

AGAINST ALL ODDS: The Survival Strategies of *Deinococcus radiodurans*

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ABSTRACT

Bacteria of the genus *Deinococcus* exhibit an extraordinary ability to withstand the lethal and mutagenic effects of DNA damaging agents—particularly the effects of ionizing radiation. These bacteria are the most DNA damage-tolerant organisms ever identified. Relatively little is known about the biochemical basis for this phenomenon; however, available evidence indicates that efficient repair of DNA damage is, in large part, responsible for the deinococci's radioresistance. Obviously, an explanation of the deinococci's DNA damage tolerance cannot be developed solely on the basis of the DNA repair strategies of more radiosensitive organisms. The deinococci's capacity to survive DNA damage suggests that (a) they employ repair mechanisms that are fundamentally different from other prokaryotes, or that (b) they have the ability to potentiate the effectiveness of the conventional complement of DNA repair proteins. An argument is made for the latter alternative.

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INTRODUCTION

The *Deinococcaceae* are distinguished by their extraordinary ability to tolerate the lethal effects of DNA damaging agents, particularly those of ionizing radiation. Although the physiological basis of the deinococci's extreme radiotolerance has never been adequately explained, it is clear that irradiated cells are not passively protected from the damaging effects of the incident radiation. Instead, available evidence (such as is presented in Figure 1) argues that the deinococci do suffer massive DNA damage following irradiation, and that extensive DNA repair is necessary if these cells are to survive such exposures. To generate panel (A) of Figure 1, a *D. radiodurans* R1 culture, grown to mid-log phase, was exposed to 3000 Gy γ radiation, a dose that introduces approximately 110 double strand breaks (dsbs) into the chromosome of each

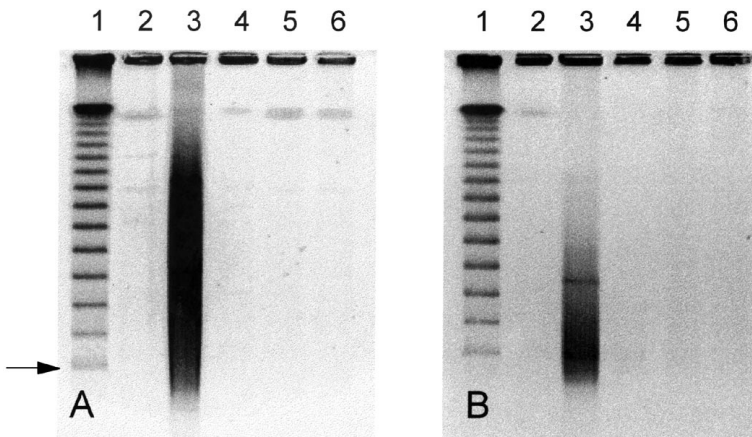


Figure 1 The ability of *D. radiodurans* R1 (*panel A*) and IRS41 (*panel B*) to survive the accumulation of chromosomal DNA double strand breaks following exposure to 3000 Gy γ radiation. For each panel: *Lane 1* is a lambda ladder size standard, *Lane 2* is chromosomal DNA prepared from an untreated culture, *lane 3* is chromosomal DNA prepared from a culture immediately after irradiation, and *lanes 4–6* are chromosomal DNA prepared from a culture 3, 6, and 9 h post-irradiation, respectively.

cell in the population.¹ At 0, 3, 6, and 9 h post-irradiation, aliquots of the irradiated culture were removed, and the chromosomal DNA was isolated for analysis. Pulsed field gel electrophoresis (PFGE) was used to provide a visual record of the cell's recovery from the dsbs. Fragmentation of the chromosome is obvious immediately after irradiation. The band of chromosomal DNA present in unirradiated cell preparations (*lane 2*) is gone, replaced by a broad smear of lower molecular weight material (*lane 3*). Within three hours, however, this smear has disappeared and the chromosome has reformed (*lane 4*). Remarkably, *D. radiodurans* survives this degree of damage without loss of viability and without evidence of induced mutation.

Deinococcus radiodurans (*D. radiodurans*) R1 was first isolated in 1956 (3) from tins of meat that had been given what was believed to have been a sterilizing dose of γ radiation. Although this organism and its relatives have been studied over the past 40 years, our knowledge of the survival strategies employed by *D. radiodurans* following DNA damage lacks the biochemical and mechanistic detail that characterizes our knowledge of the better known prokaryotic and eukaryotic organisms [e.g. *Escherichia coli* (*E. coli*) and *Saccharomyces cerevisiae* (*S. cerevisiae*)]. The absence of tractable genetics stifled the study of *D. radiodurans* for many years. *D. radiodurans* was substantially more difficult to work with and, as a consequence, few laboratories made the effort to study this organism.

Interest in *D. radiodurans* appears to be growing, as is exemplified by the number of review articles published in recent years on this organism and its DNA repair capabilities. Between 1956 and 1992, only one review on the radiobiology of *D. radiodurans* was published (52); since 1992, five such reviews have appeared (5, 46–48, 65). For this reason, it is my intent to shorten the more conventional descriptions of *D. radiodurans*' DNA damage tolerance and the enzymology of DNA repair and to focus on aspects of deinococcal biology that appear to enhance the capabilities of this organism's DNA repair proteins.

