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RADIATION RESISTANCE IN EXTREMOPHILES: FENDING OFF MULTIPLE ATTACKS

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1. Extremophiles and Radiation Resistance

Over the last few decades, studies of extremophiles have revealed an astonishing array of adaptations to the harsh environmental conditions to which those organisms are exposed (Cavicchioli et al., 2011). However, an extreme environment, from our point of view, is not extreme for the organisms that are specifically adapted to this environment. Hyperthermophilic organisms not only thrive at temperatures near the boiling point of water, but they also require those high temperatures for their cellular machinery to function. As an example, the glutamate dehydrogenase from *Pyrococcus furiosus*, a hyperthermophilic archaeon, does not function below 45 °C (Klump et al., 1992). Similarly, cells of the halophilic archaeon, *Halobacterium salinarum*, will lyse if the osmotic pressure of their aqueous environment decreases below 3 M salt (DasSarma and DasSarma, 2012).

An interesting case is that of radiation-resistant bacteria. They have garnered a great deal of attention from scientists seeking to expose the mechanisms underlying their incredible survival abilities. These microorganisms were most likely not exposed to extremes of ionizing radiation (IR) over geological times (Mattimore and Battista, 1996), raising the question of their adaptation to such high doses of radiation. Early work on the desiccation resistance of the extremely radiation-resistant bacterium, *Deinococcus radiodurans*, and more recent environmental studies have revealed that it is the adaptation to extremely dry environments and high tolerance to desiccation that impart IR resistance to these organisms (Mattimore and Battista, 1996; Fredrickson et al., 2008). In other words, the IR resistance in bacteria is an incidental mechanism evolved to resist the cellular damage induced by desiccation (Fredrickson et al., 2008). In that regard, IR-resistant organisms are true polyextremophiles.

The distribution of radiation-resistant organisms in the phylogenetic tree of life is not limited to bacteria (Fig. 1). Recent work has revealed the high level of IR resistance of several eukaryotes: the basidiomycete fungus *Ustilago maydis* (Holliday, 2004), the freshwater invertebrate animal *Philodina roseola* (Gladyshev and Meselson, 2008), the water bear *Milnesium tardigradum* (Horikawa et al., 2006), and the roundworm *Caenorhabditis elegans* (Johnson and Hartman, 1988). Among the Archaea, the halophilic archaeon *H. salinarum*, in addition to being

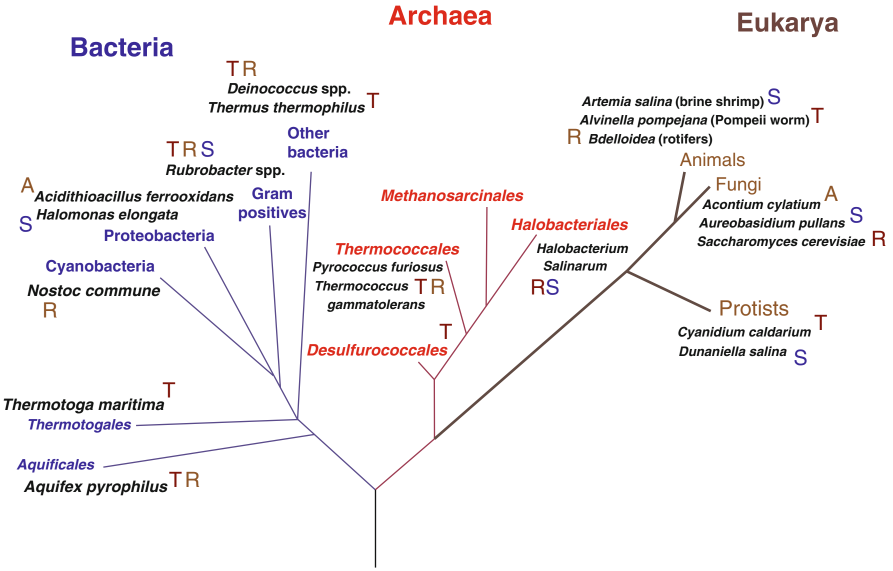


Figure 1. Phylogenetic tree of life with the distribution of extremophiles and radiation-resistant organisms. *T* thermophiles, *S* halotolerants and halophiles, *A* acidophiles, *R* radiation and desiccation resistant.

