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Transcriptomic Analysis of *Deinococcus radiodurans* During The Early Recovery Stage From Ultraviolet Irradiation*

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Abstract **Objective** *Deinococcus radiodurans* (*D. radiodurans*) is an extremophile with strong resistance to ultraviolet (UV), ionization, desiccation and chemical reagents. However, the molecular responses of this bacterium in the early recovery stage after UV irradiation are not fully understood. The aim of this work is to reveal the transcriptomic responses of *D. radiodurans* at this stage. **Methods** In this study, the transcriptomes of *D. radiodurans* under normal and UV irradiation culture conditions were determined by using RNA-seq technique. To identify the key genes and their regulatory relationships among the differentially expressed genes (DEGs), functional enrichment analysis was performed. Some key DEGs were selected and validated by real-time quantitative PCR. The transcriptome data from previous studies were adopted to find DEGs common to UV irradiation, ionizing radiation and desiccation stresses. The protein-protein interaction (PPI) network of DEGs was constructed; the hub genes and major modules in the PPI network were identified; functional enrichment analysis was performed for these hubs and modules. **Results** The results showed that the number of up-regulated genes was more than twice that of down-regulated genes in the early recovery stage after UV irradiation, and most of them were related to stress response and DNA repair. The main repair pathways in the early stage of recovery include single-strand annealing (SSA) pathway (involving genes *ddrA-D*), nonhomologous end joining (NHEJ) pathway (involving genes *ligB* and *pprA*) and nucleotide excision repair (NER) pathway (involving genes *uvrA−C*), the first two of which are for homologous recombination (HR), while the NER pathway removes pyrimidine dimers caused by UV irradiation. By comparing the transcriptome data under UV irradiation, ionizing radiation and desiccation stresses, it was found that the common responsive DEGs mainly involve *Deinococcus*-specific genes and the genes related to DNA/RNA metabolism. Several important hub genes and interaction modules were identified from the PPI network of DEGs, whose functions are concentrated in double-strand break repair, DNA topological change and replication. **Conclusion** These results indicate that in the early recovery stage after UV irradiation, a variety of genes in *D. radiodurans* undergo responses at transcriptome level, several repair pathways are initiated to cope with this stress, and some repair pathways are common to other stress conditions.

Key words *Deinococcus radiodurans*, RNA-seq, ultraviolet irradiation, DNA damage repair, protein-protein interaction (PPI) network
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Deinococcus radiodurans (*D. radiodurans*) is an extremophile, whose cells are spherical and usually live in dimer or tetramer form, and the colonies are red[1]. *D. radiodurans* has strong adaptability to various stress conditions, such as ionizing radiation, desiccation, high temperature and chemical reagents[2], which is due to its strong ability to repair DNA damage (such as chromosome double-strand breaks (DSB)). Therefore, this species has become an ideal model organism for studying tolerance and repair mechanisms under extreme stress conditions. The tolerance of *D. radiodurans* to damage by ionizing radiation, ultraviolet (UV) irradiation and desiccation may be the result of three mechanisms:

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prevention, tolerance and repair. Studies have found that ultraviolet light can damage DNA of organisms, producing cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs)[3], and such a damage can interfere with the replication and transcription mechanisms of DNA. *D. radiodurans* shows strong resistance to ultraviolet irradiation damage, and its resistance to ultraviolet irradiation is about 20 times that of *Escherichia coli* when measured from the perspective of survival rate[2]. *D. radiodurans* has a complex damage repair pathway. For example, deletion of *uvsE*, *uvrA1*, and *uvrA2* in nucleotide excision repair pathways was found to reduce but not completely eliminate the ability of this bacterium to remove CPDs and 6-4PPs from genomic DNA[3], indicating that *D. radiodurans* has multiple repair pathways to resist UV damage. What pathways are involved in the repair of UV damage remains to be further explored.

Transcriptomics studies the response of organisms to environmental stress from the aspect of gene expression, and its main techniques are microarray and mRNA sequencing (RNA-seq). Up to now, many works have studied various stress responses of *D. radiodurans* from the perspective of transcriptomes, including ionizing radiation[4], desiccation stress[5], heat stress[6], salt stress[7], cadmium stress[8], H_2O_2 stress[9], mitomycin C (MMC) stress, *etc*. *D. radiodurans* has strong tolerance to UV irradiation, but there is still a lack of systematic transcriptome study on its response to UV irradiation stress. In this work, we used RNA-seq technology to detect the transcriptome changes of *D. radiodurans* in response to UV irradiation, and compared and analyzed the differentially expressed genes (DEGs) of *D. radiodurans* under normal culture and UV irradiation conditions. Through functional analysis of the DEGs, we found key genes related to UV irradiation stress response, among which many classic damage-repair genes play a role in the early recovery stage from UV irradiation. We analyzed the similarities and differences of DEGs in response to UV irradiation, ionizing radiation and desiccation stresses, and identified common responsive genes. Moreover, the protein-protein interaction (PPI) network of DEGs was constructed and the hub nodes and major modules were identified, which enhanced our understanding on gene functions in UV damage repair. This study is the first one using RNA-seq technology to measure the transcriptomic response of *D. radiodurans* to UV irradiation, providing new data and insights for further understanding the molecular mechanism of radiation tolerance in this species.

1 Materials and methods

1.1 Strain，growth conditions and treatment

The R1 strain of *D. radiodurans* was used in this study, including two culture conditions. (1) Normal culture condition: 30℃, TGY medium, culture to the middle logarithmic stage; (2) UV irradiation condition: the bacterial solution under normal culture condition was irradiated with 2.5 $J{\cdot}m^{-2}{\cdot}s^{-1}$ UV (254 nm) for 5 min (namely, irradiation dose of 750 J/m^2) and sampled 10 min later (namely, in the early stage of recovery according to the definition in a previous work[4]). For each culture condition, three biological replicate samples were taken for RNA sequencing.

1.2 Transcriptome data analysis

1.2.1 RNA-seq data acquisition and preprocessing

The bacterial samples were sent to Tianjin Nuohe Source Biology Co., Ltd. for RNA extraction, subsequent cDNA library construction and on-machine sequencing. The sequencing standard was double-ended 150 bp, and the sequencing platform was NovA-PE150. Clean RNA-seq data from the company was used for subsequent analysis.

Clean data were checked for sequencing quality before biological analysis. The FastQC program was used for quality check, and Trimmomatic[10] was used for quality control of the data. The genome and annotation information of *D. radiodurans* R1 strain was downloaded from the NCBI genome database (Genome_Assembly_ID 300483) and used as the reference genome. The sequencing data were aligned to the reference genome by using TopHat[11] (which calls Bowtie2[12]). StringTie[13] was used for transcript assembly; the preDE.py script in StringTie was used to extract quantitative results and generate the count matrix.

1.2.2 Screening of differentially expressed genes（DEGs）

The R package DESeq2[14] was used for DEGs analysis. The gene expression levels in FPKM (fragments per kilobase of transcript per million mapped reads) obtained above were merged for the three biological replicates, and the transcriptomes under normal culture and UV irradiation conditions

were compared to obtain differentially expressed genes (DEGs). The criteria for DEG identification were: *Fold change*>2 and *False Discovery Rate* (*FDR*)<0.05.

