Gamma Radiation-induced Proteome of Deinococcus radiodurans Primarily Targets DNA Repair and Oxidative Stress Alleviation*

Bhakti Basu and Shree Kumar Apte‡

The extraordinary radioresistance of Deinococcus radiodurans primarily originates from its efficient DNA repair ability. The kinetics of proteomic changes induced by a 6-kGy dose of gamma irradiation was mapped during the post-irradiation growth arrest phase by two-dimensional protein electrophoresis coupled with mass spectrometry. The results revealed that at least 37 proteins displayed either enhanced or de novo expression in the first 1 h of post-irradiation recovery. All of the radiation-responsive proteins were identified, and they belonged to the major functional categories of DNA repair, oxidative stress alleviation, and protein translation/folding. The dynamics of radiation-responsive protein levels throughout the growth arrest phase demonstrated (i) sequential up-regulation and processing of DNA repair proteins such as single-stranded DNA-binding protein (Ssb), DNA damage response protein A (DdrA), DNA damage response protein B (DdrB), pleiotropic protein promoting DNA repair (PprA), and recombinase A (RecA) substantiating stepwise genome restitution by different DNA repair pathways and (ii) concurrent early up-regulation of proteins involved in both DNA repair and oxidative stress alleviation. Among DNA repair proteins, Ssb was found to be the first and most abundant radiation-induced protein only to be followed by alternate Ssb, DdrB, indicating aggressive protection of single strand DNA fragments as the first line of defense by D. radiodurans, thereby preserving genetic information following radiation stress. The implications of both qualitative or quantitative and sequential or co-induction of radiation-responsive proteins for envisaged DNA repair mechanism in D. radiodurans are discussed. Molecular & Cellular Proteomics 11: 10.1074/mcp.M111.011734, 1–15, 2012.

The classical proteomic approach, two-dimensional electrophoresis coupled with mass spectrometry, is a powerful tool to capture dynamics of global proteomic changes by simultaneous resolution of large number of cellular proteins. The methodology is particularly advantageous for identification of stress-induced proteins along with their post-translational modifications and to correlate altered protein abundance/modifications with physiological function(s) (1). Deinococcus radiodurans is an extremophile well known for its extraordinary DNA repair ability that renders the organism virtually resistant to DNA damage caused by variety of clastogenic agents (2). Endurance to acute exposure to ionizing radiations (10 kGy of gamma rays), UV (1 kJ/m²), or prolonged desiccation emanate from the microbe’s ability to reassemble its shattered genome with impeccable fidelity (3). The DNA repair mechanism of this organism has been envisaged to integrate prokaryotic as well as eukaryotic-like pathways of DNA repair such as homologous recombination, single strand annealing (SSA), † extended synthesis-dependent strand annealing (ESDSA), and nonhomologous end joining (4).

The genome of D. radiodurans harbors functional DNA repair pathways for RecFOR-mediated homologous recombination and nucleotide and base excision repair along with a few “hypothetical” ORFs homologous to eukaryotic DNA repair machinery but completely lacks important DNA repair pathways such as photoreactivation, SOS repair, as well as RecBCD mediated homologous recombination (5, 6). So far, the evidence for mechanistic details of DNA repair in D. radiodurans has come from transcriptome analyses, knockout mutagenesis, DNA repair kinetics of mutants, and in vitro demonstration of DNA repair-related activities of candidate proteins. Although the available information provides valuable insight into the different DNA repair pathways and important gene/protein players, the correspondence between transcript levels, protein levels, and DNA repair/radioresistance is not adequately investigated as yet.

For example, two independent transcriptome analyses carried out using D. radiodurans strains BAA-816 (7) and ATCC 13939 (8) have revealed agreement on up-regulation of 72 genes following gamma radiation stress. Of these, only a few: recA (DR2340), uvrA (DR1771), uvrB (DR2275), gyrA (DR1913), and gyrB (DR0906), are well annotated DNA repair genes/protein players, the correspondence between transcript levels, protein levels, and DNA repair/radioresistance is not adequately investigated as yet.

From the Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai 400085, India

Received June 8, 2011, and in revised form, October 4, 2011
Published, MCP Papers in Press, October 11, 2011, DOI 10.1074/ mcp.M111.011734

This is an open access article under the CC BY license.

Molecular & Cellular Proteomics 11.1 10.1074/mcp.M111.011734–1

Want to cite this article? Please look on the last page for the proper citation format.
Proteomics of Gamma Radiation Response in *D. radiodurans*

Among novel genes, *ddrA* (*DR0423*) shows homology to eukaryotic DNA repair protein Rad52 involved in SSA/ESDSA (9), and *pprA* (*DRA0346*) displays *in vitro* stimulation of ligation, an activity similar to eukaryotic Ku protein involved in nonhomologous end joining (10). Deletion of some strongly up-regulated genes such as *gyrA* (*DR1913*), *recA* (*DR2340*), *ddrA* (*DR0423*), or *ddrB* (*DR0070*) results in lethal to moderately radiosensitive phenotypes (7, 8, 11, 12). In contrast, single gene deletion mutations in *ddrP* (*DR0226*), *ddrP* (*DR0100*), or *uvrB* (*DR2275*), all identified as strongly up-regulated by transcriptomic analyses, do not affect radiation resistance (7, 8, 13). Although the deletion of *recF* (*DR1089*), *recO* (*DR0819*), *recR* (*DR0198*), *recJ* (*DR1126*), and *polA* (*DR1707*) result in severe radiation sensitivity, these genes are reportedly not induced upon irradiation (7, 8, 12, 14). Thus, radiation resistance in *D. radiodurans* involves genes that are induced upon irradiation, as well as genes that are constitutively expressed. In addition, *D. radiodurans* utilizes alternate pathways with divergent functions in the absence of a few key DNA repair proteins such as RecBC recombinases and SbcBC nucleases. For example, in the absence of RecBC, the homologous recombination in *D. radiodurans* is brought about by RecFOR pathway that utilizes helicase functions of UvrD and RecO (12).

Proteins, the final functional entities, influence cellular phenotype and stress response. In bacteria, the correlation between mRNA and protein abundance is often poor (15). Although the protein expression level at any given time is regulated through transcriptional induction, transcript stability, translational regulation, and biological half-life (16), the function or activity of the protein is often modulated by post-translational modifications, protein-protein interactions, and proteolytic processing (17). The transcriptomic abundance, knockout mutagenesis, or *in vitro* biochemistry neither reveals protein abundance nor their coordinated activities. Therefore, a systematic and comprehensive proteomic investigation is necessary to reveal the biological phenomena underlying extraordinary radioresistance of *D. radiodurans* in vivo.

