

Unlocking radiation resistance mechanisms: still a long way to go

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Recent transcriptome analysis revealed that *Deinococcus radiodurans* efficiently coordinate their recovery from ionizing radiation through a complex network of DNA repair and metabolic pathway switching. However, the additional discovery of numerous irradiation-response genes has provided new targets for the identification of genes primarily crucial to radiation resistance. Investigations based on electron microscopy suggest that the observed radiation resistance in *D. radiodurans* might be partly caused by the presence of an unusual ring-like conformation of nucleoids. Although such investigations provide useful insights into the mechanisms underlying radiation resistance, a more detailed empirical explanation of why *D. radiodurans* is so radiation resistant is still needed. Further research based on alternative genetic and biochemical approaches should help to gain a better understanding of the mechanisms involved in DNA repair.

Deinococcus radiodurans is a small, eubacteria-type bacterium that is red-pigmented and nonsporing. Members of this species are characterized by extreme resistance to both lethal and mutagenic effects of a variety of DNA-damaging agents, with an unusually high resistance to ionizing radiation. *D. radiodurans* was first isolated in 1956 from canned meat that had supposedly received a sterilizing dose of γ radiation [1]. Currently, exposure of up to 10 000 grays (Gy) of ionizing radiation is used to sterilize foods. Like other organisms, the *D. radiodurans* genome sustains over 100 DNA double-strand breaks (DSBs) after exposure to 10 kGy of γ radiation. Double-strand breaks are the most lethal form of DNA damage. Although all living organisms have DNA repair mechanisms, only a few DSBs can be repaired in most species. *D. radiodurans* is capable of repairing the fragmented genome during post-irradiation incubation. Genome sequence analysis of *D. radiodurans* has revealed that the genome encodes almost all the major prokaryotic proteins involved in DNA repair [2]. However, the molecular mechanisms underlying its radiation resistance remain unclear.

In a recent article, Liu *et al.* [3] described a transcriptome analysis of *D. radiodurans* recovering from ionizing radiation. Such a global discovery technique is expected to provide useful information for the identification of genes primarily crucial to radiation resistance. However, what specific information can be gained by transcriptome analysis? This article discusses future directions of transcriptome analysis and how this might contribute towards a better understanding of radiation resistance mechanisms.

Recently, Levin-Zaidman *et al.* [4] described an unusual ring-like conformation of nucleoids found in *D. radiodurans* and revealed intriguing new information about the morphologic transition of nucleoids during recovery from the effects of ionizing radiation. Electron microscopy analysis also highlighted the role of the toroidal nucleoid structure in radiation resistance of *D. radiodurans*. The relationship between genome conformation and DNA repair is clearly important. Here, I discuss this relationship in terms of recent advances in our understanding of the molecular mechanisms of DNA repair in *D. radiodurans*.

Transcriptome analysis

The first paper describing the remarkable capacity of *D. radiodurans* for repairing DNA DSBs was published in 1966 [5]. In the 1960s, it was reported that the highly efficient DNA DSB repair process was radiation-inducible and required *de novo* protein synthesis following irradiation [6]. Given that complex cellular responses are probably required in the recovery from exposure to ionizing radiation, an understanding of the dynamics of the expression levels of every transcript in cells recovering from radiation-induced DNA damage is particularly valuable. Microarrays provide promising tools for identification of the genes responsible for radiation resistance. Such an analysis revealed that 832 genes (28% of all genes investigated) were induced and 451 genes (15%) were repressed during recovery [3]. In total, 1283 genes displayed significant changes in their expression level in response to DNA damage. However, this result might prove to be unremarkable. In *Escherichia coli*, it was found that the expression level of 1248 genes altered following DNA-damaging treatment [7]. Liu *et al.* [3] described that, although radiation-induced genes in the *D. radiodurans* genome included functional homologs in other prokaryotic species, the majority of these genes were functionally uncharacterized and previously unsuspected. This situation was also similar to that found with *E. coli*; ~30% of the damage-inducible genes were functionally uncharacterized [7].

As expected, transcriptome analysis successfully revealed that *D. radiodurans* cells efficiently coordinate their recovery by a complex network of DNA repair and metabolic pathway switching [3]. Unfortunately, there was no substantial discussion of – or comparison with – the previously published proteome analysis of *D. radiodurans* [8]. The genes responsible for radiation resistance still need to be identified, and elucidating the biological and biochemical function of novel damage-inducible genes is of

vital importance. Liu *et al.* [3] focused in particular on the damage-inducible gene DR0070, and performed gene disruption analysis. Disruption of DR0070 rendered *D. radiodurans* sensitive to acute irradiation with γ rays. However, proteome analysis revealed that the DR0070 protein was detected only after alkaline treatment, but not under other culture conditions, including acute γ irradiation [8]. Differences such as these should not be neglected as they might provide important clues to understanding the function of novel genes.

