Comparative genomics of stress response systems in Deinococcus bacteria

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Abstract

Bacteria of the genus *Deinococcus* represent life's outer limits for the bounds of radiation and desiccation resistance. Using a comparative genomic approach, we investigated the genetic determinants of these extremophilic traits in *Deinococcus radiodurans*, *Deinococcus geothermalis* and *Deinococcus deserti*. Within this group, common evolutionary trends and a putative radiation response regulon were identified. Viewed from this perspective, contemporary hypotheses of extreme resistance were evaluated. Arguments are presented which support that the *Deinococcus* lineage emerged progressively by amassing enzymatic and non-enzymatic cell-cleaning systems, but not by acquisition of novel DNA repair systems.

Introduction

The bacterium *Deinococcus radiodurans* can typically survive acute exposures to ionizing radiation (IR) (≥12,000 Gy (gray; absorbed radiation dose)) (Daly et al. 2004), ultraviolet (UV) light (254 nm, 1,000 J per m^2) (Gutman et al. 1994), and desiccation (years) (Daly et al. 2004), and can grow under harsh oxidizing conditions of chronic irradiation (50 Gy per hour) (Daly et al. 2004). By comparison, *Escherichia coli* is killed by 200 Gy, 100 J per m^2, or a few weeks of drying (Daly et al. 2004, Gutman et al. 1994, Howard-Flanders et al. 1966). The first member of the *Deinococcaceae* to be isolated was *D. radiodurans*, originally from irradiated canned meat in the 1950s (Anderson et al. 1956). This bacterium belongs to the *Deinococcus–Thermus* group (Gupta 1998, Wolf et al. 2001). So far, the deepest branching species that belongs to *Deinococcaceae* is *Truepera radiovictrix*, which is both thermophilic and extremely IR-resistant (Albuquerque et al. 2005). To date, the natural distribution of the deinococci has still not been explored systematically. Members have been isolated worldwide but have diverse and patchy distributions (Daly 2009). Some species live in highly radioactive soils at nuclear waste sites (Fredrickson et al. 2004), some have settled on sandstone, marble and ice in Antarctica (Hirsch et al. 2004), and others are ubiquitous microbial inhabitants of the Sahara and other deserts (de Groot et al. 2005).

The survival characteristics of *D. radiodurans* and the prospects of exporting its protective processes outside of the host cell for practical purposes (Daly 2009, Makarova et al. 2001) has positioned this extremophile as a primary model to study stress response mechanisms, in particular for IR resistance. As a result, *D. radiodurans* (ATCC BAA-816) was one of the first whole-genomes to be sequenced (White et al. 1999). The annotated *D. radiodurans* genome became a platform for the earliest genome comparisons, together with attendant technologies (Lipton et al. 2002, Liu et al. 2003, Tanaka et al. 1996). High-throughput genome-based approaches for *D. radiodurans* were developed, and included

whole-transcriptome (Liu et al. 2003, Tanaka et al. 2004) and whole-proteome (Lipton et al. 2002, Tanaka et al. 1996) systems which were used to investigate gene expression in cells recovering from high-dose irradiation (Liu et al. 2003) and from desiccation (Tanaka et al. 2004).

Comparative genomics of *Deinococcaceae*

Rapid sequencing of complete genomes of organisms from the majority of known taxonomic groups has made possible the annotation of hundreds of organisms in the last decade (http://www.ncbi.nlm.nih.gov/sites/entrez?db=genome). As a scientific discipline, Comparative Genomics has begun to delineate the principles of genome organization, function and evolution, which has permitted scientists to explore and manipulate an organism's fundamental biology (Galperin and Koonin 2001, Koonin and Wolf 2008, Rodionov 2007). In this chapter, we describe the approaches and results of comparative genomic analysis of *Deinococcaceae* with an emphasis on stress response systems and their impact on contemporary models of extreme IR resistance.

The original report of the complete *D. radiodurans* genome was published in 1999, revealing a paradox. Although DNA in *D. radiodurans* is as susceptible to radiation damage as other bacteria (Blok and Loman 1973, Daly et al. 2004, Gerard et al. 2001, Gladyshev and Meselson 2008, Setlow and Duggan 1964), its genome encodes just about the same number and types of DNA repair proteins as radiation sensitive bacteria (Makarova et al. 2001, White et al. 1999). Further, a substantial number of putative stress-response genes identified in *D. radiodurans* previously had been found only in eukaryotes (White et al. 1999), and the hundreds of small DNA repeats dispersed throughout its genome seemed only to make more tenuous the prospect of understanding its repair pathways. Subsequent transcriptome and proteome analyses deepened the mystery (Lipton et al. 2002, Liu et al. 2003, Tanaka et al. 1996, Tanaka et al. 2004). Expression of genes in *D. radiodurans* after exposure to high-dose

irradiation was largely stochastic (Liu et al. 2003, Tanaka et al. 2004), and many of the novel genes implicated in IR resistance in *D. radiodurans* had little effect on survival when they were knocked out (Makarova et al. 2007). Then, a whole genome analysis of gene-gain and gene-loss between *D. geothermalis* and *D. radiodurans* showed that the number of novel genes that were thought to be implicated in recovery from IR was substantially reduced (Makarova et al. 2007). Similarly, comparisons between two *Thermus* species and *D. radiodurans* have provided many tantalizing clues in terms of genes that are shared by these organisms, to the exclusion of other organisms, and their possible functions, but so far have failed to establish an unequivocal molecular basis for thermophilicity or radioresistance.

Yet, from these seemingly irreconcilable genomic findings, a new perspective on IR resistance is emerging under the backdrop of older experimental studies. For example, the development of exceptionally high IR resistance in naturally sensitive bacteria is well-established. In 1961, Erdman *et al* (Erdman et al. 1961) first reported the directed evolution of IR-resistant *E. coli* by the repeated passage of survivors through successive sublethal doses of ^{60}Co irradiation. This work was followed in 1973 by similar studies and results published by Davies and Sinsky for *Salmonella typhimurium* (Davies and Sinskey 1973), and then in 1974 by Parisi and Antoine for *Bacillus pumilus* (Parisi and Antoine 1974). The stepwise approach to selecting bacterial radioresistance was validated once more in 2009 by Harris *et al*, followed by genome sequencing of the most IR-mutants, which revealed surprisingly few mutations (Harris et al. 2009). Collectively, these experimental results support that a relatively conventional set of DNA repair genes is sufficient for extreme IR resistance, but where subtle modifications to conventional DNA repair and metabolic pathways play an important role in promoting radiation resistance (Blasius et al. 2008, Cox and Battista 2005, Makarova et al. 2007).

General trends in evolution of Deinococcacae

Any comprehensive bioinformatics effort aimed at deciphering a complex, multi-gene phenotype using functional genomic approaches should aim to study as many closely related species as possible (Galperin and Koonin 2001, Rodionov 2007). The whole genome sequence of *Deinococcus deserti* was reported in 2009 (de Groot et al. 2009); *D. deserti* was isolated from Sahara surface sand, and is exceptionally resistant to IR, desiccation, and UV (de Groot et al. 2005, de Groot et al. 2009). Unlike the earlier *Deinococcus* annotations, the genome analysis of *D. deserti* was combined with a proteome shotgun analysis, which revealed that numerous *D. radiodurans* and *D. geothermalis* genes had been incorrectly annotated (de Groot et al. 2009). Thus, the *D. deserti* genome presents an opportunity to revisit previous *Deinococcus* annotations, which brings a fresh opportunity for comparison and experimentation.

