REVIEW ARTICLE

Physiological and molecular basis of extreme radioresistance in *Deinococcus radiodurans*

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Deinococcus radiodurans **is characterized for its extraordinary radioresistance. An efficient DNA strand-break repair and strong oxidative stress tolerance are amongst the mechanisms that contribute to its extreme phenotypes. The multipartite genome structure, recombination repair without RecBC enzymes, absence of SOS response and the roles of serine/threonine protein kinase in DNA damage response, and the small molecules protecting proteins from oxidative damage are some of the other unique features of this bacterium. Here, we review the most recent advances in our understanding on different aspects of *D. radiodurans* that are shown to be important for its extraordinary radioresistance.**

Keywords: *Deinococcus radiodurans*, DNA damage response, extreme radio resistance, oxidative stress tolerance.

Microbiological and morphological features

BACTERIA belonging to the family Deinococcaceae are known for extreme radiation resistance phenotype. Around 45 species of the genus *Deinococcus* belonging to mesophilic, thermophilic and psychrophilic groups have been isolated and identified from different sources like air, sewage water, deserts, the radioactivity contaminated sites, hot springs and Antarctica. Majority of these isolates are mesophilic and grow between 30°C and 37°C. The detailed description on microbiological, morphological and genetic aspects of these isolates has been reviewed[1,2] and therefore, kept out of discussion here. *Deinococcus radiodurans* R1, a member of the family Deinococcaceae, was earlier known as *Micrococcus radiodurans*[3] and has been studied in greater detail in the last two decades. It is a Gram-positive, pigmented, non-spore forming, nonmotile, spherical bacterium ranging from 1.5 to 3.5 µm in diameter and grows with a doubling time of ~ 80 min in a rich nutrient medium. It exists in tetrads and its genetic material is packaged in toroid form[4] (Figure 1). Cell envelope of this bacterium consists of six layers: the plasma membrane, peptidoglycan layer, compartmentalized layer, electron transparent zone, outer membrane and a hexagonally packed intermediate layer[5,6]. The cytoplasmic membrane and peptidoglycan layers participate in the septum formation, whereas the other layers are formed on daughter cells after cell division is accomplished[7]. Exponentially growing bacterium is easily transformable with dsDNA and that possibly makes it prone to horizontal gene transfer[8,9]. This bacterium is red-pigmented and rich in carotenoids[10]. Role of carotenoids in radioresistance was supported through its role in oxidative stress tolerance. *D. radiodurans* mutant defective in carotenoid biosynthetic pathway shows reduced free radical scavenging ability and therefore gamma radiation resistance[11]. As none of these features alone could make *Escherichia coli* as radiation resistant as *D. radiodurans*, the possibility of all these together with efficient double strand break (DSB) repair supporting the extreme radiation resistance in *D. radiodurans* could be suggested.

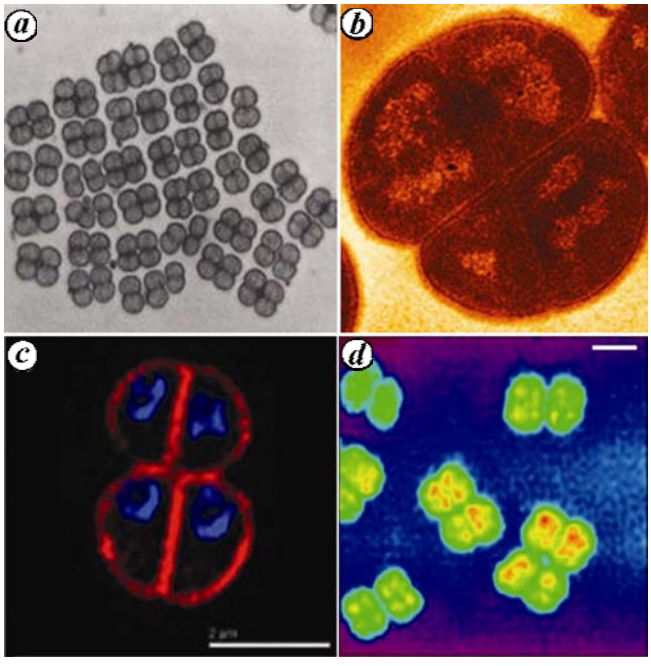

Figure 1. Microscopic features of *Deinococcus radiodurans* R1. *D. radiodurans* cells were examined microscopically under bright field[102] (***a***), electron microscope[103] (***b***), fluorescence microscope[4] (***c***) and by nanoscale X-ray imaging[104] (***d***). Images have been downloaded from webpage, http://www.google.com/search?q=deinococcus+radiodurans+images&hl=en&prmd=imvns&tbm=isch&tbo=u&source=univ&sa=X&ei=yozwTpQd6s7YBa6E_YEC&sqi=2&ved=0CCUQsAQ&biw=1226&bih=698.

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DNA damage tolerance and repair mechanisms

