinococcus radiodurans* is also highly resistant to various nitrogenous base damages including alkylation, deamination, and oxidation (see review [3]). *Deinococcus radiodurans* contains most DNA repair pathways described in other bacteria, which are briefly described below (Table 1).

Homologous recombination. The multicopy nature of the genome of *D. radiodurans* enables highly efficient double-strand break repair by homologous recombination. Homologous recombination mechanisms also play important roles in the repair of other types of DNA damages including interstrand crosslinks and photoproducts (see review [3]).

One of the most important steps in homologous recombination is the interaction of the RecA protein with single-stranded DNA. For this to occur, double-strand breaks should be processed to allow formation of free 3′-ends. In bacterial cells, such processing can be performed

Table 1. Specific features of DNA repair systems in *D. radiodurans* in comparison with *E. coli*

DNA repair component	*E. coli*	*D. radiodurans*
Processing of double-stranded breaks	RecBCD RecFOR (RecQ, RecJ)	RecFOR (UvrD, RecJ) PprA (?)
Proteins that interact with single-stranded DNA	SSB, RecA	SSB*, RecA**, DdrA, DdrB
Main pathway of double-strand break repair	SDSA	ESDSA
Photolyase	+	–
NER	UvrABC	UvrABC, UvsE***
BER	8 DNA glycosylases	12 DNA glycosylases
MMR	+	+ (low efficiency)
Dam-methylase	+	–
SOS repair	+	–

* *D. radiodurans* SSB differs in structure from its *E. coli* homolog.
** *D. radiodurans* RecA has higher affinity to double-stranded DNA than to single-stranded DNA.
*** UvsE pathway is likely specific to pyrimidine dimers.

by either the RecBCD or RecFOR system. Due to the absence of RecB and RecC proteins in *D. radiodurans*, the main role in DNA end processing is played by the RecFOR system. In *E. coli* cells, the RecFOR pathway starts with the unwinding of double-stranded DNA end by the RecQ helicase, followed by degradation of the 5′-end by the RecJ 5′-3′ exonuclease, resulting in the formation of a single-stranded 3′-end [32]. In *D. radiodurans* cells, the RecJ exonuclease is essential for DNA repair, as evidenced by the lethality of *recJ* gene deletions [33]. At the same time, the functions of the RecQ helicase are likely performed by the UvrD helicase, since *uvrD* deletion mutants are characterized by a much more pronounced delay in DNA repair after irradiation in comparison with cells lacking the *recQ* gene [33]. The UvrD helicase performs a wide range of functions in *D. radiodurans* and participates in nucleotide excision and post-replicative repair (see below). Recent studies of UvrD demonstrated that it could unwind DNA in both 3′-5′ and 5′-3′ directions, the latter activity being stimulated by DNA-bound SSB proteins [34]. It should be noted that the *D. radiodurans* genome also encodes several other helicases whose functions in DNA repair are only beginning to be explored, including RecD2 [35, 36], RecG [37], and DR1572 [38].

The newly formed single-stranded DNA ends interact with SSB proteins and the RecFOR complex, which then loads RecA onto DNA. Deletions of the *recF*, *recO*, and *recR* genes in *D. radiodurans* cells have similar phenotypes to the *recA* deletion; the bacteria become much more sensitive to radiation exposure, and the level of reparative DNA synthesis is greatly decreased [33]. In contrast to its *E. coli* homolog, the *D. radiodurans* RecA protein uniquely has a higher affinity to double-stranded DNA in comparison with single-stranded substrates [39, 40]. Under normal conditions, RecA is inactive and randomly bound to double-stranded DNA regions, and it is activated in the presence of single-stranded DNA bound with the SSB protein, whose concentration rapidly increases under damaging conditions [41]. This allows efficient alignment and repair of overlapping DNA fragments when a large number of double-stranded breaks are present in the genome [42].

In addition to RecA, homologous recombination in *D. radiodurans* involves its distant homolog, the RadA protein, which participates in processing of branched DNA structures [43, 44]. *Deinococcus radiodurans* mutants lacking the *radA* gene are more sensitive to ionizing radiation than the wild-type bacteria. It was shown that RadA together with RecA participates in the steps of homologous recombination preceding DNA synthesis but is unable to substitute for RecA in this process [45].

The next step in the DNA break repair is homology search between the 3′-ends formed at the break points and homologous chromosomes. This is followed by D-loop formation and extension of 3′-ends resulting in the formation of overlapping complementary DNA regions. This allows further gap repair and chromosome reconstitution. The DNA repair pathway that is accompanied by active DNA synthesis is known as SDSA (synthesis-dependent strand annealing). The main feature of this process in *D. radiodurans* cells is its mass character and, consequently, the ability to reconstitute DNA from many fragments resulting from DNA breaks in difference chromosome copies. This mechanism was therefore named ESDSA (extended synthesis-dependent strand annealing) [3, 45, 46].

DNA polymerases in repair. *Deinococcus radiodurans* cells contain three DNA polymerases – Pol I, Pol III, and Pol X [16]. Pol I and Pol III are homologous to corresponding enzymes of *E. coli* and perform similar functions. Deficiency of Pol I or Pol III results in increased radiation sensitivity, suggesting their key roles in the DNA break repair [45].