SUMMARY DESCRIPTION OF *DEINOCOCCUS RADIODURANS*

General Characteristics

D. radiodurans is a pigmented, nonsporeforming, nonmotile, spherical bacterium that ranges from 1.5 to 3.5 μm in diameter (59, 60). Colonies are convex, smooth, and vary from pink to red. *D. radiodurans* is gram positive but has a complex cell envelope similar to that of gram negative organisms. A

¹Calculation of this value assumes that the *D. radiodurans* genome consists of 3×10^6 bp (22) and that one double-strand break forms $10 \text{ Gy}^{-1} 5 \times 10^9$ daltons⁻¹ of double-stranded DNA (9).

thick peptidoglycan layer and outer membrane are present, and some strains exhibit a paracrystalline S layer. Cells are chemoorganotrophic with respiratory metabolism and are typically grown with aeration in TGY broth (0.5% tryptone, 0.1% glucose, 0.3% yeast extract). Under optimal conditions, the generation time of wild-type *D. radiodurans* R1 is approximately 80 min. Defined media have been described (61, 62), but their use results in slow and erratic growth. Cells divide alternately in two planes, generating pairs and tetrads in liquid culture. The optimal growth temperature is 30°C, but growth remains strong to 37°C. Growth ceases at temperatures below 4°C and above 45°C.

DNA Damage Tolerance

D. radiodurans cultures exhibit unusually high resistance to many DNA damaging agents. Most studies of this organism have focused on its tolerance of DNA damage induced by ionizing radiation, ultraviolet (UV) light, and cross-linking agents. *D. radiodurans*' earlier reported resistance to nitrous acid, hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and 4-nitroquinoline-N-oxide (66, 67) has not been further characterized by new research for 20 years. *D. radiodurans* is one of the most ionizing radiation-resistant organisms ever identified; exponential phase cultures routinely survive exposure to 15,000 Gy γ radiation (34, 59). The typical γ radiation survival curve (Figure 2) for *D. radiodurans* R1 exhibits a shoulder of resistance to approximately 5,000 Gy (57), in which there is no loss of viability. For purposes of comparison, the survival of an *E. coli* B/r culture is also plotted in Figure 2. At

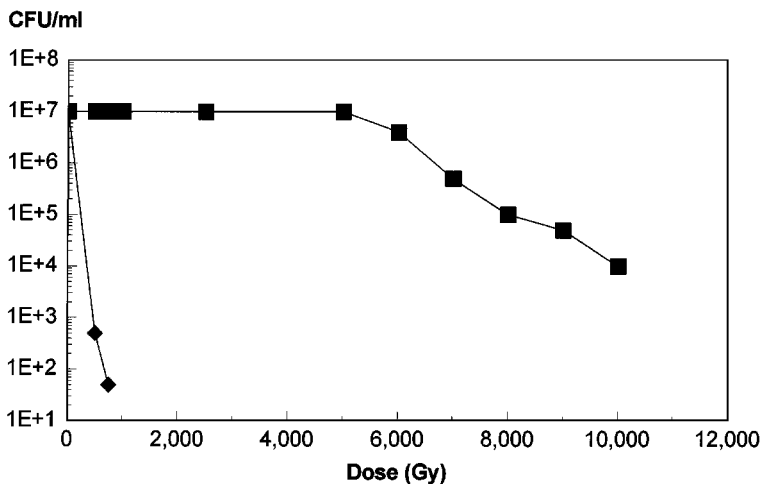


Figure 2 Representative survival curve for *D. radiodurans* R1 (squares) and for *E. coli* B/r (diamonds) following exposure to γ radiation.

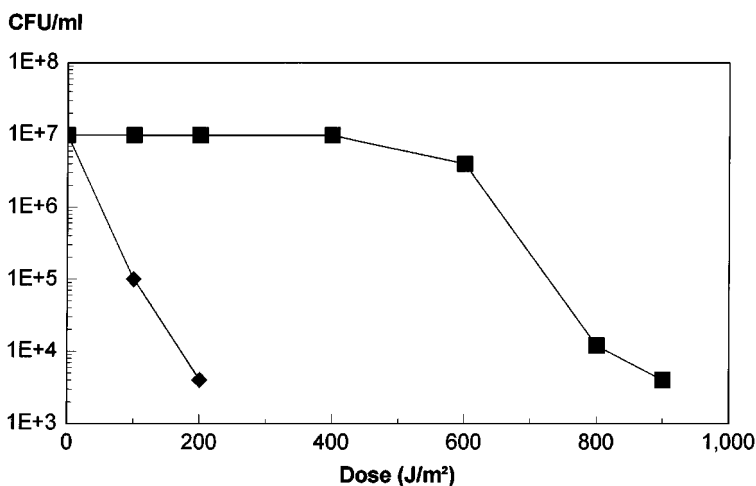


Figure 3 Representative survival curve for *D. radiodurans* R1 (squares) and for *E. coli* B/r (diamonds) following exposure to UV radiation.

doses above 5,000 Gy, however, the survival of *D. radiodurans* declines rapidly; the D_{37} dose² is approximately 6,000 Gy for exponential cultures. The D_{37} dose is 30 Gy for cultures of *E. coli* B/r exposed to ionizing radiation (67). In terms of DNA damage, 6,000 Gy γ radiation will induce approximately 200 DNA double strand breaks (9), over 3,000 single strand breaks (7), and greater than 1,000 sites of base damage per *D. radiodurans* genome (65). Although there are reports of deinococcal strains that survive as much as 50,000 Gy (4), in the author's experience viability falls to undetectable levels when exponential phase cultures of wild-type *D. radiodurans* are exposed to doses of 18,000 Gy or higher.

Wild-type *D. radiodurans* is also extremely resistant to UV radiation, surviving doses as high as 1000 J/m² (57). UV survival curves of *D. radiodurans* and *E. coli* B/r are compared in Figure 3. *E. coli* exhibits an exponential decline in viability, whereas *D. radiodurans* has a shoulder of resistance that extends to 500 J/m². The D_{37} dose for UV light is approximately 30 J/m² for *E. coli* B/r (67) and between 550 and 600 J/m² for exponentially growing cultures of *D. radiodurans* R1. The doses of UV light that *D. radiodurans* will tolerate cause an enormous amount of base damage. At 500 J/m², for example, it is estimated (6, 71) that 5000 thymine-containing pyrimidine dimers form an average of one lesion for every 600 base pairs.

