DEINOCOCCUS RADIODURANS — THE CONSUMMATE SURVIVOR

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Abstract | Relatively little is known about the biochemical basis of the capacity of *Deinococcus radiodurans* to endure the genetic insult that results from exposure to ionizing radiation and can include hundreds of DNA double-strand breaks. However, recent reports indicate that this species compensates for extensive DNA damage through adaptations that allow cells to avoid the potentially detrimental effects of DNA strand breaks. It seems that *D. radiodurans* uses mechanisms that limit DNA degradation and that restrict the diffusion of DNA fragments that are produced following irradiation, to preserve genetic integrity. These mechanisms also increase the efficiency of the DNA-repair proteins.

IONIZING RADIATION
Any electromagnetic or particulate radiation powerful enough to strip electrons from atoms to produce ions.

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In 1956, Anderson *et al.*[1] isolated a novel vegetative bacterium from canned ground meat that had been γ-irradiated at 4,000 Gray (Gy), a dose that is approximately 250 times higher than that typically used to kill *Escherichia coli*. The authors named this species *Micrococcus radiodurans* because of its superficial morphological similarity to members of the genus *Micrococcus*. However, research on *M. radiodurans* over the next 30 years resulted in reclassification of this species and its closest relatives into a distinct phylum within the domain Bacteria[2–7]. The genus name — *Deinococcus* — was based on the Greek adjective 'deinos', which means strange or unusual[8]; an apt description for an organism with an ability to survive genetic damage that sets it apart from much of the life on Earth.

Treatment of *Deinococcus radiodurans* with high levels of IONIZING RADIATION can produce hundreds of genomic double-strand breaks (TABLE 1), but the genome is reassembled accurately before initiation of the next cycle of cell division. The extraordinary capacity of *Deinococcus* spp. to reconstitute their genomes has inspired a small but growing community of researchers who are interested in the relevant mechanisms that are used to achieve this. In recent years, investigation of the biology and biochemistry of *Deinococcus* spp. has accelerated, catalysed by the availability of genome information and the development of genetic tools. Evidence has accumulated for both passive and enzymatic contributions to genome restitution (FIG. 1), which provides a framework for our discussion in this review.

Ionizing-radiation resistance

Of all the phenotypes associated with prokaryotes, resistance to ionizing radiation is one of the most difficult to rationalize in terms of natural selection. As there are no naturally occurring environments known that result in exposures exceeding 400 mGy per year[9], it is unlikely that species evolved mechanisms to protect themselves against the effects of high-dose ionizing radiation *per se*. Instead, it seems that the damage introduced by γ-irradiation shares features with the damage that results from other stresses to which bacteria have adapted. For example, desiccation introduces many DNA double-strand breaks into the genomes of *D. radiodurans*[10] and members of the cyanobacterial genus *Chroococcidiopsis*[11]. Both organisms are tolerant to desiccation and are resistant to the potentially lethal effects of ionizing radiation, which might indicate that the radioresistance of these species is a fortuitous consequence of their ability to tolerate desiccation-induced strand breaks.

TABLE 2 lists members of six bacterial phyla that are resistant to ionizing radiation. The Deinococcaceae are the best-known family on this list, and have been

Table 1 | **Double-strand breaks formed in *E. coli* and *D. radiodurans***

Species*	Genomes per cell	DNA double-strand breaks‡ at D_{37}	Average distance between lesions
Escherichia coli K12	4–5	8–9	530,000 bp
Deinococcus radiodurans R1	8–10	275	10,000 bp

*In each study, actively growing aerobic cultures were irradiated and the number of double-strand breaks determined using neutral sucrose-gradient centrifugation. ‡For *D. radiodurans* this is lesions per unit length of chromosome I (2.64 Mb). Chromosome I is used because it is the largest target within the cell. Calculations are based on a measured rate of 1 DNA double-strand break per 10 Gray per 5×10^9 Da of double-stranded DNA. D_{37}, the dose at which 37% of the cells survive.

studied for five decades. Little has been learnt about the other bacterial species listed, other than their capacity to tolerate ionizing radiation. Draft genome sequences of *Rubrobacter xylanophilus* and *Kineococcus radiotolerans* have been released in the past year, but detailed analyses of these sequences have not yet been reported.

There is no clear pattern of evolution among ionizing-radiation-resistant species (FIG. 2). The scattered appearance of ionizing-radiation resistance among distinct prokaryotic lineages indicates two possibilities. First, radioresistance could be a vestige of DNA-repair mechanisms that were present in ancestral species and have been retained in those organisms that continue to require this phenotype. This explanation assumes that the ancestor's ability to cope with DNA damage has been lost by most descendents, and predicts that the molecular mechanisms of radioresistance should be similar among ionizing-radiation-resistant species. Second, given the infrequent occurrence of ionizing-radiation resistance, it is possible that this phenotype has arisen in unrelated species through horizontal gene transfer, or possibly convergent evolution. Much as birds and bats evolved wings despite distinct evolutionary origins, it is possible that these diverse prokaryotic cells adapted differently to a similar selective pressure and that there might be multiple mechanisms of radioresistance. This latter possibility seems more probable, as fewer independent evolutionary events (the loss of resistance from each species being considered a separate event) would be required to generate the handful of known radioresistant species.

Radiation resistance is not restricted to the domain Bacteria. Several HYPERTHERMOPHILIC archaea (members of the Euryarchaeota and Crenarchaeota) show extreme ionizing-radiation resistance[12–15], in some cases (for example, *Thermococcus gammatolerans*) comparable to that of *Deinococcus radiodurans*[14].