Pol I is widely used in the repair processes. Its ability to bypass DNA lesions is increased in the presence of Mn^{2+} [47]. This may have an adaptive role since *D. radiodurans* cells accumulate Mn^{2+} under stress (see below) [48]. The Mn^{2+}-dependent stimulation of translesion Pol I activity may be important since *D. radiodurans* lacks homologs of Y-family polymerases, which participate in SOS repair in *E. coli* cells [16]. However, experiments *in vivo* demonstrate that the *polA* deletion in *D. radiodurans* can be fully compensated by expression of *E. coli polA*, resulting in the normal level of radiation resistance [49].

Pol III, the main replicase, plays the key role in DNA repair, especially in the ESDSA pathway [45]. It has a multisubunit composition; interestingly, irradiation does not induce expression of the polymerase subunit but significantly increases expression of the processivity factor and the exonuclease subunit [50]. Recently, a three dimensional structure of the sliding clamp was reported, which revealed a common two subunit composition with some specific differences in charge distribution in comparison with the *E. coli* factor. A more uniform distribution of positively and negatively charged amino acid residues on the inner clamp surface was proposed to allow less tight or less specific DNA interactions and to facilitate its sliding. This may promote DNA-coupled processes in *D. radiodurans* [51].

The Pol X activity strongly depends on the presence of Mn^{2+}, which suggests its specific activation under stress conditions. Pol X also possesses 5′-deoxyribophosphate lyase activity that may play a role in base excision repair, similarly to eukaryotic DNA polymerase β [52]. In addition, Pol X is able to exonucleolytically cleave DNA regions involved in formation of hairpin structures [53]. The SbcCD complex has a similar activity, suggesting that Pol X and SbcCD likely participate in alternative pathways of the processing of DNA ends that form hairpins and interstrand crosslinks or are covalently bound to proteins, which may be essential for efficient repair of double-strand breaks [54].

Unique proteins of DNA break repair. In addition to classical proteins that participate in DNA break repair, *D. radiodurans* contains several unique DNA-binding proteins that are also likely involved in this process. In particular, the nucleoids of irradiated bacteria become enriched with the RecA, UvrD, RecJ, and RecQ proteins, as well as with *D. radiodurans*-specific proteins DdrA, DdrB, DdrD [55], and PprA [56] within a few hours after irradiation.

The PprA protein preferentially binds to double-stranded DNA ends, thus suggesting its involvement in double-strand break repair. PprA inhibits exonucleases and activates DNA ligase *in vitro* [57], its action being dependent on concentration: at higher concentrations, PprA oligomerizes on DNA and stimulates break ligation [58]. (It should be noted that in addition to DNA ligase, 3′-5′ RNA ligase might also participate in DNA break repair in *D. radiodurans*; in particular, it was shown to ligate RNA–DNA fragments in the context of double-stranded substrates even in the presence of damaged nucleotides in the reacting molecules [59].)

The mechanism of PprA action might be similar to eukaryotic Ku proteins (also found in some bacteria), which are key components of the nonhomologous end joining (NHEJ) DNA repair pathway [57]. However, available data suggest that the NHEJ pathway is absent in *D. radiodurans* or operates very inefficiently [60]. In addition, *recA* deletants do not differ from double-deletants lacking both *pprA* and *recA* in their sensitivity to radiation [61] and reveal identical delayed kinetics of DNA repair [56], suggesting the involvement of PprA in RecA-dependent DNA repair.

The PprA protein also plays a role in cell division; in wild-type cells, it localizes at the newly formed cell septum after γ-irradiation [62], and *pprA* deletion mutants are characterized by impaired DNA distribution into daughter cells [56]. The role of PprA is probably explained by its interactions with topoisomerases, which are involved in separation of daughter DNA molecules. This hypothesis is based on two observations. First, PprA stimulates DNA topoisomerase I and increases resistance of *D. radiodurans* cells to nalidixic acid, an inhibitor of topoisomerase II [63]. Second, *D. radiodurans* mutants lacking the DNA gyrase gene *gyrA* have the same phenotype as double mutants lacking the *gyrA* and *pprA* genes, both under normal conditions and after irradiation [62].

Protein DRA0282 participates in the PprA-dependent pathway of DNA repair, likely by playing a DNA-protective role under stress conditions [64]. In the presence of Mn^{2+}, this protein preferably binds supercoiled DNA and protects it from exonuclease III in experiments *in vitro*. Interestingly, the *N*-terminal part of DRA0282 reveals homology with eukaryotic protein Ku80, a component of the NHEJ pathway. Deletion of the *dra0282* gene decreases stress resistance of *D. radiodurans*, while its expression in *E. coli* cells makes them less sensitive to ultraviolet exposure and radiation [64].

