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Deinococcus radiodurans: Does this Bug Wear a Lead Vest or what?

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Introduction

In Oregon, 1956, spoilage was found in a can of meat that had supposedly been sterilized by high doses of X-rays. Culturing of the contaminant revealed the presence of a red-pigmented bacterium that was named *Deinococcus radiodurans*. This bacterium is currently the most radioactive-resistant organism known on Earth[14,16,17]. Its tremendous ability to withstand high doses of radiation well beyond any naturally-occurring levels on the planet have caused it to become the focus of a radioactive waste clean-up initiative funded by the US Department of Energy (DOE)[3,5,11]. While incapable of degrading actual radioactive elements, genetic engineering of this organism to include genes from other organisms for the degradation or immobilization of major heavy metal and organic solvent contaminants found in radioactive dumpsites could aid the clean-up effort of these sites at a significantly reduced cost[3].

Ionizing radiation is lethal to most organisms

Ionizing radiation is caused by unstable atoms that produce charged particles or ions in matter[8]. This form of radiation is lethal to most organisms due to the damage it causes to proteins, membranes and, most importantly, DNA. The most lethal effect is the generation of double-stranded breaks (DSBs) in DNA. The fragments resulting from DSBs must be put back together in the correct order to avoid mutations and/or loss of genetic material. DSB's are difficult to repair properly because the bacterium has very little way of knowing if the ends "match" or not. Ligating one broken end to another end that was not its original partner could result in the cleavage of an important gene, leading to a nonfunctional or misfunctional product. Most bacteria are able to withstand no more than two to three DSBs in their DNA without dying from the lethal mutagenic effects[17].

How radiation-resistant is *Deinococcus radiodurans* ?

For comparison, a human exposed to less than 500 rads (or 5 Grays) of ionizing radiation suffers almost certain death[7]. *E. coli* cultures, representative of bacteria with normal resistance to radiation, are sterilized by an acute dose of 100-200 kilorads. *D. radiodurans* can survive acute exposure to more than 1,500 kilorads without dying or undergoing any kind of mutation. Foreign genes incorporated into its genome by genetic engineering are also protected from the effects of irradiation[5,6,7,11].

How did *D. radiodurans* evolve?

The extraordinary properties of this bacterium beg the question, how did such an organism evolve? More incredibly, radiation levels on the earth's surface,

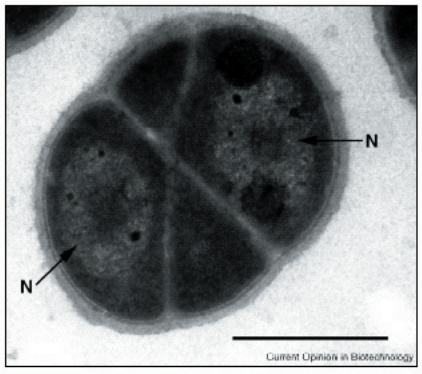

Figure 1. D. radiodurans showing the typical tetrad form of this bacterium[5].

including water sources with radionuclides dissolved in them, have only provided about 0.05 to 20 rads/years over the past 4 billion years, much less than what the bacteria can withstand[6,16]! Thus, selective pressures for the evolution of the radiation-resistant phenotype must have come from other sources. Clues to the answer come from studies demonstrating that in addition to radiation-resistance, *D. radiodurans* is also resistant to damage from UV, oxidizing agents, electrophilic mutagens, nitrous acid and other chemicals, and desiccation[5,7,11,16]. No thorough study has ever been conducted to locate the natural ecology of the bacteria; however, cultures have been isolated from granite outcrops in Antarctica to elephant trunks[6,16]. It is now thought that desiccation was the phenotype to which *D. radiodurans* evolved, as it causes very similar DNA damage to ionizing radiation[6,18]. This, the bacterium may have evolved such extraordinary properties serendipitously as a result of desiccation-resistance genes, which repair similar DNA damage caused by the two environmental agents. In addition, freezing or desiccating *D. radiodurans* cultures increases their radioactivity-resistance phenotype, providing further evidence for this hypothesis[19].

Mechanisms of radiation-resistance

There are several hypothesis as to how radiation-resistance functions in *D. radiodurans* . Unlike other bacteria, *D. radiodurans* does not form radiation-resistance spores (a differentiated form of the cell that is extremely resistance to heat and other harmful agents)[5]. Nor does *D. radiodurans* protect its DNA from double-stranded DNA breaks in the first place via a specialized system, as do some other radiation-resistant bacteria such as *Rubrobacter radiotolerans*[18]. Experiments have shown that *D. radiodurans* does suffer from double stranded breaks at high radiation doses: the 3.2 million basepair genome fragments into over 1000 pieces resulting from 100-200 DSBs[1,9,11]. Remarkably, in 12-24 hours, the DNA is fully repaired without lethality or mutagenesis[6,7]. There are various hypotheses as to how this occurs:

1) The bacterium has specialized proteins that make it very efficient at DNA repair via a process called homologous recombination.
2) The bacterium has multiple chromosomes that are aligned so that when double strand breaks occur, the bacterium has a template for putting the ends back together properly.
3) *D. radiodurans* is capable of controlling DNA degradation and exports damaged bases from the nucleus to prevent their re-incorporation into its DNA.
4) The bacteria contain other unique and uncharacterized proteins that promote radiation resistance.