D. radiodurans shows high resistance to DNA damage produced by various physical and chemical mutagens. Cells exposed to 6 Gy γ-radiation that produces ~200 DNA double strand breaks and ~3000 single strand breaks[12] do not show a measurable loss of cell viability. Although it is mainly characterized by its extraordinary resistance to γ-radiation with a D_{10} of ~8 kGy during exponential growth phase and 10–12 kGy during stationary phase, it also confers extreme resistance to other DNA damaging agents. It shows resistance to desiccation up to < 5% humidity for six weeks[13]. It was observed that the ionizing radiation-sensitive (IRS) mutants like IRS26, IRS27, IRS47, IRS7, IRS33, and IRS38 and *rec30* studied earlier, were also sensitive to desiccation[14]. In addition, it can tolerate 10 min exposure of ~20 µg/ml mitomycin C (MMC) that produces nearly 100 interstrand crosslinks and shows higher resistance to far-ultraviolet radiation (FUV; 254 nm) with D_{37} of ~600 J/m^2 for exponentially growing cultures as against 30 J/m^2 in case of *E. coli*. Surprisingly, it is highly sensitive to near-ultraviolet radiation (NUV; 300–400 nm)[15]. The molecular basis underlying the differential responses to FUV and NUV is not understood. Genome of *D. radiodurans* encodes both UvrABCD and endonuclease β (UVDE)[16] mediated UV repair pathways of nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MMR), but lacks photolyase enzyme involved in photoactivation repair[17]. UvrABCD exinuclease functions similar to classical NER pathway known in other bacteria. Mutational studies confirmed both genetic and functional interactions of both exinuclease and UV damage endonuclease β-pathways and their significance in UVC resistance[18]. *D. radiodurans* possesses the well-characterized bacterial DNA glycosylases like apurinic/apyrimidine lyase, thymine glycol DNA glycosylase[19] and multiple putative uracil–DNA glycosylases (DRB0689, DR1751, DR0022). Presence of several DNA glycosylases possibly makes this organism tolerant to various types of DNA base damage. *D. radiodurans* encodes X-family DNA polymerase that is a homologue of eukaryotic DNA polymerase β and both ATP and NAD-type DNA ligases. Roles of DNA polymerase β in UV lesion bypass and BER have been shown. Both DNA polymerase X and ATP-type DNA ligase (DRB0100) mutants of this bacterium show the significant loss of DNA damage tolerance[20–22]. The purified recombinant polymerase shows both polymerase and 5′ dRPLyase activities. *D. radiodurans* genome encodes MMR components MutS, MutL ATPases and endonuclease VII (XseA), but lacks 'Dcm' and 'Dam'-type site-specific methylases. Although the components of NER, BER and MMR pathways[23] of DNA repair in *D. radiodurans* are similar to any other bacteria, the findings show the involvement of these DNA repair pathways in radiation resistance of this bacterium. The 3D structure of DR0715, a mismatch-specific uracil–DNA glycosylase (MUG) solved recently, showed that this enzyme contains a novel catalytic residue (Asp93), which could be responsible for its wide substrate specificity compared to its *E. coli* homologue[24]. Comparison of genome sequences of four species of *Deinococcus*, such as *D. radiodurans*[25], *D. geothermalis*[26], *D. deserti*[27] and *D. maricopensis*[28] published recently argued that in spite of their similar levels of radioresistance, they share very little similarity at the genetic level. A large number of genes of *D. radiodurans* are absent in the genome of *D. geothermalis*, *D. deserti*, *D. radiopugnans*, *D. proteolyticus* and *D. radiophilus*. Certain unique genes of *D. radiodurans* such as *pprI* (DR0167, Dgeo0395)[29] and *pprA* (DRA0346, Dgeo2628)[30] and four of the most highly ionizing radiation and desiccation inducible genes like *ddrA* (DR0423), *ddrB* (DR0070), *ddrC* (DR0003) and *ddrD* (DR0326) are subjected to positive selection during evolution of radiation-resistant bacteria[31]. Some of the putative DNA repair and/or hypothetical proteins of *D. radiodurans* that have been studied for their possible involvement in γ-radiation resistance and DNA repair are summarized in Table 1.

Recombination repair in *D. radiodurans*

The reassembling of shattered genome during post irradiation recovery (PIR) utilizes biphasic kinetics[32] and requires the RecA activity. Moreover, the absence of RecBC enzyme offers better survival. *Deinococcus* contains most of the orthologues of recombination repair genes, except RecBC. An aberrant RecD with 200 amino acids extension at the N-terminal is characterized as a weak helicase *in vitro*[33]. Interestingly, it was found that the cell-free extract of wild type as well as transgenic *D. radiodurans* expressing RecB and RecC together from *E. coli*, did not show ATP-stimulated exonuclease (exonuclease V) activity[34], indicating that deinococcal RecD failed to constitute RecBCD complex with recombinant RecBC. *D. radiodurans* genome encodes the complete system of RecF homologous recombination pathway. The loss of γ-resistance in *D. radiodurans* expressing SbcB, a protein that is shown to destabilize the RecF pathway of recombination in *E. coli*[35] indicated that the radiation resistance in *D. radiodurans* is most likely supported by RecF recombination. Further studies provided strong evidence in support of RecFOR involvement in higher radiation resistance and DSB repair[36,37]. Thus, the extraordinary radiation resistance and the DSB repair in *D. radiodurans* are supported by RecF recombination and the role of RecBC in DSB repair seems to be organism-specific and not necessarily universal.

Recombination repair in most bacteria is taken as a synonym to SOS repair, where the co-protease activity of RecA inactivates LexA and activates UmuD. LexA inactivation eventually derepresses many recombination

Table 1. Some of the DNA replication, recombination and repair genes studied for their roles in radiation resistance and double strand break (DSB) repair in *Deinococcus radiodurans*. Details within parenthesis are names of corresponding open reading frames (ORFs) annotated in genome of this bacterium