DdrB is another unique *D. radiodurans* protein that is able to bind single-stranded DNA and is therefore an SSB protein from the functional point of view. However, it is not homologous to regular bacterial SSB proteins and, in contrast to homotetrameric SSBs, has a pentameric structure. This puts it in a separate family of SSB proteins [65]. Standard SSB of the *Deinococcus–Thermus* phylum also has a unique dimeric structure, while

remaining a functional tetramer since each subunit has two oligonucleotide-binding domains [66]. These domains are not identical in their structure and functions; the *C*-terminal domain is mainly responsible for DNA binding, while the *N*-terminal part is involved in multimerization. Both domains participate in disruption of secondary structures in single-stranded DNA [67]. Such structure, as well as high concentration of SSB in the cells of *D. radiodurans*, likely promotes more efficient SSB–DNA interactions in comparison with other bacteria [66].

Deletants of *D. radiodurans* lacking the *ddrB* gene are less resistant to irradiation but viable [61]. At the same time, deletion of the *ssb* gene is lethal, and decrease in its expression results in a significant decrease in radio-resistance [68]. The kinetics of DNA repair in the double *recA/ddrB* deletant suggests the involvement of DdrB in RecA-independent repair mechanisms and, in particular, in the single-strand annealing (SSA) pathway [69, 70]. To date, the structure of DdrB in complex with single-stranded DNA has been reported, and the mechanisms of its action are being discussed [71].

DdrA is a distant homolog of eukaryotic protein Rad52 that participates in homologous recombination. DdrA binds DNA 3′-ends *in vitro* and likely protects them from degradation [72]. Similarly to DdrB, it is involved in RecA-independent DNA repair [61, 72].

Analysis of *D. radiodurans* mutants lacking the *ddrA*, *ddrB*, *pprA*, and *ddrD* genes and their different combinations suggested the roles for these proteins in stress resistance. Deletions of these genes are not lethal and result in various degrees of cell sensitivity to different stress factors. For example, the *pprA/ddrD* deletants are much more sensitive to ultraviolet exposure in comparison with the *pprA/ddrA* mutants, but both strains have the same resistance to ionizing radiation and mitomycin C (an agent that stimulates interstrand DNA crosslinks). These data suggest that these proteins participate in repair processes that are specific to different types of DNA damages [73]. Despite the involvement of the unique *D. radiodurans* proteins in homologous recombination repair, their absence does not affect the natural competence of this bacterium, which also depends on homologous recombination. This observation suggests that these proteins play specific roles in stress resistance, likely by protecting damaged DNA from degradation and giving the cell more time for DNA repair [73].

Excision repair. The general mechanisms of base excision repair (BER) in *D. radiodurans* are similar to other bacteria. Analysis of its genome revealed 12 DNA glycosylases that are specific to uracil, thymine glycol, methyl adenine, formamidopyrimidine, modified guanine, and noncomplementary guanine–guanine, guanine–adenine, and guanine–thymine pairs [16, 21]. For comparison, the *E. coli* genome encodes eight DNA glycosylases [74].

Deinococcus radiodurans has two nucleotide excision repair (NER) systems – UvrABC and UvsE. The UvsE protein is a Mn^{2+}-dependent endonuclease (endonuclease β) that is specific to pyrimidine dimers whose formation is induced by ultraviolet irradiation [75, 76]. This probably explains the absence of photolyase in *D. radiodurans*, which plays the main role in direct repair of thymine dimers in *E. coli* [16]. The high GC-content of the *D. radiodurans* genome (67%) also decreases the number of thymine dimers that are formed after irradiation [77].

Mismatch repair (MMR). Post-replicative repair of mismatched nucleotides in *D. radiodurans* involves proteins MutS1, MutL, and UvrD, which are homologous to the corresponding proteins of *E. coli*. *Deinococcus radiodurans* lacks a MutH protein, and the Dam DNA-methylation system is absent [78]. Thus, similarly to most other bacteria, the recognition of the daughter strand for repair depends on some other, poorly characterized mechanism, which is different from *E. coli*. The frequency of spontaneous mutations per round of replication in *D. radiodurans* is much higher than in *E. coli*, revealing a low efficiency of MMR in *D. radiodurans* cells. This is further corroborated by weak effects of MutS1 and MutL inactivation on the mutation frequency in *D. radiodurans* [78]. It should be noted that yet another homolog of *E. coli* MutS in *D. radiodurans*, MutS2, is not involved in MMR but was shown to participate in RecA-independent repair of oxidative DNA damages. Molecular mechanisms underlying the functions of this protein remain unknown, but its Smr domain was shown to have a Mn^{2+}-dependent endonuclease activity [79].

Repair of interstrand crosslinks. Interstrand crosslinks prevent DNA melting and can completely block DNA replication. *Deinococcus radiodurans* is resistant to mitomycin C, which generates such crosslinks. In bacteria, interstrand crosslinks can be repaired by homologous recombination and NER. Recent studies demonstrated that in *D. radiodurans* two proteins are involved in the repair process, the products of the *ygjD* and *yeaZ* genes that have homologs in many bacterial genomes. These proteins were proposed to possess helicase and endonuclease activities, which might contribute to the bypass of interstrand crosslinks, but the exact mechanisms of their action remain unclear [80].

