Bacterial and archaeal resistance to ionizing radiation

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Bacterial and archaeal resistance to ionizing radiation

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Abstract. Organisms living in extreme environments must cope with large fluctuations of temperature, high levels of radiation and/or desiccation, conditions that can induce DNA damage ranging from base modifications to DNA double-strand breaks. The bacterium Deinococcus radiodurans is known for its resistance to extremely high doses of ionizing radiation and for its ability to reconstruct a functional genome from hundreds of radiation-induced chromosomal fragments. Recently, extreme ionizing radiation resistance was also generated by directed evolution of an apparently radiation-sensitive bacterial species, Escherichia coli. Radioresistant organisms are not only found among the Eubacteria but also among the Archaea that represent the third kingdom of life. They present a set of particular features that differentiate them from the Eubacteria and eukaryotes. Moreover, Archaea are often isolated from extreme environments where they live under severe conditions of temperature, pressure, pH, salts or toxic compounds that are lethal for the large majority of living organisms. Thus, Archaea offer the opportunity to understand how cells are able to cope with such harsh conditions. Among them, the halophilic archaean Halobacterium sp and several Pyrococcus or Thermococcus species, such as Thermococcus gammatolerans, were also shown to display high level of radiation resistance. The dispersion, in the phylogenetic tree, of radioresistant prokaryotes suggests that they have independently acquired radioresistance. Different strategies were selected during evolution including several mechanisms of radiation byproduct detoxification and subtle cellular metabolism modifications to help cells recover from radiation-induced injuries, protection of proteins against oxidation, an efficient DNA repair tool box, an original pathway of DNA double-strand break repair, a condensed nucleoid that may prevent the dispersion of the DNA fragments and specific radiation-induced proteins involved in radioresistance. Here, we compare mechanisms and discuss hypotheses suggested to contribute to radioresistance in several Archaea and Eubacteria.

1. Introduction
Extreme environments are a permanent challenge for living organisms. However, a large diversity of microorganisms belonging to the three domains of life (bacteria, archaea, eukaryotes) were isolated from harsh environments such as hydrothermal vents, surface sands of hot arid deserts exposed to intense ultraviolet (UV) radiation, cycles of extreme temperatures, and desiccation [1]. Artificial environments, such as radiation and toxic chemical waste dumps, also provide intense selection pressure for extremophiles.
Among these highly stress resistant organisms, bacteria belonging to the *Deinococccaeae* family are known for their ability to survive exposure to ionizing radiation and *Deinococcus radiodurans* is, by far, the best characterized. It was first isolated in canned meat exposed to 4,000 Gy γ-irradiation in order to achieve sterility [2]. This bacteria is characterized by an exceptional ability to withstand the lethal effects of DNA-damaging agents, including ionizing radiation, ultraviolet light and desiccation [3]. *D. radiodurans* can survive extremely high doses of radiation such as 5,000 Gy γ-irradiation generating approximately 200 DNA double-strand breaks, 3000 DNA single-strand breaks and more than 1000 damaged bases per genome, without loss of viability. Survival is also not affected by a dose of 500 J / m² UV-light that generates up to 5,000 pyrimidine dimers in DNA, and *D. radiodurans* shows 85% viability after 2 years in the presence of less than 5% humidity. *D. radiodurans* is also able to deal with high oxidative stress. However, resistance to ionizing radiation is not a common trait of all *Deinococcus* species. Indeed, some *Deinococcales* species, such as *Deinococcus radiomollis*, *Deinococcus claudionis*, *Deinococcus altitudinis* and *Deinococcus alpinitundrae*, isolated recently from alpine environments, were shown to be sensitive to ionizing radiation [4]. Moreover, a survey of bacteria identified eleven phyla that contain species with unusually high resistance to the lethal effects of ionizing radiation. These phyla are not closely related to each other and do not share a recent common lineage [5].

Radioresistance is also widespread among hyperthermophilic archaea. Among them, *Thermococcus gammatolerans* is almost as radioresistant as *D. radiodurans* (figure 1). This anaerobic organism was isolated after a γ-ray exposure at a dose of 30 kGy, of an enriched culture of microorganisms collected in a North Pacific hydrothermal vent [6]. When cells grow in a rich medium, they can withstand a dose of 5000 Gy without lethality [7] (figure 1). This property is not surprising since at high temperature, which corresponds to the natural environment of this archaeon, DNA accumulates many types of damage (e.g., hydrolytic depurination, deamination of cytosine and adenine, and single- or double-strand breaks) that have to be very rapidly repaired to avoid complete denaturation of DNA. However, a 5,000 Gy dose of ionizing radiation drastically decreases the viability of *Pyrococcus abyssi*, a hyperthermophilic archaean phylogenetically close to *T. gammatolerans* with optimal growth temperatures approaching the boiling point of water (figure 1). Consequently, the radioresistance of hyperthermophilic organisms cannot be explained only through their adaptation to high temperatures. Despite the differences observed in the *Thermococcales* order, *Pyrococcales* species, compared to most living species, can be considered as radioresistant, showing 100 % viability at a dose of 2,000-2,500 Gy.

