

Why is *Deinococcus radiodurans* so resistant to ionizing radiation?

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The publication of the fully assembled and annotated sequence of the *Deinococcus radiodurans* R1 genome is expected during 1999 and, if the anecdotal information released to the press¹ thus far is accurate, analysis of the sequence has not revealed much that can be used to explain this organism's extraordinary capacity to tolerate DNA damage. It appears that most, if not all, of the typical complement of prokaryotic DNA-repair proteins are found in *D. radiodurans*. This observation suggests two equally intriguing possibilities: (1) *D. radiodurans* uses the same DNA-repair strategies as other prokaryotes but does so in a manner that is somehow more effective than in other species or (2) *D. radiodurans* uses a DNA-repair system that has novel components. Nevertheless, the precise mechanisms of radiation resistance in *D. radiodurans* are not obvious and are possibly without precedent. Here, we will discuss several reports from the past 30 years that are relevant to understanding ionizing-radiation resistance in *D. radiodurans* but are not generally well known. It is our intent to encourage further investigation into the phenomena described in these reports.

Tolerating ionizing-radiation-induced DNA damage

To fully appreciate the remarkable ability of *D. radiodurans* to survive ionizing radiation (Box 1), one only needs to examine the pulsed-field gel shown in Fig. 1. A sublethal, 3000-Gray (Gy) (one Gy is a unit of absorbed dose equal to 100 rads) dose of γ radiation causes enormous DNA damage: the chromosomes of every *D. radiodurans* cell are cleaved into multiple, subgenomic fragments. For most species, this level of DNA damage is irreparable and lethal, but *D. radiodurans* has the capacity to reform its chromosomes from these fragments in less than three hours without any loss of viability or evidence of mutation².

In addition to DNA double-strand breaks, ionizing radiation introduces other types of DNA damage including single-strand breaks, DNA-protein cross-links and a myriad of different base damages³. Although there has never been a comprehensive effort

When exponential-phase cultures of *Deinococcus radiodurans* are exposed to a 5000-Gray dose of γ radiation, individual cells suffer massive DNA damage. Despite this insult to their genetic integrity, these cells survive without loss of viability or evidence of mutation, repairing the damage by as-yet-poorly-understood mechanisms.

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to document all forms of ionizing-radiation-induced DNA damage occurring in *D. radiodurans*, it is assumed that all of these types of damage occur. Certainly, measurements of single-strand breaks⁴⁻⁶ and of thymine-glycol production^{7,8} appear to be at levels commensurate with the dose of ionizing radiation administered.

D. radiodurans does not passively protect its genome from the incident radiation. Rather, all the available evidence argues that this organism rapidly and accurately repairs

DNA damage. Given the scale of the capacity of *D. radiodurans* to survive massive DNA damage, we assume that this organism has evolved specific and distinctive mechanisms to deal with such damage. We have identified several observations that hint at possible mechanisms.

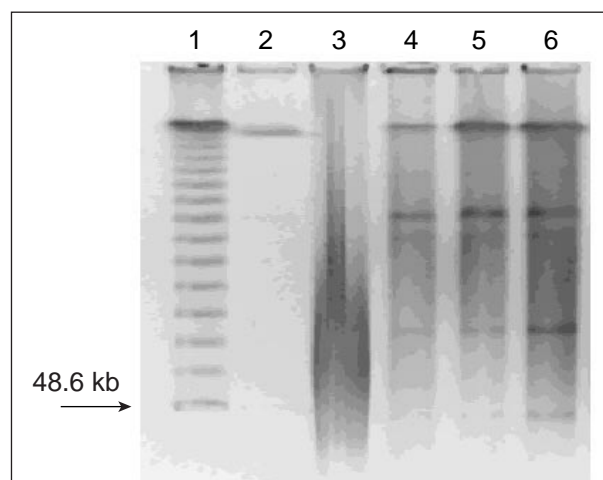


Fig. 1. The ability of *Deinococcus radiodurans* R1 to survive the accumulation of DNA double-strand breaks following exposure to a 3000-Gray (Gy) dose of γ radiation. Lane one contains a lambda size standard; lane two contains chromosomal DNA prepared from an untreated culture; lane three contains chromosomal DNA prepared from a culture immediately after irradiation; lanes four to six contain chromosomal DNA prepared from a culture three, six and nine hours post-irradiation, respectively. Assuming that the size of the *D. radiodurans* genome is 3.2 Mb²², 3000 Gy generates 120 dsbs per genome or, on average, one dsb for every 27 kb²¹.

