Extremophiles (2006)
DOI 10.1007/s00792-006-0002-9

ORIGINAL PAPER

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Microarray analysis of the hyperthermophilic archaeon *Pyrococcus furiosus* exposed to gamma irradiation

Received: 21 March 2006 / Accepted: 16 May 2006

Abstract The remarkable survival of the hyperthermophilic archaeon *Pyrococcus furiosus* to ionizing radiation was previously demonstrated. Using a time course study and whole-genome microarray analyses of mRNA transcript levels, the genes and regulatory pathways involved in the repair of lesions produced by ionizing irradiation (oxidative damage and DNA strand breaks) in *P. furiosus* were investigated. Data analyses showed that *radA*, encoding the archaeal homolog of the RecA/Rad51 recombinase, was moderately up regulated by irradiation and that a putative DNA-repair gene cluster was specifically induced by exposure to ionizing radiation. This novel repair system appears to be unique to thermophilic archaea and bacteria and is suspected to be involved in translesion synthesis. Genes that encode for a putative Dps-like iron-chelating protein and two membrane-bound oxidoreductases were differentially expressed following gamma irradiation, potentially in response to oxidative stress. Surprisingly, the many systems involved in oxygen detoxification and redox homeostasis appeared to be constitutively expressed. Finally, we identified several transcriptional regulators and protein kinases highly regulated in response to gamma irradiation.

Electronic Supplementary Material Supplementary material is available to authorised users in the online version of this article at http://dx.doi.org/10.1007/s00792-006-0002-9.

Communicated by K. Horikoshi

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Keywords Archaea · Hyperthermophile · DNA repair · Ionizing radiation · Oxidative stress · Transcriptional analysis

Introduction

Pyrococcus furiosus is an hyperthermophilic archaeon that grows optimally at 100°C under anaerobic conditions (Fiala and Stetter 1986). Its genome has been sequenced along with two other *Pyrococcus* species, *P. abyssi* and *P. horikoshii*, providing a basis for comparative genome analyses (Maeder et al. 1999). We demonstrated that exposure of *P. furiosus* cells to ionizing radiation—a substitute to heat in inducing DNA strand breaks (Peak et al. 1995)—at 2,500 Grays (Gy) resulted in chromosome fragmentation (DiRuggiero et al. 1997). Similar results were obtained with *P. abyssi* (Gerard et al. 2001) and within 2–4 h of incubation at 95°C the chromosomes of both *Pyrococcus* species were reassembled, producing actively growing cells and demonstrating highly effective DNA repair systems (DiRuggiero et al. 1997). Chromosome reassembly following fragmentation by ionizing radiation was also reported for the halophilic archaeon *Halobacterium salinarium* strain NRC-1 (Kottemann et al. 2005) and the bacterium *Deinococcus radiodurans* (Cox and Battista 2005) suggesting that adaptation to environmental conditions such as high temperature and desiccation may result in enhanced radiation resistance (DiRuggiero et al. 1997; Kottemann et al. 2005; Cox and Battista 2005).

More than 80% of the deleterious effects of gamma radiation are the result of hydroxyl radical production via radiolysis of water, imparting severe oxidative stress to all the cell's components (Hutchinson 1985). Lesions to the DNA include bases modifications, DNA backbone breakage and cross-linking of proteins to DNA (Hutchinson 1985; Riley 1994). Clustered lesions are converted into double-strand breaks when two opposing strand breaks are formed or during attempted base

excision repair (Dianov et al. 2001). Therefore, DNA repair systems and cellular defense mechanisms against oxidative stress are key components of radiation resistance.

Maintenance of genome stability is critical to the survival of organisms and in particular to those organisms living at high temperature. Surprisingly, our understanding of the pathways and proteins involved in DNA repair in hyperthermophiles, and in archaea in general, is still very limited (Kelman and White 2005). Comparative genome analyses have shown that archaeal DNA repair proteins are more closely related to their eukaryal than to their bacterial counterparts and that many proteins, and sometimes entire pathways, are missing in some or in all the archaea (DiRuggiero and Robb 2004; Kelman and White 2005). Homologs of the conserved mismatch proteins MutS and MutL have only been found in the genomes of a few archaea (Grogan 2004), and while most archaeal genomes contain homologs of eukaryal nucleotide excision repair proteins, several archaea also have bacterial UvrABC homologs (Kelman and White 2005). Archaeal proteins involved in homologous recombination, one of the major pathways for the repair of double-strand breaks, are structurally and functionally similar to eukaryotic proteins (Ishino et al. 2006) but several proteins involved in key steps of this pathway remain to be identified in both the Archaea and Eukarya (DiRuggiero and Robb 2004; Ishino et al. 2006).

In this work we investigated the genes and regulatory pathways in *P. furiosus* involved in the repair of DNA strand breaks and oxidative damage produced by ionizing irradiation, using a full-genome microarray. We found that the physiological response of *P. furiosus* during recovery from gamma irradiation involved several DNA repair processes, a down-regulation of metabolic functions and a concerted attempt to reverse and minimize oxidative damage. Surprisingly, many of the oxygen detoxification systems appeared to be constitutively expressed.

Materials and methods

Cell growth and treatments

P. furiosus strain (DSMZ 3638) was grown in 100 ml serum bottles at 90°C under anaerobic conditions in the absence of sulfur and with 100 μM Na_2WO_4 and 0.5% (wt/vol) maltose (Robb et al. 1992). Cultures grown to approximately 5×10^6 cells/ml were chilled to 4°C and exposed to 2,500 Gy of gamma radiation using a 26,000-curie (9.6×10^{14} Bq) ^{60}Co gamma source at the University of Maryland College Park Gamma Test Facility, at a dose rate of 73 Gy/min. Cultures were further incubated at 90°C and samples for RNA extraction were taken at 0, 20, 40, 60, 120 and 240 min after the temperature of the cultures reached 90°C (DiRuggiero et al. 1997). Cells were treated with H_2O_2 and ethyl methanesulfonate (EMS) by adding 0.001% of H_2O_2 or 0.1 M EMS (final concentrations) to cultures grown to 5×10^6 cells/ml (Watrin and Prieur 1996; Praul and Taylor 1997). Cultures were incubated at room temperature for 30 min under anaerobic conditions. Sodium thiosulfate was added to the EMS treated cultures to a final concentration of 200 mM to inactivate EMS. Cells from both treatments were washed twice, resuspended in reduced culture medium and incubated at 90°C for 40 min. Controls were treated in the same way without addition of H_2O_2 or EMS, or without gamma irradiation.

RNA extraction

Cultures were centrifuged at 4,000*g* for 4 min at 4°C and RNA was extracted from the pellet using the TRI reagent (SIGMA T9424, St Louis, MO) as previously described (Chomczynski and Sacchi 1987). RNA samples were analyzed with a Beckman DU640 spectrophotometer (Beckman Coulter, Fullerton, CA) and gel electrophoresis for quality and quantification.

Array design and printing

The *P. furiosus* full-genome microarray represented 2,065 protein encoding genes. DNA fragments for spotting were produced by PCR. The array was constructed by designing oligonucleotide primers to span the most unique portions of each gene, averaging 350 nucleotides in length. A custom Perl program was written to select primers with the most desirable thermodynamic conditions possible for each gene using the Whitehead Institute's Primer3 program (version 3.09) as its core analysis engine. The polymerase chain reaction was performed in 100 μl reactions with 1 μM of each primer, 2 mM $MgCl_2$, 1.0 mM dNTPs, 1.75 U AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA) 1x AmpliTaq buffer, and 20 ng genomic DNA for 30 cycles (94°C 30 s, 50°C 1 min, 72°C 2 min). All PCR products were checked by agarose gel electrophoresis and failures were repeated or new primers designed. Each PCR amplicon was spotted on poly-*L*-lysine coated glass slides twice, each time by a different print pin (print plates were rotated 180° for second spotting) using a 16-pins ArrayMaker Version 1.0 (http://www.cmgm.stanford.edu/pbrown/mguide/index.html).