![Figure 1](image.png)

**Figure 1.** Survival curves for *Pyrococcus furiosus*, *T. gammatolerans*, *D. radiodurans*, *Halobacterium* and *E. coli*, following exposure to γ irradiation.
In contrast to *Thermococcales*, the halophilic archaean *Halobacterium* sp. NRC-1 is aerobic, mesophilic and obligatory halophilic, requiring a salt concentration 10 times higher than those found in sea-water (250 g/L versus 20 g/L) for growth. A high intracellular KCl concentration together with a membrane pigmented by bacterioruberin provide protection from DNA damaging agents [8]. It has a capacity to cope with desiccation, UV radiation and ionizing radiation, its cell survival being 80% after irradiation at a dose of 2500 Gy and 10% at 5000 Gy [9].

More recently, Harris *et al* [10] have isolated 5 independent derivatives of the model *Escherichia coli* bacteria almost as radioresistant as *D. radiodurans* by exposure of the radiosensitive bacteria to multiple cycles of increasing radiation doses and selection of the survivors after each cycle of irradiation. The existence of so many unrelated radioresistant species suggests that the molecular mechanisms that protect against ionizing radiation-induced damage evolved independently in these organisms and that they combine a variety of physiological tools that are tightly coordinated to achieve radioresistance. In this review, we will discuss and compare different features that have been proposed to contribute to radioresistance in these Bacteria and Archaea.

### 2. Effects of ionizing radiation on living cells

Ionizing radiation, produced by the decay of radioactive elements, leads to the formation of multiple ions and electrons. γ-rays are photons modifying many molecules in a cell and producing highly reactive hydroxyl radicals (ROS), in particular OH\(^*\) by ionization of water molecules. These free radicals cause, directly or indirectly, a variety of DNA damage products, such as base modifications, DNA single-strand and double-strand breaks. It is generally admitted that 80% of the DNA damage result indirectly by the action of ROS and only 20% from direct interaction between γ photons and DNA [11]. Bacteria or Archaea highly resistant to ionizing radiation, are not protected against DNA damage [12] and the number of DNA double strand breaks per cell at a given dose is proportional to the size of the genome. However, we did not notice a correlation between resistance to radiation and the size of the genome, the number of chromosomes or the number of genes encoded by these organisms (table 1 and figure 1).

#### Table 1. Genomic information in different microorganisms [13-17] tested for their sensitivity to ionizing radiation (see figure 1)

<table>
<thead>
<tr>
<th>Species</th>
<th><em>Escherichia coli</em></th>
<th><em>Pyrococcus furiosus</em></th>
<th><em>Halobacterium NRC-1</em></th>
<th><em>Thermococcus gammatolerans</em></th>
<th><em>Deinococcus radiodurans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Domain</td>
<td>Bacteria</td>
<td>Archaea</td>
<td>Archaea</td>
<td>Archaea</td>
<td>Bacteria</td>
</tr>
<tr>
<td>Genome size (Mebabases)</td>
<td>4.64</td>
<td>1.91</td>
<td>2.57</td>
<td>2.04</td>
<td>3.28</td>
</tr>
<tr>
<td>Chromosomes and plasmids</td>
<td>1 chromosome</td>
<td>1 chromosome</td>
<td>1 chromosome</td>
<td>1 chromosome</td>
<td>2 chromosomes, 1 megaplasmid and 1 plasmid</td>
</tr>
<tr>
<td>Number of open reading frames</td>
<td>4289</td>
<td>2065</td>
<td>2630</td>
<td>2157</td>
<td>3187</td>
</tr>
<tr>
<td>% GC</td>
<td>50,8</td>
<td>40,7</td>
<td>65,9</td>
<td>54,0</td>
<td>66,6</td>
</tr>
</tbody>
</table>
In addition to causing DNA injuries, elevated doses of ionizing radiation also provoke damage to many other cell components.