1.2.3 GO and KEGG enrichment analysis for DEGs

GO database (http://geneontology.org/) was used for mapping and enrichment analysis of the obtained DEGs before and after UV irradiation. Based on the KEGG database, the R package clusterProfiler[15] was used for KEGG pathway enrichment analysis. In this process, the *D. radiodurans* R1 genome was used as background with threshold of *FDR*<0.05.

1.3 Validating RNA−seq results by real−time quantitative PCR （RT−qPCR）

For each of the two above-mentioned culture conditions (normal and UV irradiation), three biological replicate samples were taken for RT-qPCR analysis to validate the RNA sequencing results. Using housekeeping gene DR_1343 (glyceraldehyde 3-phosphate dehydrogenase) as internal reference, three key DEGs (DR_B0100, DR_1262, DR_2275) were selected for RT-qPCR experiments and the designed primer sequences are listed in Table S1. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of DEGs. *T*-test was used to evaluate the statistical significance (*P*-value) in data comparison.

1.4 Construction of the protein−protein interaction （PPI） network for DEGs

The PPI network of *D. radiodurans* was downloaded from STRING database (https://cn.string-db.org/)[16], and the subnetworks related to DEGs were screened out and imported into Cytoscape V3.9.1[17] for visualization. Unconnected nodes were deleted from the network. In the visualized PPI network, nodes represent proteins, and edges between nodes represent interactions. The color of node shows the regulation direction of a DEG (red for up-regulation, green for down-regulation, and yellow for insignificant change). The node size reflects the connectivity degree of a node.

1.5 Identification of hubs and modules in the PPI network of DEGs

The Centiscape V2.2 plug-in[18] in Cytoscape was used to analyze the PPI network topology. For each node in the network, the values of three network centrality indexes (degree, betweenness, eigenvector) were calculated. For each centrality index, the average value over the whole PPI network was calculated, and the nodes with higher values than the average were selected as high-centrality subset. Therefore, three high-centrality subsets were obtained, corresponding to the above three centrality indexes. The VENNY 2.1 software (https://bioinfogp.cnb.csic.es/tools/venny/) was used to draw the Venn diagram; the genes in the intersection of three high-centrality subsets were selected as the main gene set. The main gene set was uploaded to STRING database again to construct PPI network, and the PPIs with weighted score≥0.4 (namely, medium confidence) were screened out as the PPI network of main genes. CytoHubba plug-in[19] was used to calculate the connectivity of each main gene, and the top 15 genes with the highest connectivity were considered as hub nodes in the PPI network. The MCODE plug-in[20] was used to identify the major modules (*K-Score*>5) from the PPI network of main genes.

2 Results and discussion

2.1 Growth status and viability of *D. radiodurans* cells under UV irradiation stress

After UV stress, the recovery of *D. radiodurans* usually goes through three stages: early recovery, middle recovery and late recovery[4]. To investigate the adaptation mechanism of *D. radiodurans* to UV stress, we measured the survival rates for the Normal and UV irradiation culture conditions (Table 1). The survival rate (UV/Normal) of *D. radiodurans* cells was about 76.6% after UV irradiation of dose 750 J/m^2.

Table 1 The viable counts per 100 μl of broth before and after UV irradiation

Culture	Duplication_A	Duplication_B	Duplication_C
Normal	5.9×10^7	5.1×10^7	5.4×10^7
UV irradiation	4.2×10^7	4.5×10^7	4.3×10^7

2.2 Key functional genes related to UV irradiation stress

Through transcriptome data analysis, we found that a total of 750 genes had significant changes (*Fold change*>2 and *FDR*<0.05) in expression level after UV irradiation. Table 2 lists the top 30 most significantly up-and down-regulated DEGs. Interestingly, the fold changes of the top 30 up-regulated genes were all more than 8 times ($\log_2FC$>3), while the fold changes of the top 30

down-regulated genes were all less than 8 times ($\log_2 FC$>−3), indicating that positive responses are more likely taken by *D. radiodurans* under UV irradiation. In addition, many previously reported genes that may be involved in damage repair in *D. radiodurans* were also identified in our study (Table 3), suggesting that cells utilize many repair processes in response to UV stress. We hope to further investigate the roles of these genes in the early repair of UV damage.

Table 2 The 30 genes with most significant differences in expression after UV irradiation

Gene ID	$\log_2 FC$	*P*-adj	Function description
DR_B0100	7.549	3.19×10^{-132}	RNA ligase family protein
DR_B0099	7.032	4.07×10^{-186}	TIGR02452 family protein
DR_0003	6.773	2.50×10^{-60}	DNA damage response protein DdrC
DR_1263	6.541	5.44×10^{-180}	NADAR family protein
DR_1262	6.530	1.45×10^{-136}	TROVE domain-containing protein
DR_0203	6.442	4.80×10^{-103}	ABC-2 family transporter protein
DR_1264	6.383	1.75×10^{-108}	tRNA-OTHER
DR_B0098	6.088	2.29×10^{-154}	AAA family ATPase
DR_0430	6.011	4.93×10^{-156}	RtcB family protein
DR_0204	5.889	1.58×10^{-99}	ABC transporter permease
DR_t1264	5.710	6.95×10^{-77}	tRNA-Tyr
DR_C0012	5.608	7.57×10^{-34}	LuxR C-terminal-related transcriptional regulator
DR_1143	5.594	5.63×10^{-20}	Hypothetical potein
DR_RS02180	5.592	1.29×10^{-31}	Hypothetical potein
DR_0205	5.573	4.68×10^{-114}	ATP-binding cassette domain-containing protein
DR_0423	5.502	2.35×10^{-34}	Sngle-stranded DNA-binding protein DdrA
DR_RS05900	5.486	3.12×10^{-8}	Hypothetical protein
DR_1977	5.448	2.29×10^{-62}	Hypothetical protein
DR_0394	5.328	2.40×10^{-47}	Phosphotransferase enzyme family protein
DR_t23	5.223	2.72×10^{-91}	tRNA-Asp
DR_0206	5.190	6.39×10^{-67}	Hypothetical protein
DR_0431	5.127	7.12×10^{-123}	Class I SAM-dependent methyltransferase
DR_1354	4.966	2.48×10^{-80}	Excinuclease ABC subunit UvrC
DR_1978	4.876	3.52×10^{-55}	GNAT family N-acetyltransferase
DR_1267	4.843	1.78×10^{-60}	Tetratricopeptide repeat protein
DR_RS05895	4.782	4.60×10^{-6}	Hypothetical protein
DR_0798	4.608	1.32×10^{-23}	GNAT family N-acetyltransferase
DR_2273	4.606	1.19×10^{-23}	DNA topology modulation protein FlaR
DR_0422	4.570	5.25×10^{-18}	Trans-aconitate 2-methyltransferase
DR_0233	4.544	2.26×10^{-58}	Winged helix-turn-helix domain-containing protein
DR_0152	−2.302	1.86×10^{-7}	Acetoacetate decarboxylase family protein
DR_1886	−2.192	1.75×10^{-5}	SCO family protein
DR_1133	−2.154	9.34×10^{-6}	Uroporphyrinogen decarboxylase
DR_1082	−2.137	6.30×10^{-11}	Ribosome-associated translation inhibitor RaiA
DR_1885	−2.129	4.38×10^{-7}	Copper chaperone PCu(A)C
DR_1367	−2.128	2.70×10^{-4}	Pyridoxal 5'-phosphate synthase lyase subunit PdxS
DR_2382	−2.120	3.98×10^{-9}	Hypothetical protein
DR_1374	−2.067	4.40×10^{-22}	Type I DNA topoisomerase
DR_2024	−2.007	5.87×10^{-7}	DoxX family protein
DR_A0114	−1.984	9.28×10^{-3}	Hypothetical protein

