ELSEVIER
SAUNDERS

Clin Lab Med ■ (2006) ■–■

CLINICS IN
LABORATORY
MEDICINE

# Modulating Radiation Resistance: Insights Based on Defenses Against Reactive Oxygen Species in the Radioresistant Bacterium *Deinococcus radiodurans*

Michael J. Daly, PhD

*Department of Pathology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda 20814-4799, Maryland, USA*

Humans and other mammals are remarkably sensitive to acute doses of ionizing radiation (IR) (eg, x and γ rays, neutrons, and α and β particles) [1], in that a whole-body exposure of just 5 Gy is lethal; the gray (Gy) is the amount of energy absorbed by a tissue or substance and applies to all types of radiation. Low-dose chronic IR is a well-established human carcinogen and is omnipresent on Earth. Background sources of terrestrial IR include radioisotopes present in essentially all materials in the environment and cosmic sources, including the sun and extremely distant galactic events, such as supernovae. Collectively, these natural sources of IR deliver only about 0.0024 Gy/y [2]. In this context, the evolution of bacteria that are able to grow under high-dose chronic IR (60 Gy/h) [3] or survive acute irradiation exposures [4] of 15,000 Gy is truly extraordinary given the apparent absence of highly radioactive habitats on Earth over geologic times. Human-made sources of IR, developed over the last 60 years, have contributed significantly to low-dose radiation, including occupational exposures associated with developing and maintaining nuclear weaponry and power sources; environmental release of radionuclides, such as occurred during atomic bomb tests during the Cold War (1945–1986); and nuclear power plant accidents, such as in Chernobyl, Ukraine (1986) [5]. A recent National

This work was supported by grant DE-FG02-04ER63918 from The United States Department of Energy under the Environmental Remediation Sciences Program of the Office of Biological and Environmental Research.

*E-mail address:* mdaly@usuhs.mil


doi:10.1016/j.cll.2006.03.009 *labmed.theclinics.com*

Research Council report [6] concluded that whereas risks of low-dose IR are small, there is no safe level. This finding has grown stronger over the last 15 years, dismissing the notion that there is a threshold of exposure to IR below which there is no health threat. Currently, the most significant, widespread human-made sources of IR are medical devices and procedures, including diagnostic radiographs and therapeutic radiation. Natural background IR remains the most significant source of exposure for humans, however, representing more than 80% of the total dose of a typical resident of the United States [6]. Although the biological hazards of IR have been evident for a century [1], a clear understanding of the molecular mechanisms underlying IR toxicity and resistance remains controversial despite decades of molecular research dedicated to this field. Perhaps paramount, the catastrophic terrorist events of September 11, 2001 underscore the possibility of radiological terrorism and the pressing need to understand further how to prevent radiation injury. This article presents a new view of IR-resistance mechanisms in extremely radiation-resistant bacteria and questions some longstanding assumptions regarding the causes of IR toxicity. The extrapolation of recent experimental findings [7] to the potential development of approaches to modulate radiation resistance is intentionally speculative, aimed at contributing to the groundwork of new ideas needed to address the emerging threat of nuclear terrorism.

## The classical dogma

The classical dogma of radiation biology [8] asserts that the cytotoxic and mutagenic effects of IR are principally the result of genetic damage caused during or immediately after transfer of radiation energy to genomic DNA [1] with the following characteristics: (1) the damage is mediated indirectly by short-lived reactive oxygen species (ROS), predominantly hydroxyl radicals ($HO^{\bullet}$) generated by the radiolysis of water; (2) to a lesser extent, the damage is the result of direct interaction of DNA with radiation; and (3) mutations and other genetic alterations are set on genome replication. Much evidence has accumulated that cannot be explained by this classical dogma. Among these heretical results are (1) extreme radiation sensitivities observed in bacteria that encode a full compliment of DNA repair and protection systems [7,9–11]; (2) evidence that proteins likely are the first major class of molecules attacked during irradiation [12,13]; and (3) IR-induced bystander effects (BSEs), defined as effects elicited in cells that are not directly traversed by radiation [14]. The pathway connecting the formation of ROS with endpoint biologic damage is far from clear, largely because the identity of the first critical molecular targets of ROS still is not established [13]. In the hierarchy of cellular targets for reproductive cell death following irradiation, DNA almost always is placed at the top [1]. This DNA-centric view of radiation toxicity is pervasive, a consequence of

DNA being the least complex biologically active material that can be tested with respect to its radiation response [1].

## Ionizing radiation resistance in bacteria

Until recently, there have been no clear physiologic predictors of a cell's ability to recover from IR. In general, most of the resistant bacteria reported have been Gram-positive and the most sensitive have been Gram-negative [9,10,15]. There are several reported exceptions to this paradigm, however; the Gram-negative cyanobacterium *Chroococcidiopsis* is extremely resistant to IR [16], whereas the Gram-positive *Micrococcus luteus* (*Sarcinia lutea*) is sensitive [17,18]. Recently, a relationship between intracellular Mn/Fe concentration ratios and bacterial IR resistance was reported, in which very high and very low Mn/Fe ratios correlated with very high and very low resistances, respectively, and restricting Mn(II) in the famously IR-resistant *Deinococcus radiodurans* sensitized this eubacterium to IR [7]. For example, *D radiodurans* (Mn/Fe ratio: 0.24) accumulates greater than 300 times more Mn than the extremely IR-sensitive *Pseudomonas putida* (Mn/Fe ratio: $<0.0001$), and *P putida* accumulates 4.6 times more Fe than *D radiodurans* [7,9]. Fig. 1 presents the relationship between IR resistance and intracellular Mn/Fe concentration ratios for eight bacterial species [7,19]. These bacteria were selected for investigation because they have been subjected to whole genome sequencing and annotation, revealing that they all appear to encode a similarly complex set of DNA repair and protection functions [7,19]. Most recently it was shown that *Shewanella oneidensis* (Fig. 1), which has a low intracellular Mn/Fe ratio and is extremely sensitive to IR [7], strongly induced gene systems controlling the expression of prophages on exposure to 40 Gy. Induction by IR of the lytic cycle of prophages in bacteria might also contribute to cell killing [11].