adapted to high salt, also shows a high level of resistance to desiccation, high pressure, UV radiation, and IR (Kish et al., 2012; Robinson et al., 2011; Kottemann et al., 2005). This is also true for a number of thermophilic archaea, including the sulfate-reducing *Archaeoglobus fulgidus*, methanogens such as *Methanocaldococcus jannaschii*, and the hyperthermophiles *P. furiosus*, *Thermococcus radiotolerans*, and *Thermococcus gammatolerans* (Beblo et al., 2011; DiRuggiero et al., 1997; Jolivet et al., 2003, 2004). Despite the seeming prevalence of radiation-resistant thermophiles, it would be unjustified to assume this is true of all thermophiles; several have been shown to be radiation sensitive, such as the archaeon *Sulfolobus solfataricus* (Rolfsmeier et al., 2011). Table 1 lists radiation-resistant organisms with their D_{10} value – the dose of radiation in gray (Gy) that reduces the survival of a population by 90 %. Radiation resistance is strongly linked to genome size and the number of DNA double-strand breaks (DSBs) resulting from exposure to IR, which is approximately 0.004–0.01 DSB/Gy/Mbp. Because of the large difference in genome size, at a dose of IR of 1 kGy, the roundworm *C. elegans* experiences 400 DNA DSBs, whereas the bacterium *D. radiodurans* faces only 158 DSBs at 12 kGy (Daly, 2012). As a result, the D_{10} of eukaryotes is much lower than that of bacteria, but those organisms are considered to be highly resistant to IR.

While IR-resistant organisms are distributed across the three domains of life (Fig. 1), this distribution can vary dramatically between organisms of the same family and even between species. For example, *Thermus thermophilus* is as radiation sensitive as *Escherichia coli* (D_{10} 0.7 kGy) but belongs to the same clade as one of the most IR-resistant bacterium known to date, *D. radiodurans* (D_{10} 12 kGy) (Omelchenko et al., 2005; Table 1). This raises an important question regarding the evolution of radiation resistance and whether or not the mechanisms underlying IR resistance are shared between the three domains of life.

In this chapter, we first discuss the cellular effects of IR and the parallels with desiccation, we follow by the current concepts regarding radioprotection and damage repair and the role of Mn antioxidants in radiation resistance, and finally we discuss the mechanisms underlying the radiation resistance of polyextremophilic archaea.

2. Cellular Effects of Ionizing Radiation

Ionizing radiation damages cellular components by direct and indirect effects (Riley, 1994). While direct ionization within the cell results in molecular damage, the vast majority of cellular insults under aqueous conditions are caused by indirect effects, through the actions of reactive oxygen species (ROS) formed by the radiolysis of water (Fig. 2) (Riley, 1994). Water radiolysis generates hydroxyl radicals ($HO^{\bullet}$), protons, and free electrons (Eq. 1).

$$H_2O \rightarrow HO^{\bullet} + e^{-}_{aq} + H^{+} \tag{1}$$

$$2HO^{\bullet} \rightarrow H_2O_2 \tag{2}$$

$$O_2 + e^{-}_{aq} \rightarrow O_2^{\bullet -} \tag{3}$$

Hydroxyl radicals react indiscriminately with all macromolecules in the cell and with each other to form hydrogen peroxide (H_2O_2) (Eq. 2), and free electrons react with dissolved oxygen to form superoxide ($O_2^{\bullet -}$) (Eq. 3). DNA-associated water molecules that undergo radiolysis become an immediate threat for nucleic acids, generating oxidized DNA bases and sugar moieties, abasic sites, strand breaks, and cross-links to proteins. These damages often produce complex clustered lesions resulting in DNA DSBs from attempted repair (Dianov et al., 2001; Regulus et al., 2007; Kish and DiRuggiero, 2008). DNA is further damaged through its association with free iron in the cells (Dianov et al., 2001; Ward, 1994). Proteins are attacked by hydroxyl radicals introducing carbonyl residues, amino acid radical chain reactions, cross-linking, and ultimately resulting in protein inactivation and denaturation (Daly, 2009). By analogy to chemical oxidative stress, it is hypothesized that the low reactivity and high specificity of superoxide and H_2O_2 for iron-sulfur and heme groups produce consequential damage to [4Fe-4S] clusters of labile dehydratases (Imlay, 2006). This results in the release of free Fe^{2+} in the cytoplasm and enzyme inactivation, failure of metabolic pathways, and the synthesis of aromatic and sulfur amino acids (Imlay, 2006, 2008).

Table 1. Examples of IR-resistant polyextremophiles.