²This is the dose of radiation required to reduce the number of individuals in the irradiated population to 37%, i.e. the dose that, on average, is required to inactivate a single colony-forming unit of the irradiated population.

Wild-type *D. radiodurans* is more resistant to the cross-linking agent mitomycin C than are most vegetative bacteria, as it can survive incubation in the presence of 20 $\mu\text{g/ml}$ mitomycin for 10 min at 30°C without loss of viability. After 40 min of incubation at this concentration, 1% of the culture still remains viable (35). Kitayama (35) has reported that, given cultures that are treated for 10 min at this dose, greater than 90% of the isolated chromosomal DNA exists as fragments of nondenaturable double-stranded DNA, with an average molecular mass of 2×10^7 Da. This indicates that at least 100 mitomycin C-induced crosslinks form per genome at this dose.

Phylogeny and Habitat

Four species make up the genus *Deinococcus*: *D. radiodurans*, *D. proteolyticus*, *D. radiopugnans*, and *D. radiophilus*. *D. radiodurans* is the type species for the genus (8, 59, 60). The deinococci were originally classified as members of the genus *Micrococcus*. However, chemotaxonomic studies of the *Micrococcaceae* suggest that this classification is incorrect, and subsequent phylogenetic analysis of deinococcal 16S and 5S rRNA sequences confirm that the deinococci are not related to the *Micrococcaceae* (33, 70, 73). The genus *Deinococcus* is specifically related to the gram negative genera *Deinobacter* ($S_{AB} = 0.58$ to 0.68) and *Thermus* ($S_{AB} = 0.22$ to 0.29). *Deinobacter* strains are radiation-resistant rod-shaped organisms that exhibit chemotaxonomic characteristics very similar to those of *Deinococcus*. In contrast, the thermophiles of the genus *Thermus* do not exhibit any obvious phenotypic relationship to *Deinococcus*.

The natural habitat of the deinococci has not been defined, even though members of this family have been isolated from a variety of locations worldwide. The environments from which these isolations have been made vary greatly in terms of their overall ecology and geographic distribution. This variability has undoubtedly contributed to the uncertainty concerning the deinococci's habitat. Two ecological studies have suggested that the deinococci are widely distributed soil organisms (8, 59). It is consistent with this conclusion that most deinococcal isolates have been recovered from environments that are rich in organic nutrients, including soil (8, 59, 60), animal feces (34), processed meats (14, 21), and sewage (34).

Deinococcal strains have also been recovered from more unforgiving environments. Successful isolation of deinococci from dried foods (41, 45), room dust (10), medical instruments (10), and textiles (38) suggests that this family has the capacity to survive in dry, nutrient-poor surroundings, which greatly expands the number of niches that this organism could exploit. These isolations are significant because they suggest that the deinococci could have evolved, much as the spore-forming organisms did, to survive periods of prolonged environmental stress. It is noteworthy that *D. radiodurans* is exceptionally resistant

to desiccation: According to one anecdotal account (59), it can survive for six years in a desiccator with 10% viability.

Mattimore & Battista (43) have recently provided evidence of a connection between the ionizing radiation resistance of *D. radiodurans* and its desiccation resistance. They evaluated the ability of 41 ionizing radiation-sensitive (IRS) strains of *D. radiodurans* to survive six weeks in a desiccator and demonstrated that every IRS strain was sensitive to desiccation. In addition, they established that, during dehydration *D. radiodurans* accumulates DNA damage—including accumulating DNA double-strand breaks. It appears that *D. radiodurans* is an organism that has adapted to dehydration and that its DNA repair capability is one manifestation of that evolutionary adaptation.

The Genetics of D. Radiodurans

D. radiodurans is multigenomic (28, 32), with stationary phase cells that each carry an estimated four genome equivalents. Each chromosome is a single covalently closed circular molecule that contains approximately 3×10^6 base pairs (22). The base composition of all deinococcal species is characterized by a high GC content, ranging from 65 to 71 mol%. The genome of *D. radiodurans* R1 is currently being sequenced, and publication of that sequence is expected by November, 1997 (O White, The Institute for Genomic Research, personal communication).

Stable naturally occurring plasmids have been found in all species examined to date (63). Most are larger than 20 kb and are present in low copy number. These plasmids have not been extensively studied, and their function in cellular metabolism is unknown.

The phylogenetic isolation of *D. radiodurans* makes genetic studies of this organism difficult. In general, *D. radiodurans* genes are not expressed in other bacteria, and the genes of other bacteria are not expressed in *D. radiodurans* (39, 64). Presumably, those structural features that signal the initiation of transcription in *D. radiodurans* evolved differently than they did in other prokaryotes. As a consequence, attempts at defining the function of a *D. radiodurans* gene product by interspecies complementation are effectively blocked unless hybrids are made—either by fusing the *D. radiodurans* gene to an appropriate promoter, or by fusing a foreign gene to a *D. radiodurans* promoter. Once transcribed, however, heterologous sequences are translated. Shuttle plasmids have been generated that are capable of replicating in *D. radiodurans* and *E. coli* by inserting an *E. coli* plasmid into a naturally occurring *D. radiodurans* plasmid, and by removing sequences that are not needed for plasmid replication (63). Expression of promoterless *cat* and *tet* genes from these vectors in *D. radiodurans* requires that an uncharacterized segment of *D. radiodurans* DNA (presumably containing a recognized promoter) be located immediately upstream of the drug

resistance gene. Minton and colleagues have exploited this finding and developed methods that utilize this putative promoter to create selectable, insertional mutations in the *pol* (25) and *rec* (23) genes of *D. radiodurans* and to express *E. coli* genes in *D. radiodurans*.