Three detailed studies on radiation-responsive proteomes have been carried out in *D. radiodurans*. Single time point expression analyses carried out in *D. radiodurans* strains KD8301 (18) and ATCC13939 (19), in the pl range of 4–7, demonstrated induction of RecA, Ssb, and PprA at 1 h following a 1-kGy sublethal dose of gamma irradiation. However, these studies did not reveal the underlying dynamics of radiation-responsive protein expression or their relevance to cell physiology. A multiple time point study carried out in 6 kGy of irradiated *D. radiodurans* during 0–18 h of post-irradiation recovery using tube gel system did not report any radiation-inducible protein expressions (20). The radiation-responsive induction of DdrB has been immunodetected in one-dimensional SDS-PAGE (21). In *D. radiodurans* BAA-816, a strain whose complete genome has been sequenced (5) and wherein radiation-responsive transcriptome has been profiled (7), the radiation stress proteomics has remained unexplored.

The present study systematically investigated the kinetics of radiation-induced protein expression and processing during the entire growth arrest phase of 4 h following 6 Gk of gamma irradiation of *D. radiodurans* R1 strain BAA-816. The proteomic changes in response to irradiation were visualized by gel based two-dimensional resolution and identified by mass spectrometry. The observed profile of DNA repair proteins suggests early activation of ssDNA and ssDNA end protection mechanisms followed by induction of proteins possibly involved in SSA and ESDSA in the initial 1 h of post-irradiation recovery (PIR). Transient elevated expression of PprA was observed at 1 h along with moderate induction in RecA levels. Concurrent with DNA repair, induction of several key oxidative stress alleviating proteins, and proteins involved in translation and protein folding were also evident. This work integrates information on proteomic changes observed in the present study, revelations from previous transcriptome analyses (7, 8), and phenotypic outcome of several knockout mutagenesis studies (8, 11–14) to elucidate mechanism(s) underlying the radiation resistance of *D. radiodurans*.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strain, Growth, and Irradiation Conditions—** *D. radiodurans* strain R1 ATCC BAA-816 was grown aerobically in TGY medium (1% bacterotryptone, 0.1% glucose, and 0.5% yeast extract) at 32 °C and 150 rpm. Radiation stress was produced by measuring turbidity *A* 600 or by determining colony-forming units on TGY agar plates (1.5% bactoagar), after 48 h of incubation at 32 °C. The deino-coccal cells were grown to an *A* 600 of 2.5 ± 0.2, resuspended in fresh TGY broth (*A* 600 adjusted to 3.0), and exposed (on ice) to 6 kGy of 60Co γ-rays, at a dose rate of 5 Gy/min. Another aliquot, kept outside the radiation source, served as the sham control. Subsequently, the irradiated and control cell suspensions were centrifuged (5000 rpm, 5 min, 4 °C), transferred to fresh TGY broth at a starting cell density of 600 adjusted to 3.0, and incubated under normal growth conditions to allow recovery. The experiment was repeated three times, and the observed variation in viability was less than 10%.

**Preparation of Protein Samples for Two-Dimensional Electrophoresis—** At appropriate times during PIR, the cells were harvested by centrifugation (5000 rpm, 10 min, 4 °C), washed twice with sterilized and chilled distilled water, and resuspended in lysis buffer (1 mM Tris-HCl, pH 8.0, containing 1 mM PMSF). All further steps were carried out at 4 °C. The cells were lysed by sonication (Branson Digital Sonifier, model 250), and the cell free extracts were obtained by centrifugation at 25,000 × g for 30 min at 4 °C. The protein content was measured by a Lowry protein estimation kit (Sigma). The extracts were treated with DNase I and RNase I (10 μg/ml each) for 1 h on ice. Protein extracts containing the required amount of protein were concentrated under vacuum to about 3 μl and then solubilized in rehydration buffer (8 M urea, 1% thiourea, 2% CHAPS, 15 mM DTT, 2% IPG buffer, traces of bromphenol blue) for 1 h at room temperature.

**Two-Dimensional Electrophoresis—** Protein samples were applied to immobilized pH gradient strips, either nonlinear pH 3–10, 17 cm or pH 4–7, 11 cm (Bio-Rad) for 1 h, and the strips were covered with mineral oil for 15 h at room temperature. First dimensional protein separation was performed with the protein IEF cell (Bio-Rad) at 20 °C as per the manufacturer’s protocol. The 11- and 17-cm IPG strips were focused till 30,000 and 50,000 volt hours were achieved, re-
spectively. The IPG strips were stored at −70 °C until used for second dimensional separation. For the second dimensional separation, IPG strips were equilibrated initially in solution I (6 u urea, 50 mM Tris, pH 6.8, 30% glycerol, 4% SDS, 20 mM DTT) for 15 min and then in solution II (6 u urea, 50 mM Tris, pH 6.8, 30% glycerol, 4% SDS, 4.5% iodoacetamide) for another 15 min. The strips were embedded in 0.5% agarose on top of 12% or 14% polyacrylamide slab gels for the second dimensional separation by SDS-PAGE. After electrophoresis, the gels were removed from the cast, briefly rinsed in deionized water, and stained with Coomassie Brilliant Blue G250 stain for 30 min followed by destaining overnight. Typically, 300 and 500 μg of proteins were applied to pH 4–7, 11-cm and pH 3–10NL, 17-cm IPG strips, respectively. Each experiment was repeated at least three times, and at least one two-dimensional gel for each time point was analyzed in each experiment.

**Gel Imaging and Spot Analysis**—Images of Coomassie Brilliant Blue-stained gels were acquired by Dyeversion-6 gel imager (Syngene, UK) using GeneSnap software (Syngene, UK). The gel images were cropped to remove dye front, molecular weight markers or any other artificially stained area before subjecting the gels to comparative analysis. Dymension-3 two-dimensional image analysis software (Syngene, UK) was used to compare two-dimensional gel sets with respect to up-regulated and down-regulated proteins. Densitometry was used to quantify relative expressions levels of proteins using GelQuant software (Biosystematica). The protein fold induction was expressed as the ratio of densimetric values obtained for protein expression in irradiated/control cells. The protein spots of interest were manually excised from the gel and processed for identification.