MECHANISMS OF OXIDATIVE STRESS RESISTANCE IN *D. radiodurans*

Overall, analysis of DNA repair systems in *D. radiodurans* does not give a clue about the nature of radioresistance, because: (1) analogous systems are widespread among radiosensitive organisms, and (2) individual components of the *D. radiodurans* repair systems are not necessarily present in radioresistant bacteria. Moreover, lab-

oratory experiments on evolution of radioresistant *E. coli* strains revealed that the selected traits are connected not only with DNA repair systems [81].

Recent studies have shown that the high resistance of *D. radiodurans* to various damaging conditions can be explained by highly efficient antioxidative cell protection.

Oxidative stress results from formation of three main reactive oxygen species (ROS): hydroxyl radicals, superoxide radicals, and hydrogen peroxide. Hydroxyl radicals can be formed in the reaction of water radiolysis or in Fenton's reaction of hydrogen peroxide with iron ions. Hydroxyl radicals induce macromolecule degradation with the formation of superoxide radicals and hydrogen peroxide. Superoxide radical in turn can free Fe^{2+} from iron-sulfur clusters of protein molecules [82]. This results in the formation of a positive feedback between these reactions.

It was shown that both radioresistant and desiccation-resistant bacteria have high levels of oxidative stress resistance [83]. Cell damage caused by ionizing radiation mainly results from the formation of ROS, which can destroy not only DNA, but also other macromolecules [5]. And while the number of DNA breaks that are induced by ionizing radiation does not differ between radioresistant and radiosensitive bacteria, the level of proteome damage is much higher in the latter case [6]. Furthermore, it was shown that the level of protein carbonylation that is caused by oxidative stress is inversely proportional to the stress resistance [84]. Therefore, efficient proteome protection under oxidative stress conditions may help the cells to survive both desiccation and high radiation levels. Revealing of the primary importance of protein protection under stress conditions, because the proteins determine the efficiency of DNA repair, has changed the paradigm in studies of the nature of radioresistance [4].

In addition to efficient DNA repair, *D. radiodurans* cells employ several strategies to prevent oxidative stress and overcome its consequences, including [3]: (1) prevention of the formation of endogenous ROS; (2) activation of antioxidant defense systems; (3) selective protection of some proteins from oxidation; (4) removal and degradation of damaged macromolecules. These defense mechanisms are briefly outlined below.

Antioxidative systems in *D. radiodurans*. The genome of *D. radiodurans* encodes two peroxidases, three catalases, four superoxide dismutases, and two Dps proteins [3]. The activity of the enzymes utilizing ROS, including catalase and superoxide dismutase, is several-fold higher than in *E. coli* cells [85, 86]. Catalase activity is increased in response to the increase in hydrogen peroxide and Mn^{2+} concentrations [87], and ionizing radiation [88]. The Dps proteins participate in DNA compaction and are important components of nucleotides. They protect DNA from oxidative damage through direct interactions, by chelating iron ions, and by reducing hydrogen peroxide [89]. However, deletions of the genes encoding all the above-mentioned proteins have only mild effects on radioresistance [90]. This may be explained by significant contribution of nonenzymatic mechanisms to the resistance of *D. radiodurans* (see below).

The production of ROS in the *D. radiodurans* cells may be also decreased due to decrease in the number of oxidative chain proteins and proteins containing iron-sulfur clusters in comparison with radiosensitive bacteria [5]. In particular, the presence of the glyoxylate pathway decreases the number of some enzymes of the Krebs cycle. Furthermore, in response to oxidative stress, *D. radiodurans* releases a part of its cell wall polysaccharides [91]. This probably decreases the formation of ROS by removing polysaccharide-associated water molecules [92]. Membrane-bound carotenoids may also play a protective role (see above).

Role of Mn^{2+} in oxidative stress resistance. Manganese ions play important roles in the response of *D. radiodurans* to stress conditions. Bacteria growing on Mn^{2+}-depleted medium are several-fold more sensitive to ionizing radiation and oxidative stress [48]. The content of Mn^{2+} in *D. radiodurans* cells reaches millimolar concentrations [93]. The increase in intracellular Mn^{2+} concentrations under stress conditions likely results from activation of an ABC-transporter [24], whose expression is induced by irradiation and during a post-irradiation period [94].

Manganese ions stimulate activities of several enzymes involved in DNA repair and replication, including endonuclease β, superoxide dismutase, DNA polymerase X, and RNA ligase by replacing Mn^{2+} in their active sites (see above). At higher concentrations, Mn^{2+} is able to substitute for iron ions in Fe-containing enzymes, thus preventing their destruction in Fenton's reaction because Mn^{2+}, in contrast to iron and copper ions, does not react with hydrogen peroxide and does not produce hydroxyl radicals [95].

Manganese ions can also act as antioxidants in complexes with various compounds, including orthophosphate, nucleotides, amino acids, and peptides [92]. Stress-dependent induction of nucleotide synthesis may therefore contribute to the antioxidative function by increasing concentration of nucleotide–manganese complexes. In addition, *D. radiodurans* cells secrete a nuclease that participates in degradation of extracellular DNA and increases radiation cell resistance, probably by producing nucleoside monophosphates [96]. Activation of proteases in *D. radiodurans* cells observed under stress conditions may also serve as a mechanism of antioxidant cell defense. The efficiency of the antioxidant function depends on the amino acid composition of peptides, the most active components being manganese complexes with sulfur-containing and aromatic amino acid residues [97]. At the same time, recent studies demonstrated that

a significant part of manganese ions in *D. radiodurans* cells is bound to water molecules, and only a small part is found in low molecular weight complexes [98]. At stationary phase, the concentrations of low molecular weight manganese complexes are further decreased, and manganese is mainly complexed with superoxide dismutase and another unidentified protein [98, 99]. However, these cells are radiation resistant, which questions the role of low molecular weight manganese complexes in stress resistance, at least at the stationary phase of cell growth [99].