Clusters of orthologous genes (COGs) are the most capable framework for comparative genomics (Tatusov et al. 2000). COGs for the *Deinococcus*/*Thermus* group (tdCOGs) have already been constructed using sequence data from the complete genomes of *D. radiodurans* and *D. geothermalis*, and two *Thermus* species (HB8 and HB27), which were compared and used to reconstruct the major evolutionary trends of gene-loss, gene-gain and family expansion in the *Deinococcus* lineage (Makarova et al. 2007, Omelchenko et al. 2005). We have now assigned the proteins of *D. deserti* to tdCOGs, which has reinforced our view of the proliferation of *Deinococcus* genes involved in stress response pathways. The first trend supported by the inclusion of *D. deserti* is the acquisition of a set of genes involved in transcriptional regulation and signal transduction. Examples of acquired transcriptional regulators include proteins of the AsnC, GntR, and IclR families, which are likely to be involved in amino acid degradation and metabolism (Gerischer 2002, Molina-Henares et al. 2006, Yokoyama et al. 2006). Further, the *Deinococcus* lineage acquired TetR and MerR family regulators dedicated to diverse stress

response pathways (Hobman et al. 2005, Ramos et al. 2005); and three groups of two-component regulators of the NarL family, involved in the regulation of a variety of oxygen and nitrate-dependent pathways (Bearson et al. 2002). A second evolutionary trend in *Deinococcus* is the acquisition of genes encoding proteins involved in nucleotide metabolism, in particular, degradation and salvage (Knofel and Strater 1999, Sandrini et al. 2006). For example, this group includes genes for deoxynucleoside kinases, thymidine kinase, FlaR-like kinase, and two UshA family 5'-nucleotidases. A third trend is the expansion of several families by gene acquisition and specific duplication (Table 1). Such *Deinococcus*-specific expanded families include the Yfit/DinB family of proteins, acetyltransferases of the GNAT family, Nudix hydrolases, α/β superfamily hydrolases, calcineurin family phosphoesterases, and others. Many of these expansions are for predicted hydrolases, phosphatases in particular, with unknown substrate specificities, which are proposed to facilitate the degradation of nucleic acids, proteins and lipids, and/or stress-induced cytotoxins (Galperin et al. 2006). To avoid autolysis, the proliferation of degradative functions for cellular macromolecules in *Deinococcus* certainly must be countered by special regulatory control mechanisms. Such degradative functions in *Deinococcus* were previously ascribed mainly to cell-cleaning and the removal of damaged macromolecules following irradiation or desiccation (Makarova et al. 2001). More recently, it has been proposed that the degradative functions might also contribute to a large depot of intracellular small molecules including nucleosides, peptides and inorganic phosphate, which together with divalent manganese ions are needed to protect proteins from oxidative damage (MJD, unpublished).

Deinococcus radiation response genes and regulation

Gene expression in *D. radiodurans* recovering from high-dose irradiation has been investigated using whole-genome microarrays, which identified hundreds of *D. radiodurans* genes that were

upregulated during recovery (Liu et al. 2003, Tanaka et al. 2004). From this group of upregulated genes, we previously used a comparative genomic approach based on *D. radiodurans* and *D. geothermalis* to delineate a set of genes involved in extreme resistance. Genes which were unique to both organisms were ruled out, whereas shared genes were pooled as candidates for involvement in resistance. Within the group of shared genes, we searched for a potential radiation-desiccation response regulon and a corresponding regulator (Makarova et al. 2007). First identified in *D. radiodurans*, the upstream regions of several upregulated genes contained a strong palindromic motif, designated the radiation/desiccation response motif (RDRM) (Makarova et al. 2007). Then, a genome survey using a RDRM position-specific matrix picked up a similar motif in the upstream regions of several *D. geothermalis* genes (Table 2). The RDR regulon was predicted to consist of at least 29 genes in *D. radiodurans*, and 25 genes in *D. geothermalis*, which were contained within 20 operons in both species. An equivalent search in the *D. deserti* genome revealed at least 13 genes shared with two other deinococci which featured RDRM upstream sites (Table 2) (de Groot et al. 2009).

The RDR regulon is dominated by DNA repair genes, including the recombinational repair proteins RecA and RecQ (Kunkel and Erie 2005, Kuzminov 1999); the mismatch repair proteins MutS and MutL (in two species); and the UvrB and UvrC proteins, which are involved in nucleotide excision repair (Table 2). In all three *Deinococcus* species the predicted RDR regulon also includes the transketolase gene, an enzyme of the pentose-phosphate pathway, which is known to be induced by a variety of stress conditions and mutagens that trigger the SOS response in other bacteria (Touati et al. 1996, Zhang et al. 2003). This finding has reinforced the notion that a coordinated metabolic response and a high level of respiratory control is a critical determinant of *D. radiodurans* survival (Bruce and Berner 1976, Ghosal et al. 2005, Liu et al. 2003).

Despite the parallels with SOS regulons in other bacteria, it is unlikely that LexA repressor is responsible for RDRM binding. Several experimental studies in *D. radiodurans* have demonstrated that its *lexA* genes are not induced by IR (Liu et al. 2003, Tanaka et al. 2004); its *lexA* genes are not involved in the induction of RecA (Narumi et al. 2001); and its *lexA* genes are not preceded by RDRM sites (Makarova et al. 2007). Another candidate regulator is the IrrE/PprI protein (Earl et al. 2002, Hua et al. 2003), which was originally considered as a signal for initiating the recovery response in *D. radiodurans*. However, IrrE/PprI was later shown to be constitutively expressed in *D. radiodurans*, showing no post-irradiation induction (Gao et al. 2006, Liu et al. 2003, Tanaka et al. 2004), and it did not bind the promoter region of *recA* or other genes induced by IR (Gao et al. 2006). Instead, there is some evidence that IrrE/PprI is a global regulator for the toxin-antitoxin systems of *D. radiodurans,* and could be responsible for cell stasis before the onset of DNA replication in acutely irradiated cells (Makarova et al. 2009). The only potential regulatory gene in *D. radiodurans*, *D. geothermalis* and *D. deserti* which contains the upstream RDRM site, encodes the *xre*-like DNA-binding protein DdrO (DR2574) (Tanaka et al. 2004). DdrO is currently the most plausible candidate for the global regulator of the RDR regulon.

Impact of the comparative-genomic analysis of *Deinococcus* genomes on resistance hypotheses

For a given dose of IR, the number of DNA double strand breaks (DSBs) inflicted per unit length of DNA in diverse organisms is similar. Values approximating 0.005 DSB $Gy^{-1}Mbp^{-1}$ have been reported for extremely IR-sensitive and extremely IR-resistant bacteria (Daly et al. 2004, Gerard et al. 2001); for IR-resistant archaeal species (Gerard et al. 2001, Kish et al. 2009); for yeast (Argueso et al. 2008); and for invertebrate animals (Gladyshev and Meselson 2008). *D. radiodurans* contains 4-8 identical copies of its genome per cell (Minton 1996). Yet, this level of genetic redundancy is not nearly

sufficient to impart to *D. radiodurans* its DNA damage-resistance (Minton 1996). For example, all eukaryotic cells in G2 are tetraploid, but typically are very IR-sensitive. Most bacteria with multiple chromosomes are also very IR-sensitive. For example, *Escherichia coli* contains 4-8 haploid genomes per cell but cannot survive 200 Gy which cause only 5 DSBs per genome (Daly et al. 2004); yet, *E. coli* can survive high levels of genome fragmentation under non-oxidizing conditions (Heitman et al. 1989). Early research demonstrated that DNA repair enzymes (*e.g.*, RecA, UvrA and PolA), which are central to recovery of irradiated bacteria in general, were equally important to *D. radiodurans* survival. The possibility that *D. radiodurans* encoded distinctly different versions of these enzymes, however, was ruled out. Several IR-sensitive *D. radiodurans* DNA repair mutants were fully complemented by expression of orthologous DNA repair genes from IR-sensitive bacteria ((Gutman et al. 1994) and covered/reviewed in (Makarova et al. 2007)). Thus, the extreme resistance phenotype appeared to be dependent, at least in part, on a conventional set of DNA repair functions (Daly et al. 2004). This has left the tantalizing question how a seemingly conventional set of DNA repair proteins in *D. radiodurans* is able to escape oxidative damage and proceed to reconstitute a genome shattered into hundreds of DSB fragments by IR. The impact of genome comparisons on three prevailing hypotheses of extreme IR resistance in *Deinococcaceae* follows.