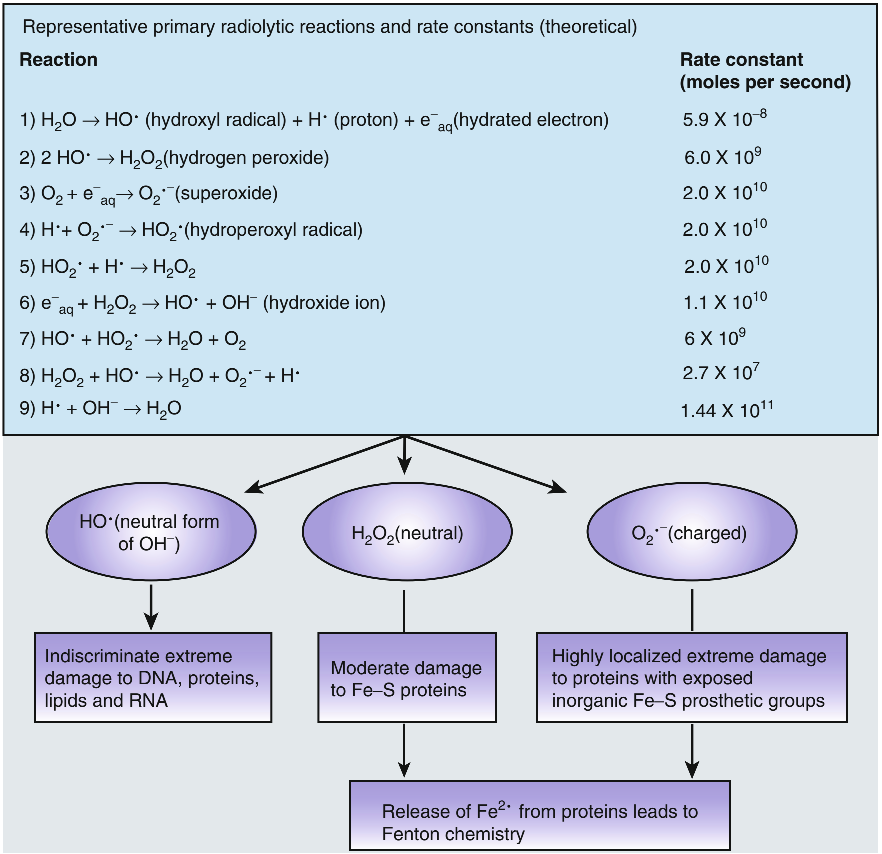
Extremophile	D_{10} (kGy)[a]	Haploid genome size (Mbp)[b]	Characteristics	References
Eukarya				
Adineta vaga	1	180	Desiccation resistant	Gladyshev and Meselson (2008)
Philodina roseola	1	180	Desiccation resistant	Gladyshev and Meselson (2008)
Caenorhabditis elegans	1.2	100	Thermotolerant	Johnson and Hartman (1988)
Ustilago maydis	6	20	Thermotolerant, halotolerant	Holliday (2004)
Milnesium tardigradum	1	75–800[c]	Thermotolerant, desiccation resistant	Horikawa et al. (2006)
Archaea				
Methanocaldococcus jannaschii	1	1.7	Thermophile	Beblo et al. (2011)
Archaeoglobus fulgidus	1	2.2	Thermophile	Beblo et al. (2011)
Haloferax volcanii	1.5	4.0	Halophile	Delmas et al. (2009)
Pyrococcus furiosus	3	1.9	Thermophile, halotolerant	DiRuggiero et al. (1997)
Halobacterium salinarum	5	2.7	Halophile, desiccation and pressure resistant	Kottemann et al. (2005)
Thermococcus gammatolerans	6	2.1	Thermophile	Jolivet et al. (2003)
Bacteria				
Aquifex pyrophilus	2.8	1.6[d]	Thermophile	Beblo et al. (2011)
Rubrobacter xylanophilus	6	3.2	Thermophile, halotolerant, desiccation resistant	Carreto et al. (1996)
Rubrobacter radiotolerans	10	3.4	Thermophile, desiccation resistant	Suzuki et al. (1988)
Deinococcus geothermalis	10	3.3	Thermophile, desiccation resistant	Daly (2009)
Deinococcus radiodurans	12	3.3	Desiccation resistant	Daly (2009)

[a] D_{10} value – the dose of radiation in gray (Gy) that reduces the survival of a population by 90 %.

[b] Genome sizes at http://www.ncbi/nlm.nih/gov/genome

[c] Genome size unknown, range given from http://www.genomesize.com/

[d] Estimated from the closely related *Aquifex aeolicus*.



Representative primary radiolytic reactions and rate constants (theoretical)

Reaction	Rate constant (moles per second)
1) $H_2O \rightarrow HO^{\bullet}$ (hydroxyl radical) + $H^{\bullet}$ (proton) + e^{-}_{aq}(hydrated electron)	5.9×10^{-8}
2) $2\ HO^{\bullet} \rightarrow H_2O_2$(hydrogen peroxide)	6.0×10^{9}
3) $O_2 + e^{-}_{aq} \rightarrow O_2^{\bullet -}$(superoxide)	2.0×10^{10}
4) $H^{\bullet} + O_2^{\bullet -} \rightarrow HO_2^{\bullet}$(hydroperoxyl radical)	2.0×10^{10}
5) $HO_2^{\bullet} + H^{\bullet} \rightarrow H_2O_2$	2.0×10^{10}
6) $e^{-}_{aq} + H_2O_2 \rightarrow HO^{\bullet} + OH^{-}$ (hydroxide ion)	1.1×10^{10}
7) $HO^{\bullet} + HO_2^{\bullet} \rightarrow H_2O + O_2$	6×10^{9}
8) $H_2O_2 + HO^{\bullet} \rightarrow H_2O + O_2^{\bullet -} + H^{\bullet}$	2.7×10^{7}
9) $H^{\bullet} + OH^{-} \rightarrow H_2O$	1.44×10^{11}

Figure 2. Theoretical cellular reactions generating ROS following IR. *Top*: expected reactions resulting from the radiolysis of water and their rate constants. *Bottom*: cellular targets of ROS (From Daly 2009, reproduced with permission from Macmillan Publishers Ltd.).