**Sample Preparation for Mass Spectrometry Analysis**—Gel plugs chosen for mass spectrometric analysis were processed as described earlier (18) with a few modifications. In brief, protein gel plugs were washed and destained with 50% acetonitrile (ACN) in 100 mM ammonium bicarbonate. The concentration of ammonium bicarbonate was gradually reduced to 10 mM and then the gel plugs became clear of Coomassie Brilliant Blue stain, 100% ACN was used to dehydrate the gel plugs. The ACN was evaporated from destained gel plugs by vacuum concentration. The gel plugs were then reduced in the presence of 25 mM DTT (Sigma) and alkylated in presence of 50 mM iodoacetamide (Sigma) followed by a cycle of dehydration using ACN. In-gel proteolytic digestion was carried out using either 20 ng of trypsin (Trypsin Gold in ProteaseMax buffer, 20 ng/μl, Promega, UK) at 37 °C for 3 h or 20 ng of trypsin (Promega, UK) at 37 °C for 16 h. The polypeptides were eluted from the gel piece in 2.5% trifluoroacetic acid in 50% ACN by vortexing for 15 min. Extracted peptides were vacuum-concentrated, if necessary, and stored at −20 °C until use.

**Mass Spectrometry and Protein Identification**—Most of the protein samples were analyzed by mass spectrometry at a commercial facility of The Centre for Genomic Applications (New Delhi). The eluted polypeptides were co-crystallized with α-cyano-4-hydroxycinnamic acid (2.5 mg/ml in 50% ACN) matrix on the target plate (384-well ground steel plate; Bruker Daltonics, Bremen, Germany). External peptide mass calibration was applied (Peptide calibration mix I; Bruker Daltonics) as per the manufacturer’s instructions. MALDI-TOF/TOF-MS analysis was performed using (UltraFlex II MALDI-TOF/TOF mass spectrometer; Bruker Daltonics) in positive ion reflection mode. The samples were analyzed with standard TOF-MS protocol, and the laser was set to fire 250 times/spot. The mass spectra were acquired in a mass range of 800–4500 Da. Peak list was generated using FlexAnalysis software 2.2 (Bruker Daltonics). The human keratin and trypsin autodigest peptide ions were excluded. The mass spectra were imported into the database search engine (BioloTools v2.2 connected to Mascot, version 2.2.04; Matrix Science). Mascot searches were conducted using the NCBI nonredundant database (released May 29, 2010; 11,111,565 entries actually searched) with the following settings: number of miss cleavages permitted was 1; fixed modifications such as carbamidomethyl on cysteine, variable modification of oxidation on methionine residue (or acetyl on lysine for DR1473); peptide tolerance of 100 ppm, MS/MS tolerance of 0.5 Da; enzyme used as trypsin; a peptide charge setting as +1; and the known contaminating peaks excluded in the MS mode were those of matrix, keratin, trypsin, cytokeranine, Coomassie, and zinc finger. For two proteins (DdrB and DdrB1, DR0070), Mascot searches were conducted using the NCBI nonredundant database (release September 30, 2011; 15,377,270 sequences actually searched) with the aforesaid settings. A match with D. radiodurans protein with the best score in each Mascot search was accepted as successful identification. A Mascot score of >65 with a minimum of 10 peptide matches was considered to be a significant identification (p < 0.05). For a few low molecular mass proteins (~<23 kDa), a Mascot score in the range of 35–65 was accepted as a positive identification with minimum of five peptide matches and ≥39% sequence coverage.

Four protein samples were also analyzed by mass spectrometry at a commercial facility of The Centre for Genomic Applications (New Delhi). The eluted polypeptides were co-crystallized with α-cyano-4-hydroxycinnamic acid (2.5 mg/ml in 50% ACN) matrix on the target plate (384-well ground steel plate; Bruker Daltonics, Bremen, Germany). External peptide mass calibration was applied (Peptide calibration mix I; Bruker Daltonics) as per the manufacturer’s instructions. MALDI-TOF/TOF-MS analysis was performed using (UltraFlex II MALDI-TOF/TOF mass spectrometer; Bruker Daltonics) in positive ion reflection mode. The samples were analyzed with standard TOF-MS protocol, and the laser was set to fire 250 times/spot. The mass spectra were acquired in a mass range of 800–4500 Da. Peak list was generated using FlexAnalysis software 2.2 (Bruker Daltonics). The human keratin and trypsin autodigest peptide ions were excluded. The mass spectra were imported into the database search engine (BioTools v2.2 connected to Mascot, version 2.2.04; Matrix Science). Mascot searches were conducted using the NCBI nonredundant database (released May 29, 2010; 11,111,565 entries actually searched) with the following settings: number of miss cleavages permitted was 1; fixed modifications such as carbamidomethyl on cysteine, variable modification of oxidation on methionine residue; peptide tolerance of 100 ppm, enzyme used as trypsin and a peptide charge setting as +1. A match with D. radiodurans protein with the best score in each Mascot search was accepted as successful identification. A Mascot score of >90 with a minimum of 13 peptide matches was considered to be a significant identification (p < 0.05).

**RESULTS AND DISCUSSION**

Following 6 kGy of gamma irradiation on ice (for 20 h), D. radiodurans cells entered immediate growth arrest phase (~4 h) when compared with unirradiated control cells, consistent with the data reported previously (20). Assuming that the growth would resume only after the repair of DNA and other essential biomolecules, the growth arrest (lag) phase was considered appropriate for proteomic investigations. The expression dynamics of radiation-responsive proteins were established by comparing two-dimensional protein profiles of control and irradiated cells at different stages of PIR. Representative examples of two-dimensional resolution in pl ranges of 3–10 and 4–7 are shown in

**Molecular & Cellular Proteomics 11.1**

10.1074/mcp.M111.011734-3
supplemental Figs. S1 and Fig. S2, respectively. A total of 70 proteins were analyzed by mass spectrometry in either MS or MS-MS mode (supplemental mass spectrometry data), and their identities are listed in supplemental Table 1. Among the identified proteins, 28 prominent radiation-inducible proteins belonged to major functional categories of DNA repair, oxidative stress response, protein translation/folding, and general housekeeping.