Transposon mutagenesis has never been reported in *D. radiodurans*, probably because the genes required for transposition are not expressed in this organism. *D. radiodurans* is, however, mutable when treated with simple alkylating agents such as MNNG, and most mutant strains are produced by chemical mutagenesis (51, 54, 69). Typically, cultures are treated with a mutagen, diluted into fresh media, and allowed to grow (usually overnight) before identification of the desired mutant phenotype is attempted. The outgrowth of mutagenized cells is necessary because *D. radiodurans* is multigenomic, and recessive mutations need the opportunity to segregate and form cells homozygous for that mutation before the mutant phenotype can be expressed.

The study of *D. radiodurans* has been hampered by a lack of genetic methods appropriate for use with this organism. Many of the tools that prokaryotic geneticists take for granted are not available when studying *D. radiodurans*. There is no evidence of conjugation, and no phage capable of infecting *D. radiodurans* has been identified (52, 65). Fortunately, *D. radiodurans* is relatively easy to manipulate using natural transformation, and experimental methods exploiting this property have been developed. *D. radiodurans* is fully competent throughout exponential growth (68). This species readily takes up and incorporates transforming DNA into its chromosomes, with marker-specific efficiencies from 0.01 to 3.0%, when cells are transformed in liquid culture in the presence of calcium (68). *D. radiodurans* strains are the only members of the deinococci that are naturally transformable. It is largely for this reason that *D. radiodurans* is the only species routinely used when studying the extreme radioresistance of the *Deinococcaceae*. The ability to transform *D. radiodurans* with high efficiency allows for relatively rapid isolation of DNA fragments that carry genes involved in DNA damage resistance. A genomic library generated from the wild-type strain can be efficiently screened for clones that are capable of restoring the wild-type phenotype to mutant strains. As part of the transformation process, the mutant allele is replaced by the wild-type sequence in a recombinational event. Moseley and coworkers (2) were the first to use this technique when they cloned the wild-type gene necessary for *D. radiodurans*' mitomycin resistance from a cosmid library. They transformed individual cosmids into *D. radiodurans* 302, a mitomycin-sensitive strain, and they plated transformants onto media containing mitomycin C. The restoration of mitomycin resistance identified the cosmid that carries the wild-type sequence that corresponds to the allele responsible for mitomycin C sensitivity. Once cosmids capable of restoring mitomycin resistance were identified, the gene of interest was isolated by transforming restriction fragments, obtained from that cosmid, into strain 302.

The efficiency of transformation remained high until fragment size dropped below 1.2 kb.

The large window of competence found in strains of *D. radiodurans* also permits the investigator to use in situ or "dot" transformation when manipulating these strains (69). In this protocol, exponential phase *D. radiodurans* cells are simply plated onto a rich medium, dotted with transforming DNA, and allowed to grow into a lawn. The lawn is replica plated and selective pressure is applied. Successful transformants appear within the area where the transforming DNA was dotted. Dot transformation has the advantage of avoiding most of the tedium associated with conducting the large number of transformations needed to screen a genomic library.

The genetic methods available for the study of *D. radiodurans* are, at present, relatively primitive in comparison with that of many other prokaryotes. This should change rapidly, however, when the sequence of the R1 genome becomes available.

DNA DAMAGE-SENSITIVE STRAINS OF *D. RADIODURANS*

Screens for mitomycin C (54), UV (51), and ionizing radiation (69) sensitive strains of *D. radiodurans* following chemical mutagenesis have been described. Table 1 lists characterized genotypes that exhibit damage-sensitive phenotypes.

Mutational inactivation of the *pol* or *rec* loci of *D. radiodurans* R1 results in strains that are sensitive to all three agents. The *pol* and *rec* gene products of *D. radiodurans* have been shown to be homologues of *E. coli*'s DNA polymerase I (25) and RecA protein (23), respectively. The deinococcal proteins appear to play roles similar to those that their *E. coli* counterparts play in DNA repair.

The *uvrA* gene product also plays a part in the cell's tolerance to these three types of DNA damage, but its contribution to UV and ionizing radiation resistance is redundant and, therefore, not essential in wild-type *D. radiodurans*.

Table 1 *D. radiodurans* strains sensitive to DNA damage

| Mitomycin C | Ultraviolet radiation | Ionizing radiation |
|-------------|-----------------------|--------------------|
| <i>pol</i> | <i>pol</i> | <i>pol</i> |
| <i>recA</i> | <i>recA</i> | <i>recA</i> |
| <i>uvrA</i> | <i>uvrA, uvsC</i> | <i>uvrA, irrB</i> |
| <i>mtcC</i> | <i>uvrA, uvsD</i> | <i>irrI</i> |
| <i>mtcD</i> | <i>uvrA, uvsE</i> | |
| <i>mtcE</i> | <i>irrI</i> | |
| <i>mtcF</i> | | |
| <i>mtcG</i> | | |

A *uvrA* strain is quite sensitive to mitomycin C, however (54). The first *uvrA* strains were derived from *D. radiodurans* R1, and the loci inactivated were designated *mtcA* and *mtcB* (54). Clones of these “loci” were recently sequenced and found to be two parts of a single gene that encodes a protein that is 60% identical to the UvrA proteins of *E. coli* and *Micrococcus luteus* (1). The *uvrA* strains of *D. radiodurans* only become sensitive to UV light when a second locus, designated *uvs*, is also inactivated (20, 56). Three *uvs* loci—*uvsC*, *uvsD*, and *uvsE*—have been identified (20). In a wild-type background *uvs* mutants exhibit near wild-type levels of UV resistance, indicating that the *uvrA* and *uvs* gene products encode functionally redundant proteins (18, 19). In addition, *uvs* mutants exhibit wild-type resistance to mitomycin C and γ radiation. The *uvrA uvs* strain is sensitive to mitomycin C, but it is nearly as resistant to γ radiation as is wild-type *D. radiodurans* (20, 26).