Inhibition of DNA replication

Immediately after cultures of *D. radiodurans* are irradiated, DNA replication ceases⁹⁻¹¹. At sublethal doses of ionizing radiation, there is a linear relationship between the duration of this stoppage and the administered dose. Although it is certain that this replication inhibition is caused by DNA damage, it is not clear whether the movement of the DNA polymerase is blocked by lesions or whether an undefined regulatory process specifically prevents chromosome replication until repairs are made. We favor the latter because of reports indicating that DNA replication only resumes after repair of DNA damage has been

completed⁹⁻¹². If the inhibition of DNA synthesis was solely the result of lesions blocking the progress of DNA polymerase, we would expect the delay to be alleviated as these lesions are removed. These reports imply a level of regulation that involves proteins with the ability to sense DNA damage and relay that information to the cell's replication machinery – a regulatory system not unlike the DNA-damage checkpoints found in eukaryotic cells¹³. There is no published evidence of such checkpoints operating in *D. radiodurans* but, to our knowledge, a formal search for this type of regulation has not been undertaken. Given the extent of the DNA damage caused by high-dose irradiation,

Box 1. A brief description of *Deinococcus radiodurans*

Deinococcus radiodurans, the type species of the family *Deinococcaceae*^a, is a mesophilic, non-spore-forming, non-motile, spherical bacterium that forms pairs and tetrads when grown in rich liquid medium. Cells divide alternately in two planes and optimal growth occurs at 30°C. The sequence of the *D. radiodurans* R1 3.2-Mb^b genome has been determined in its entirety and will be released in 1999 (O. White, The Institute for Genomic Research, pers. commun.; <http://www.tigr.org>). Cells grown in rich media are 'multigenomic', containing no less than four genome equivalents per cell^{c,d}. The natural habitat of this species has not been defined^a and, although it has been found in a variety of locations worldwide, there is no obvious connection between these locales.

D. radiodurans is distinguished by its ability to survive the lethal effects of ionizing radiation^{e-i}. A typical survival curve obtained when aerated, exponential-phase cultures of *D. radiodurans* R1 are γ -irradiated is shown in Fig. 1, along with the survival curve obtained for *Escherichia coli* B/r cultures irradiated under the same conditions. In contrast to *D. radiodurans*, the *E. coli* culture demonstrates an exponential loss of viability with no evidence of resistance. As the isolation of high-radiation-resistant bacteria from natural microflora is a rare occurrence^a, it is assumed that the survival of most vegetative bacteria will parallel that of *E. coli*.

The ability of *D. radiodurans* to survive ionizing radiation raises an intriguing evolutionary question: why would this capability evolve in any species? Ionizing-radiation resistance does not provide a selective advantage, as there are no terrestrial environments that generate a radiation flux in excess of 200 mGray (mGy) per year^l. It must, therefore, be assumed that the ability of *D. radiodurans* to repair DNA damage was created by a selective pressure unrelated to ionizing radiation.

Mattimore and Battista^k have provided evidence suggesting that the radiation resistance of *D. radiodurans* is a consequence of its ability to survive prolonged dehydration. They evaluated 41 ionizing-radiation-sensitive strains of *D. radiodurans* for their ability to survive desiccation: all exhibited a loss of viability upon rehydration compared with wild type. They also demonstrated that desiccation caused a time-dependent increase in DNA double-strand breaks, which are the most deleterious lesions formed by ionizing radiation. Taken together, these observations suggest that *D. radiodurans* is resistant to ionizing radiation because it has the capacity to repair the DNA damage introduced during dehydration.