Proteins and ORFs	Characterized functions	Reference
UvsE (DR_1819)	UV-endonuclease β	18
DNA polymerase I (DR_1707)	DNA-dependent DNA synthesis required in ESDSA	35
Fpg (DR0493)	Excision of modified DNA bases FapyGua, FapyAde and 8-OH-Gua	19
RecA (DR_2340)	Recombinase, role in ESDSA and required for recombination repair and genome stability	40, 92
TopIB (DR_0690)	Type IB DNA topoisomerases, relaxes supercoiled DNA	95
IrrE/PprI (DR_1067)	Master regulator of gamma radiation-inducible expression of various genes.	29, 61
PQQ synthase (DR_C0034)	Pyrroloquinoline quinone synthesis, antioxidant and a protein kinase activity inducer	66, 96
RecD (DR_1902)	DNA helicase, acts on short DNA duplexes with 5′-tail or a forked end in 5′–3′ direction	33
LexA2 (DR_A0074)	SOS response regulator	38
UDG (DR_1751)	Uracil–DNA glycosylase	97
Ung (DR_0689)	Uracil–DNA N-glycosylase	97
PprA (DR_A0346)	DNA protection, stimulation of DNA ligase	30
UvrD (DR_0065)	ATP-dependent helicase	43
MutL (DR_1696)	DNA mismatch repair protein	43
MutS1 (DR_1976)	DNA mismatch repair protein	43
PolX (DR_0167)	Short patch BER DNA polymerase	20, 21
HU protein (DR_A0065)	Histone-like protein	98
SSB (DR_0100)	Single-stranded DNA binding protein, with four OB folds; it functions as a homodimer	41
RecX (DR_1310)	Involved in regulating RecA expression	64
RecO (DR_0819)	RecFOR recombination repair protein	37, 77
RecQ (DR_1289)	DNA helicase with three tandem HRDC domains	37
LigB (DR_B0100)	ATP-type DNA ligase involved in the joining of DNA ends	22, 47
LigA (DR_2069)	NAD (+)-dependent DNA ligase	47
DR1572	DNA helicase	44
SbcC (DR_1922) SbcD ((DR_1921)	Mre11–Rad50-type functions in prokaryote	83
DdrB (DR_0070)	SSB-type function	70–72
RadA (DR_1105)	Priming DNA ends during recombination repair	36
DNA polymerase III, α-subunit DR_0507)	DNA-dependent DNA synthesis with anticipated roles in ESDSA	36
RecG (DR_1916)	DNA helicase	96
Rqk (DR_2518)	Gamma radiation responsive eSTPK with a role in DNA damage tolerance	66
DR0505	5′ Nucleotidase, a phosphoesterase with 3′ → 5′ exonuclease activity	46
Rec J	5′ → 3′ Exonuclease	36, 44
RadR/RadS (DR_B0091/DR_B0090)	A two-component system having roles in radiation resistance and DSB repair	67
DRA0282	DNA protection and topology-specific DNA-binding protein	78
DR2417	A novel member of beta CASP family DNA processing enzyme	55

repair genes under LexA repression and *recA*. Expression of recombination repair genes leads to DSB repair. Although the genome of *D. radiodurans* encodes three diverged copies of LexA[38] having helix–turn–helix (HTH) DNA-binding motif and autoprotease domain as known in LexA of *E. coli*, the DNA damage-induced expression of *recA* seems to be independent of LexA[39]. Furthermore, the deinococcal RecA (drRecA) prefers dsDNA to ssDNA[40] and deinococcal SSB (drSSB) functions in dimeric form with each monomer containing two QB folds[41]. Therefore, the possibility of drRecA being regulated differently at both synthesis and function levels may be speculated and requires further studies. Recently, a novel DSB repair mechanism called extended synthesis-dependent strand annealing (ESDSA)[42] is suggested to be the key mechanism for efficient DSB repair in this bacterium. ESDSA involves extensive DNA synthesis, DNA processing, homology search and ssDNA annealing with complementary strands and joining of the nicks during early phase of DSB assembly. During this process, the overlapping homologies of chromosomal fragments act as both primers and templates for synthesis of longer complementary single strands. Annealing of the newly synthesized complementary ssDNA occurs with high precision, resulting in joining of adjacent DNA fragments to form the long and linear dsDNA intermediates. Replicative DNA polymerases are essential for ESDSA and DNA synthesis occurs more efficiently on damaged DNA than the normal rate of DNA replication[36]. Even though the ESDSA is found to be a mechanism that supports the efficient DSB repair in *D. radiodurans*, the molecular devices operating several steps of ESDSA, including the

higher rate of DNA synthesis and its processivity on damaged DNA are not understood and require independent studies. DNA polymerase III plays a role in repair synthesis, whereas both DNA polymerases I and III are required for efficient elongation. Remarkably, the *Deinococcus* encodes three small nucleotidyltransferases (DR1806, DR0679 and DR0248), which are present in only a few other bacteria[43]. Out of several functions involved in ESDSA, the enzymes required for DNA processing and resection are not fully characterized, except that the roles of classical RecFOR proteins and RecJ nuclease along with UvrD helicase[44] are suggested. Recently, several proteins have been characterized with $3' \rightarrow 5'$ and $5' \rightarrow 3'$ exonuclease activities[45,46], which could be checked for their roles in ESDSA. The genome of this bacterium encodes both NAD type (*ligA*) and ATP type (*ligB*) DNA ligases encoded from DR_2069 and DR_B0100 ORF respectively[47]. Although the purified LigB did not show ligase activity *in vitro*[47], the deletion mutant of *ligB* becomes sensitive to γ-radiation and the complementation of this function requires the expression of the entire *ligB* operon (*drb0100–drb0099–drb0098*)[22]. The possible involvement of LigB in ESDSA would be worth knowing. As ESDSA has been suggested to be a mechanism that mostly makes *D. radiodurans* efficient in DSB repair and radioresistant, the existence of a similar mechanism in other species of genus *Deinococcus* while its absence in radiation-sensitive bacteria might provide further insight into the ESDSA mechanism and its universal role in higher DNA damage tolerance in bacteria.