The *uvrA* mutation can influence ionizing radiation resistance in *D. radiodurans* as well. Udupa et al (69) screened 45,000 MNNG-treated colonies of the *uvrA* strain *D. radiodurans* 302 for ionizing radiation sensitivity, and they identified 49 putative ionizing radiation-sensitive (IRS) strains in this screen. All of the IRS strains are, therefore, double mutants. Subsequent characterization of one of these strains, IRS18, showed that ionizing radiation resistance was largely restored when the strain was transformed to mitomycin C resistance with the appropriate *uvrA*⁺ sequence. In other words, the *irrB* mutant was only ionizing radiation sensitive when the background was also *uvrA*, indicating that the *uvrA* gene product can, when necessary, contribute significantly to ionizing radiation resistance.

In addition to the *pol*, *recA*, and *uvrA* mutants, there are six other mutations that will render *D. radiodurans* more sensitive to at least one of the DNA damaging agents listed in Table 1. Inactivation of any of five independent loci, designated *mtcC* through *mtcG*, enhances *D. radiodurans*' sensitivity to mitomycin C-induced DNA damage (36). The functions of these *mtc* gene products have not been described. Inactivation of the *irrI* locus (69) results in a significant loss of resistance to ionizing radiation, and the *irrI uvrA* double mutant and the *irrI* mutant are equally sensitive to ionizing radiation. The *irrI* mutations also reduce *D. radiodurans* resistance to ultraviolet radiation, but this effect is only observed in *uvrA*⁺ backgrounds. The function of the IrrI protein is unknown, but there is preliminary evidence that IrrI may be a regulatory protein.

THE ENZYMOLOGY OF DNA REPAIR IN *D. RADIODURANS*

Only a handful of studies in the last 40 years has attempted to detail the biochemistry of any process associated with DNA repair in *D. radiodurans*. As

Table 2 Characterized DNA repair proteins of *D. radiodurans*

| Repair process | Repair protein | Associated loci | Comments | Reference |
|----------------------------|------------------------------|-------------------------|---|-----------|
| Nucleotide excision repair | Endonuclease α | <i>uvrA</i> | Shares 60% amino acid sequence identity with the UvrA protein of <i>E. coli</i> | (1) |
| Base excision repair | Endonuclease β | <i>uvsC, uvsD, uvsE</i> | Appears to be a pyrimidine-dimer DNA glycosylase | (19, 26) |
| | Uracil DNA glycosylase | ? | Activity detected in extracts of <i>D. radiodurans</i> , | (42) |
| | Thymine glycol glycosylase | ? | Activity detected in extracts of <i>D. radiodurans</i> | (58) |
| | AP endonuclease | ? | Activity detected in extracts of <i>D. radiodurans</i> | (42) |
| | Deoxyribosephosphodiesterase | ? | Activity detected in extracts of <i>D. radiodurans</i> | (58) |
| Recombinational repair | RecA | <i>rec</i> | Shares 56% amino acid sequence identity with RecA protein of <i>E. coli</i> | (23) |
| Accessory proteins | DNA polymerase I | <i>pol</i> | Shares 51% amino acid sequence identity with DNA Polymerase I protein of <i>E. coli</i> | (24) |

a consequence, our knowledge of these processes is rudimentary. Three types of DNA repair have been described: (a) nucleotide excision repair, (b) base excision repair, and (c) recombinational repair. A list of those *D. radiodurans* proteins that have been at least partially characterized, and that appear to function in DNA repair, is presented in Table 2. Since a detailed description of the biochemistry of *D. radiodurans* DNA repair proteins has been published in two recent reviews (5, 46), it will not be repeated here.

Nucleotide excision repair is mediated by the activity of a protein identified as endonuclease α (56). Endonuclease α recognizes a broad range of DNA damage, and it incises the DNA at or near the site of that damage. It appears to be a functional homologue of the *uvrABC* endonuclease of *E. coli* (1). Mutational inactivation of the deinococcal *uvrA* locus results in loss of endonuclease α activity. Given that a UvrA homologue has been identified as part of endonuclease α , it is not unreasonable to expect that *D. radiodurans* expresses homologues of the *uvrB* and *uvrC* gene products of *E. coli* as well. There is no direct evidence, however, that these homologues are present in *D. radiodurans*.

Base excision repair has also been detected in *D. radiodurans*. There have been reports describing an apurinic/aprimidinic (AP) endonuclease activity (42), a uracil-N-glycosylase activity (42), a DNA deoxyribosephosphodiesterase

(dRPase) activity (58), and a thymine glycol glycosylase activity (58) in cell extracts of *D. radiodurans*. The genes that encode the proteins responsible for these activities have not been identified. Thus, the influence of these repair activities on DNA damage resistance has not been assessed.

Endonuclease β is a pyrimidine-dimer DNA glycosylase (PD DNA glycosylase) analogous to the PD DNA glycosylases isolated from *Micrococcus luteus* or bacteriophage T4 (26). Expression of the *denV* gene of T4 in *uvrA uvs* and *uvs* strains of *D. radiodurans* partially restores UV resistance to these strains, which indicates that endonuclease β has specificity for pyrimidine dimers. This protein appears to be involved exclusively in the repair of UV-induced DNA damage. Three mutations, designated *uvsC*, *uvsD*, and *uvsE*, inactivate endonuclease β (20), which renders *uvrA* strains of *D. radiodurans* UV sensitive. Strains with wild-type *uvs* resist crosslinking agents and ionizing radiation.