Studies have shown that for a given dose of IR, delivered aerobically or anaerobically, the number of DNA double strand breaks (DSB) in *D radiodurans* compared with sensitive bacteria is about the same and that genomic DNA of *D radiodurans* is not endowed with unusual protection from in vivo irradiation [7,20]. The possibility that extreme IR resistance in *D radiodurans* is determined by novel genes also has been explored. At least 20 predicted genes of *D radiodurans*, which were identified by transcriptional profiling following IR as the most highly induced, have been disrupted and the corresponding mutants have been characterized for IR resistance [21–24]. Remarkably, the resistances of these novel mutants remained high, indicating that survival of irradiated *D radiodurans* might depend on a conventional set of repair functions. This conclusion is supported partially by earlier research that showed that several *Escherichia coli* DNA repair genes, which do not differ greatly from their *D radiodurans* counterparts, fully restored corresponding *D radiodurans* mutants to wild-type levels of

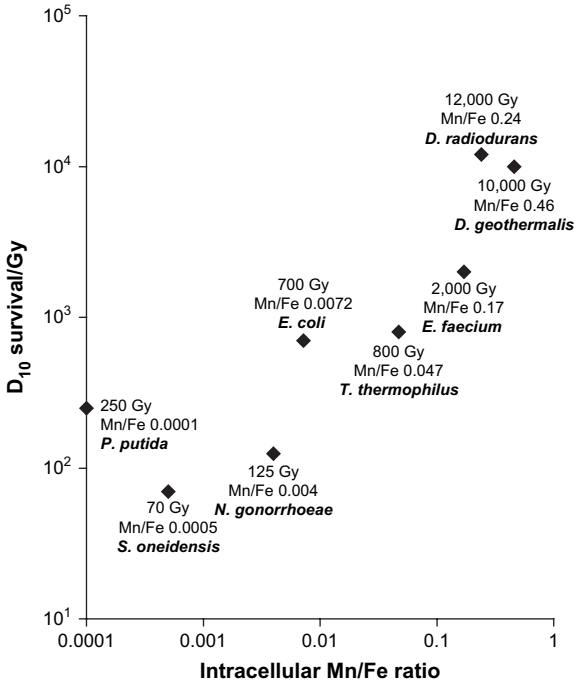


Fig. 1. Relationship between intracellular Mn and Fe contents of various bacteria and their survival following exposure to IR [7,19]. $D_{10}$, IR dose that reduces the number of colony-forming units by 90%. Standard growth, irradiation ($^{60}$Co, 0°C), and recovery conditions were as described previously [7].

*D radiodurans* DNA damage resistance [25]. Also significant, when the *E coli* genome was subjected to extensive DSB fragmentation without IR (ie, by endogenous expression of a restriction enzyme in vivo) survival remained high [26].

*D radiodurans* contains 4 to 10 identical copies of its genome per cell, and when irradiated to a dose of 10 kGy generates 400 to 1000 genomic DSB fragments per cell [4,7]. This amount of DNA damage in *D radiodurans* typically does not lead to cell death, induced levels of mutation, or genomic rearrangements. Previous reports on homologous recombination in *D radiodurans* following IR are consistent with the canonical version of the DSB repair model [27], which additionally supported the existence of single-stranded annealing in *D radiodurans* [28], but nonhomologous end-joining was not observed [28]. It was also shown that chromosomal fragments released by IR-induced DSB in *D radiodurans* appear to be diffusible within condensed nucleoids, whereby DSB fragments in stationary-phase cells recombined with high efficiency at homologous DSB sites whether the sites were located adjacently on the same chromosome [28], separated on different chromosomes [27], or present on a chromosome and a plasmid

[29]. Recent use of cryoelectron microscopy of vitreous sections of *D radiodurans* supports the conclusion that DNA fragments in *D radiodurans* are mobile and that the arrangement of its nucleoids does not play a key role in radioresistance [30]. Collectively, these broad-based studies have converged on the conclusion that *D radiodurans* uses a conventional set of DNA repair and protection functions but with far greater efficiency than in IR-sensitive bacteria [7,9].

## The role of Mn in ionizing radiation resistance

It has been shown recently that intracellular accumulation of Mn(II) in *D radiodurans* facilitates IR resistance; *D radiodurans* accumulates $\geq 2$ mmol/L Mn(II) [7]. How Mn contributes to IR resistance is the subject of ongoing experimentation, which currently is examining the intracellular distribution of Mn relative to Fe and other elements. One possible Mn-dependent mechanism for scavenging ROS was reported by Archibald and Fridovich [31] in 1982, who showed that at high concentrations in nonradiolytic systems, Mn(II) acts as a potent scavenger of superoxide ($O_2^{\bullet -}$) ions, with Mn cycling between the divalent and trivalent states [31]. They investigated Mn(II,III) redox cycling in light of the bacterium *Lactobaccillus plantarum*, which accumulates high intracellular concentrations of Mn(II) and is radiation resistant [7] but does not encode any superoxide dusmutases (SODs) [7]. Although SODs are estimated to be approximately 65 times more efficient than the most efficient nonenzymic Mn redox-cycling complexes, Mn typically is present in millimolar concentrations in lactobacilli compared with micromolar levels of SODs in those organisms possessing the enzymes [31].

