Supplemental Information

Materials and Methods

Strains and Culturing

The wild type strain of *Halobacterium salinarium strain NRC-1* was used for the gamma radiation experiments (1) and the Δ*ura3* mutant strain was from Dr. M.P. Krebs (Illinois State University, Normal, Illinois). Culturing of all strains was done in a liquid Complete Medium (CM; (2); at 42 °C with shaking at 220 rpm (Innova 4080, NewBrunswick Scientific, Edison, NJ).

Gamma Radiation and Response Time Course

γ-irradiation experiments were conducted as follows: Cell pellets from two 180mL cultures (control and experimental) of *Halobacterium NRC-1* (OD_{600nm}= 0.4) were resuspended in 1/20 volume in a basal salt solution (CM without peptone) and exposed to 2500 Gy of gamma-ray at 22°C (measured with an Omega Engineering Model HH 611PL4F Type K logging thermometer during irradiation) using a 26,000-curie (9.6E14 Bq) ^{60}Co gamma source at Univ. of Maryland College Park Gamma Test Facility at a dose rate of 62.01 Gy/min. Irradiated and control cultures were resuspended in the original volume of CM, split into 20mL aliquots in baffled flasks and incubated at 42°C and 220rpm shaking. Time course samples were placed on ice, pelleted (5000 x *g*, 4°C, 5 min) (Supplemental Fig. 1) and flash-frozen in a dry ice/ethanol bath after decanting the supernatant. RNA extractions were performed using the Stratagene Absolute RNA kit (La

Jolla, CA) and RNA quality checked with the Agilent Bioanalyzer (Palo Alto, CA) and with PCR.

Microarrays

Microarrays were fabricated at the Institute for Systems Biology Microarray Facility. The arrays contain 4 spots per unique 70-mer oligonucleotides for each of 2400 non-redundant genes in *Halobacterium NRC-1*. Labeling, hybridization and washing have been previously described (3). Bias in dye incorporation was accounted for by reversing the labeling dyes (dye-flip). Raw data was processed and converted into $\log_{10}$ ratios with lambda (λ) values determined by the maximum likelihood method (4).

Quantitative proteomics: iTRAQ Reagent Labeling, μLC-MS/MS and data analysis

Proteomic analysis was conducted at three time points (30, 40 and 60 min) for both control and γ irradiated cultures. Relative quantitation was achieved using shotgun isobaric tagging with iTRAQ reagents (Applied Biosystems, Foster City, CA) (5, 6). The primary amine-specific iTRAQ reagent tags virtually all proteins/peptides except those lacking both lysine and reactive N-terminal amino acids. Quantitation is achieved upon tandem MS, which fragments the iTRAQ reagents unevenly to release daughter products of differing mass (m/z 114, 115, 116 and 117). For direct comparison across multiple runs a common reference sample derivatized with the 114 mass tag was included in each four-plex experiment.

Soluble proteins were recovered by centrifugation of cell lysate (prepared by resuspending the cell pellet in 1mL of water + 1mM of PMSF (protease inhibitor)) and the insoluble

proteins were dissolved in 3mL of 10% SDS and centrifuged again. The combined (soluble + insoluble) protein preparation was treated with Benzoase nuclease (25 U/µl, 37 °C, 45 min) to remove nucleic acids and acetone precipitated. iTRAQ labeling was conducted as per manufacturer's instructions (Applied Biosystems). In brief, in each experiment 4 different protein samples (100 µg each) were separately denatured and reduced with tributylphosphine (TBP; 60°C, 1hr), blocked with Cysteine Blocking reagent, trypsinized (37°C, 1hr), labeled with one of four isobaric reagents (114,115, 116 and 117) (25°C, 10min) and mixed in equimolar ratios. The combined preparation was desalted by cation exchange, diluted with Buffer A (1:1) and acidified (4.5% of H_2PO_4 to pH = 2.9), fractionated by HPLC (Integral 100 Q, Applied Biosystems) and further desalted using UltraMicroSpin columns (Nest Group, Southborough, MA). The fractions were dried, dissolved in 0.4% acetic acid and analyzed by µLC/MS/MS analysis on an Applied Biosystems API QSTAR Pulsar I mass spectrometer, equipped with an in-house nanospray device using standard procedures.

Peptide and protein identification was achieved with COMET, SEQUEST, PeptideProphet and ProteinProphet and relative quantitation was conducted using the Libra algorithm (7) (8) (9). Libra integrates signal intensities of the reagent mass/charge (m/z), normalizes each peptide for a given protein by the sum of its channel intensities (114, 115, 116 and 117 isobars), removes values more than 2 standard deviations (σ) from the mean, recalculates the mean, and lastly calculates the 1- σ errors to improve the consistency of quantitation (6). The data for the two iTRAQ sets were then merged and loaded into the Gaggle suite (10).