The released Fe^{2+} participates in the Fenton reaction (Eq. 4), which is the electron transfer from ferrous ion to H_2O_2 and the formation of superoxide (Imlay, 2008). The resulting $HO^{\bullet}$ inflicts a barrage of oxidative damage upon all cellular components.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \qquad (4)$$

The mechanistic link between desiccation and IR resistance can be found in the formation of ROS resulting from both stresses (Nauser et al., 2005). During desiccation, loss of control of the electron transport chain, decrease membrane integrity compromising gas diffusion, impaired antioxidant systems, and macromolecule distortions as the result of volume changes contribute to the accumulation of ROS. This high level of ROS, in particular hydroxyl and peroxyl radicals, causes a major oxidative stress to the cell (Kranner, 2002; Kranner and Birtic, 2005). Thus, both desiccation and IR inflict severe oxidative damage to macromolecules in the cell that must either be prevented or repaired in order for the cell to survive.

3. Radioprotection and Damage Repair

In the 1960s, DNA was considered to be the principal target of radiation, and DNA damage was responsible for its lethal effects (Hutchinson, 1966). Scientists believed that IR-resistant microorganisms survived high-level radiation because they possessed unique and highly efficient DNA repair mechanisms. Several pathways for the repair of DNA DSBs after ionizing radiation have been proposed for bacteria, including homologous recombination; single-strand annealing; extended synthesis-dependent strand annealing, where cells need to contain another intact copy of the damaged DNA region; and nonhomologous end joining, which does not require a second homologous copy of DNA to join two contiguous fragments (Blasius et al., 2008; Confalonieri and Sommer, 2011; Slade and Radman, 2011). Recent work showed that the steps of DNA repair from IR damage were surprisingly ordinary in contrast to the extreme nature of the chromosome fragmentation and that the DNA repair proteins involved were not unique to IR-resistant organisms (Confalonieri and Sommer, 2011; Daly, 2012; Gutman et al., 1994).

In contrast to bacteria, DSB repair in the Archaea is less well characterized. In *P. furiosus*, DNA end processing is carried out by Rad50/Mre11 complexes that attach to the DNA ends and recruit nuclease and helicase proteins (NurA and HerA, respectively) to form 3′ overhangs (Hopfner et al., 2001). This in turn recruits RadA, a RecA homolog (Constantinesco et al., 2004; Hopfner et al., 2001). In *H. salinarum*, *nurA* and *herA* homologs are missing, and while Rad50/Mre11 proteins are present, Rad50 is not required for homologous recombination (Kish and DiRuggiero, 2008). Additionally, mutants of *H. salinarum* deficient in both Rad50 and Mre11 (Δ*rad50*-Δ*mre11*) are just as IR resistant as the wild type, though the repair of DNA DSBs occurs less efficiently (Kish and DiRuggiero, 2008). These facts together with the demonstration that IR-sensitive and IR-resistant organisms suffer the same number of DNA DSBs for an equivalent dose of IR (~0.01 DSB/Gy/Mbp) depart from the dogma that DNA damage and in particular DNA DSBs are the most cytotoxic lesions resulting from exposure to IR. From early studies in 1940 (Dale, 1940), work from Gebicki's group (Du and Gebicki, 2004; Nauser et al., 2005), and recent reports from Daly's group (Daly, 2009; Daly et al., 2007), it is now established that proteins are the major targets for oxidation following exposure to IR. Thus, the idea that protein protection might govern radiation resistance has severely challenged the conventionally held view that DNA damage is paramount in radiation toxicity. The current model is that by protecting protein function from the damages inflicted by IR, DNA can be repaired by competent proteins, and the cell can survive (Fig. 3).

