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Mini review

Death by protein damage in irradiated cells

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ABSTRACT

A founding concept of radiobiology that deals with X-rays, γ-rays and ultraviolet light is that radiation indiscriminately damages cellular macromolecules. Mounting experimental evidence does not fit into this theoretical framework. Whereas DNA lesion-yields in cells exposed to a given dose and type of radiation appear to be fixed, protein lesion-yields are highly variable. Extremely radiation resistant bacteria such as *Deinococcus radiodurans* have evolved extraordinarily efficient antioxidant chemical defenses which specifically protect proteins and the functions they catalyze. In diverse prokaryotes, the lethal effects of radiation appear to be governed by oxidative protein damage, which inactivates enzymes including those needed to repair and replicate DNA. These findings offer fresh insight into the molecular mechanisms of radiation resistance and present themselves as new opportunities to study and control oxidative stress in eukaryotes, including mammalian cells and their cancer cell counterparts.

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Contents

1. Introduction

For seventy years, ionizing (X-rays, γ-rays) and non-ionizing (ultraviolet light) forms of radiation have been used as a primary tool to study oxidative stress responses in organisms spanning prokaryotes to higher eukaryotes. Molecular insights into how reactive oxygen species (ROS) generated by radiation elicit their toxic effects have served as the foundation for investigating the nature of mutagenesis, disease, aging, and a myriad of biological processes ending in cell death. Recently, experimental evidence from several independent groups has converged on the conclusion that proteins in mammalian cells [1] and prokaryotes [2–5] are more probable initial targets of cellular radiation damage than DNA. While classical radiation toxicity models identify DNA damage as the universal critical lesion in cells [6,7], studies now support that the survival of many organisms is governed by the level of oxidative

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protein damage caused during irradiation [2–5], which limits the functionality and efficiency of enzymes, including those needed to repair and replicate DNA.

In this review, experimental studies are presented which demonstrate that the critical "target" molecules in irradiated bacteria are proteins. Whereas the levels of DNA damage inflicted in naturally radiation-sensitive and radiation-resistant bacteria exposed to a given dose of radiation are very similar, resistant bacteria have evolved highly efficient antioxidant chemical defenses which specifically protect proteins and the functions they catalyze. The thesis that radiation toxicity is determined mainly by the level of protein oxidation caused during irradiation rather than the amount of DNA damage, developed from comparisons between the extremely radiation resistant bacterium *Deinococcus radiodurans* [8] and other bacteria representing the full range of resistances encountered in the natural world [2,9]. The trends which now support a critical role of protein oxidation in the survival of irradiated bacteria and archaea, the predominant forms of life on Earth, parallel the trends developing for irradiated mammalian cells – and the inferences carry with them the prospects of new strategies to combat oxidative stress in humans.

2. *D. radiodurans*

The bacterium *D. radiodurans* is capable of surviving huge doses of X-rays or γ-rays (12,000 Gy), 20 times greater than the bacterium *Escherichia coli*, and 3000 times greater than most human cells in liquid culture (Fig. 1) [8]. Survival curves for *D. radiodurans* display very large shoulders (Fig. 1), but the mutation frequency of the cells does not increase significantly until very high doses [8]. Reasoning that DNA in *D. radiodurans* might be unusually protected, early studies compared the amount of DNA damage in *D. radiodurans* and *E. coli* exposed to ionizing radiation or ultraviolet C (UVC) (254 nm) radiation [8], and later with other organisms [9–11]. For a given dose of X-rays or γ-rays, or UVC, the relatively small differences in DNA breaks and DNA base damages between the bacteria were not nearly sufficient to explain the great differences in their resistance [12]. *D. radiodurans* exposed to γ-rays can survive ~160 DNA double strand breaks (DSBs) per haploid genome whereas highly radiosensitive bacteria can survive only a few γ-ray-induced DSBs

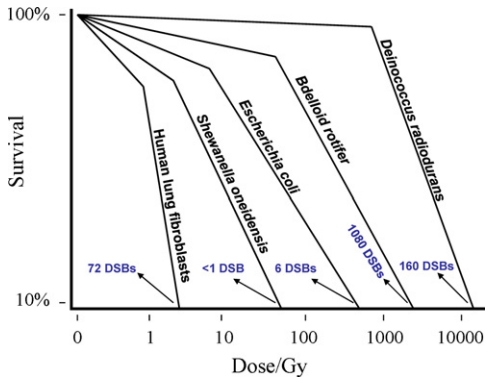

Fig. 1. Survival curves of representative organisms exposed to γ-radiation. The arrows indicate the approximate number of DSBs inflicted per haploid genome at the dose which kills 90% of the organisms. Yields of DSBs were determined by pulsed field gel electrophoresis (Table 1). Bacteria: *S. oneidensis* (ATCC 700550), *E. coli* (K12, MG1655), *D. radiodurans* (ATCC BAA-816); human lung fibroblasts in liquid culture: wild-type (European Collection of Cell Culture, MRC-5); bdelloid rotifer: *A. vaga* (Table 1). Many explanations of the cause of the shoulders on cell survival curves have been proposed. The most favored hypotheses begin with the premise that the yield of DSBs is linear with dose and that the non-linearity of the cell survival curves is caused by dose-dependent changes in the efficiency/accuracy of enzymatic repair [30]. The vast majority of organisms on Earth are radiation sensitive, killed by doses less than 500 Gy [8,12].

(Table 1). The paradoxical survival of irradiated *D. radiodurans* and other extremely radiation resistant organisms has been rationalized under the hypothesis of enhanced DNA repair [8,12].