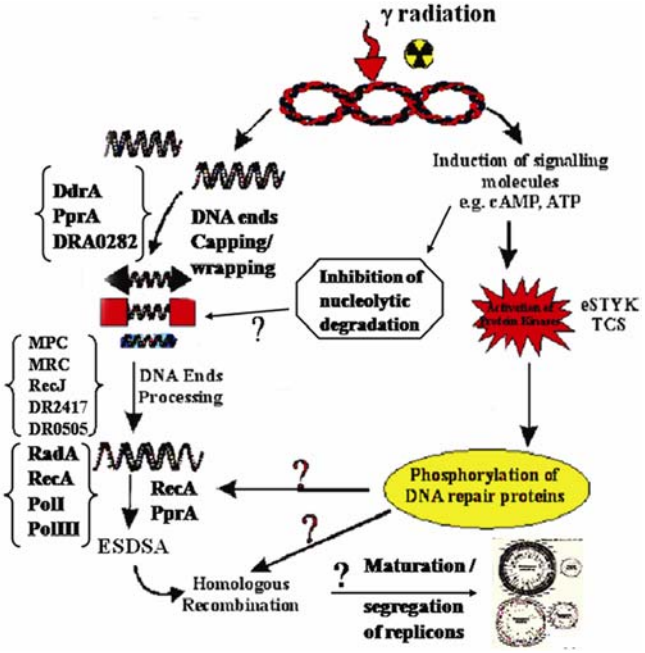

Figure 2. Diagrammatic representation of various processes discovered during post-irradiation recovery of *D. radiodurans*. Cells exposed to γ radiation produce DNA double strand breaks (DSB) and induced the synthesis of signalling nucleotides (cAMP and ATP) and protein kinase (a eukaryotic type Ser/Thr/Tyr kinase (eSTYK)) and sensor kinase and response regulator (TCS). DSBs are protected by DNA capping (DdrA and PprA) and DNA wrapping (DRA0282) proteins and by protein kinase-dependent ATP-regulated process. Different proteins in the form of multiprotein complex (MPC), Mre11-Rad50 complex (MRC) and in isolation like RecJ, DR2417, DR0505, expressing nucleases activities are anticipated to be involved in DSBs processing. These function in tandem with RadA/RecA and PolI/PolIII enzymes regulating recombination and DNA synthesis. A eSTYK induced during early phase of post-irradiation recovery could phosphorylate PprA and RecA of this bacterium. (These are based on the results published[30,44,46,52,54,78,83] and unpublished results of H. S. Misra and colleagues.)

Ploidy and its possible role in extreme phenotypes

D. radiodurans R1 is a multigenomic and multipartite genome containing bacteria. Its genome is made up of chromosome I (2,648,638 bases), chromosome II (412,348 bases), megaplasmid (177,466 bases) and small plasmid (45,704 bases)[25]. Genome multigenomicity creates higher order of genetic redundancy, which not only provides functional redundancy but also act as a source of undamaged substrates for recombination repair of damaged DNA. Therefore, the ploidy was debated to be one of the factors responsible for efficient DSB repair in *D. radiodurans*. However, there are exceptions where mere ploidy does not suffice for extreme radiation resistance. Also, *Truepera radiovictrix* a bacterium belonging to Deinococcus–Thermus phylum, contains single circular chromosome of 3,260,398 bp and shows an extraordinary resistance to radiation and other DNA damaging agents[48]. Although ploidy alone may not be sufficient, the possibility of it complementing with the unique DSB repair mechanism in *D. radiodurans* to its extreme phenotypes cannot be ruled out. Earlier, it was hypothesized that chromosomes in *Deinococcus* are joined to each other at thousands sites[49], most likely through homologous sequences present on different genome units and that would have made it efficient in DSB repair. Experimental evidence that ploidy has any role in extreme phenotypes of this bacterium would first require understanding of mechanisms of genome maintenance.

Bacterial genome maintenance and segregation have been studied largely in bacteria harbouring single circular chromosome and low copy plasmid[50]. Mechanisms underlying the maintenance and inheritance of multipartite genome are not studied in detail. *D. radiodurans* harbours a multipartite genome system, which is annotated with putative genome partitioning proteins like ParA and ParB expressing in *parAB* operons, one each on chromosome I and chromosome II and two on megaplasmid[17]. Small plasmid does not encode ParA and ParB proteins and the centromeres were not identified in this bacterium till recently. Now, the chromosome I partitioning system has been functionally characterized and for the first time, the centromeric sequences were identified in the genome of

D. radiodurans[51]. Chromosome I of *D. radiodurans* has chromosomal-type centromeric sequences and its partitioning system follows the pulling mechanism of genome segregation[51]. We further showed that the mutants of *parB* encoded on chromosome I, chromosome II and megaplasmid showed less tolerance to DNA damage, indicating the roles of multipartite genome system in conferring DNA damage tolerance of this bacterium.

DNA protection and regulated processing of damaged genome

Protection of damaged genome from the nucleolytic degradation could be an important feature contributing to efficient DSB repair and radioresistance in *D. radiodurans*. Cell-free extract of this bacterium showed several-fold less specific nuclease activity compared to *E. coli*[34], indicating that either the levels of total soluble nucleases are less in *D. radiodurans* or its broken DNA is protected from nucleolytic degradation. DNA protection can occur by either making DNA not accessible by nucleases or by regulated expression of nuclease activity. Two proteins, namely DdrA (DR0423) and PprA (DRA0346), encoded on the genome of *D. radiodurans* were characterized with DNA ends protection activity *in vitro*[30,52]. Recently, another protein DRA0282 was identified from a pool of DNA-binding proteins exhibiting ATP-sensitive nucleolytic degradation[53]. This protein is now characterized for having higher affinity toward superhelical DNA and ssDNA than linear dsDNA. However, it protects dsDNA from exonuclease III degradation. DRA0282 could support higher UVC tolerance in *E. coli*. *Deinococcus* lacks single-stranded DNA exonucleases like SbcA and SbcB and RecBCD, which functions as a χ (chi)-regulated dsDNA exonuclease. Except few of these examples, the other known recombination nucleases are the same in both *E. coli* and *D. radiodurans*. Nucleases are the important genetic armour required for DNA ends processing during genetic recombination ensuring genome integrity. Regulation of nuclease activity during post-irradiation recovery of *D. radiodurans* has been reported from independent studies. It was observed that the nuclease activity present in a multiprotein complex isolated from this bacterium[54] and in a pool of DNA-binding proteins[55] could be inhibited by ATP and this inhibition was not due to ATP-mediated metal-ion chelation. Subsequently, DR2417 and DR0505 proteins were characterized as ATP-sensitive nucleases *in vitro*[46,55]. ATP inhibition of nuclease activity has been reported in enzymes belonging to β-CASP family proteins from eukaryotes whereas ATP stimulation of exonuclease activity of RecBCD and SbcCD complexes is the core of homologous recombination in *E. coli*. DR2417 was found to be a novel member of β-CASP family enzyme in *D. radiodurans*. Contrasting effect of ATP on nuclease(s) functions in *D. radiodurans* and *E. coli* could be seen as an evolutionary consequence of these bacteria exhibiting the opposite response to γ-radiation and DNA damage. Thus, there seems to be two types of DNA protection mechanisms in this bacterium. One that supports the protection of dsDNA by making DNA inaccessible to degradation and the other that modulates nuclease functions during PIR. As DNA protection and DNA processing are mutually incompatible processes, regulation of these processes during post-irradiation recovery would be worth studying. The possibility of switching the polarity of exonucleolytic end processing and/or the modulation of nucleolytic activity upon interaction with other proteins and/or DNA sequence *in vivo* may be envisaged. Based on these findings, the different possible mechanisms that could associate with the protection of DNA from nucleolytic degradation and the regulated processing of dsDNA ends in the context of *D. radiodurans* are summarized in Figure 3.

