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Deinococcus radiodurans: What Belongs to the Survival Kit?

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***Deinococcus radiodurans*, one of the most radioresistant organisms known to date, is able to repair efficiently hundreds of DNA double- and single-strand breaks as well as other types of DNA damages promoted by ionizing or ultraviolet radiation. We review recent discoveries concerning several aspects of radioresistance and survival under high genotoxic stress. We discuss different hypotheses and possibilities that have been suggested to contribute to radioresistance and propose that *D. radiodurans* combines a variety of physiological tools that are tightly coordinated. A complex network of regulatory proteins may be discovered in the near future that might allow further understanding of radioresistance.**

Keywords *Deinococcus radiodurans,* radiation resistance, DNA repair, desiccation, oxidative stress

DEINOCOCCUS RADIODURANS RESISTANCE TO IONIZING RADIATION AND PROLONGED DESICCATION

Deinococcus radiodurans belongs to the *Deinococcaceae*, a family of bacteria characterized by an exceptional ability to withstand the lethal effects of DNA-damaging agents, including ionizing radiation, ultraviolet (uv) light, and desiccation (Rainey *et al*., 1997; Battista and Rainey, 2001). *Deinococcaceae* were isolated from diverse environments after exposure to high doses of ionizing radiation (Table 1). Among this family, *D. radiodurans* is by far the best characterized and was first isolated in canned meat that had been irradiated at 4,000 Grays (Gy) in order to achieve sterility (Anderson *et al*., 1956).

D. radiodurans cells are nonmotile and non-spore-forming. They are pigmented and appear pink-orange. They stain Gram-positive, but the cell wall differs from other Gram-positive bacteria in respect to its lipid composition and to the types of cell layers. *D. radiodurans* is very easy to handle: it grows optimally at 30°C in rich medium and is an obligate aerobe. Cells are naturally competent and genetic tools are available for conditional expression or complete inactivation of genes (Funayama *et al*., 1999; Lecointe *et al*., 2004a; Meima and Lidstrom, 2000).

D. radiodurans can survive the extremely high dose of 5000 Gy without loss of viability (Moseley and Mattingly, 1971). The radioresistance of *D. radiodurans* cannot be related to prevention of DNA damage, because DNA double-strand breaks are formed at the same rate in *Escherichia coli* and *D. radiodurans* when cells are irradiated under identical conditions (Gerard *et al*., 2001). Ionizing radiation leads to the formation of highly reactive radicals (e.g., hydroxyl radicals) and causes a variety of damage, such as DNA single- and double-strand breaks and base modifications. A 5000 Gy dose introduces approximately 200 DNA double-strand breaks per *D. radiodurans* genome equivalent (Cox and Battista, 2005). In comparison only a few DNA double-strand breaks can kill an *E. coli* cell (Krasin and Hutchinson, 1977).

Several links between radioresistance and desiccation tolerance have been described (Dose *et al*., 1991; Dose *et al*., 1992; Battista *et al*., 2001; Mattimore and Battista, 1996; Billi *et al.,* 2000; Tanaka *et al*., 2004; Rainey *et al*., 2005). Both desiccation and ionizing radiation lead to DNA double-strand breaks (Mattimore and Battista, 1996), and desiccation, like ionizing

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TABLE 1
Deinococcaceae species by geographic location and study

Species	Identified in	Reference
D. apachensis	Sonoran desert, North America	(Rainey *et al*., 2005)
D. cellulosilyticus	Air from Jeju Island, Korea	(Weon *et al*., 2007)
D. deserti	Sahara desert, North Africa	(de Groot *et al*., 2005)
D. ficus	Ficus rhizosphere, Taiwan	(Lai *et al*., 2006)
D. frigens	Antarctica	(Hirsch *et al*., 2004)
D. geothermalis	Hot springs, Italy and Portugal	(Ferreira *et al*., 1997)
D. grandis	Intestine of Cyprinus carpio	(Oyaizu, 1987; Rainey *et al*., 1997)
D. hohokamensis	Sonoran desert, North America	(Rainey *et al*., 2005)
D. hopiensis	Sonoran desert, North America	(Rainey *et al*., 2005)
D. indicus	Aquifer, West Bengal, India	(Suresh *et al*., 2004)
D. maricopensis	Sonoran desert, North America	(Rainey *et al*., 2005)
D. marmoris	Antarctica	(Hirsch *et al*., 2004)
D. murrayi	Hot springs, Portugal	(Ferreira *et al*., 1997)
D. navajonensis	Sonoran desert, North America	(Rainey *et al*., 2005)
D. papagonensis	Sonoran desert, North America	(Rainey *et al*., 2005)
D. peraridilitoris	coastal desert, Chile	(Rainey *et al*., 2007)
D. pimensis	Sonoran desert, North America	(Rainey *et al*., 2005)
D. proteolyticus	Faeces of Lama glama	(Brooks and Murray, 1981; Kobatake, 1973)
D. radiodurans	Irradiated meat cans	(Anderson *et al*., 1956)
D. radiophilus	Irradiated Bombay duck	(Brooks and Murray, 1981)
D. radiopugnans	Irradiated Haddock	(Brooks and Murray, 1981)
D. saxicola	Antarctica	(Hirsch *et al*., 2004)
D. sonorensis	Sonoran desert, North America	(Rainey *et al*., 2005)
D. yavapaiensis	Sonoran desert, North America	(Rainey *et al*., 2005)
D. yunnanensis	Agar plate, China	(Zhang *et al*., 2007)
T. radiovictrix	Hot springs, Azores	(Albuquerque *et al*., 2005)

radiation, leads to oxidative damage (Potts, 1994), suggesting that a combination of pathways that can deal with DNA double-strand breaks and oxidative damage plays a crucial role in resistance to both genotoxic stresses. Possible passive and enzymatic contributions to its radioresistance have been soundly reviewed and discussed and it was suggested that *D. radiodurans* combines a variety of physiological tools that are tightly coordinated and that many different mechanisms contribute to radiation resistance (Cox and Battista, 2005), but several factors contributing to radioresistance still remain controversial, and more probably remain to be discovered.

GENOMIC ANALYSES

The *D. radiodurans* genome was sequenced in 1999 (White *et al*., 1999). The genome consists of two chromosomes, a megaplasmid and a smaller plasmid encoding for a total of approximately 3200 proteins. *D. radiodurans* contains multiple genome copies per cell (Hansen, 1978), but since this had been proposed, no correlation seems to exist between the number of genome copies and the degree of radioresistance (Harsojo *et al*., 1981). It is likely however, that two or more genomic copies are a necessary, but not sufficient, condition for radioresistance.

So far, the genomes of two relatives of *D. radiodurans* have been sequenced as well, and their genomes have been compared to the genome of *D. radiodurans*. One was *Thermus thermophilus*, a radiosensitive thermophile from the same phylum (Henne *et al*., 2004). The other, *Deinococcus geothermalis*, is a radioresistant moderate thermophile from the same genus as *D. radiodurans* (Makarova *et al*., 2007). Interesting insights into their evolution was gained from both comparisons, such as indications that horizontal gene transfer played a major role for the evolution of bacteria and archaea (Makarova *et al*., 2001; Nelson *et al*., 1999). However, a simple explanation for a radioresistant phenotype could not be obtained.

The analysis of the genome of *D. geothermalis* showed that many genes that had been implicated in radioresistance in *D. radiodurans* are absent, reducing the group of genes that might be essential for radioresistance (Makarova *et al*., 2007). However, several presumptions have to be made, in order to derive a set of resistance-genes from genome comparisons: (1) the exact function of a gene can be predicted solely based on its DNA sequence and (2) the radioresistant phenotype of *D. geothermalis* is caused by the same combination of genes. In particular, it could be difficult to determine what genes are important for radiation resistance if the relative contributions to radioresistance of many different mechanisms are not the same in each radioresistant organism and if these organisms use a partially different set of genes involved in radioresistance.

Both *D. radiodurans* and *D. geothermalis* do not seem to have acquired novel DNA repair systems, but progressively expanded their systems involved in cell cleaning and salvage, an important process that prevents, for example, incorporation of damaged nucleotides (Galperin *et al*., 2006), as well as genes involved in transcriptional regulation and signal transduction (Makarova

et al., 2000). In summary, the high levels of stress resistance observed in *D. radiodurans* have probably evolved continuously by gene duplications and through various events of horizontal gene transfer (Makarova *et al*., 2001; Makarova *et al*., 2007).