Superinduction of Ssb in Response to Gamma Radiation Stress—Single-stranded DNA-binding protein is vital for cell survival because it is involved in DNA replication, recombination, and repair (22). Moderate induction of deinococcal Ssb in response to gamma radiation has been reported earlier at transcript as well as protein levels (7, 18, 19, 23). We identified 10 protein spots, in the molecular mass range of 27–32 kDa and pl range of 5.4–8.8, in irradiated samples to correspond to Ssb protein of D. radiodurans (Fig. 1, A and B, supplemental Table 1). The spots followed a dynamic pattern of appearance indicative of progressive processing of its C-terminal acidic tail. The protein spot marked as Ssb (molecular mass of 32.5 and pl of 5.4), an expected primary product of the reannotated ssb ORF (GenBank™ accession number JN571419), showed only marginal enhancement in its level, but the PIR was accompanied by sequential appearance of spots Ssb1 to Ssb9. The spot Ssb1 corresponds to the radiation-induced Ssb spot identified by Lu et al. (19) earlier.

Fig. 1. Kinetics of post-irradiation cellular levels and processing of Ssb protein in D. radiodurans. A and B, isoelectric focusing of soluble cellular proteins was performed on pH 4–7, 11-cm (A) or pH 3–10NL, 17-cm IPG strips (B), followed by second dimensional electrophoretic resolution by 14% SDS-PAGE. The appearance of several processed forms of Ssb protein, indicated by arrows and names, is shown immediately after irradiation (0 h) and at specified time points (h) of PIR in 6 kGy-irradiated D. radiodurans cells when compared with unirradiated (control) cells. The acidic (+) and basic (−) sides of the electrophoretogram are marked. C, C-terminal processing of Ssb protein in D. radiodurans following gamma irradiation. The Ssb protein is represented by a black line marked with the number of amino acid residues involved, on a scale. The N and C termini of the Ssb protein are indicated. The highlighted chain of amino acids at the C terminus represents the acidic tail of Ssb protein. The dotted arrows above the Ssb protein indicate the C terminus of in silico processed Ssb forms with their molecular mass and pl calculated using Compute pl/MW software. The arrows below the Ssb protein indicate observed and identified Ssb proteins shown in A and B. The underlined Ssb proteins exhibiting acidic shift in their pl were predicted to arise from one (1P) or two (2P) phosphorylation events in their precursor proteins Ssb2 and Ssb3. All other identified Ssb forms correspond to estimated pl and molecular mass.
Although the spots Ssb1, Ssb2, and Ssb3 were observed immediately after irradiation (0 h); the spots Ssb4, Ssb5, Ssb6, and Ssb8 were first seen at 0.5 h of PIR. The spots Ssb7 and Ssb9 appeared only at 1 h of PIR. The levels of all of the Ssb spots reached a maximum at 2 h.

The C-terminal acidic tail of Ssb protein is structurally dynamic, competes with ssDNA for binding to oligonucleotide/oligosaccharide-binding fold in vitro and is implicated in recruitment of several important proteins involved in DNA replication, recombination, and repair (22, 24). In vitro, the acidic tail of Ssb is prone to proteolytic processing; more so when Ssb binds to ssDNA, whereas the oligonucleotide/oligosaccharide-binding fold core domain is resistant to degradation (22, 24). Although processing of Ssb protein in vivo has not been reported, enhanced helix destabilizing activity and higher affinity for ssRNA have been reported in vitro for Escherichia coli Ssb protein lacking the C-terminal 40 and 25–60 amino acid residues, respectively (25, 26). Our observation of several processed forms of deinococcal Ssb gives the first indication of in vivo processing of C-terminal acidic tail of Ssb protein, perhaps upon its interaction with ssDNA.

The observed pI and molecular mass of deinococcal Ssb forms (Fig. 1, A and B) agreed well with the in silico predicted pI and molecular mass of the Ssb proteins shortened from the C-terminal end (Fig. 1C) using Compute pI/MW software (27). When the Ssb protein was shortened in silico from the N terminus, the same software instead predicted acidic shift in the resultant Ssb forms (data not shown). A further acidic shift in Ssb2 and Ssb3 was also observed (Fig. 1C) and has been ascribed to a single or double phosphorylation event of these proteins, as predicted by Scansite 2.0 software (28). Recently, in vivo phosphorylation of bacterial Ssb and increased affinity of phosphorylated Ssb toward ssDNA has been demonstrated (29). Whether the processed and/or phosphorylated forms of deinococcal Ssb possess altered ssDNA binding efficiencies that cooperate with various DNA repair pathways or they represent routine turnover of induced Ssb, possibly displaced from ssDNA as DNA repair progresses, is worth exploring. Nevertheless, the data presented in Fig. 1 clearly established enhanced expression and rapid processing of Ssb protein during PIR of D. radiodurans.

De Novo Induction of Proteins Implicated in Strand Annealing in the Early Phase of DNA Repair—In D. radiodurans, the five maximally radiation-induced transcripts include those of novel genes ddrA (DR0423) and ddbB (DR0070) (7, 8). We observed de novo induction of both DdrA and DdrB proteins in the radiation-stressed cells of D. radiodurans (Fig. 2, A and B, and supplemental Table 1) within 30 min of PIR. The induction of GyrA and GyrB was also seen in the radiation-stressed cells of D. radiodurans at the same time point (Fig. 2, C and D, and supplemental Table 1). The levels of DdrA and GyrA proteins reached a peak at 1 h, decreased by 2 h and completely disappeared by 4 h. The levels of DdrB and GyrB proteins peaked at 2 h. An in vivo processing of DdrB, perhaps of its unstructured C-terminal acidic tail, was also observed similar to that of Ssb protein. The proteolytic processing of Ssb as well as DdrB was concomitant with their induction. Based on DdrB expression analysis and comparisons among three deinococcal species, Norais et al. (21) reannotated the start of D. radiodurans DdrB protein from the second methionine, thereby excluding the initial 11 amino acids from the protein. However, both the N-terminal pep...
tides, one (MCYVIYVRR) as annotated by White et al. (5) and second (MLQIEFITDLGAR) as reannotated by Norais et al. (21), have been identified for the DdrB protein. The observation therefore suggests a possible utilization of both the initiation codons for post-irradiation expression of DdrB. Functional significance of DdrB proteins differing in the initial 11 amino acids remains unknown.