D. radiodurans also is very resistant to desiccation, showing 85% viability after two years in the presence of less than 5% humidity [9,13]. It has long been recognized that extreme radiation resistance and desiccation tolerance are closely aligned [8]. Desiccation-tolerant bacteria are very resistant to protein oxidation caused during drying, and studies support that the mutual nature of radiation and desiccation resistance resides in cytosolic Mn-dependent antioxidant processes which selectively protect proteins from ROS [14]. Since life on earth most likely did not commonly encounter extremes of ionizing radiation over geologic times, the extreme radiation resistance phenotypes frequently observed in desert soil-inhabiting organisms likely evolved in response to oxidative stress caused during cycles of drying and rehydration [15]. Based on functional and comparative genomics, it has been proposed that the extreme resistance phenotypes of the family *Deinococcaceae* stem from a subtle regulatory interplay between diverse processes including Mn homeostasis, metabolite regulation, respiratory control, macromolecular degradation, and other oxidative stress response pathways [8,12,16]. In *Deinococcus* bacteria, these functions manifest themselves as protein protection, which preserves the high efficiency of its DNA repair enzymes during irradiation or desiccation [2,4,12]. In contrast, irradiated or desiccated bacteria lacking these antioxidant processes are readily overwhelmed by protein oxidation, which renders even minor DNA damage irreparable [2,8,12,14]. This model was offered on the basis that a system which protected and preserved the activity of diverse repair enzymes would more likely be evolved to provide multiple resistances than would a series of separate repair mechanisms be evolved for each extremophilic character noted for *D. radiodurans* [2,12].

3. The role of DNA damage and repair in radiation-induced toxicity

The biological effects of ionizing radiation on DNA are usually ascribed to the sum of two indiscriminately destructive processes. 'Direct action' refers to the unavoidable damaging effects of energy deposited by photons, damage which predominates in deeply frozen (−80 °C) or dry preparations [17,18]. In contrast, the overwhelming majority of lesions in cells irradiated in the aqueous state are caused by the 'indirect action' of ROS – principally short-lived hydroxyl radicals ($HO^{\bullet}$) formed from water that react with DNA at near diffusion-limited rates [12]. The yields of DSBs and single-strand breaks (SSBs) in DNA by γ-rays in aqueous solution are typically 2–3 orders of magnitude greater than those for dry DNA [18] (Fig. 2). Yet, at high concentrations, potent $HO^{\bullet}$-scavenging agents such as dimethyl sulfoxide (DMSO) prevent less than 80% of damage to purified DNA in aqueous preparations exposed to ionizing radiation [19] (Fig. 2). The non-scavengable indirect effects on DNA by ionizing radiation are presumed to be caused by proximal $HO^{\bullet}$ formed from water molecules which are bound tightly to DNA [17,18], and also by ultrashort-lived prehydrated electrons [20,21], which are not easily scavenged due to their extremely short lifetimes. In cells and viruses, DNA is bound and condensed by proteins, and is highly protected from ROS [17,22,23] (Fig. 2). As the yields of ionizing radiation-induced DSBs are very similar across phylogenetically diverse cell-types with greatly differing antioxidant statuses (Table 1), it is clear that DSBs in irradiated cells and viruses are caused mainly by non-scavengable indirect effects.

The nature of the "target" molecules in cells – the alteration of which by radiation leads to cell death – was first studied in radiation-sensitive bacteria, and the conclusions were broadly

Table 1
Relative efficiency of DNA DSB repair in representative organisms following exposure to ionizing radiation.

Organism	[a]Haploid genome size (Mbp)	D_{10} survival (10% survival)	DSB/Gy/Mbp (approximate linear density of DSBs *in vivo*)	Number of survivable DSBs per haploid genome	[Reference]
Adineta vaga (tetraploid rotifer)	180	[b]1200 Gy	0.005 [PFGE]	1080	[95]
Ustilago maydis (diploid fungus)	20	[c]6000 Gy	ND	[d]480	[96]
Caenorhabditis elegans (diploid roundworm)	100	[b]1000 Gy	ND	[d]400	[97]
D. radiodurans (polyploid bacteria)	3.3	[c]12000 Gy	0.004 [OM]	158	[98]
D. radiodurans (polyploid bacteria)	3.3	[c]12000 Gy	0.003 [PFGE]	118	[9]
Human cells (diploid, ECCC MRC-5)	3000	[c]4 Gy	0.006 [PFGE and γ-H2]	72	[87]
Mouse leukemia cells (diploid, L1210)	3000	[c]4 Gy	0.004–0.008 [PFGE]	48–96	[92]
Saccharomyces cerevisiae (diploid yeast)	12.1	[c]800 Gy	0.006 [PFGE]	58	[99]
Enterococcus faecium (polyploid bacteria)	3.0	[c]2000 Gy	ND	[d]24	[9]
Halobacterium salinarum (polyploid archaeon)	2.6	[c]4000 Gy	0.002 [PFGE]	21	[10]
T. thermophilus (polyploid bacteria)	2.1	[c]800 Gy	ND	[d]7	[32]
E. coli (MG1655) (polyploid bacteria)	4.6	[c]700 Gy	0.002 [PFGE]	6	[9]
E. coli (AB2497) (polyploid bacteria)	4.6	[c]200 Gy	0.006 [SGC]	6	[100]
Pseudomonas putida (polyploid bacteria)	6.2	[c]100 Gy	ND	[d]3	[9]
Shewanella oneidensis (polyploid bacteria)	5.1	[c]70 Gy	0.002 [PFGE]	<1	[9]
Intracellular SV40 virus (circular dsDNA: 5.2 kbp)	0.0052	NA	0.004 [CGE]	NA	[22]
Purified bacteriophage λ (linear dsDNA: 48.5 kbp)	0.0485	NA	0.005 [CGE]	NA	(Fig. 2)
***E. coli* plasmid** (*in vivo*, 28 kbp; ~50 copies/cell)	NA	NA	0.002 [CGE]	NA	[23]
***D. radiodurans* plasmid** (*in vivo*, 28 kbp; ~6 copies/cell)	NA	NA	0.002 [CGE]	NA	[23]