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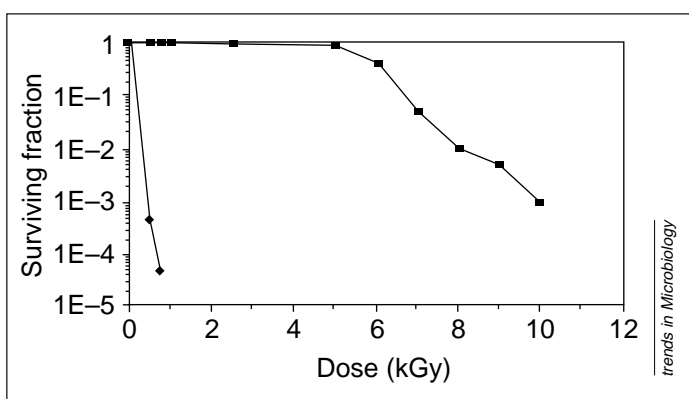


Fig. 1. Representative survival curves for *Deinococcus radiodurans* R1 (squares) and *Escherichia coli* B/r (diamonds) following exposure to γ radiation. $1E-1$ is 1×10^{-1} , or 0.1; each designation on the y-axis therefore represents a reduction in viability by a factor of 10. The D_{37} dose (i.e. the average dose of ionizing radiation that is required to inactivate a single colony-forming unit) for the *E. coli* culture is 30 Gray (Gy), approximately 200 times lower than that of *D. radiodurans*. *D. radiodurans* has a characteristic shoulder of resistance to approximately 5000 Gy, in which there is no loss of viability. Above 5000 Gy, there is an exponential decline in viability and a D_{37} dose of between 6000 Gy–7000 Gy for cultures in exponential phase^{l,m}.

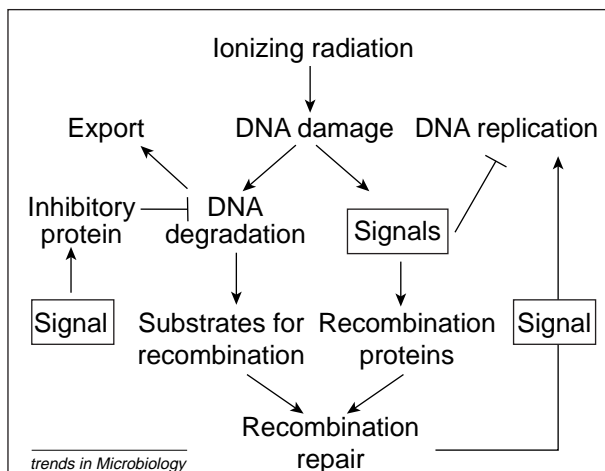


Fig. 2. Schematic representation of the response of *Deinococcus radiodurans* to ionizing-radiation-induced DNA damage. As DNA replication, degradation and recombination repair are coordinately regulated, it is proposed that these processes are sensitive to, or responsible for the generation of, intracellular signals. It is believed that the ability of *D. radiodurans* to survive ionizing-radiation-induced DNA damage involves recombination repair, the regulation of DNA replication and the export of damaged nucleotides. The nature of the inhibitory protein controlling DNA degradation is unknown.

it seems likely that *D. radiodurans* can coordinately regulate cellular functions and prioritize DNA repair.

DNA degradation and the export of damaged DNA

When exposed to ionizing radiation, bacterial chromosomal DNA is degraded by a process that is presumably initiated by cellular exonucleases at the sites of DNA-strand breaks. For most species, this degradation has lethal consequences, but, in *D. radiodurans*, DNA degradation appears to play an integral role in the cell's defense against DNA damage. The extent of DNA degradation in *D. radiodurans* is determined by the dose of ionizing radiation administered: the higher the dose, the greater the loss of chromosomal DNA^{9,10,14}. Unlike other species, *D. radiodurans* seems to be able to control the exonucleases that are responsible for DNA degradation by producing a protein that prevents protracted chromosomal digestion¹⁵. DNA degradation continues unchecked in irradiated cultures incubated with chloramphenicol, resulting in extensive destruction of the chromosome and a dramatic reduction in cell

Questions for future research

- Are there DNA-damage checkpoints in *Deinococcus radiodurans*?
- Is the process of exporting damaged DNA critical for radiation resistance in *D. radiodurans* and/or for preventing excessive mutation following exposure to ionizing radiation?
- Does the process of DNA degradation provide substrates for homologous recombination?
- Is genome multiplicity necessary for ionizing-radiation resistance?
- Is there a central regulatory system controlling DNA-damage repair in *D. radiodurans*?

viability^{6,16}. The ability of *D. radiodurans* to limit the extent of DNA degradation could give the cell some protection against the lethal effects of ionizing radiation.