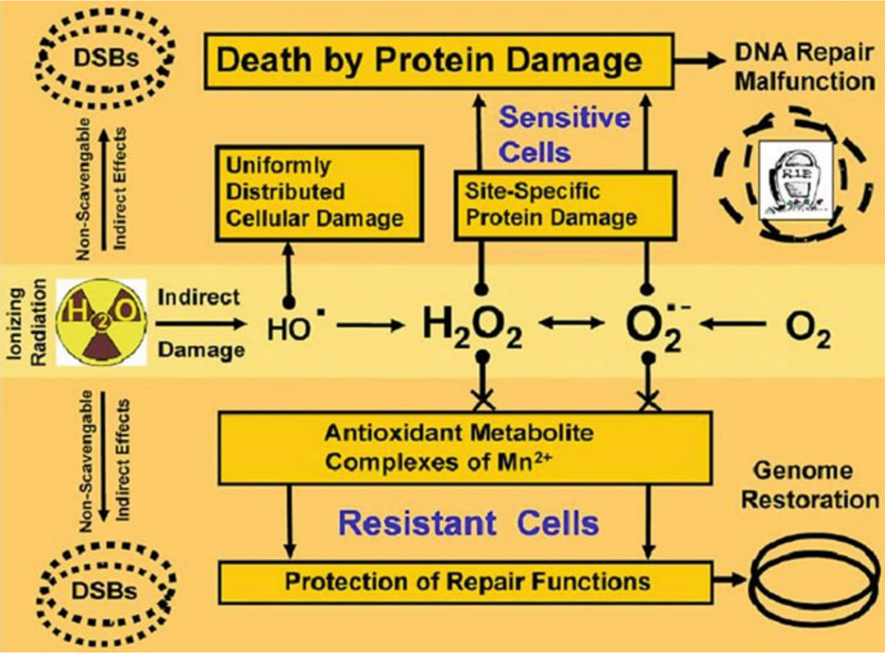


Figure 3. Model for death by protein damage in IR-sensitive cells and damage avoidance in IR-resistant cells (From Daly 2012, reproduced with permission from Elsevier).

4. Enzymatic Defense and IR

The major defenses of the cell against oxidative stress from exposure to H_2O_2 and redox cycling drugs – producing superoxide – are enzymatic (Imlay, 2008). Superoxide dismutases (SODs), catalases, and peroxidases are highly induced upon oxidative stress, and mutants of those proteins are greatly sensitive to chemical oxidants (Imlay, 2008). Surprisingly, SOD and catalase mutants of *H. salinarum* showed the same level of survival to IR as strains with active SODs and catalases (Fig. 4, Robinson et al., 2011), suggesting that those enzymes were not required for the survival of *H. salinarum* to IR; similar results were obtained with SOD and catalase mutants of *D. radiodurans* and *E. coli* (Markillie et al., 1999; Scott et al., 1989). This is quite paradoxical since the major stress from IR is oxidative stress (Daly, 2009) and both SOD and catalase are major ROS detoxification enzymes (Imlay, 2008). Furthermore, in bacterial systems and in *H. salinarum*, SODs and catalases were induced by several orders of magnitude in response to redox cycling drugs and H_2O_2 (Imlay, 2008; Kaur et al., 2010), but no increase in mRNA or protein levels for SODs, catalases, or peroxidases was detected in *H. salinarum* after IR (Whitehead et al., 2006). The question then is as follows: What protects the macromolecules of IR-resistant organisms since the enzymatic defenses of the cell against oxidative stress are not engaged by IR exposure?

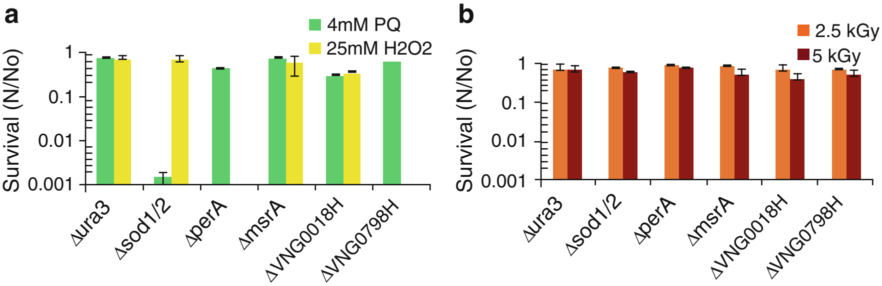


Figure 4. Survival of *H. salinarum Δura3* and mutant strains exposed to H_2O_2, paraquat, and IR. Survival was calculated as the average ratio (N/No) of surviving colony-forming units from treated (N) compared to untreated (No) cultures. (**a**) Doses of chemical oxydants: paraquat (PQ) and H_2O_2; (**b**) doses of ionizing radiation (From Robinson et al. 2011, reproduced with permission).