Labeling and hybridization

First strand cDNA synthesis and labeling was carried out with total RNA (3 μg), 200 U Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA), random hexamers, 0.5 mM dATP, dGTP, dCTP, 0.2 mM dTTP and 0.3 mM Cy3-dUTP or Cy5-dUTP (Amersham Biosciences, Piscataway, NJ) for 110 min at 42°C.

Labeled cDNAs were cleaned up with alkaline treatment and Cyscribe GFX columns (Amersham Biosciences) according to the manufacturer's instructions. Hybridization was carried out in a sealed Corning hybridization chamber (Acton, MA) at 60°C for 18 h. For each experimental time point four slides, with at least two features per open reading frame (ORF), were hybridized producing eight replicates or more per ORF.

Slide scanning, feature detection, and normalization

Slides were scanned using an Axon 4100a Genepix personal slide scanner and the Genepix software V5 (Axon, Union City, CA). The background-normalized intensities were normalized by median ratio normalization as follows: median values were calculated for the ratios of the intensities for the two channels, channel one was divided by the square root of the median and channel two was multiplied by the square root of the median. This effectively centralizes the median close to a value of one.

Quantitative RT-PCR

Primers were generated from the *P. furiosus* genome sequence (Genbank accession number NC_003413) using Primer Express V.1.5 (Applied Biosystems, Foster City, CA). RNA was treated with RQ1 RNase free DNase according to the manufacturer's instructions (Promega, Madison, WI) and cDNAs were generated from 1 μg of total RNA using the TaqMan Reverse Transcription Reagents with random hexamers according to the supplied protocol (Applied Biosystems). PCR was performed as described before (Komori et al. 2000). Cycling was carried out with an ABI Prism 7900 and data were collected with Sequence Detector V.1.7 (Applied Biosystems). Three experimental replicates were used for each treatment. Expression levels were calculated using standard curves and normalized with the expression level of ORF PF1722 (histone) that was constitutive under our experimental conditions.

RT-PCR

Regions joining ORFs PF0639 through PF0643 and PF1118 through PF1122, were amplified using Primer Express V.1.5 (Applied Biosystems). Amplification targets resulted in approximately 50 nucleotides from each adjoining ORFs. Reverse transcription and PCR were carried out as described above. Non-reverse transcriptase controls were processed in the same way.

Data analysis and databases searches

For microarray analyses, two types of replicates were included: (1) technical replicates: each gene-specific PCR product was spotted at two spatially different locations onto the slides, (2) biological replicates: each comparison was performed from four independently processed cultures and hybridized to four different slides to account for inherent biological variation occurring independently of the experimental perturbations, giving a total of eight data points per condition per gene. All RNA samples were compared with a reference RNA extracted from an aliquot of the same culture prior to treatment. Ratio of fluorescence intensity between sample and reference RNA were calculated as $\log_{10}$ values. To evaluate the statistical significance of the mRNA changes observed over the time course of the experiment, we performed a maximum likelihood analysis for each gene over eight replicates (Ideker et al. 2000). The maximum likelihood statistic lambda (λ) is a function of the variation in absolute intensity values within a microarray experiment. A λ value < 20 for over 99% of all genes in the array was consistently obtained when RNA preparations from independently processed but identically grown cultures were compared. Changes in gene expression associated with $\lambda > 20$, which correlate to a 99% confidence level, were considered statistically significant (Ideker et al. 2000). Hierarchical clustering was performed using the Hierarchical clustering Explorer (HCE 3.0) and time series were analyzed using TimeSearcher 2, both developed by the Human Computer Interaction Lab at the University of Maryland (http://www.cs.umd.edu/hcil/bioinfovis/). Similarity searches were performed against the non-redundant protein sequence database at the National Center for Biotechnology Information (NCBI, Bethesda, MD) using standard BLAST (blastp) (Altschul et al. 1997) with default parameters. Position-specific iterative (PSI) BLAST (Altschul et al. 1997) searches were performed with the inclusion threshold of 0.01 (*E* value). Conserved domain and motif searches were performed using reverse-position-specific (RPS) BLAST (Marchler-Bauer et al. 2005), the Conserved Domain Architecture Retrieval Tool (CDART) (Geer et al. 2002), the Conserved Domain Database for protein classification (CDD) (Marchler-Bauer et al. 2005) at NCBI, SMART (Letunic et al. 2004) and Pfam (Bateman et al. 2002) with HMMER default parameters, and by searching the ProDom database with blastp at a *E* value cutoff of 0.01 (Corpet et al. 2000).

The complete microarray data has been deposited in the Gene Expression Omnibus database (GEO) at NCBI under accession number GPL3926, and is also available at the UCSC Genome Browser at http://www.archaea.ucsc.edu/.

Results

The remarkable survival of *P. furiosus* to ionizing irradiation prompted us to investigate the global regulatory response of cells subjected to 2,500 Gy of ^{60}Co gamma ray using microarray analyses. At this radiation dose,

P. furiosus showed 75% survival (DiRuggiero et al. 1997). Following irradiation, cells were allowed to recover at 90°C and total RNA was extracted at six time points during recovery, from 0 to 240 min. Out of 2,065 ORFs the level of mRNA for 321 ORFs changed significantly ($\lambda > 20$; see Material and methods) by two-fold or more for at least one of the time points after irradiation (Table S1, published on line in supplemental material). Those ORFs encoded for proteins of all metabolic pathways including carbohydrate, amino acid and nucleotide metabolism, transcription, translation, DNA repair, transport and a large number of hypothetical proteins. Most changes in mRNA levels occurred at 20 min following irradiation with 162 differentially expressed ORFs ($\lambda > 20$; > 2-folds) whereas at 120 min only 39 ORFs showed significant changes in their mRNA levels (Table S1).

DNA repair and replication

We found up to 2.5-fold increase in the mRNA level of *radA*, a functional homolog of the *recA*/*rad51* recombinases, following gamma irradiation (Fig. 1a). In contrast, the *radB* gene, encoding a protein with sequence similarity to recombinases (Komori et al. 2000), did not show increased expression level after exposure of the cells to gamma ray. Biochemical characterization and structural studies revealed that RadB is not a recombinase and may instead be involved in the regulation of homologous recombination (Komori et al. 2000; Guy et al. 2006). In *P. furiosus*, *radB* and the two subunits of DNA polymerase D (*dp1* and *dp2*) constitute an operon (Komori et al. 2000). Our data showed that those three genes were co-regulated following gamma irradiation validating our microarray experiment (Fig. 1b).