DdrA, a distant homologue of eukaryotic Rad52 protein, protects 3′ ssDNA ends from nuclease degradation in vitro (9). DdrB binds to ssDNA on its surface (30) and promotes SSA reaction (31) but fails to displace Ssb from ssDNA (31) and to load RecA on ssDNA (21) in vitro. These activities of DdrB are apparently similar to eukaryotic Rad59 protein (32). In eukaryotes, the Rad52 and Rad59 cooperatively promote single strand annealing (SSA) reaction between two Ssb-coated homologous ssDNAs in vivo (32). It would be appropriate to investigate the cooperative activities of DdrA and DdrB in strand annealing reactions in D. radiodurans. The presence of gyrase proteins may facilitate SSA and ESDSA as well as RR. Our findings substantiate the transcriptome-based speculation that ESDSA and SSA repair mechanisms precede the RecA-mediated homologous recombination in D. radiodurans.

Co-induction of PprA and RecA at 1 h of PIR—Following induction of DdrA and DdrB at 30 min of PIR, up-regulation of PprA (DRA0346, pleiotropic protein promoting DNA repair) and RecA (DR2340, recombinase A) levels was observed at 1 h of PIR in D. radiodurans cells (Fig. 3, A and B, supplemental Table 1). Although induction of PprA was transiently observed at 1 h, the level of RecA protein remained high up to 2 h of PIR, indicating that the activity of PprA may complete before that of RecA.

Induction of RecA following radiation stress has been shown at both transcriptional and translational levels, irrespective of differences in strain, irradiation, and culture conditions (7, 8, 18, 19, 33) and is vital for DNA repair in D. radiodurans (34). The induction of RecA hallmarks the phase of homology-driven DNA repair, a slow process that ensures high fidelity. We report substantial up-regulation of RecA protein levels at 1 and 2 h of PIR followed by their return to basal level by 4 h of PIR, the time by which the cells resume active growth. RecA promotes homologous recombination, resulting in accurate assembly of chromosome structure. But in the absence of RecBC proteins, the RecFOR pathway has been shown to be vital for RecA-mediated DNA repair by homologous recombination in D. radiodurans (12). The RecFOR proteins were not among the radiation-induced proteins detected (18, 19), nor do the corresponding genes show up-regulation at transcriptome level (7, 8). The basal levels of RecFOR along with elevated RecA levels might be sufficient to carry out homology-based recombination necessary for final assembly of intact chromosomes. It would be interesting to investigate whether DdrA possesses RecA loading activity, as was found in case of its homologue Rad52 (35).

In vitro, PprA has been shown to interact with open circular and linear dsDNA, protect linear DNA from exonucleolytic digestion, and stimulate DNA end joining by ATP-dependent ligase (10). These activities are similar to that of eukaryotic Ku protein that promotes error prone nonhomologous end joining in the absence of homologous sequences in a “fight or flight” situation (10, 36). In vivo, whether PprA actually carries out nonhomologous end joining or its role is limited to protecting DNA nicks and ends remains to be ascertained. The in vivo
role of PprA in error-free DNA repair in \textit{D. radiodurans} is still hypothetical, although induction of PprA has been consistently reported earlier \cite{7, 8, 18, 19}.

\textit{Up-regulation of Nucleoside Diphosphate Kinase and Uridylate Kinase during PIR—}Extensive \textit{de novo} synthesis of DNA during ESDSA mode of DNA repair would create a huge demand for nucleotide triphosphates. Indeed the levels of nucleoside diphosphate kinase (Ndk, DR2499) and uridylate kinase (PyrH, DR1511) were found to be up-regulated during PIR (Fig. 3, \textit{C} and \textit{D}, supplemental Table 1). Ndk mediates synthesis of nucleoside triphosphates from nucleoside diphosphates by using ATP as a source of phosphate, whereas uridylate kinase mediates reversible phosphorylation of UMP to UDP, also utilizing ATP as a source of phosphate. Up-regulation of these proteins probably ensures adequate availability of nucleotide triphosphates for DNA replication, recombination, and repair.

\textit{Oxidative Stress Proteins during PIR—}Oxidative stress is an integral component of ionizing radiation stress. An efficient oxidative stress-alleviating system consisting of enzymatic and nonenzymatic components has been implicated in protecting deinococcal proteome from oxidative damage \cite{37–39}.

The present study revealed up-regulation of several enzymatic components of oxidative defense system, indicating their importance in radiation resistance of \textit{D. radiodurans}. The catalase (KatA, DR1998) and superoxide dismutase (SodA, DR1279) were present constitutively at high levels but showed further up-regulation following radiation stress (Fig. 4, \textit{A} and \textit{B}, and supplemental Table 1). Oxidative stress-responsive proteins such as TerB (DR2220), TerD (DR2221), and protein showing homology to thioredoxin reductase (TrxR, DR2623) were all found to be up-regulated immediately after irradiation (Fig. 4, \textit{C}–\textit{E}, and supplemental Table 1). The levels of thiosulfate sulfur transferase (CysA-1, DR0217), peroxidase associated protein (DRA0144), organic hydroperoxide resistance protein (DR1857), and cysteine desulfurase activator ATPase (SufC, DR2107) significantly increased at 1 h of PIR (Fig. 4, \textit{F}–\textit{H}, and supplemental Table 1). With the exception of tellurium resistance proteins (TerB and TerD) and SufC, the elevated levels of all oxidative stress proteins returned to normal by 2 h of PIR, indicating possible alleviation of oxidative stress in \textit{<}2 h. Our findings confirm that in response to radiation stress, \textit{D. radiodurans} further up-regulates its enzymatic de-
fense system in addition to its novel nonenzymatic oxidative stress-alleviating cellular components, thereby protecting its proteome from oxidative inactivation (37–39).

Proteins Involved in Protein Translation and Folding during PIR—Timely protein induction response of *D. radiodurans* to radiation stress indicates intact and functional translation machinery. Several components of translation process were found induced throughout the growth arrest phase following irradiation. Although the levels of DnaK, GroEL, EF-Tu, EF-G, SerS, and a few ribosomal proteins (such as L1, L9, L10, L7/12, L15, etc.) did not change significantly (supplemental Fig. S2 and supplemental Table 1), the levels of BipA (DR1198), HflX (DR0139), peptidyl-prolyl *cis-trans*-isomerase (PPlase, DR0237), GlyRS (DR2059), and EF-Ts (DR1512) were found to be elevated at 1–2 h of PIR (Fig. 5 and supplemental Table 1). BipA is an alternate elongation factor that has been implicated in the regulation of different pathways by translation efficiency of DNA repair proteins further. The HflX protein is a GTPase that binds ribosomal RNA and is implicated in ribosome biogenesis under stress conditions (41–42). Whereas glycyl-tRNA synthetase could aid in incorporation of glycine in the freshly synthesized proteins, the PPlase might help in protein folding.