Continued to Table 2

Gene ID	$\log_2FC$	*P*-adj	Function description
DR_1240	−1.968	4.40×10^{-20}	CoA ester lyase
DR_1887	−1.929	1.69×10^{-4}	Cytochrome c oxidase assembly protein
DR_1366	−1.925	2.43×10^{-3}	Pyridoxal 5'-phosphate synthase glutaminase subunit PdxT
DR_2476	−1.922	4.74×10^{-6}	Hypothetical protein
DR_1072	−1.918	1.11×10^{-10}	Acetyl-CoA C-acetyltransferase
DR_2132	−1.915	3.50×10^{-10}	Universal stress protein
DR_1481	−1.910	3.79×10^{-5}	Chlorite dismutase family protein
DR_2547	−1.846	3.97×10^{-6}	Glutamyl-tRNA reductase
DR_1749	−1.762	2.11×10^{-3}	Peptidoglycan endopeptidase
DR_0805	−1.750	1.14×10^{-5}	Twin-arginine translocase TatA/TatE family subunit
DR_1100	−1.730	1.54×10^{-4}	Rhodanese-related sulfurtransferase
DR_0151	−1.707	7.45×10^{-4}	Cysteine desulfurase-like protein
DR_1239	−1.677	3.38×10^{-11}	MaoC family dehydratase
DR_A0256	−1.675	1.08×10^{-4}	Phenylacetate-CoA ligase
DR_A0146	−1.665	9.71×10^{-5}	Catalase family protein
DR_0932	−1.655	1.34×10^{-5}	Polyprenyl synthetase family protein
DR_0189	−1.646	3.19×10^{-9}	TlpA family protein disulfide reductase
DR_2475	−1.628	4.90×10^{-4}	Hypothetical protein
DR_1542	−1.617	8.31×10^{-5}	Acyl-CoA carboxylase subunit beta
DR_2597	−1.617	1.61×10^{-4}	Nitric oxide synthase oxygenase

Table 3 The DEGs involved in the known damage repair pathways

Gene ID	Function	$\log_2FC$	*P*-adj	Damage repair pathway
DR_B0100	RNA ligase family protein LigB	7.549	3.19×10^{-132}	NHEJ
DR_A0346	DNA repair protein PprA	3.910	7.23×10^{-24}	NHEJ/HR/SSA
DR_0423	Single-stranded DNA-binding protein DdrA	5.502	2.35×10^{-34}	SSA
DR_0070	Single-stranded DNA-binding protein DdrB	3.300	2.24×10^{-10}	SSA
DR_0003	DNA damage response protein DdrC	6.773	2.50×10^{-60}	SSA
DR_0326	DNA damage response protein DdrD	2.977	2.69×10^{-10}	SSA
DR_0100	Single-stranded DNA-binding protein	1.993	1.05×10^{-6}	SSA
DR_1771	Excinuclease ABC subunit UvrA	2.444	1.06×10^{-17}	NER
DR_2275	Excinuclease ABC subunit UvrB	3.072	4.51×10^{-50}	NER
DR_1354	Excinuclease ABC subunit UvrC	4.966	2.48×10^{-80}	NER
DR_1976	Endonuclease MutS2	1.663	3.05×10^{-13}	MMR
DR_1039	DNA mismatch repair protein MutS	1.056	1.72×10^{-2}	MMR
DR_0906	Type IIA DNA topoisomerase subunit B	2.318	2.98×10^{-11}	—
DR_1913	DNA gyrase subunit A	2.315	1.57×10^{-11}	—
DR_0928	EndonucleaseIII, Nth	1.156	2.47×10^{-5}	BER
DR_1262	TROVE domain-containing protein Rsr	6.530	1.45×10^{-136}	—
DR_A0344	Transcriptional repressor LexA	1.550	0.000 248	SOS
DR_2340	Recombinase RecA	1.050	3.28×10^{-2}	HR/ESDSA/SOS
DR_0596	Holliday junction branch migration DNA helicase RuvB	2.177	8.65×10^{-6}	HR
DR_0440	Crossover junction endodeoxyribonuclease RuvC	1.346	9.60×10^{-9}	HR

2.2.1 Response of the four replicons to UV irradiation stress

The percentage of responsive genes in each of the four genomic components (replicons) of Chr1, Chr2, pMP1 and pSP1 was compared, and the percentage of responsive genes in pSP1 was the highest (40%). Among the 40 genes of pSP1, the expression levels of 16 genes were significantly changed and all of them were up-regulated, indicating that the small plasmid played an important role in the damage repair after UV irradiation. This point was also found in the repair after ionizing radiation, where most genes on pSP1 were significantly up-regulated in the middle and late stages of post-radiation recovery, and almost all genes were activated in the late stage of recovery[4].

2.2.2 Non-homologous end joining (NHEJ) pathway participates in early UV damage repair

DR_A0346 (*pprA*), DR_B0100 (*ligB*), DR_B0098 and DR_B0099 were significantly induced after UV irradiation. It was found that *pprA* and *ligB* were involved in the non-homologous end joining (NHEJ) pathway in *D. radiodurans*. As observed by atomic force microscopy (AFM), PprA protein preferentially binds to DNA double-stranded ends, promotes the ligation of DNA ends catalyzed by ATP-dependent DNA ligase (DR_B0100), and protects it from degradation by exonuclease[21]. DR_B0098–DR_B0100 are three genes encoded in an operon, which may be directly involved in DNA repair[4]. DR_B0098 contains a phosphatase domain of the HD hydrolase family and a polynucleotide kinase domain, and resembles a human protein with similar structure that plays an important role in DSB repair[22]. Studies have shown that in the presence of DR_B0098, PprA can stimulate LigB DNA ligase activity several times, and PprA and DR_B0098 proteins are essential for LigB function[23]. At 1.5 to 5 h of ionizing radiation, the expression level of each of these genes was induced 5 to 10 times[4]. Therefore, this pathway plays an important role in both UV damage repair and ionization damage repair.

2.2.3 Single-strand annealing (SSA) pathway participates in early UV damage repair

The genes involved in the single-stranded annealing (SSA) pathway were found to be: DR_0423 (*ddrA*), DR_0070 (*ddrB*), DR_0003 (*ddrC*), DR_0326 (*ddrD*), DR_A0346 (*pprA*) and DR_0100 (*ssb*). These genes were significantly induced in the early stage of UV irradiation. Some studies have found that the reaction products consistent with annealing mainly appear in the early stage after irradiation (*i.e.*, 1.5 h)[4], which was also proved in this study, namely, genes related to SSA were significantly up-regulated in the early stage after UV irradiation, indicating that the SSA pathway was initiated for repair. DdrB protein can bind to single-stranded DNA and promotes annealing, and single-stranded binding protein (SSB) plays a promoting role in this pathway[24]. Alternatively, DdrB may facilitate the accurate assembly of countless small fragments generated by extreme radiation exposure *via* SSA, thereby generating suitable substrates for subsequent genomic reconstruction by the ESDSA pathway[25].