Recently, a novel species has been isolated, which belongs to the phylum *Deinococcus/Thermus* but represents a new family, the *Trueperaceae* (Table 1). *Truepera radiovictrix* is thermophilic and extremely ionizing radiation resistant, whereas all *Thermus* family members are thermophilic and radiosensitive. The genome of this organism is likely to be sequenced in the near future and may allow to further narow down candidate genes for radioresistance. Further comparisons of different genomes, proteomes, and transcriptomes of radioresistant species could possibly help define a minimal set of genes that can contribute the extreme radioresistance.

Analysis of the transcriptome of *D. radiodurans* revealed an exciting group of genes that are up-regulated in response to either desiccation or ionizing radiation (Tanaka *et al*., 2004). Among the 72 genes up-regulated during the first hour after a sublethal dose of ionizing radiation, 33 are also highly expressed in *D. radiodurans* cultures recovering from desiccation (Tanaka *et al*., 2004). Only a limited number of well defined DNA repair genes including *recA* (*DR2340*), *uvrA* (*DR1771*), *uvrB* (*DR2275*), *gyrA* (*DR1913*) and *gyrB* (*DR0906*) are found among these genes. The five genes that are most highly induced in response to each stress include *ddrA* (*DR0423*), *ddrB* (*DR0070*), *ddrC* (*DR0003*), *ddrD* (*DR0326*), and another gene called *pprA* (*DRA0346*) encoding proteins of unknown function. Inactivation of the corresponding genes indicates that they play a role in radioresistance (Tanaka *et al*., 2004). None of the mutant strains exhibits a decrease in the efficiency of natural transformation by genomic DNA relative to the R1 wild-type strain, indicating that the most highly induced genes do not seem to be involved in a RecA-dependent recombinational repair pathway (Tanaka *et al*., 2004).

However, many genes that are strongly induced upon irradiation are not necessarily directly involved in recovery (Makarova *et al*., 2007). For example, expression of DRB0100, a homolog of eukaryotic DNA ligase III, is highly induced by ionizing radiation or desiccation (Tanaka *et al*., 2004) but a mutant *D. radiodurans* strain devoid of DRB0100 is as radioresistant as the wild type R1 strain (S. Sommer, unpublished results).

NUCLEOTIDE AND BASE EXCISION REPAIR

D. radiodurans is very resistant to UVC-radiation (254 nm) and can sustain doses that cause enormous amounts of base damage. Pyrimidine dimers are the most abundant type of UV-induced damage. It has been estimated that exposure of *D. radiodurans* to a UV dose of 500 J/m2, which is nonlethal for this organism, as many as 5000 thymine-containing pyrimidine dimers could be formed per genome (Battista, 1997). *D. radiodurans* lacks photolyases for light-stimulated reversion of dimers (Makarova *et al*., 2001), but contains two excision repair pathways for efficient removal of pyrimidine dimers: first, the classical nucleotide excision repair (NER) and second, the alternate UV damage endonuclease (UVDE) repair pathway (Minton, 1994; Moseley and Evans, 1983). The NER pathway involves a protein complex (the UvrABC exci-nuclease), which recognizes the structural changes caused by UV damage in DNA and proceeds by creating dual incisions 5′ and 3′ to the damaged site. This pathway is involved in removal of many other bulky lesions such as mitomycin-C adducts (Petit and Sancar, 1999). *D. radiodurans* encodes homologs of UvrA (DR1171), UvrB (DR2275), and UvrC (DR1354). UvrA mutants (formerly called endo α) have been isolated as mitomycin-C-sensitive cells (Agostini *et al*., 1996; Narumi *et al*., 1997). The UvrA protein from *E. coli* can restore mitomycin-C resistance in UvrA deficient *D. radiodurans* cells, suggesting that the two bacteria may have very similar NER systems (Agostini *et al*., 1996).

The *D. radiodurans uvrA* mutants retain full UV-resistance because of the presence of an alternate UVDE (UV damage endonuclease) repair pathway. UVDE (formerly called UV endonuclease β) was initially identified as mutants called *uvsC*, *uvsD*, and *uvsE* that confer UV sensitivity in an *uvrA*-deficient background (Earl *et al*., 2002b). It has been now established that *uvsC*, *uvsD*, and *uvsE* are mutated alleles of a unique gene (DR1819) that encodes endonuclease β (Earl *et al*., 2002, Kitayama *et al*., 2003). DR1819 has 40% similarity and 30% identity with the UVEp protein from *Schizosaccharomyces pombe* (Earl *et al*., 2002b). This enzyme introduces a nick immediately 5' to a UV-lesion that is then processed by other proteins for damage excision and repair replication (see Yasui and McCready, 1998, for review). The UV endonuclease β of *D. radiodurans* has been purified. It is a 36,000 Da protein with endonuclease activity and uses Mn(II) as cofactor (Evans and Moseley, 1985, 1988). The respective contribution of the NER and the UVDE pathways to eliminate pyrimidine dimers from UV-irradiated *D. radiodurans* DNA has been evaluated: UVDE efficiently removes both cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone dimers (6-4 PPs), whereas NER seems more specific for 6–4 PPs (Tanaka *et al*., 2005). Moreover, inactivation of the two pathways does not completely abolish the ability to eliminate CPDs and 6-4 PPs from DNA suggesting the presence of another back-up pathway (Tanaka *et al*., 2005). *D. radiodurans* encodes a second UvrA-related protein (UvrA2, DRA0188). However, inactivation of uvrA2 in an *uvsE* or *uvrA* background has no effect on UV sensitivity or on DNA repair kinetics, suggesting that UvrA2 plays no role in resistance to UV (Tanaka *et al*., 2005). UvrA1 and UvrA2 belong to the family of ABC transporters. UvrA2 might be implicated in export of damaged oligonucleotides, a process that is known to take place in irradiated *D. radiodurans* (Battista *et al*., 1999).

As *D. radiodurans* is also very resistant to hydrogen peroxide or methyl methane sulfonate, both leading to severe base modifications and base damage, an efficient base excision repair machinery is anticipated to be active in *D. radiodurans*. Multiple genes encoding uracil-DNA glycosylases have been identified so far. Among them are the uracil-DNA glycosylase DRB0689, which shows similarity to the *E. coli* UNG and

removes uracil from U:G and U:A mismatches as well as from single-stranded DNA, and the unrelated DR1751, which also removes uracil from U:G and U:A mismatches, but clearly prefers single-stranded DNA as substrates (Sandigursky *et al*., 2004). The structure of the main UNG enzyme (DRB0689) has been solved. The elevated catalytic efficiency of the DRB0689 glycosylase compared to the *E. coli* and human homologs may be caused by an increased positive surface at the site of DNA binding (Leiros *et al*., 2005a). Another putative uracil-DNA glycosylase (DR0022) did not show any substantial activity so far (Sandigursky *et al*., 2004). *D. radiodurans* also possesses a formamido pyrimidine glycosylase, Fpg (DR0493), which, unlike its *E. coli* homolog, prefers formamido pyrimidines to 8-OH guanine (Bauche and Laval, 1999; Senturker *et al*., 1999). *D. radiodurans* encodes a MutY (DR2285) protein, which can complement *E. coli mutY* mutants and shows a substrate specificity similar to the *E. coli* homolog (Li and Lu, 2001). Recently, the structure of a mismatch-specific uracil-DNA glycosylase (MUG, DR0715) has been solved. The enzyme contains a novel catalytic residue (Asp93), which is probably responsible for a broadened substrate specificity compared to the *E. coli* homolog (Moe *et al*., 2006). In summary, the excision repair machineries from *D. radiodurans* do not appear to differ greatly from the *E. coli* pathways. However, several adaptations to deal with numerous different lesions at the same time might have been acquired by *D. radiodurans*, such as modulated substrate specificities and redundant enzymatic activities.

DNA DOUBLE-STRAND BREAK REPAIR PROTEINS

D. radiodurans can sustain gamma irradiation doses that introduce hundreds of double-strand breaks in its genome. The kinetics of DNA double-strand breaks repair is very rapid as an intact genome complement is reconstructed from a myriad of fragments in few hours (Figure 1). Several mechanisms have been proposed to account for such an efficient repair (Figure 2). These mechanisms will be reviewed below together with key intervening proteins.