We also found a cluster (cluster 1, Fig. 2a) of five conserved hypothetical genes with up to a 10-fold increase in mRNA levels following gamma irradiation (Fig. 2b). Those five genes were co-regulated with maximum mRNA levels at 40 and 60 min after irradiation. Using RT-PCR, we demonstrated that they are found on the same transcriptional unit (result not shown). Comparative sequence analysis allowed us to assigned putative functions to four of the five genes present in the cluster. PF0639 (COG 2254) has a predicted HD-nuclease domain and is a member of a superfamily of metal-dependant phosphorylases; PF0640 (COG 1203) is typical of a superfamily II of helicases, the DEAD/DEAH box helicases (pfam00270); PF0641 (COG1688) is an uncharacterized protein predicted to be involved in DNA repair and recombination. It belongs to the RAMP superfamily [Repair Associated Mysterious Protein, (Makarova et al. 2002)]; PF0642 (COG 1857) belongs to a protein family of putative negative autoregulators that includes the DevR protein from *Myxococcus xanthus*; PF0643 is a conserved hypothetical protein with no predicted function. ORFs PF0637 and PF0638 encode for unknown CRISP-associated proteins that are only similar to each other and to *M. jannaschii* MJ0385. Sequence analysis (http://www.archaea.ucsc.edu/) predicts that they belong to the same operon as PF0639. The DNA-damage induced gene cluster 1 is similar to another gene cluster (cluster 2, Fig. 2a) previously described in *P. furiosus* (Makarova et al. 2002). However, this second gene cluster did not show any significant changes in gene expression level following gamma irradiation ($\lambda < 20$; Fig. 2c). The main differences in cluster 2 are (1) the presence of two putative nucleases: PF1118 (COG1518) with a pattern of conserved acidic residues often present in the catalytic site of nucleases, and PF1119 (COG1468) that belongs to the RecB family of exonucleases involved in DNA repair and recombination in bacteria; and (2) the ORF PF1120 is a typical superfamily II helicase domain (COG1203) fused to a predicted HD-nuclease domain (with similarity to COG 2254), whereas these domains are present as separate proteins in cluster 1 (HD-nuclease PF0639 and DNA helicase PF0640, respectively) (Fig. 2a). In the other two *Pyrococcus* strains sequenced so far there is one copy of cluster 1 in *P. abyssi*, and 1 copy of cluster 1 and 2 copies of cluster 2 in *P. horikoshii*. We used quantitative RT-PCR probes against

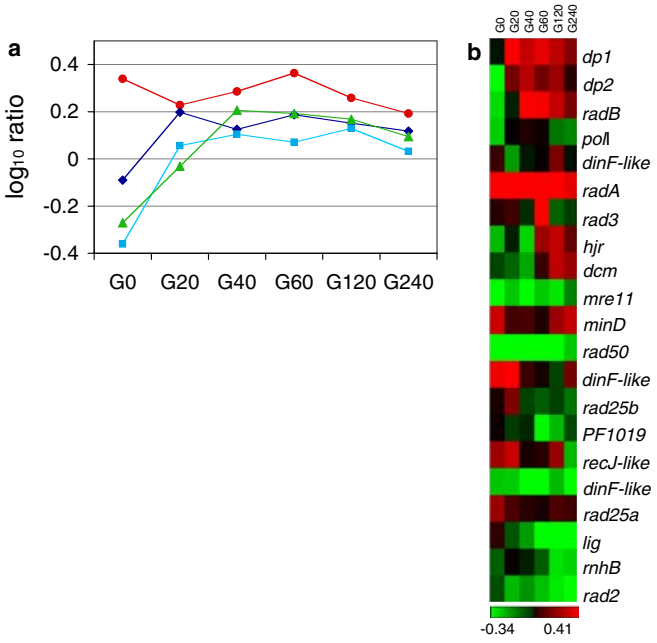


Fig. 1 Transcript levels for selected DNA repair and replication genes in *P. furiosus* following gamma irradiation. **a** *radA*, DNA polymerase D subunits *dp1* and *dp2*, and *radB* recombinase genes. The *y*-axis indicates $\log_{10}$ ratios of mRNA changes with respect to the reference RNA, and the *x*-axis indicates incubation time at 90°C after gamma irradiation in min (G0–240). The *lines connecting the dots* are for the benefit of the reader to correlate mRNA changes for a given gene in the six experimental time points. *Red circles*, *radA* PF1926; *green triangles*, *radB* PF0021; *blue diamonds*, *dp1* PF0018; *light blue squares*, *dp2* PF0019. **b** Hierarchical clustering of $\log_{10}$ ratios of mRNA changes for *P. furiosus* DNA repair genes with associated lambda values above 20 for the six experimental time points. *Red* denotes up-regulation and *green* denote down-regulation; *at the top*, time course in min (G0–240)

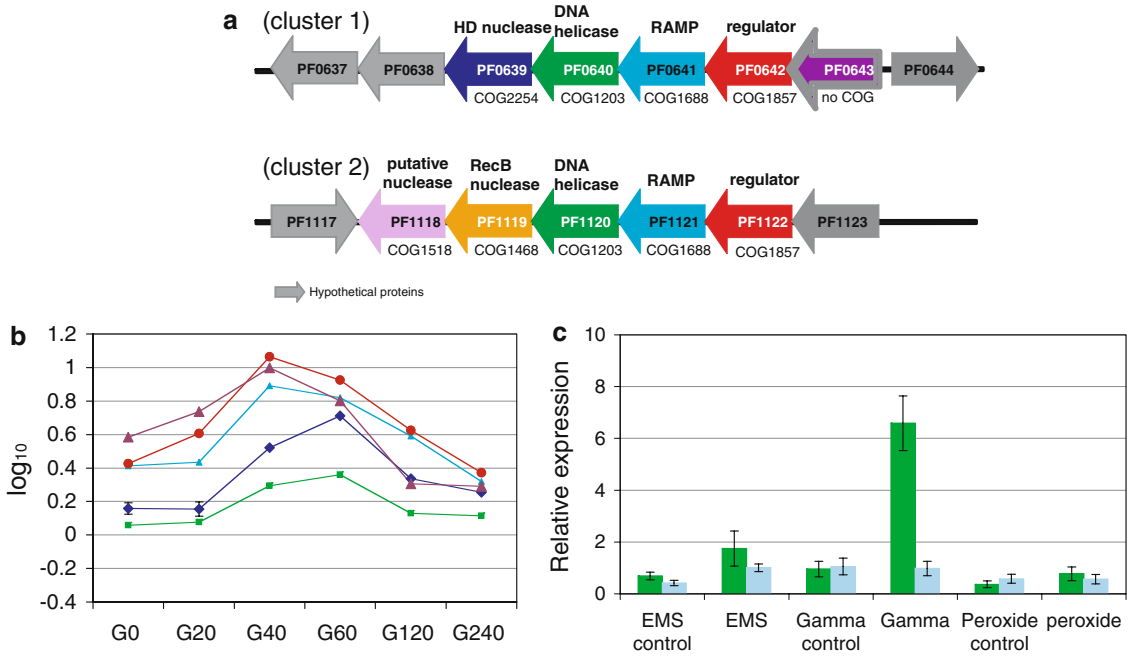


Fig. 2 Analysis of the "thermophile-specific DNA repair system" constituted of two gene-clusters in *P. furiosus*. **a** Schematic showing the genomic organization of clusters 1 and 2. *Arrows* indicate ORFs with transcripts orientation, ORF numbers are *inside arrows* and COG numbers are *below arrows*; *color code* indicates homologous ORFs; *in grey* are hypothetical protein encoding genes. **b** Transcript levels for genes from cluster 1; The *y*-axis indicates $\log_{10}$ ratios of mRNA changes with respect to the reference RNA, and the *x*-axis indicates incubation time at 90°C after gamma irradiation in min (G0–240). *Blue diamonds*, PF0639; *green squares*, PF0640; *light blue triangles*, PF0641; *red circles*, PF0642; *purple triangles*, PF0643. **c** mRNA levels of PF0641 (cluster 1) and PF1121 (cluster 2) genes in *P. furiosus* cells exposed to gamma irradiation, H_2O_2 and EMS treatments. Cells were grown to mid exponential phase, exposed to treatments and incubated at 90°C for 30 min. Real time quantitative reverse transcriptase-PCR was performed with the ABI Prism 7700 detection system and the SYBR Green I dye (Perkin Elmer). ORF PF1722 was found to be constitutively expressed and was used to normalize expression levels. *Dark green* and *light blue colored bars* indicate relative expression levels for PF0641 (cluster 1) and PF1121 (cluster 2), respectively

PF0641 (cluster 1) and PF1121 (cluster 2) and RNA extracted from cells exposed to sublethal doses of gamma irradiation (75% survival), H_2O_2 (70% survival) and EMS (58% survival) to determined expression pattern of genes from cluster 1 and 2. Figure 2c shows that PF0641 (cluster 1) was induced in cells subjected to gamma irradiation when compared to non-irradiated cells, whereas PF1121 (cluster 2) was expressed at the same level in the irradiated and non-irradiated cells. Neither ORF was significantly induced in cells treated with H_2O_2 or EMS when compared to non-treated cells.