2.2.4 Nucleotide excision repair (NER) pathway participates in early UV damage repair

D. radiodurans has a strong tolerance to UV irradiation. Pyrimidine dimers produced by UV damage can interfere with the processes of DNA replication and transcription, while nucleotide excision repair (NER) can remove pyrimidine dimers to ensure normal cell life activities. *Mfd*, *uvrA*, *uvrB*, *uvrC*, *uvrD*, *polA*, *ligA*, *uveE*, *yejH* / *rad25* and *sms*/*radA* are genes involved in the NER pathway, among which DR_1771 (*uvrA1*), DR_2275 (*uvrB*) and DR_1354 (*uvrC*) are significantly different in expression level after UV irradiation. UvrABC complex plays an important role in nucleotide excision repair in *D. radiodurans*, and the NER pathway has a unique ability to clear DNA damage[26]. This complex recognizes and binds to the damage site and then cleaves, with about 4 nucleotides cleaved at the 3' end and 8 nucleotides cleaved at the 5' end. Finally, DNA is synthesized and the gap is completed under the actions of DNA polymerase and DNA ligase to complete DNA damage repair[27]. Compared with the previous results on the response to ionizing radiation[4], UvrA1 in the NER pathway was expressed at the early stage of UV irradiation and at 1.5 h after ionizing radiation; UvrA2 (DR_A0188) was not expressed at the early stage of UV irradiation, but expressed at 5 h after ionizing radiation; UvrB and UvrC in NER pathway were expressed after UV irradiation and at 3 h after ionizing radiation; UvrD was involved in NER, methylation-dependent mismatch repair (mMM) and SOS repair, and was not

expressed after UV irradiation, but was expressed at 1.5 h after ionizing radiation. Two possible pathways for pyrimidine dimer clearance in *D. radiodurans* are ultraviolet damage endonuclease UvsE-dependent excision repair (UVER) and nucleotide excision repair (NER). These two pathways make recovery from UV damage redundant. Some studies have shown that UvrA2 plays a minor role in UV resistance, and NER is more related to UV resistance than UVER[3], which is consistent with our transcriptomic findings, that is, no significant difference was found for UvrA2 and UvsE expression after UV irradiation. One possible explanation is that the contribution of NER and UVER pathways to UV resistance is related to the growth stage[28], which needs to be further investigated.

2.2.5 Superhelix related genes play a role in the repair of early UV damage

GyrA and GyrB are two DNA-gyrase subunits in *D. radiodurans*, both of which are significantly induced after UV irradiation, indicating that the regulation of DNA supercoiling is important for DNA repair, and GyrA and GyrB may be nucleoid associated proteins (NAPs) of *D. radiodurans*[29]. Both subunits were also found to be induced after ionizing radiation[4], in which GyrA protein is essential for DNA replication and genome reconstruction after severe ionizing radiation exposure[30].

2.2.6 Mismatch repair (MMR) system participates in the repair of early UV damage

MutS2 and MutS1 are involved in the base mismatch repair system and are significantly induced after UV irradiation. MutS recognizes and binds to base mismatch[31]. MutS1 is a key protein involved in mismatch repair system, which can ensure the replication and recombination fidelity of *D. radiodurans*[32], while MutS2 is involved in RecA-independent repair mechanism in *D. radiodurans*, which enhances the resistance of cells to oxidative stress-induced DNA damage, so as to make organisms exhibit remarkable DNA repair capabilities[33].

2.2.7 Homologous recombination (HR) repair is involved in DNA repair

RecA protein is a recombinant enzyme with unwinding ability, which plays an important role in homologous recombination (HR) and extended synthesis-dependent strand annealing (ESDSA). RecA is essential for genome recovery after irradiation, and its induction is considered to be a major marker for the initiation of homologous recombination[34]. In the present study, RecA protein was only induced more than two-fold at the early stage of UV irradiation (Table 3). Previous studies have found that RecA-dependent homologous recombination occurs 5 h or more after irradiation[4], so it is possible that this protein is not sufficiently induced in the early stage of UV irradiation, and other genes related to homologous recombination are also rarely induced. In addition, there are RuvA/B/C proteins in *D. radiodurans*, whose functions are similar to the DNA branch transferase and dissociase involved in homologous recombination of DNA in human cells, and among them two proteins (RuvB, RuvC) were significantly induced (Table 3).

2.2.8 Other key functional genes related to UV irradiation stress

In addition, our study also found that DR_1262 (*rsr*), an important gene against UV stress, was significantly up-regulated. Rsr protein is an RNA-binding protein. Studies have shown that Rsr protein can bind some small RNA produced in cells after UV irradiation and improve the resistance of cells to UV irradiation[35].

DR_0928 in the base excision repair (BER) pathway, which is expressed in the early and middle stages of ionizing radiation[4], is also induced after UV irradiation (Table 3). RecA and LexA proteins are involved in SOS repair[22], which are induced at 1.5 h after ionizing radiation[4], are also induced after UV irradiation (Table 3). However, *D. radiodurans* does not possess a functional SOS response system, so this remains to be investigated.

ABC transporters (DR_1356-DR_1359) are highly induced and may be involved in the transshipment of damaged products, and these transporters are also highly induced after ionizing radiation[4]. Up-regulated kinases of unknown characteristics (DR_2467, DR_0394) may be involved in a variety of cellular processes, and when expressed in *Escherichia coli*, these uncharacterized kinases affect DNA topology[22]. Several genes encoding proteins from the extended family of stress response (DinB family) were also induced in early UV irradiation, including DR_1263 and two members of this family (DR_0053, DR_0841), which were

strongly induced in a RecA-like manner, supporting their important role in UV irradiation response.

From Table 3, it can be found that among the 20 DEGs, there are 6 genes involved in the SSA pathway, 3 genes involved in the NER pathway, 2 genes involved in the NHEJ pathway and 4 genes involved in the HR pathway. Among the top 30 up-regulated genes (Table 2), in addition to genes with known functions, some genes with unknown functions expressed, including DR_1143, DR_RS02180, DR_RS05900, DR_1977, DR_0206, and DR_RS05895, indicating that these genes also play an important role in the response to UV irradiation, which should be the focus of future research.