Abbreviations: [PFGE]: pulsed field gel electrophoresis; [OM]: optical mapping; [γ-H2]: γ-H2AX foci analysis; [SGC]: sucrose gradient centrifugation; [CGE]: conventional gel electrophoresis; ND: not determined; dsDNA: double-stranded DNA; NA: not applicable.

[a] Genome sizes at http://www.ncbi.nlm.nih.gov/sites/entrez?db=genomeprj.
[b] Survival based on the normal lifespan of animal, but with loss of fecundity.
[c] Survival based on the ability of cells to proliferate and form colonies.
[d] Predicted number of survivable DSBs based on 0.004 DSB/Gy/Mbp (the average DSB lesion-yields for presented organisms).

applied to formulating general models of radiation toxicity [6,7]. Survival curves for radiation-sensitive organisms (Fig. 1) display near-exponential killing, which seemed to correspond to the requirement that only a few events are necessary to produce inactivation. As radiation was deemed to damage cellular macromolecules indiscriminately, and as genes exist at far lower abundance in cells than their products, DNA assumed the role of the most important target. For all cell-types, chromosomal DNA is an indispensable molecule whose integrity must be conserved following exposure to radiation to ensure survival [6–8,12]. Early on, the most significant inactivation events were ascribed to the production of the DSB, the most severe and least frequent form of DNA damage in cells exposed to ionizing radiation [12]. As most of the organisms studied were killed by low doses of ionizing radiation, the DSB was ranked at the top in the hierarchy of radiation-induced lesions most responsible for lethality.

Impaired DSB repair currently provides the best available correlation with radiation-induced cell-killing, as shown, for example, by the greatly increased radiosensitivity of specific repair-deficient mutants [8]. Thus, the functionality of DSB repair proteins (*e.g.*, RecA) ultimately determines if an irradiated cell lives or dies, even for the most radiation-resistant cell-types [8,12]. In this context, the recent demonstration – that DNA damage is uncoupled from protein damage in irradiated *Deinococcus* cells but not in naturally sensitive bacteria, and protein oxidation is quantifiably related to radiation survival [2–5] – clearly associated proteome damage to radiation toxicity [12]. The hypothesis that protein inactivation by ROS may be responsible for radiation toxicity is not new – first proposed by Walter Dale in the 1940s [24,25]. Later, James Watson and others demonstrated that *in vitro* inactivation of virus particles by ionizing radiation proceeds primarily through damage to proteins when antioxidants are limited [26,27].

4. Protein oxidation as the basis for the efficiency of DSB repair

It is generally assumed that X-rays and γ-rays indiscriminately damage cellular macromolecules [7,28]. This, however, is not the case. Extreme radiation resistance in bacteria and archaea consistently coincides with a greatly diminished susceptibility to protein oxidation compared to sensitive species [2,4,5,8,10,12,29], but DNA in resistant and sensitive prokaryotes, and all other cell-types examined, is similarly susceptible to ionizing radiation-induced DSBs (Table 1). Indeed, DSB lesion-yields in cells and viruses exposed to ionizing radiation (~0.004 DSB/Gy/Mbp) are typically