Unique DNA damage response mechanism

D. radiodurans cells treated with high doses of γ-radiation postpone growth until DNA is repaired. This observation might indicate the possibility of some checkpoint regulation amongst DNA repair, replication and cell division. *D. radiodurans* exposed to lethal doses of γ-radiation overcomes its deleterious effects by adjusting its transcriptome and proteome[56–58]. SOS response is a well-characterized DNA damage response mechanism in prokaryotes. As the DinP/UmuC family of nonprocessive DNA polymerases are absent and the LexA inactivation failed to induce *recA* expression in response to DNA damage, the possibility of a functional SOS response system is ruled out in *D. radiodurans*[39,59]. However, the LexA binding motif in the form of SOS box-like consensus sequences ($GTTCN_7GTTC$) is found in 61 DNA repair genes, 145 stress responsive genes and 41 clusters of unusual predicted operons in *D. radiodurans*[60]. The regulation of *recA* and *pprA* was shown to be under the control of an ionizing radiation responsive protein IrrE (also called as PprI)[29,61]. But, many differentially regulating genes reported in different studies[56,62] were found to be independent of PprI. These findings might suggest the possibility of some alternate DNA damage response mechanisms in this bacterium. Genome of *D. radiodurans* encodes various stress-responsive proteins. Some of these include two OxyR homologues having a role in oxidative stress response[63], RecX that downregulates the transcriptional activation of *recA* expression[64], a large number of uncharacterized putative transcription factors, type IIA group stress response regulators, histidine kinases and Hank type STPKs[17] and a mammalian homologue of nitric oxide synthase having a role in upregulation of *obgE* (a GTPase)[65]. An eukaryotic-type Ser/Thr

protein kinase (eSTPK) DR2518 has been characterized from *D. radiodurans* R1 and showed that both the synthesis and phosphorylation of this kinase are induced in response to γ-radiation[66]. DR2518 is a membrane protein kinase that could phosphorylate a number of DNA metabolic proteins, including PprA and drRecA *in vitro*. Similarly, the two-component systems like RadS–RadR that induce in response to γ-radiation[67] and PhoR–PhoB in response to phosphate starvation[68] were shown for their role in radiation resistance. Transcriptome analysis of *dr2518* deletion mutant and its comparison with wild type showed the differential expression of a large number of genes important for normal metabolism, growth and maintenance of bacterium and DNA repair[69]. When the proteome of *D. radiodurans* was scanned for the presence of putative phospho-motifs for eSTPKs, a large number protein including those involved in DNA metabolism were found containing sites for STPK phosphorylation (Table 2). Recently, it has been shown that the levels of high-energy phosphates, signalling nucleotide like cAMP and stress responsive enzymes like protein kinases and adenylate cyclase were enhanced during PIR in *D. radiodurans*[53]. This indicated that *D. radiodurans* has a mechanism of adjusting its signalling components in response to γ-radiation and thus the involvement of signalling molecules and kinases in response to radiation-induced DNA damage could suggest an alternative DNA damage response mechanism in this bacterium.

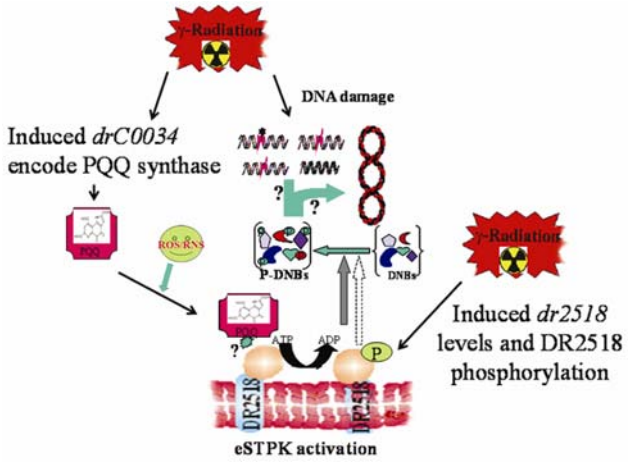

Figure 3. Schematic representation on the regulation of DR2518 kinase activity in response to gamma radiation. Gamma radiation induced the expression of *drC0034*, a gene encoding pyrroloquinoline quinone (PQQ) synthase enzymes responsible for PQQ synthesis, and also the levels of transcription as well as phosphorylation of DR2518 kinase. PQQ is known for antioxidant behaviour and it forms nonreactive species. It can interact with DR2518 *in vitro* and induce its kinase activity. Higher activity of DR2518 could phosphorylate a number of DNA metabolic proteins having putative phosphorylation motifs as shown in Table 2, including PprA, ParB1 and RecA (H. S. Misra and Y. S. Rajpurohit, unpublished data) and improve their activities. The role of these phosphorylations in efficient DSB repair could be hypothesized. (The representation is made on the basis of published findings[56,66,85] and unpublished results of H. S. Misra and colleagues.)