2.2.9 PprI/DdrO mediated regulatory relationship

A large number of proteins in *D. radiodurans* were significantly up-regulated in the early recovery from UV irradiation, among which PprI/DdrO mediated regulatory proteins were the most prominent. RDRM is a conserved palindromic sequence that exists upstream of many radiation-induced genes (*e.g.*, *ddrO, ddrA, ddrB, ddrC, ddrD, ddrF, ddrR, recA, pprA, gyrA, gyrB, ssb, recQ, ruvB, uvrA, uvrB, uvrD*). It has been found that *ddrO* acts as a repressor by binding to RDRM, the promoter that appears to be located at or very close to radiation-induced genes, including *ddrO* itself. However, PprI (IrrE) is a metalloproteinase required for the hydrolytic inactivation of DdrO protein to deactivate the suppressor gene, a process that is somehow activated after exposure of *D. radiodurans* to radiation[36]. IrrE-dependent cleavage of the DdrO, and hence up-regulation of RNA-dependent RNA polymerase (RDR), is essential for radiation tolerance. The genes that were significantly induced include the *Deinococcus*-specific genes (*ddrO, ddrA, ddrB, ddrC, ddrD, ddrF, ddrR, pprA*), DNA repair genes (*recA, recQ, ruvB, uvrA, uvrB, uvrD, ssb*), DNA superhelix genes (*gyrA, gyrB*) (Figure 1), among which *pprA* is inhibited by *pprM* and *lexA2*[37-38], and *recA* is inhibited by *recX*[39].

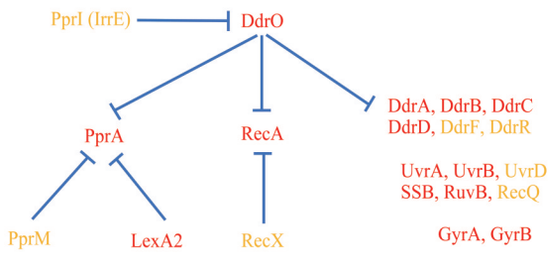

Fig. 1 PprI/DdrO mediated regulatory relationships in *D. radiodurans*

Red color indicates significant up-regulation after UV irradition, while yellow color indicates no significant difference in expression level after UV irradition.

2.3 Functional enrichment analysis of DEGs under UV irradiation

For the selected criteria ($\log_2$ (*Fold change*) >1 and *FDR*<0.05), there were 750 DEGs (Figure 2a), among which 544 genes were up-regulated and 206 genes were down-regulated, and the number of up-regulated genes was more than twice that of down-regulated genes. It seems that the tolerance of *D. radiodurans* to UV irradiation mainly depends on the enhancement of most stress response activities under irradiation stress to maintain cell survival and repair damage, rather than the slowing down or even stagnation of normal physiological activities. To determine the biological functions of DEGs under UV irradiation stress, we performed KEGG (Figure 2b) and GO (Figure 2c, d) functional enrichment analysis. To ensure the accuracy of the results, the hypothetical proteins in the protein functional annotation of *D. radiodurans* were removed before enrichment analysis.

According to the GO enrichment results, damage repair responses, including SOS response and DNA recombination repair, appeared in *D. radiodurans* when exposed to UV irradiation stress. The translation process was enhanced, the translocation of translation-related carboxylic acid and amino acid was enhanced, and the ribosome, rRNA, tRNA and other related genes were significantly up-regulated, presumably due to the massive synthesis of damage repair related proteins. ATP-binding and ATP-dependent activities were enhanced to maintain normal physiological status and to supply more energy for damage repair pathways. DNA binding is enhanced, due to the proteins in the damage repair pathway, such as DNA ligase LigB, DNA damage repair protein DdrB, single-strand binding protein SSB, UvrABC component protein, and mismatch repair protein MutS, need to bind to DNA for damage repair. When exposed to UV irradiation, the number of down-regulated genes was significantly less than that of up-regulated genes, and mainly concentrated on the reduction of redox reactions: oxidoreductase activity in redox reactions

decreased, cytochrome complex assembly decreased, and heme synthesis decreased. Cytochrome is involved in redox reactions with heme as a prosthetic group. Redox reactions provide energy for life activities, which is essentially the transfer of electrons and an important way of energy transfer. Therefore, UV irradiation has an impact on the energy metabolism of *D. radiodurans*.

KEGG pathway enrichment analysis was performed on DEGs to investigate the involved metabolic pathways (Figure 2b). Genes related to the ABC transporters pathway were significantly up-regulated, while genes related to the biosynthesis of ubiquinone and other terpenoid-quinone and phenylalanine metabolism were significantly down-regulated. Studies have found that ABC transporters in *D. radiodurans* play an important role in the supply of exogenous amino acids, and ABC transporters can be used for the uptake of amino acids and peptides[40]. When adapting to irradiation pressure, bacterial cells need various ions and amino acids to synthesize corresponding proteins, which can explain the significant up-regulation of this metabolic pathway. On the other hand, it also indicates that *D. radiodurans* has a certain ability to adapt to UV irradiation pressure. Ubiquitin, also known as coenzyme Q, is a hydrogen transporter in the respiratory chain and plays a role in electron transport. The decrease of ubiquitin synthesis corresponds to the decrease of redox reactions in the GO enrichment analysis, which jointly indicates that UV irradiation has an impact on the energy metabolism of *D. radiodurans*.