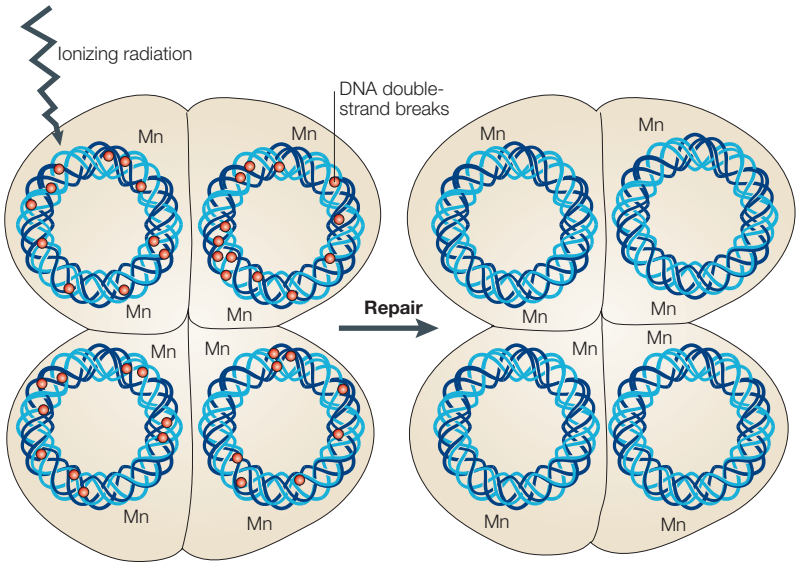


Figure 1 | **Potential contributions to the recovery from radiation damage in *Deinococcus radiodurans*.** The schematic depicts a *D. radiodurans* tetracoccus. The nucleoid in each compartment is highly condensed and maintains its overall architecture after irradiation. High levels of Mn(II) might contribute to the recovery from DNA damage. A wide range of enzymes probably also contribute to genome reconstitution.

The Deinococcaceae

The Deinococcaceae family comprises 11 validly described species — *D. radiodurans*, *Deinococcus proteolyticus*, *Deinococcus radiopugnans*, *Deinococcus grandis*, *Deinococcus radiophilus*, *Deinococcus geothermalis*, *Deinococcus murrayi*, *Deinococcus indicus*, *Deinococcus frigens*, *Deinococcus saxicola* and *Deinococcus marmoris* — all grouped in a single genus, *Deinococcus*[16–18]. Deinococci do not form spores and are non-motile. Most species grow best in rich media at temperatures between 30 and 37°C, with a doubling time between 1.5 and 3 hours. However, *D. geothermalis* and *D. murrayi* are true thermophiles, with optimal growth temperatures of 45–55°C. With the exception of *D. grandis*, which is rod-shaped, all *Deinococcus* species are spherical cells that exist singly or as pairs and tetrads in liquid culture.

To date, *D. radiodurans* has received more attention than the other deinococci. The genome of *D. radiodurans* strain R1 (ATCC BAA-816) has been sequenced[19,20] and can be accessed at the TIGR Comprehensive Microbial Resource database (see Online links box). The *D. radiodurans* chromosome is 3.28 Mb, with a GC content of 66.6%. There are nine types of short nucleotide repeats, ranging in size from 60 to 215 bp, found at 295 sites that are randomly scattered in the genome. The genome is segmented and consists of a 2.64-Mb chromosome (chromosome I), a 0.41-Mb chromosome (chromosome II), a 0.18-Mb megaplasmid and a 0.045-Mb plasmid[21]. *D. radiodurans* has between 4 and 10 genome copies per cell, depending on the stage of the bacterial growth phase[22,23].

The γ-irradiation survival curves of actively growing cultures of *D. radiodurans* R1 have a shoulder of resistance up to 5,000 Gy[24], and until this dose is achieved there is no measurable loss of viability in the irradiated culture. Under these conditions, the D_{37} dose (BOX 1) for *D. radiodurans* R1 is approximately 6,500 Gy. Assuming that there are eight genome copies per cell in these cultures, a 5,000-Gy dose will introduce approximately 1,600 double-strand breaks per cell. As this dose is sublethal, it can be inferred that potentially catastrophic deletions and genome rearrangements occur at low frequencies. Although there is no formal proof, it seems that the process of double-strand-break repair in *D. radiodurans* is error-free.

Table 2 | **Species of ionizing-radiation-resistant bacteria**

Species	Representative D_{10} value*	Phylum	Refs
Methylobacterium radiotolerans	1,000 Gray	α-Proteobacteria	80,81
Kocuria rosea	2,000 Gray	Actinobacteria	4,8
Acinetobacter radioresistens	2,000 Gray	γ-Proteobacteria	82
Kineococcus radiotolerans	2,000 Gray	Actinobacteria	83
Hymenobacter actinosclerus	3,500 Gray	Flexibacter–Cytophaga–Bacteroides	84
Chroococcidiopsis spp.	4,000 Gray	Cyanobacteria	11
Rubrobacter xylanophilus	5,500 Gray	Actinobacteria	85
Deinococcus radiodurans R1	10,000 Gray	Deinococcus–Thermus	86
Rubrobacter radiotolerans	11,000 Gray	Actinobacteria	85

*The D_{10} defines the dose that is needed to eradicate 90% of the irradiated population. The D_{10} values for a given species vary substantially depending on growth conditions. The values provided are for actively growing oxygenated cultures.

TABLE 1 compares the number of double-strand DNA breaks that occur in the large chromosome of *D. radiodurans* R1 (REF. 25) and the *E. coli* K12 (REF. 26) genome at the D_{37} radiation dose. The difference is striking: compared with *E. coli* K12, *D. radiodurans* tolerates approximately 30-fold more DNA double-strand breaks before succumbing to the damage. Clearly, *D. radiodurans* R1 cells have mechanisms to avoid the lethal effects of double-strand breaks that are absent in *E. coli*.

Whatever the mechanisms that *D. radiodurans* uses, they do not prevent DNA damage. Recent evidence from two groups of researchers has shown that measurable double-strand breaks form at the same rate in *E. coli* and *D. radiodurans* if cultures are irradiated under identical conditions[27,28], which makes the existence of a mechanism that blocks the formation of strand breaks in the *D. radiodurans* genome unlikely. In contrast to most other cells, *D. radiodurans* has the ability to efficiently and accurately repair that damage.

The observation that *D. radiodurans* cannot passively prevent DNA damage does not rule out the possibility that other passive mechanisms contribute to the capacity of the cell to tolerate DNA damage. Experimental evidence indicates that the recovery of *D. radiodurans* from substantial DNA damage relies on both passive features of deinococcal physiology and a robust complement of repair enzymes. There are several mechanisms that have the potential to contribute to ionizing-radiation resistance. For example, there is a clear requirement for RecA-dependent homologous recombination — this process has been examined and reviewed extensively[29–36].

In this review, we do not attempt to attribute the contribution to recovery from DNA damage that is made by each different mechanism, and the reader should bear in mind that new recovery mechanisms might still await discovery.