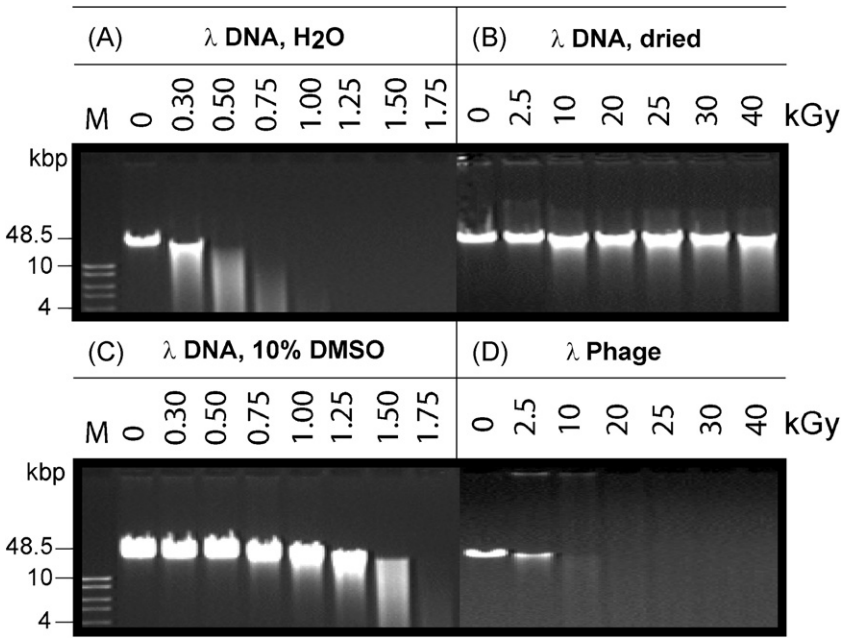

Fig. 2. Approximate DSB lesion-yields in bacteriophage λ DNA (48.5 kbp) by γ-radiation (^{60}Co). (A) Purified λ DNA in water (no scavengers) (0.2 DSB/Gy/Mbp); (B) λ DNA dried (<0.001 DSB/Gy/Mbp); (C) λ DNA in 10% DMSO (0.05 DSB/Gy/Mbp); (D) λ DNA native in bacteriophage particles (0.005 DSB/Gy/Mbp). Gy, dose; M, DNA size standards. Purified λ DNA is a linear molecule (48.5 kbp) and was irradiated at 50 μg/ml. Bacteriophage λ particles were irradiated at 10^{10} particles/ml. DSB damage was determined by conventional agarose gel electrophoresis as described previously [23].

only 2–4 times greater than DSB lesion-yields for purified DNA irradiated at −80 °C [22] or when dried [18], where 'non-scavengable indirect effects' and 'direct effects' are believed to predominate (Fig. 2). In marked contrast, the amount of protein damage in irradiated cells is strongly influenced by their antioxidant status, where yields of radiation-induced protein oxidation can be more than one hundred times greater in hypersensitive bacteria than in extremely resistant bacteria [2,12].

An organism's radiation resistance is typically defined by the maximum dose it can survive. This metric, however, fails to consider that cells with small genomes suffer proportionally fewer DSBs than cells with large genomes, and a ranking of organisms by the number of survivable DSBs carries some surprises (Table 1). The DSB repair capacities of the bdelloid rotifer *Adineta vaga*, the fungus *Ustilago maydis*, and the roundworm *Caenorhabditis elegans* appear to be substantially greater than *D. radiodurans* (Table 1). Clearly, the fate of irradiated cells rests not on the number of DSBs caused during irradiation, but rather on their capacity to accurately mend DSBs [12,30] (Fig. 1).

The debate over the molecular basis of efficient DSB repair manifested in radiation-resistant organisms has sought answers in the realm of DNA repair proteins [8,13,15,16,31]. As reviewed previously in detail, molecular genetic and functional genomic studies for numerous representative resistant species have revealed nothing distinctly unusual about the nature of their DNA repair proteins [8,9,31–38]. The main strategy to delineating a minimal set of genes involved in extreme resistance has been to compare the whole-genome sequences of phylogenetically related but distinct *Deinococcus* species that are equally resistant, whereby genes that are unique are ruled out, whereas shared genes are pooled as candidates for involvement in resistance. This approach eliminated almost all the novel genes first implicated in the extreme radiation resistance of *D. radiodurans* [31,33], and only a few unique deinococcal genes remain implicated in contributing mildly to its remarkable DNA repair capacity [16,33,39]. Indeed, the conserved set of radiation resistance determinants of *Deinococcus* consists mainly of genes present in many other organisms [16,31–34].

At one end of the bacterial resistance spectrum, representatives of *Deinococcus* are extremely resistant to ionizing radiation-induced protein oxidation and survive hundreds of DSBs per cell (Table 1) [2,4,8,9,12,23]. At the other end, extremely radiation-sensitive bacteria such as *Shewanella* and *Pseudomonas* species are hypersensitive to ionizing radiation-induced protein oxidation and killed by doses of ionizing radiation which cause few, if any, DSBs (Table 1) [2,9,12,40]. Other model prokaryotes (*e.g.*, *E. coli* and *Halobacterium salinarum*) display intermediate levels of ionizing radiation resistance, and proportionally lower yields of radiation-induced protein oxidation [2,4,5,10]. All of these species appear to encode similar DNA repair systems [9,31,38] and all succumb to radiation if DNA remains unrepaired. Using Occam's razor, my simplest explanation is that the major defense against radiation damage in *D. radiodurans* and other resistant organisms (Table 1) is a greatly enhanced capacity for scavenging ROS, and that the proteins thereby protected include DNA repair and replication enzymes [2,4,12,29] (Fig. 3). Within my conceptual framework, *D. radiodurans* is not an exception, but rather represents a new paradigm for the role of intracellular antioxidants in protecting the DNA repair functions of diverse resistant cell-types (Fig. 3). Equally, the findings for *E. coli*, *P. putida*, and *S. oneidensis* represent a new paradigm for the role of protein oxidation in the inhibition of DNA repair processes (Fig. 3) [2,9,12,40]. The possibility that oxidative protein damage might also govern or strongly influence the functionality and efficiency of DNA repair proteins in lower and higher eukaryotes exposed to radiation has not yet been explored – the verdict is still out.