Variations in manganese content have an interesting effect on the cell cycle of *D. radiodurans*: at low Mn^{2+} concentrations, the culture ceases growth even before the exhaustion of broth resources, and it resumes growing after addition of manganese salts [87]. It was proposed that bacteria secrete in the environment a low molecular weight factor that inhibits cell growth, and this inhibition is prevented by Mn^{2+} [100].

Systems of cell cleaning from toxic compounds. Damaged oligonucleotides that appear during DNA repair in *D. radiodurans* cells are actively exported from the cells, likely by the UvrA2 transporter that is related to ABC transporters [21]. In addition, the *D. radiodurans* cells contain specialized Nudix-hydrolases (nucleoside diphosphate linked to some other moiety x), which can remove diphosphate groups from damaged nucleoside triphosphates, thus preventing their incorporation into DNA. The *D. radiodurans* genome contains genes for 23 Nudix hydrolases, and transcription of five of them is induced by ionizing radiation [50]. Damaged nucleoside monophosphates can be further dephosphorylated and removed from the cell (see review [3]).

Proteolytic activity is greatly increased in *D. radiodurans* cells under stress conditions, which facilitates utilization of damaged and misfolded proteins [92]. The key activator of this process may be aconitase, which acts as an oxidative stress sensor in cells of other organisms [101]. The *D. radiodurans* genome encodes homologs of Lon proteases that participate in degradation of damaged proteins; however, deletion of these genes does not decrease radiation resistance. At the same time, such decrease is observed in the case of the ClpXP protease gene deletion [102]. ClpXP was proposed to participate in the production of peptides that form manganese complexes.

While certain proteins, including citrate synthase, aconitase, and some chaperones are actively recycled under oxidative stress conditions, others avoid degradation. The latter include some translation factors, serine proteases, and β and β′ subunits of RNA polymerase [103]. The particular mechanisms underlying the increased stability of these proteins remain unclear, but selective protection of the transcription and translation systems is likely essential for rapid recovery of cellular functions after stress.

REGULATION OF GENE EXPRESSION IN *D. radiodurans*

As discussed above, the radioresistance of *D. radiodurans* depends on the activity of various DNA protection and repair systems, but even more important is its resistance to oxidative stress. *Deinococcus radiodurans* reveals complex stress response by inducing expression of many proteins and noncoding RNAs that result in global changes in cell metabolism and changes in activities of particular molecular pathways. The detailed mechanisms of gene regulation in *D. radiodurans* are only beginning to be studied. The following sections briefly describe the existing data on transcription regulation in *D. radiodurans* cells under normal and stress conditions.

Changes of transcriptome under stress conditions. Transcriptomic and proteomic methods revealed significant changes in gene expression in *D. radiodurans* under stress. Depending on the radiation dose, the method of analysis, and the stringency of the criteria used, from ~100 to ~1000 genes increase their expression during γ-irradiation and in the post-irradiation period [50, 61]. Irradiation induces genes involved in the processes of DNA repair, antioxidative defense, and utilization of damaged biopolymers. The activation of gene expression goes through several steps; some proteins, such as superoxide dismutase, are induced during stress, some immediately after stress, while activation of others is delayed until later steps of cell recovery [50, 94]. Stress induces transcription of not only mRNAs, but also noncoding RNAs including antisense RNAs and tRNAs [94]. The expression of seven small noncoding RNAs that are conserved in bacteria of the *Deinococcus* genus is also induced in a closely related *D. geothermalis* species [104]. These data reveal an important role of noncoding RNAs in the regulation of stress response in this genus.

Similar analyses have been performed for other stress conditions including osmotic stress [105] and cadmium stress [106]. In both cases, experiments revealed changes in the expression of hundreds of genes. Cadmium stress induces the same repair pathways and repair proteins as radiation exposure [106]. Differential changes in gene expression observed during osmotic stress also overlap with those characteristic for radiation stress, suggesting the existence of common adaptation mechanisms for both conditions [105].

Analysis of the proteome dynamics demonstrated that irradiation leads to an increase in intracellular concentrations of dozens of proteins in *D. radiodurans*, and some of the proteins are completely absent in unexposed cells. Identified proteins include factors of replication, DNA repair, transcription, and translation, proteins involved in transport and metabolism of inorganic ions, nucleotides, and carbohydrates, and chaperones [107]. Not all the proteins are induced at the same time after

irradiation but appear in several steps, which is in agreement with the transcriptomic data [108].