Passive contributions to radioresistance

Genome copy number. Cells with increased numbers of genome copies have enhanced resistance to ionizing radiation[26,37]. The extra genetic material is thought to protect the cell in two ways. First, when multiple genomes are present, there are additional copies of crucial loci that improve the chance of the cell surviving irradiation. The probability of inactivating a specific gene in an organism is given by $P=1-(1-1/M)^L$ (where M is the total number of genes present, and L is the number of inactivating lesions per genome). For a specific dose of radiation, the probability of inactivating all the copies of a specific gene is equivalent to P^N, where N is the number of gene copies present (assuming that

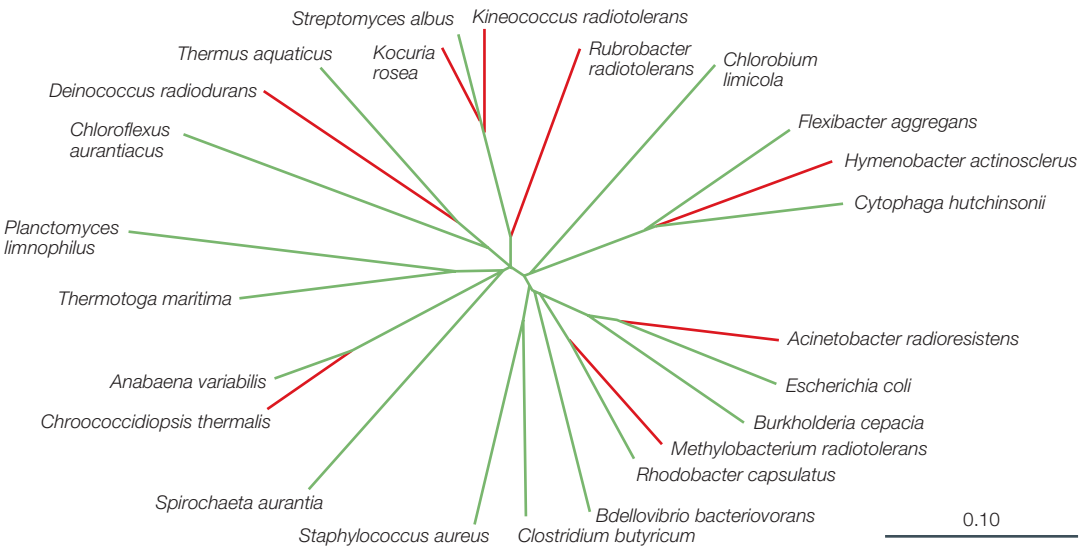


Figure 2 | **A 16S-rRNA-gene-sequence-based phylogeny of the main lineages of the domain Bacteria.** The branches in red are those in which ionizing-radiation-resistant taxa have been described. The scale bar represents 10 inferred nucleotide substitutions per 100 nucleotides.

HYPERTHERMOPHILIC
Organisms that have an optimal growth temperature above 80°C.

Box 1 | **High-energy radiation and its effect on DNA**

Ionizing radiation is radiation with sufficient energy to ionize molecules. There are two types of ionizing radiation, both produced by the decay of radioactive elements: electromagnetic (X- and γ-radiation that form part of the electromagnetic spectrum that includes visible light and radio waves) and particulate (α- and β-particles). Different types of ionizing radiation deposit energy in matter at different rates. α- and β-particles produce ionization by collisions, depositing their energy within a short range after entering matter. γ-rays are photons that generate ions by several types of energy-absorption events (most commonly by the Compton effect, an increase in the wavelength of electromagnetic radiation when it collides with electrons in matter, see the figure) and can penetrate deeply into a cell or tissue. Ion production is accompanied by the release of energetic electrons (see the figure), and multiple ions and electrons can be generated in one event. The figure shows the tracks of three different types of ionizing radiation. Small dots indicate energy deposition events. The inset depicts the ejection of an electron from an atom to generate an ion, mediated by an encounter with a γ-ray photon. The γ-ray transfers part of its energy to a valance electron, which is thereby ejected from the nucleus to create an ion. The scattered γ-ray can undergo additional Compton effects within the matter.

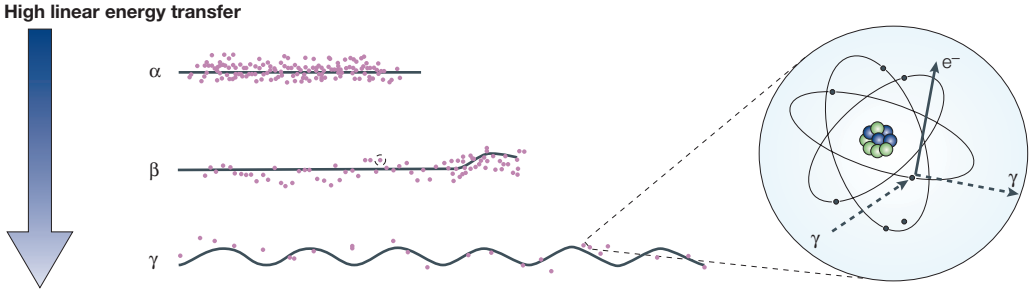
Ions react with other molecules to produce free radicals on a nanosecond timescale. In water, the products include highly reactive hydroxyl radicals. Typically, each energy deposition event can generate 2–5 radical pairs within a radius of a few nm.

The energy deposited (the dose absorbed by the cell, tissue or other matter) is measured in rads or in Gray (SI measurement; 100 rad = 1 Gray). The radioresistance of microorganisms is compared by measurement of the D_{37} dose (the dose at which 37% of the cells survive). At the D_{37} dose, each cell on average has experienced a lethal event (those that survive are balanced by other cells with two or more lethal events).

The ions and free radicals produced as radiation passes through matter react rapidly and modify molecules. Of all the effects, genome damage probably has the greatest impact on cell viability. The reasons for this are threefold. First, genomic DNA occupies the largest fraction of the cell volume, and will therefore be 'hit' most often. Second, the genome is present in lower copy numbers than other molecules — there is little redundancy. Third, and most importantly, the genome regulates all cellular functions, so loss of any portion is catastrophic for a single-celled organism.

Ionizing radiation generates multiple types of DNA damage. As much of the damage results from the action of hydroxyl radicals, the spectrum of damage is similar to that produced by oxidative damage associated with endogenous aerobic metabolism. In DNA, the nucleobase is most often modified, and many dozens of different structural modifications to the bases have been characterized. However, 10–20% of the time the sugar-phosphate moiety is affected, which can lead to a single-strand break. The 5′ and 3′ ends at these breaks are usually phosphorylated, indicating that one or more nucleotides have been excised at the break site. The 3′ ends sometimes include a glycolate moiety derived from fragmentation of the deoxyribose.