3. Protection against oxidative stress

The first line of protection against oxidative stress is the presence of antioxidant enzymes such as superoxide dismutases and catalases that protect biomolecules from ROS-mediated damage (figure 2A). Superoxide dismutase catalyzes the conversion of oxygen superoxide to hydrogen peroxide which is subsequently transformed into \( \text{H}_2\text{O} \) by either catalases or peroxidases (figure 2A). The latter enzymes are coupled to redox pathways such as the ascorbate/glutathione cycles found in plants [18]. 

*D. radiodurans*, encodes 3 predicted superoxide dismutases and 3 predicted catalases [19] induced after exposure to ionizing radiation.

In Archaea, superoxide dismutase is mostly encoded by aerobic organisms (like *Halobacterium*) and few anaerobic organisms such as *Methanosarcina barkeri* [20]. In other anaerobes, this enzyme is replaced by a superoxide reductase (SOR) (figure 2B) [21-23]. Superoxide reductase oxidized during the processing of ROS needs to be reduced by rubredoxin which is in turn re-oxidized by NAD(P)H rubredoxin oxidoreductase (NROR). Finally hydrogen peroxide is most likely converted into H\(_2\)O by the action of the rubrerythrin and possibly by as-yet uncharacterized peroxidases. In addition, some anaerobic organisms can use another pathway to eliminate superoxides without production of hydrogen peroxide (figure 2C) [24]. A crystallographic study revealed that superoxide reductase forms a complex with ferrocyanides [25]. This complex reacts very efficiently with oxygen radicals, the end product of the reaction being not hydrogen peroxide, but oxidized formate and H\(_2\)O (figure 2C).

**Figure 2.** Proposed pathways for detoxification of oxygen superoxide in : (A) aerobic and (B and C) anaerobic organisms. Cyt bd: cytochrome bd ubiquinol oxidase. Prx: peroxiredoxin, Rd: rubredoxin, SOD: superoxide dismutase, SOR: superoxide reductase, NROR: NAD(P)H rubredoxin oxidoreductase.
A second line of protection against oxidative stress is the presence of an enhanced [Mn]/[Fe] ratio that confers an increased capacity to prevent the formation of iron-dependent ROS through the Fenton reaction (\( \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow 2\text{OH}^{-} + \text{Fe}^{3+} \)) [26]. Intracellular Mn can also be protective by scavenging ROS [27]. The most radioresistant species contain an intracellular concentration of Mn about 300 times higher and an intracellular concentration of Fe about 3 times lower than the most-sensitive species [28]. When \( \text{D. radiodurans} \) cells were grown in defined medium without Mn supplementation, a 1000-fold reduction in survival was observed at 10,000 Gy \( \gamma \)-irradiation. Mn(II) does not directly prevent DNA double-strand breaks [29], but it can mimic the activities of catalase and superoxide dismutase [30].

An inverse correlation between the [Mn]/[Fe] concentrations ratios and the levels of protein oxidation was shown, with no protein oxidation being detected in cells with the highest [Mn]/[Fe] ratios [28]. It was also reported that desiccation resistant dry-climate soil bacteria accumulate high intracellular manganese and low iron concentrations compared to desiccation sensitive bacteria. Moreover, their proteins are protected from oxidation during drying [31].

It is interesting to note that a high intracellular [Mn]/[Fe] ratio was also found in the radioresistant archaeon \( \text{Halobacterium NRC-1} \) [32]. \( \text{P. furiosus} \) increased the expression of a ferritin/Dps-like gene after irradiation, and the protein by chelating iron, could limit the production of hydroxyl radicals by the Fenton reaction [33]. Therefore, the decrease of iron levels in a cell could be a common strategy between some radioresistant bacteria and archaea to counteract protein oxidation. However, this feature cannot be generalized since the [Mn]/[Fe] ratio in several \( \text{E. coli} \) radioresistant mutants is the same than those in wild type cells [10].

Other ions also play a protective role against oxidative stress. A high concentration of NaBr (1.7 M) enhances cell survival of \( \text{Halobacterium} \) NRC-1 to ionizing radiation [32]. This improved ability to respond to ionizing radiation is due to bromine ions, which traps hydroxyl radicals, thereby significantly reducing the oxidation of nucleotides and proteins when cells are irradiated.

However, whereas the levels of protein oxidation are not the same in the radioresistant \( \text{D. radiodurans} \) bacteria and the radiosensitive \( \text{Escherichia coli} \) bacteria, the number of DNA double strand breaks generated in their DNA is the same even if 80% of the DNA damage result indirectly by the action of ROS [12]. This led Daly and collaborators to postulate that protection of proteins could be a major feature of radioresistance, as cells require functional proteins present immediately after irradiation for DNA repair [28]. Interestingly, efficient protection against protein carbonylation produced by cellular metabolism, or induced by radiation, was also observed in \( \text{E. coli} \) strains generated by directed evolution of \( \text{E. coli} \) K-12 and specifically adapted to survive exposure to high doses of ionizing radiation [34].