Protein abundance changes were observed for key cellular processes including DNA repair and replication (RadA1, UvrD and GyrA), ribosome biogenesis (35 proteins detected), protease activity (PsmA1 and VNG0557H), transcriptional regulation (TfbB, TfbF, TfbG, Boa3, PrrC and Prrlv2), and several enzymatic steps from various biochemical pathways (e.g. ArcC, PyrG, YafB, CbiC) including 6 of the 8 downregulated dehydrogenases. All proteomic changes are consistent with our interpretations of physiological responses to γ radiation solely on the basis of transcriptional analysis. Due to the limited number of samples analyzed (three time points) and paucity of data for some individual proteins we are unable to cluster the protein data in a statistically meaningful manner. We further detected gene products for 4 ORFs not included among the initial gene predictions during the *Halobacterium NRC-1* genome annotation stressing the potential for proteomic analysis to add to the gene enumeration of classical methods for gene prediction. All four new ORFs have pI $\leq$ 4.5 typical for halobacterial proteins (11) and putative functions for two are given in Supplemental Table 3.

Data Integration and Visualization

Data analysis was performed using the Gaggle (12) and several of its inter-linking components including Cytoscape (13), Data Matrix Viewer (DMV), a 'kegg wbi' for metabolic pathways and Tile Viewer to examine ChIP/chip data (14). Statistical analysis was conducted with the TIGR MeV program (15) and the R package (www.r-project .org).

γ radiation survival of Δ*ura3* strain

Observed gene expression changes could be associated with direct repair of cellular damage, with pathways that manufacture necessary metabolites for the repair and recovery process or with an attempt to energetically accommodate costly repair processes. We tested the hypothesis that increased synthesis of nucleotide biosynthesis genes post-γ irradiation was necessary to provide the nucleotide pool required for DNA repair and replication activity by assaying the phenotype of a Δ*ura3* strain. This strain is deficient in orotidine 5'-phosphate decarboxylase and therefore defective in *de novo* uracil biosynthesis. Cell survival was evaluated in triplicate at 5000 Gy ($N/N_o = 0.11$ for wild-type cells) by counting survivor colonies on agar plates (16, 17). Average N/N_o of the *Δura3* strain was 0.095 ± 0.007 while wild type cells have an average N/N_o of 0.276 ± 0.024. Reduced survival of Δ*ura3* strain (34.4% lower than wildtype) is consistent with the importance of *de novo* nucleotide biosynthesis during recovery from γ-radiation damage.

Multiple mechanisms are triggered to minimize and repair γ radiation damage in *Halobacterium NRC-1*

Below we discuss in more detail the specific aspects of the γ radiation response involved in repair or avoidance of damage including (A) restoration of genome integrity, (C) modulation of dehydrogenases, redoxins and cytochromes to minimize ROS reactions, (D) inhibition of cell division, and (E) coordination of a response regulatory circuit.

A. DNA Repair: glycosylase activity and homologous recombination

Oxidative damage to nucleotides (18) can result in deleterious mutations during subsequent DNA replication. Damaged nucleotides are recognized and removed by glycosylases as part of the base excision repair (BER) pathway. Transcriptional upregulation of the DNA glycosylase Ogg was observed during the *Halobacterium NRC-1* response to γ radiation as well as an increase in Gap expression. While Ogg is an A/G specific DNA glycosylases function in BER based on primary sequence matches (COG0122; PF00730), Gap, on the other hand, is believed to participate in many cellular processes and has been shown to have uracil DNA glycosylase activity in eukaryotes (19, 20) and may have a similar multifunctional role in haloarchaea.

Archaeal proteins of the Homologous Recombination repair (HR) pathway are structurally and functionally similar those eukaryotes (21). The steps in HR repair include detection of the strand break and end processing, strand invasion and formation of heteroduplex DNA, branch migration and resolution of the Holliday junction. Similar to previous observations in bacteria (*recBCD)* (22) and eukaryotes (*mre11*/*rad50*) (23), the two putative subunits of the complex for DSB detection and processing in *Halobacterium NRC-1*, VNG0512G (*mre11*) and VNG0514C (*rad50*), were not differentially regulated after γ irradiation. Of the two RecA/Rad51 homologs in archaea, RadA (also called RadA1) and RadB (also called RadA2), only RadA can catalyze strand exchange (24). In *Halobacterium NRC-1* only RadA1 mRNA and protein levels increased during early γ response which parallels similar DNA damage-responsive regulation of this gene in other organisms (22). Likewise, of the two putative branched structure-specific endonucleases

(*hjr*, Holliday junction resolvase and *hef*, nuclease/helicase) only *hjr* was up regulated during the γ radiation response.