Most of the lesions are accurately repaired by robust DNA-repair systems present in all cells, but double-strand breaks are the most difficult to repair, and therefore the most lethal. They can arise when single-strand breaks occur by chance in close proximity on opposite strands, when a cluster of hydroxyl radicals introduce strand breaks in both strands at one location, or when the cell attempts to enzymatically excise damaged bases present in close proximity on both strands. Double-strand breaks can result in significant loss of genetic information and, if not repaired, will prevent replication of the prokaryotic genome. How an organism deals with double-strand breaks lies at the heart of that organism's capacity to survive radiation exposure.



there is one copy of each gene on each genome copy, N is equal to the number of genome copies). If 100 inactivating lesions are randomly introduced into a single copy of the *D. radiodurans* genome (2,897 genes), the probability of inactivating any specific gene on that genome copy is 3.4%. The probability of inactivating all the copies of the same gene is reduced to 0.12% when two genomes are present, and to 0.004% when there are three genomes.

Redundant genetic information also functions as a reserve that can be used to repair DNA segments that are damaged (or degraded) beyond repair. In *E. coli* K12, repair of DNA double-strand breaks only occurs during the exponential growth phase in rich media, when cells contain multiple chromosomes[26], and diploid and tetraploid yeast are more resistant to ionizing radiation than isogenic haploid strains[37]. It has been hypothesized that genetic redundancy contributes

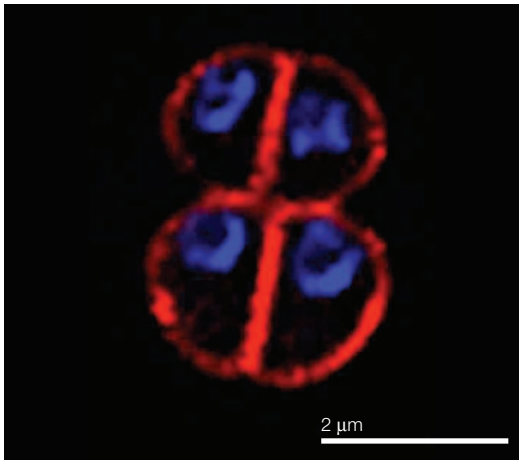


Figure 3 | **A tetrad of *Deinococcus radiodurans*.** An epifluorescence image of stationary-phase *D. radiodurans* R1. DNA is stained with DAPI and appears blue in the figure; the membrane is stained with the dye FM4-64 and appears red.

to the radioresistance of *Deinococcus* spp., although this idea has not been tested. The number of genome copies in *D. radiodurans* has never been reported to be less than four, and cells have the same survival rate whether they contain four or ten genome copies[23]. The contribution of genome redundancy to radioresistance in *D. radiodurans* (relative to other mechanisms) is unclear. Many bacteria contain more than one genome copy, particularly during exponential growth. For example, *E. coli* contains between five and 18 genome equivalents during exponential-phase growth[38], and *Azotobacter vinelandii* can accumulate more than 100 genome copies[39]. Neither species is radioresistant, so genetic redundancy alone does not account for radioresistance. Nevertheless, repair of DNA double-strand breaks by recombination requires the presence of more than one genome[26,37], so it seems probable that genome redundancy is necessary for genome repair.

Nucleoid organization. The nucleoids of stationary-phase *D. radiodurans* cells (FIG. 3) are arranged as a tightly structured ring[40] that remains unaltered by high-dose irradiation[41]. Minsky and colleagues[40] have suggested that this structure passively contributes to *D. radiodurans* radioresistance by preventing the fragments that are formed by double-strand breaks from diffusing apart during repair, which maintains the linear continuity of the genome even when it is fragmented. Although intellectually appealing, this hypothesis is controversial and has been criticized because the prevalence of ring-like nucleoids under different growth conditions does not always correlate with ionizing-radiation resistance[27,41]. A recent examination of the nucleoids of members of the radioresistant genera *Deinococcus* and *Rubrobacter* revealed a high degree of genome condensation — regardless of nucleoid shape — relative to the more radiosensitive species *E. coli* and *Thermus aquaticus*[41], which could indicate that species with a condensed genome might be better protected from ionizing radiation.

Manganese content. Daly and colleagues examined the effects of the high concentrations of Mn(II) that can accumulate in *D. radiodurans*[27] on the capacity of these cells to survive irradiation. When *D. radiodurans* cultures are starved of Mn(II), their resistance to ionizing radiation decreases[27]. The numbers of DNA double-strand breaks formed are the same for a specific dose of ionizing radiation both in the presence or absence of Mn(II), so Mn(II) does not prevent DNA damage[27]. Instead, cellular damage that results from exposure to high radiation doses is better tolerated if Mn(II) is present.

Most DNA damage that occurs after exposure to ionizing radiation results from the generation of reactive oxygen species (ROS) and the hydrolytic cleavage of water. Intracellular Mn(II) can be protective by scavenging ROS. For example, *Lactobacillus plantarum* lacks the protective enzyme superoxide dismutase, and instead substitutes with intracellular Mn(II) concentrations of 20–25 mM[42,43]. As the levels of double-strand breaks in *D. radiodurans* seem to be unaffected by Mn(II), any scavenging of ROS must protect macromolecules other than DNA. Daly *et al.*[27,44] have proposed that Mn(II) accumulation prevents superoxide and related ROS that are produced during irradiation from damaging proteins. If this is the case, bacteria that do not accumulate sufficient Mn(II) might succumb to ionizing-radiation-induced protein damage before DNA is significantly damaged[44].

Alternatively, the increased Mn(II) concentration could contribute to the condensation of the *D. radiodurans* genome[40,45]. DNA can be condensed *in vitro* by adding multivalent cations to an aqueous solution of DNA — the cations neutralize the repulsion of phosphate groups in the DNA backbone[46]. In this way, the proposals of Daly[27,44] and Minsky[40,45] could be related and have similar positive consequences in the context of genome reconstitution.

Regulation of cellular responses to extensive radiation damage. When *D. radiodurans* is exposed to ionizing radiation, a well characterized sequence of physiological events take place, including rapid cessation of DNA replication. At sublethal doses, the duration of the replication delay always exceeds the time that is required to repair the DNA damage that caused inhibition of replication[47–49]. The capacity to inhibit DNA replication is not unlike the DNA-damage checkpoints of eukaryotes — mechanisms that sense DNA damage and initiate a delay in the cell cycle until the damage is repaired[50]. However, the existence of a DNA-damage checkpoint operating in *D. radiodurans* has not been formally established.