A conceptual problem in attempting to link Mn-based $O_2^{\bullet -}$-scavenging to extreme IR resistance is that $O_2^{\bullet -}$ generally is not implicated as a significant ROS during irradiation when atmospheric $O_2$ is limited [1]. The solubility of $O_2$ in water is low, reaching only 5 to 6 ppm at 30° to 40°C under normal atmospheric conditions, and $O_2^{\bullet -}$ does not cross membranes easily because it is charged [32,33]. Under IR, therefore, exogenously generated $O_2^{\bullet -}$ likely does not pose a great threat to cells. $O_2^{\bullet -}$ generated inside cells during irradiation is dangerous, however, because it can become trapped, leading to intracellular accumulation of $O_2^{\bullet -}$. Regarding the high Mn and low Fe contents of several phylogenetically distinct IR-resistant bacteria [7,16,17] (Fig. 1), reactions implicated in ROS production and removal are presented here.

Reaction 1: Water radiolysis: $H_2O \rightarrow HO^{\bullet} + H^+$ [1,34]
Reaction 2: A primary radiolytic reaction: $2\ HO^{\bullet} \rightarrow H_2O_2$ [1,34]
Reaction 3: Fenton reaction: $H_2O_2 + Fe(II) \rightarrow HO^{\bullet} + OH^- + Fe(III)$ [1,32]
Reaction 4: IR-induced superoxide: $O_2 + e^-_{aq}$ (IR-induced solvated electron) $\rightarrow O_2^{\bullet -}$ [1,34]

Reaction 5: Mn oxidation: $Mn(II) + O_2^{\bullet -} + 2\,H^+ \rightarrow H_2O_2 + Mn(III)$ [31]
Reaction 6: Mn reduction: $Mn(III) + H_2O_2 \rightarrow O_2 + 2H^+ + Mn(II)$ [31]
Reaction 7: Fe-cycling: $H_2O_2 + Fe(III) \rightarrow O_2 + Fe(II) + 2H^+$ [1], then reaction 3

Ferrous iron [Fe(II)] catalyses the rapid reduction of radiolysis-induced hydrogen peroxide ($H_2O_2$) (reactions 1 and 2) [34] to hydroxide ions ($OH^-$) and $HO^\bullet$ (reaction 3); $HO^\bullet$ are extremely damaging to all biomolecules [1,32]. Superoxide, formed during irradiation from dissolved $O_2$ (reaction 4) [34], does not damage DNA but can oxidize and deactivate specific Fe-containing proteins [32]. In contrast to Fe(II), Mn(II) does not react with $H_2O_2$. Instead, Mn(II) selectively reacts with $O_2^{\bullet -}$ and hydrogen ions ($H^+$) to form $H_2O_2$ and Mn(III) (reaction 5) [31]; Mn(III) is known to react with $H_2O_2$ to form $O_2$ (reaction 6) [31], which would rapidly reform $O_2^{\bullet -}$ under IR (reaction 4). One predicted consequence of Mn redox-cycling reactions is the conversion of $O_2^{\bullet -}$ to $H_2O_2$, which could diffuse out of cells, thereby limiting the number of intracellular reactions involving ROS during irradiation. In contrast, the Fenton reaction yields Fe(III) which reacts with $H_2O_2$ to form $O_2$ (reaction 7), and generates $O_2^{\bullet -}$ under IR (reaction 4). Under IR, Fe(II,III) redox cycling thus is predicted to give rise to $HO^\bullet$ and $O_2^{\bullet -}$, whereas Mn(II,III) redox cycling is predicted to favor $O_2^{\bullet -}$ scavenging with intermediate release of $H_2O_2$, without $HO^\bullet$ formation. If proteins are major initial targets of IR, as proposed by Du and Gebicki [12], then cells lacking adequate $O_2^{\bullet -}$ defenses might be particularly prone to protein damage before DNA is significantly affected; in contrast, lethality in Mn-accumulating, $O_2^{\bullet -}$-resistant cells might be determined by $HO^\bullet$-mediated DNA damage at higher doses.

## Proteins are initial targets of ionizing radiation

Although the primary radiolytic reaction products $H_2O_2$ and $O_2^{\bullet -}$ are relatively stable, $HO^\bullet$ are extremely toxic to cells because of their high reactivity [1]. $H_2O_2$ and $O_2^{\bullet -}$ also are generated during cellular metabolism when electrons leak from the substrate side of the respiratory chain [9,32] and $HO^\bullet$ consequently are generated by Fe(II)-dependent reduction of $H_2O_2$ (reaction 3). The production of $O_2^{\bullet -}$ in cells exposed to IR is extremely rapid [34]; the theoretical rate constant for reaction 4 is $2 \times 10^{10}$ $m^{-1}s^{-1}$, whereas metabolism-induced $O_2^{\bullet -}$ take a comparatively long time before they become manifest [32]. Metabolism-induced ROS (oxidative stress) can have profoundly sensitizing effects on bacterial IR resistance, even for extremely IR-resistant bacteria. For example, exposure of metabolically active *D radiodurans* at 30°C on minimal medium to 3 kGy (60 Gy/h) is 100% lethal, whereas at 0°C, in the absence of metabolism, >12 kGy is needed to sterilize an equivalent number of *D radiodurans* cells exposed in the same medium [35].