It was also recently proposed that proteins with abundant hydrophilic low complexity (LC) regions participate to the recovery processes of \( \text{D. radiodurans} \) to DNA damage and to its resistance to desiccation [35].

4. Efficient DNA double-strand break repair

4.1. Kinetics of DNA double-strand break repair

DNA double-strand breaks are considered the most dangerous and difficult to repair DNA lesions. However, \( \text{D. radiodurans} \) bacteria and \( \text{T. gammatolerans} \) archaea can sustain \( \gamma \)-irradiation doses that introduce hundreds of DNA double-strand breaks in their genome. The kinetics of \( \text{D. radiodurans} \) DNA double-strand break repair after exposure to 6,800 Gy \( \gamma \)-irradiation is very rapid as an intact genome complement is reconstructed from a myriad of fragments in two to three hours when cells where incubated post-irradiation in a rich-nutrient medium (figure 3).
Figure 3. Kinetics of genome reconstitution in *D. radiodurans* and *T. gammatolerans* cells exposed to γ-ray. *D. radiodurans* and *T. gammatolerans* exponentially growing cells were exposed to 6,800 Gy or 5000 Gy γ-irradiation, respectively, and then incubated in a rich culture medium at 30°C for *Deinococcus* or at 85°C for *Thermococcus* with agitation for recovery. To avoid double-strand breaks generated by mechanical manipulation of the DNA and not present *in vivo*, cells were immobilized and lysed in agarose plugs at different post-irradiation times. The plugs were then incubated with proteinase K, an enzyme that destroys proteins, and RNase to eliminate the RNA. Finally, the purified DNA was digested using a rare cutting restriction enzyme (Not1 or SwaI) before being analyzed by pulsed field gel electrophoresis. Detailed protocols are available in [7,36].

### 4.2. Mechanisms involved in DNA double-strand break repair

Several mechanisms have been proposed to account for such an efficient DNA double-strand break repair (figure 4). To repair DNA double strand breaks by homologous recombination (HR), single strand annealing (SSA), or extended synthesis dependent strand annealing (ESDSA), cells need to contain another intact copy of the damaged DNA region. In contrast, non-homologous end joining (NHEJ) does not require a second homologous copy of DNA to join two contiguous fragments [37].

#### 4.2.1. Homologous recombination (HR)

HR is the main pathway involved in DNA double-strand break repair in bacteria [39] and in the yeast *Saccharomyces cerevisiae* [40]. HR uses an intact homologous DNA molecule to restore the correct DNA sequence at damaged sites. The first step of DNA double-strand break repair by homologous recombination is the processing of the DNA ends to generate single stranded 3’ overhangs on which the recombinase RecA in bacteria, RadA in Archaea [41] and Rad51 in eukaryotic cells are recruited to form a nucleoprotein filament. The next steps of HR are invasion of a homologous overlapping fragment, exchange of DNA strands, extension of the nascent DNA heteroduplex and resolution of the resulting crossover structure.
4.2.2. Single-Strand Annealing (SSA). In addition to homologous recombination, SSA has been proposed to occur 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 [42]. The process of SSA can occur if two DNA double-strand breaks are located in the same region of two copies of the same chromosome. After exonuclease-catalysed resection of DNA ends, single-stranded overhangs are produced. If the overhangs contain complementary sequences, they can anneal. Then, single-stranded regions present in the reconstituted chromosome are filled in by DNA synthesis (figure 4).