Potential enzymatic contributions to repair

Ultimately, DNA strand breaks must be enzymatically repaired, and *Deinococcus* spp. can use novel repair processes. In 1996, Daly and Minton provided evidence for a genome-repair process that involved a temporal progression through at least two distinct stages[31]. Substantial chromosome repair occurs during the first

Table 3 | **Selected *Deinococcus radiodurans* proteins with putative functions in DNA repair**

Protein	Region of homology	% identity	% similarity	Comments
Ligase (DnlJ)	11–669/671 *Escherichia coli*, 29–681/708 *D. radiodurans*	42	57	-
PolA	5–924/928 *E. coli*, 43–952/965 *D. radiodurans*	35	49	-
PriA	202–729/731 *E. coli*, 404–922/924 *D. radiodurans*	26	42	-
RecA	4–324/353 *E. coli*, 16–336/362 *D. radiodurans*	57	72	-
RecD	30–598/609 *E. coli*, 218–705/716 *D. radiodurans*	27	40	N-terminal extension in *D. radiodurans*
RecF	3–330/358 *E. coli*, 6–326/360 *D. radiodurans*	28	43	-
RecG	6–693/694 *E. coli*, 107–777/785 *D. radiodurans*	39	53	N-terminal extension in *D. radiodurans*
RecJ	68–570/579 *E. coli*, 3–461/685 *D. radiodurans*	34	51	C-terminal extension in *D. radiodurans*
RecN	2–553/553 *E. coli*, 34–564/564 *D. radiodurans*	31	49	-
RecO	7-157/242 *E. coli*, 10-159/224 *D. radiodurans*	*18*	*34*	Low homology; required PSI-BLAST
RecQ	9–600/608 *E. coli*, 8–605/824 *D. radiodurans*; 557–605/608 *E. coli*, 680–728/824 *D. radiodurans;* 549–606/608 *E. coli*, 768–825/824 *D. radiodurans*	46, 36, 33	63, 64, 59	HRDC domain repeated three times in *D. radiodurans*
RecR	1–199/202 *E. coli*, 1–196/220 *D. radiodurans*	42	55	C-terminal extension in *D. radiodurans*
RuvA	1–199/203 *E. coli*, 1–197/201 *D. radiodurans*	33	49	-
RuvB	13–332/337 *E. coli*, 2–321/333 *D. radiodurans*	56	75	A second orthologue with weaker similarity is present
RuvC	4–168/174 *E. coli*, 3–166/179 *D. radiodurans*	33	51	-
SbcC	27–1032/1049 *E. coli*, 22–896/909 *D. radiodurans*	21	35	-
SbcD	1–293/400 *E. coli*, 24–319/417 *D. radiodurans*	28	46	C-terminal changes

For each protein, the region of homology is indicated as residue numbers, followed by the total number of residues in the protein. The gene for the single-stranded-DNA-binding protein (SSB) is described in the text. No homology found for DnaC, DnaT, PriB, PriC, RecB, RecC, RecE, RecT or SbcB.

1.5 hours after *D. radiodurans* is treated with a high dose of ionizing radiation, through RecA-independent repair processes. Almost one-third of the double-strand breaks were repaired in this phase. RecA-dependent recombinational DNA repair becomes important several hours after irradiation, and predominates in the later stages of genome reconstitution[31]. Proteins that are known to be potentially important in genome-repair processes are listed in TABLE 3.

A search for novel genes that are induced in response to ionizing radiation and desiccation, using genome-based microarrays, provided new evidence for both RecA-independent and RecA-dependent pathways of double-strand-break repair[51]. In exponentially growing cells, 72 genes are induced three-fold or more after γ-irradiation. Seventy-three loci were induced during recovery from extended desiccation, and 33 of these genes were also induced following irradiation. The five genes most highly induced in response to both stresses were identical and encode proteins of unknown function. Inactivation of these loci — *ddrA*, *ddrB*, *ddrC*, *ddrD* and *pprA* — produces phenotypes that are relevant for genome repair. Genetic analyses defined three EPISTASIS GROUPS that affect ionizing-radiation resistance, and established that two of the loci (*ddrA* and *ddrB*) contribute to radioresistance through different RecA-independent processes. The *pprA* and *recA* loci form a third epistasis group, indicating that the *pprA* gene product interacts with RecA. Identification of these novel loci indicates that there are new mechanisms with an important role in genome repair of *D. radiodurans* post-irradiation.

EPISTASIS GROUP
This occurs when two or more genes control a phenotype. The combined effect of mutations in these genes on a phenotype deviates from the sum of their individual effects.