5. Protein damage: cause or effect of radiation toxicity?

The discovery that oxidative protein lesion-yields in irradiated bacteria are quantitatively related to survival [2] raised the question of whether protein damage is causative or merely correlative in radiation toxicity. A study of the killing of sensitive and resistant *E. coli* strains and *D. radiodurans* exposed to γ-rays or UVC radiation [4], which cause distinctly different types and

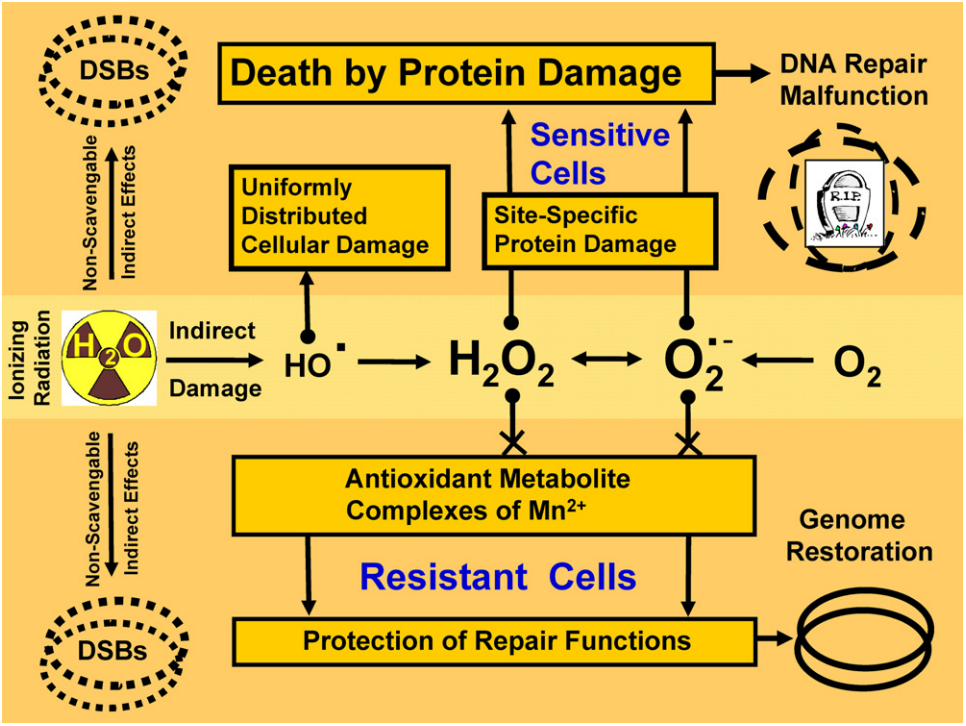

Fig. 3. Model of death by protein damage in irradiated cells. DSBs in the genomes of cells exposed to X-rays or γ-rays are caused mainly by non-scavengable indirect effects, yielding ~0.004 DSB/Gy/Mbp (Table 1). Proteins in sensitive cells are the principal, early targets of ROS [1,2]. Hydroxyl radicals ($HO^{\bullet}$) generated by the radiolysis of water react indiscriminately with small and macromolecular organic molecules, but also with each other to generate hydrogen peroxide (H_2O_2). Superoxide ($O_2^{\bullet-}$) in irradiated cells is generated from dissolved dioxygen (O_2), which is derived from the decomposition of H_2O_2 by metal-catalyzed or enzymatic processes, and from the atmosphere [12]. $O_2^{\bullet-}$ and H_2O_2 are relatively inert, long-lived, and diffusible throughout the cell, do not react with DNA directly, but are extremely damaging to some proteins [12]. $O_2^{\bullet-}$ is particularly dangerous because it is charged, does not readily cross membranes, and becomes trapped in irradiated cells [12]. The most consequential damage by $O_2^{\bullet-}$ and H_2O_2 in cells is site-specific, to proteins which contain exposed iron–sulfur or haem groups, to proteins which contain cysteine residues, and mononuclear iron enzymes in general [12,48–50]. As radiation doses increase, cytosolic proteins – particularly those with solvent-accessible Fe^{2+} groups – are increasingly at risk for oxidative inactivation by site-specific ROS. In contrast, the active sites of enzymes which bind Mn^{2+} instead of Fe^{2+} are resistant to $O_2^{\bullet-}$ and H_2O_2. Surplus Mn^{2+}, *i.e.*, Mn^{2+} not bound to proteins, forms Mn^{2+}-orthophosphate metabolite complexes, which provide highly delocalized (global) protein protection [29]. When the antioxidant properties of Mn^{2+} complexes are exhausted, ROS would become pervasive and compromise the activity/efficiency of DNA repair proteins, which would lead to mutations and cell death [2,81]. The radioprotective benefits of ROS-scavenging complexes in haploid cells which lack systems which heal DSBs directly without a homologous template are limited, as one DSB would be lethal [12,52].

levels of DNA damage, investigated the relationship between protein oxidation, loss of cellular repair activities, and survival. For DNA repair-proficient *E. coli* and *D. radiodurans* cells, the quantitative relationship between protein oxidation and cell death was the same, but independent of DNA damage. The study also used lambda phage production as a metric for global cellular biosynthetic efficacy in irradiated *E. coli*, and demonstrated that bacteriophage maintenance systems in *E. coli* were even more sensitive to inactivation by radiation-induced protein oxidation than the systems needed for cell survival [4].

The conclusion that protein oxidation is causative in killing of irradiated cells is reinforced by studies on *E. coli* inactivated by UVA (350 nm) radiation [3], and also by studies on the radiation resistant archaeon *H. salinarum* NRC-1 exposed to γ-rays [5,10]. From these studies and others, it is evident that oxidation of the proteomes of irradiated sensitive cells is specific, where some proteins are readily oxidized while others are not [2,3,5,10,12,29,41–43]. Again, these findings underscore that the indirect effects of radiation are highly discriminating. Radiation resistance in prokaryotes appears to be dependent on how essential the function of a targeted protein is, its abundance, and how susceptible it is to carbonylation, a severe and permanent form of oxidation [41–43]. It is hypothesized that the decay of cellular robustness culminating in cell death is a direct result of the progressive accumulation of oxidative damage to the proteome [4], where the accumulation of oxidative damage to proteins diminishes their catalytic activities and interactions [8] (Fig. 3). It follows that the recovery of an irradiated cell is limited by the oxidative sensitivity and rate of turnover of a subset of enzymes needed for DNA maintenance and growth in a given environment.