4.2.3. Extended Synthesis-Dependent Strand Annealing (ESDSA). Recently, Zharadka et al (2006) [43] proposed a variant of the SSA model called ESDSA to account for their findings that (i) DNA fragments assemble coincides with a massive DNA synthesis which occurs at a much higher rate in irradiated cells than in unirradiated growing cultures (ii) high amount of single-stranded DNA are transiently present in the damaged cells (iii) the reassembled genome appears to be composed of a patchwork of contiguous blocks of old (in black on figure 4) and newly synthesized DNA (in green on figure 4) [43]. According to the ESDSA model, the single-stranded tail of a recessed fragment invades a partially overlapping fragment and primes DNA synthesis. However, contrary to what was observed during classical DNA replication of double-stranded DNA, newly synthesized single-stranded DNA tails are generated by displacement of the D-loop in a manner similar to that observed during transcription, when newly-synthesized RNA is progressively liberated from the transcription machinery during the progression of RNA polymerase on the DNA template. The strand extension can proceed to the end of the DNA template. The newly synthesized single-stranded DNA tails generated by this process (in green in figure 4) can anneal if they contain complementary sequences thereby facilitating the precise reconstruction of long double-stranded DNA intermediates. These long intermediates subsequently recombine to reform a circular chromosome. Many enzymes play a key role in this process.
role in the ESDSA pathway, DNA polymerases Pol I and Pol III are required for the massive DNA synthesis step [44] whereas RecA is required for homologous strand invasion to prime DNA synthesis and for the maturation of the linear intermediates into full-size circular chromosomes through a classical recombination process. It was shown that ESDSA plays a major role in *D. radiodurans* radioresistance. A massive DNA synthesis beginning 2 h after γ-irradiation at a dose of 2,500 Gy was observed in *P. abyssi* [45] suggesting that ESDSA may also take place in Archaea. However, the presence of an efficient ESDSA mechanism in Archaea has not yet been investigated.

4.2.4. Non-homologous end-joining (NHEJ). It is the major pathway of DNA double-strand break repair in eukaryotes and it has only recently been identified and characterized in bacteria [46-48]. It was proposed that NHEJ could also take place in *D. radiodurans* because of the presence of proteins consistent with this process [49,50]. In particular, PprA, a protein specific from *Deinococcus* and highly induced by ionizing radiation or desiccation [51] has been shown to preferentially bind to double-stranded DNA carrying strand breaks, to inhibit degradation of DNA by exonuclease, and to stimulate the DNA end-joining reaction catalyzed by DNA ligases [50,52]. Moreover, cells devoid of PprA are highly radiosensitive [50,51]. However, evidence for an active NHEJ process involved in DNA double-strand break repair in *D. radiodurans* remains to be discovered.

4.3. Processing of DNA ends

In Bacteria and Archaea, the three main pathways involved in DNA double-strand break repair require the formation of single-stranded DNA tails. This processing can be achieved through the activities of multiple helicases and nucleases (for review, see [53]). The *E. coli* RecBCD complex and AddAB, its *B. subtilis* functional analog, with their helicase and ATP-dependent nuclelease activities play the major role in resection of DNA ends and in the loading of the RecA recombinase on SSB covered single-stranded DNA tails (figure 5A). However, if the RecBCD pathway is inactivated, an alternate pathway, the RecF pathway, promotes recombinational DNA double-strand break repair in *E. coli* [54-56]. The *E. coli* RecF pathway depends on the RecQ helicase and the RecJ exonuclease to resect duplex DNA, whereas RecO and RerR act with RecF to load RecA onto single-strand DNA covered by SSB [57] (figure 5A).

**Figure 5.** Main pathways involved in the processing of DNA ends.
D. radiodurans, like D. geothermalis and D. deserti, is naturally devoid of the RecB and RecC enzymes but encodes all the components of the alternate RecF pathway. We have recently shown that, in D. radiodurans, RecFOR proteins are essential for DNA double-strand break repair through ESDSA whereas RecJ protein is essential for cell viability [58]. In contrast, cells devoid of RecQ, the major helicase implicated in repair through the RecF pathway in E. coli, are resistant to γ-irradiation and display a wild type DNA double-strand break repair capacity. In contrast, uvrD mutants showed a markedly reduced radioresistance and a slow rate of fragment assembly suggesting that UvrD might be involved in the processing of double-stranded DNA ends (figure 5B).