**Efficient Turnover of Radiation-induced Proteome**—Proteome modifications during PIR were found to be under tight proteolytic control in *D. radiodurans*. The up-regulated levels of DdrA, PprA, RecA, GyrA, etc., returned to normal before the organism resumed active growth following PIR. The Ssb and DdrB proteins were also found to be quickly processed following their up-regulation in response to radiation stress. The Ssb protein was processed so quickly that the native Ssb spot did not *per se* appear to be induced at all during PIR (Fig. 1). Similarly, elevated levels of several proteins involved in oxidative stress alleviation and protein translation/folding returned to basal levels before resumption of active growth. Efficient turnover of stress-induced proteome is essential to degrade proteins that are no longer required once the stress.
ATP synthesis was found to be elevated from 1–2 h of PIR level of V-type ATP synthase, A subunit (DR0700), involved in mental Table 1), consistent with the previous finding (19). The described in the legend to Fig. 1.

Figure 6: Kinetics of post-irradiation cellular levels of proteins involved in general housekeeping functions in D. radiodurans. Isoelectric focusing of proteins was performed using pH 4–7, 11-cm IPG strips, followed by second dimensional resolution by 14% SDS-PAGE. The acyl-CoA dehydrogenase (DR2361) (A), cell division GTPase FtsZ (B), V-type ATP synthase subunit A (DR0700) (C), and electron transfer flavoproteins (EtfA and EtfB: arrows with straight and dotted line, respectively) (D) proteins are indicated by arrows. Other details are as described in the legend to Fig. 1.

is alleviated and homeostasis is achieved (43). It may also help maintain the intracellular pool of peptide-derived amino acids (38). Indeed, efficient recycling of several proteins has been demonstrated in the lag phase of PIR earlier (20) and may involve pre-existing or de novo induced proteases. The present study identified a few constitutive proteases such as serine protease (DRA0283), oligopeptidase A (DR1659), and aminopeptidase (DR2188) (supplemental Fig. S2 and supplemental Table 1), which could be involved in proteolytic processing. Also, several proteases such as Lon2 (DR0349, DR1656, DR2189), FtsH (DRA0290), serine proteases (DR0812, DR1459, DR1491, DR2322, DR2325, DRA0341, DRB0069), and metalloproteases (DR0982, DR1659, DR2055, DR2516, DRA0249) have been reported earlier as induced at transcript levels, in response to radiation stress (7, 8). In view of turnover of radiation-induced proteome and recycling of radiation exposed proteins, the proteolytic activities of these radiation-inducible/activated proteases deserves further exploration.

**Proteins Involved in Housekeeping Functions**—The proteins involved in carbohydrate metabolism such as, fructose bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, enolase, pyruvate kinase, and endoglucanase (supplemental Fig. S2 and supplemental Table 1) did not change significantly in their levels during PIR. The tricarboxylic acid cycle enzymes aconitate hydratase and isocitrate dehydrogenase were identified (supplemental Fig. S2 and supplemental Table 1), of which the latter displayed marginal elevated levels during PIR. Acyl-CoA dehydrogenase (DR2361) was found to be at higher level in irradiated cells and supple-

Despite differences in the D. radiodurans strains used, irradiation conditions, radiation doses and dose rates applied, and different protein resolution and staining methods used, gamma radiation-induced expression of several genes/proteins have been reported in all D. radiodurans strains (7, 8, 18, 19, 21, 33). Among the 28 gamma radiation-induced proteins reported in the present study, transcript level induction of 16 and 10 genes was reported in D. radiodurans strains BAA-816.
Proteomics of Gamma Radiation Response in *D. radiodurans*

![Dynamic graphs showing post-irradiation cellular levels of proteins related to DNA repair](image)

**Fig. 7.** Dynamics of post-irradiation cellular levels of proteins related to DNA repair as compared with corresponding proteomic/transcriptomic fold induction. A, Ssb, DdrB, and DdrA. B, GyrA, GyrB, RecA, PprA, Ndk, and PyrH. The intensities of all of the forms of Ssb or DdrB and other protein spots were measured from a single gel of control and irradiated samples, at the indicated time of PIR, using GelQuant software. The radiation-induced protein level was calculated by subtracting the spot intensities of control sample from corresponding spot intensities of irradiated sample. C, comparison of relative fold induction of DNA repair proteins (this study, black bars) with fold induction of corresponding genes at transcript level reported by Liu et al. (7) (gray bars) and Tanaka et al. (8) (white bars) at the indicated time points of PIR. The actual fold induction values are provided in supplemental Figs. S3–S5.

(7) and 13939 (8), respectively, whereas protein level induction of six and seven genes was reported in *D. radiodurans* strains KD8301 (18) and 13939 (19), respectively (supplementary Table 2). The qualitatively best agreement was found for transcript and protein level expression of DNA repair genes/proteins across the various strains of *D. radiodurans*.

The transcriptome/proteome changes are often reported as fold induction, which do not reveal (i) the relative abundance of various cellular proteins and (ii) the correspondence of fold induction with maximum abundance of a protein at a given time point. For example, the cellular levels of Ssb and DdrB, including their truncated forms, were found to be at least 10- and 4.5-fold higher, respectively, than those of DdrA, GyrA, GyrB, PprA, and RecA in irradiated *D. radiodurans* cells during PIR (Fig. 7, A and B). However, when the fold induction values were compared, *de novo* induced DdrA and DdrB proteins showed 47.8- and 128.7-fold induction, respectively, although the cellular levels of these proteins were 13.6- and 3.08-fold lower than that of Ssb protein, respectively (Fig. 7, A and C). Ssb expression was maximally induced (8.93-fold) at 0.5 h, although its maximum abundance was observed at 2 h. Qualitatively, the few best transcriptionally induced genes such as *ssb*, *ddrA*, *ddrB*, *gyrA*, *gyrB*, and *pprA* (Fig. 7C) were also found to be significantly elevated at proteomic level (Fig. 7). An exception was observed for the *recA* gene, which has been reported to be highly induced at transcript levels (7, 8) but was only moderately enhanced at proteomic levels (Fig. 7, B and C). Lu et al. (19) also reported a moderate 4-fold induction of RecA in *D. radiodurans* ATCC 13939 stain recovering from gamma radiation stress. All of the induced DNA repair proteins detected in this study have homologues in two other radioresistant deinococcal species, *Deinococcus geothermalis* (44) and *Deinococcus deserti* (45), indicating their importance for radioresistance. Their corresponding genes harbor radiation and desiccation response motifs in their promoter sequences (44, 45), suggesting their possible regulation by a common regulator. Indeed, the induction of Ssb, RecA, and PprA proteins are known to be affected in a *pprI* knockout mutant of *D. radiodurans* (19).
Nine important oxidative stress proteins, five proteins involved in protein translation and folding and at least five proteins with general housekeeping functions were found at elevated levels (Fig. 8, A–D). When the fold induction levels were compared between irradiated and control cells, the proteomic level induction (Fig. 8, E and F) was found to be consistent with the transcriptomic level induction (7, 8). However, proteomic level induction of DR1857, DRA0144, BipA, HflX, FtsZ, EtfA, and EtfB was found to be considerably higher (Fig. 8, E and F) than what was revealed by transcriptomic studies (7, 8). Our proteomic data therefore suggest that the radiation-responsive gene expression in *D. radiodurans* is perhaps significantly regulated at translational level, and the radiation-responsive proteome is rather focused on DNA damage repair and oxidative stress alleviation.