Mutational inactivation of the *rec* locus of *D. radiodurans* R1 will result in strains that are sensitive to all forms of DNA damage, presumably because this mutant cannot carry out homologous recombination. The *rec* gene has been cloned and sequenced, and its gene product has been shown to be a homologue to *E. coli*'s RecA protein, which shares 56% identity with *E. coli*'s RecA (23). Despite this similarity, the deinococcal RecA will not restore recombinational repair to an *E. coli recA* null mutant, which distinguishes the deinococcal gene product from the majority of prokaryotic RecA homologues. Evidence has been presented that suggests that when the deinococcal RecA is expressed in *E. coli*, even at low levels, it is toxic (23). The reasons for the toxicity are unknown.

Gutman et al (25) have cloned and sequenced a gene that encodes a DNA polymerase and that is necessary for DNA damage resistance in *D. radiodurans*. It appears that the *pol* gene product of *D. radiodurans* and *E. coli*'s DNA polymerase I have similar, if not identical, functions. The *D. radiodurans*' *pol* gene product shares 51.1% identity with DNA polymerase I of *E. coli*. In addition, a *pol* strain of *D. radiodurans* can be restored to DNA damage resistance by the intracellular expression of a clone of *E. coli*'s DNA polymerase I (24).

An evaluation of the enzymology of DNA repair in *D. radiodurans* reveals that each deinococcal DNA repair protein that has been identified, to date, has a functional homologue in other prokaryotes. Further, with the possible exception of the RecA homologue, nothing exceptional has been reported concerning the catalytic abilities of this group of proteins. Although Table 2 defines an admittedly short list of repair proteins, the similarity between the deinococcal proteins and those found in *E. coli* is striking, which suggests that *D. radiodurans* may use the same complement of DNA repair proteins as does this much more DNA damage-sensitive organism. If this is true, it seems unlikely that an exhaustive characterization of the enzymology of deinococcal DNA repair will provide insight into this organism's extreme DNA damage tolerance. Instead, it is perhaps more pertinent to ask whether the context in which these proteins

are utilized in *D. radiodurans* is somehow different from the context in which they are used in more radiosensitive organisms.

D. RADIODURANS' STRATEGIES FOR SURVIVAL

Redundant Genetic Information

As stated earlier, *D. radiodurans* is multigenomic (28,32). Stationary phase cells contain four copies of their chromosome, and actively dividing cells contain from four to ten copies. Since multiple copies of the genome provide the cell with a reservoir of genetic information, it is expected that DNA repair processes will be more efficient in organisms with higher chromosome multiplicity. Mortimer (50) demonstrated in 1958 that diploid and tetraploid forms of *S. cerevisiae* are more radiation resistant than are haploid strains. From these results, it was assumed that the additional genetic information "protected" the polyploid cells from radiation-induced lethality, because the presence of multiple genomes offered the possibility of restoring the DNA sequence that had been damaged or lost during irradiation through homologous recombination. Similar results were obtained by Krasin & Hutchinson (37) in their studies of *E. coli*. They showed that *E. coli* cultures grown in minimal media were more radiation sensitive than were exponential phase cultures grown in rich media. When growing under optimal conditions, the *E. coli* chromosome replicates faster than the cell septates, which results in multigenomic cells. In contrast, in minimal media the cell replicates slowly, and individual cells are haploid. Thus, in *E. coli* and *S. cerevisiae*, chromosome multiplicity confers enhanced radiation resistance. Clearly, just as the materials necessary to build a house don't assemble themselves, the availability of redundant genetic information is of little use if the mechanisms are not in place to take advantage of that information. Simply being multigenomic cannot, in and of itself, provide additional protection. As discussed below, *D. radiodurans* exhibits extremely efficient recombinational repair and it is here that the availability of redundant genetic information appears to play a critical role in cell survival.

It should be noted that, at this time, there is no physical evidence that redundant genetic information is necessary for the extreme radioresistance of *D. radiodurans*. Attempts at reducing chromosomal copies to less than four have been unsuccessful (32). Defined media have been used to vary chromosome multiplicity between 5 and 10 copies in *D. radiodurans*, but these studies fail to demonstrate any correlation between the number of copies and the radioresistance of *D. radiodurans* (32).

Interchromosomal Recombination

When an exponential phase culture of *D. radiodurans* is exposed to 5000 Gy γ radiation, more than 150 DNA double-stranded breaks (dsbs) are introduced

into the chromosome (9). Given the number of dsbs generated, it is remarkable that this level of damage is repaired without loss of genetic integrity. Cellular exonucleases, acting at strand breaks, should rapidly destroy overhanging ends, with a concomitant loss of sequence information. Even so, *D. radiodurans* is apparently able to reassemble an intact chromosome from the fragments produced following γ radiation in a way that conserves the linear continuity of its genome. This ability undoubtedly requires chromosome multiplicity. Because strand breaks are generated randomly, the probability of losing genetic information at the same site on every chromosome is very low at sublethal doses of radiation. Although each chromosome will suffer strand breaks, the distribution of that damage will, on average, be different from chromosome to chromosome. In principle, the total complement of fragments that remain after sublethal damage and repair should be sufficient to form an intact chromosome, provided that the cell has a way of mediating this reassembly.

Daly & Minton (11–13, 48) have suggested that *D. radiodurans* uses interchromosomal recombination to take advantage of the additional information present in multiple chromosomal copies. They have provided physical evidence that interchromosomal recombination occurs, reporting that γ irradiation induces as many as 600 crossovers per four-chromosome nucleoid (11). Approximately one third of these crossovers were identified as nonreciprocal, which suggests that *D. radiodurans* is, at least in part, restoring its fragmented chromosomes by piecing available sequences together.