Consistent with the proteome analysis is the significant induction of the *recA* gene during recovery from exposure to γ radiation. The RecA recombinase protein is believed to play a crucial role in the homologous recombination repair of radiation-induced DNA DSBs in *D. radiodurans* [9,10]. However, recent molecular genetic and biochemical studies with *D. radiodurans* RecA mutants suggest that radiation resistance by RecA recombination activity plays a lesser role than its co-protease activity [11]. Transcriptome analysis revealed that many genes implicated in DNA repair, recombination and replication – in addition to several uncharacterized genes – exhibited a *recA*-like activation pattern. The products of these genes seem to comprise the core radiation response regulon of *D. radiodurans*. Unlike *E. coli*, *D. radiodurans* LexA is not involved in RecA induction following γ irradiation [12,13].

Transcriptome analysis also revealed that several genes putatively encoding transcriptional regulators (including a *lexA* paralog) exhibit significant induction during recovery, and can be considered as candidates for regulating the radiation response in *D. radiodurans*. However, recent work on a DNA-damage-sensitive strain of *D. radiodurans* identified a novel regulatory protein (DR0167; IrrE protein, also called PprI protein) that stimulates transcription of the *recA* gene following γ irradiation [14,15]. This protein might act as a general switch in the radiation response of *D. radiodurans*. As the transcriptome analysis did not make particular reference to DR0167, this is likely to be a constitutively expressed gene. It would be interesting to compare gene expression profiles in the wild type and DR0167-deficient mutant in an effort to evaluate the potential role of DR0167-controlled genes in DNA repair.

Ring-like structure of genome

In general, the only known mechanism for accurate DNA DSB repair is homologous recombination. In 1978, Hansen [16] demonstrated that *D. radiodurans* contains at least four genomic copies per cell in stationary growing cells and up to 10 copies in exponentially growing cells. The redundant genome copies appear to be advantageous in DNA DSB repair by homologous recombination. However, there is no correlation between the number of copies and the radiation resistance of *D. radiodurans* [17]. Minton's group [18] reported that RecA recombinase was not detectable in *D. radiodurans* during normal growth, and can only be observed after substantial exposure to DNA damage. However, recent studies [8,11–13] have detected a steady level of RecA during normal growth. In *D. radiodurans*, constitutive and induced RecA concentrations are estimated to be ~11 000 and 44 000

monomers per cell, respectively [13]. The latter value is twofold less than the RecA level found in fully induced *E. coli* cells. This raises the question of how RecA can manage hundreds of radiation-induced DSBs. Minton and Daly [19,20] proposed that the *D. radiodurans* genome has a special form of redundancy wherein the different genome copies exist in pairs. These are linked to each other by thousands of four-stranded (Holliday) junctions. This alignment of genome structure enables one genome fragment to find an intact homologous neighbor, thus facilitating the involvement of RecA in the repair reaction by mediating DNA strand exchange. However, there is no experimental evidence to support this hypothesis. Optimal mapping analysis, which enables a direct observation of individual DNA molecules, failed to find DNA molecules containing Holliday junctions in the *D. radiodurans* genome [21].

Recently, two separate groups purified the *D. radiodurans* RecA protein and characterized its biochemical properties [11,22]. It was found that the *D. radiodurans* RecA protein shared similar properties to the *E. coli* RecA protein, although the *D. radiodurans* RecA protein had a higher affinity for double-stranded DNA than the *E. coli* RecA protein. Kim and Cox [23] further examined the pathway for *D. radiodurans* RecA-mediated DNA-strand exchange and found that the pathway proceeded in an inverse manner compared with the *E. coli* RecA protein. It was assumed from this result that the peculiar property of RecA might account for its efficiency in the repair of DNA DSBs in *D. radiodurans*. However, accurate homologous recombination alone cannot account for the extraordinary radiation resistance of *D. radiodurans* because a *D. radiodurans* *recA* mutant strain completely lacking the recombination activity of RecA has been found to be only slightly sensitive to γ irradiation compared with the wild type strain [11]. This implies that *D. radiodurans* possesses a RecA-independent recombination repair mechanism and/or a DNA repair mechanism other than homologous recombination. The RecA-independent recombination repair pathway might include a single-strand annealing reaction that was proposed by Daly and Minton [24]. However, a gene involved in the RecA-independent recombination repair has yet to be identified.

Recently, a eukaryote-type DNA repair pathway by non-homologous end-joining (NHEJ) was discovered in *Bacillus subtilis* and *Mycobacterium tuberculosis* [25]. *D. radiodurans* might contain an as-yet undiscovered NHEJ pathway for the repair of radiation-induced DNA DSBs. Such a pathway must be error-free because DNA ends produced by irradiation probably receive clustered damages, the removal of which results in mutation. It is also necessary to consider special genome conformations that might regulate the NHEJ repair process. Hypotheses concerning repair mechanisms of radiation-induced DNA DSBs in *D. radiodurans* are summarized in Fig. 1. A detailed investigation of genome conformations found in the *D. radiodurans* cells during recovery from γ radiation is of vital importance to clarify the DNA repair mechanism of this bacterium.