5. Manganese (Mn) Antioxidants

"Mn antioxidants" were first discovered in *Lactobacillus plantarum* where accumulation of millimolar concentrations of Mn suppressed oxidative stress and substituted for the lack of superoxide dismutase (SOD) (Archibald and Fridovich, 1981, 1982b). High levels of Mn also rescued *E. coli* and *Saccharomyces cerevisiae* SOD-deficient mutants (Al-Maghrebi et al., 2002; Chang and Kosman, 1989), and small molecule complexes of Mn have been shown to exhibit superoxide-scavenging activity in vitro (Archibald and Fridovich, 1982a; Barnese et al., 2008). Recently, the high Mn/Fe ratio found in IR-resistant bacteria and archaea revealed a direct link between Mn and protection of proteins from oxidative damage by ROS (Fredrickson et al., 2008; Daly, 2009; Kish et al., 2009). Work with *D. radiodurans* elegantly established the key role played by Mn-peptide complexes in the extreme radiation resistance of this organism (Daly et al., 2010), and in yeast, in vivo studies showed the important function of Mn-orthophosphate complexes in oxidative stress (McNaughton et al., 2010) (Fig. 5). In *H. salinarum* enzyme-free cell extracts rich in Mn, phosphate, amino acids, and peptides provided a great level of enzyme protection against the deleterious effect of IR (Robinson et al., 2011), and recent studies with *Rubrobacter* species showed that the association of Mn and trehalose was essential for the extreme radiation resistance observed in these organisms (Webb and DiRuggiero, 2012). High levels of intracellular concentration of trehalose were also reported in the IR-resistant cyanobacterium *Chroococcidiopsis* (Billi et al., 2000). Investigating the mechanisms of catalytic removal of superoxide by Mn compounds, Barnese et al. (2012) found that Mn phosphate and Mn carbonate, but not Mn pyrophosphate and citrate, can catalyze superoxide disproportionation in vitro at rates sufficient to mimic enzymatic SOD. They also noted that carboxylate and phosphate motifs, found in amino acids and nucleotides, are the most commonly available ligands for Mn in vivo (Fig. 5). In addition to its antioxidant

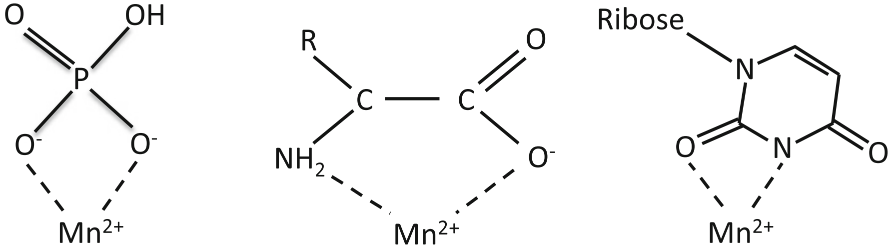


Figure 5. Model for manganese antioxidants. Mn^{2+} in complex with orthophosphate (*left*), with free amino acids or peptides (*center*) and with nucleosides (*right*) catalytically scavenges superoxide radicals ($O_2^{\bullet-}$). Mn^{2+} in complex with free amino acids or peptide and orthophosphate catalytically decompose hydrogen peroxide (H_2O_2). Nucleosides, free amino acids, peptides, and other small organic metabolites scavenge hydroxyl radicals ($HO^{\bullet}$) (Daly et al., 2010).

activity, Mn may also act by functionally substituting for Fe in the Fe-S cluster of enzymes and thereby mitigating the deleterious effects of Fenton chemistry during oxidative stress (Sobota and Imalay, 2011).

In *Bacillus,* spores resistant to wet and dry heat benefited from the accumulation of Mn coordinated with small molecules including dipicolinic acid (DPA), and possibly α-/β-type small, acid-soluble proteins (Ghosh et al., 2011). DPA also formed antioxidant complexes with Ca^{2+} and phosphate, indicating that other divalent metal ions may contribute to protection from IR (Granger et al., 2011). Mn-mycosporine complexes were also attributed to facilitating radiation and desiccation resistance in cyanobacteria (Oren and Gunde-Cimerman, 2007; Rastogi et al., 2010). Cellular accumulation of Mn together with a variety of organic and inorganic ligands may be a widespread mechanism to surviving oxidative stress, and there is evidence that this may extend also to simple animals such as rotifers (Krisko et al., 2012).

6. What Is the Basis for the Radiation Resistance of the Polyextremophile *H. salinarum*?

While *H. salinarum* is highly polyploidic, with 15–25 copies of its chromosome per cell (Breuert et al., 2006), no connection has been established between chromosome copy numbers and radiation resistance (Daly et al., 2004; Gladyshev and Meselson, 2008), nor does the presence of eukaryotic-like proteins involved in the repair of DNA DSBs account for the high level of survival of this organism to IR (Kish and DiRuggiero, 2008). However, recent work revealed the critical role played by nonenzymatic antioxidant processes in the resistance of *H. salinarum* to IR (Robinson et al., 2011; Kish et al., 2009). Scavenging of ROS by intracellular halides in *H. salinarum* resulted in increased protection against nucleotide modification and carbonylation of protein residues (Kish et al., 2009). Measurements of *H. salinarum* cell interior revealed a high Mn/Fe ratio similar to that of *D. radiodurans* and other radiation-resistant microorganisms, underlying the role of Mn in radiation resistance (Kish et al., 2009; Daly, 2009). Protein-free cell extracts from *H. salinarum* provided a high level of protection for protein activity against IR in vitro. Compared with cell extracts of radiation-sensitive bacteria, *H. salinarum* extracts were enriched in manganese-antioxidant complexes, supporting an essential role in ROS scavenging for those small molecules in vivo (Robinson et al., 2011).