Hypothesis I*: Chromosome Alignment and Nucleoid Morphology Facilitate Genome Reassembly*. *D. radiodurans* can recover from 180 IR-induced DSBs per haploid genome within 12 hours following an exposure to 12 kGy (Daly et al. 2004). In an early model, the alignment of its multiple identical chromosomes was tacitly assumed as the launching point for DSB repair (Minton and Daly 1995). This model made two major predictions: first, *recA*-dependent recombination between homologous DSB fragments originating from widely separated genomic locations should show strong positional effects

upon irradiation; and second, transmission electron microscopy (TEM) of chromosomal DNA in *D. radiodurans* should reveal evidence of structures linking chromosomes. Both predictions were tested and refuted: molecular studies showed high levels of recombination between homologous DSB fragments irrespective of their genomic origin (Daly et al. 1994, Daly and Minton 1996, Daly and Minton 1995); and no linking structures were observed by TEM-based optical mapping (Lin et al. 1999). Another model proposed that high levels of chromosomal condensation observed in *D. radiodurans* grown in rich medium facilitated repair by holding proximal DSB ends together; and that manganese promoted the condensation of its nucleoids into ringlike structures (Levin-Zaidman et al. 2003). This model is also generally discounted: *D. radiodurans* grown in defined minimal medium (DMM) did not display condensed nucleoids but remained extremely IR resistant; and *D. radiodurans* which was depleted in manganese displayed condensed ringlike nucleoids but was rendered IR-sensitive (Daly et al. 2004). Thus, IR-induced DSB fragments in irradiated *D. radiodurans* are not immobilized, and the structural form of its nucleoids does not play an important role in radioresistance. Within these conceptual frameworks, it has been shown that *D. radiodurans* contains numerous, unusual, mosaic-type small nuclear repeats (SNRs) (Makarova et al. 2001, Makarova et al. 1999, White et al. 1999) and G-quadruplex sequences (Makarova et al. 2007); both types of sequence potentially could contribute to genome structure and reassembly (Lin et al. 1999). However, shared SNRs and G-quadruplex sequences were not identified in the genomes of *D. geothermalis* (Makarova et al. 2007) or *D. deserti* (KSM, unpublished). In summary, we did not detect any distinctly unusual features which were conserved in the genomes of *D. radiodurans, D. geothermalis* and *D. deserti*. Thus, there is currently no functional genomic evidence supporting Hypothesis 1.

Hypothesis II*: A Subset of Uncharacterized Genes Encodes Novel Proteins that Enhance the Efficiency of DNA repair*. Experimental evidence supporting that *D. radiodurans* relies, at least in part, on a core

set of ordinary DNA repair proteins is now well-established (Blasius et al. 2008, Cox and Battista 2005, Makarova et al. 2007, Slade et al. 2009). This has left the question how repair enzymes in heavily irradiated *D. radiodurans* remain functionally active. The idea that a group of novel genes might facilitate recombination in some way was introduced soon after the *D. radiodurans* genome was published (Makarova et al. 2001, White et al. 1999). Whole-transcriptome studies on irradiated *D. radiodurans* were used to identify novel genes induced during recovery (Liu et al. 2003, Tanaka et al. 2004); there are only approximately 150 uncharacterized genes that are shared between the three *Deinococcus* genomes. Among those which were induced in irradiated *D. radiodurans*, only a few have a discernible functional relevance to the preservation of genome integrity (Table 3). One moderately IR-sensitive *D. radiodurans* mutant which has been constructed is *ddrB*$^-$ (DR0070), which encodes an extremely diverged single-strand binding protein (Norais et al. 2009). Another moderately IR-sensitive *D. radiodurans* mutant is *pprA*$^-$ (DRA0346), which is a putative DNA-binding protein (Kota and Misra 2006, Misra et al. 2006). However, for most of the mutants derived from this subset of novel genes there was no drastic change in the level of IR resistance, indicating that few of the putative resistance proteins, at least individually, make a substantial contribution to the recovery of irradiated *D. radiodurans*. Thus, functional genomic evidence supporting Hypothesis II has grown progressively weaker (Makarova et al. 2007).

Hypothesis III*: The level of Oxidative Protein Damage during Irradiation Determines Bacterial Radioresistance*. Hydroxyl radicals are the primary reactive oxygen species (ROS) generated by IR (Fig. 1), and indiscriminately damage all macromolecules. As individual proteins in a cell typically exist at much higher levels than their corresponding genes, IR-induced cell death has been attributed mainly to DNA damage (Daly 2009). However, extreme resistance among bacteria consistently coincides with a

greatly diminished susceptibility to IR-induced protein oxidation (Daly et al. 2007). It has been proposed that naturally sensitive bacteria are killed by IR mainly owing to protein oxidation, whereas manganese complexes in extremely resistant bacteria protect enzymes needed to repair DNA and allow survival (Daly et al. 2007, Daly et al. 2004). The correlation between protein oxidation and bacterial survival also extends to the ratio of intracellular manganese to iron concentrations. Bacteria with high manganese to iron ratios are extraordinarily resistant to IR-induced protein oxidation, whereas bacteria with low manganese to iron ratios are hypersensitive to protein oxidation (Daly 2009). The effects of radiation, desiccation and various other oxidizing agents are all mediated principally through ROS. The role of accumulated manganese in the chemical removal of ROS has been ascribed to the formation of small complexes. Inorganic phosphate and Mn^{2+} form complexes which catalytically remove superoxide (Barnese et al. 2008); and amino acids and peptides form complexes with Mn^{2+} which catalytically decompose hydrogen peroxide (Berlett et al. 1990) (Fig. 1).

In agreement with this hypothesis, it has been shown that the genes encoding Mn transporters are essential to the IR-resistant phenotype of *D. radiodurans* (Chang et al. 2009, Makarova et al. 2007). However, comparative genomic analysis has shown that the oxidative stress response systems of *D. radiodurans*, including Mn transport genes, cannot be considered as a specific acquisition in the *Deinococcus* lineage; most of the systems are ubiquitous and present in all bacteria (Makarova et al. 2007). The formation of Mn complexes is highly dependent on the availability of inorganic phosphate and free amino acids or peptides. Thus, the strong trend in the *Deinococcus* genomes of genes encoding phosphatases, nucleases and proteases are predicted to support the formation of Mn complexes (Ghosal et al. 2005, Makarova et al. 2001, Makarova et al. 2007). Regarding iron acquisition, *D. radiodurans* lacks most of the Fe-chelating and Fe-transport systems identified in IR-sensitive bacteria (Ghosal et al. 2005, Makarova et al. 2007); most iron in *D. radiodurans* is sequestered outside of the cytosol in the

septum between dividing cells (Daly 2009, Daly et al. 2007). It is also known that at least some of the desiccation related genes present in all three *Deinococcus* species (DRB0118/ DRA0258 orthologs) belong to a ferritin family (Omelchenko et al. 2005) which likely are involved in the storage of iron in a non-reactive state, which would attenuate intracellular Fenton chemistry (Fig. 1). Thus, functional genomic evidence is mounting in favor of hypothesis III.

Conclusion

The prospect of comparative genomics helping researchers resolve the seemingly paradoxical mechanism of extreme IR resistance in *Deinococcaceae* is good. Two additional whole-genome sequencing projects for *Deinococcus* are underway at the US Department of Energy's Joint Genome Institute: *Truepera radiovictrix* (http://genomesonline.org/GOLD_CARDS/Gi02949.html) and *Deinococcus grandis* (http://www.jgi.doe.gov/sequencing/cspseqplans2010.html) are expected to be completed in 2010. Based on historical and contemporary research, it now seems evident that the extreme IR resistance phenotype of *Deinococcaceae* stems from a subtle regulatory interplay between diverse but widespread systems including Mn homeostasis (Daly 2009), metabolite regulation (Ghosal et al. 2005), respiratory control (Bruce and Berner 1976, Liu et al. 2003), macromolecular degradation (Makarova et al. 2001, Sweet and Moseley 1976), and other oxidative stress response pathways (Makarova et al. 2001). In *Deinococcus* bacteria, these systems manifest themselves as protein protection, which preserves the activity of enzymes during irradiation (Daly 2009, Daly et al. 2007) or desiccation (Fredrickson et al. 2008). In contrast, irradiated or desiccated bacteria lacking these functions appear to be easily overwhelmed by protein oxidation (Daly 2009, Daly et al. 2007, Fredrickson et al. 2008), which renders even minor DNA damage unrepairable (Daly 2009, Makarova et al. 2007). The present comparative analysis serves as a background for our holistic view of IR

resistance: i) a reconstruction of the general evolutionary events which led to these three bacteria demonstrates the proliferation of redundant stress response systems and cell-cleaning protein families, and corresponding regulators; ii) the radiation and desiccation response regulon (RDR) is conserved and likely reflects the gene set which is important in the early stages of recovery; iii) the conserved set of radiation resistance determinants has been further refined, and contains many genes present in other organisms; iv) a small set of uncharacterized proteins specific to *Deinococcus* species has been delineated, but it cannot be ruled out that that these genes are involved in novel repair pathways or perhaps complement already known repair mechanisms; and v) numerous species-specific characteristics have been identified that illustrate the broad genetic context in which extreme resistance evolved. Collectively, these features represent new targets for investigation using classical and modern genetic approaches.