Oxidative stress response

Most cellular damages from ionizing radiation result from reactive oxygen species (ROS) production by water hydrolysis (Hutchinson 1985). The generation of ROS can be amplified by the presence of transition metal ions, such as Fe^{2+}, via the Haber–Weiss and Fenton reaction (Riley 1994). In this reaction, unbound Fe^{2+} catalyzes the generation of reactive hydroxyl radicals ($^{\cdot}OH$) from hydrogen peroxide. This in turn produces secondary clustered damages in various cellular compartments. In the cell, ferric iron binding and storage, iron ion homeostasis and iron detoxification are catalyzed by ferritin and ferritin-like proteins, which are major non-heme iron storage proteins found in the three domains of life (Pulliainen et al. 2005; Theil 1987). *P. furiosus* ORF PF1193 displayed up to 12-fold increase in mRNA level at 20 min following irradiation (Table S1). PF1193 contains a ferritin-like di-iron motif found in ferritin- and Dps-like proteins and bacterioferritins and was found to belong to a new subclass of ferritin-like di-iron carboxylate superfamily (Ramsay et al. 2006; Tatur et al. 2005).

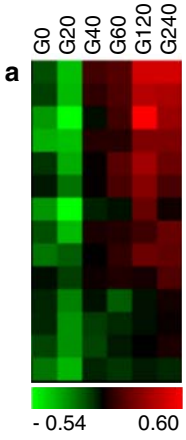
Hydrogenases are typically iron-sulfur proteins that catalyze the reversible oxidation of hydrogen gas; they are essential in energy metabolism, fermentation and H_2 respiration (Vignais and Colbeau 2004). The *P. furiosus* genome contains genes encoding two soluble sulfhydrogenases (SuDH-I and SuDH-II) and two membrane-bound oxidoreductase complexes, a hydrogenase complex (MBH) and a NADH:ubiquinone oxidoreductase complex (MBX) (Ma et al. 2000; Sapra et al. 2000; Silva et al. 2000). The MBH and MBX protein complexes are very similar in sequence and structure (Silva et al. 2000), but the catalytic subunit of the MBX complex lacks the two conserved CxxC nickel binding motifs that are characteristic of hydrogenase catalytic subunits (Deppenmeier 2002; Silva et al. 2000). We found that the two membrane-bound complexes MBH and MBX displayed antagonistic expression patterns

immediately following gamma irradiation of *P. furiosus* cells, with an increase in the level of mRNA up to 10-fold for the 13-gene operon of MBX, 20 min after irradiation (Fig. 3b). Similarly to the other membrane bound complex, MBH, the increase in mRNA for the soluble SuDH-I sulfhydrogenase genes occurred later in the recovery period at 90°C (Fig. 3a, c).

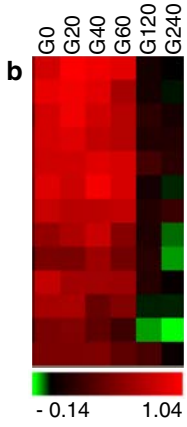
Transcriptional and post-translational regulation of the stress response

Although the archaeal basal transcription apparatus is eukaryotic-like in nature, the transcriptional regulators characterized so far are mostly bacterial-like with both activators and repressors members of the helix–turn–helix (HTH) superfamily of DNA binding proteins (Ouhammouch et al. 2004). Here we found that two putative transcriptional regulators showed up to four-fold increases ($\lambda > 20$) in mRNA levels following gamma irradiation (Table S1). The first one, PF0250, belongs to the family of bacterial regulatory proteins AsnC/Lrp (for asparagine and leucine; Pfam) that also include LrpA from *P. furiosus* (Brinkman et al. 2000). LrpA was found to bind to its own regulatory region and to exhibit negative autoregulation independently of leucine or any other amino-acids (Brinkman et al. 2000). The second putative transcriptional regulator, PF1072, belongs to the bacterial regulatory protein ArsR (for arsenic; Pfam) family; these regulatory proteins act as transcriptional repressor and their DNA binding properties are modulated by metal ions. One example in the archaea is the MDR1 metal-dependent repressor from *Archaeoglobus fulgidus* (Bell et al. 1999). The targets of both PF0250 and PF1072 still remain to be determined.

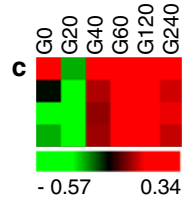
Phosphorylation and dephosphorylation mechanisms for regulating protein functions have been found in the three domains of life, including the archaea (Eichler and Adams 2005; Kennelly 2003). The examination of archaeal genome sequences has identified putative protein kinase and phosphatase ORFs in nearly all the genomes analyzed, however only a few of the encoded proteins have been characterized (Kennelly 2003). In *P. furiosus* ORF PF0364 belongs to the family of RIO-like protein-serine/threonine kinases. This family contains eukaryotic and archaeal proteins characterized by several sub-domains with highly conserved residues (Hanks and Hunter 1995). These sub-domains are 4–20 residues in length and located in the N-terminal nucleotide-binding domain (sub-domains I–IV), in a linker domain (sub-domain V) and in phosphotransferase and protein binding domains at the C-terminal of the protein (sub-domains VIa–XI). Table 1 shows the conserved residues of the PF0364 encoded protein for the most highly conserved sub-domains of the eukaroytic protein kinase superfamily. Furthermore, PF0364 is 88% identical to the PH0512 protein kinase from *P. horikoshi* (Tahara et al. 2004). PH0512 was found to phosphorylate in vitro Ser48 of the α subunit of the translation initiation factor



ORF number	Gene name	Max. fold change
PF1423	*mbhA*	3.1
PF1424	*mbhB*	2.6
PF1426	*mbhD*	1.5
PF1428	*mbhF*	4.0
PF1436	*mbhN*	1.8
PF1435	*mbhM*	2.0
PF1430	*mbhH*	1.7
PF1429	*mbhG*	1.7
PF1427	*mbhE*	1.1
PF1425	*mbhC*	1.0
PF1431	*mbhI*	1.1
PF1432	*mbhJ*	0.9
PF1433	*mbhK*	2.0
PF1434	*mbhL*	2.4



ORF number	Gene name	Max. fold change
PF1449	*mbhF*	9.1
PF1448	*mbhG*	4.5
PF1441	*mbhN*	3.1
PF1450	*mbhD*	3.5
PF1451	*mbhC*	5.7
PF1453	*mbhA*	8.2
PF1452	*mbhB*	6.1
PF1446	*mbhH'*	9.3
PF1442	*mbhL'*	11.0
PF1447	*mbhH*	7.1
PF1445	*mbhM*	7.5
PF1443	*mbhK*	5.8
PF1444	*mbhJ*	9.7



ORF number	Gene name	Max. fold change
PF0892	*hydG*	3.7
PF0893	*hydD*	3.2
PF0891	*hydB*	3.5
PF0894	*hydA*	2.2