6. The role of Mn antioxidants and metabolites

Interspecies comparisons of irradiated bacteria and archaea show that the levels of protein damage are not only quantitatively related to the efficiency of DNA repair and survival, but are mechanistically linked to the accumulation of divalent manganese ions (Mn^{2+}) [2,5,9,12,44]. Notably, extreme radiation resistance in prokaryotes, and associated protein protection, is not dependent on the presence of antioxidant enzymes [5,9,12,45]. Proteins in *D. radiodurans* are not inherently radiation resistant – *D. radiodurans* proteins lose their resistance when the cells are grown under conditions which limit Mn^{2+} uptake or prevent Mn^{2+} redox-cycling, and when the proteins are extracted [2]. In contrast, proteins in naturally sensitive bacteria are as susceptible to oxidation as when they are purified [2,29]. Ensuing studies showed that protein-free cell extracts of *D. radiodurans* [29] and *H. salinarum* [5] are armed with low-molecular-weight ROS-scavenging Mn^{2+} complexes which consist mainly of peptides bound to Mn^{2+} and orthophosphate (Pi). Mn^{2+} and orthophosphate form complexes, which catalytically remove superoxide via a disproportionation mechanism [29,46]; and amino acids and peptides, which scavenge hydroxyl radicals very efficiently, form complexes with Mn^{2+} which catalytically decompose hydrogen peroxide [29,47]. When reconstituted *in vitro* at physiologically relevant concentrations,

these constituents interacted synergistically in preventing the inactivation of enzymes during high-dose irradiation [29]. At 50,000 Gy, Mn^{2+}–peptide–Pi complexes preserved 50% activity of the dodecameric enzyme glutamine synthetase (466 kDa), which is normally inactivated by 150 Gy; however, Mn–peptide–Pi did not significantly protect DNA from DSBs [29]. Evidently, the quaternary structures of proteins and their functions can be preserved in aqueous solution by Mn^{2+}–metabolite complexes at doses of ionizing radiation which destroy similarly treated DNA [29]. In summary, the action of Mn^{2+} in protecting cytosolic proteins from ROS appears to occur at two levels: (i) by replacing Fe^{2+} and other divalent cations (*e.g.*, Mg^{2+} and Cu^{2+}) with Mn^{2+} as mononuclear cofactors in enzymes, active sites are protected from oxidative damage [48–50]; and (ii) surplus Mn^{2+} (*i.e.*, the portion of a cell's Mn^{2+} budget which is not bound to proteins) forms ROS-scavenging complexes with various metabolites [29,51–53], which provide global protein protection and preserve the quaternary structures of irradiated enzymes [29] (Fig. 3). It is important to note, based on *in vitro* enzyme studies, that high (not extreme) levels of radiation resistance are predicted to occur in cells which accumulate secondary metabolites without Mn^{2+} [29]; Mn^{2+} accumulation is not a singular determinant of radiation resistance. Rather, Mn^{2+} boosts protein protection in cells by interacting synergistically with the pool of small-molecule metabolites built up in cells.

7. Evolution of radiation resistance

The distribution of extreme radiation resistance in the phylogenetic tree of life is not domain specific. Surprisingly, there are dramatic differences in radiation resistance among organisms from the same order and even between species which share a large core of genes and which evolved from a proximal common ancestor [13,32]. Notably, *D. radiodurans* forms a clade in the gene-content tree with *Thermus thermophilus* (Fig. 4), which is as radiation sensitive as *E. coli* (Fig. 1). The genetic basis for the great differences in radiation resistance within the *Deinococcus–Thermus* group is unknown, but appears not to be the result of acquisition or loss of DNA repair genes [16,32,33], nor by elevated expression levels of the corresponding repair proteins in *Deinococcus* [54,55].

Over the last fifty years, members of the family *Deinococcaceae* have been isolated worldwide, from very diverse nutrient-poor environments [8,12]. It was, therefore, surprising to find that extremely radiation-resistant deinococcal isolates display severe metabolic defects [56]. Could radiation resistance develop through the loss of metabolic functions? *D. radiodurans* is predicted to accumulate a large pool of small molecules based on its metabolic configuration, which includes defects in biosynthesis, and expanded gene families encoding phosphatases and proteases [16,31,33,56]. *D. radiodurans* also displays a remarkable shift in the regulation of metabolic flux through the tricarboxylic acid (TCA) cycle following irradiation [57]. Potent antioxidant complexes consisting of Mn^{2+}, Pi, and small organic molecules specifically protect proteins from oxidation in *D. radiodurans* [29]. This raises the possibility that a major route to extreme radiation resistance in cells which express Mn^{2+} uptake systems is via metabolite accumulation, which may represent a widespread strategy for efficiently combating oxidative stress [29,51,52]. Numerous organisms which accumulate "compatible solutes" fit this model, including representative archaea, cyanobacteria, lichens, black yeast and fungi, and tardigrades [58–65], which are well-known for their radiation and desiccation resistance.