Although Archaea are prokaryotes, most of the mechanisms and associated proteins involved in DNA metabolism (replication of DNA, transcription, translation, DNA repair and recombination pathways) are close to those found in eukaryotic cells. Eukaryotic cells use two main pathways to repair DNA double-strand breaks: HR and NHEJ depending on the phase of the cell cycle and the nature of the DNA ends. A key step where pathway choice is exerted is in the “licensing” of DNA ends to produce recombinogenic single-stranded tails. The conserved MRX/MRN complex, consisting of proteins Rad50, Mre11 and Xrs2/Nbs1 (in yeast/human) appears to be a major regulator of DNA end processing (for review, see [59]). Archaea encode proteins homologous to Rad50 and Mre11, but not to Xrs2/Nbs1. In hyperthermophilic archaea, Rad50 and Mre11 genes form a well conserved operon with two other genes encoding a nuclease (NurA) and a helicase (HerA/MlaA) [60-62]. In vitro, NurA and HerA proteins from Pyrococcus furiosus form a complex exhibiting helicase/nuclease activities stimulated by the presence of Rad50/Mre11 [63]. The Rad50/Mre11 complex generates short 3’ ends before recruitment of the NurA/HerA complex. This allows degradation of the 5’ end of DNA and generates a 3’ protruding end which may be invaded by RadA (figure 5C). In vivo studies conducted in Sulfolobus acidocaldarius revealed that when cells were exposed to γ-rays at a dose of 1000 Gy, Mre11 was recruited on DNA [64]. HerA protein was also detected on the chromosome and formed a complex with Rad50 and Mre11. In contrast, NurA was not detected on the chromosome after ionizing irradiation and was not found associated within the Rad50/Mre11/HerA complex. Complementary approaches are required to elucidate in vivo the role of NurA in thermophilic Archaea DNA repair mechanism. The role of Rad50 and Mre11 was also analyzed in vivo in a polyploid archaeon Halofexx volcanii, which does not encode NurA and HerA proteins [65]. Unlike the yeast Saccharomyces cerevisiae, deletion of the rad50 and mre11 genes leads to a higher resistance of this archaeon to DNA damaging agents. In H. volcanii, homologous recombination is the primary pathway of DNA double strand break repair in cells devoid of Rad50 and Mre11 proteins, but not in the wild type where, as in mammalian cells, DNA double-strand break repair is mainly performed by NHEJ. However, the RadA protein remains essential for cell survival after DNA-damaging treatments [65]. RadA-dependent HR would probably be required late in the restoration of an intact circular chromosome, when most of the fragments are already reassembled through an NHEJ process. Further studies are required to identify the complete set of archaeal proteins involved in the NHEJ pathway.

As in bacteria, accessory proteins are required in eukaryotic cells to favor HR. Rad52 and Rad55/57 were shown to promote the loading of the Rad51 recombinase on DNA single-stranded tails covered by the SSB-like complex RPA in irradiated cells. Rad54 is a motor protein that translocate along double-stranded DNA, promotes chromatin remodeling and strongly stimulates the Rad51 DNA strand exchange activity. Together with rad51 and rad52, rad54 is one of the three most ionizing radiation sensitive single mutant in yeast S. cerevisiae [66]. In Archaea, proteins found by sequence homology with RadA may act as accessory proteins to promote HR. They include RadA2, RadB, RadC, KaiC/aRadC and Sms proteins (reviewed in [67]). The deletion of RadB in H. volcanii leads to a decrease of cell growth, a high UV sensitivity and recombination defects. In vitro studies of RadB from P. furiosus showed that the protein is able to interact with DNA, but does not catalyze the exchange of DNA strands required for homologous recombination. Other mediators may be involved in homologous recombination in Archaea, such as the homolog of the eukaryotic Rad54 protein. In Sulfolobus solfataricus, SsoRad54 is able to interact in vitro with DNA double strands, and was
proposed to be a translocase. In addition, SsoRad54 also interacts with RadA protein to stimulate DNA strand exchange [68].

5. Nucleoid structure

Bacterial chromosomes form in association with proteins, condensed structures called nucleoids. The nucleoids of members of the radioresistant genera Deinococcus and Rubrobacter revealed a high degree of genome condensation and remain unaltered after high-dose $\gamma$-irradiation [69, 70]. Restricted diffusion of the DNA ends would be a reasonable explanation for efficient repair of the numerous DNA double-strand breaks generated by exposure to high doses of ionizing radiation. Moreover, it was proposed that the 4 to 10 copies of the D. radiodurans genome were prealigned [71], facilitating the search of homology to favor efficient error-free DNA double-strand break repair (HR and ESDSA). However, prealignment of the copies of homologous chromosomes has not been demonstrated.

The very abundant histone-like protein HU from E. coli is associated with the bacterial nucleoid and shown to be involved in cell survival after $\gamma$-irradiation [72]. In D. radiodurans, HU protein is essential for cell viability and, when expressed from a thermosensitive plasmid, its progressive cellular depletion at the non-permissive temperature generates decondensation and fractionation of the nucleoid preceding cell lysis, suggesting that the HU protein plays a major architectural role in the structure of the D. radiodurans nucleoid [73].

Archaea belonging to the Euryarchaeota lineage, such as Pyrococcus and Thermococcus species, as well as Methanogenic archaea, encode histone-like proteins displaying structural homology with those found in eukaryotes (reviewed in [74]). The archaeal nucleosome is not an octamer as in eukaryotes. In solution, archaeal histone-like proteins form dimers that can subsequently become tetramers to bind DNA. Several other small proteins have been characterized in some Euryarchaea species as the Methanogenic archaea that may possibly be involved in chromosome compaction [75, 76].