None of these hypotheses for the mechanisms of radiation-resistance have been conclusively proven, and it is possibly a combination of several of them are responsible for the phenotype[5,18].

The RecA protein in *D. radiodurans* has special properties:

RecA is an essential protein found in virtually all bacteria, and its homologues are found in humans and other eukaryotes. It has a central role in maintaining DNA integrity via recombination repair[15]. In the event of DNA damage, if no complementary undamaged strand is available to act as a template for repair, then the information required must come from a separate, homologous chromosome. RecA promotes all the central steps in this process, known as homologous genetic recombination (Figure 2). Homologous recombination repair occurs in the following way: the broken ends of the DNA are bound by SSB (single-stranded binding protein). Next, RecA protein aligns one of the broken strands of DNA with a nearly identical region on another copy of the chromosome that will act as the template for repair. RecA forms a complex between the single, broken strand and the homologous target double-stranded DNA (dsDNA). Then, it inserts the single strand of DNA into dsDNA, displacing one preexisting strand. Lastly, it forms a heteroduplex, Holliday-type structure that can migrate along the dsDNA strand, transcribing as it goes and filling in the missing information on the single strand, based on the genetic information in the homologous chromosome[15].

The importance of RecA to *D. radiodurans* viability under radiation is shown by strains of *D. radiodurans* lacking RecA (*recA* strains) that are radiation sensitive, similar to *E. coli*. Introducing the gene for *E. coli recA* into *D. radiodurans* strains lacking their own RecA cannot restore radiation- resistant phenotype, suggesting that the deinococcal RecA has special properties.

Multiple chromosomes of the bacteria are aligned

The genome of *D. radiodurans* is comprised of 2 large chromosomes (2.65 Mega basepairs and 412 kilo basepairs in size), one megaplasmid (177 kilo basepairs) and one plasmid (46 kilo basepairs)[3,16]. There are normally four copies of the chromosomes in stationary phase of growth (when cells are not dividing), and up to 10 in exponential phase of growth (when cells are actively dividing)[16,17]. One group of researchers

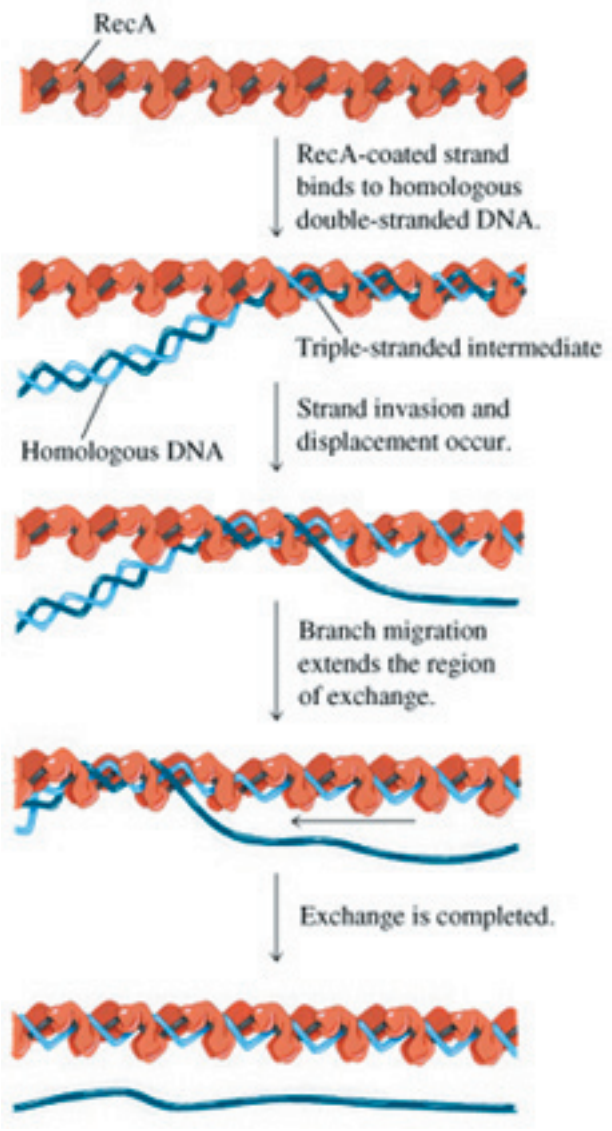

Figure 2. Repair of double strand breaks by homologous recombination repair (from cwx.prenhall.com/horton/medialib/ media_portfolio/ 20.html)

has proposed that the deinococcal chromosomes are joined to each other at thousands of sites[6]. Presumably, the close proximity of identical genetic sequences can facilitate rapid homologous recombination and account for the ability of the bacteria to repair so many DSBs[17]. There is still little evidence to support this theory, however. A model is shown in Figure 3.