The idea that interchromosomal recombination reassembles an entire chromosome from a myriad of small, randomly generated fragments implies that *D. radiodurans* is able to efficiently bring homologous regions of those fragments together by an unprecedented mechanism. In an elegant theoretical discussion of this concept, Minton & Daly (48) point out that a search for homology among hundreds of fragments is a logistical nightmare—a problem that is perhaps most easily explained by the following analogy. Assume that the fragments of each *D. radiodurans* chromosome are pieces of a jigsaw puzzle scattered on a table and, because *D. radiodurans* is multigenomic, there are four identical and intermixed sets of puzzle pieces. Putting the puzzle together requires that each piece be examined individually and fit to other compatible pieces. Since there is no obvious way of determining whether a piece has already been used, solving the puzzle will require an extensive and frequently futile search for compatibility. The *D. radiodurans* cell must, therefore, not only have the means for assembling an intact chromosome from component parts, but it must also have a mechanism for avoiding these potentially fruitless homology searches. Without such a mechanism, it is difficult to envision how the process of chromosome restitution could be completed within a reasonable time frame.

Minton & Daly (48) have suggested that the problem of futile searches for homology can be overcome by physically eliminating the need for such searches.

They have proposed that there is a pre-existing alignment between homologous regions on the different *D. radiodurans* chromosomes, and that this alignment facilitates the association of homologous sequences after irradiation. However, there is no experimental evidence to support this hypothesis.

Coordinate Regulation of DNA Repair Functions

When microorganisms are treated with DNA damaging agents, such as UV light, the lesions that are introduced will stop DNA replication by blocking movement of the polymerase along the template strand (27, 49). While this phenomenon might contribute to the inhibition of DNA replication that is observed following DNA damage in *D. radiodurans*, it does not appear to be the primary reason for the inhibition. Numerous studies (15, 40, 55) have shown that the DNA damage-induced delay in *D. radiodurans*' chromosomal DNA replication is dose dependent, and that the length of the delay always exceeds the time required to repair the DNA damage that caused the inhibition. These observations suggest that the process of DNA replication in *D. radiodurans* is sensitive to the level of intracellular DNA damage, and that the cell has a mechanism for sensing the completion of DNA repair (i.e. the level of DNA damage) and relaying that information to the replication machinery. In other words, these observations suggest that there is a regulatory checkpoint in *D. radiodurans* that controls chromosome replication and, as a consequence, controls cell division.

Mattimore et al (44) have provided genetic evidence supporting the existence of a DNA damage checkpoint in *D. radiodurans*. This group identified three mutant strains of *D. radiodurans*, designated SLR2, SLR4, and SLR5, that exhibit a slow recovery phenotype following exposure to ionizing radiation. The SLR mutants are as resistant to γ radiation as the wild-type organism is, but after irradiation they require from 48 to 72 h longer than the wild type does to form colonies on agar. These strains do not express a growth defect, however, because they have generation times identical to wild-type *D. radiodurans*. While they are obviously able to repair the DNA damage that is introduced following irradiation, the SLR strains appear to be defective in control of the reinitiation of DNA repair following irradiation. The SLR strains have been divided into two classes (NC Shank & JR Battista, unpublished observations), based on the rate at which they repair ionizing radiation-induced DNA damage. Figure 4 illustrates how the SLR strains recover from exposure to 5000 Gy γ radiation, a dose at which all three strains exhibit 100% viability. Using the restitution of DNA dsbs as an endpoint, recovery was followed using PFGE. Samples of each culture were removed immediately after irradiation and at 24 h intervals thereafter for the next 72 h. Restoration of chromosomal DNA was not observed in SLR2 until 48 h after irradiation, whereas the chromosomal DNA of R1, SLR4, and SLR5 had reassembled within 24 h. (SLR4 and SLR5 recover with

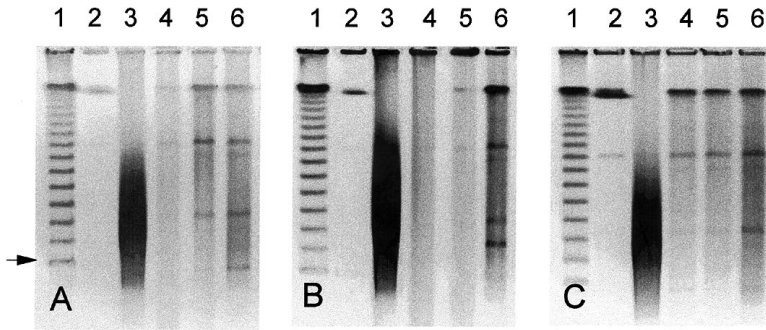


Figure 4 The ability of *D. radiodurans* R1 (panel A), SLR2 (panel B), and SLR5 (panel C) to survive the accumulation of chromosomal DNA double strand breaks following exposure to 5000 Gy γ radiation. For each panel: Lane 1 is a lambda ladder size standard, lane 2 is chromosomal DNA prepared from an untreated culture, lane 3 is chromosomal DNA prepared from a culture immediately after irradiation, and lanes 4–6 are chromosomal DNA prepared from a culture at 24, 48, and 72 h post-irradiation, respectively.

the same kinetics. Only SLR5 is represented in Figure 4.) In fact, for these strains, recovery is complete in less than 12 h (data not shown). SLR2 mends dsbs slowly relative to wild type, and this obvious delay in DNA repair correlates with the delay in colony formation (cell division?) noted by Mattimore et al (44). It appears that SLR2 cells have the ability to suspend DNA replication until repairs are complete, even though that delay is 36 h longer than what would normally occur following 5000 Gy γ radiation.