There are also data to support the argument that chromosomal DNA degradation is an essential part of DNA repair in *D. radiodurans*¹⁷. By progressively replacing the thymine in the *D. radiodurans* chromosome with 5-bromouracil (5-BU), it is possible gradually to inhibit the extent of DNA degradation. As thymine substitution increases, the 'shoulder' of the survival curve becomes smaller. Complete loss of this shoulder corresponds with the point where 5-BU incorporation is maximal and DNA degradation can no longer be detected. As 5-BU incorporation does not increase the extent of DNA damage, we can assume that the inhibition of DNA degradation results in increased sensitivity to ionizing radiation. The exact function that DNA degradation performs is unknown but, given that this process generates long regions of single-stranded DNA, it could promote homologous recombination.

DNA degradation is evident immediately after *D. radiodurans* is exposed to ionizing radiation^{9,10,14}. The degradation of the *D. radiodurans* chromosome is accompanied by the export of damaged and undamaged nucleotides from the cell. Among the first degradation products to be formed are large oligonucleotides, approximately 2000 bp in length, which are rapidly exported from the cell and then digested into nucleotides¹⁴. In cells treated with sublethal doses of γ radiation, oligonucleotide production is limited and ends within one hour. The extent of free-nucleotide release is linearly related to the dose of radiation administered. The rate at which DNA degradation proceeds is independent of the dose and approximately 0.1% of the chromosome is lost every minute¹⁰. The importance, if any, of this phenomenon to ionizing-radiation resistance has never been explored. Removing damaged bases from the intracellular nucleotide pool could be part of the explanation for how *D. radiodurans* avoids an increase in mutation frequency, despite suffering high levels of base damage².

Genome multiplicity and interchromosomal recombination

Moseley *et al.*¹¹ identified and characterized *ts1*, a temperature-sensitive *D. radiodurans* mutant that gradually loses the ability to carry out homologous recombination when its growth temperature is shifted from 30°C to 39°C. The resistance of *ts1* cultures grown at 30°C is identical to that of wild-type cultures, but when cultures are held at 39°C they become sensitive to ionizing radiation and the shoulder of the survival curve becomes smaller as a function of time. The reduction in the size of the shoulder correlates with the inactivation of recombination, suggesting that homologous recombination contributes to ionizing-radiation resistance.

D. radiodurans is 'multigenomic', having from four to ten identical copies of its genome per cell^{18,19} and it has been suggested that *D. radiodurans* uses

this redundant genetic information to repair DNA²⁰. Daly and Minton¹² provided support for this idea by establishing that *D. radiodurans* carries out inter-chromosomal recombination. These authors estimate that as many as 700 crossovers are formed per four-chromosome nucleoid when cultures are irradiated at their D₃₇ dose (i.e. the average dose of ionizing radiation that is required to inactivate a single colony-forming unit), indicating that, following irradiation, *D. radiodurans* pieces together an intact genome from the remnants of its chromosomes. Although this notion is appealing, it must be noted that there is no direct evidence that multiple copies of the genome are needed for ionizing-radiation resistance. In fact, Harsojo *et al.*¹⁸ have reported that, when the genome number is varied from four to ten copies per cell, there is no change in the radiation resistance – a fact that is inconsistent with the hypothesis that additional chromosomal copies enhance such resistance.

Conclusions

We believe that *D. radiodurans* has a DNA-repair strategy that is quite different from that of other, better-characterized prokaryotes. The limited information available argues that the survival of *D. radiodurans* (1) relies on homologous recombination, thus taking advantage of its multiple chromosomes, (2) uses post-irradiation DNA degradation to provide single-stranded DNA for recombination, (3) regulates DNA replication and post-irradiation DNA degradation to maximize repair efficiency and (4) might use the export of damaged nucleotides from the cell to avoid mutation. DNA replication, degradation and recombination repair appear to be coordinately regulated, suggesting that these processes are sensitive to intracellular signals (Fig. 2). We have identified three positions within this regulatory scheme where it is likely that the cell senses the extent of DNA damage or DNA repair. The physical nature of such signals is

unknown. Obviously, a more complete analysis is required before it can be said with certainty that these phenomena contribute to radiation resistance in *D. radiodurans*.

Acknowledgements

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