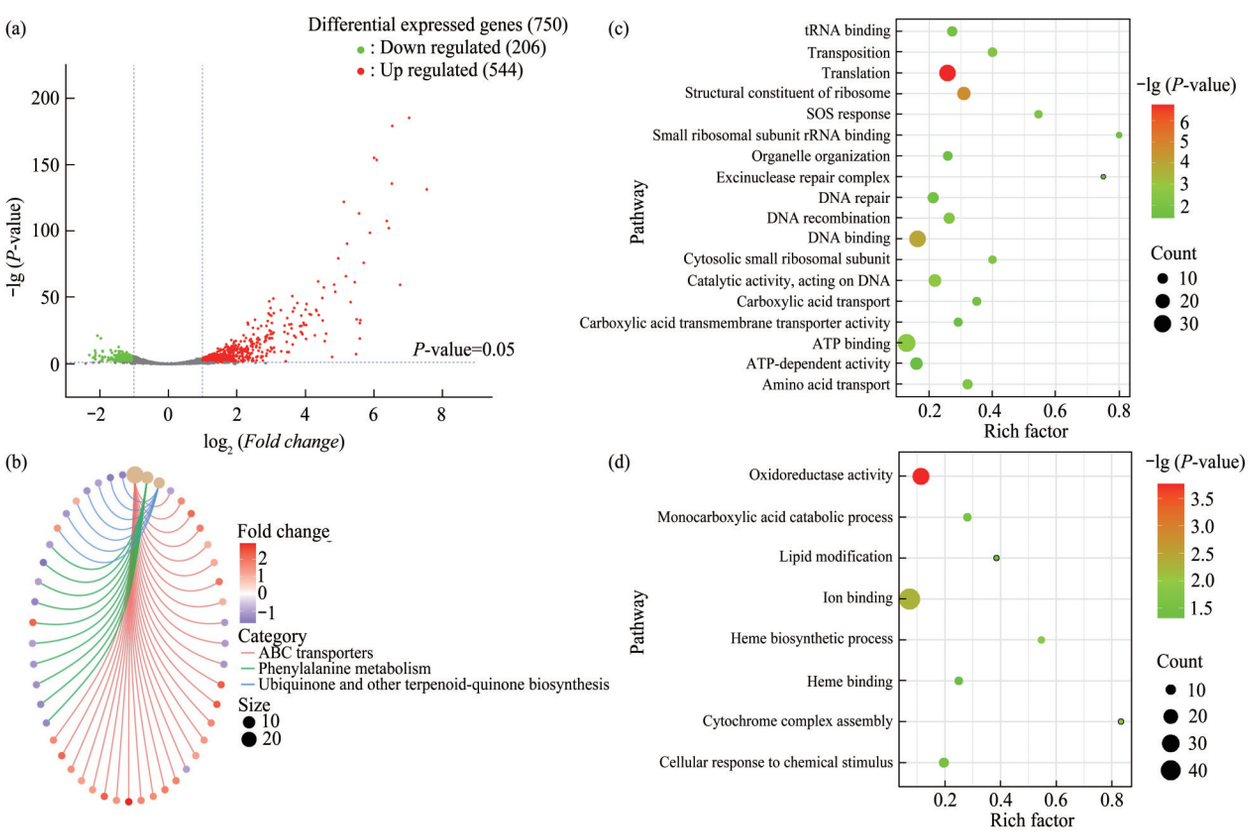

Fig. 2 Differentially expressed genes and their enrichment

(a) Distribution of DEGs before and after UV irradiation. (b) KEGG pathway enrichment. (c) Bubble chart of GO enrichment results of up-regulated genes. (d) Bubble chart of GO enrichment results of down-regulated genes.

2.4 Validation of DEGs by RT−qPCR

To further validate the expression level changes of DEGs in response to UV irradiation, 3 key functional genes in *D. radiodurans*, namely, DR_B0100 (RNA ligase family protein LigB), DR_1262 (TROVE domain-containing protein Rsr) and DR_2275 (excinuclease ABC subunit UvrB), were selected for RT-qPCR analysis. The results showed that the changes in expression level of the 3 genes after UV irradiation are consistent with the

transcriptome sequencing results (Figure 3), indicating that the transcriptome data are reliable.

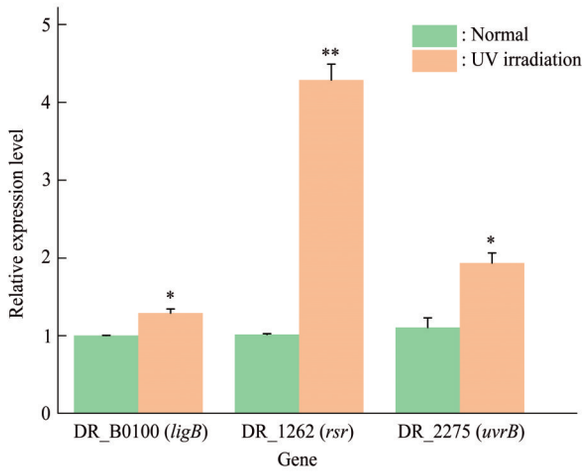

Fig. 3 RT-qPCR results of 3 DEGs in response to UV irradiation

Error bars indicate SD; *P <0.05; **P <0.01.

2.5 Similarities and differences of DEGs under the three stress conditions of UV irradiation, ionizing radiation and desiccation

The intersection (Figure 4) of DEGs (*Fold change*>3) under UV irradiation with ionizing radiation and desiccation stresses was taken to obtain the genes listed in Table S2. These genes can be broadly classified into the following categories: DNA metabolism, energy acquisition, putative regulatory proteins, RNA metabolism, protein synthesis, transport, specific genes of the *Deinococcus* genus, and other genes[41]. We identified the common responsive genes (shown in bold fonts in Table S2) under UV irradiation, ionizing radiation and desiccation stresses. These genes are mainly concentrated in DNA metabolism, RNA metabolism and *Deinococcus*-specific genes. It seems that these are common molecular responses of *D. radiodurans* to UV, ionization and desiccation stresses. DNA metabolism includes some repair genes and superhelix related genes. Some genes were common response genes of UV irradiation and ionizing radiation but did not participate in the response to desiccation stress, while others were common response genes of UV irradiation and desiccation but did not participate in the response to ionizing radiation. These genes need to be further analyzed.

It has been reported that there is a strong correlation between desiccation resistance and γ-radiation resistance in *D. radiodurans*[5]; both radiation (3 kGy) and desiccation stresses induced 5 genes, and we found that UV irradiation induced high expression of the same 5 genes: DR_0423 (*ddrA*), DR_0070 (*ddrB*), DR_0003 (*ddrC*), DR_0326 (*ddrD*) and DR_A0346 (*pprA*). These 5 genes correspond to GO terms: “Response to Gamma radiation (GO: 0010332)” and “Cellular response to Desiccation (GO: 0071465)”. This indicates a strong correlation between UV irradiation and resistance to desiccation and γ-radiation, and since these five proteins are involved in the SSA pathway, it is speculated that the common properties of resistance to desiccation, γ-radiation and UV irradiation may be related to the SSA pathway.

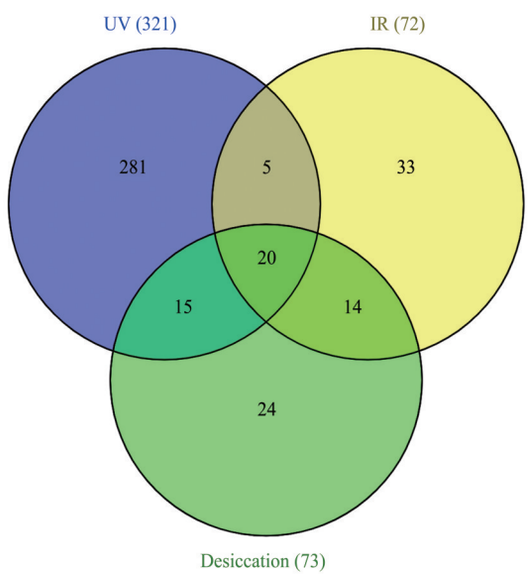

Fig. 4 Venn diagram of DEGs in response to UV irradiation, ionizing radiation and desiccation stresses

2.6 Hub and module analysis in the PPI network of DEGs

The protein-protein interaction (PPI) network of *D. radiodurans* was downloaded from the STRING database[16], and the DEGs related subnetwork was screened out. Unconnected nodes were deleted from the network. The initial PPI network consisted of 2 747 nodes and 100 965 edges (Figure 5). From the initial network, we can see that there are many genes, although they are not DEGs themselves, but they interact with DEGs, and these genes may still play an important role in the response to UV irradiation.

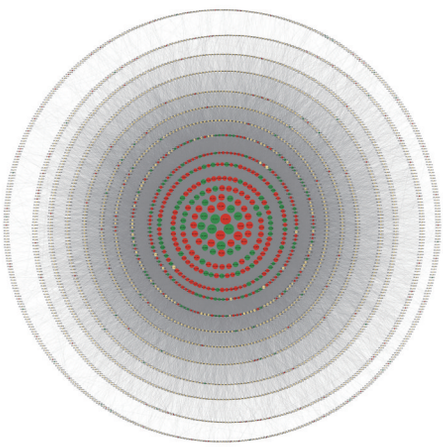

Fig. 5 The initial PPI network of DEGs after UV irradiation of *D. radiodurans* constructed by using the STRING database

Red nodes represent up-regulated genes; green nodes represent down-regulated genes; yellow nodes represent genes with no significant difference in expression. The node size represents the degree of connectivity in the PPI network.