Homologous Recombination (HR)

This is the main pathway involved in DNA double-strand break repair in bacteria (Wyman *et al*., 2004) and in the yeast *Saccharomyces cerevisiae* (Paques and Haber, 1999). Homologous recombination uses an intact homologous DNA molecule to restore the correct DNA sequence at sites of damages. In the model bacterium *E. coli*, the RecBCD complex, with its helicase and ATP-dependent nuclease activities, produces a single-stranded 3' DNA overhang and loads the RecA protein. The nuclease activity needs to be tightly controlled to prevent further DNA degradation. The single-stranded DNA invades a homologous DNA molecule and new stretches of DNA are synthesized by DNA polymerase I using the intact DNA strand as a template. The remaining nicks are then sealed by DNA ligase and branch migration can occur before the so called "Holliday junctions" are resolved.

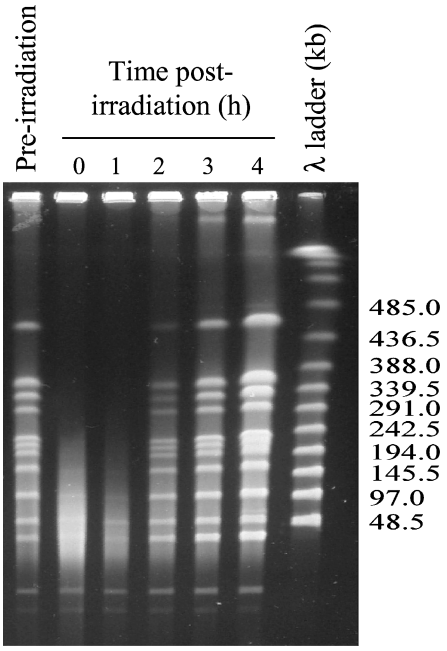

FIG. 1. Kinetics of DNA double-strand break repair in *D. radiodurans* cells exposed to 6,800 Gy γ-irradiation. *D. radiodurans* R1 bacteria were grown in TGY2X to an OD650 of 2, concentrated ten times, exposed to 6,800 Gy γ-irradiation, diluted in TGY2X to an OD650 of 0.2 and then incubated at 30°C with agitation for recovery. DNA agarose plugs were prepared at the post-irradiation times indicated on the abscissa and digested with Not1 before being analyzed by PFGE.

D. radiodurans, like *D. geothermalis*, does not encode RecB and RecC homologs (Makarova *et al*., 2007). It does, however, encode RecD (DR1902). RecD protein was shown to be present in the absence of RecBC, not only in *D. radiodurans*, but also in mollicutes, firmicutes and *Streptomyces* (Rocha *et al*., 2005). Deinococcal RecD protein contains an extended N-terminus, is expressed and active as a DNA helicase (Wang and Julin, 2004). As to the *in vivo* role of RecD in *D. radiodurans*, conflicting data have been published. Zhou *et al*. (2007) created a *recD* deletion strain, which was sensitive to hydrogen peroxide, but was normally resistant to UV and ionizing radiation. They found that the *D. radiodurans* RecD protein is able to stimulate catalase activity, suggesting a role in an antioxidant pathway rather than in a DNA double-strand break repair pathway (Zhou *et al*., 2007). In contrast to these findings, Servinsky and Julin (2007) describe a *recD* mutant, which is sensitive to γ-rays, UV and hydrogen peroxide, but resistant to methyl methane sulfonate and mitomycin-C. In addition, the *recD* mutant showed a greater efficiency of transformation by exogenous DNA, an effect that has also been observed in *E. coli* (Russell *et al*., 1989). As the *recD* mutant was not sensitive to mitomycin-C, which causes DNA strand-breaks, a role in the repair of oxidative damage seems more likely. Further work is required to assign RecD protein to a specific DNA repair pathway. The effect of the overexpression

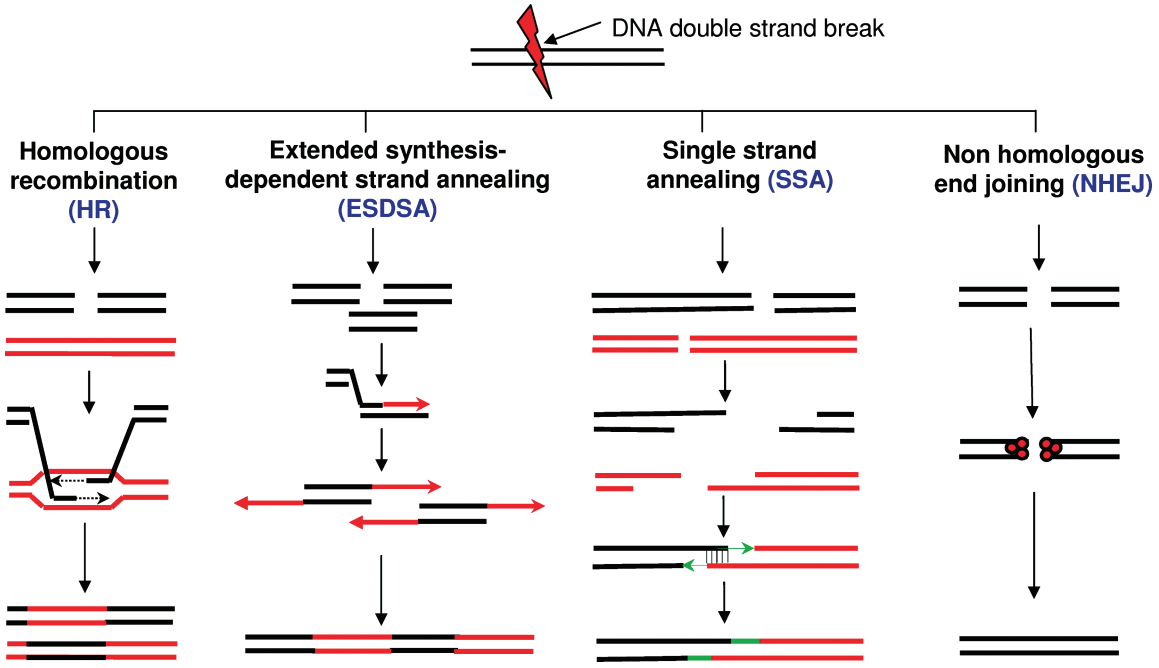

FIG. 2. Different pathways for DNA DSB repair in *D. radiodurans*. Adapted from (Featherstone and Jackson, 1999), and (Zahradka *et al*., 2006). For details see text.

of the *E. coli* RecBC complex in *D. radiodurans* has also recently been investigated (Khairnar *et al*., 2008). RecBC overexpression leads to a reduction in resistance to ionizing radiation (Khairnar *et al*., 2008). This effect was independent of RecD. RecBC might interfere with a DNA repair pathway by competing with another nuclease or might participate in extensive degradation of DNA ends via its exonuclease activity or create a single-strand DNA overhang, which could be a problematic substrate for *D. radiodurans* RecA.

In *Bacillus subtilis*, RecN was proposed to be one of the first recombination proteins detected as a discrete focus in live cells in response to double-strand breaks (Sanchez *et al*., 2006). It binds and protects 3' single strand DNA extensions in the presence of ATP (Sanchez and Alonso, 2005). A RecN homolog (DR1477) is present in *D. radiodurans* and a *recN* disruptant mutant strain shows substantial sensitivity to γ-rays, MMC and UV, suggesting a role of the deinococcal RecN protein in DNA repair and a possible involvement in the initiation of recombinational repair (Funayama *et al*., 1999). RecA protein is a key protein required for radioresistance of *D. radiodurans* strains. It is required for recombinational repair of double-strand breaks (Gutman *et al*., 1994a). Biochemical characterization of RecA protein shows a higher affinity of RecA to double-strand DNA than to single-strand DNA (Kim *et al*., 2002). Moreover, the RecA proteins of *D. radiodurans* and *E. coli* promote DNA strand exchange via inverse pathways (Kim and Cox, 2002). Indeed, the RecA protein of *Escherichia coli*, and filament-forming archaeal or eukaryotic homologs, promote DNA strand exchange by a common, ordered pathway. A filament is first formed on single-stranded DNA, followed by uptake of the duplex substrate. The observed course of DNA strand exchange promoted by the deinococcal RecA protein is the exact inverse of this established pathway.