C. Mechanisms to minimize oxidative damage

Electron transport systems are especially sensitive to increased ROS production (25). Downregulation of 8 dehydrogenases (e.g. *adh2*, *adh3*, *sdh* and *mdh*), as was observed during halobacterial γ response and may reflect the depletion of intracellular reducing equivalents during severe oxidative stress (26) and an attempt to minimize ROS production by further oxidation reactions (25). Increased protein abundances for superoxide dismutase Sod2 might serve to scavenge the free radicals.

D. Coordinated expression between cell division and DNA replication and repair genes

Communication between DNA replication, repair and cell cycle progression is imperative to maintain genomic stability (27). Minichromosome maintenance proteins (Mcm) play essential roles in replication, and in humans, Mcm proteins appear to be recruited to HR sites by hRad51/52 interactions to initiate replication for repair (28). The functional similarity between archaeal Mcm/RadA1 and human Mcm/Rad51 (29) (30), and the upregulation of both components in *Halobacterium NRC-1* after γ irradiation are suggestive of a similar repair relationship in archaea (29). Furthermore, an inverse relationship was observed between mRNA changes of Mcm and Cdc48c, a CdcH ortholog putatively involved in cell division (Fig. 1B). This relationship is also observed under several other stress conditions (31); (32). This observation implies a pause in the cell

division cycle as has been observed in other organisms (27, 33, 34) and is believed to ensure completion of DNA repair prior to cell division.

E. The γ response regulatory network

Transcriptional regulation in haloarchaea is an amalgam of eukaryotic basal machinery and bacterial transcription regulators (35). The archaeal preinitiation complex consists of a homolog to eukaryotic RNA polymerase II enzyme (RNAP), TATA-binding proteins (TBPs) and Transcription Factor IIB homologs (TFBs) (35). Transcription is further modulated by regulatory proteins orthologous to bacterial regulators (36) of which there are approximately 130. During the γ response at least 9 regulators were upregulated along with one TBP (*tbpE*) and two TFBs (*tfbB* and *tfbF*). Downregulation of 5 regulators was observed, two which were of unknown function and have been newly annotated (Supplemental Table 3). Besides the 5 regulators, one TFB, *tfbG*, was also repressed during early gamma response.

We have used a protein-DNA interaction map for Halobacterium (14) to create a rudimentary regulatory network for the γ response to better understand the interaction between TFBs and protein regulators. Binding sites for TfbB, TfbF, TfbD or TfbG were identified upstream of 45% of the 216 γ responsive genes (Supplemental Fig. 5). The transcription factors bind promoters of over half of the regulators and protein kinases that were also differentially regulated during the γ response (grey nodes, Supplemental Fig. 5) several of which are also differentially regulated in response other stressor (31) (32), indicating a central roles in regulating stress responses.