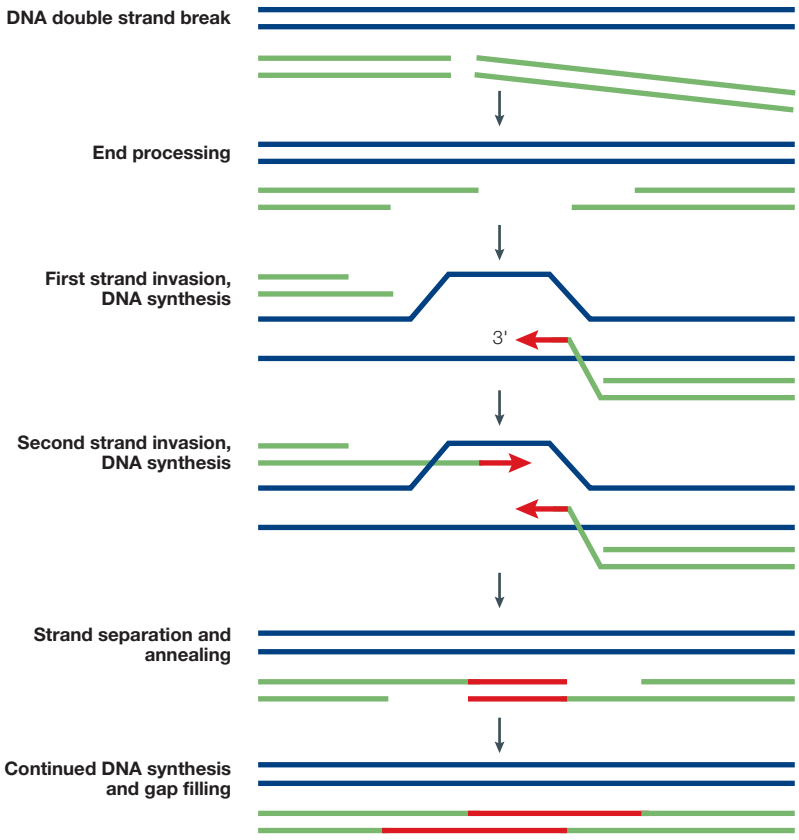


Figure 4 | **Synthesis-dependent strand annealing.** A mechanism of error-free double-strand-break repair that is initiated by creating 3′ overhangs from the ends of the broken DNA duplex (green in the figure). One of these 3′ ends invades a homologous region on an undamaged sister duplex (blue in the figure), priming DNA synthesis and creating a D-loop that acts as a template or DNA synthesis primed by the other 3′ end. If displaced, the newly synthesized DNA can anneal, closing the double-strand break. Newly synthesized DNA is coloured red.

DNA end protection. The *ddrA* locus was the first of the five new genes to be characterized[52]. Deletion of *ddrA* function results in a modest increase in radiation sensitivity in cells grown in rich media. However, when cells are irradiated and then starved, deletion of *ddrA* results in a 100-fold loss of viability over 5 days compared with wild-type cells. The loss in viability of the *ddrA* mutant is accompanied by a dramatic decrease in genomic DNA content by nucleolytic degradation. The DdrA protein binds to the 3′ ends of single-stranded DNA *in vitro* and protects them from nucleolytic degradation[52].

DdrA seems to function as a DNA-end-protection system. As double-strand breaks occur, DdrA (and perhaps other proteins) binds to the exposed DNA ends and prevents nuclease digestion of the chromosomal DNA. This strategy is particularly useful in the genome repair that occurs after desiccation. DNA repair uses a lot of metabolic energy, but cells recovering after desiccation in an environment that lacks nutrients would not have the opportunity to repair DNA damage. As DNA strand breaks occur, nuclease action could degrade genomic DNA. By protecting the broken DNA ends, cells could preserve genomic DNA until conditions become suitable for cell growth and DNA repair.

NON-HOMOLOGOUS END JOINING
One of several pathways that can be used to repair chromosomal double-strand DNA breaks. The process is non-homologous because adjacent broken strands are fused by direct end-to-end contact without regard to sequence homology. Therefore, non-homologous end joining is error-prone because it results in joining of the breaks without a template.

Bioinformatics initially failed to identify any DdrA homologues, but further bioinformatics investigation revealed that DdrA was distantly related to the eukaryotic Rad52 protein and to the prokaryotic Redβ, Erf and RecT proteins[52,53], so additional functions for DdrA have not been ruled out.

RecA-independent double-strand-break repair. Both NON-HOMOLOGOUS END JOINING (NHEJ) and single-strand annealing (SSA) pathways have been hypothesized to function in *D. radiodurans*. Minsky and colleagues[40,45] suggested that NHEJ would be a useful process for the repair of double-strand breaks in the context of a condensed chromosome, in which ends might not be free to diffuse away from each other. An NHEJ system has been identified in *Bacillus subtilis*[54] and is probably present in other bacteria. Two other laboratories also recently suggested that NHEJ occurs in *D. radiodurans*[55,56]. PprA and PolX are two proteins with predicted activities that are consistent with the existence of NHEJ[55,56]. However, classical NHEJ systems are generally error-prone[57] and seem unsuited to the accurate genome repair that is observed in *Deinococcus* spp. Patterns of recombination between plasmids and the re-circularization of integrated plasmids in irradiated *Deinococcus* cells are not consistent with NHEJ[31].

Plasmid repair and re-circularization of genome-integrated plasmids during the RecA-independent phase of DNA double-strand-break repair in *D. radiodurans* is dependent on homology, indicating that SSA might have a role[31]. Recent research has suggested a compelling model for genome restitution in this species, in which the related but more efficient process called synthesis-dependent single-strand annealing (SDSA) plays a necessary part (M. Radman, personal communication). It provides evidence that *D. radiodurans* R1 uses SDSA as a first step in genome re-assembly. During SDSA, the 3′ end of a strand derived from a DNA double-strand break invades the homologous region of a sister duplex (FIG. 4). The invading 3′ end is used to prime DNA synthesis, unwinding the sister duplex and enlarging the D-loop. The displaced strand in the undamaged complex anneals to the remaining free 3′ end created by the double-strand break. As each 3′ end primes complementary DNA synthesis, the resulting newly synthesized strands can anneal, sealing the breach in the damaged duplex in an error-free manner. This work shows that all RecA-independent genome assembly requires extensive *polA*-dependent DNA synthesis, and that the pattern of nucleotide incorporation, as measured by density labelling post-irradiation, indicates a distributive mode of replication that is consistent with SDSA. The amount of newly synthesized DNA recorded during this phase of post-irradiation recovery indicates that the tails are longer than expected based on precedent in other species that exhibit SDSA. The authors suggest that the long tails assure precise annealing, ensuring an error-free recovery.

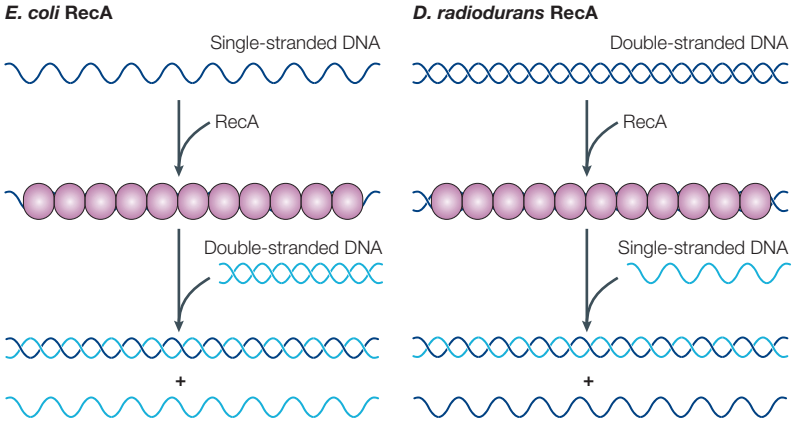


Figure 5 | **Inverse DNA-strand exchange promoted by the *Deinococcus radiodurans* RecA protein.** Instead of forming a filament on single-stranded DNA and then reacting with a homologous duplex, strand exchange is initiated by a filament formed on duplex DNA.