There is general agreement that lipids, DNA, and proteins are major targets of ROS. There is disagreement, however, on which group of molecules is the most vulnerable [12,13]. Regarding the first group, there is strong evidence that lipid peroxidation can be dissociated from lethal damage [36]. In the case of chromosomal DNA, it is clear that it is an indispensable molecule whose integrity must be conserved. DNA is likely a secondary rather than a primary target of ROS, however [12,13]. Irrespective of source, purified DNA is approximately 40 times more sensitive to IR than DNA packaged in cells [4,7] where it is complexed with proteins and shielded from $HO^{\bullet}$. Instead, the first major class of molecules damaged by ROS during irradiation seems to be proteins [12,13]. The following assumption thus is tenuous: if a dose of IR is sufficient to cause only minor DNA damage it should damage a similarly small fraction of proteins present in the same cell.

There is a growing body of evidence from different laboratories that proteins are significant targets for ROS in vivo. For example, Dr. James Imlay's group [32] (University of Illinois, Urbana, IL, USA) has reported studies on how $O_2^{\bullet-}$ ions damage Fe-containing proteins, resulting in the intracellular release of Fe and oxidative stress [32]; and Dr. Janusz Gebicki's group [12,13] (Macquarie University, Sydney, Australia) has been investigating the early formation of protein radicals generated by $HO^{\bullet}$. Although their results provide strong evidence of damage to proteins, for many years proteins were not considered to be significant targets of IR- or metabolism-induced ROS because there was little evidence that proteins could pass the damage to other molecules, either indirectly or directly. In the case of $O_2^{\bullet-}$-dependent transmissible protein damage, $O_2^{\bullet-}$ ions are known to damage some [4Fe-4S] cluster-containing proteins in vivo. In bacteria containing high concentrations of such proteins [9,11,32], $O_2^{\bullet-}$ would be expected to result in a significant release of intracellular bound ferrous ions [Fe(II)], followed by intracellular oxidation of unbound Fe(II) by $H_2O_2$ (Fenton reaction), generating $HO^{\bullet}$, $OH^{-}$, and Fe(III). For $HO^{\bullet}$-dependent transmissible protein damage, $HO^{\bullet}$ can give rise to protein peroxyl radicals, which are precursors of protein hydroperoxides [13]. Protein hydroperoxides decompose in the presence of Fe to give new radicals, including oxidizing alkoxyl radicals, which are even more reactive than peroxyl radicals [13]. Although the focus of such ROS research has not been in vivo IR damage, the findings generally appear relevant to developing a comprehensive view of the chain of molecular events in cells during irradiation.

The theoretical IR-induced reaction scheme presented above (reactions 1–6) might ultimately determine if protein-mediated damage is transmitted, because the ratio of Mn to Fe in a cell would be expected to affect significantly the stoichiometry of intermediates and end-products of radiolysis. For example, (1) although $O_2^{\bullet-}$ ions do not produce protein radicals [13], scavenging of $O_2^{\bullet-}$ by Mn redox cycling might help prevent release of Fe(II) from the subset of Fe-S proteins vulnerable to $O_2^{\bullet-}$, thereby reducing the number of intracellular Fenton-type reactions; and (2) although Mn(II)

does not scavenge $HO^{\bullet}$, Mn redox cycling might facilitate removal of $H_2O_2$ from the cell before it is subjected to intracellular Fenton-type chemistry. Irradiated Fe-rich cells not able to cycle Mn(II,III) thus might be prone to extensive intracellular decomposition of radiogenic $H_2O_2$, and protein hydroperoxides could yield other more toxic radicals. Unfortunately, there are no techniques currently capable of measuring directly the myriad of radiolytic reactions in cells because of their multitude of targets, and the existence of IR-driven Mn(II,III) cycling remains unexplored.

## Possible role of mitochondria in ionizing radiation toxicity

Mitochondria are likely the descendants of endosymbiotic eubacteria, an idea strengthened recently by whole genome sequence comparisons. For example, phylogenetic tree analyses have shown that the accessory subunit animal mitochondrial DNA polymerase emerges as a result of horizontal transfer of the gene encoding glycyl-tRNA synthetase from a bacterium of the *Thermus-Deinococcus* group into the animal nuclear genome [37]. This acquisition by a distinct eukaryotic lineage of a gene encoding a mitochondrial protein from a *Thermus-Deinococcus* bacterial source underscores the relevance of exploring possible links between bacterial IR responses and the behavior of irradiated mitochondria. Until about a decade ago, it was assumed widely that nuclear DNA is the main target for IR-induced genotoxicity and carcinogenesis in mammalian cells. The recent use of microbeam technology has challenged this paradigm, however, showing that IR traversal through the nucleus of a cell is not a prerequisite for genetic damage. This biological phenomenon has been termed the radiation-induced BSE [14]. BSEs have been observed in various mammalian cell types exposed to either low linear energy transfer (LET) x or $\gamma$ rays or high-LET $\alpha$ particles. BSEs have been reported following doses as low as 5 mGy of IR [14].