Fig. 3 Transcript levels for *P. furiosus* oxidoreductases after gamma irradiation. Hierarchical clustering of $\log_{10}$ ratios of mRNA changes for *P. furiosus* oxidoreductase gene complexes with associated lambda values above 20. Time course is in minutes of incubation at 90°C following irradiation (G0–240); *red* denotes up-regulation and *green* denote down-regulation. *Each row* represents a gene with, in the corresponding row of the table, its ORF number, gene name and maximum fold mRNA change during the time course. The order of the ORFs in the tables matches that of the clustering in the figures. **a** membrane-bound dehydrogenase complex MBH; **b** membrane-bound NADH:ubiquinone oxidoreductase complex MBX; **c** sulfhydrogenase suDH-I

elF2 suggesting a role in regulation of protein synthesis (Tahara et al. 2004). We found that the mRNA of the *P. furiosus* homolog (PF0364) was significantly decreased 40 min after gamma irradiation in contrast to that of another putative protein kinase (PF1581), which showed an increase at the same time point (Table S1). PF1581 is also a member of the RIO-like serine/threonine protein kinase family (Table 1); it is 85% identical to the *P. horikoshi* homolog (PH1567) for which no target protein has yet been identified. Both proteins belong to COG1718 that contain proteins involved in

Table 1 Comparison of sequences of putative archaeal kinases with the consensus sequence of highly conserved subdomains of eukaryotic protein kinases as described in Hanks and Hunter (1995)

organisms	ORFs	Length	Subdomains									
			I	II	III	IV	V	VIb	VII	VIII	IX	XI
Consensus			ogxGxogxv	oaoKxo	Exxoo	hxxooxoxxxo	oooo*oo	oohrDok + xNooo	oko + Dfgo +	+ pEoo	Doo + ogoooo	Rx +
P. furiosus	PF0364	311aa	iGvGkdadV	vAiKfn	Ealvl	Haivmefidgv	eilgkvl	ivHgDmsefNivi	iliiDwaqy	nPEsl	Disvllnafr	Rfy
P. horikoshi	PH0512	313aa	iGvGkdadV	vAvKfn	Ealvl	Haivmeyisgv	eilgkil	ivHgDmsefNvvl	iliiDwaqy	nPEsl	Ditvllnafr	Rfy
P. furiosus	PF1581	266aa	lstGkeanV	iAvKiy	Eylaa	rklvfvwtrre	vlimeyi	mvHgDlseyNili	pviiDwsqa	nPEek	_[a]	_[a]
P. horikoshi	PH1567	266aa	lstGkeanV	iAvKiy	Eylaa	rklvfvwtrre	ilimefi	mvHgDlseyNili	pviiDwsqa	dPEvk	_[a]	_[a]
M. jannaschi	MJ0444	290aa	vnsGkeavV	rAvKvy	Efrnl	_[a]	vlvmdfv	lvHgDlseyNilv	pvfiDFGqs	_[a]	Dcnykdlyk	_[a]

Symbols include upper case letters for universal functionally essential residues, lowercase letters for highly conserved amino acid residues, *o* for positions conserving nonpolar residues, # for positions conserving charged residues, * for positions conserving polar residues, + for positions conserving small residues with near neutral polarity, and *x* for positions showing no clear pattern of conservation

[a]No domain found

cell cycle control, signal transduction, cell division and chromosome partitioning.

Hypothetical proteins

Among the hypothetical protein encoding genes either conserved or specific to *P. furiosus* that exhibited differential expression following gamma irradiation two ORFs, PF0926 and PF2025, showed the largest changes in mRNA levels. PF0926 mRNA level was decreased 4.5 folds at 40 min following irradiation (Table S1). This ORF encodes for a 75 amino acid protein unique to *P. furiosus* and with no matches in protein databases. In contrast, the mRNA level of PF2025 was increased 15-fold 40 min after gamma irradiation (Table S1). PF2025 is found in other archaea and bacteria, and the structure of a homologous protein from *Methanothermobacter thermautotrophicus* MTH1175 (44% identity) was solved (Christendat et al. 2000). MTH1175 belongs to a family of iron-molybdenum cluster-binding proteins that includes nitrogenase iron-molybdenum cofactors (Christendat et al. 2000). In a previous study PF2025 was found to be the most significantly up-regulated gene in *P. furiosus* grown in presence of sulfur along with another ORF, PF2026. Their products were renamed SipA and SipB (for sulfur-induced proteins), respectively, and Schut et al. (2001) suggested that they might constitute a membrane-associated complex involved in the reduction of S° in H_2S. In our study the mRNA level of PF2026 did not increased significantly following irradiation (Table S1).

Discussion

We investigated the transcriptional response of *P. furiosus* to ionizing radiation at six time points during the recovery period after irradiation. Following a dose of 2,500 Gy of ^{60}Co gamma ray, resulting in oxidative stress and DNA strand breaks, we found that about 15% of all *P. furiosus* ORFs were more than twofold up or down regulated for at least one time point during the recovery period (Table S1). At 20 min following irradiation a large fraction of the changes are decreases in mRNA levels suggesting a down-regulation of metabolic functions following stress induction. This trend was also observed in *H. salinarium* strain NRC-1 (Baliga et al. 2004), *Sulfolobus solfataricus* (Salerno et al. 2003), yeast (Gasch et al. 2000) and *E. coli* (Courcelle et al. 2001; Khil and Camerini-Otero 2002) following DNA damaging treatments and might represent an adaptation to stress common to the three domains of life.

To maintain a constant temperature during the irradiation procedure, we irradiated *P. furiosus* cells in presence of ice (Baliga et al. 2004; DiRuggiero et al. 1997). As a consequence, the cultures took 20 min to reach the incubation temperature of 90°C following irradiation. At this point in time (T_0) we found that the

mRNA of 65 ORFs changed significantly (> 2-folds, $\lambda > 20$), with mostly un-annotated ORFs. Among the ORFs that showed an increase in mRNA levels, we found the small heat shock protein *shsp* (PF1883), *radA* (PF1926), several ribosomal protein subunit encoding genes and membrane-bound oxidoreductase MBX genes, whereas the mRNA for a putative endonuclease (PF1298) and a *dnaG*-like primase (PF1725) showed a significant decrease. Although the rapid increase in temperature of the cultures (from 4 to 90°C in 20 min) could be equated to a form of heat shock, the temperature did not reach the upper limit for growth for *P. furiosus* and as a consequence many of the significant mRNA changes that we observed during the 240 min recovery period after irradiation differed from the mRNA changes reported in previous heat shock studies in *P. furiosus* and *A. fulgidus* (Rohlin et al. 2005; Shockley et al. 2003).

Repair of DNA damages

Very few DNA repair genes displayed significant differential expression in *P. furiosus* following gamma irradiation. For example, the genome of *P. furiosus* encodes for several DNA glycosylases and dGTPases specialized in the removal of oxidative damage, i.e. *nth* (thymine glycol glycosylase, PF1229), *alkA* (methyladenine DNA glycosylase/8-oxoguanine DNA glycosylase, PF0511) and *mutT* (8-oxo dGTPase, PF1590) but surprisingly none of those genes were induced after gamma exposure and the subsequent production of ROS. We found no evidence of SOS-type response to DNA damage and only a moderate increase in the mRNA level of the recombinase *radA* after irradiation. This is in contrast to *E. coli* and other bacteria where *recA* expression level increases up to 10-fold following DNA damaging treatments (Courcelle et al. 2001; Liu et al. 2003), but it is in agreement with previous studies of other mesophilic and hyperthermophilic archaea (Baliga et al. 2004; Komori et al. 2000; Reich et al. 2001). Furthermore, studies with *P. abyssi* did not show any significant increase in protein expression after exposure to ionizing radiation (Jolivet et al. 2003), although those studies only analyzed a limited number of proteins. In *S. solfataricus* cells exposed to UV radiation and actinomycin D, two proteins with a putative role in chromosome packaging were found to be inducible (Salerno et al. 2003), and only a few genes involved in the repair of photo-oxidative damage were up-regulated when *H. salinarium* strain NRC-1 was exposed to UV radiation (Baliga et al. 2004). Pretreatments of *Halobacterium halobium* and *S. solfataricus* to H_2O_2 and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (Praul and Taylor 1997), and *H. salinarium* strain NRC-1 to gamma irradiation (Kottemann et al. 2005) failed to elicit an inducible response to the deleterious effects of those treatments. Those studies taken together with the data we reported here suggest that DNA repair proteins in *P. furiosus* and several other archaea are constitutively expressed and that they may be present in the cell at a level sufficient to maintain the integrity of the cell's genetic material (Kottemann et al. 2005).