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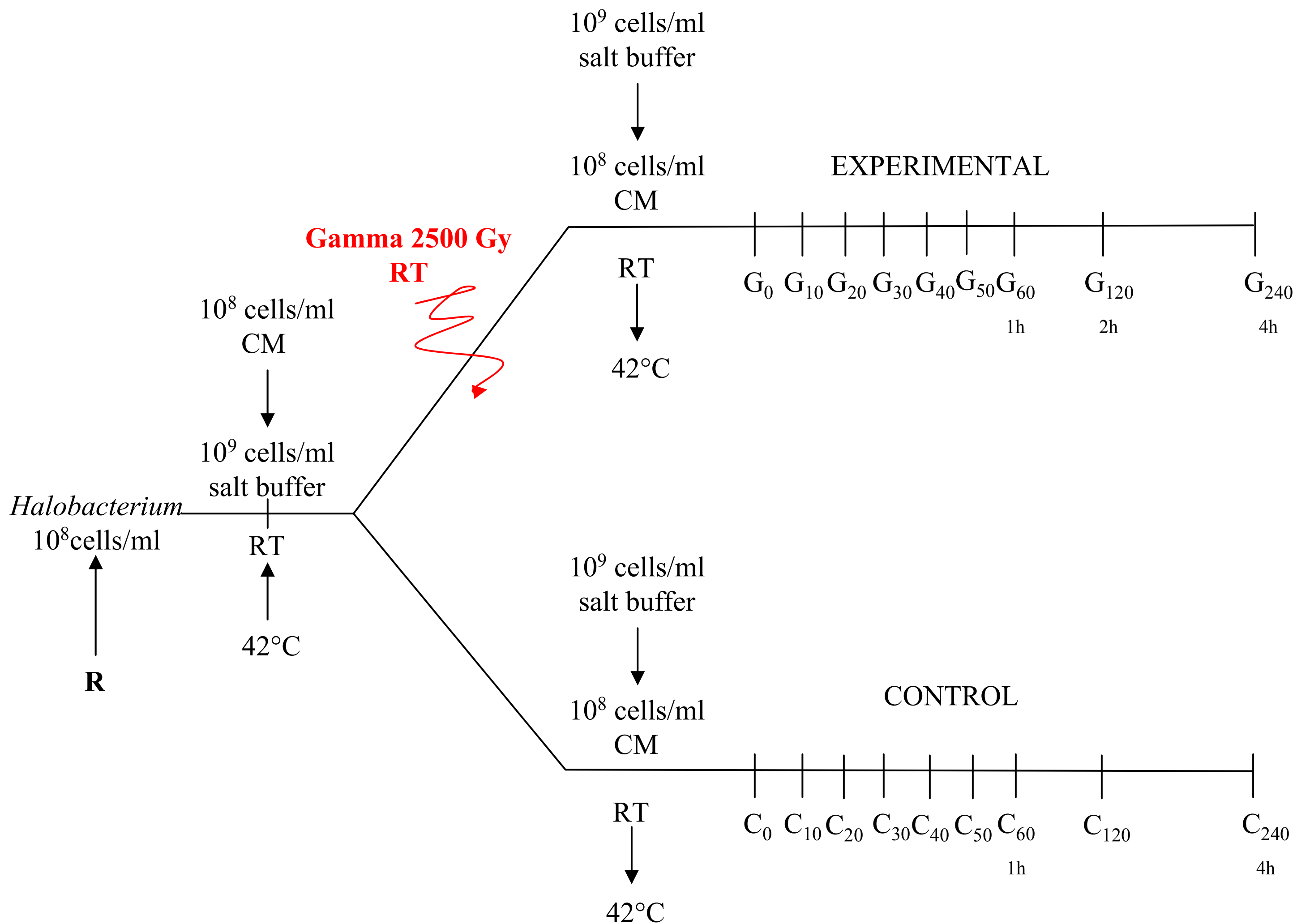
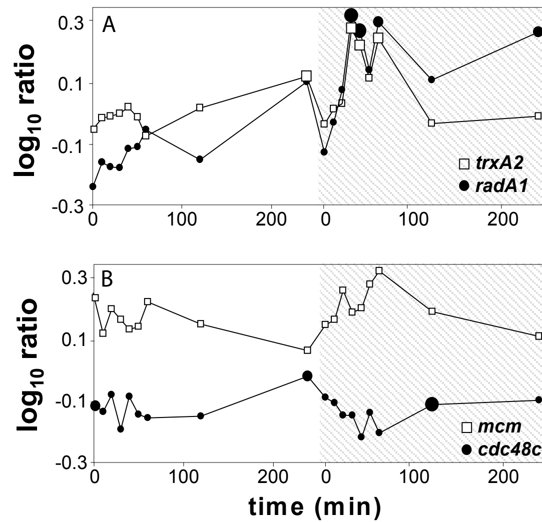
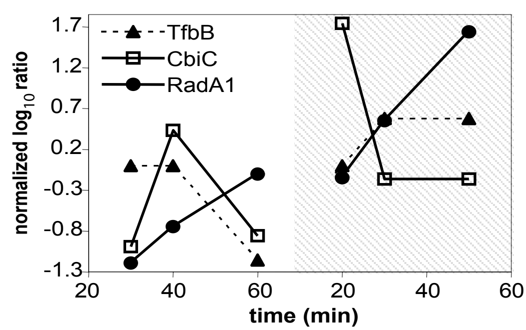
Supplemental Fig. 1. Experimental design for the global analysis of gamma response in *Halobacterium NRC-1*. Cells were harvested and concentrated via centrifugation before gamma exposure. After γ irradiation at 2500 Gy, cells were diluted back to their original volume in CM. Time points were taken as the cultures recovered. Both the control and irradiated samples were treated in the same manner. RT: room temperature.



R; reference RNA; C: control; G: experimental



Supplemental Figure 2. Expression profiles (log_{10} ratios) for *radA1*, *trxA2*, *mcm* and *cdc48C* from one of the two replicate experiments of control and gamma irradiated (shaded area) cultures over time (min). The size of the symbol relates to the statistical significance of the change (λ).



Supplemental Figure 3. Normalized $\log_{10}$ ratios of protein abundance of RadA1, TfbB and CbiC for both the control and γ irradiated (shaded area) cultures over time (min). The size of the symbol relates to the statistical significance of the change.

Supplemental Figure 4. Cytoscape view of the Halobacterium NRC-1 genome 40 min after gamma irradiation showing global repression with the exception of ribosome biogenesis clusters and DNA repair associated functions (for description of symbols, see legend Figure 1).