The chromatin structure of some species from the Crenarchaeota lineage, such as Sulfolobus, differs from those observed in Pyrococcus and Thermococcus archaea since to date, none of them have been found to encode histones. These organisms use at least two other DNA-binding proteins called AlbA and Sul7d to achieve DNA compaction (reviewed in [77]).

In eukaryotic cells, DNA repair requires local changes in chromatin structure to facilitate the accessibility of the lesions to the DNA repair machinery. Histone acetylases and histone desacetylases were shown to play a major role in the regulation of transcription by modulation of the compaction of the chromatin [78]. As in eukaryotes, there are several lines of evidence that Archaea could adjust chromatin compaction. In Sulfolobus, the DNA-binding affinity of AlbA is modulated by Pat and Sir2 enzymes through acetylation and deacetylation processes [79, 80]. Deacetylation of AlbA results in repression of transcription. The observed decrease of histones Htz1 and Htz2 concentration in Thermococcus zilligii when cells enters stationary phase cells may have a considerable effect on the overall structure of genomic DNA and consequently on gene expression [81]. Unfortunately, the data illustrating the global structure of archaeal genome during DNA repair remains scarce in the literature and the involvement of the nucleoid structure in archaeal radioresistance is still an open question.

6. Induced response to $\gamma$-irradiation

6.1. Transcriptome analysis

Analysis of the transcriptome of D. radiodurans revealed a novel group of genes that are up-regulated in response to either dessication or ionizing radiation [51]. 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 dessication. Only a limited number of well defined DNA repair genes including recA are found among these genes. The five genes whose expression is most highly
induced in response to each stress include ddrA, ddrB, ddrC, ddrD and pprA. These genes are conserved in *D. geothermalis* and *D. deserti* but are not found outside of the *Deinococcus* lineage. Three of them were shown to play a major role in *D. radiodurans* radioresistance [51]. DdrA protects the single-stranded DNA ends against degradation [82] and is probably involved in protection of the recombinogenic DNA substrates [83] whereas DdrB binds to single-strand DNA [84] and PprA protects DNA double-stranded DNA ends against degradation and stimulates the activity of DNA ligases [50] suggesting a role of PprA in a putative NHEJ pathway. It is also interesting to note that the 3 predicted superoxide dismutases and the 3 predicted catalases which can protect biomolecules from ROS mediated damage are induced after exposure to ionizing radiation [51].

A transcriptional study was undertaken with *P. furiosus* exponentially growing cells irradiated with γ-Rays at a dose of 2500 Gy [33]. Under these conditions, 321 ORFs showed changes in gene expression in the time needed to repair all cell damage. In *P. furiosus*, the response to γ-irradiation affects various metabolic pathways as well as transcription, translation, various membrane transporters and also a large number of genes of unknown function. Interestingly, most of the systems involved in oxygen detoxification and redox homeostasis appeared to be highly and constitutively expressed. Moreover, the up-regulation of a gene encoding a ferritin/Dps-like protein in *P. furiosus* could trap free iron for limiting the production of hydroxyl radicals by Fenton chemistry [33]. The radA, radB, dp1 and dp2 genes encoding the two subunits of DNA polymerase D were shown to be slightly induced under these conditions but most genes known to be involved in DNA repair or in DNA replication do not show significant variation in gene expression. Unlike *P. furiosus*, RadA protein seems present at a constant level in *P. abyssi* after irradiation at 2,500 Gy [45]. One can assume that, due to the deleterious effects of temperature on DNA, repair genes are constitutively expressed in hyperthermophilic organisms.

Transcriptomic coupled with proteomic analysis was also performed in the halophilic archaeon *Halobacterium* sp. NRC-1 [85]. This analysis was conducted during 240 minutes post-irradiation incubation in cells exposed to 2500 Gy γ-irradiation. Genes encoding proteins involved in DNA repair (RadA recombinase, Hjr resolvase, UvrD helicase) or nucleotide biosynthesis (CTP synthase, cytidylate kinase) were found among the up-regulated genes. An inverse relationship was observed between mRNA changes of some genes involved in DNA replication and cell division suggesting that a pause occurred to ensure completion of DNA repair prior cell division. A significant increase of superoxide dismutase (SOD2) and thioredoxin (trxA2) levels was also observed after γ- irradiation to probably scavenge free radicals. Furthermore, eight dehydrogenases are down-regulated [85] probably to minimize ROS production by subsequent auto-oxidation reactions [26].