Distinct features of proteins in *D. radiodurans*

Structural genomics contributes in understanding the function of proteins and their possible interacting partners *in vivo*. Genome of *D. radiodurans* encodes several proteins that are structurally different from their homologues in other bacteria. For example, the RecD of *D. radiodurans* contains ~200 amino acids extension at the N-terminal and shows a weak DNA helicase activity in isolation[33]. Unlike most of the other bacteria, the RecQ helicases in *D. radiodurans* have an unusual domain architecture containing three tandem copies of the C-terminal helicase–RNase D (HRD) domain. *Deinococcus* encodes a protein (DR2444) that contains an HRD domain and a domain homologous to cystathionine γ-lyase, that is not associated with either a helicase or a nuclease and implicated to contribute in the repair phenotype[17]. The drSSB is a homodimer with functionally essential two OB folds per monomer[41], whereas SSB from other bacteria is functionally active as a homotetramer. Another ssDNA binding protein, DdrB comprises OB fold, but is structurally and topologically distinct from all other SSBs characterized till date. The DdrB involved in protein recruitment or DNA architecture maintenance in response to extreme conditions has been suggested[70–72]. Similarly, drRecA is different from *E. coli*, which has been attributed to extraordinary DNA damage tolerance in *D. radiodurans*. The possibility of the unique features of drRecA and drSSB contributing to efficient ESDSA mechanism of DSB repair in *D. radiodurans* may be suggested. *D. radiodurans* contains several proteins which are involved in different cellular recovery processes and have large hydrophilic regions. Some of these are similar to desiccation-responsive/resistant proteins of plants and animals[73], which are absent in desiccation-sensitive species. The proteome of *D. radiodurans* also shows a group of proteins having significantly disordered regions, which are absent in non-extremophile homologues. Molecular dynamics simulations predict that the disordered tails minimize the hydration free energy of the whole protein, which helps them to function and to remain stable in the low water site in cells under desiccation[74]. *D. radiodurans* proteins have also been found containing internal peptides structurally similar to 'inteins', which led to speculation that protein splicing also regulates enzyme functions and protein diversity in this bacterium. The Snf2/Rad54 helicase-related protein and Snf2/Rad54 helicase encoded from DR_1258 and DR_1259 ORFs have been identified from *D. radiodurans*[75]. The mechanism of Snf2 splicing is similar to class 3 mechanisms of protein splicing[76]. These findings may therefore suggest that the structural differences of these proteins from their homologues could have been an added advantage to the extreme phenotypes in *D. radiodurans*. How these structural differences contribute to extreme phenotypes is not known and would be

Table 2. Some of the important proteins in *D. radiodurans* showing putative phosphorylation motif searched using (–S/T–Q–X-hydrophobic–hydrophobic–) motifs, where T is phosphoacceptor and X can be any amino acid residues except the positively charged[100] and ('X-X-T-Q-α-X/V-$-X-$'), where T is phosphoacceptor site and α is an acidic residues, $ a large hydrophobic residue and X any amino acid[101]

Protein Id (ORFs)	Protein name (annotated)	Putative phosphomotifs	No. of motif presents	Molecular weight (kDa)
DR_0002	DnaA	M**SQEIW**AD	1	52
DR_0012	ParB1	RA**SQLAG**L TGT**T**QVQTL	2	31.8
DR_0198	RecR	LEY**T**DEVTLG	1	23.7
DR_0400	FtsK-like protein	MM**SQVGA**K	1	107
DR_0440	RuvC	LT**T**ESAWLMP	1	19.6
DR_0493	Fpg (MutM)	RN**T**ERAHGRQ	1	30.8
DR_0507	DNA Pol III α subunit	LAM**T**DHGNM	1	149.2
DR_0689	UDG	EL**T**EDIPGFVA	1	27.7
DR_0911	DNA-directed RNA polymerase subunit β (RpoC)	LM**SQGAP**D KPK**T**QAVVAD RSL**T**DLLGGK	3	171.8
DR_0912	RpoB	VVLQ**T**QDLHLPEA GDI**T**EVIPLP	2	128.7
DR_0939	Rex	LQ**T**QDLHLPE	1	25.09
DR_1089	RecF	GE**T**EAYVRA LG**T**EIMLFRR	2	39.14
DR_1354	UvrC	GDK**T**DLIEMAQ	1	68.9
DR_1424	DnaJ	VE**T**QQVCPTC	1	40.23
DR_1696	MutL	TV**SQLF**AR	1	57.9
DR_1771	UvrA	SEV**T**DRLLAG	1	112.1
DR_1922	SbcC	DIE**T**QAAEAGR	1	100
DR_1984	Thymidine kinase	ATR**TQRLI**GG	1	22.3
DR_2069	DNA ligase	LD**T**DDFTFTG AE**T**EAAPAES LV**T**QLLHEG	3	75.5
DR_2263	DNA protecting protein	DAR**T**QVADLV	1	23.03
DR_2340	RecA	VN**T**DELLV	1	38.14
DR_2417	Putative β CASP family nuclease	FA**SQVYRI** PA**SQAHP**D	2	68
DR_2509	Hypothetical	RFT**T**QRARALGA	1	14.86
DR_A0065	HU protein	VAK**T**QLVEMV	1	12.289
DR_A0282	Ku80 type DNA binding protein	PY**SQVAF**AG GE**T**QILSNLQG	2	54.8
DR_A0344	LexA	QV**T**DRARAA	1	22.3
DR_A0346	PprA	GL**SQWAA**LGEG VD**SQIAA**LA AAL**TQSL**QEA	3	32.2
DR_B0002	ParB type protein from megaplasmid (M1B)	IQ**SQGIL**QP GL**T**EVPVIV	1	32