An exception to this paradigm is a set of genes highly inducible by gamma irradiation in *P. furiosus* and that constitutes a novel "thermophile-specific" DNA repair system (Makarova et al. 2002). Two homologous gene clusters are present in the genome of *P. furiosus* but the mRNA of only one of them, cluster 1, was found to greatly increase following irradiation. We did not find any significant increase in the mRNA of cluster 1 or 2 after treatment with EMS or H_2O_2 suggesting that cluster 1 might be specifically induced by DNA strand breaks resulting from direct and indirect effects of ionizing radiation (Riley 1994). Those lesions are significantly different from the DNA alkylation damage resulting from EMS treatments (Friedberg et al. 1995) and exposure of the cells to H_2O_2, which may induce a general oxidative stress to all the cellular components of this anaerobe in addition to damage to its DNA (Imlay 2003; Kultz 2005). Nevertheless it is remarkable that only *P. furiosus* cluster 1 is induced following gamma irradiation whereas cluster 2, composed of proteins very similar in sequence (18–26% identity) and in organization to cluster 1, did not show any change in expression level. The above gene clusters along with a novel-type of DNA polymerase (COG 1353; PF1129) were mostly found in thermophilic bacteria and archaea (Makarova et al. 2002). This finding prompted Makarova et al. (2002) to suggest that they might represent a "thermophile-specific DNA repair system" possibly involved in DNA translesion synthesis.

Protection against oxidative damage

P. furiosus is well equipped to face oxidative stress with systems including a superoxide reductase-dependent system for the removal of ROS, peroxiredoxin and thioredoxin systems involved in the elimination of H_2O_2 and the maintenance of redox balance, as well as enzymes for the removal of alkyl hydroperoxides and the chelation of ferric ions (Harris et al. 2005; Ward et al. 2001; Weinberg et al. 2004). Surprisingly, most of the systems involved in oxygen detoxification and redox homeostasis appeared to be constitutively expressed after gamma irradiation. This is supported by the observation that superoxide reductase, rubredoxin and rubrerythrin, along with two putative alkyl hydroperoxide reductases (AhpC, PF1033 and PF0722), were among the most highly expressed genes in our study. It is also in agreement with previous studies with *P. furiosus* that found superoxide reductase, rubredoxin and rubrerythrin to be highly expressed (Schut et al. 2003), and preliminary data that showed no significant effect on the expression level of those genes when H_2O_2 was added to the cells (Weinberg et al. 2004). In contrast, peroxiredoxins in *P. horikoshii* and *S. solfataricus* were induced

by exposure of the cells to exogenous oxygen (Kawakami et al. 2004) and H_2O_2 (Limauro et al. 2006) respectively, indicating possible induction of oxygen species detoxification systems in the archaea under specific conditions.

The very large increase in mRNA of a ferritin/Dps-like protein following gamma irradiation indicates its critical role in removing free iron from solution and thereby limiting the production of hydroxyl radicals by Fenton chemistry. Daly et al. (2004) suggested that in the extremely radioresistant bacterium, *D. radiodurans*, the high intracellular manganese/iron (Mn/Fe) ratio allowed for limited oxidative damage after gamma irradiation because Mn does not participate in the Fenton-type chemistry. In contrast, bacteria with low Mn/Fe intracellular ratio were found to be highly radiation sensitive (Daly et al. 2004), underlying the requirement for low intracellular free iron in radiation resistance. Recombinant *P. furiosus* Dps-like protein was found to self-assemble into a dodecameric cage-like quaternary structure that is analogous to multimeric assemblies of Dps and ferritin proteins and to catalyze Fe(II) oxidation using H_2O_2 as an oxidant, demonstrating a critical role for this protein in the reduction of H_2O_2 in the cell as well (Ramsay et al. 2006).

In response to oxidative stress we also found that the expression patterns of two membrane-bound oxidoreductase complexes were antagonistic suggesting different cellular functions for those two multimeric enzymes. MBH is a 14-subunit enzyme complex (*mbhABCDEFGHIJKLMN*) containing the two subunits of a Ni–Fe hydrogenase (Sapra et al. 2000). In Schut et al. study (2001), all 14-subunit encoding genes (with the exception of *mbhG* missing from the study) showed decreased expression level of more than fivefold in presence of sulfur, confirming the operon structure for the genes of this membrane-bound complex and the role of the enzyme in H_2 metabolism. Similarly, we observed a decrease in the mRNA level of all 14 genes shortly after gamma irradiation. This is not unexpected since down regulation of dehydrogenases under severe oxidative stress has been reported as the result of depletion of reducing equivalents used as co-factors by those enzymes, leading to loss of function (Golden and Ramdath 1987). The operon for the other membrane-bound hydrogenase complex, MBX was identified by Silva et al. (2000) using sequence comparison. It is a 13-subunit hydrogenase (*mbhABCDFGHH'MJKL'N*) with 45–72% similarity to 11 of the 14 subunits of MBH, but its catalytic subunit lacks the conserved cysteine residues found in all dehydrogenases. The finding that MBX mRNA increased by 10-fold less than 20 min after irradiation suggests that the enzyme might be involved in a response to oxidative stress. In *Desulfovibrio vulgaris* cytoplasmic [Fe] hydrogenase was up-regulated by exposure of the cells to oxygen and chromate and the [Fe] hydrogenase mutant was more sensitive to oxygen exposure than the wild type (Fournier et al. 2004). Van Ooteghem et al. (2002) reported similar findings with *Thermotoga neapolitana* supporting a transcriptional regulation for increased production of the enzyme when cells are under oxidative stress. One possible explanation for this up regulation of specific oxidoreductases during oxidative stress is the necessity for the cell to generate reducing equivalents for cellular antioxidant systems and for energy production, providing the energy necessary for other stress related functions such as DNA repair and proteins turnover (Kultz 2005).

This whole-genome study, along with those of *H. salinarium* strain NRC-1 (Baliga et al. 2004; Whitehead et al. 2006), clearly demonstrated that the transcriptional response to DNA damage in archaea differs from that of bacteria and that several stress response systems known to be inducible in mesophiles might be constitutively expressed in hyperthermophilic archaea (Gerard et al. 2001; Jolivet et al. 2003; Kottemann et al. 2005). The global mRNA changes we observed after gamma irradiation may be mediated by at least two transcriptional regulators and our results suggest that post-translational modification by phosphorylation/dephosphorylation may be significant in the regulation of the stress response in this microorganism. This has also been shown in several other archaea (Eichler and Adams 2005; Kottemann et al. 2005; Whitehead et al. 2006). The present work does not elucidate the mechanisms for extreme radiation resistance in *P. furiosus*, but similarly to *D. radiodurans*, a combination of factors is probably responsible for this physiological property (Cox and Battista 2005). Among those, efficient DNA repair pathways that are constitutively expressed as the result of life at high temperature, high level of oxygen detoxification enzymes in the cell, sequestration of soluble iron to prevent increase ROS production and potentially a novel thermophile-specific DNA repair system involved in translesion synthesis. Furthermore, the data presented here provide a foundation for comparing DNA damage response in the three domains of life and it generates hypotheses that can be experimentally tested using biochemical characterization of *P. furiosus* proteins, or genetic methods possibly in *P. furiosus* (Lucas et al. 2002).