Transcription activators and repressors. Significant changes in the profile of gene expression observed under stress conditions raise the question about regulatory pathways involved in these changes. To date, several transcription regulators of *D. radiodurans* involved in cell stress response have been characterized to different extents (Table 2).

Promoters of many stress-response operons in *D. radiodurans* contain a palindromic regulatory element that was named RDRM (radiation/desiccation response motif). The repressor protein that interacts with RDRM is DdrO (also known as DR2574) [109], whose expression is induced under stress [50]. RDRM-dependent regulation was predicted for at least 29 genes of *D. radiodurans* and 25 genes of *D. geothermalis*. These genes include *ddrA*, *ddrB*, and *ddrD*, which are among the most strongly stress-induced genes (see above). The expression of these genes is induced as a result of DdrO cleavage by the PprI protein (see next paragraph). The insufficiency of DdrO results in decrease in cell viability, which is accompanied by DNA fragmentation, membrane blebbing, and problems with cell division [110].

The unique *Deinococcus*-specific protein PprI (also known as IrrE) is a broad-spectrum transcription factor. The absence of this factor significantly decreases cell resistance to ionizing radiation and ultraviolet exposure, as well as mitomycin C resistance [111]. PprI regulates synthesis of at least 31 proteins, including stress-activated proteins involved in DNA repair, such as PprA, RecA, and SSB [112]. It was shown that PprI differentially regulates gene expression both during stress and at different stages of post-stress cell recovery [113]. Expression of PprI in *E. coli* cells significantly increases their resistance to osmotic stress by inducing a wide spectrum of genes responsible for metabolism of carbohydrates, nucleotides, and amino acids [114]. The mechanism of PprI action is not completely understood. On one hand, its specific binding to promoter regions of the target genes is essential for its activity as a transcription factor [113]. On the other hand, PprI from *D. deserti* is a metalloprotease that cleaves transcription factor DdrO *in vitro*, which may likely induce transcription of DdrO-repressed genes [115]. The same mechanism of action was recently demonstrated for PprI from *D. radiodurans*, and it was shown that the proteolytic activity of PprI depends on Mn^{2+} [116]. PprI was also proposed to be involved in

Table 2. Previously studied factors that participate in transcription regulation in *D. radiodurans*

Factor	Function
DdrO	repression of operons containing RDRM, including the *ddrA*, *ddrB*, *ddrD* genes
PprI (IrrE)	regulation of expression of 31 proteins, including activation of some DNA repair factors
PprM	PprI-dependent regulation; repression of expression of *pprA* and, probably, other genes
DrRRA	transcription activation of dozens of genes, including those encoding stress response factors
OxyR	activation and repression of genes depending on the redox potential of the cytoplasm
IrrI (DR0171)	activation or expression of more than 100 proteins, including stress response factors
Mur	transcription activation of genes encoding proteins that participate in the transport of metal ions across the cell membrane
RecX	repression of genes encoding proteins involved in post-irradiation response
LexA2	repression of *pprA*
CsoR	repression of the cluster of copper resistance genes
HucR	repression of urate oxidase synthesis
HspR	repression of heat-shock protein genes
Two-component systems	transcription regulation of genes involved in stress response
NO	stimulation of cell growth and division after irradiation
FMN-riboswitch	regulation of riboflavin biosynthesis; antioxidative protection

post-translational modification (probably, proteolysis) of the PprM protein, which regulates the expression of *pprA* and likely other genes [117].

Other studied transcription factors that affect expression of many genes under stress conditions include DrRRA [118], OxyR [119], and DR0171 (also known as IrrI) [120]. Each of these factors controls expression of dozens of genes, and mutants lacking these factors are more sensitive to various stress conditions. *Deinococcus radiodurans* has two homologous OxyR proteins, which may act as activators or repressors of gene expression. The interactions of OxyR with promoters depend on the redox potential of the cytoplasm. The OxyR molecule contains a cysteine residue that is oxidized in the presence of ROS, resulting in either activation or repression of the target promoters. OxyR-controlled processes include regulation of intracellular Mn^{2+} concentration [119].

Concentrations of metal ions in *D. radiodurans* cells is also regulated by transcription factor Mur (DR0865) that affects expression of genes involved in ion transport. The *dr0865* mutants are characterized by increased stress sensitivity and significantly changed intracellular concentrations of manganese, iron, zinc, and copper ions. Detailed mechanisms of transcription regulation by Mur and its interactions with other transcription regulators remain to be studied [121].

Studies of the mechanisms that regulate RecA activity revealed that both transcription of the *recA* gene and function of the RecA protein are inhibited by the RecX protein [122]. Comparative proteomic analysis also demonstrated that RecX participates in inhibition of a wide spectrum of genes whose products are involved in post-irradiation recovery. Based on these observations, RecX was proposed to play a role in the transition from stress to normal cell phenotype [123].

The *D. radiodurans* genome encodes two homologs of the repressor protein LexA but, in contrast to *E. coli*, neither of them participates in the regulation of RecA expression. At the same time, LexA2 represses the *pprA* gene. Interestingly, bacteria lacking *lexA1* do not differ in their stress response from wild-type cells, while *lexA2* mutants are even more stress resistant [124].