Remarkably, BSEs can be induced in nonirradiated cells as far as 1 mm away from irradiated cells [38]. There are two major types of bystander effects: the first depends on cell–cell communication and contact and the second results from substances released from the exposed cells [14]. Studies on the mechanisms underlying radiation-induced BSEs are beginning to elucidate the nature of the mediating factors. Among those factors, a role of ROS has been inferred from the finding that various BSEs can be inhibited by the addition of catalase or SOD [39]. It is not clear, however, how extracellular ROS-scavenging enzymes could significantly affect intracellular ROS, unless ROS were released from cells traversed by IR. Actual measurements postirradiation indicate that much higher ROS amounts are produced than predicted and suggest possible amplification mechanisms [40].

A number of studies also have provided indirect evidence for a role of respiratory control in radiation resistance, including mitochondria [41] and bacteria [9,11,22,42], whereby the mechanism may be part of general cellular response pathways to oxidative stress; less respiratory control is correlated

with lower resistance to IR. Definitive evidence is lacking, however. Regarding target size, the mitochondrial volume of a typical mammalian cell is 4% to 30% of total cellular volume [14]. In light of the hypothetical mechanism of Mn-facilitated bacterial IR resistance proposed above, mitochondria might be expected to increase IR toxicity in mammalian hosts. Mitochondria [43] accumulate 10 to 20 mmol/L Mn(II), which is 5 to 10 times more Mn(II) than accumulated by *D radiodurans* [7], and might be potential sites for Mn(II,III) cycling in eukaryotes [43,44]. Mn redox cycling in mitochondria may conserve IR-induced $H_2O_2$ (see previous discussion), but unlike free-living bacteria might release $H_2O_2$ into a host cell. Fig. 2 shows that rat brain tissue sections (0.5 g) exposed anaerobically to 10 kGy in deionized $H_2O$ (6 mL) at 0°C released $H_2O_2$ ($\sim 4 \times 10^{-5}$ M) (see Fig. 2). If $H_2O_2$ that diffused out of the nonmetabolizing (0°C) irradiated brain samples originated in mitochondria, then these organelles and their hosts would be particularly prone to oxidative stress, which could trigger mitochondrial apoptosis (programmed cell death) [14,45]. This assay (see Fig. 2) supports estimates that rat brain ($9.2 \times 10^7$ neurons/g and $1.85 \times 10^9$ glia cells/g) generated at least 14,000 $O_2^{\bullet -}$/cell/Gy as precursors of $H_2O_2$; the amount of $H_2O_2$ released by the irradiated brain sections, not including $H_2O_2$ decomposed by radiolysis [34] and Fenton-type chemistry [32] is (6/1000) × ($4 \times 10^{-5}$ M) × ($6.02 \times 10^{23}$ molecules/mole)/$9.7 \times 10^8$ cells/$10^4$ Gy. The apparent production of $H_2O_2$ by irradiated brain tissue (see Fig. 2) thus implicates mitochondrial IR-driven Mn redox cycling [31,43,44] as a possible protagonist in mammalian IR injury, including IR-induced bystander responses [14]. Although high concentrations of Mn(II) accumulated by mitochondria [43] normally might be engaged in scavenging $O_2^{\bullet -}$ produced by respiration,

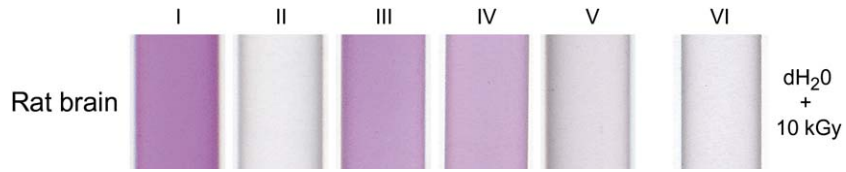


Fig. 2. IR-driven $H_2O_2$ generation in irradiated rat brain. (*I*) No IR, aerobic. (*II*) No IR, anaerobic; preconditioned by purging with argon (Ar, ultra-high purity). (*III*) 10 kGy, anaerobic. (*IV*) As for panel III, but re-purged with Ar after irradiation to remove $O_2$. (*V*) As for panel III, but treated with catalase (15,000 units) after irradiation and then re-purged with Ar. (*VI*) 10 kGy, anaerobic, deionized water ($dH_2O$), no brain. (*I–V*) Freshly harvested rat brain segments were rinsed (0°C) twice in 0.9% NaCl followed by $dH_2O$ to remove blood, transferred to $dH_2O$ (0°C) (0.5 g brain/6 mL $dH_2O$), and sealed in tubes after purging (11 min) with 99.999% pure Ar. Irradiations ($^{60}$Co) were at 0°C, and all tubes were centrifuged before testing by the Rhodazine D assay (RDA) (CHEMetrics, Calverton, VA). Color development in a sample that was re-purged with Ar after IR (panel *IV*), but not following catalase/purging treatment (panel *V*), indicates $H_2O_2$ accumulation. $H_2O_2$ concentrations were determined by comparison to RDA standards. Dissolved $O_2$ and $H_2O_2$ react with the pale yellow–colored leuco form of Rhodazine D to produce a deep rose color, with the color proportional to the dissolved $O_2$ or $H_2O_2$ concentration. The RDA is suitable for measuring 0.05–1 mg $L^{-1}$ $O_2$, and 1–10 × $10^{-5}$ M $H_2O_2$.