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Figure 1. Model of ionizing radiation-driven manganese and iron redox cycling. Water is the most abundant chemical found in living cells, and the primary ROS known to arise during the radiolysis of H_2O are hydroxyl radicals ($H_2O \rightarrow HO^{\bullet} + H^{+}$ [proton] + e^{-}_{aq} [hydrated electron]) (Daly 2009, von Sonntag 1987); hydrogen peroxide ($2\ HO^{\bullet} \rightarrow H_2O_2$) (Daly 2009, von Sonntag 1987); and superoxide anions ($O_2 + e^{-}_{aq} \rightarrow O_2^{\bullet-}$) (Daly 2009, von Sonntag 1987). Immediate cellular damage during exposure to IR is typically attributed to $HO^{\bullet}$. Whereas $HO^{\bullet}$ radicals are extremely reactive and short-lived, $O_2^{\bullet-}$ and H_2O_2 are relatively inert and long-lived (Daly 2009, von Sonntag 1987); this, however, does not imply that $HO^{\bullet}$ will display greater toxicity. For ROS, high reactivity without specificity is distributed

uniformly across cell targets; low reactivity with high specificity is focused on particular cellular targets (Omar et al. 1992). A secondary source of $HO^{\bullet}$ in cells during irradiation is the Fenton reaction, which is one of the most powerful oxidizing reactions known and involves the catalytic decomposition of H_2O_2 by ferrous ions (H_2O_2 + Fe(II) → Fe(III) + OH^- + $HO^{\bullet}$); the analogous reaction with Mn(II) does not occur (Daly et al. 2007). The most consequential damage by $O_2^{\bullet-}$ and H_2O_2 in cells is to proteins which contain exposed iron-sulfur or haem groups (Imlay 2008, Imlay 2006), to proteins which contain cysteine residues (Omar et al. 1992, Yan 2009), and to proteins containing cation-binding sites where an iron-catalyzed site-specific oxidation occurs (Stadtman and Levine 2006). It follows that the survival of irradiated enzymes and their hosts rests on preventing both non-specific ($HO^{\bullet}$) and site-specific ($O_2^{\bullet-}$ and H_2O_2) forms of ROS damage. Under IR, Fe(II,III) redox cycling is predicted to generate $HO^{\bullet}$ and $O_2^{\bullet-}$, whereas Mn(II,III) redox cycling is predicted to favor $O_2^{\bullet-}$ scavenging without $HO^{\bullet}$ production. Thus, manganese complexes are predicted to prevent the proliferation of iron-dependent ROS and protect diverse cellular functions (Daly 2009, Daly et al. 2007).

References

Albuquerque, L., C. Simoes, M. F. Nobre, N. M. Pino, J. R. Battista, M. T. Silva, F. A. Rainey, and M. S. da Costa. 2005. Truepera radiovictrix gen. nov., sp. nov., a new radiation resistant species and the proposal of Trueperaceae fam. nov. FEMS Microbiol Lett **247:**161-9.

Anderson, A., H. Nordan, R. Cain, G. Parrish, and D. Duggan. 1956. Studies on a radoresistant micrococcus. I. Isolation, morphology, cultural charchteristoics, and resistance to gamma radiation. Food Technology **10:**575-578.

Argueso, J. L., J. Westmoreland, P. A. Mieczkowski, M. Gawel, T. D. Petes, and M. A. Resnick. 2008. Double-strand breaks associated with repetitive DNA can reshape the genome. Proc Natl Acad Sci U S A **105:**11845-50.

Barnese, K., E. B. Gralla, D. E. Cabelli, and J. S. Valentine. 2008. Manganous phosphate acts as a superoxide dismutase. J Am Chem Soc **130:**4604-6.

Battista, J. R., M. J. Park, and A. E. McLemore. 2001. Inactivation of Two Homologues of Proteins Presumed to Be Involved in the Desiccation Tolerance of Plants Sensitizes Deinococcus radiodurans R1 to Desiccation. Cryobiology **43:**133-9.

Bearson, S. M., J. A. Albrecht, and R. P. Gunsalus. 2002. Oxygen and nitrate-dependent regulation of dmsABC operon expression in Escherichia coli: sites for Fnr and NarL protein interactions. BMC Microbiol **2:**13.

Berlett, B. S., P. B. Chock, M. B. Yim, and E. R. Stadtman. 1990. Manganese(II) catalyzes the bicarbonate-dependent oxidation of amino acids by hydrogen peroxide and the amino acid-facilitated dismutation of hydrogen peroxide. Proc Natl Acad Sci U S A **87:**389-93.

Blasius, M., S. Sommer, and U. Hubscher. 2008. Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol **43:**221-38.

Blok, J., and H. Loman. 1973. The effects of gamma-radiation in DNA. Curr Top Radiat Res Q **9:**165-245.

Bruce, A. K., and J. D. Berner. 1976. Respiratory activity as a determinant of radiation survival response. Can J Microbiol **22:**1336-44.

Chang, S., H. Shu, Z. Li, Y. Wang, L. Chen, Y. Hua, and G. Qin. 2009. Disruption of manganese ions [Mn(II)] transporter genes DR1709 or DR2523 in extremely radio-resistant bacterium Deinococcus radiodurans. Wei Sheng Wu Xue Bao **49:**438-44.

Cox, M. M., and J. R. Battista. 2005. Deinococcus radiodurans - the consummate survivor. Nat Rev Microbiol **3:**882-92.

Daly, M. J. 2009. A new perspective on radiation resistance based on Deinococcus radiodurans. Nat Rev Microbiol **7:**237-45.

Daly, M. J., E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, and M. Zhai. 2007. Protein Oxidation Implicated as the Primary Determinant of Bacterial Radioresistance. PLoS Biol **5:**769-779.

Daly, M. J., E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, A. Venkateswaran, M. Hess, M. V. Omelchenko, H. M. Kostandarithes, K. S. Makarova, L. P. Wackett, J. K. Fredrickson, and D. Ghosal. 2004. Accumulation of Mn(II) in Deinococcus radiodurans facilitates gamma-radiation resistance. Science **306:**1025-8.

Daly, M. J., O. Ling, and K. W. Minton. 1994. Interplasmidic recombination following irradiation of the radioresistant bacterium Deinococcus radiodurans. J Bacteriol **176:**7506-15.

Daly, M. J., and K. W. Minton. 1996. An alternative pathway of recombination of chromosomal fragments precedes recA-dependent recombination in the radioresistant bacterium Deinococcus radiodurans. J Bacteriol **178:**4461-4471.

Daly, M. J., and K. W. Minton. 1995. Interchromosomal recombination in the extremely radioresistant bacterium Deinococcus radiodurans. J Bacteriol **177:**5495-505.

Davies, R., and A. J. Sinskey. 1973. Radiation-resistant mutants of Salmonella typhimurium LT2: development and characterization. J Bacteriol **113:**133-44.

de Groot, A., V. Chapon, P. Servant, R. Christen, M. F. Saux, S. Sommer, and T. Heulin. 2005. Deinococcus deserti sp. nov., a gamma-radiation-tolerant bacterium isolated from the Sahara Desert. Int J Syst Evol Microbiol **55:**2441-6.

de Groot, A., R. Dulermo, P. Ortet, L. Blanchard, P. Guerin, B. Fernandez, B. Vacherie, C. Dossat, E. Jolivet, P. Siguier, M. Chandler, M. Barakat, A. Dedieu, V. Barbe, T. Heulin, S. Sommer, W. Achouak, and J. Armengaud. 2009. Alliance of proteomics and genomics to unravel the specificities of Sahara bacterium Deinococcus deserti. PLoS Genet **5:**e1000434.

Earl, A. M., M. M. Mohundro, I. S. Mian, and J. R. Battista. 2002. The IrrE protein of Deinococcus radiodurans R1 is a novel regulator of recA expression. J Bacteriol **184:**6216-24.

Erdman, I. E., F. S. Thatcher, and K. F. Macqueen. 1961. Studies on the irradiation of microorganisms in relation to food preservation. II. Irradiation resistant mutants. Can J Microbiol **7:**207-15.

Fredrickson, J. K., S. M. Li, E. K. Gaidamakova, V. Y. Matrosova, M. Zhai, H. M. Sulloway, J. C. Scholten, M. G. Brown, D. L. Balkwill, and M. J. Daly. 2008. Protein oxidation: key to bacterial desiccation resistance? Isme J **2:**393-403.

Fredrickson, J. K., J. M. Zachara, D. L. Balkwill, D. Kennedy, S. M. Li, H. M. Kostandarithes, M. J. Daly, M. F. Romine, and F. J. Brockman. 2004. Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the hanford site, washington state. Appl Environ Microbiol **70:**4230-41.