Like RecA from *E. coli*, RecA from *D. radiodurans* forms right-handed helical filaments on DNA. The crystal structure of the deinococcal RecA in complex with ATPγS reveals a significant reorientation of the double-strand DNA-binding C-terminal domain, an increased positive electrostatic potential along the inner surface of the filament and structural changes in the flexible β6-β7 hairpin that has also been implicated in DNA binding (Rajan and Bell, 2004). The particular structure of the deinococcal RecA DNA binding sites may favour its direct binding to the double strand DNA ends and play a significant role in the inverse DNA strand exchange pathway to promote the repair of DNA double strand breaks. However, it is not yet clear whether the deinococcal RecA protein itself exhibits any specificity for DNA ends or whether another protein is required for this function. In *D. radiodurans*, the basal level of RecA has been estimated to about 11,000 monomers per cell and the induced level to 44,000 monomers per cell (Bonacossa de Almeida *et al*., 2002). Interestingly, when RecA was expressed out of its normal regulation from an IPTG-inducible promoter, bacteria producing RecA at low concentration (2500 molecules per cell) were as radioresistant as the wild-type bacteria expressing radiation-induced concentrations of RecA (Jolivet *et al*., 2006). This result suggests that a limited number of RecA molecules are sufficient to repair several hundred DNA double strand breaks. However, limiting the concentration of RecA protein increased radiation sensitivity of *ddrA* deficient cells suggesting that, under these conditions,

a functional *ddrA* gene product is required for survival. DdrA is involved in protection of single-strand DNA with 3′ ends (Harris *et al*., 2004) and presumably ensures long-lived recombinational substrates and recycling of RecA protein. The additive effect of a defect in the *ddrA* gene and of a limiting concentration of RecA also suggests that DdrA could be involved in a *recA*-independent repair mechanism. As DdrA is distantly but specifically related to the Rad52 family of eukaryotic proteins, it was suggested that DdrA could be a component of a single-strand annealing system that functions in conjunction with RecA-dependent homologous recombination (Harris *et al*., 2004). High concentrations of RecA protein can partially suppress radiation sensitivity of *ddrA*-deficient cells (Jolivet *et al*., 2006) suggesting that (i) by polymerization on DNA, RecA can protect DNA ends from degradation, or (ii) more rapid recombinational repair could partially compensate for defects in alternative DSB repair mechanisms.

Various DNA repair enzymes were also proposed to play a role in recombinational repair. The *D. radiodurans* SSB (DR0099) differs from its standard bacterial homologs. It is double the size of *E. coli* SSB, forms dimers instead of tetramers and contains two OB-folds per monomer instead of one (Eggington *et al*., 2004; Witte *et al*., 2005). The same kind of homodimer has been found in *Deinococcus murrayi* (Filipkowski *et al*., 2007), but the reason for this change from a tetra- to a homodimer is not clear given that Deinococcal SSB can replace SSB in *E. coli* cells (Filipkowski *et al*., 2007). The deinococcal SSB protein, however, seems to have a more robust capacity to displace a short DNA strand from a DNA duplex consistent with a relevance in DNA double-strand break repair (Eggington *et al*., 2006). Recently, it has been shown that bacterial SSB proteins are phosphorylated on tyrosine residues and that this modification can increase the DNA binding by approximately 200 fold (Mijakovic *et al*., 2006). It would be interesting to analyze the deinococcal SSB with respect to tyrosine phosphorylation and look for possible changes in response to ionizing radiation.

Orthologs of SbcD (Mre11) and SbcC (Rad50) proteins exist in all kingdoms of life and are involved in a wide variety of DNA repair and maintenance functions, including initiation of homologous recombination and nonhomologous end-joining. In *E. coli*, the SbcCD complex stimulates repair of double-strand breaks generated by a restriction endonuclease (Cromie and Leach, 2001), and in *B. subtilis* the SbcC protein appears to play a role in the repair of DNA inter-strand cross-links (Mascarenhas *et al*., 2006). Homologs of the Rad50/SbcC (DR1922) and Mre11/SbcD (DR1921) proteins have been identified in the *D. radiodurans* genome. Knock-out cells devoid of SbcCD activity display increased sensitivity to high doses of γ-rays, altered kinetics of DSB repair and a delay in resumption of cell division following γ-irradiation (Bentchikou *et al*., 2007). However, it is unlikely that the SbcCD complex plays a major role in recombinational repair of double-strand breaks, since survival of cells devoid of the SbcCD complex was not dramatically affected by γ-radiation at doses up to 10 kGy.

Although *D. radiodurans* lacks the RecBCD helicase/nuclease that processes DNA double-strand breaks in *E. coli*, it encodes all the components of the alternate RecF pathway, RecF (DR1089), RecR (DR0198), RecO (DR0819) and RecJ (DR1126) that form an alternative system for initiation of recombination in *E. coli* (Kowalczykowski *et al*., 1994). This pathway is inhibited by the SbcB nuclease (Kowalczykowski *et al*., 1994) and *D. radiodurans* is naturally devoid of the SbcB protein. Moreover, it was shown that expression *in trans* of the SbcB protein from *E. coli* renders *D. radiodurans* cells radiation-sensitive (Misra *et al*., 2006). Thus, a RecF-like pathway might operate in *D. radiodurans* to generate 3′ overhangs of single-stranded DNA. Nevertheless, the SbcCD complex might be required for processing a subset of DSBs with secondary structures or 5′ single stranded overhangs. This can be inferred from the biochemical activities of the prototype *E. coli* SbcCD complex that possesses a structure-specific endonuclease cleaving DNA hairpin loops, and a 3′->5′ single-strand exonuclease that may function in rendering DNA ends flush (Connelly *et al*., 1999).

RecR, which together with RecF and RecO helps to replace SSB by RecA, plays a role in the bacterial homologous recombination RecFOR pathway. It was proposed that the deinococcal RecR protein (DR0198) plays a role in homologous recombination and DNA interstrand cross-link repair (Kitayama *et al*., 2000) and the RecR crystal structure revealed a ring-shaped tetramer that is able to open and close (Lee *et al*., 2004; Koroleva *et al*., 2007). Each monomer consists of an N-terminal helix-hairpin-helix motif essential for DNA binding, a C-terminal domain with a Cys4 zinc-finger motif, a Toprim domain and a Walker B motif (Lee *et al*., 2004). The Deinococcal RecO (DR0819) supports DNA annealing and RecA-mediated recombination similar to eukaryotic Rad52 (Kantake *et al*., 2002). It was recently shown that *D. radiodurans* bacteria devoid of RecO exhibited a growth defect and extreme sensitivity to γ or UV-irradiation (Xu *et al*., 2008) suggesting an important role of the RecFOR pathway in double-strand break repair. The *in vivo* effect of a *recR* or a *recF* deletion on *D. radiodurans* radioresistance has to be investigated. The crystal structure of RecO showed a novel alpha-helical domain, a zinc-binding domain and an OB-fold that is structurally similar to the OB-fold of single-strand DNA-binding proteins such as RP-A, SSB or BRCA2 (Leiros *et al*., 2005b). Structural studies showed that RecO and RecR form a heterohexameric complex with a stoichiometry of 2:1 that preferentially binds to 3′ DNA overhangs (Timmins *et al*., 2007). The structure of *D. radiodurans* RecF, the third protein of the recombination mediator complex, has recently been solved (Koroleva *et al*., 2007). The protein shows a strong structural similarity to the head domains of Rad50 and SMC proteins suggesting a conserved mechanism of DNA recognition and DNA binding. The protein forms dimers in the presence of ATP and a clamp-loader function for the tetrameric RecR-ring has been proposed (Koroleva *et al*., 2007).

In *E. coli*, the RecJ exonuclease acts during gap repair to enlarge the single-strand DNA region for RecFOR binding (Lovett

and Clark, 1984). A RecJ homolog (DR1126) is present in *D. radiodurans* as in most of bacterial species except those lacking RecA, mollicutes and *mycobacteria*. Its function in DNA repair has to be characterized.

RecQ helicase (DR1289) contains 3 HRD domains involved in regulation of the helicase activity (Huang *et al*., 2007; Huang *et al*., 2006; Killoran and Keck, 2006). Another putative RecQ helicase, containing only one HRD domain, has been identified (DR2444) but its enzymatic activity still needs to be confirmed.

The homologs of recombinational proteins involved in branch migration or resolution of Holliday junctions RuvA (DR1274), RuvB (DR0576), RuvC (DR0440), and RecG (DR1916) are also present in *D. radiodurans* (White *et al*., 1999). The putative RuvB protein has a conserved ATP binding domain characteristic of DNA helicases. The *ruvB* mutant was transformed with exogenous DNA at the same rate as the wild-type cells, but it was moderately sensitive to UV, gamma-rays and to interstrand cross-linking reagents (Kitayama *et al*., 1997).