low doses of IR in metabolically active cells might increase the flux of $O_2^{\bullet-}$ sufficiently to accelerate mitochondrial Mn redox cycling, precipitating the release of $H_2O_2$ into host cells containing hundreds to thousands of mitochondria per cell before their enzymatic ROS defenses are induced. A cascade of $H_2O_2$ or related membrane-permeable ROS might diffuse out of irradiated cells into neighboring cells. Under normal physiologic conditions, $H_2O_2$ would certainly be reduced to $O_2$ by endogenous catalase or glutathione peroxidase, perhaps yielding $O_2^{\bullet-}$ during chronic IR exposures (reaction 4), which in turn could generate peroxynitrite in the presence of nitric oxide [33]. Peroxynitrite, like $O_2^{\bullet-}$, can damage some Fe-S-containing proteins but unlike $O_2^{\bullet-}$ is not scavenged by SOD and can easily cross membranes [33]. In summary, these preliminary data provide an intriguing, but speculative, insight into how mitochondrial Mn(II), $O_2^{\bullet-}$, and $H_2O_2$ might exacerbate mammalian IR toxicity.

## Radiation resistance modulators based on superoxide scavengers

Superoxide generally is not implicated as a significant ROS during anaerobic irradiations [1]. For $O_2$-free deionized water exposed to IR this assertion is correct, but not for cells. Preconditioning cells to be anaerobic does not preclude the intracellular formation of significant levels of $O_2^{\bullet-}$ during irradiation because the presence of intracellular Fe or $H_2O_2$-reducing enzymes favors $O_2$ formation, which even under low-dose $\gamma$ radiation rapidly yields $O_2^{\bullet-}$ and related ROS [34]. Although $O_2^{\bullet-}$ does not damage DNA [32], the fact that $O_2^{\bullet-}$ can oxidize at least some [4Fe-4S] proteins [32] suggests that $O_2^{\bullet-}$-scavenging agents might be effective radioprotectors. The accumulation of high intracellular levels of Mn in extremely radiation-resistant bacteria but not sensitive bacteria generally supports this view [9]. Two groups of stable SOD-mimetic compounds that have been shown to protect against the toxicity of ROS in vitro and in vivo are nitroxides [46] and manganese porphyrin complexes [47]. Tempol (4-hydroxy-2,2,6,6,-tetramethylpiperidine-*N*-oxyl) [46] and manganese (III) tetrakis(N-methyl-2-pyridyl)porphyrin (MnTMPyP) [47] are prototypes of these antioxidant groups, respectively, and both compounds are promising radioprotectors in animals. The identity of molecular targets first protected by these compounds has not been established, however, although proteins would appear to rank high on a list of possible candidates.

Radiotherapy is used commonly to treat humans with head and neck cancers. For such patients, the scalp and salivary glands in the radiation field are affected dramatically by this procedure [46,48]. Tempol recently has been shown to confer protection against radiation. In humans, for example, dramatic protection against IR-induced alopecia occurs when patients apply topical Tempol to the scalp during and after whole brain radiation therapies [48]. A phase II study that uses a gel formulation to increase the exposure of scalp to Tempol has begun [48]. In mice, oral treatments of the mouth or intravenous delivery of Tempol during irradiation also provided significant

radioprotection, preventing salivary hypofunction and other side effects of the oral cavities. Both studies strongly suggest that Tempol is a promising candidate for clinical applications in patients undergoing radiotherapies. Because ROS also appear to be mediators of apoptosis in mammalian cells [14], compounds that modulate the fate of ROS may serve as radioprotectors. For example, Mn-Sod exists exclusively in the mitochondria, which are the dominant sites of ROS generation in normal cells. In this context, a recent study concluded that MnTMPyP might be useful in regulating apoptosis induced by IR, presumably through scavenging ROS [47]. Unfortunately, these studies have not yet addressed comprehensively the underlying mechanisms of protection, and an important concern regarding such potential treatments remains; a radioprotective agent administered during therapy could result in tumor radioprotection.

## Summary

There is an enormous divide separating the IR resistances of bacteria such as *D radiodurans* and the IR sensitivities of mammalian cells. Bringing together radiation biologists from both sides, including representatives of their respective funding agencies, could help bridge a chasm between these groups and benefit development of novel strategies for the prevention and treatment of radiation injury. This article presents a new view of IR resistance emerging for bacteria that have high Mn and low Fe contents (Fig. 1), and considers how bacterial intracellular Mn redox cycling [31] might be induced by IR, thereby limiting the number of intracellular reactions involving ROS during irradiation. This could occur first by scavenging $O_2^{\bullet -}$, which likely is generated rapidly at high levels in cells exposed to IR even in the absence of atmospheric $O_2$, and second by preserving $H_2O_2$, which could diffuse out of cells and limit intracellular Fenton-type chemistry. Proteins appear to be the first major targets of IR [12] and might be protected by Mn redox cycling. If $O_2^{\bullet -}$ is a major protagonist in mammalian IR-induced toxicity also, this could explain why the $O_2^{\bullet -}$ scavengers Tempol [46,48] and MnTMPyP [47] are effective radioprotectors. In this context, the high levels of Mn(II) accumulated in mitochondria [43] might exacerbate IR sensitivities of mammalian cells. Unlike free-living Mn-accumulating bacteria, mitochondria engaged in IR-induced Mn redox cycling might release $H_2O_2$ or related membrane-permeable ROS into their hosts (Fig. 2). Collectively, the views expressed here might justify further research, including: (1) identifying approaches that inhibit IR-induced Mn redox cycling and their potentially sensitizing effects on bacterial IR resistance. Such knowledge might be applied to IR-based sterilization of highly IR-resistant Mn-dependent bacteria, such as *Baccilus anthracis* [49], or future potential threats, such as genetically engineered pathogens based on *D radiodurans* [50]. The possibility also exists of modulating Mn and Fe homeostasis as a mechanism to increase the resistance of potentially useful bacteria for