Funayama, T., I. Narumi, M. Kikuchi, S. Kitayama, H. Watanabe, and K. Yamamoto. 1999. Identification and disruption analysis of the recN gene in the extremely radioresistant bacterium Deinococcus radiodurans. Mutat Res **435:**151-61.

Galperin, M. Y., and E. V. Koonin. 2001. Comparative genome analysis. Methods Biochem Anal **43:**359-92.

Galperin, M. Y., O. V. Moroz, K. S. Wilson, and A. G. Murzin. 2006. House cleaning, a part of good housekeeping. Mol Microbiol **59:**5-19.

Gao, G., D. Le, L. Huang, H. Lu, I. Narumi, and Y. Hua. 2006. Internal promoter characterization and expression of the Deinococcus radiodurans pprI-folP gene cluster. FEMS Microbiol Lett **257:**195-201.

Gerard, E., E. Jolivet, D. Prieur, and P. Forterre. 2001. DNA protection mechanisms are not involved in the radioresistance of the hyperthermophilic archaea Pyrococcus abyssi and P. furiosus. Mol Genet Genomics **266:**72-8.

Gerischer, U. 2002. Specific and global regulation of genes associated with the degradation of aromatic compounds in bacteria. J Mol Microbiol Biotechnol **4:**111-21.

Ghosal, D., M. V. Omelchenko, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, A. Venkateswaran, M. Zhai, H. M. Kostandarithes, H. Brim, K. S. Makarova, L. P. Wackett, J. K. Fredrickson, and M. J. Daly. 2005. How radiation kills cells: survival of Deinococcus radiodurans and Shewanella oneidensis under oxidative stress. FEMS Microbiol Rev **29:**361-75.

Gladyshev, E., and M. Meselson. 2008. Extreme resistance of bdelloid rotifers to ionizing radiation. Proc Natl Acad Sci U S A **105:**5139-44.

Gupta, R. S. 1998. Protein phylogenies and signature sequences: A reappraisal of evolutionary relationships among archaebacteria, eubacteria, and eukaryotes. Microbiol Mol Biol Rev **62:**1435-91.

Gutman, P. D., P. Fuchs, and K. W. Minton. 1994. Restoration of the DNA damage resistance of Deinococcus radiodurans DNA polymerase mutants by Escherichia coli DNA polymerase I and Klenow fragment. Mutat Res **314:**87-97.

Gutman, P. D., P. Fuchs, L. Ouyang, and K. W. Minton. 1993. Identification, sequencing, and targeted mutagenesis of a DNA polymerase gene required for the extreme radioresistance of Deinococcus radiodurans. J Bacteriol **175:**3581-90.

Harris, D. R., S. V. Pollock, E. A. Wood, R. J. Goiffon, A. J. Klingele, E. L. Cabot, W. Schackwitz, J. Martin, J. Eggington, T. J. Durfee, C. M. Middle, J. E. Norton, M. C. Popelars, H. Li, S. A. Klugman, L. L. Hamilton, L. B. Bane, L. A. Pennacchio, T. J. Albert, N. T. Perna, M. M. Cox, and J. R. Battista. 2009. Directed evolution of ionizing radiation resistance in Escherichia coli. J Bacteriol **191:**5240-52.

Harris, D. R., M. Tanaka, S. V. Saveliev, E. Jolivet, A. M. Earl, M. M. Cox, and J. R. Battista. 2004. Preserving genome integrity: the DdrA protein of Deinococcus radiodurans R1. PLoS Biol **2:**e304.

Heitman, J., N. D. Zinder, and P. Model. 1989. Repair of the Escherichia coli chromosome after in vivo scission by the EcoRI endonuclease. Proc Natl Acad Sci U S A **86:**2281-5.

Hirsch, P., C. A. Gallikowski, J. Siebert, K. Peissl, R. Kroppenstedt, P. Schumann, E. Stackebrandt, and R. Anderson. 2004. Deinococcus frigens sp. nov., Deinococcus saxicola sp. nov., and Deinococcus marmoris sp. nov., low temperature and draught-tolerating, UV-resistant bacteria from continental Antarctica. Syst Appl Microbiol **27:**636-45.

Hobman, J. L., J. Wilkie, and N. L. Brown. 2005. A design for life: prokaryotic metal-binding MerR family regulators. Biometals **18:**429-36.

Howard-Flanders, P., R. P. Boyce, and L. Theriot. 1966. Three loci in Escherichia coli K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53:**1119-36.

Hua, Y., I. Narumi, G. Gao, B. Tian, K. Satoh, S. Kitayama, and B. Shen. 2003. PprI: a general switch responsible for extreme radioresistance of Deinococcus radiodurans. Biochem Biophys Res Commun **306:**354-60.

Huang, L., X. Hua, H. Lu, G. Gao, B. Tian, B. Shen, and Y. Hua. 2007. Three tandem HRDC domains have synergistic effect on the RecQ functions in Deinococcus radiodurans. DNA Repair (Amst) **6:**167-76.

Imlay, J. A. 2008. Cellular defenses against superoxide and hydrogen peroxide. Annu Rev Biochem **77:**755-76.

Imlay, J. A. 2006. Iron-sulphur clusters and the problem with oxygen. Mol Microbiol **59:**1073-82.

Kish, A., G. Kirkali, C. Robinson, R. Rosenblatt, P. Jaruga, M. Dizdaroglu, and J. DiRuggiero. 2009. Salt shield: intracellular salts provide cellular protection against ionizing radiation in the halophilic archaeon, Halobacterium salinarum NRC-1. Environ Microbiol **11:**1066-78.

Kitayama, S., M. Kohoroku, A. Takagi, and H. Itoh. 1997. Mutation of D. radiodurans in a gene homologous to ruvB of E. coli. Mutat Res **385:**151-7.

Kitayama, S., I. Narumi, M. Kikuchi, and H. Watanabe. 2000. Mutation in recR gene of Deinococcus radiodurans and possible involvement of its product in the repair of DNA interstrand cross-links. Mutat Res **461:**179-87.

Knofel, T., and N. Strater. 1999. X-ray structure of the Escherichia coli periplasmic 5'-nucleotidase containing a dimetal catalytic site. Nat Struct Biol **6:**448-53.

Koonin, E. V., and Y. I. Wolf. 2008. Genomics of bacteria and archaea: the emerging dynamic view of the prokaryotic world. Nucleic Acids Res **36:**6688-719.

Kota, S., and H. S. Misra. 2006. PprA: A protein implicated in radioresistance of Deinococcus radiodurans stimulates catalase activity in Escherichia coli. Appl Microbiol Biotechnol **72:**790-6.

Kunkel, T. A., and D. A. Erie. 2005. DNA mismatch repair. Annu Rev Biochem **74:**681-710.

Kuzminov, A. 1999. Recombinational repair of DNA damage in Escherichia coli and bacteriophage lambda. Microbiol Mol Biol Rev **63:**751-813, table of contents.

Lecointe, F., I. V. Shevelev, A. Bailone, S. Sommer, and U. Hubscher. 2004. Involvement of an X family DNA polymerase in double-stranded break repair in the radioresistant organism Deinococcus radiodurans. Mol Microbiol **53:**1721-30.

Levin-Zaidman, S., J. Englander, E. Shimoni, A. K. Sharma, K. W. Minton, and A. Minsky. 2003. Ringlike structure of the Deinococcus radiodurans genome: a key to radioresistance? Science **299:**254-6.

Lin, J., R. Qi, C. Aston, J. Jing, T. S. Anantharaman, B. Mishra, O. White, M. J. Daly, K. W. Minton, J. C. Venter, and D. C. Schwartz. 1999. Whole-genome shotgun optical mapping of Deinococcus radiodurans. Science **285:**1558-62.

Lipton, M. S., L. Pasa-Tolic, G. A. Anderson, D. J. Anderson, D. L. Auberry, J. R. Battista, M. J. Daly, J. Fredrickson, K. K. Hixson, H. Kostandarithes, C. Masselon, L. M. Markillie, R. J. Moore, M. F. Romine, Y. Shen, E. Stritmatter, N. Tolic, H. R. Udseth, A. Venkateswaran, K. K. Wong, R. Zhao, and R. D. Smith. 2002. Global analysis of the Deinococcus radiodurans proteome by using accurate mass tags. Proc Natl Acad Sci U S A **99:**11049-54.