To further elucidate the metabolic routes instrumental to this enhanced radiation resistance, IR-"super"-resistant mutants (IR$^+$) of *H. salinarum* were evolved from the wild-type strain over multiple cycles of exposure to high doses of IR (Webb et al., 2013; DeVeaux et al., 2007). Proteomic analysis of IR$^+$ mutants revealed overexpression of enzymes from central carbon metabolism, channeling a substantial flux of carbon into pyruvate and therefore the generation of energy and reducing equivalents (Webb et al., 2013). The corresponding IR$^+$ mutants also had increased intracellular Mn concentration, compared to the wild type, supporting the case of an important role for Mn in central carbon metabolism, via strictly Mn-dependent enzymes or enzymes highly stimulated by Mn (Kehres and Maguire, 2003; Liedert et al., 2012; Ogunniyi et al., 2010). Maintenance of redox homeostasis was also activated by the overexpression of coenzyme biosynthesis pathways involved in redox reactions. These findings support the idea that increased IR tolerance is most likely achieved by a "metabolic route" and underscore the physiological importance in aerobic fitness of Mn antioxidants.

Recent studies regarding single-strand DNA-binding protein (SSB) in halophiles suggested a key role for these proteins in radiation resistance. Single-strand DNA-binding proteins (SSBs), also called replication protein A (RPAs), bind to ssDNA with high affinity and provide protection against nuclease and chemical attacks. These proteins are essential for DNA metabolism including DNA replication, recombination, and repair in all domains of life (Wold, 1997). The basic architecture of RPAs is based on the oligonucleotide-/oligosaccharide-binding (OB) fold, a five-stranded β-sheet coiled into a closed barrel, but the number of OB-folds present varies from species to species (Bochkarev et al., 1999). Unlike in bacteria and eukaryotes, there is a wide diversity in the architecture of RPAs present in archaea. Two operons, RPA1 (with the genes *rfa2* and *rfa7* in *H. salinarum*) and RPA3 (with the genes *rfa3* and *rfa8* in *H. salinarum*), and a single gene, *rpa2* (*rfa1* in *H. salinarum*), have been found to encode RPA proteins in the halophilic archaea (Fig. 6). *H. salinarum* IR$^+$ mutants all showed overexpression of the RPA3 operon (Webb et al., 2013; DeVeaux et al., 2007). The same operon was upregulated in previous studies following irradiation of *H. salinarum* (Whitehead et al., 2006), and more recently two independent studies (Skowyra and Macneill, 2011; Stroud et al., 2012) reported hypersensitivity to DNA damaging agents of *rpa3* mutants in *Haloferax volcanii*. Furthermore, *H. volcanii* constructs overexpressing the Rpa2 protein exhibited increased resistance to DNA damage (UV, MMS, and phleomycin) (Skowyra and MacNeill, 2011). These data clearly implicate RPA proteins in enhanced IR tolerance.

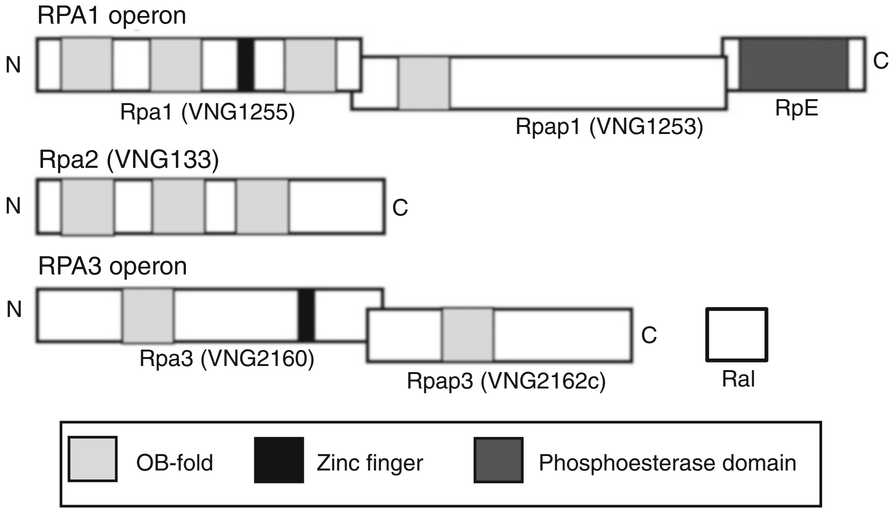
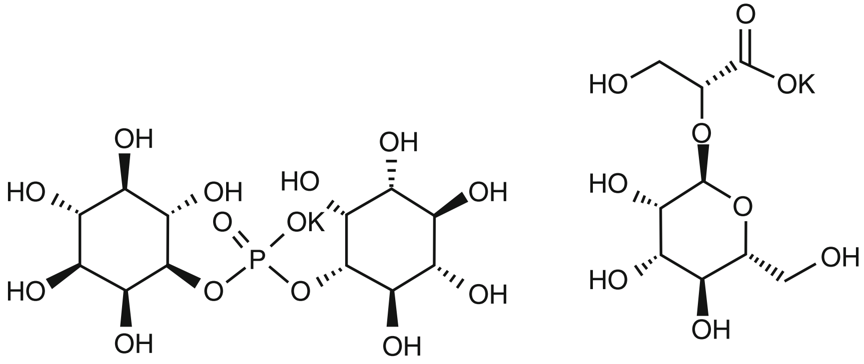


Figure 6. Operon organization and domain structures of *H. volcanii* and *H. salinarum* (gene names in *parenthesis*) of single-strand DNA-binding proteins (Adapted from Stroud et al. 2012, reproduced with permission).