worth studying. Recently, the role of drSSB in RecO recruitment was studied and it was found that the C-terminal of drSSB interacted strongly with EcRecO, but not with RecO of *D. radiodurans* (drRecO). drSSB shows weak interaction with the peptide of RecF[77]. The possibility of other homologues of SSB interacting with drRecO just as of *E. coli* SSB does with EcRecO cannot be ruled out. As this bacterium is dependent upon the RecF pathway of homologous recombination for its efficient DSB repair, the mechanism of action of drRecA and drSSB in the RecFOR pathway is intriguing. Further, there are more proteins characterized for having higher affinity to ssDNA compared to dsDNA[78] and they need to be studied for their involvement in recombination repair. Because majority of recombination and repair proteins in *D. radiodurans* are nearly similar to the respective homologues present in radiation sensitive bacteria, the possibilities of these enzymes working differently in different microenvironments could be hypothesized. A recent finding that might accommodate the unique features of DNA repair proteins from *D. radiodurans* was that a DNA processing multiprotein complex of 24 proteins, including DRB0100 and PprA was identified from this bacterium[59]. DRB0100 was found to be inactive in pure form. However, co-incubation of purified PprA with pure DRB0100 could help this enzyme regain its DNA ligase activity in the presence of ATP[22]. This suggests that enzymes in this bacterium work differently both in terms of specificity

and catalytic efficiency when they are present with their interacting partners. Such kind of functional interaction of proteins requires the evolution of better protein structure than the existing homologues in other bacteria. The structural biology of the proteins of this bacterium therefore becomes more interesting to study.

Possible involvement of small molecules in radiation resistance

Effects of small molecules in the regulation of gene expression, protection of biomolecules from oxidative damage and regulation of DSB repair have gained significant importance in this bacterium. It has been shown that *D. radiodurans* accumulates high levels of [Mn] and this contributes to its radioresistance and oxidative stress tolerance[79,80]. Higher cellular concentration of [Mn] is shown to support the ordered DNA condensation and DNA toroid formation in other systems[4]. However, the different isolates of *Deinococcus* containing different levels of [Mn] ions do not show correlation with the levels of their oxidative stress tolerance and γ-radiation resistance[81]. Further, the *Bacillus subtilis* spores lacking (α(–)β(–))-type, DNA-binding, small acid-soluble proteins (SASP), in spite of having higher levels of [Mn] to [Fe] ratio fail to show higher protection to DNA and proteins from γ-radiation than wild-type spores. Surprisingly, the α(–)β(–) spores were more sensitive to H_2O_2 when they had high levels of [Mn][82]. It appears that it is not the mere presence of [Mn] per se, but the presence of novel Mn^{2+}-requiring proteins and the formation of [Mn] complexes with phosphate might require for Mn^{2+} roles in higher γ-radiation resistance. Earlier a number of Mn^{2+} requiring proteins have been characterized from this bacterium[20,83] and their roles in radiation resistance have been shown. Apart from metal ion homeostasis, a number of other small molecules with antioxidant properties have been reported from this bacterium. For examples, deinoxanthin, a unique carotenoid[10]; bacillithiol, a GSH-like α-anomeric glycoside of l-cysteinyl-*d*-glucosamine with l-malic acid[84] and pyrroloquinoline quinone[85,86] have been identified from *D. radiodurans* and their roles as an antioxidant were shown independently. All these components working together in support of higher oxidative stress tolerance could be important in radiation resistance of this bacterium (Figure 4).

Roles of small RNAs in cellular response to abiotic stresses including oxidative stress have gained significant importance in other systems. The effects of γ-radiation on the stability of RNA molecules and their roles in regulation of radiation-responsive genes, etc. have not been studied in detail in *D. radiodurans*. The *D. radiodurans* genome encodes Ro protein Rsr, an orthologue of an RNA-binding protein Ro60-kD autoantigen that is shown to regulate ribosomal RNA (rRNA) degradation during stationary phase. Its role in UV resistance by binding to several small RNAs, which are known to accumulate following UV irradiation, has been demonstrated. The interaction of Rsr with the 3′ to 5′ exoribonuclease like polynucleotide phosphorylase (PNP) and the additional nucleases, in the degradation of rRNA has been demonstrated. Rsr functions in conjunction with other exonucleases during stress conditions, suggesting the possibility of small RNAses regulating the extreme abiotic stress in this bacterium[87]. These findings together indicated a significant role of small metabolites, [Mn] complexes and RNA metabolism in stress tolerance of this bacterium.

Is there a paradigm shift?

The possibility of the potentially catastrophic deletions and genome rearrangements occurring at lower frequencies has not been completely ruled out in *D. radiodurans*[1]. Factors like complex genetic make-up and the absence of SOS response and error-prone DNA polymerases might allow us to conceive it conveniently. As the genome of *D. radiodurans* is enriched with insertion sequence (IS)-like transposons and small intergenic repeats[17], the absence of genomic rearrangement that has the functional transposition machineries is still being debated in *D. radiodurans*. It was demonstrated that *D. radiodurans* has the smallest known IS-type transposase of IS200/IS605 family and the transposition by one particular family member ISDra2, is dramatically stimulated upon massive γ-irradiation[88,89]. Hickman *et al.*[90] had monitored mutational profile in the

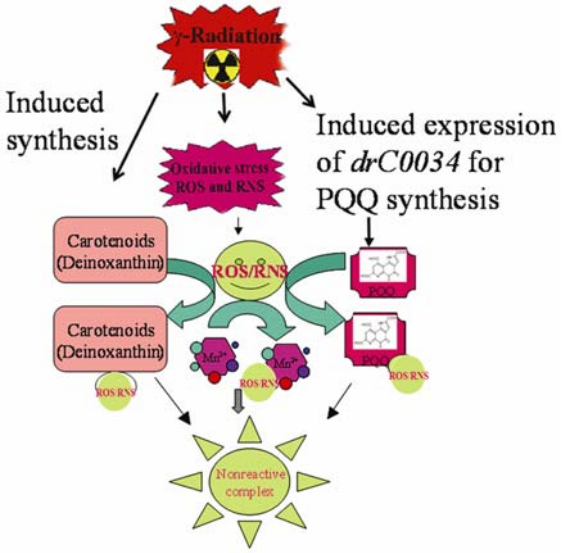

Figure 4. Known processes of oxidative stress management in *D. radiodurans*. Gamma radiation induces the expression of carotenoid biosynthesis, PQQ synthesis and metal ion, mainly Mn transporters. All three components like carotenoids, PQQ and Mn have been shown for neutralizing the reactive species and producing adducts that are non-reactive to biological molecules. Model is based on published findings[2,10,80,85,96].