Based on a series of transmission electron micrographs, Levin-Zaidman *et al.* [4] recently argued that the *D. radiodurans* genome assumes an unusual toroidal morphology that might contribute to its radiation resistance. Using six-day-old stationary-state cells, they observed that the ring-like shape of the nucleoid was maintained for 1 hour after exposure to 15 kGy of γ radiation. During continuous post-irradiation incubation, the toroidal DNA structure underwent a transition into an open S-like morphology, followed by progressive spreading of the DNA between two compartments through a cell wall septum. After 3 hours, this morphologic reorganization culminated in the fusion of two nucleoids. They suggested that these structural features play a crucial role in the radiation resistance of *D. radiodurans*.

It was also proposed that homologous recombination cannot function during the first phase of DNA DSB repair in *D. radiodurans* because a single copy of the genome is tightly packed in the toroidal nucleoid and is extensively shattered by cell wall septa that divide the cell into four compartments. Furthermore, it was proposed that the first phase of DNA repair involves template-independent – yet error-free – joining of DNA fragments with DNA toroids. This hypothesis assumes that each tetracoccus is a single cell with four compartments. However, previous studies that determined the number of genome copies per cell were based on the assumption that each diplococcus contained two cells or that each tetracoccus contained four cells [16,17]. Kikuchi *et al.* [26] reported a multimeric formation of chromosome II and the megaplasmid in *D. radiodurans*. Similarly, repeated DNA molecules consisting of chromosome II have been observed by optical mapping analysis [21]. Together, this evidence does not support the idea that each compartment contains a single genome copy. Therefore, the hypothesis of Levin-Zaidman *et al.* seems controversial at this time, and requires further empirical evidence.

Future prospects

Although comprehensive transcriptome analysis and genome conformation analysis in *D. radiodurans* have provided clues to the mechanisms underlying radiation resistance, there is still no empirically based explanation of why *D. radiodurans* is so radiation resistant. Further research based on alternative genetic and biochemical approaches is required to obtain a better understanding of the DNA repair mechanisms. Previously developed gene disruption methods will prove to be invaluable tools to elucidate the function of each deinococcal gene involved, as indicated by transcriptome analysis [27–29]. In addition, there are a large number of DNA-damage sensitive mutants that have been previously isolated and these might help to elucidate the molecular mechanisms of radiation resistance in *D. radiodurans* [30–32]. These mutants will be valuable for transcriptomic and morphologic analyses. Progress in our understanding of the DNA repair mechanism of *D. radiodurans* is not only important for basic science but also has a great potential to promote novel DNA technologies. Further progress might provide guidelines for activating the DNA repair

mechanism in humans to act as an effective treatment for protection against cancer and aging.

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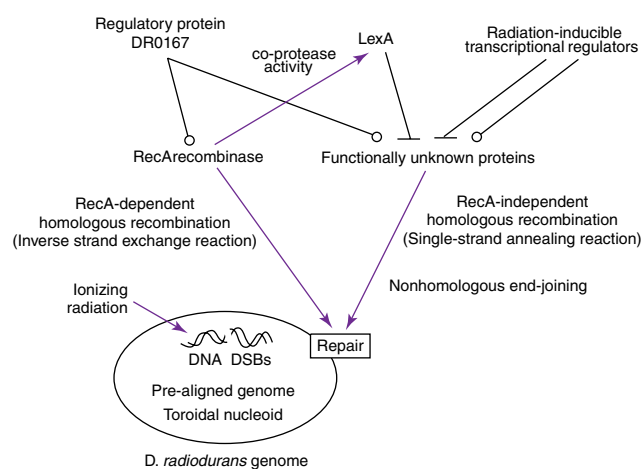
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Fig. 1. Hypothesized repair mechanisms of radiation-induced DNA double-strand breaks (DSBs) in *Deinococcus radiodurans*. The *D. radiodurans* genome sustains >100 DNA DSBs after exposure to 10 kilograys (kGy) of γ radiation. Using the *D. radiodurans*-specific genome structure, a pre-aligned double genome structure and toroidal nucleoid structure are proposed. DR0167 is involved in the stimulation of *recA* expression following γ irradiation. RecA recombinase engages with DNA DSB repair via an inverse-strand exchange reaction. DR0167, LexA repressor and other putative transcriptional regulators can be involved in the regulation of functionally unknown proteins. RecA-independent homologous recombination (including a single-strand annealing reaction) and/or non-homologous end-joining (NHEJ) might contribute significantly to radiation-induced DNA DSB repair in *D. radiodurans*.