If there is a mechanism that is both sensitive to DNA damage and capable of restricting DNA replication, it must be assumed that there is also a mechanism for alleviating that inhibition, once DNA damage has been removed. The phenotypes observed in SLR4 and SLR5 may reflect a breakdown in the cell's ability to "realize" that DNA repair is complete. In contrast to SLR2, SLR4 and SLR5 do not exhibit a delay in double strand break repair. Once repairs are finished, however, these strains seem unable to reinitiate DNA synthesis and/or cell division. This defect could be in the strain's ability to detect removal of the signal that triggers the inhibition of DNA replication, or, alternatively, the strain may not be able to clear that signal from the cell with the same kinetics as the wild-type organism.

One of the most pronounced physiological effects observed following the administration of ionizing radiation to *D. radiodurans* is an immediate and extensive breakdown of chromosomal DNA (40, 53, 71). This degradation seems to be caused by the introduction of single and double strand breaks into the chromosome that serve as substrates for exonucleases present in the cell. For

most species, the loss of genetic information that accompanies this breakdown is considered one of the lethal consequences of ionizing radiation-induced DNA damage. In *D. radiodurans*, however, the DNA degradation observed appears to be part of the DNA repair process. There is evidence of degradation within 5 min of the administration of 5000 Gy γ radiation (72).

The duration of postirradiation DNA degradation is determined by the dose of radiation administered: The larger the dose, the longer the degradation continues and the greater the loss of chromosomal DNA (40, 53, 71). The rate of degradation is independent of dose, with an estimated 0.1% of the total genomic DNA lost per minute. The degradative process, once started, must be "turned off" by an uncharacterized inhibitory protein that seems to be induced by DNA damage (29). The termination of DNA degradation requires protein synthesis post-irradiation. Administration of either chloramphenicol (16, 17) or actinomycin D (16) to cultures prior to irradiation results in extensive loss of chromosomal DNA, and it ultimately results in cell death. The observation that the extent of DNA degradation is governed by the dose of radiation administered indicates that this process is regulated, and that this regulation keys on the level of intracellular DNA damage. It therefore appears that the signal(s) that control DNA replication and the process of DNA degradation (and DNA repair?) are interrelated. DNA replication is held in check until DNA repair is complete, and DNA repair ends before DNA replication is reinitiated, suggesting that these phenomena are coordinately regulated. If this is true, it is not unreasonable to expect that the same signal affects each process. The nature of the proposed signal is unknown.

The protein that inhibits DNA degradation appears to be a component of the cell's mechanism for sensing DNA damage. As noted, this protein is responsible for stopping DNA degradation. Presumably, it is sensitive to DNA damage and, when damage drops to an "acceptable" level, it relays that information to the proteins catalyzing the degradation. The recently described IrrI protein of *D. radiodurans* (69) is a candidate for this inhibitor of DNA degradation. *irrI* strains are extremely sensitive to ionizing radiation, exhibiting a dramatic reduction in survival following doses that are sublethal to the wild-type organism. As illustrated in Figure 1 (*panel B*), the chromosome of the *irrI* strain, IRS41, is completely destroyed by 3000 Gy γ radiation and fails to recover (MD Manuel & JR Battista, unpublished observations). Only 0.1% of an IRS41 culture will survive this dose. In contrast, the wild-type *D. radiodurans* restores its genome in less than 3 h (Figure 1, *panel A*), and exhibits no loss of viability at this dose. Following irradiation, an *irrI* mutant exhibits the same unrestricted DNA degradation that typifies wild-type cultures that are pre-treated with chloramphenicol, which suggests that IrrI is the protein needed to stop DNA degradation.

DNA Damage Export

UV and γ radiation-induced DNA degradation are accompanied by export of DNA damage. Initially, the products formed are oligonucleotides approximately 2000 bp long, and a mixture of damaged and undamaged nucleotides and nucleosides (72). These products are found in the cytoplasm and the surrounding growth medium, which indicates that *D. radiodurans* exports the degradation products once they are formed (6, 30, 31). Release of degradation products continues throughout this stage in the cell's recovery, ending shortly after degradation ends. While the release of DNA damage has been exploited for many years as a convenient means of following the repair of base damage in *D. radiodurans*, the relationship of this phenomenon to DNA damage resistance has never been explored. The removal of damaged nucleotides from the intracellular nucleotide pool, and their subsequent conversion to nucleosides, could represent a survival strategy. Two possibilities seem likely: 1. Moving damaged nucleotides outside the cell might protect the organism from elevated levels of mutagenesis by preventing the reincorporation of damaged bases into the genome during DNA synthesis subsequent to repair. 2. Removal of nucleotides from the cell is part of the signal that coordinates the DNA repair functions described above.

CONCLUDING REMARKS

Despite 40 years of investigation, our knowledge of *D. radiodurans*' extraordinary ability to survive DNA damage is still limited. Even so, evidence is accumulating that indicates that *D. radiodurans* uses a set of proteins not unlike those of other prokaryotes to carry out DNA repair functions. The picture that emerges suggests that *D. radiodurans* influences the effectiveness of an "ordinary" complement of DNA repair proteins by changing the conditions in which they operate. Many of the unintended consequences of the DNA repair process observed in other organisms seems to be avoided. Redundant genetic information is always available and through interchromosomal recombination a new genome can be built, from fragments if necessary, without loss of genetic integrity. In effect, this system seems to prevent the loss of genetic information through deletion during DNA repair. Also, the process of DNA repair appears to be coordinately regulated with the cell cycle, providing adequate time for repair and averting a potentially deleterious attempt to replicate a damaged genome. Finally, damaged nucleotides are sequestered by expulsion from the cell, which prevents their reincorporation during DNA synthesis subsequent to their removal during repair. In the author's estimation, appreciating how this combination of characteristics influences the activity of DNA repair proteins is key to understanding the extreme DNA damage tolerance of *D. radiodurans*.

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