Liu, Y., J. Zhou, M. V. Omelchenko, A. S. Beliaev, A. Venkateswaran, J. Stair, L. Wu, D. K. Thompson, D. Xu, I. B. Rogozin, E. K. Gaidamakova, M. Zhai, K. S. Makarova, E. V. Koonin, and M. J. Daly. 2003. Transcriptome dynamics of Deinococcus radiodurans recovering from ionizing radiation. Proc Natl Acad Sci U S A **100:**4191-6.

Makarova, K. S., L. Aravind, Y. I. Wolf, R. L. Tatusov, K. W. Minton, E. V. Koonin, and M. J. Daly. 2001. Genome of the extremely radiation-resistant bacterium Deinococcus radiodurans viewed from the perspective of comparative genomics. Microbiol Mol Biol Rev **65:**44-79.

Makarova, K. S., M. V. Omelchenko, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, A. Lapidus, A. Copeland, E. Kim, M. Land, K. Mavrommatis, S. Pitluck, P. M. Richardson, C. Detter, T. Brettin, E. Saunders, B. Lai, B. Ravel, K. M. Kemner, Y. I. Wolf, A. Sorokin, A. V. Gerasimova, M. S. Gelfand, J. K. Fredrickson, E. V. Koonin, and M. J. Daly. 2007. Deinococcus geothermalis: the pool of extreme radiation resistance genes shrinks. PLoS One **2:**e955.

Makarova, K. S., Y. I. Wolf, and E. V. Koonin. 2009. Comprehensive comparative-genomic analysis of type 2 toxin-antitoxin systems and related mobile stress response systems in prokaryotes. Biol Direct **4:**19.

Makarova, K. S., Y. I. Wolf, O. White, K. Minton, and M. J. Daly. 1999. Short repeats and IS elements in the extremely radiation-resistant bacterium Deinococcus radiodurans and comparison to other bacterial species. Res Microbiol **150:**711-24.

Mattimore, V., and J. R. Battista. 1996. Radioresistance of Deinococcus radiodurans: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J Bacteriol **178:**633-7.

Minton, K. W. 1996. Repair of ionizing-radiation damage in the radiation resistant bacterium Deinococcus radiodurans. Mutat Res **363:**1-7.

Minton, K. W., and M. J. Daly. 1995. A model for repair of radiation-induced DNA double-strand breaks in the extreme radiophile Deinococcus radiodurans. Bioessays **17:**457-64.

Misra, H. S., N. P. Khairnar, S. Kota, S. Shrivastava, V. P. Joshi, and S. K. Apte. 2006. An exonuclease I-sensitive DNA repair pathway in Deinococcus radiodurans: a major determinant of radiation resistance. Mol Microbiol **59:**1308-16.

Molina-Henares, A. J., T. Krell, M. Eugenia Guazzaroni, A. Segura, and J. L. Ramos. 2006. Members of the IclR family of bacterial transcriptional regulators function as activators and/or repressors. FEMS Microbiol Rev **30:**157-86.

Narumi, I., K. Satoh, M. Kikuchi, T. Funayama, T. Yanagisawa, Y. Kobayashi, H. Watanabe, and K. Yamamoto. 2001. The LexA protein from Deinococcus radiodurans is not involved in RecA induction following gamma irradiation. J Bacteriol **183:**6951-6.

Norais, C. A., S. Chitteni-Pattu, E. A. Wood, R. B. Inman, and M. M. Cox. 2009. DdrB protein, an alternative Deinococcus radiodurans SSB induced by ionizing radiation. J Biol Chem **284:**21402-11.

Omar, B. A., S. C. Flores, and J. M. McCord. 1992. Superoxide dismutase: pharmacological developments and applications. Adv Pharmacol **23:**109-61.

Omelchenko, M. V., Y. I. Wolf, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, M. J. Daly, E. V. Koonin, and K. S. Makarova. 2005. Comparative genomics of Thermus thermophilus and Deinococcus radiodurans: divergent routes of adaptation to thermophily and radiation resistance. BMC Evol Biol **5:**57.

Parisi, A., and A. D. Antoine. 1974. Increased radiation resistance of vegetative Bacillus pumilus. Appl Microbiol **28:**41-6.

Ramos, J. L., M. Martinez-Bueno, A. J. Molina-Henares, W. Teran, K. Watanabe, X. Zhang, M. T. Gallegos, R. Brennan, and R. Tobes. 2005. The TetR family of transcriptional repressors. Microbiol Mol Biol Rev **69:**326-56.

Rodionov, D. A. 2007. Comparative genomic reconstruction of transcriptional regulatory networks in bacteria. Chem Rev **107:**3467-97.

Sandrini, M. P., A. R. Clausen, B. Munch-Petersen, and J. Piskur. 2006. Thymidine kinase diversity in bacteria. Nucleosides Nucleotides Nucleic Acids **25:**1153-8.

Satoh, K., H. Ohba, H. Sghaier, and I. Narumi. 2006. Down-regulation of radioresistance by LexA2 in Deinococcus radiodurans. Microbiology **152:**3217-26.

Setlow, D. M., and D. E. Duggan. 1964. The resistance of Micrococcus radiodurans to ultraviolet radiation: Ultraviolet-induced lesions in the cell's DNA. Biochim. Biophys. Acta **87:**664-8.

Slade, D., A. B. Lindner, G. Paul, and M. Radman. 2009. Recombination and replication in DNA repair of heavily irradiated Deinococcus radiodurans. Cell **136:**1044-55.

Stadtman, E. R., and R. L. Levine. 2006. Chemical Modification of Proteins by Reactive Oxygen Species, p. 123-168. *In* I. Dalle-Donne, A. Scaloni, D. A. Butterfield, D. M. Desiderio, and N. M. Nibbering (ed.), Redox Proteomics: From Protein Modifications to Cellular Dysfunction and Diseases. Wiley Interscience, New York, USA.

Sweet, D. M., and B. E. Moseley. 1976. The resistance of Micrococcus radiodurans to killing and mutation by agents which damage DNA. Mutat Res **34:**175-86.

Tanaka, A., H. Hirano, M. Kikuchi, S. Kitayama, and H. Watanabe. 1996. Changes in cellular proteins of Deinococcus radiodurans following gamma- irradiation. Radiat Environ Biophys **35:**95-9.

Tanaka, M., A. M. Earl, H. A. Howell, M. J. Park, J. A. Eisen, S. N. Peterson, and J. R. Battista. 2004. Analysis of Deinococcus radiodurans's transcriptional response to ionizing radiation and desiccation reveals novel proteins that contribute to extreme radioresistance. Genetics **168:**21-33.

Tatusov, R. L., M. Y. Galperin, D. A. Natale, and E. V. Koonin. 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res **28:**33-36.

Touati, E., C. Laurent-Winter, P. Quillardet, and M. Hofnung. 1996. Global response of Escherichia coli cells to a treatment with 7-methoxy-2-nitronaphtho[2,1-b]furan (R7000), an extremely potent mutagen. Mutat Res **349:**193-200.

Udupa, K. S., P. A. O'Cain, V. Mattimore, and J. R. Battista. 1994. Novel ionizing radiation-sensitive mutants of Deinococcus radiodurans. J Bacteriol **176:**7439-46.

von Sonntag, C. 1987. The chemical basis of radiation biology. Taylor & Francis, London, United Kingdom.

White, O., J. A. Eisen, J. F. Heidelberg, E. K. Hickey, J. D. Peterson, R. J. Dodson, D. H. Haft, M. L. Gwinn, W. C. Nelson, D. L. Richardson, K. S. Moffat, H. Qin, L. Jiang, W. Pamphile, M. Crosby, M. Shen, J. J. Vamathevan, P. Lam, L. McDonald, T. Utterback, C. Zalewski, K. S. Makarova, L. Aravind, M. J. Daly, K. W. Minton, R. D. Fleischmann, K. A. Ketchum, K.

E. Nelson, S. Salzberg, H. O. Smith, J. C. Venter, and C. M. Fraser. 1999. Genome Sequence of the Radioresistant Bacterium Deinococcus radiodurans R1. Science **286:**1571-1577.

Wolf, Y. I., I. B. Rogozin, N. V. Grishin, R. L. Tatusov, and E. V. Koonin. 2001. Genome trees constructed using five different approaches suggest new major bacterial clades. BMC Evol Biol **1:**8.

Xu, G., L. Wang, H. Chen, H. Lu, N. Ying, B. Tian, and Y. Hua. 2008. RecO is essential for DNA damage repair in Deinococcus radiodurans. J Bacteriol **190:**2624-8.

Yan, L. J. 2009. Analysis of oxidative modification of proteins. Curr Protoc Protein Sci **Chapter 14:**Unit 14 4.