Intermediary organic metabolites are ordinarily present at extremely low intracellular concentration. However, mutations which block biosynthetic reactions present themselves as routes

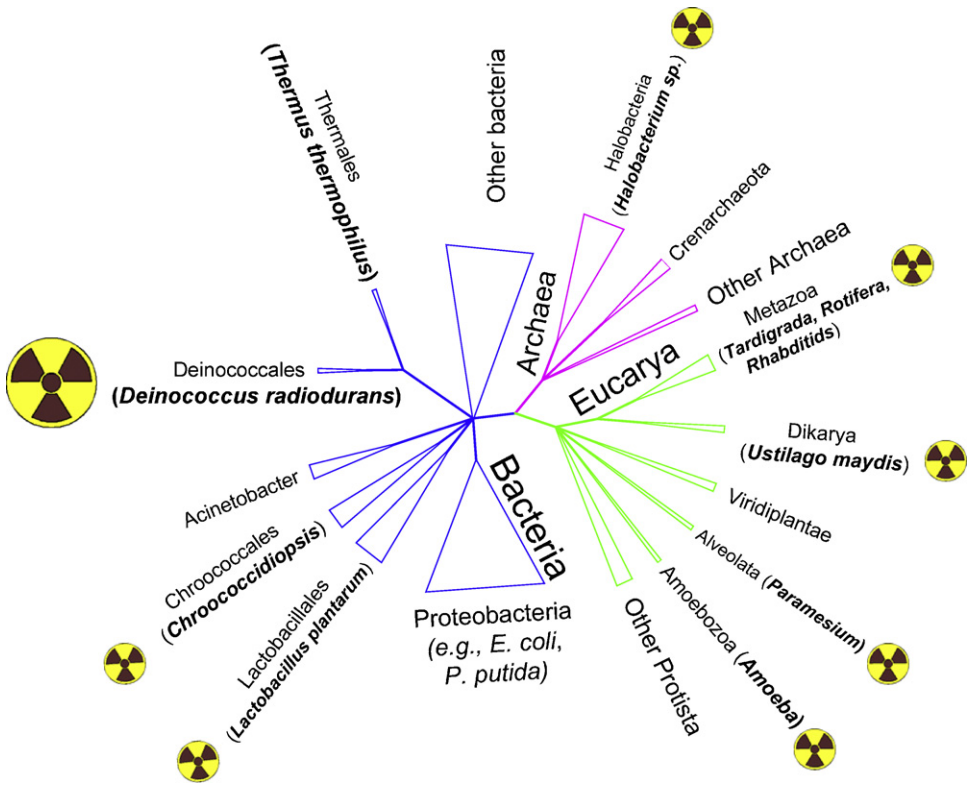

Fig. 4. Phylogenetic distribution of radiation resistant organisms. Among bacteria, *D. radiodurans* and *T. thermophilus* share a large core of genes, which evolved from a proximal common ancestor, forming a clade in the gene-content tree [32]. Yet, *T. thermophilus* is as sensitive to radiation as *E. coli* (Fig. 1). Trefoils indicate radiation resistant members. The existence of so many unrelated radioresistant species suggests that the molecular mechanisms that protect against ionizing radiation-induced damage evolved independently in these organisms [13].

to radiation resistance by promoting the constitutive accumulation of precursors which precede the defective reactions. This idea links not only naturally radiation resistant prokaryotes, but also highly radiation-resistant mutants evolved from radiation-sensitive bacteria in the laboratory. For example, for *Bacillus pumilus*, *Salmonella typhimurium* and *E. coli*, directed evolution of highly radiation-resistant mutants has been achieved by the successive passage of cells through fractionated sublethal exposures to γ-rays [66–68]. The most radiation resistant mutants of *B. pumilus* displayed multiple amino acid auxotrophies and a requirement for nicotinamide adenine dinucleotide (NAD) [66] – similar to the natural metabolic deficiencies of many *Deinococcus* species [56]. In contrast, the most resistant *S. typhimurium* mutants displayed no requirements for amino acids or NAD, and grew in minimal medium with glucose as the sole carbon-energy source [67]. However, the most resistant *S. typhimurium* mutants lost their ability to grow on non-glucose carbohydrates in minimal medium. Recent whole-genome sequence comparisons between radioresistant *E. coli* mutants and their sensitive founder strain (K-12) also lend early support to this hypothesis – (i) many mutations in metabolic genes of the resistant mutants were reported, but they have not yet been characterized comprehensively for their effects on substrate utilization or metabolite accumulation [68]; and (ii) consistent with a role of protein oxidation in the efficiency of DSB repair, the resistant *E. coli* mutants were somewhat less susceptible to radiation-induced protein oxidation than their sensitive parent [4].

As for bacteria, there are now several lines of evidence that radioresistance in cancer cells can develop during fractionated exposures to γ-rays [69]. Failure to control tumor growth and recurrence remains a major obstacle to recovery in many cases following radiation therapy. One can reasonably extend the inferences from metabolic defects in naturally and evolved resistant bacterial strains to the development of resistant cancer cells. The resistance of tumors to both radiotherapy and chemotherapy can often be attributed to its aberrant metabolism [70]. Precursor accumulation has been reported in cancer cells including grossly elevated concentrations of succinate, lactate and citrate [71,72], which can form catalytic ROS-scavenging Mn-complexes [53]; ceramide precursors are accumulated in multidrug-resistant cancer cell lines [73]; and in many adenocarcinomas, precursor accumulation also occurs [74]. The extent to which metabolism plays a role in radioresistance and tumorigenesis should not be underestimated [56,70].