Two independent radioresistant derivatives of *Halobacterium* NRC-1, LH5 and LH7a, isolated by step doses of electron-beam radiation and selection of survivors [86], displayed a LD_{50} greater than 11,000 Gy. To understand how these mutants have acquired the ability to better cope with radiation, a transcriptomic analysis was performed to compare whole expression profile of the two mutants versus wild-type strain under standard growth conditions, [86]. The LH5 mutant showed 119 deregulated genes, 47 up-regulated and 72 down-regulated, compared to the wild strain. The genes encoding the RadA recombinase, the RPA protein complex (rfa3, rfa8 and ral genes) and the superoxide dismutase enzymes (sod1 and sod2) were shown to be induced, confirming their important role in the cellular DNA repair and detoxification. The LH7a mutant showed 47 deregulated genes including 11 that were up-regulated and 36 that were down-regulated. The comparison between LH5 and LH7a expression patterns showed that, only the 3 genes encoding the trimeric RPA protein were induced in both independent mutants suggesting that the complex is critical for the radioresistance.

### 6.2. Regulatory network

In *E. coli*, DNA damaging treatments result in the induction of SOS functions, a variety of cellular processes mediated by the RecA-dependent cleavage of LexA, the repressor of SOS genes. *D. radiodurans* possesses two LexA homologs that undergo, as in *E. coli*, RecA-dependent cleavage after DNA damage. However, no regulon under the control of LexA1 or LexA2 proteins has been identified.
to date in *D. radiodurans*. In particular, *recA* induction following γ-irradiation is not controlled by LexA1 or LexA2 but depends on a *Deinococcus* specific regulatory protein IrrE, also called PprI, that was shown to be a positive effector that enhances the expression of some DNA repair genes following exposure to radiation [50, 87, 88]. Recent crystallographic data combined with a site-directed mutagenesis study on the *D. deserti* IrrE protein, suggest that IrrE possesses a putative proteolytic activity essential for radiotolerance [89]. The presence of a common radiation/desiccation response motif (RDRM) found in the upstream region of *recA*, *pprA* and other highly radiation-induced genes in *D. radiodurans* and of their homologs in *D. geothermalis* and *D. deserti* suggests that a transcription regulator would target this common RDRM sequence [90, 91]. Direct binding of IrrE to the RDRM sequence has not, to our knowledge, been demonstrated. DdrO, another protein specific to *Deinococcaceae*, has been proposed to be this global regulator [91]. However, nothing is known about the precise role of IrrE and DdrO in the response to γ-irradiation or desiccation.

In Archaea, the transcription of several genes encoding general transcription factors, as well as regulators, was found to be modulated during cell recovery [33, 85]. These activation and repression are responsible of other gene regulation and coordinate the network of repair pathways to face cell injuries. Unfortunately, most of their targets and their hierarchical expression during repair have yet to be elucidated, thus limiting our understanding of the variety of archael responses to stress.

7. Conclusion

Different strategies were independently selected during evolution in Bacteria and Archaea to help cell to recover from radiation-induced damage. It is now generally accepted that the Archaea share many similarities in their information-processing pathways with Eukarya and could be used as simpler models of the much more complex eukaryal ones. Comparison of radioresistant bacteria and archaea has revealed several common strategies such as (i) limitation of ROS production or scavenging of free radicals (ii) efficient DNA double-strand break repair. However, other strategies are only found in some species (i) several radioresistant bacteria exhibit a low iron concentration to prevent production of OH· but not *E. coli* radioresistant mutants (ii) the protection of *D. radiodurans* and *Halobacterium* proteins against oxidation plays a critical role for their ability to repair cell injuries (iii) several radioresistant bacteria and archaea, but not all, encode pigments (iv) many genes induced during post irradiation cell recovery, and involved in radioresistance, are specific for a particular phylum (v) *D. radiodurans* combines DNA double-strand break repair with massive DNA synthesis, also observed in *P. abyssi*, suggesting that ESDSA may also take place in Archaea. However, the presence of an efficient ESDSA mechanism in Archaea has not yet been investigated (vi) a condensed nucleoid structure could favor error-free genome reassembly by limiting the diffusion of DNA fragments. However, the proteins involved in the organization of the nucleoid are not the same in radioresistant bacteria and archaea. Radioresistance is the result of complex cell networks and further work is required to understand the combination of pathways that contributes to radioresistance at the molecular level.

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References


[77] White M F and Bell S D 2002 Trends Genet. 18 621.