Yokoyama, K., S. A. Ishijima, L. Clowney, H. Koike, H. Aramaki, C. Tanaka, K. Makino, and M. Suzuki. 2006. Feast/famine regulatory proteins (FFRPs): Escherichia coli Lrp, AsnC and related archaeal transcription factors. FEMS Microbiol Rev **30:**89-108.

Zhang, Y. M., J. K. Liu, and T. Y. Wong. 2003. The DNA excision repair system of the highly radioresistant bacterium Deinococcus radiodurans is facilitated by the pentose phosphate pathway. Mol Microbiol **48:**1317-23.

Table 1. Protein family expansions specific for the *Deinococcus* lineage

Description	**COG Numbers[A]**	**Number of Representatives in DR[B]**	**Number of Representatives in DG[B]**	**Number of Representatives in DD[B]**	**Number of Representatives in TT[B] (HB27)**
Nudix (MutT-like) phosphohydrolases	COG0494 COG1051	17 5	7 7	5 15	5 5
Lipase-like alpha/beta hydrolase	COG0596 COG0400 COG1073 COG1075 COG0657	10 1 8 2 4	8 1 4 0 3	19 3 5 2 2	6 1 1 0 0
Subtilisin-like protease	COG1404	10	7	22	2
Acetyltrasferases GNAT family	COG0454 COG0497 COG1610	26 10 11	11 8 8	14 1 0	2 3 3
DinB/YfiT family	COG2318; no COG	3 10	2 6	6 13	1 1
Calcineurin like phosphoesterase	COG0639 COG1408	9 1	8 1	9 2	2 0
AcrR-like transcriptional regulators	COG1309	15	7	13	4
WD-40 repeats	COG1520	6	2	3	1
PR1 family	COG2340	5	2	5	1

[A]COG information: http://www.ncbi.nlm.nih.gov/COG/grace/uni.html
[B]Abbreviations: DR, *D. radiodurans*; DG, *D. geothermalis* ; DD, *D.deserti*; TT, *T.thermophilus*

Table 2. The predicted radiation and desiccation resistance regulon of *Deinococci*

[A]DR gene	Site in DR	[A]DG ortholog	Site in DG	[A]DD ortholog	Site in DD	[B]Tanaka *et al*	[C]Liu *et al*	Gene product name	Description and Comments
DR0070*	yes	Dgeo_0295	yes	Deide_02990	yes	yes	yes	DdrB	*Deinococcus* specific distant homolog of Single-stranded DNA-binding protein
DR0099	yes	Dgeo_0165	yes	Deide_00120	yes	no	yes	Ssb	Single-stranded DNA-binding protein
DR0219*	yes	no	-	no	-	yes	yes	DdrF	Predicted protein
DR1913*	yes	Dgeo_1016	yes (2)	Deide_12520	yes	yes	yes	GyrA	DNA gyrase (topoisomerase II) A subunit
DR0906*	yes	Dgeo_0546	yes	Deide_15490	yes	yes	yes	GyrB	DNA gyrase (topoisomerase II) B subunit
DR0423*	yes (2)	Dgeo_0977	-	Deide_09150	yes	yes	no	DdrA	Predicted DNA single-strand annealing protein, containing a HHH motif, Rad22/RecT family
DR0326*	yes	Dgeo_2186	yes	Deide_01160	yes	yes	no	DdrD	Predicted low complexity protein
reverse DR0003	yes	reverse Dgeo_0047	n/a	Deide_23280	yes	yes	yes	DdrC	Distant DinD homolog of DNA-damage-inducible protein
DRA0346*	yes	Dgeo_2628	yes	Deide_2p01380	yes	yes	no	PprA	PprA protein, involved in DNA damage resistance mechanisms
DR2256	yes	Dgeo_2283	yes (2)	Deide_00600	yes	no	yes	Tkt	Transketolase, Tkt
DR1039	yes	Dgeo_1537	yes (2)	Deide_15540	no	no	no	[D]MutS	DNA mismatch repair ATPase MutS
DR1696	yes	Dgeo_1538	yes	Deide_15600	no	no	no	[D]HexB/MutL	DNA mismatch repair enzyme, Hexb/MutL
DR1289	yes	Dgeo_1226	yes	Deide_11320	yes	no	no	RecQ	RecQ helicase
DR1775	yes	Dgeo_0868	yes	Deide_12100	yes	no	yes	UvrD	UvrD Superfamily I helicase
DR2275	yes	Dgeo_1890	yes	Deide_03120	yes	no	yes	UvrB	Helicase subunit of the DNA excision repair complex, UvrB
DR0596	yes	Dgeo_0404	yes	Deide_18350	no	yes	yes	RuvB	Holliday junction resolvasome, helicase subunit, RuvB
DR2338	yes	Dgeo_2136	yes	Deide_19450 Deide_1p01260 Deide_3p00210	yes yes yes	yes	yes	CinA LigT RecA	CinA ortholog, MoeA family, first gene in operon containing RNA ligase ligT and RecA; *D.deserti* has a RecA specific duplication
DR1771	yes	Dgeo_0694	yes	Deide_12760	yes	yes	yes	UvrA	Excinuclease ATPase subunit, UvrA
DR2574	yes	Dgeo_0336	yes	Deide_02843	yes	yes	yes	DdrO	HTH transcription factor, phage type
DRA0151	yes	Dgeo_2735	yes	Deide_15250	no	no	yes	HutUHIG	Urocanate hydratase; histidine degradation
DR1921	yes	Dgeo_0824	yes	Deide_16180	no	no	no	SbcD	SbcD, DNA repair exonuclease
no	-	Dgeo_2035	yes	Deide_04721	yes	no	no		Zinc finger protein, function unknown

[A]Abbreviations: DR, *D. radiodurans*; DG, *D. geothermalis;* DD, *D.deserti*;

[B]Induction in whole-genome microarrays reported by Tanaka *et al* (Tanaka et al. 2004) and [C] in Liu *et al* (Liu et al. 2003).

[D]In *D. geothermalis*, MutS and MutL are in the same operon, therefore RDRM information is shown only for Dgeo_1537 (the first gene in the operon).

* RDRM sites included in the final profile were used to scan the genomes of *D. radiodurans* and *D. geothermalis*.

Table 3. Selected *D. radiodurans* genes implicated in radiation resistance

[A]DR gene	[A]DG ortholog	[A]DD ortholog	Homologs in other organisms (COG number)[B]	[C]Reported Induction in Microarrays	[D]Reference	Description and Comments
Strong effect on radioresistance						
DR2340 (recA)	Dgeo_2138	Deide_19450	COG0468	+/+	(Liu et al. 2003, Tanaka et al. 2004)	RecA recombinase.
DR1707 (polA)	Dgeo_1666	Deide_15130	COG0258/ COG0749	-/-	(Gutman et al. 1993, Mattimore and Battista 1996)	DNA Polymerase A, PolA.
DR0819 (recO)	Dgeo_0855	Deide_13810	COG1381	-/-	(Xu et al. 2008)	DNA annealing during homologous recombination
DRA0346 (pprA)	Dgeo_2628	Deide_2p01380	-	+/+	(Liu et al. 2003, Tanaka et al. 2004)	PprA protein, involved in DNA damage resistance mechanisms.
DR0423 (ddrA)	Dgeo_0977	Deide_09150	COG4712	+/-	(Liu et al. 2003, Tanaka et al. 2004); (Harris et al. 2004)	Predicted DNA single-strand annealing protein, containing HHH motif, Rad22/RecT family.
DR0167 (irrE)	Dgeo_0395	Deide_03030	COG2856	-/-	(Earl et al. 2002)	Regulatory Zn-dependent protease fused to HTH transcriptional regulator domain.
DR0070 (ddrB)	Dgeo_0295	Deide_02990	-	+/+	(Liu et al. 2003, Tanaka et al. 2004)	Uncharacterized conserved protein.
DR1477 (recN)	Dgeo_1194	Deide_12310	COG0497	-/-	(Funayama et al. 1999)	DNA repair protein
Moderate effect on radioresistance						
DR0596 (ruvB)	Dgeo_0404	Deide_18350	COG2255	+/+	(Kitayama et al. 1997, Liu et al. 2003, Tanaka et al. 2004)	Holliday junction resolvasome, helicase subunit, RuvB.
DR1289	-	Deide_06510	COG0514	-/-	(Huang et al. 2007)	DNA helicase of RecQ family
DR1771 (uvrA)	Dgeo_0694	Deide_12760	COG0178	+/+	(Liu et al. 2003, Tanaka et al. 2004)	Excinuclease ATPase subunit, UvrA.
DR1709	Dgeo_0709	Deide_3p02300	COG1914	+/-	(Chang et al. 2009, Tanaka et al. 2004)	NRAMP family membrane transporter
DR0003 (ddrC)	Dgeo_0047	Deide_23280	-	+/+	(Liu et al. 2003, Tanaka et al. 2004)	Uncharacterized conserved protein
DR0194 (ddrE)	Dgeo_1282	Deide_11220	COG2738	+/-	(Tanaka et al. 2004)	Zn-dependent protease, HTPX superfamily
DR0326 (ddrD)	Dgeo_2186	Deide_01160	-	+/NA	(Tanaka et al. 2004)	Predicted low-complexity protein.