Bacterial chromosomal DNA is degraded after exposure to ionizing radiation, presumably by cellular exonucleases that chew back DNA from starting at the ends of DSBs[2,14]. While this phenomenon is lethal for most bacteria, it may have a defensive role in *D. radiodurans*. The bacteria appear to be able to control the exonucleases responsible for DNA degradation, perhaps by producing a novel protein that prevents complete chromosomal digestion. Furthermore, degradation of the chromosome is followed by export of damaged nucleotides from the cell. Damage to bases (such as base loss, deamination, methylation, oxidation and dimerization) is very common after exposure to ionizing radiation. It is possible that removing damaged bases from the intracellular nucleotide pool could prevent their re-incorporation into the genome, partly explain how *D. radiodurans* avoids mutations despite suffering from a high degree of base damage[2,14].

Other, uncharacterized proteins unique to *D. radiodurans*

The genome of this bacterium was recently sequenced[16]. However, there is no evidence so far for any unique repair mechanism; in fact, the known repair systems appear to be less complex and sophisticated than those found in *E. coli* [9,18]. Nevertheless, analysis is not complete, and of the 3187 genes identified, only 1493 could be assigned a function based on similarity to other gene products from the protein database. Of the other 1694 unknown genes, 1002 are unique to *D. radiodurans*[1,16]. Thus, the possibility of novel mechanisms of radiation resistance cannot be ruled out.

Bioremediation of radioactive mixed waste sites

Bioremediation is defined as the use of biological agents to contain or control environmental contaminants by reducing toxic substances to less toxic or less soluble states to facilitate their immobilization and removal[10]. This is generally accomplished by introducing or enhancing already-present bacterial populations that have enzymes capable of degrading or controlling these substances. Bioremediation can be a viable and relatively inexpensive clean-up option[10].

Between 1945 and 1986, huge volumes of radioactive

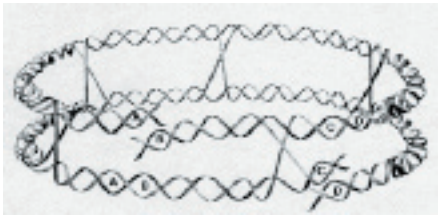

Figure 3. Hypothetical double chromosome structure showing dsDNA breaks (at loci A-B and C-D) held in alignment by virtue of persistent Holliday junctions[7].

waste from US nuclear weapons programs were disposed of directly in the ground at thousands of sites, where they now threaten large portions of surface and subsurface soil. The waste includes not only radionuclides (^{235}Uranium, ^{238}Plutonium, ^{99}Technetium, ^{90}Strontium, ^{137}Cesium), but also heavy metals, acids and bases, and organic solvents[3,5]. The cost of cleaning these sites has been estimated at over 250 billion US dollars[11]. The US Department of Energy (DOE) has sponsored studies on the use of *D. radiodurans* as a bioremediation agent to remove heavy metals and organic solvents such that the subsequent radionuclide isolation is easier and safer[3,5].

D. radiodurans has several properties beyond its inherent radioactive-resistance that make it a viable candidate for such a role. First, unlike some other radiation-resistant bacteria, *D. radiodurans* is not pathogenic and therefore will not create further environmental damage[5]. Second, its genome is highly plastic and is capable of supporting DNA inserts up to 2,000 kilobasepairs in size, which are also protected from radiation damage[4,5]. This is ample space for the introduction of degradative enzyme genes. Lastly, the bacterium itself is already capable of reducing some contaminant metals found at the DOE sites, including Cr, Te and U, to less soluble species[3,5,14].

Recently, the *merA* gene from *E. coli* was successfully introduced into the genome of *D. radiodurans*. This gene encodes an enzyme that reduces highly toxic Hg(II) to the inert and much less toxic form, Hg (0)[3,5]. Additionally, the *tod* gene from *Pseudomonas putida* was also introduced into the same genetically-engineered strain. This gene encodes toluene dioxygenase (TDO), which degrades organic solvents known collectively as BTEX (benzene, toluene, ethylbenzene, xylenes) to forms that readily polymerize to form insoluble polymers, which are far less toxic and more easily removed[3,5,11]. Both mercury and BTEX contaminants are found at high levels in DOE sites. Further genetically engineering strains may hold potential for degradation or detoxification of other contaminants as well.

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