Based on the observed kinetics of radiation-induced proteins and previously published data of *D. radiodurans* DNA repair proteins (18, 19), we propose a model (Fig. 9) of repair of radiation fragmented DNA (I) comprising the following steps: (i) An early phase of ssDNA and ssDNA end protection by Ssb and DdrA (II) that may act as the first line of defense by protecting all ssDNA and ssDNA ends that are vulnerable to exonucleolytic/endonucleolytic attack and by removing secondary structures. (ii) Preparation of DNA for strand annealing by end resection possibly by RecJ (0–30 min) (III). In *E. coli*, Ssb has been shown to promote RecJ-mediated 5′-3′ end

**Fig. 8.** Dynamics of post-irradiation cellular levels of oxidative stress alleviating proteins, proteins involved in protein translation/folding and general housekeeping functions as compared with corresponding proteomic/transcriptomic fold induction. A, KatA, SodA, DR1857 and TrxR. B, TerB, TerD, DRA0144, SulC and CysA-1. C, EF-Ts, GlyRS, PPlase, BipA, and HflX. D, DR2361, FtsZ, DR0700, EtfA, and EtfB. E and F, comparison of relative fold induction of proteins involved in oxidative stress, protein translation/folding, and general housekeeping genes (this study, black bars) with fold induction of corresponding genes at transcript level reported by Liu et al. (7) (gray bars) and Tanaka et al. (8) (white bars), at the indicated time points of PIR. Actual fold induction values are provided in supplemental Figs. S6–S9.
resection (48). End resection generates 3′ ssDNA ends necessary for subsequent strand annealing by SSA and ESDSA pathways. (iii), strand annealing (SSA and ESDSA) with the help of Ssb, DdrB, GyrA, GyrB, RecA, and possibly by DdrA proteins (30 min to 1 h) (IVa and IVb). A functional cooperation between co-induced DdrA and DdrB is speculated to ensure strand annealing between homologous DNA sequences by SSA and ESDSA to generate exhaustive scaffold of DNA templates, which might be ligated upon stimulation by PprA in an end joining reaction (V). (iv) A final phase of DNA repair by RR with induced levels of RecA and gyrase (VI). The induction of RecA hallmarks the phase of recombination repair to ensure high fidelity for final assembly of intact chromosomes.

The model involves a few proteins that were not detected as induced in response to radiation in this study (e.g., RecJ, RecFOR, Pol, Lig, etc.) but are considered essential for DNA repair. It must also be mentioned that for DdrA, a distant homologue of eukaryotic Rad52 protein, a strand annealing activity is yet to be demonstrated (9). Similarly, PprA is known to stimulate ligase activity in vitro (10), but occurrence of nonhomologous end joining in D. radiodurans in vivo and role of PprA therein remains to be proven. The involvement of DdrA and PprA in SSA and end joining, respectively, is therefore speculated and indicated by a question mark in Fig. 9.

The integration of our proteomic data with the transcriptomic data (7, 8) and data on radioresistance of various knockout mutants of D. radiodurans (Table I) reveal that (i) some of the proteins involved are essential for DNA repair, (ii) the model is consistent with the observed gene expression patterns, and (iii) the participation of DdrA and PprA in SSA and end joining, respectively, is supported by our proteomic data.

**Fig. 9. A proteome-based model for genome restitution during PIR in D. radiodurans.** The fragmented genome following irradiation (I) is shown in black. The de novo DNA synthesized during SSA, ESDSA, or RR are shown in gray highlights. The dotted lines indicate fresh DNA synthesis, whereas the arrowhead indicates 3′ end of DNA. The various steps of DNA repair are underlined, and the proteins involved are indicated. The proteins marked with an asterisk were identified as induced in our data set. The proteins involved are represented by symbols, indicated in parentheses. The model proposes an initial phase of ssDNA protection by Ssb (II) and end resection by RecJ (0–0.5h) (III), the next phase of strand annealing by SSA (IVa) and ESDSA (IVb) mechanisms to generate exhaustive overlapping templates to be further acted upon by PprA-mediated end joining (1 h) (V) and the final phase of RecA-mediated RecFOR pathway of recombination repair (1–3 h) (VI) to reconstitute complete chromosomes (VII).
Comparison of post-irradiation expression of DNA repair genes/proteins at transcriptomic and proteomic levels and the phenotype of D. radiodurans strains mutated in the corresponding genes