8. Fresh insights into the induction of DNA repair and mutagenesis

Within the Death by Protein Damage model (Fig. 3), DNA remains the sovereign molecule which needs to be rebuilt following irradiation to ensure survival, but the level of protein oxidation ultimately determines the functionality and efficiency of DNA repair proteins. Generally, any process which inhibits the activity of DNA repair – by mutation of repair genes [8], by compounds which specifically inhibit repair enzymes [17], by mechanisms which sequester repair proteins away from chromosomal DNA [75,76], by epigenetic inactivation of repair genes [77], or by oxidative damage to repair enzymes [2,4] – will limit a cell's ability to recover from DNA damage caused by radiation. The finding that proteins are more probable initial targets of cellular ionizing radiation damage than DNA in mammalian cells [1] and sensitive prokaryotes [2] also raises the possibility that protein oxidation might trigger cellular responses involved in DNA repair. Although there is no direct evidence in the literature that damage to proteins is directly involved in the induction of DNA damage responses, results of existing studies do not rule out this possibility [78–80].

A distinctive feature of radiation sensitive bacteria is error-prone DNA repair. For example, *E. coli* is readily mutated by X-rays and UVC over the entire range of survival, but *D. radiodurans* exposed to far harsher radiation treatments displays approximately the same low level of mutagenesis that occurs during one normal round of replication [8,81]. Unlike *D. radiodurans*, upon exposure to radiation, *E. coli* induces low-fidelity DNA polymerases as part of the SOS response [78]. This is mediated by the cleavage of the LexA protein and its release from DNA–protein repressor complexes located upstream of several DNA repair and replication genes. While DSBs are strongly implicated among the primary lesions which trigger the SOS response, SOS functions in bacteria have been induced by doses of ionizing radiation as low as 1 Gy, which do not generate DNA breaks in bacteria [40,82,83]. As DNA repair proficient radiation-sensitive bacteria are highly susceptible to protein oxidation, it is conceivable that protein damage primes the induction of the SOS response – perhaps by promoting the destabilization of LexA binding to its DNA consensus sequence. DNA–protein complexes are readily disrupted by ionizing radiation, mainly due to oxidative protein modifications [1,84–86]. This could help explain how bacteria manage to mount a strong SOS response under conditions which are only minimally genotoxic [40,79]. By contrast, in human and other mammalian cells, which have genomes on the order of 3 billion bp, DSBs do occur at doses less than 0.5 Gy (0.004 DSB/Gy/Mbp), but those DSBs are not repaired efficiently [87,88]. Based on ideas presented here, it is conceivable that the levels of protein oxidation induced in mammalian cells exposed to doses (∼10 mGy) which cause few DSBs are too low to trigger DNA repair responses mediated by protein damage.

9. Conclusion and future perspectives

Two definitive insights into the reparability of broken genomes were gained recently by comparisons of DNA and protein damage in irradiated bacteria. First, the yields of DSBs per dose among naturally sensitive and extremely resistant bacteria, and for other cell types with very different antioxidant statuses are relatively constant (Table 1) – this supports that DSBs in irradiated cells are caused mainly by 'non-scavengable indirect effects' (Fig. 2). Second, the yields of protein oxidation in sensitive and resistant bacteria exposed to radiation are highly variable and quantitatively related to survival [2,4,12] – this supports that variations in radiosensitivity and efficiency of DNA repair in wild-type bacteria may be determined mainly by protein oxidation, which is governed by the antioxidant status of a cell (Fig. 3) [12]. Indeed, for many oxidative stress conditions, DNA is no longer considered the principal target of ROS that accounts for their toxicity [2–4,44,89–91]. These trends parallel some emerging for irradiated mammalian cells. For example, the relationship between DSBs and γ-ray dose in human cells is about the same as in other cell-types [87,92] (Table 1). In cultured mouse cells exposed to γ-rays, protein oxidation precedes DNA damage, and is implicated as a critical and very early event in radiotoxicity [1]. Moreover, mouse cells which maintain low levels of ROS, either naturally or by treatment with antioxidants, are consistently more resistant to ionizing radiation than cells with high ROS levels, but with no overt effects on DSB yields [93]; and, highly radiation resistant cancer cells (*e.g.*, osteosarcoma cells) display high ROS-scavenging capacities and highly efficient DNA repair compared to normal mammalian cells [94]. However, the degree to which protein oxidation is expected to influence recovery of irradiated mammalian cells at low doses is greater than for bacteria because of the impact of genome size (Table 1). For example, 16 Gy does not cause DSBs in an *E. coli* genome (4.6 Mbp), and any oxidative damage to DSB repair proteins in *E. coli* exposed to 16 Gy would be inconsequential. Not so

for most human cells in liquid culture, where a typically lethal dose of 16 Gy is expected to cause approximately 190 DSBs per haploid genome [87,92]. In this context, it has been demonstrated that human Jurkat T cells exposed to 16 Gy in liquid culture can be rescued by *Deinococcus* protein-free cell extracts, which are highly enriched in Mn^{2+}–peptide–phosphate complexes [29]. Thus, the level of protein protection may also set the efficiency of DSB repair and radiation resistance in human cells exposed to higher doses (0.5–20 Gy).

Since the 1960s the overriding goal of the field radiobiology was to develop medical countermeasures against ionizing radiation – for medical purposes and national defense. Unfortunately, few advances have been made in radioprotection in the last several decades and large gaps persist in the treatment of radiation exposure. Today, radiation resistant prokaryotes stand poised to help expand radiation countermeasures in diverse settings, from pre-exposure prophylactic interventions to post-exposure therapeutics.

Conflict of interest statement

Michael J. Daly declares that there are no conflicts of interest.

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