Single-strand Annealing (SSA) and Extended Synthesis-Dependent Strand Annealing (ESDSA)

In addition to homologous recombination, it has been proposed that a SSA reaction occurs at early times in irradiated *deinococcal* cells to account for the observation that part of the radiation-induced double-strand breaks can be mended in a recombination-defective *recA* mutant (Daly and Minton, 1996). The process of SSA can occur between different copies of broken chromosomes and be facilitated by the multiplicity of the deinococcal genome equivalents. This might constitute a preparatory step for further DNA repair (Daly and Minton, 1996).

Recently, Zahradka and coworkers (2006) proposed a variant of the SSA model, called ESDSA, to account for their findings that (i) DNA fragment assembly coincides with a massive DNA synthesis that occurs at a much higher rate in irradiated cells than in unirradiated growing cultures (ii) the reassembled genome appears to be composed of a patchwork of contiguous blocks of old and newly synthesized DNA (Zahradka *et al*., 2006). According to the ESDSA model, the single-stranded tail of a recessed fragment invades a partially overlapping fragment and primes DNA synthesis through a moving D-loop. Dissociation of the newly synthesized DNA from the template produces long stretches of single-strand DNA that anneal to complementary sequences thereby facilitating the precise reconstruction of long double-strand DNA intermediates. These intermediates subsequently recombine to reform a circular chromosome. Two enzymes play a key role in the ESDSA model, PolA, which participates in the initial DNA synthesis-step, and RecA, that ensures the maturation of the linear intermediates into full-size circular chromosomes through a classical recombination process. Accordingly, *polA* and *rec*A mutants are highly radiation-sensitive (Zahradka *et al*., 2006). However, many aspects of the ESDSA mechanism have not been clarified, such as the priming step in DNA strand elongation. Since this step does not require RecA, it must be carried out by other, not yet identified, proteins with a RecA-like activity. The RadA protein might be a candidate to promote the strand invasion of a fragment by the recessed end of a partially overlapping DNA fragment or annealing of the newly generated single-strand complementary sequences. RadA is highly conserved in bacteria and shares sequence similarity with both RecA strand transferase and Lon protease. A role was proposed for RadA in the stabilization or processing of branched DNA molecules (Beam *et al*., 2002). Disruption of the *D. radiodurans radA* (*DR1105*) gene resulted in a modest sensitization to γ and UV radiations (Zhou *et al*., 2006). This suggests that other SSA proteins could be involved in ESDSA steps. In eukaryotic cells, Rad52 protein possesses a SSA activity (Mortensen *et al*., 1996; Sugiyama *et al*., 1998). Since *D. radiodurans* DdrA is distantly but specifically related to the Rad52 family of eukaryotic proteins, as well as a family of phage-associated proteins that mediate SSA (Iyer *et al*., 2002), it was proposed that DdrA could be a component of a SSA system (Harris *et al*., 2004). It was reported that *E. coli* RecO protein anneals single-strand DNA complexed with SSB (Kantake *et al*., 2002). Therefore the deinococcal RecO could also be involved in SSA.

Nonhomologous End-joining (NHEJ)

In contrast to HR or SSA, nonhomologous end-joining does not require another copy of the DNA strand (Hefferin and Tomkinson, 2005). It is the major pathway of DNA double-strand break repair in eukaryotes and has only recently been identified and characterized in bacteria (Shuman and Glickman, 2007; Weller *et al*., 2002). In eukaryotes, DNA ends are first recognized and bound by the Ku heterodimer. Next, proteins involved in end-processing are recruited such as the Mre11 complex, the nuclease Artemis, the Pols μ and λ and the polynucleotide kinase/3′ phosphatase (PNKP). Finally, the DNA ligase IV/XRCC4 complex joins the two DNA ends (Hefferin and Tomkinson, 2005). Very recently CtIP (also called RB binding protein 8) was found to be important for promoting DNA end resection (Sartori *et al*., 2007). Nonhomologous end-joining also seems to provide an important function in at least some prokaryotes (Weller *et al*., 2002; Bowater and Doherty, 2006; Pitcher *et al*., 2007a, 2007b). It was proposed that nonhomologous end-joining could also play an important role in *D. radiodurans* double-strand break repair (Kobayashi *et al*., 2004; Narumi *et al*., 2004; Lecointe *et al*., 2004b). Although, nonhomologous end-joining has never been established experimentally in *D. radiodurans* bacteria, it could take place in *D. radiodurans* because of the presence of proteins consistent with this process. In particular, it was proposed that PprA from *D. radiodurans* (Murakami *et al*., 2006; Narumi *et al*., 2004) or Gam proteins present in some bacteria that contain Mu-like prophages (d'Adda di Fagagna *et al*., 2003) might possess Ku-like functions. Although the genome of *D. radiodurans* contains a Mu-like prophage, no Gam-homolog has been identified (Morgan *et al*., 2002), making the latter possibility unlikely for *D. radiodurans*. In contrast, PprA, whose expression is highly induced

by ionizing radiation or desiccation (Tanaka *et al*., 2004) has been shown to preferentially bind to double-strand DNA carrying strand breaks, to inhibit *E. coli* exonuclease III activity, and to stimulate the DNA end-joining reaction catalyzed by ATP-dependent and NAD-dependent DNA ligases (Murakami *et al*., 2006; Narumi *et al*., 2004). Moreover, cells devoid of PprA are highly radiosensitive (Narumi *et al*., 2004; Tanaka *et al*., 2004).

Various bacteria encode more than one DNA ligase (Wilkinson *et al*., 2001). In addition to the standard bacterial NAD^+-dependent DNA ligase (DR2069), an ATP-dependent DNA ligase can be found, often in an operon with a Ku homolog. Several bacterial ATP-dependent DNA ligases have been implicated in nonhomologous end-joining. *D. radiodurans* encodes a standard NAD^+-dependent DNA ligase, which preferentially uses Mn(II) as a cofactor (Blasius *et al*., 2007). A small predicted ATP-dependent DNA ligase (DRB0100) has also been identified in *D. radiodurans* (Liu *et al*., 2003), but so far no ligation activity could be shown *in vitro* (Blasius *et al*., 2007). The putative ATP-dependent DNA ligase from *D. radiodurans* has most similarity with bacterial LigC, but is lacking the OB-fold present in LigC (Shuman and Glickman, 2007). It might be that this putative DNA ligase requires another subunit, such as XRCC4 in eukaryotes, but a bacterial counterpart of XRCC4 has not been identified so far. However, a significant contribution of this putative ATP-dependent DNA ligase to radioresistance seems unlikely, because cells devoid of DRB0100 are as radioresistant as wild-type R1 (S. Sommer, unpublished results; Makarova *et al*., 2007).

In addition, the presence of a PolX domain in some bacteria has been implicated in DNA double-strand break repair (Pitcher *et al*., 2005). A $PolX_{Dr}$ (DR0467) protein containing a polymerase beta-like PolX domain in combination with a PHP (polymerase and histidinol phosphatase) domain has been identified in the genome of *D. radiodurans*. The presence of a PolX domain is rather uncommon in bacteria and archaea, but homologs have been identified in *Bacillus subtilis* and *Methanothermobacter thermoautotrophicum*. Deletion of the *$PolX_{Dr}$* gene leads to an increased radiosensitivity, ranging from three-fold at 13.6 kGy to more than 300-fold at 20.4 kGy (Lecointe *et al*., 2004b) and surprisingly the enzyme shows a structure-modulated nuclease activity (Blasius *et al*., 2006). Deletions of both SbcCD and the structure-modulated nuclease PolX have an additive effect, suggesting complementary roles in processing damaged DNA ends (Bentchikou *et al*., 2007). However, the enhanced sensitivity of the double mutant cells deleted of *sbcCD* and *$polX_{Dr}$* is observed only at elevated γ-irradiation doses where the survival of the wild-type cells also began to decline. This suggests that these proteins might be part of a back-up repair system acting to rescue cells containing excessively numerous DNA lesions or lesions that are particularly difficult to repair.

FIDELITY OF DNA REPAIR

The *D. radiodurans* genome contains repeated sequences, including families of transposases (Makarova *et al*., 2001). Since extensive intra- and inter-chromosomal recombination between these sequences can be potentially highly deleterious, *D. radiodurans* must possess regulatory mechanisms that negatively control these recombination events, in particular, during the reconstruction of its genome after DNA damage. One such mechanism might concern a particular organization of its genome limiting ectopic recombination events (Levin-Zaidman *et al*., 2003; Minton and Daly, 1995).