NHEJ, Non-homologous end joining; NER, Nucleotide excision repair; NA, data not available; NI, not induced during the post-irradiation lag phase; ND, not found among the detectable radiation induced proteins in the post-irradiation lag phase.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Function/pathway</th>
<th>Transcriptomic fold induction (reference)</th>
<th>Proteomic fold induction (reference)</th>
<th>Phenotype of knockout mutant (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transcriptionally induced genes indispensable for radioresistance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gyrA</td>
<td>DNA unwinding</td>
<td>3.3-fold/0.5 h (7), 13-fold/0 h (8)</td>
<td>5.61-fold/1 h$^a$</td>
<td>Lethal (11)</td>
</tr>
<tr>
<td>ddrA</td>
<td>SSA? 3' end ssDNA protection</td>
<td>4.5-fold/0.5 h (7), 18-fold/0 h (8)</td>
<td>47.8-fold/1 h$^a$</td>
<td>Radiosensitive beyond 3 kGy (8)</td>
</tr>
<tr>
<td>ddrB</td>
<td>SSA? ssDNA binding</td>
<td>3.3-fold/1.5 h (7), 13-fold/0 h (8)</td>
<td>128.7-fold/2 h$^a$ (21)</td>
<td>Radiosensitive beyond 1 kGy (8)</td>
</tr>
<tr>
<td>pprA</td>
<td>NHEJ?</td>
<td>3.5-fold/0.5 h (7), 15-fold/0 h (8)</td>
<td>6.13-fold/0.5 h$^a$ (18, 19)</td>
<td>Radiosensitive to 1 kGy (8)</td>
</tr>
<tr>
<td>recA</td>
<td>RR</td>
<td>7.9-fold/1.5 h (7), 10-fold/0.5 h (8)</td>
<td>2.14-fold/1 h$^a$ (18, 19, 33)</td>
<td>Radiosensitive to 1 kGy (12)</td>
</tr>
<tr>
<td>ruvB</td>
<td>RR</td>
<td>3.2-fold/0.5h (7), 11-fold/0.5 h (8)</td>
<td>ND</td>
<td>Radiosensitive beyond 3 kGy (46)</td>
</tr>
<tr>
<td>uvrD</td>
<td>NER</td>
<td>3-fold/1.5 h (7)</td>
<td>ND</td>
<td>Radiosensitive to 1 kGy (12)</td>
</tr>
<tr>
<td><strong>Constitutively transcribed genes indispensable for radioresistance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>polA</td>
<td>DNA synthesis</td>
<td>NI (7)</td>
<td>ND</td>
<td>Radiosensitive to 1 kGy (14)</td>
</tr>
<tr>
<td>recFOR</td>
<td>RR</td>
<td>NI (7)</td>
<td>ND</td>
<td>Radiosensitive to 1 kGy (12)</td>
</tr>
<tr>
<td>recJ</td>
<td>RR</td>
<td>NI (7)</td>
<td>ND</td>
<td>Lethal (12)</td>
</tr>
<tr>
<td>sbcCD</td>
<td>RR</td>
<td>NI (7)</td>
<td>ND</td>
<td>Radiosensitive beyond 4 kGy (47)</td>
</tr>
<tr>
<td><strong>Transcriptionally induced genes dispensable for radioresistance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ddrP</td>
<td>NHEJ</td>
<td>14.4-fold/3 h (7), 5-fold/0.5 h (8)</td>
<td>ND</td>
<td>Wild type radioresistance (13)</td>
</tr>
<tr>
<td>uvrB</td>
<td>NER</td>
<td>5-fold/3 h (7), 7-fold/0 h (8)</td>
<td>ND</td>
<td>Radiosensitive to 9 kGy (13)</td>
</tr>
<tr>
<td>uvrA</td>
<td>NER</td>
<td>3.5-fold/1.5 h (7), 7-fold/0.5 h (8)</td>
<td>ND</td>
<td>Radiosensitive (49)</td>
</tr>
<tr>
<td>recG</td>
<td>RR</td>
<td>2.6-fold/0.5 h (7)</td>
<td>ND</td>
<td>Radiosensitive to 9 kGy (13)</td>
</tr>
<tr>
<td><strong>Constitutively transcribed genes dispensable for radioresistance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recD</td>
<td>RR</td>
<td>NI (7)</td>
<td>ND</td>
<td>Wild type radioresistance (12)</td>
</tr>
<tr>
<td>recQ</td>
<td>RR</td>
<td>NA</td>
<td>ND</td>
<td>Wild type radioresistance (12)</td>
</tr>
<tr>
<td><strong>Transcriptionally induced/constitutive genes with no data available on dispensability for radioresistance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ssb</td>
<td>ssDNA protection</td>
<td>3-fold/0.5 h (7)</td>
<td>8.93-fold/0.5 h$^a$ (18, 19, 23)</td>
<td>NA</td>
</tr>
<tr>
<td>gyrB</td>
<td>DNA unwinding</td>
<td>4.4-fold/0.5 h (7), 8-fold/0h (8)</td>
<td>21.38-fold/0.5 h$^a$</td>
<td>NA</td>
</tr>
<tr>
<td>uvrC</td>
<td>NER</td>
<td>3.8-fold/3 h (7)</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>ruvA</td>
<td>RR</td>
<td>NI (7)</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>ruvC</td>
<td>RR</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
</tr>
</tbody>
</table>

$^a$ Gamma radiation-induced proteins identified in this study.
proteins that are essential for radioresistance were found induced in our study, irrespective of their induction at transcriptome level, and (ii) a few genes that influence radioresistance of *D. radiodurans* (polA, recD, recF, recO, recR, recJ, ruvA, sbcC, sabcD, etc.) are neither up-regulated at transcriptomic level (7, 8) nor detected at proteomic level (in the present study and Refs. 18 and 19), suggesting that their basal expression level might be sufficient for DNA repair. The up-regulation of *ruvB* and *uvrD* genes was not observed at proteomic level, although they are important for radioresistance of *D. radiodurans* and were also moderately up-regulated at transcriptional level (7, 8). This could be due either to very low abundance of corresponding proteins or to their very transient expression. Similarly, transcriptionally up-regulated genes, *ddrP*, *uvrA*, *uvrB*, and *recG* (7, 8) were not induced at a proteomic level. These genes were also found dispensable for radiation resistance of this organism (13, 49).

The present work integrates data from transcriptome/proteome analyses, mutagenesis, and DNA repair kinetics to evolve a comprehensive picture of events underlying post-irradiation recovery in *D. radiodurans*. The observed pattern of induction and processing of DNA repair proteins offers insight into functional cooperation of various proteins during PIR, resulting in the radioreistant phenotype of this superbug.

**Acknowledgments—** *D. radiodurans* strain R1 ATCC BAA-816 was kindly provided by K. W. Minton and M. J. Daly (Uniformed Services University of the Health Sciences, Bethesda, MD).

*The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.*

[S] This article contains supplemental Tables 1 and 2 and Figs. S1–S9 and supplemental mass spectrometry data.

**D** To whom correspondence should be addressed: Molecular Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India. Tel: 91-22-25505342; Fax: 91-22-25505189/25505326; E-mail: aptesk@barc.gov.in, sksmbd@barc.gov.in.

# REFERENCES


vivo function of the C-terminus of *Escherichia coli* single-stranded DNA binding protein. *Nucleic Acids Res.* 24, 2706–2711