At least one additional RecA-independent pathway mediated by the DdrB protein is present in *D. radiodurans*[51]. Although DdrB has not yet been characterized *in vitro*, deletion of *ddrA* and *ddrB* produces a mutant that is significantly more sensitive to ionizing radiation than either *ddrA* or *ddrB* mutants, indicating that DdrA and DdrB have complementary activities.

Recombinational DNA repair. Any ends that lack overlapping sequence or other means to guide accurate repair require recombinational repair using redundant genome information. Studies of the roles of the classical proteins involved in bacterial recombinational DNA repair in *D. radiodurans* have been initiated (TABLE 3), including studies of recombinase A (RecA), single-strand-binding protein (SSB), recombinase D (RecD), DNA polymerase I, recombinase R (RecR) and recombinase O (RecO).

The *D. radiodurans* RecA protein (361 amino acids, M_r 38,013) is 57% identical (72% similar) to the *E. coli* RecA protein (352 amino acids, M_r 37,842). *In vitro*, the protein promotes all of the key recombinogenic activities of RecA-class recombinases. It forms filaments on DNA, hydrolyses ATP and dATP in a DNA-dependent fashion and promotes DNA-strand exchange[58]. However, the *D. radiodurans* RecA protein has one distinct function. The DNA strand-exchange reactions of the *E. coli* RecA protein, and all other homologues examined to date, are ordered so that the single-stranded DNA is generally bound first, before the double-stranded DNA is bound. This order of DNA binding makes sense from a biological standpoint, as the RecA protein must be targeted to single-strand gaps at stalled replication forks and other damaged DNA sites. By contrast, the *D. radiodurans* RecA protein promotes an obligate inverse DNA-strand-exchange reaction[59], binding the duplex DNA first and the homologous single-stranded DNA substrate second (FIG. 5). It is probable that this reaction pathway reflects the function of *D. radiodurans* RecA in double-strand-break repair, although its significance is not yet clear.

Based on the properties of an E142K mutant of *D. radiodurans* RecA (also known as RecA424), Satoh *et al.*[60] suggested that the effect of *D. radiodurans* RecA on double-strand-break repair primarily reflected a regulatory rather than a recombination function. The mutant protein does not complement the null phenotype of a *recA* knockout in *E. coli*, but does have residual regulatory functions associated with RecA. Strains harbouring the E142K mutation seem to retain resistance to γ-irradiation. However, the E142K mutation does retain significant DNA-strand-exchange activity in some assays, and more work is needed to define the function of RecA in *D. radiodurans*. Assuming that *D. radiodurans* must locate and splice together overlapping segments of its chromosomes to reconstruct a functional genome, a DNA-pairing activity such as that provided by RecA would be at the centre of such a process.

The *ssb* gene in *D. radiodurans* was originally annotated as a tripartite gene, with two frameshifts required to form a functional open reading frame (ORF) for translation[20,61–63]. However, this locus seems to be specific to the *D. radiodurans* R1 strain that was sequenced (ATCC BAA-816), as it differs substantially from that of the *D. radiodurans* R1 type strain (ATCC 13939). In the type strain, the *ssb* locus is a single continuous ORF that encodes the largest bacterial SSB polypeptide identified to date. It has two oligonucleotide/oligosaccharide-binding (OB) folds rather than the one present in most bacterial SSB proteins, and it functions as a dimer rather than a tetramer[64]. The gene is highly homologous to *ssb* genes found in *Thermus* species, to which *D. radiodurans* is closely related. The *D. radiodurans* SSB protein is efficient at stimulating the DNA-strand exchange that is promoted by RecA proteins from both *E. coli* and *D. radiodurans*, being more active than the *E. coli* SSB in both cases. The atomic structure of *D. radiodurans* SSB has been solved[65]. This protein could be important in chromosome repair. The concentration of the *D. radiodurans* SSB is an order of magnitude higher than the normal *in vivo* levels of the SSB protein in *E. coli*.

Less is known about other classical repair proteins in *D. radiodurans*. In the absence of a *recB* or *recC* gene, the *recD* gene product has been characterized. The *D. radiodurans* RecD protein has a 5′ to 3′ helicase activity, in common with the RecD subunit of the *E. coli* RecBCD enzyme[66]. It can unwind short (20 bp) duplexes if a 5′ single-strand tail is adjacent. The atomic structure of the *D. radiodurans* RecO protein has been solved[67], but nothing is known about its function. Similarly, the structure of the *Deinococcus* RecR protein has been determined[68].

Narumi and colleagues have shown that purified PprA protein can bind to duplex DNA with strand breaks *in vitro*, protect the strands from nuclease digestion and facilitate the ligation of duplex DNA fragments[56]. They speculate that PprA is part of a deinococcal NHEJ system, although this role seems inconsistent with the absence of mutations observed after irradiation in this species. As with most *D. radiodurans* proteins, PprA characterization is at an early stage.

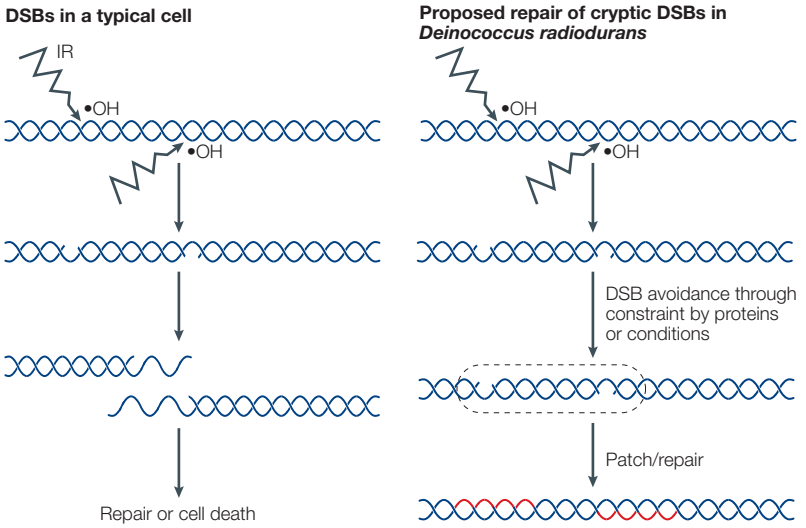


Figure 6 | **A proposed mechanism that might contribute to the tolerance of radiation damage in *Deinococcus radiodurans*.** Potential double-strand breaks (DSBs) are constrained, held together by proteins and/or their local environment, so that many breaks scored as DSBs in *in vitro* assays are cryptic in the cell. IR, ionizing radiation; •OH, hydroxyl radical.