DR0171 (irrI)	-	Deide_22910	A/B	-/+	(Udupa et al. 1994) (Liu et al. 2003)	HTH transcriptonal regulator
DR0467	Dgeo_1609	Deide_07030	COG1796/ COG1387	-/-	(Lecointe et al. 2004)	DNA polymerase of the X family
No effect on radioresistance						
DR2221	-	-	COG2310	-/-	(Liu et al. 2003, Makarova et al. 2007)	Tellurium resistance protein TerZ/TerD.
DR1262 (rsr)	-	-	B/E	+/-	(Tanaka et al. 2004)	Ro-like RNA binding protein
DR1172	Dgeo_1473 Dgeo_1798	Deide_01434	B/E	-/-	(Makarova et al. 2001); (Battista et al. 2001)	LEA76/LEA26-like desiccation-induced protein. Mutant sensitive to desiccation but not to radiation
DR0140	-	-	-	-/+	(Makarova et al. 2001, Makarova et al. 2007)	Hypothetical protein.
DRB0118	Dgeo_0097 Dgeo_1323	Deide_07540	A/B/E	-/-	(Battista et al. 2001, Makarova et al. 2001)	Desiccation-induced protein. The mutant is resistant to radiation but sensitive to desiccation.
DRB0100 (ddrP)	-	-	A/B/E	+/+	(Liu et al. 2003, Makarova et al. 2007, Tanaka et al. 2004)	Homolog of eukaryotic DNA ligase III.
DRA0344	Dgeo_1366	Deide_01180	COG1974	-/-	(Satoh et al. 2006)	LexA ortholog.
DR0189 (recR)	Dgeo_1248	Deide_08290	COG0353	-/-	(Kitayama et al. 2000)	RecR, the mutant is sensitive to DNA interstrand cross-linking agents but resistant to UV and IR.
Up-regulated after irradiation, unknown effect on radioresistance						
DR2574 (ddrO)	Dgeo_0336	Deide_02843	COG1396	+/+	(Liu et al. 2003, Tanaka et al. 2004)	HTH transcription factor, phage type.
DR0438 (ddrH)	-	Deide_20641	-	+/-	(Tanaka et al. 2004)	Uncharacterized conserved protein, probably secreted.
DR0219 (ddrF)	-	-	-	+/+	(Liu et al. 2003, Tanaka et al. 2004)	Predicted protein
DR1263 (ddrJ)	-	-	COG3236	+/+	(Liu et al. 2003, Tanaka et al. 2004)	Uncharacterized protein conserved in bacteria.
DR1264 (ddrK)	-	-	-	+/+	(Liu et al. 2003, Tanaka et al. 2004)	Predicted protein.

[A]Abbreviations: DR, *D. radiodurans*; DG, *D. geothermalis* ; DD, *D. deserti*; TT, *T. thermophilus*

[B]COG information: http://www.ncbi.nlm.nih.gov/COG/grace/uni.html; In not in COGs the lineages where homologs are found are listed as follows: A– homologs in archaea, B – bacteria, E –eukaryotes.

[C]Induction in DR whole-genome microarrays reported by Tanaka *et al* (Tanaka et al. 2004) versus results by Liu *et al* (Liu et al. 2003); +, induced; -, not induced; NA, microarray result is not available.

[D] References include original papers where the gene was inferred to be involved in radiation resistance or the corresponding mutant of the gene has been studied.

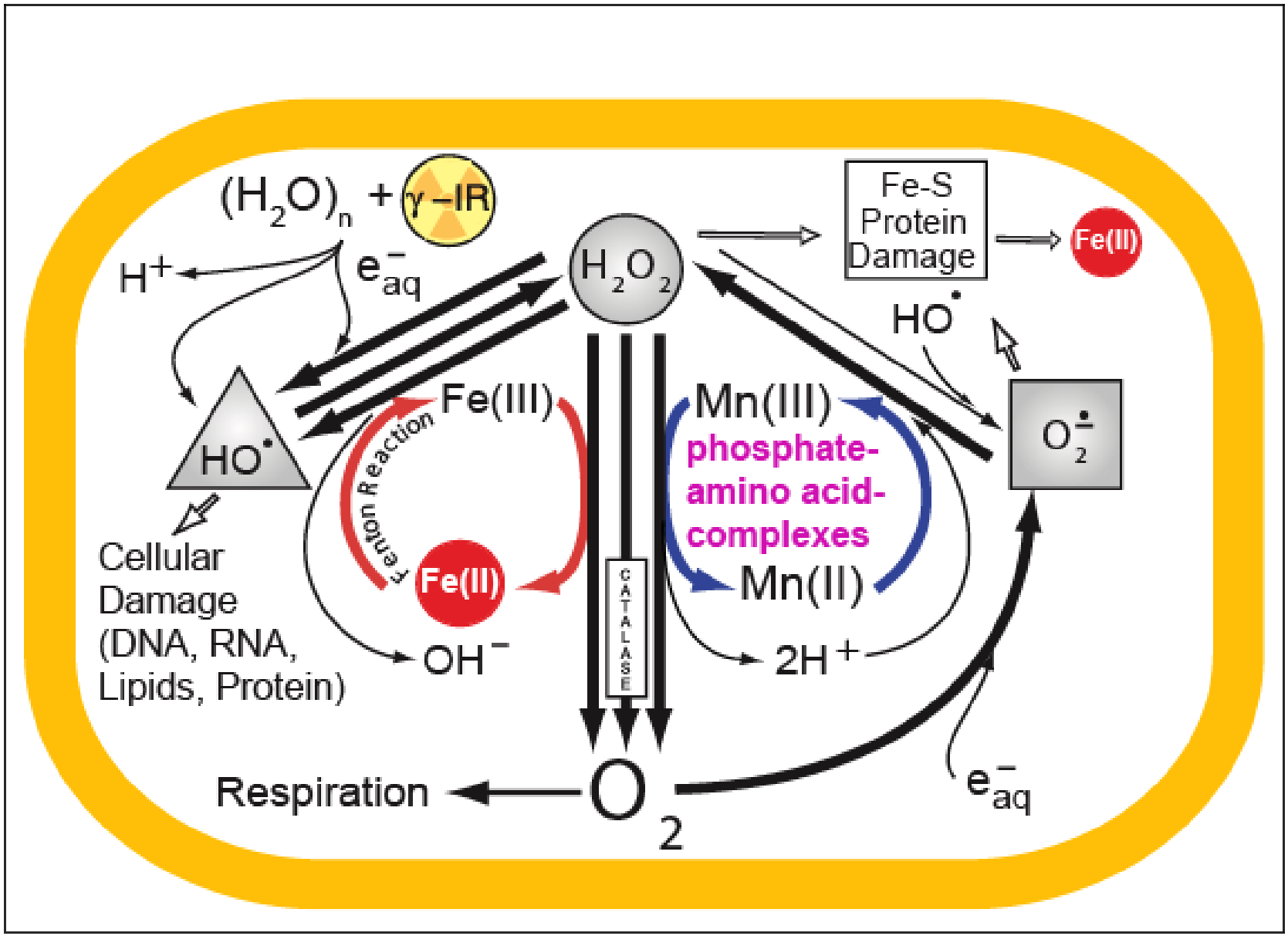
$(H_2O)_n$ + γ –IR
H^+
e^-_{aq}
H_2O_2
Fe-S Protein Damage
Fe(II)
HO•
HO•
Fe(III)
Fenton Reaction
Fe(II)
Mn(III)
phosphate-amino acid-complexes
Mn(II)
$O_2^{\bullet-}$
Cellular Damage (DNA, RNA, Lipids, Protein)
OH^-
$2H^+$
CATALASE
Respiration
O_2
e^-_{aq}

Figure 1