Acknowledgments We thank Peter Kennelly for help in analyzing the protein kinase sequences from *P. furiosus*, and Rhonda Holley-shank for technical support. This work was supported by funds from NASA (NCC9147 to JDR) and the Human Frontier Science Program (RG522002 to JDR).

References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Baliga NS, Bjork SJ, Bonneau R, Pan M, Iloanusi C, Kottemann MCH, Hood L, DiRuggiero J (2004) Systems level insights into the stress response to UV radiation in the halophilic archaeon *Halobacterium* NRC-1. Genome Res 14:1025–1035

Bateman A, Birney E, Cerruti L, Durbin R, Etwiller L, Eddy SR, Griffiths-Jones S, Howe KL, Marshall M, Sonnhammer EL

(2002) The Pfam protein families database. Nucleic Acids Res 30:276–280
Bell SD, Cairns SS, Robson RL, Jackson SP (1999) Transcriptional regulation of an archaeal operon in vivo and in vitro. Mol Cell 4:971–982
Brinkman AB, Dahlke I, Tuininga JE, Lammers T, Dumay V, de Heus E, Lebbink JH, Thomm M, de Vos WM, van Der Oost J (2000) An Lrp-like transcriptional regulator from the archaeon *Pyrococcus furiosus* is negatively autoregulated. J Biol Chem 275:38160–38169
Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal Biochem 162:156–159
Christendat D, Yee A, Dharamsi A, Kluger Y, Savchenko A, Cort JR, Booth V, Mackereth CD, Saridakis V, Ekiel I, Kozlov G, Maxwell KL, Wu N, McIntosh LP, Gehring K, Kennedy MA, Davidson AR, Pai EF, Gerstein M, Edwards AM, Arrowsmith CH (2000) Structural proteomics of an archaeon. Nat Struct Biol 7:903–909
Corpet F, Servant F, Gouzy J, Kahn D (2000) ProDom and ProDom-CG: tools for protein domain analysis and whole genome comparisons. Nucleic Acids Res 28:267–269
Courcelle J, Khodursky A, Peter B, Brown PO, Hanawalt PC (2001) Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. Genetics 158:41–64
Cox MM, Battista JR (2005) *Deinococcus radiodurans*—the consumate survivor. Nature Rev Microbiol 3:882–892
Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, Venkateswaran A, Hess M, Omelchenko MV, Kostandarithes H M, Makarova KS, Wackett LP, Fredrickson JK, Ghosal D (2004) Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. Science 306:1025–1028
Deppenmeier U (2002) The unique biochemistry of methanogenesis. Prog Nucleic Acid Res Mol Biol 71:223–283
Dianov GL, O'Neill P, Goodhead DT (2001) Securing genome stability by orchestrating DNA repair: removal of radiation clustered leions in DNA. Bioessays 23:745–749
DiRuggiero J, Santangelo N, Nackerdien Z, Ravel J, Robb FT (1997) Repair of extensive ionizing-radiation DNA damage at 95°C in the hyperthermophilic archaeon *Pyrococcus furiosus*. J Bacteriol 179:4643–4645
DiRuggiero J, Robb FT (2004) Early evolution of DNA repair mechanisms. In: Ribas de Pouplana L (eds) The genetic code, the origin of life. Landes Biosciences pp 474–485
Eichler J, Adams MWW (2005) Posttranslational protein modification in archaea. Microbiol Mol Biol Rev 69:393–425
Fiala G, Stetter KO (1986) *Pyrococcus furiosus* sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100°C. Arch Microbiol 145:56–61
Fournier M, Dermoun Z, Durand MC, Dolla A (2004) A new function of the *Desulfovibrio vulgaris* Hildenborough [Fe] hydrogenase in the protection against oxidative stress. J Biol Chem 279:1787–1793
Friedberg EC, Walker GC, Siede W (1995) DNA repair and mutagenesis. ASM, Washington
Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO (2000) Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell 11:4241–4257
Geer LY, Domrachev M, Lipman DJ, Bryant SH (2002) CDART: protein homology by domain architecture. Genome Res 12:1619–1623
Gerard E, Jolivet E, Prieur D, Forterre P (2001) DNA protection mechanisms are not involved in the radioresistance of the hyperthermophilic archaea *Pyrococcus abyssi* and *P. furiosus*. Mol Genet Genom 266:72–78
Golden MH, Ramdath D (1987) Free radicals in the pathogenesis of kwashiorkor. Proc Nutr Soc 46:53–68
Grogan DW (2004) Stability and repair of DNA in hyperthermophilic archaea. Curr Issues Mol Biol 6:137–144
Guy CP, Haldenby S, Brindley A, Walsh DA, Briggs GS, Warren MJ, Allers T, Bolt EL (2006) Interactions of RadB, a DNA repair protein in archaea, with DNA and ATP. J Mol Biol 358:46–56
Hanks SK, Hunter T (1995) Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. FASEB J 9:576–596
Harris DR, Ward DE, Feasel JM, Lancaster KM, Murphy RD, Mallet TC, Crane III EJ (2005) Discovery and characterization of a Coenzyme A disulfide reductase from *Pyrococcus horikoshii*. FEBS J 272:1189–1200
Hutchinson F (1985) Chemical changes induced in DNA by ionizing radiation. Prog Nucl Acid Res Mol Biol 32:115–154
Ideker T, Thorsson V, Siegel AF, Hood LE (2000) Testing for differentially-expressed genes by maximum-likelihood analysis of microarray data. J Comput Biol 7:805–817
Imlay JA (2003) Pathways of oxidative damage. Ann Rev Microbiol 57:395–418
Ishino Y, Nishino T, Morikawa K (2006) Mechanisms of maintaining genetic stability by homologous recombination. Chem Rev 106:324–339
Jolivet E, Matsunaga F, Ishino Y, Forterre P, Prieur D, Myllykallio H (2003) Physiological responses of the hyperthermophilic archaeon "*Pyrococcus abyssi*" to DNA damage caused by ionizing radiation. J Bacteriol 185:3958–3961
Kawakami R, Sakuraba H, Kamohara S, Goda S, Kawarabayasi Y, Ohshima T (2004) Oxidative stress response in an anaerobic hyperthermophilic archaeon: presence of a functional peroxiredoxin in *Pyrococcus horikoshii*. J Biochem 136:541–547
Kelman Z, White MF (2005) Archaeal DNA replication and repair. Curr Opin Microbiol 8:669–676
Kennelly PJ (2003) Archaeal protein kinases and protein phosphatases: insights from genomics and biochemistry. Biochem J 370:373–389
Khil PP, Camerini-Otero RD (2002) Over 1000 genes are involved in the DNA damage response of *Escherichia coli*. Mol Microbiol 44:89–105
Komori K, Miyata T, DiRuggiero J, Holley-Shanks R, Hayashi I, Cann I K, Mayanagi K, Shinagawa H, Ishino Y (2000) Both RadA and RadB are involved in homologous recombination in *Pyrococcus furiosus*. J Biol Chem 275:33782–33790
Kottemann M, Kish A, Iloanusi C, Bjork S, Diruggiero J (2005) Physiological responses of the halophilic archaeon *Halobacterium* sp. strain NRC1 to desiccation and gamma irradiation. Extremophiles 9:219–227
Kultz D (2005) Molecular and evolutionary basis of the cellular stress response. Ann Rev Physiol. 67:225–257
Letunic I, Copley RR, Schmidt S, Ciccarelli FD, Doerks T, Schultz J, Ponting CP, Bork P (2004) SMART 4.0: towards genomic data integration. Nucleic Acids Res 32:D142–D144
Limauro D, Pedone E, Pirone L, Bartolucci S (2006) Identification and characterization of 1-Cys peroxiredoxin from *Sulfolobus solfataricus* and its involvement in the response to oxidative stress. FEBS J 273:721–731
Liu Y, Zhou J, Omelchenko MV, Beliaev AS, Venkateswaran A, Stair J, Wu L, Thompson DK, Xu D, Rogozin IB, Gaidamakova EK, Zhai M, Makarova KS, Koonin EV, Daly MJ (2003) Transcriptome dynamics of *Deinococcus radiodurans* recovering from ionizing radiation. Proc Natl Acad Sci USA 100:4191–4196
Lucas S, Toffin L, Zivanovic Y, Charlier D, Moussard H, Forterre P, Prieur D, Erauso G (2002) Construction of a shuttle vector for, and spheroplast transformation of, the hyperthermophilic archaeon *Pyrococcus abyssi*. Appl Environ Microbiol 68:5528–5536
Ma K, Weiss R, Adams MW (2000) Characterization of hydrogenase II from the hyperthermophilic archaeon *Pyrococcus furiosus* and assessment of its role in sulfur reduction. J Bacteriol 182:1864–1871
Maeder DL, Weiss RB, Dunn DM, Cherry JL, Gonzalez JM, DiRuggiero J, Robb FT (1999) Divergence of the hyperthermophilic archaea *Pyrococcus furiosus* and *P. horikoshii* inferred from complete genomic sequences. Genetics 152:1299–1305