To obtain the main PPI network from the initial network, we performed the following analysis. Firstly, nodes whose connectivity degree, betweenness and eigen vector values were higher than the average of the network were selected and their intersection was taken as the main gene set, resulting in a total of 336 nodes (Figure 6a). Next, these 336 main genes were submitted to the STRING database to construct a PPI network. Finally, unconnected nodes were removed from this network and nodes with interaction scores greater than 0.4 (medium confidence) were selected to obtain the main PPI network (Figure 6b), which consists of 314 nodes and 3 924 edges.

To analyze the main PPI network, we used the CytoHubba plug-in[19] for Cytoscape[17] to identify the top 15 hub genes with high connectivity (Figure 6c) and rank them as follows: *polA* (degree=164), *metG* (degree=138), *guaA* (degree=136), DR_0603 (degree=134), *dnaK* (degree=134), *guaB* (degree=128), *pheT* (degree=126), DR_0183 (degree=122), DR_2168

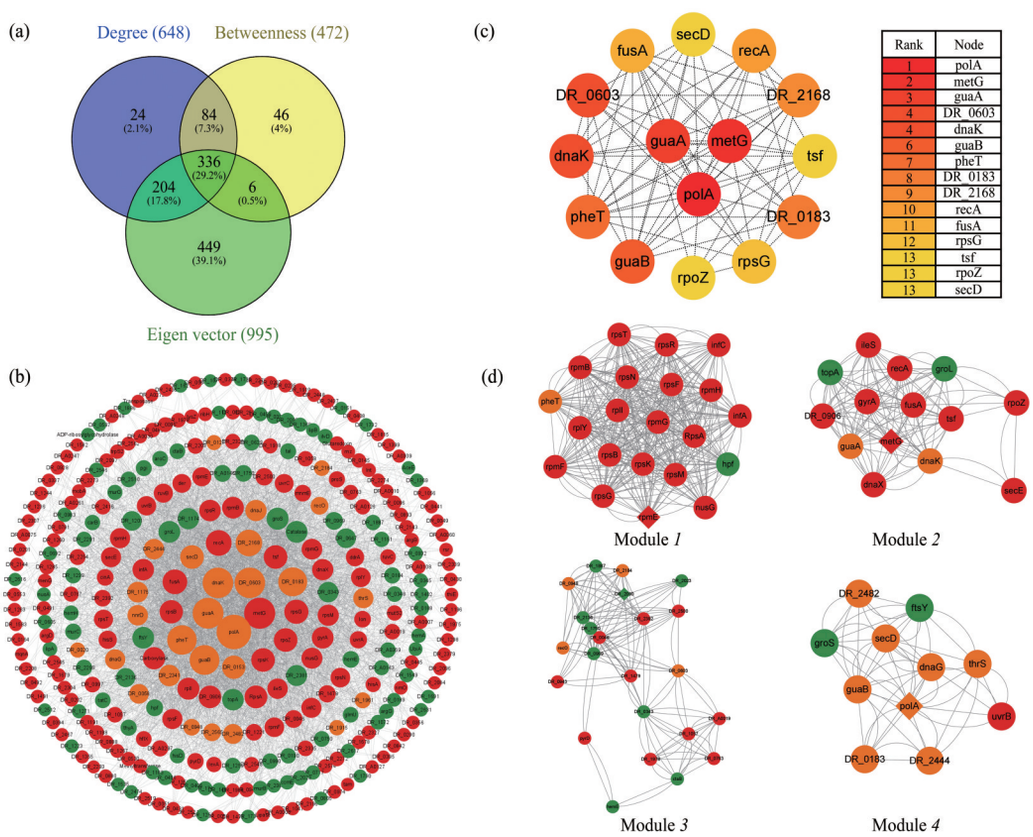

Fig. 6 Hubs and modules in the main PPI network of DEGs

(a) Centrality analysis of the initial PPI network. The Venn diagram shows the number of nodes with centrality (degree, betweenness, and eigen vector) values above the mean. (b) The main PPI network of DEGs. (c) The top 15 hub genes of the main PPI network. Node color reflects connectivity, with red representing the highest connectivity. (d) MCODE analysis of the main PPI network. Module *1*: Score=19.1, Module *2*: Score=10, Module *3*: Score=6.545, Module *4*: Score=6. Diamonds represent hub genes. Node size reflects connectivity. Red indicates up-regulation, green indicates down-regulation, and yellow indicates non-DEGs.

(degree=104), *RecA* (degree=98), *fusA* (degree=90), *rpsG* (degree=86), *tsf* (degree=82), *rpoZ* (degree=82), *secD* (degree=82). The names and functions of these hub genes are shown in Table 4. Subsequently, GO functional enrichment analysis was performed for these hub genes (Figure 7b), and according to the results, the most enriched GO biological processes (BP) include: GMP biosynthetic process, double-strand break repair, translation; the most enriched GO molecular functions (MF) include: catalytic activity, acting on a nucleic acid, nucleic acid binding.

Table 4 The functions of 15 hub genes

Gene_ID	Name	$\log_2FC$	*P*-adj	Function
DR_1707	*polA*	−0.033	9.05×10^{-1}	DNA polymerase I
DR_1433	*metG*	1.491	2.04×10^{-10}	Methionine--tRNA ligase
DR_1874	*guaA*	−0.667	4.05×10^{-3}	Glutamine-hydrolyzing GMP synthase
DR_0603	DR_0603	−0.561	1.85×10^{-1}	NUDIX domain-containing protein
DR_0129	*dnaK*	−0.401	1.56×10^{-1}	Molecular chaperone DnaK
DR_1878	*guaB*	−0.640	5.30×10^{-3}	IMP dehydrogenase
DR_2357	*pheT*	−0.592	4.40×10^{-2}	Phenylalanine--tRNA ligase subunit beta
DR_0183	DR_0183	−0.546	1.83×10^{-1}	Glutamate synthase subunit alpha
DR_2168	DR_2168	−0.428	1.22×10^{-1}	Methyltransferase domain-containing protein
DR_2340	*recA*	1.050	3.28×10^{-2}	Recombinase RecA
DR_0307	*fusA*	1.044	1.15×10^{-2}	Elongation factor G
DR_0306	*rpsG*	1.180	9.92×10^{-3}	30S ribosomal protein S7
DR_1512	*tsf*	1.177	3.92×10^{-3}	Translation elongation factor Ts
DR_2494	*rpoZ*	2.061	1.76×10^{-15}	DNA-directed RNA polymerase subunit omega
DR_1822	*secD*	−0.152	5.92×10^{-1}	Protein translocase subunit SecD