7. What About Thermophiles?

While halophilic archaea and bacteria are adapted to desiccating conditions, imparting resistance to IR, it is not the case for many thermophiles and hyperthermophiles found to be radiation resistant. In fact, no direct correlation was found between desiccation tolerance and radiation resistance among (hyper)-thermophilic archaea (Beblo et al., 2009, 2011).

Archaea are of particular interest because they synthesize unusual, low-molecular-weight organic compounds such as ß-amino acids, N$^{\varepsilon}$-acetyl-ß-lysine, mannosylglycerate (MG), and di-*myo*-inositol phosphate (DIP) known as compatible solutes (Fig. 7) (Martins et al., 1997; Santos and da Costa, 2002). These compounds are typically negatively charged in contrast to compatible solutes from mesophiles. Compounds such as DIP (found in *Pyrococcus*/*Thermococcus*, *Archaeoglobus*, and *Aquifex* species), di-glycerol-phosphate (in *Archaeoglobus* species), and MG (in *Pyrococcus*/*Thermococcus* and *Archaeoglobus* species) accumulate in the cell in response to supraoptimal growth temperature and osmotic shock, which are stress conditions likely to generate ROS (Müller et al., 2005). In addition, compounds such as DIP and MG have been shown to play a role in protein thermostabilization by protecting model enzymes against heat-induced denaturation, aggregation, and inactivation (Faria et al., 2004; Lamosa et al., 2003; Müller et al., 2005; Ramos et al., 1997; Scholz et al., 1992). The ability of



di-*myo*-inositol phosphate (DIP)

Mannosylglycerate (MG)

Figure 7. Compatible solutes in hyperthermophiles. Mannosylglycerate (*MG*) and di-*myo*-inositol phosphate (*DIP*) are anions and depicted as potassium salts, K^+ being the main counterion in the organisms from which they originate.

such compounds to provide IR resistance is currently under investigation using *P. furiosus* and *Thermococcus gammatolerans* as model systems (Webb and DiRuggiero, unpublished), two hyperthermophiles highly resistant to IR (DiRuggiero et al., 1997; Jolivet et al., 2003; Table 1).

8. Relevance to Astrobiology

The understanding that adaptation to extreme environments might provide protection again high radiation levels is particularly relevant to the field of astrobiology. Lacking an atmosphere and magnetic shield to reduce the surface solar irradiance, microorganisms on the surface of Mars are exposed to far greater levels of UV-C (Cockell et al., 2000) and high-energy radiation than are microorganisms on Earth. Furthermore, there is evidence of evaporitic deposits containing high concentrations of chloride and bromide at both Meridiani Planum (Rieder et al., 2004) and Gusev crater (Haskin et al., 2005) gathered by the Mars Exploration Rovers and evidence of other evaporite deposits, possibly containing chloride, in the southern highlands of Mars reported by the Mars Odyssey Orbiter (Osterloo et al., 2008). The findings that the salt environment itself may be a protective factor for potential microbial life on the surface of Mars (Davila et al., 2008) indicate that chloride and bromide evaporite deposits showing water modification are excellent areas for surface investigations looking for evidence of life on Mars.

9. Conclusion

The study of extremophiles and how they meet the physical and chemical challenges found in the environmental extremes they inhabit lead to new insights on the mechanisms of stress response. Many extremophiles are found to be resistant to IR, suggesting that radiation resistance is a fortuitous consequence of a high tolerance to other environmental stressors (e.g., desiccation). Given the diversity of IR-resistant extremophiles and their natural environments, we do not know yet if there are universal features of IR resistance, such as high intracellular concentration of Mn (Daly et al., 2010; Robinson et al., 2011). The IR resistance found in *H. salinarum* is attributed to high intracellular concentrations of salts and Mn-antioxidant complexes that protect proteins from oxidative damage (Robinson et al., 2011; Kish et al., 2009). However, little is known regarding their physiology in the context of cellular adaptation to stress. The variety of Mn complexes found so far (Daly et al., 2010; Ghosh et al., 2011; Granger et al., 2011; Robinson et al., 2011) and the potential for compatible solutes from thermophiles to provide ROS-scavenging activity in the cell suggest that the adaptations of extremophiles to their environments provide a tremendous reservoir for novel radioprotective molecules and antioxidants against the deleterious effect of IR.

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11. References

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