A new proposal for radiation tolerance

To the list above, we add one more mechanism, which is a new proposal (FIG. 6). It is particularly difficult to explain the accurate repair of the hundreds of scattered repeat elements in the *D. radiodurans* genome[20,63,69] after the introduction of hundreds of double-strand breaks. How does *D. radiodurans* avoid the potentially disruptive recombination events that could occur between these repeated elements to maintain the continuity of its genome? We speculate that *D. radiodurans* uses a strategy that potentially combines elements of many of the systems described in this review to avoid this problem — including the condensed nucleoid structure first highlighted by Minsky[40,45] and the high concentrations of Mn(II) ions discussed by Daly[27,44] — thereby improving the cell's capacity to tolerate ionizing radiation.

Many DNA double-strand breaks arise from the juxtaposition of two single-strand breaks that form as a function of the distance in base pairs between these breaks (BOX 1). In general, the further apart the single-strand breaks, the less likely it is that the DNA ends will separate to form a double-strand break. We suggest that a significant contribution to the observed tolerance of ionizing radiation in *D. radiodurans* could arise if many of the measured double-strand breaks that occur are cryptic *in vivo*. In other words, some fraction of the measured double-strand breaks could actually be held together so that the separation of the DNA ends never really occurs in the cell. This proposal assumes that the organism has a mechanism to stabilize opposed breaks, constraining the intervening base pairs so that actual separation of the two DNA ends does not occur. This idea is distinct from proposals[40,45] in which the ends separate and are repaired by NHEJ. The cryptic breaks we propose would be scored as double-strand breaks during the processing and analysis of genomic DNA because the conditions necessary to stabilize the paired single-strand breaks are lost when the cell is disrupted. However, in *situ* the DNA strands are not separated. Unlike classical NHEJ, repair of these cryptic breaks would be effectively templated, as genomic continuity would never be lost.

This proposal envisions a specialized system operating in the condensed chromosome that prevents the formation of double-strand breaks at those sites where some base pairing is present. Opposed single-strand breaks would be repaired using a set of enzymes that could deal with the close proximity of the breaks in opposing strands without affecting this base pairing. By this mechanism, the linear continuity of the genome sequence, including regions rich in short nucleotide repeats, would be preserved at many potential sites of double-strand breaks in a manner that is error-free. Experimentally, this process would be indistinguishable from a non-mutagenic type of NHEJ. If such a system exists, the repair would be accurate and RecA-independent, passively reducing the cell's dependence on recombinational DNA repair and the accompanying homology search at any break site that is stabilized in this manner.

Examples from the literature indicate that the cell might use two alternative, but not mutually exclusive, mechanisms to stabilize base pairing between opposed single-strand breaks. First, *D. radiodurans* might encode proteins that hold the DNA together. Second, the intracellular ionic composition could be sufficient to physically limit dissociation of DNA base pairs. If proteins are responsible, we assume that they will be functionally analogous to the structural-maintenance-of-chromosomes (SMC) proteins that are present in many eukaryotic and prokaryotic species[70–72]. In eukaryotes, these proteins are referred to as cohesins and condensins, and the significance of their role in genome stabilization and DNA repair is becoming apparent[73]. The stability of annealed complementary DNA is dependent on the ionic strength of the medium in which the DNA is dissolved, and increased intracellular Mn(II) concentrations[27] might help to hold DNA that contains several single-strand breaks together. Freifelder and Trumbo[74] have shown that high-ionic-strength media stabilize opposed breaks separated by as little as two base pairs

A system for the repair of cryptic double-strand breaks might exist in many organisms. Conceptually, the damage that is caused by high-dose ionizing radiation is similar to the damage that would occur if a type II restriction enzyme was expressed *in vivo*. A large number of opposed single-strand breaks would be formed and would produce double-strand breaks unless stabilized. In yeast, the prolonged expression of *Eco*RI produces thousands of breaks but results in a surprisingly modest (2–3 fold) loss of viability[75–77]. Similarly, prolonged artificial expression of the yeast mating-type-specific HO endonuclease results in only a 35% loss of viability[78] in strains that have four HO-sensitive chromosomal loci. Survival

in these strains does not depend on homologous-recombination systems. Lethal fragmentation of chromosomes only occurs in cells that have defective NHEJ or checkpoint-control systems. Even *E. coli* cells are surprisingly resistant to *Eco*RI-mediated chromosomal cleavage as long as the appropriate DNA ligases are not inactivated[79]. Once the cryptic breaks are repaired, breaks where the ends have separated could be repaired by additional processes such as SSA and recombinational DNA repair.

Conclusion

The extraordinary phenotypes of *D. radiodurans* have encouraged a host of rather fanciful descriptions of the origin of this organism. Stories of the arrival of this species on earth on a comet, or arising through mutations owing to mankind's attempts to harness nuclear power, are readily available on the internet (See Online links box). These ideas are likely to flourish until this remarkable organism is better understood. *D. radiodurans* has a readily documented evolutionary origin within the domain Bacteria. It is clearly related to the rest of life on this planet, carrying out all of the fundamental processes that have been characterized in prokaryotes.

Our interpretation of the literature published to date on the Deinococcaceae suggests no extraordinary survival strategies, or at least no single 'magic bullet' that provides a complete explanation for the phenomenon. Instead, enhanced radioresistance seems to be the consequence of an evolutionary process that has coordinated various passive and active mechanisms, enabling survival from stresses such as desiccation. The almost ubiquitous classical DNA-repair pathways, perhaps with some specialized properties, seem to be augmented by novel features of deinococcal nucleoid structure and metabolism. It is currently difficult to predict which mechanism(s) will be most important in radioresistance, or even whether all of the contributing mechanisms have been discovered. Each of the many enhancements could individually have a modest effect, but collectively they mediate radioresistance. These changes need not be unique to *D. radiodurans*, but might be present in other organisms.

Therefore, the capacity of the deinococci to deal with stresses that cause massive genetic damage might not be as strange or as unusual as it once seemed. The combination of repair strategies is sophisticated and effective, but the individual strategies delineated so far are based on mechanisms present in many other organisms.

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Competing interests statement

The authors declare no competing financial interests

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