bioremediation of extremely toxic radioactive waste sites, of which there are many thousands left over from the Cold War [3,50]; and (2) developing protocols to modulate mitochondrial respiration and Mn redox cycling as a possible treatment to control the IR resistance of mammalian cells. In summary, the possibility that DNA is not the first major class of molecules damaged by IR warrants careful investigation, especially because it may come to affect estimates of risk, models of IR-induced toxicity, approaches to modulating IR resistance, and the identification of new targets to control recovery from IR injury [9,14]. Such research is critical to mounting an effective response to radiological nuclear terrorism.

## Acknowledgments

The author is grateful to Dr. Vera Y. Matrosova and Dr. Min Zhai for their assistance in conducting Rhodazine D assays presented in Figure 2.

## References

[1] von Sonntag C. The chemical basis of radiation biology. London: Taylor & Francis; 1987.
[2] Sorenson JA. Perception of radiation hazards. Semin Nucl Med 1986;16(3):158–70.
[3] Brim H, McFarlan SC, Fredrickson JK, et al. Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. Nat Biotechnol 2000;18(1):85–90.
[4] Daly MJ, Ling O, Fuchs P, et al. *In vivo* damage and recA-dependent repair of plasmid and chromosomal DNA in the radiation-resistant bacterium *Deinococcus radiodurans*. J Bacteriol 1994;176(12):3508–17.
[5] Dubrova YE. Long-term genetic effects of radiation exposure. Mutat Res 2003;544(2–3):433–9.
[6] National Research Council. Health risks from exposure to low levels of ionizing radiation. BEIR VII Phase 2. Washington, DC: The National Academies Press; 2005.
[7] Daly MJ, Gaidamakova EK, Matrosova VY, et al. Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. Science 2004;306(5698):1025–8.
[8] Lea DE. Actions of radiation on living cells. Cambridge, MA: University Press; 1946.
[9] Ghosal D, Omelchenko MV, Gaidamakova EK, et al. How radiation kills cells: survival of *Deinococcus radiodurans* and *Shewanella oneidensis* under oxidative stress. FEMS Microbiol Rev 2005;29(2):361–75.
[10] Thornley MJ. Radiation resistance among bacteria. J Appl Bacteriol 1963;26(3):334–45.
[11] Qiu X, Daly MJ, Vasilenko A, et al. Transcriptome analysis applied to survival of *Shewanella oneidensis* MR-1 exposed to ionizing radiation. J Bacteriol 2006;188(3):1199–204.
[12] Du J, Gebicki JM. Proteins are major initial cell targets of hydroxyl free radicals. Int J Biochem Cell Biol 2004;36(11):2334–43.
[13] Nauser T, Koppenol WH, Gebicki JM. The kinetics of oxidation of GSH by protein-bound radicals. Biochem J 2005;392:693–701.
[14] Bystander effects and the dose response, Belle Newsletter Vol. 11, No. 3, October 2003. Available at: http://www.belleonline.com/newsletters/volume11/vol11-3.pdf. Accessed November 20, 2005.
[15] Van Gerwen SJ, Rombouts FM, van't Riet K, et al. A data analysis of the irradiation parameter D10 for bacteria and spores under various conditions. J Food Prot 1999;62(9):1024–32.
[16] Billi D, Friedmann EI, Hofer KG, et al. Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. Appl Environ Microbiol 2000;66(4):1489–92.