Besides multifunctional transcription factors, several operon-specific protein regulators were also studied in *D. radiodurans*. CsoR is a repressor of a gene cluster responsible for copper resistance. At low copper ion concentrations, this protein is bound to target promoters, while at high concentrations it binds copper and loses affinity to DNA [125]. The HucR protein belongs to the MarR family of transcription regulators and represses transcription of its own gene as well as the gene of urate oxidase [126]. In the presence of uric acid, its affinity to the DNA operator is decreased, resulting in gene activation. The three dimensional structure of HucR has been reported, and the mechanism of its interactions with uric acid has been studied in detail [127, 128]. The *dr0265* gene encodes a transcription factor belonging to the GntR family. Its dysfunction was shown to decrease stress resistance, but its target genes remain unknown [129].

Regulation of protein activity and gene expression under stress conditions involves the action of protein kinases. It was shown that the total level of protein phosphorylation in *D. radiodurans* cells is increased after irradiation [130]. Mutations in gene *dr2518* that encodes a serine-tyrosine protein kinase result in increased sensitivity to γ-irradiation and altered profile of gene expression, suggesting a possible role for this protein kinase in transcription regulation [131].

Two-component signal systems play important roles in the resistance of *D. radiodurans* cells to various stress factors. Such systems consist of sensor histidine kinases and downstream response regulators (which usually act as transcription factors). The *D. radiodurans* genome encodes 20 putative histidine kinases and 25 response regulators [132]. Mutations of the DrRRA regulator (see above, [118]), RadS kinase, and its RadR regulator [133] decrease cell resistance to oxidative stress and DNA damages. Systematic analysis of mutants with deletions of 12 histidine kinase genes demonstrated that many of them are important for cell resistance to radiation and ultraviolet exposure, mitomycin C, and hydrogen peroxide [132].

The gene of NO synthase was found among genes whose deletion decreases stress resistance of *D. radiodurans* [134]. It was shown that NO production in cells of *D. radiodurans* is increased after UV exposure. NO induces expression of the *obgE* gene, which encodes a GTPase with unknown functions. Homologs of this GTPase in other bacteria were shown to be involved in the control of cell cycle and cell growth. It is therefore possible that NO stimulates cell growth and division after irradiation and performs some additional functions [134].

Another molecule that is involved in the stress-induced regulation of gene expression in *D. radiodurans* is flavin mononucleotide, which acts through a riboswitch present in the 5′-untranslated region of the operon responsible for riboflavin metabolism. Deletion of this riboswitch increases the concentration of ROS in the cell and decreases catalase activity, resulting in decreased cell viability under oxidative stress conditions [135]. It was proposed that hydrogen peroxide could interact with the flavin riboswitch, thus directly affecting transcription.

RNA polymerase and associated factors. In recent years, individual components of the transcription apparatus of *D. radiodurans* were studied in some detail, including analysis of *D. radiodurans* RNA polymerase and the major σ subunit (σ^A) responsible for recognition of most cell promoters. Promoters recognized by the major σ^A subunit reveal high level of homology with σ^{70} promoters of *E. coli* [136]. However, in contrast to *E. coli* RNA polymerase, the holoenzyme of *D. radiodurans* RNA polymerase forms unstable promoter complexes and poorly

melts DNA around the starting point of transcription, which largely depends on the properties of the σ^A subunit [137, 138]. In these properties, *D. radiodurans* RNA polymerase is similar to RNA polymerase from the phylogenetically related bacterium *Thermus aquaticus* [138]. Possible roles of these features in stress resistance remain unknown.

In comparison with *E. coli* RNA polymerase, *D. radiodurans* RNA polymerase is much more sensitive to the antibiotic streptolydigin and less sensitive to rifampicin [138]. At the same time, rifampicin resistance mutations can still be used as a marker to estimate the rate of mutagenesis in *D. radiodurans* cells [139]. Transcriptome analysis of one rifampicin resistant mutant revealed significant changes in the profile of gene expression. It was shown that the mutant RNA polymerase forms less stable promoter complexes, thus favoring transcription of genes with AT-rich promoters [140].

In addition to the major σ^A subunit (DR0916) that belongs to the σ^{70} family, the genome of *D. radiodurans* encodes at least three alternative σ factors – DR0180 (Sig1), DR0804 (Sig2), and DR2482. Expression of Sig1 and DR2482 is significantly increased after irradiation, while expression of Sig2 does not change [50]. Analysis of *D. radiodurans* mutants with deletions of the *sig1* and *sig2* genes demonstrated that both alternative σ factors are involved in heat-shock response, and the loss of *sig1* significantly decreases cell viability [141]. Transcriptomic and proteomic analyses of the *sig1* deletion mutant under heat-shock conditions characterized promoters recognized by this factor. It was shown that Sig1 recognizes two types of promoters, which resemble promoters of *E. coli* σ^{70} and *B. subtilis* σ^W, respectively [142]. Sig1 participates in transcription of mRNAs for 31 proteins, including heat-shock proteins with cytosolic localization. Transcription of heat-shock proteins in *D. radiodurans* cells under normal conditions is repressed by transcription factor HspR [143]. No such studies were performed for Sig2 and DR2482.