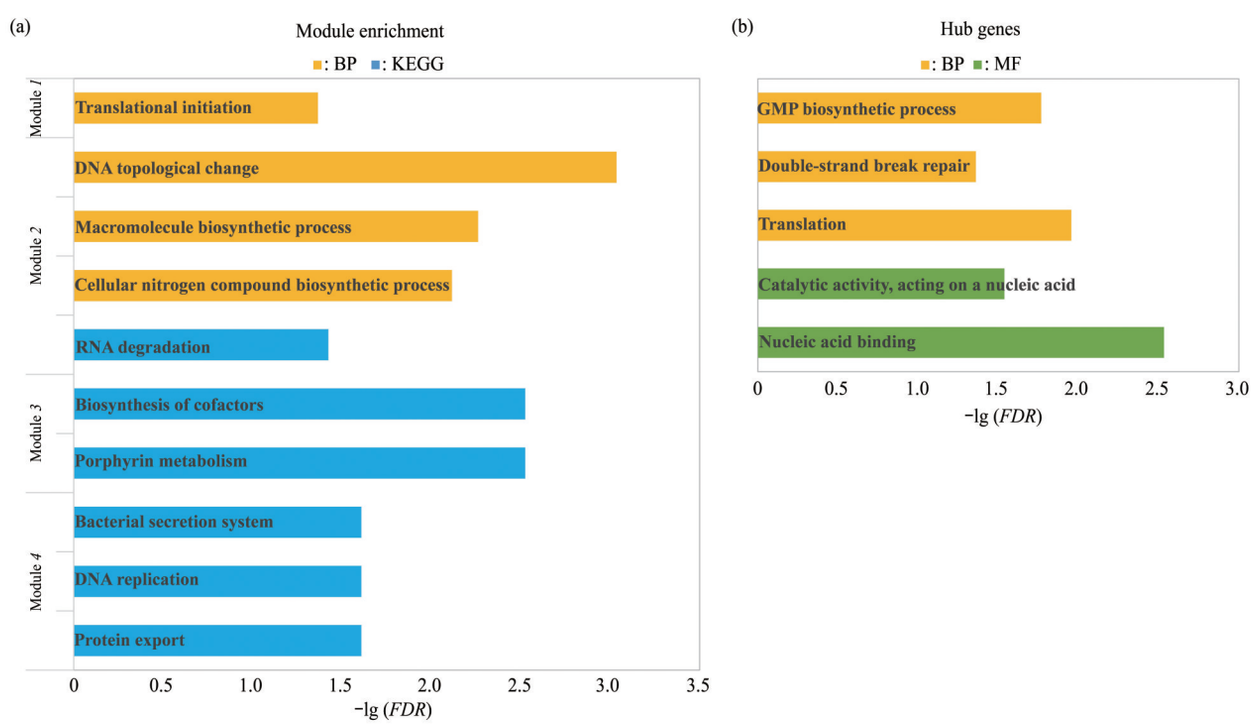

Fig. 7 Function enrichment analysis

(a) Module enrichment. The results of GO (biological process) function and KEGG pathway enrichment analysis for the genes in the 4 modules of the PPI network discovered by the MCODE plug-in of Cytoscape. (b) Hub genes. The results of GO functional enrichment analysis for the 15 hub genes in Table 4.

Four major modules were discovered through the MCODE plug-in of Cytoscape (Figure 6d). Subsequently, GO function and KEGG pathway enrichment analysis were performed for genes in these four modules (Figure 7a), and according to the results, the most enriched GO biological processes include: translational initiation, DNA topological change, macromolecule biosynthetic process, cellular nitrogen compound biosynthetic process; the most enriched KEGG pathways include: RNA degradation, biosynthesis of cofactors, porphyrin metabolism, bacterial secretion system, DNA replication, protein export.

3 Conclusion

D. radiodurans is the most radiation-resistant organism ever discovered on earth. Our study found that *D. radiodurans* not only has the common DNA damage repair genes of general prokaryotes and eukaryotes, but also has some special multidomain gene families in the repair of UV irradiation damage. Through transcriptomic analysis, according to different DNA repair pathways, we explored genes or enzymes related to DNA repair in the early response to UV irradiation, especially the genes that may play a key role in DNA repair, such as *pprA*, *ddrA-D*, *rsr*, *etc*. Our results show pathways (SSA, NHEJ, NER, HR) that may be involved in early damage repair and genes that play important roles, and suggest that these genes may be regulated by PprI/DdrO. By comparing with ionizing radiation and desiccation stresses, the common molecular responses to the three stresses were found, which mainly involved DNA metabolism, RNA metabolism and *Deinococcus*-specific genes. The PPI network of DEGs was constructed, and hub genes including *polA*, *metG*, *guaA*, DR_0603, *dnaK*, *guaB*, *pheT*, DR_0183, DR_2168, *recA*, *fusA*, *rpsG*, *tsf*, *rpoZ*, *secD* were identified. These genes also played important roles in the response to UV irradiation stress. At the same time, some highly expressed genes with unknown functions may also play a key role in UV irradiation resistance. Therefore, further elucidation of the functions and regulatory mechanisms of these genes is crucial for understanding the extreme resistance and damage repair mechanisms of *D. radiodurans*.

Supplementary Available online (http://www.pibb.ac.cn or http://www.cnki.net):
PIBB_20220471_Table S1.pdf
PIBB_20220471_Table S2.pdf

Data Accessibility The transcriptome data generated in this work were deposited in the National Genomics Data Center (NGDC) with accession number CRA009109. The data were also uploaded to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession numbers SRR22460685 to SRR22460690.

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紫外辐照恢复早期耐辐射奇球菌的转录组学分析*

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摘要　目的　耐辐射奇球菌是一种对紫外线、电离、干燥和化学试剂具有较强抗性的极端微生物。然而，该菌在紫外辐照后恢复早期的分子响应还不完全清楚。本文的目的是揭示耐辐射奇球菌在这一阶段的转录组响应。**方法**　本研究采用RNA-seq技术，测定了正常和紫外辐照培养条件下耐辐射奇球菌的转录组。为确定关键的差异表达基因及其调控关系，进行了功能富集分析。选取部分关键差异表达基因，进行实时定量PCR实验验证。利用以往研究中的转录组数据，寻找紫外辐照、电离辐射和干燥胁迫条件下公共的差异表达基因。构建了蛋白质-蛋白质相互作用网络；对蛋白质互作网络中的枢纽基因和主要模块进行了鉴定；对这些枢纽基因和模块进行了功能富集分析。**结果**　紫外辐照后的恢复早期，上调基因数量是下调基因数量的2倍以上，且多数与应激反应和DNA修复有关。恢复早期的修复途径主要有单链退火（SSA）途径（涉及基因：*ddrA-D*）、非同源端连接（NHEJ）途径（涉及基因：*ligB*、*pprA*）和核苷酸切除修复（NER）途径（涉及基因：*uvrA-C*），前两种途径为同源重组（HR）做准备，而NER途径去除紫外线照射带来的嘧啶二聚体。通过比较紫外辐照、电离辐射和干燥胁迫下的转录组数据，发现公共的差异表达基因主要涉及奇球菌属特异性基因和DNA/RNA代谢相关基因。从差异表达基因的蛋白质互作网络中发现了几个重要的枢纽基因和相互作用模块，其功能集中在双链断裂修复、DNA拓扑改变和复制。**结论**　在紫外辐照后的早期恢复阶段，耐辐射奇球菌的多种基因发生了转录组水平的分子响应，并启动了多个修复途径来应对这种胁迫，其中一些修复途径在其他应激条件下同样存在。

关键词　耐辐射奇球菌，RNA-seq，紫外辐照，DNA损伤修复，蛋白质-蛋白质相互作用网络

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