Another, and nonmutually exclusive, mechanism may occur through the operation of an efficient mismatch repair (MMR) system (Spell and Jinks-Robertson, 2003; Worth *et al*., 1994).

MMR proteins are able to recognize mismatches in recombination intermediates and thus to inhibit recombination between moderately divergent (homologous) sequences. *D. radiodurans* possesses homologs of *mutL* (*DR1696*), *mutS* (*DR1039, DR1976*), and *uvrD* (*DR1775*) and a functional MMR system participates in the fidelity of DNA replication and recombination (Mennecier *et al*., 2004). Indeed, inactivation of *mutS1* (*DR1039*) or *mutL* genes results in a seven-fold increase in the frequency of spontaneous Rif^R mutagenesis and in a 10-fold increase in the efficiency of integration by recombination of a donor point mutation marker. In contrast, inactivation of MutS2 (DR1976) protein, encoded by the second *mutS*-related gene present in *D. radiodurans*, has no effect on mutagenesis or recombination (Mennecier *et al*., 2004). Cells devoid of MutS1 or MutL proteins are as resistant to γ-rays, mitomycin-C or UV-irradiation as wild-type bacteria, suggesting that the MMR system plays no role in the reconstitution of a functional genome after DNA damage.

Insertion sequences (ISs) are abundant in the radioresistant bacterium *D. radiodurans* (Makarova *et al*., 1999). By developing a forward mutagenesis assay to detect any inactivation events in *D. radiodurans*, it was found that IS-transposition plays a major role in spontaneous mutagenesis (Mennecier *et al*., 2006). Indeed, IS insertion into the *thyA* coding region represent 75% of the mutations to trimethoprim-resistance (Tmp^R) in cells proficient for MMR. Analysis of their distribution among the spontaneous Tmp^R mutants indicated that five different ISs were transpositionally active. A type II miniature inverted-repeat transposable element (MITE), related to one of the deinococcal ISs, was also discovered as an insertion into *thyA*. Seven additional genomic copies of this MITE element were identified by BLASTN. Transposition of one of these IS elements, IS*Dra2*, was markedly enhanced by γ-rays or UV-irradiation. These data indicate that DNA transposition may constitute a major mutagenic event induced by DNA lesions (Mennecier *et al*., 2006). A transiently enhanced transposition activity during the recovery process in heavily DNA-damaged cells might endow them with the ability to rapidly respond and adapt to changing environmental conditions.

Point mutations to rifampicin resistance are also induced by γ-irradiation (Mennecier *et al*., 2006). *D. radiodurans* lacks homologs of SOS translesion polymerases (Makarova *et al*., 2001). The enhancement of point mutagenesis in the irradiated cells

could be related to a transient inhibition of the MMR system in cells recovering from DNA damage. The MMR system, and in particular MutS (the mismatch recognition and binding protein) has been shown to be down-regulated under some stress conditions such as starvation (Bregeon *et al*., 1999), resulting in enhanced stationary phase mutagenesis (for review, see Matic *et al*., 2004). Alternatively, induced point mutations might result from some inaccuracy in repair synthesis carried out by DNA polymerases such as PolA or PolX (Gutman *et al*., 1993, 1994b) during the reconstitution of a functional genome after γ-irradiation. Notably, it has been shown that *D. radiodurans* PolA can bypass some forms of radiation-induced DNA damage and that this bypass is facilitated by Mn(II) (Heinz and Marx, 2007).

NUCLEOID STRUCTURE

Bacterial chromosomes usually form condensed structures—the so-called nucleoid. In *D. radiodurans*, donut-like DNA structures were detected by transmission electron microscopy. (Englander *et al*., 2004; Levin-Zaidman *et al*., 2003; Minsky, 2003). *D. radiodurans* cells adopt a condensed ring-like nucleoid structure that remains unaltered after high-dose γ-irradiation, suggesting that this structure may passively contribute to radioresistance by preventing the dispersion of free DNA ends (Levin-Zaidman *et al*., 2003). Such a condensed genome may provide suitable scaffolds for DNA repair through single-strand annealing, recombinational and/or DNA end joining processes. A recent examination of nucleoids in members of the radioresistant genera *Deinococcus* and *Rubrobacter* revealed a high degree of genome condensation, whereas the nucleoid is uniformly distributed in *E. coli* cells (Zimmerman and Battista, 2005). The nucleoid structure of the radioresistant species *Deinococcus radiopugnans, Deinococcus geothermalis*, and *Rubrobacter radiotolerans* do not adopt a fixed shape (Zimmerman and Battista, 2005), suggesting that strong nucleoid condensation, rather than the shape of the nucleoid, may be the common trait among radioresistant organisms. Recently, Eltsov and Dubochet (2005, 2006) performed cryoelectron microscopy using vitreous sections of *D. radiodurans* and were unable to detect any particular order of the DNA, suggesting that DNA ends would still remain mobile, even though the DNA can condense in liquid crystalline phases.

Nevertheless, restricted diffusion would be a reasonable explanation for efficient repair of double-strand breaks. Prealignment of the copies of the chromosomes has never been shown, but was proposed to favor efficient and error-free double-strand break repair (Minton, 1994).

More work is required to characterize the factors involved in the organization of the *D. radiodurans* nucleoid and to investigate the role of the condensed structure of the nucleoid in *Deinococcus* radioresistance. DNA compaction could be achieved via specialized proteins. *D. radiodurans* encodes various proteins that bind and protect DNA, stabilise repair intermediates or help maintaining the structure of the chromosome.

One such protein is Dps-1 (DNA protection during starvation, DR2263). Identified initially in *E. coli*, Dps was shown to protect DNA by its ability to chelate ferrous iron, and by its direct association with DNA (Almiron *et al*., 1992; Ilari *et al*., 2002; Zhao *et al*., 2002). Moreover, Dps has an important function in compaction of the *E. coli* nucleoid (Kim *et al*., 2004). The structure of *D. radiodurans* Dps-1 has been solved (Kim *et al*., 2006; Romao *et al*., 2006) and the protein possesses a unique N-terminal extension, which is important for DNA-binding and the formation of protein dodecamers. It has been suggested that interaction of two N-termini in successive DNA major grooves would lead to stacked protein-DNA layers and compaction of the DNA (Bhattacharyya and Grove, 2007). Another Dps protein (Dps2, DRB0092) is encoded by *D. radiodurans* and the gene's expression is induced upon γ-irradiation (Tanaka *et al*., 2004). The crystal structure of Dps2 has recently been solved (Cuypers *et al*., 2007). Like other Dps family members, Dps2 from *D. radiodurans* assembles as a spherical dodecamer. Deletion of Dps-1 and Dps2 however, does not lead to an increased radiosensitivity of *D. radiodurans* cells (S. Sommer, unpublished observations). Nevertheless, the Dps-1 protein possesses five iron-binding sites and a nonspecific metallic binding site, which might have a role in regulating the iron uptake and release from the protein. It was shown that dimeric Dps-1 protects DNA from both hydroxyl radical cleavage and from DNase I-mediated cleavage (Grove and Wilkinson, 2005) and it was recently shown that a *dps2* mutant strain is sensitive to H_2O_2, suggesting a role of Dps2 in protection from hydroxyl radicals (Yan *et al*., 2007).

The very abundant histone-like protein HU (for *h*eat *u*nstable) from *E. coli* is associated with the bacterial nucleoid and was shown to be involved in cell survival after γ-irradiation (Boubrik and Rouviere-Yaniv, 1995). HU can strongly bend DNA and is known to play an architectural role for the nucleoid, although it is still under debate whether HU binding leads to compaction or decondensation of the chromosome (Dame and Goosen, 2002). It has also been suggested that *E. coli* HU regulates gene expression, presumably by producing an open nucleoid complex (Dorman and Deighan, 2003). More recently, a role for HU in DNA repair has emerged, as *E. coli* HU preferentially binds nicks, gaps and abasic sites and harbours AP lyase activity (Kow *et al*., 2007) and is involved in the repair of lesions that are in close proximity (Hashimoto *et al*., 2003).