Makarova KS, Aravind L, Grishin NV, Rogozin IB, Koonin EV (2002) A DNA repair system specific for thermophilic Archaea and bacteria predicted by genomic context analysis. Nucleic Acids Res 30:482–496

Marchler-Bauer A, Anderson JB, Cherukuri PF, DeWeese-Scott C, Geer LY, Gwadz M, He S, Hurwitz DI, Jackson JD, Ke Z, Lanczycki CJ, Liebert CA, Liu C, Lu F, Marchler GH, Mullokandov M, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Yamashita RA, Yin JJ, Zhang D, Bryant S H (2005) CDD: a conserved domain database for protein classification. Nucleic Acids Res 33:D192–D196

Ouhammouch M, Werner F, Weinzierl ROJ, Geiduschek EP (2004) A fully recombinant system for activator-dependent archaeal transcription. J Biol Chem 279:51719–51721

Peak JG, Ito T, Robb FT, Peak MJ (1995) DNA damage produced by exposure of supercoiled plasmid DNA to high- and low-LET ionizind radiation: effects of hydroxyl radical quenchers. Int J Radiat Biol 67:1–6

Praul CA, Taylor WD (1997) Responses of *Halobacterium halobium* and Sulfolobus solfataricus to hydrogen peroxide and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine [correction of *N*-methyl-*N*-nitrosoguanidine] exposure. Microbiol Res 152:257–260

Pulliainen AT, Kauko A, Haataja S, Papageorgiou AC, Finne J (2005) Dps/Dpr ferritin-like protein: insights into the mechanism of iron incorporation and evidence for a central role in cellular iron homeostasis in *Streptococcus suis*. Mol Microbiol 57:1086–1100

Ramsay B, Wiedenheft B, Allen M, Gauss GH, Martin Lawrence C, Young M, Douglas T (2006) Dps-like protein from the hyperthermophilic archaeon *Pyrococcus furiosus*. J Inorg Biochem 100(5–6):1061–1068

Reich CI, McNeil LK, Brace JL, Brucker JK, Olsen GJ (2001) Archaeal RecA homologues: different response to DNA-damaging agents in mesophilic and thermophilic Archaea. Extremophiles 5:265–275

Riley PA (1994) Free radicals in biology:oxidative stress and the effects of ionizing radiation. Int J Radiat Biol 65:27–33

Robb FT, Park JB, Adams MWW (1992) Characterization of an extremely thermostable glutamate dehydrogenase: a key enzyme in the primary metabolism of the hyperthermophilic archaebacterium *Pyrococcus furiosus*. Biochim Biophys Acta 1120:267–272

Rohlin L, Trent JD, Salmon K, Kim U, Gunsalus RP, Liao JC (2005) Heat shock response of *Archaeoglobus fulgidus*. J Bacteriol 187:6046–6057

Salerno V, Napoli A, White MF, Rossi M, Ciaramella M (2003) Transcriptional response to DNA damage in the archaeon *Sulfolobus solfataricus*. Nucl Acids Res 31:6127–6138

Sapra R, Verhagen MFJM, Adams MWW (2000) Purification and characterization of a membrane-bound hydrogenase from the hyperthermophilic archaeon *Pyrococcus furiosus*. J Bacteriol 182:3423–3428

Schut GJ, Zhou J, Adams MWW (2001) DNA microarray analysis of the hyperthermophilic archaeon *Pyrococcus furiosus*: evidence for a new type of sulfur-reducing enzyme complex. J Bacteriol 183:7027–7036

Schut GJ, Brehm SD, Datta S, Adams MWW (2003) Whole-genome DNA microarray analysis of a hyperthermophile and an archaeon: *Pyrococcus furiosus* grown on carbohydrates or peptides. J Bacteriol 185:3935–3947

Shockley KR, Ward DE, Chhabra SR, Conners SB, Montero CI, Kelly RM (2003) Heat shock response by the hyperthermophilic archaeon *Pyrococcus furiosus*. Appl Environ Microbiol 69:2365–2371

Silva PJ, van den Ban ECD, Wassink H, Haaker H, de Castro B, Robb FT, Hagen WR (2000) Enzymes of hydrogen metabolism in *Pyrococcus furiosus*. Eur J Biochem 267:6541–6551

Tahara M, Ohsawa A, Saito S, Kimura M (2004) In vitro phosphorylation of initiation factor 2 alpha (aIF2 alpha) from hyperthermophilic archaeon *Pyrococcus horikoshii* OT3. J Biochem (Tokyo) 135:479–485

Tatur J, Hagedoorn P-L, Overeijnder ML, Hagen WR (2005) A highly thermostable ferritin from the hyperthermophilic archaeal anaerobe *Pyrococcus furiosus*. Extremophiles 10(2):139–148

Theil EC (1987) Ferritin: structure, gene regulation, and cellular function in animals, plants, and microorganisms. Annu Rev Biochem 56:289–315

Van Ooteghem SA, Beer SK, Yue PC (2002) Hydrogen production by the thermophilic bacterium *Thermotoga neapolitana*. Appl Biochem Biotechnol 98–100:177–189

Vignais P, Colbeau A (2004) Molecular biology of microbial hydrogenases. Curr Issues Mol Biol 6:159–188

Ward DE, Donnelly CJ, Mullendore ME, van der Oost J, de Vos WM, Crane EJ 3rd (2001) The NADH oxidase from *Pyrococcus furiosus*. Implications for the protection of anaerobic hyperthermophiles against oxidative stress. Eur J Biochem 268:5816–5823

Watrin L, Prieur D (1996) UV and ethyl methanesulfonate effects in hyperthermophilic archaea and isolation of auxtrophic mutants of *Pyrococcus* strains. Curr Microbiol 33:377–382

Weinberg MV, Jenney FE Jr, Cui X, Adams MWW (2004) Rubrerythrin from the hyperthermophilic archaeon *Pyrococcus furiosus* is a rubredoxin-dependent, iron-containing peroxidase. J Bacteriol 186:7888–7895

Whitehead K, Kish A, Pan M, Kaur A, King N, Hohmann L, DiRuggiero J, Baliga NS (2006) Stress management: using a systems approach to understand stress response to gamma radiation. Mol Syst Biol (in press)