thyA gene following irradiation. They report that the majority of *thyA* mutants resulted from transposition of one particular IS element, ISDra2 (ref. 90). *D. radiodurans* also has several other types of IS elements, which have not been characterized in details[91]. The roles of several enzymes in maintaining error-free DSB repair and control of genomic rearrangement have been demonstrated recently. *D. radiodurans* cells lacking *sbcC*/*sbcD* (ref. 83), *recA* (ref. 92) and a two-component system RadS–RadR (ref. 70) did not recover the typical wild-type *Not*I pattern of genome. These cells also showed reduced resistance to γ-radiation compared to wild type, although the molecular mechanism was not known. Recently, it has been shown that *D. ficus* which has high tolerance to DNA damage could accumulate UV-induced mutation and this function was implicated to an operon *lexA–imuB–dnaE2*, known to support translesion synthesis (TLS) in UV-induced mutagenesis[93]. These findings could indicate that there can be the possibility of genomic rearrangement, which apparently could not be detected because of ploidy and functional redundancy that would have compensated the phenotypic loss due to genomic rearrangement.

A relationship of proteins with radiation resistance and DSB repair has been repeatedly discussed. The proteins have been annotated based on a set rule of paradigms, where the translational initiation site (TIS) has been searched with respect to the vicinity of Shine–Delgarno sequences. Bioinformatics analysis suggested that the bacterial genome encodes three main categories of genes, namely SD-led, TA-led and atypical genes that do not follow any of the other two rules in expression. A large number of genes (nearly 20%) in the Deinococcus–Thermus phyla are leaderless, i.e. they have TA sequences around 10 bp upstream of TIS[94]. Bacteria belonging to the Deinococcaceae family contain a large number of leaderless genes belonging to important DNA metabolic functions (Figure 5). Correlation of these genes to radiation resistance and DSB repair might be a far-fetched assumption. However, the kind of protein diversity one would anticipate from such type of translation programming and its functional relevance would be an interesting area that would lead to a logical shift in paradigm and worth strengthening in future.

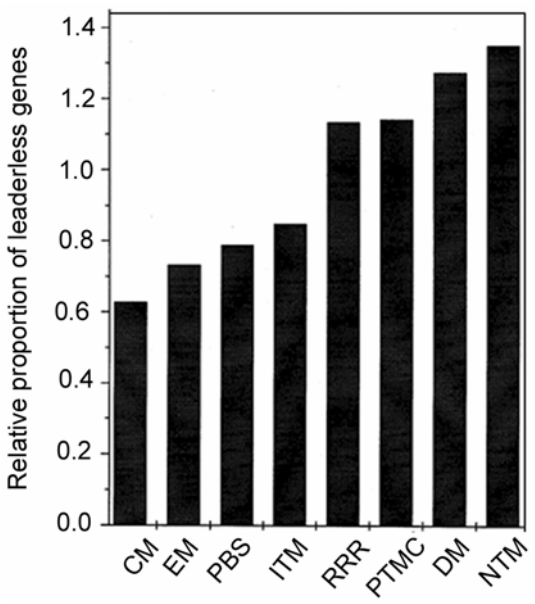

Figure 5. COG functional categories that are significantly enriched (FVOL) or lack (PJCN) leaderless genes in the *Deinococcus–Thermus* group. Leaderless genes encoding various functions like cell motility (CM), energy metabolism (EM), protein biosynthesis (PBS), ion-transport metabolism (ITM), replication, recombination and repair (RRR), post-translation modification and chaperone (PTMC), defence mechanisms (DM) and nucleotide transport and metabolism (NTM) were analysed. Relative proportions of leaderless genes were calculated by the ratio of the proportion of leaderless in certain category to the overall proportion of leaderless genes. $P < 0.05$ for all listed categories and $*P < 0.0001$ is for Fisher exact test. (Courtesy: Xiaobin Zheng and Huaiqiu Zhu, and published in Zheng *et al.*[94]).

Conclusion

Studies on molecular mechanisms underlying extreme radiation resistance in *D. radiodurans* have grown exponentially after its genome sequence was published. The main emphasis had been on the mechanisms contributing to efficient DSB repair and the ESDSA mechanism is an outcome of these studies. ESDSA could accommodate several DNA metabolic steps such as DNA ends processing, DNA synthesis, homology search and strand exchange reactions, ends joining and subsequently leading to maturation of individual genome by the slow crossover event in homologous recombination. Interestingly, all these DNA metabolic functions have also been known in many bacteria, which failed to withstand even moderate doses of DNA damage. Then the most obvious question of how these common DNA metabolic processes could favour ESDSA in *D. radiodurans* and not in other bacteria, if it is true, would be worth addressing. The involvement of small metabolites and molecular complexes in the protection of biomolecules from oxidative damage has been recently emphasized as other features that could contribute to extraordinary radioresistance of this bacterium. In this review, we have reported several facts to support that extreme phenotypes of this bacterium might not be possible by any one single process, but the coordinated role of many unique components present in this organism. Some of the notable ones are unique mechanism of action of deinococcal proteins, a possibility of protein diversity, regulation of protein functions by small molecules, absence of error-prone SOS repair, but Ser/Thr protein kinase-based DNA damage response mechanism and their involvement in radiation resistance and DSB repair. Further studies on these new aspects would strengthen the growth of interdisciplinary science in the area of molecular microbiology.

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