The HU-homolog encoded by *D. radiodurans* (DRA0065) was expected to be involved in stabilizing DNA repair intermediates and DNA architecture in general, but the specific functions of *D. radiodurans* HU are not yet fully clear. *D. radiodurans* HU binds preferentially to DNA junctions. Unlike its *E. coli* counterpart, it does not prefer nicks or gaps to undamaged duplex DNA and it is unable to circularize linear double-stranded DNA molecules (Ghosh and Grove, 2004). In addition, the HU protein from *D. radiodurans* possesses a 47 amino acid extension at the N-terminus that modulates the binding to 4-way junctions, which represent a recombination intermediate. HU seems to be important for the stabilization of such junctions and has

therefore been proposed to play a role in DNA recombination rather than in maintenance of a general DNA structure (Ghosh and Grove, 2006). A role for *D. radiodurans* HU in transcriptional regulation remains possible, other histone-like proteins might be important as well.

D. radiodurans encodes also an SMC family protein (DR1471), which might be involved in DNA architecture. SMC proteins are conserved in all domains of life (Losada and Hirano, 2005) and disruption of the SMC gene in *B. subtilis* causes decondensation and missegregation of chromosomes (Britton *et al*., 1998), indicating similar or even identical functions of bacterial SMC proteins in comparison to the eukaryotic SMC complexes. Some evidence suggests that *B. subtilis* SMC has cohesin-like functions and promotes DNA repair (Dervyn *et al*., 2004; Lindow *et al*., 2002) and it would be interesting to look closer at the role of *D. radiodurans* SMC in DNA packing and DNA repair processes.

The *D. radiodurans* DNA gyrase, consisting of GyrA (DR1913) and GyrB (DR0906), has not fully been characterized, but preliminary data show DNA-dependent ATPase and DNA helicase activities for this enzyme (I. Shevelev, personal communication). Both subunits are strongly induced upon ionizing radiation (Liu *et al*., 2003; Tanaka *et al*., 2004). Conditional *gyrA* mutants show a conditional lethal phenotype and aberrant morphologies suggesting defects in DNA segregation (Lecointe *et al*., 2004a). DNA type IB topoisomerases, found in all known eukaryotes, are very rare in bacteria. *D. radiodurans* contains one operon encoding two eukaryotic-type enzymes, a uracil DNA glycosylase (DRB0689) and a DNA topoisomerase IB (DRB0690), suggesting an acquisition by horizontal transfer. The first DNA topoisomerase IB discovered in a prokaryote was found in *Methanopyrus kandleri* and was shown to possess associated apurinic/apyrimidic (AP) site-processing activities, suggesting a potential role in base excision DNA repair (Belova *et al*., 2002).

REGULATORY MECHANISMS

A novel regulatory protein IrrE (DR0167), also called PprI, has been shown to be a positive effector that maximally increases the expression of RecA protein and of PprA, a protein which interacts with DNA ends, protects them from DNA degradation and stimulates DNA ligase activity (Earl *et al*., 2002a; Hua *et al*., 2003; Narumi *et al*., 2004). The loss of the *irrE* gene product increases the cell's sensitivity to UV, ionizing radiation and mitomycin-C. Constitutive high expression of RecA does not compensate the absence of IrrE for the resistance to γ-irradiation (Jolivet *et al*., 2006). IrrE protein appears to play a crucial role in regulating multiple DNA repair and protection pathways to radiation exposure and to be part of a putative signal transduction pathway in response to DNA damage in *D. radiodurans* (Earl *et al*., 2002a; Hua *et al*., 2003).

Interestingly, *D. radiodurans* possesses two LexA homologs, LexA1 (DRA0344) and a more divergent protein called LexA2 (DRA0074). As in *E. coli*, LexA1 and LexA2 undergo RecA-stimulated self-cleavage after DNA damage in *D. radiodurans* (Narumi *et al*., 2001; Sheng *et al*., 2004). However, no regulon under the control of LexA1 or LexA2 has been identified to date in *D. radiodurans*. In particular, LexA1 and LexA2 proteins do not regulate RecA induction following γ-irradiation (Bonacossa de Almeida *et al*., 2002; Narumi *et al*., 2001). Neither the *lexA1* gene deletion nor a mutation leading to a non cleavable LexA1 derivative affected repair of double-strand breaks or cell survival suggesting that LexA1 do not play a major role in radioresistance (Jolivet *et al*., 2006). However, inactivation of LexA1 caused cells to aggregate, suggesting that LexA1 may control the activity or expression of as yet undefined membrane functions (Bonacossa de Almeida *et al*., 2002).

In contrast, disruption of LexA2 enhances cell survival at elevated γ-irradiation doses (8 to 15 kGy) (Satoh *et al*., 2006). This can be in part explained by an enhancement of PprA expression in cells devoid of LexA2 (Satoh *et al*., 2006).

There are also indications for a distinct *Deinococcus* response regulon, as a common DNA motif has been identified in a conserved set of genes associated with radioresistance and DdrO (DR2574) has been proposed to be the global regulator (Makarova *et al*., 2007). Many questions still remain: what is the phenotype of a DdrO mutant? By which mechanism does DdrO recognize radiation damage and how does it induce gene expression? What are the relations between DdrO and IrrE? Does such a regulon exist also in other radioresistant bacteria?

PUTATIVE CELL-CYCLE CHECKPOINTS

Irradiated *D. radiodurans* cultures show a dose-dependent growth lag. The isolation by Mattimore and colleagues (Mattimore *et al*., 1995) of SLR mutants (Slow recovery) presenting no defect in DSB repair but a delay in restarting cell growth after irradiation suggests that *D. radiodurans* possesses mechanisms for sensing the completion of DNA repair to allow reinitiation of replication and/or of cell division (Battista, 1997; Battista *et al*., 1999). Interestingly, cells expressing limiting concentration of RecA protein show a dose-dependent commitment to die during post-irradiation incubation (Jolivet *et al*., 2006). These cells, when exposed to 6800 Gy γ-irradiation, were able to reconstitute an intact genome but showed no increase in the cellular DNA content during further incubation, suggesting that they have a defect in replication reinitiation (Jolivet *et al*., 2006). *D. radiodurans* encodes a protein containing a SARP domain (DR0724), which in eukaryotes is involved in apoptosis-related signalling (Melkonyan *et al*., 1997). It could be interesting to investigate the involvement of this gene in the loss of viability of irradiated cells expressing a limited concentration of RecA protein.

The growth lag after exposure to γ-rays is also particularly long in mutant cells devoid of the ClpP (DR1972) or the ClpX (DR1973) subunits of the ClpPX protease (Servant *et al*., 2007). These cells can perform DNA repair and replication reinitiation but show a very abnormal morphology (enlarged cells, decondensed nucleoid) during post-irradiation recovery. These results

suggest that ClpPX is involved in a damage-responsive cell division checkpoint (Servant *et al*., 2007).

PROTECTION OF CELL MEMBRANE, PROTEINS AND DNA

Ionizing radiation leads to the formation of multiple ions and electrons. Then, reactions with other molecules in a cell produce free radicals, which in turn can harm and modify many molecules of a cell. In particular, reaction with water molecules forms highly reactive oxygen species (ROS). *D. radiodurans* is able to deal with high oxidative stress. To battle oxidative agents, living organisms have developed antioxidant systems as the first line of protection. In *D. radiodurans*, 3 predicted superoxide dismutases (DR1279, DR1546, and DRA0202) and 3 predicted catalases (DR1998, DRA0259, and DRA0146) (Omelchenko *et al*., 2005), which can protect biomolecules from ROS-mediated damage, are induced after exposure to ionizing radiation. These enzymes do not seem to be very different from their homologs in *E. coli*. Indeed, the *D. radiodurans* KatA (DR1998) is a typical monofunctional heme-containing catalase (Kobayashi *et al*., 2006) and the structure of the manganese superoxide dismutase (Mn-SOD, DR1279) was shown to be essentially identical to the Mn-SOD from *E. coli* (Dennis *et al*., 2006). In *D. radiodurans*, insertional inactivation of catalase and superoxide dismutase genes results in an increased sensitivity to ionizing radiation compared with the wild-type (Markillie *et al*., 1999). Interestingly, it was shown that, when expressed in *E. coli*, the *Deinococcal* PprA protein enhances oxidative stress tolerance and stimulates by 2.8-fold the KatE catalase activity (Kota and Misra, 2006), suggesting that PprA could also play an important role in protection against oxidative stress in *D. radiodurans*. It was also shown that other *Deinococcal* proteins such as IrrE (DR0167, also called PprI) expressed in *E. coli* increases KatG activity (Gao *et al*., 2003).