[17] Leibowitz PJ, Schwartzberg LS, Bruce AK. The *in vivo* association of manganese with the chromosome of *Micrococcus radiodurans*. Photochem Photobiol 1976;23(1):45–50.
[18] Merrick TP, Bruce AK. Radiation response of potassium efflux in *Micrococcus radiodurans* and *Sarcina lutea*. Radiat Res 1965;24:612–8.
[19] Omelchenko MV, Wolf YI, Gaidamakova EK, et al. Comparative genomics of *Thermus thermophilus* HB27 and *Deinococcus radiodurans* R1: Divergent paths of adaptation to thermophily and radiation resistance. BMC Evol Biol 2005;5:57.
[20] Gerard E, Jolivet E, Prieur D, et al. DNA protection mechanisms are not involved in the radioresistance of the hyperthermophilic archaea *Pyrococcus abyssi* and *P. furiosus*. Mol Genet Genomics 2001;266(1):72–8.
[21] Tanaka M, Earl AM, Howell HA, et al. Analysis of *Deinococcus radiodurans's* transcriptional response to ionizing radiation and desiccation reveals novel proteins that contribute to extreme radioresistance. Genetics 2004;168(1):21–33.
[22] Liu Y, Zhou J, Omelchenko MV, et al. Transcriptome dynamics of *Deinococcus radiodurans* recovering from ionizing radiation. Proc Natl Acad Sci USA 2003;100(7):4191–6.
[23] Harris DR, Tanaka M, Saveliev SV, et al. Preserving genome integrity: the DdrA protein of *Deinococcus radiodurans* R1. PLoS Biol 2004;2(e304):304–13.
[24] Gaidamakova EK, Matrosova VY, Zhai M, et al. Resistance phenotypes of novel *Deinococcus radiodurans* mutants. 2005. Available at: http://www.usuhs.mil/pat/deinococcus/index_20.htm. Accessed November 22, 2005.
[25] Minton KW. Repair of ionizing-radiation damage in the radiation resistant bacterium *Deinococcus radiodurans*. Mutat Res 1996;363(1):1–7.
[26] Heitman J, Zinder ND, Model P. Repair of the *Escherichia coli* chromosome after *in vivo* scission by the *Eco*RI endonuclease. Proc Natl Acad Sci USA 1989;86(7):2281–5.
[27] Daly MJ, Minton KW. Interchromosomal recombination in the extremely radioresistant bacterium *Deinococcus radiodurans*. J Bacteriol 1995;177(19):5495–505.
[28] Daly MJ, Minton KW. An alternative pathway of recombination of chromosomal fragments precedes recA-dependent recombination in the radioresistant bacterium *Deinococcus radiodurans*. J Bacteriol 1996;178(15):4461–71.
[29] Daly MJ, Minton KW. Recombination between a resident plasmid and the chromosome following irradiation of the radioresistant bacterium *Deinococcus radiodurans*. Gene 1997;187(2):225–9.
[30] Eltsov M, Dubochet J. Fine structure of the *Deinococcus radiodurans* nucleoid revealed by cryoelectron microscopy of vitreous sections. J Bacteriol 2005;187(23):8047–54.
[31] Archibald FS, Fridovich I. The scavenging of superoxide radical by manganous complexes: *in vitro*. Arch Biochem Biophys 1982;214(2):452–63.
[32] Imlay JA. Pathways of oxidative damage. Annu Rev Microbiol 2003;57:395–418.
[33] Liochev SI, Fridovich I. Cross-compartment protection by SOD1. Free Radic Biol Med 2005;38(1):146–7.
[34] Lin LH, Slater GF, Lollar BS, et al. The yield and isotopic composition of radiolytic $H_2$, a potential energy source for the deep subsurface biosphere. Geochim Cosmochim Acta 2004;69(4):893–903.
[35] Venkateswaran A, McFarlan SC, Ghosal D, et al. Physiologic determinants of radiation resistance in *Deinococcus radiodurans*. Appl Environ Microbiol 2000;66(6):2620–6.
[36] Wolters H, Konings AWT. Radiation effects on membranes. III. The effect of X irradiation on survival of mammalian cells substituted by polyunsaturated fatty acids. Radiat Res 1982;92(3):474–82.
[37] Wolf YI, Koonin EV. Origin of an animal mitochondrial DNA polymerase subunit via lineage-specific acquisition of a glycyl-tRNA synthetase from bacteria of the *Thermus-Deinococcus* group. Trends Genet 2001;17(8):431–3.
[38] Belyakov OV, Mitchell SA, Parikh D, et al. Biological effects in unirradiated human tissue induced by radiation damage up to 1 mm away. Proc Natl Acad Sci USA 2005;102(40):14203–8.

[39] Azzam EI, De Toledo SM, Spitz DR, et al. Oxidative metabolism modulates signal transduction and micronucleus formation in bystander cells from alpha-particle-irradiated normal human fibroblast cultures. Cancer Res 2002;62(19):5436–42.
[40] Leach JK, Van Tuyle G, Lin PS, et al. Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen. Cancer Res 2001;61(10):3894–901.
[41] Gudz TI, Pandelova IG, Novgorodov SA. Stimulation of respiration in rat thymocytes induced by ionizing radiation. Radiat Res 1994;138(1):114–20.
[42] Bruce AK, Berner JD. Respiratory activity as a determinant of radiation survival response. Can J Microbiol 1976;22(9):1336–44.
[43] Puskin JS, Gunter TE. Ion and pH gradients across the transport membrane of mitochondria following Mn ++ uptake in the presence of acetate. Biochem Biophys Res Commun 1973;51(3):797–803.
[44] Gunter TE, Miller LM, Gavin CE, et al. Determination of the oxidation states of manganese in brain, liver, and heart mitochondria. J Neurochem 2004;88(2):266–80.
[45] Sohal RS, Dubey A. Mitochondrial oxidative damage, hydrogen peroxide release, and aging. Free Radic Biol Med 1994;16(5):621–6.
[46] Cotrim AP, Sowers AL, Lodde BM, et al. Kinetics of tempol for prevention of xerostomia following head and neck irradiation in a mouse model. Clin Cancer Res 2005;11(20):7564–8.
[47] Lee JH, Lee YM, Park JW. Regulation of ionizing radiation-induced apoptosis by a manganese porphyrin complex. Biochem Biophys Res Commun 2005;334(2):298–305.
[48] Metz JM, Smith D, Mick R, et al. A phase I study of topical Tempol for the prevention of alopecia induced by whole brain radiotherapy. Clin Cancer Res 2004;10(19):6411–7.
[49] Mizak L, Mierzejewski J. Gamma radiation resistance of *Bacillus anthracis* spores. Med Dosw Mikrobiol 2003;55(4):315–23 [Polish.].
[50] Daly MJ. The emerging impact of genomics on the development of biological weapons. Threats and benefits posed by engineered extremophiles. Clin Lab Med 2001;21(3):619–29.