The mechanisms of regulation of transcription elongation in *D. radiodurans* remain insufficiently studied. It was shown that, in comparison with *E. coli*, *D. radiodurans* RNA polymerase could much more efficiently cleave RNA in transcription complexes [144-146]. The reaction of RNA cleavage was proposed to play an important role in transcription proofreading and in reactivation of elongation complexes that backtracked along the DNA template [147]. In particular, backtracked complexes can likely form in the presence of various lesions in the transcribed DNA template. Importantly, transcription complex backtracking was shown to play an important role in transcription–replication conflicts, which are one of the main causes of DNA damage and genomic instability in *E. coli* [147-149]. Therefore, the increased rate of RNA cleavage by *D. radiodurans* RNA polymerase might play a role in transcription fidelity and in resolution of transcription–replication conflicts under stress conditions.

The genome of *D. radiodurans* contains homologs of several well-known factors that modulate RNA polymerase activity during transcription elongation, including GreA, Gfh, Mfd, and UvrD. The GreA protein activates RNA cleavage in the RNA polymerase active site and is important for reactivation of backtracked transcription complexes in *E. coli* cells [147, 148, 150]. The expression of this protein (DR1162) in cells of *D. radiodurans* is induced after irradiation [50]. Genomes of several representatives of the *Deinococcus–Thermus* phylum also encode homologs of the GreA protein: protein Gfh1 (Gre-factor homolog 1) in *T. thermophilus* and proteins Gfh1 and Gfh2 in *D. radiodurans*. The Gfh1 factor of *T. thermophilus* has been studied in detail. In contrast to GreA, this factor was shown to inhibit all RNA polymerase activities, including RNA synthesis, endonucleolytic RNA cleavage, and RNA pyrophosphorolysis [151-153]. It was demonstrated that Gfh1 can directly affect the structure of the active site of RNA polymerase and change the overall enzyme conformation, thus inhibiting its activity [152, 154-156]. Similar changes might play a role in switching the activity of *D. radiodurans* RNA polymerase by corresponding Gfh factors.

In *E. coli* cells, the Mfd and UvrD proteins participate in reactivation of arrested transcription complexes and in the coupling of transcription with DNA repair. The *E. coli* Mfd protein binds stalled transcription complexes and causes their dissociation through forward translocation along the DNA template, followed by recruitment of NER enzymes to the site of DNA damage [157]. The *E. coli* UvrD helicase also interacts with RNA polymerase but, in contrast, stimulates backward translocation of the complex (wherein RNA polymerase remains bound to DNA), thus clearing the damaged DNA region for the action of NER factors [158]. The transcription functions of orthologous factors in *D. radiodurans* have not been studied, but it can be proposed that they may play key roles in transcription of damaged DNA regions under stress conditions.

CONCLUSION

The bacterium *D. radiodurans* is renowned for its exceptional resistance to ionizing radiation and other factors that induce oxidative stress in cells. To protect and repair its DNA, *D. radiodurans* has a full set of the major DNA repair pathways that are found in other bacteria, and it possesses some unique proteins that complement them. Despite the presence of some specific features of the DNA repair systems, the main factor that determines the high efficiency of the repair processes in *D. radiodurans* is likely high stress resistance of its proteome, including DNA repair proteins. *Deinococcus radiodurans* harbors a number of adaptations that help to preserve proteome integrity under oxidative stress conditions, includ-

ing highly efficient enzymatic and protective antioxidant systems.

Very interesting is the question on the evolutionary origin of radioresistance in *D. radiodurans*, since no natural habitats on Earth have such high radiation levels as *D. radiodurans* can survive. An extraterrestrial origin or at least temporary "training" of radioresistant bacteria at increased radiation levels were proposed as possible mechanisms explaining the origin of such resistance [159]. In addition, the possibility of rapid evolution of radioresistance of *D. radiodurans* in artificial environments created by humans was also considered. However, many unrelated radioresistant bacterial species were found in *Chroococcidiopsis*, *Methylobacterium*, *Rubrobacter*, and other genera in addition to *D. radiodurans* [160], making it unlikely that all of them developed the resistance in such a way.

At present, the most plausible hypothesis on the origin of radioresistance is its appearance because of adaptation to desiccation conditions. Indeed, *D. radiodurans* is found ubiquitously in nature, and deserts are natural habitats for several members of this genus. Both radiation and desiccation lead to oxidative stress, and the ability to survive oxidative stress likely underlies the resistance to both stress conditions. It is likely that the *Deinococcus* bacteria evolved toward increased desiccation resistance by improving the mechanisms that prevent reactive oxygen species formation and eliminate the consequences of oxidation of macromolecules [161]. Since oxidative stress underlies the damaging action of many adverse conditions, the adaptations that *D. radiodurans* has developed allow it to survive a wide spectrum of impacts [4].

Molecular mechanisms of the regulation of gene expression in *D. radiodurans* under stress conditions are only beginning to be investigated, but it is already clear that the ability of this bacterium to adapt to various stress factors and the presence of unique transcription regulators make this system different from classical bacterial models. From this point of view, analysis of the mechanisms of transcription and its regulation in this bacterium will be an exciting topic of further studies.

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