In addition to the uncommon lipid composition of the cell envelope, a variety of proteins and carotenoids play a role for its integrity and the resistance to oxidative stress (Carbonneau *et al*., 1989; Rothfuss *et al*., 2006). Recent data suggest an important role of carotenoids in stress resistance. Deinoxanthin, a major product of the carotenoid synthesis pathway shows an especially strong ability to scavenge H_2O_2 and singlet oxygen, thus exhibiting a protective effect on DNA (Tian *et al*., 2007). Mutation of the phytoene desaturase CrtI (DR0861) leads to cells without pigmentation that are sensitive to desiccation, oxidative stress and γ-irradiation (Xu *et al*., 2007). This finding was confirmed by Zhang and colleagues (2007), who showed that *crtB* (DR0682) or *crtI* (DR0861) deletion mutants are colourless and more sensitive to ionizing radiation, UV, and H_2O_2 than wild-type cells (Zhang *et al*., 2007). However, pigmentation of the cells is not a prerequisite for radioresistance in the *Deinococcaceae*. Indeed, *D. deserti* bacteria are unpigmented and as radioresistant as *D. radiodurans* (de Groot *et al*., 2005). Moreover, some *D. radiodurans* unpigmented mutants isolated on TGY plates after γ-irradiation are as radioresistant as the wild type (S. Sommer, unpublished results).

D. radiodurans DNA is associated with high levels of Mn(II) (Leibowitz *et al*., 1976) and the presence of Mn(II) has been shown to be important for radioresistance (Daly *et al*., 2004). Mn(II) does not directly prevent DNA double-strand breaks (Daly *et al*., 2004), but it can mimic the activities of catalase and superoxide dismutase (Seib *et al*., 2004). Recently, Daly and colleagues (2007) compared radiosensitive and radioresistant bacteria with respect to their cytosolic ion concentrations. They found that *D. radiodurans*, as well as other radioresistant bacteria, have high intracellular Mn/Fe concentration ratios, and they could show a link between Mn(II) ions and protein protection against oxidation (Daly *et al*., 2007). Thus, proteins involved in DNA repair could function with far greater efficiency during recovery in radioresistant bacteria than in radiosensitive bacteria. Interestingly, desiccation-resistant isolates that inhabit dry-climate soils also accumulate high intracellular manganese and low iron concentrations compared to sensitive bacteria (Fredrickson *et al*., 2008).

Mn(II) is globally distributed throughout the cytosol, but X-ray fluorescence microprobe analysis revealed an accumulation of Fe(II) at the septum between dividing cells and high Mn(II) concentrations at electron-dense granules in the centre of the nucleoids (Daly *et al*., 2007). The mechanism by which Mn(II) could protect proteins and the reason for cellular ion distribution remain unclear.

Even though *D. radiodurans* proteins are protected from oxidative damage by an elevated Mn/Fe concentration ratio (Daly *et al*., 2007), the removal of detrimentally damaged proteins may complement the DNA repair capabilities of *D. radiodurans* and contribute to its extreme radioresistance. *D. radiodurans* encodes an unusually high number of putative proteases (Makarova *et al*., 2001) and several of these enzymes are induced after γ-irradiation (Tanaka *et al*., 2004). Moreover, it was shown that in *D. radiodurans* an extensive degradation of proteins takes place during the lag period of post-irradiation recovery (Joshi *et al*., 2004). Recently, all ATP-dependent proteases belonging to the Clp or Lon families have been separately inactivated (Servant *et al*., 2007). Inactivation of Lon1 (DR1974) or Lon2 (DR0349) did not affect radioresistance of *D. radiodurans*, whereas it highly increased sensitivity to puromycin. It was previously shown that sensitivity to puromycin is related to a reduced degradation of abnormal proteins in different bacteria (Frees and Ingmer, 1999; Kock *et al*., 2004; Missiakas *et al*., 1996; Thomsen *et al*., 2002). In contrast to Δ*lon1* and Δ*lon2* mutants, cells devoid of ClpX (DR1973) or ClpP (DR1972) proteins have only a minor role in degradation of abnormal proteins but showed increased radiosensitivity. These results suggest that bulk proteolysis of damaged proteins seems to have only a minor impact on the survival of heavily irradiated Deinococcal cells. In addition, *D. radiodurans* may also efficiently repair damaged proteins. For instance, the multiple chaperones that are induced after irradiation (Tanaka *et al*., 2004) may restore activity

TABLE 2
Selected genes with likely involvement in *D. radiodurans* radiation resistance

Gene	Protein	Function	Remarks	Reference
		Important effect on radioresistance		
DR2340	$RecA_{Dr}$	homologous recombination	inverse DNA strand exchange pathway	(Kim *et al*., 2002; Kim and Cox, 2002)
DR1707	PolA	DNA polymerase, probably involved in translesion synthesis	important role in ESDSA DSB repair pathway, modulated by Mn(II)	(Zahradka *et al*., 2006; Heinz and Marx, 2007)
DR0819	RecO	DNA annealing during homologous recombination	strong defect in growth and increased radiosensitivity of strain devoid of RecO	(Xu *et al*., 2008)
DRA0346	PprA	DNA binding and tethering of termini, stimulation of DNA ligases and catalase	strongly induced upon ionizing radiation	(Kota and Misra, 2006; Murakami *et al*., 2006; Narumi *et al*., 2004; Ohba *et al*., 2005; Tanaka *et al*., 2004)
DR0423	DdrA	protection of 3′ ends of single-stranded DNA	strongly induced upon ionizing radiation, homolog of Rad52	(Harris *et al*., 2004; Tanaka *et al*., 2004)
DR0167	IrrE (PprI)	positive regulator of *recA* and other gamma irradiation-induced genes		(Earl *et al*., 2002a; Hua *et al*., 2003 ; Narumi *et al*., 2004)
DR0070	DdrB	unknown function	strongly induced upon ionizing radiation	(Tanaka *et al*., 2004)
DR1477	RecN	DNA repair protein	mutant shows general repair deficiency	(Funayama *et al*., 1999)
		More modest effect on radioresistance		
DR0467	$PolX_{Dr}$	DNA polymerase/ 3′-5′ exonuclease	nuclease is structure-modulated, uses Mn(II)	(Blasius *et al*., 2006; Lecointe *et al*., 2004)
DR1921	SbcD	homologue of Mre11, DNA end processing	additive effects of SbcCD and PolX deficiencies	(Bentchikou *et al*., 2007)
DR1922	SbcC	homologue of Rad50, DNA end processing	additive effects of SbcCD and PolX deficiencies	(Bentchikou *et al*., 2007)
DR0003	DdrC	unknown function	strongly induced upon ionizing radiation	(Tanaka *et al*., 2004)
DR0326	DdrD	unknown function	strongly induced upon ionizing radiation	(Tanaka *et al*., 2004)
DR0576	RuvB	resolution of Hollyday junctions		(Kitayama *et al*., 1997)
DR1105	RadA	RecA-like function		(Zhou *et al*., 2006)
DR1972	ClpP	proteolytic subunit of ATP-dependent Clp protease	not involved in degradation of abnormal proteins	(Servant *et al*., 2007)
DR1973	ClpX	ATP-binding subunit of ATP-dependent Clp protease	not involved in degradation of abnormal proteins	(Servant *et al*., 2007)
DR0682	CrtB	phytoene synthase		(Zhang *et al*., 2007)
DR0861	CrtI	phytoene desaturase		(Zhang *et al*., 2007)

of conformationally damaged proteins, and other inducible enzymes may directly reverse certain forms of protein alteration, such as repair of oxidized Met residues by the MsrA/B system (Grimaud *et al*., 2001; Tanaka *et al*., 2004).

CONCLUSIONS

Considering all factors that contribute to the radioresistant phenotype of *D. radiodurans*, it seems most likely that radioresistance is achieved by a combination of various proteins (Table 2) and mechanisms. The radioresistant bacteria are dispersed in a phylogenetic tree suggesting that they have acquired radioresistance independently (Cox and Battista, 2005). It seems rather unlikely that one repair or protection system exists in all radioresistant organisms, but rather that different strategies have evolved in parallel. Taken together, a single key to radioresistance, be it a special DNA repair system, a high Mn/Fe ratio or a condensed nucleoid, does not seem to exist. Further work has to be done to understand the complete survival kit components that lead to the full intricacy of *D. radiodurans* radioresistance.

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