Conservation and diversity of radiation and oxidative stress resistance mechanisms in *Deinococcus* species

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One sentence summary: The authors reviewed the mechanisms and factors involved in the extreme radiation and oxidative stress resistance in *Deinococcus radiodurans* in comparison with 10 other resistant *Deinococcus* species, and highlighted not only conserved pathways but also a large diversity of the repair, protection and regulation toolbox among the different deinococci.

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**ABSTRACT**

*Deinococcus* bacteria are famous for their extreme resistance to ionising radiation and other DNA damage- and oxidative stress-generating agents. More than a hundred genes have been reported to contribute to resistance to radiation, desiccation and/or oxidative stress in *Deinococcus radiodurans*. These encode proteins involved in DNA repair, oxidative stress defence, regulation and proteins of yet unknown function or with an extracytoplasmic location. Here, we analysed the conservation of radiation resistance-associated proteins in other radiation-resistant *Deinococcus* species. Strikingly, homologues of dozens of these proteins are absent in one or more *Deinococcus* species. For example, only a few *Deinococcus*-specific proteins and radiation resistance-associated regulatory proteins are present in each *Deinococcus*, notably the metallopeptidase/repressor pair IrrE/DdrO that controls the radiation/desiccation response regulon. Inversely, some *Deinococcus* species possess proteins that *D. radiodurans* lacks, including DNA repair proteins consisting of novel domain combinations, translesion polymerases, additional metallorepressors, redox-sensitive regulator SoxR and manganese-containing catalase. Moreover, the comparisons improved the characterisation of several proteins regarding important conserved residues, cellular location and possible protein–protein interactions. This comprehensive analysis indicates not only conservation but also large diversity in the molecular mechanisms involved in radiation resistance even within the *Deinococcus* genus.

**Keywords:** DNA repair; oxidative damage protection; regulatory proteins; stress response; metal homeostasis; biodiversity

**INTRODUCTION**

In 1956, scientists described a bacterium that was found as a contaminant in a can of ground meat. This bacterium had survived exposure to a high dose of ionising radiation (IR) that was supposed to sterilise the canned meat (Anderson et al. 1956). Now known as *Deinococcus radiodurans*, this bacterial species is not only extremely tolerant to gamma radiation, but also to other DNA damage- and oxidative stress-generating conditions such as UV and desiccation (Battista 1997). Exposure to high doses of IR generates massive DNA damage, including hundreds of double-strand breaks, but *D. radiodurans* is able to reconstitute its genome completely within hours after irradiation. Therefore,
D. radiodurans is a good model organism to study DNA repair, DNA damage and oxidative stress response, and radiation resistance.

Deinococcus radiodurans and other Deinococcus species show no loss of viability after exposure to IR doses up to 5 kGy. For comparison, a few hundred Gy will kill most known bacterial species, including Escherichia coli and Thermus thermophilus, and 5–10 Gy are lethal to most vertebrates, including humans (Daly 2012). Nevertheless, IR resistance is not unique to Deinococcus, and several organisms tolerating more than 1 kGy have been described, including not only bacteria (e.g. Chroococcidiopsis of the phylum Cyanobacteria) and archaea (e.g. Thermococcus gammatolerans), but also some small eukaryotes (e.g. tardigrades and bdellidoid rotifers) (Cox and Battista 2005; Daly 2012). Of these IR-resistant species, D. radiodurans has been studied most extensively, which was accelerated after obtaining its genome sequence (White et al. 1999) and by the development of techniques for its genetic manipulation. Characterisation of the mechanisms underlying IR resistance in Deinococcus is also useful to understand IR resistance, or sensitivity, in other organisms.

The various in vivo and in vitro approaches used in recent years to study D. radiodurans have indicated that its tolerance to radiation, desiccation and oxidative stress results from a combination of different physiological determinants and well-regulated molecular mechanisms (Fig. 1) (Cox and Battista 2005; Confalonieri and Sommer 2011; Slade and Radman 2011; Daly 2012; Agapov and Kubachinskiy 2015; Timmins and Moe 2016). Compared to radiation-resistant species such as E. coli, proteins in D. radiodurans and other radiation-resistant organisms are much better protected against oxidative damage (Daly et al. 2007, 2010; Krisko and Radman 2010). Radiation and desiccation lead to generation of reactive oxygen species (ROS), but D. radiodurans has developed efficient enzymatic and non-enzymatic antioxidant systems to remove ROS and limit protein damage. Sufficient proteome protection is crucial for survival after irradiation because protein activity is required for essential processes including transcription, translation and DNA repair. Compared to IR-sensitive bacteria, the nucleoid of Deinococcus species appears more condensed, which may contribute to radiation resistance by limiting diffusion of DNA fragments (Levin-Zaidman et al. 2003; Zimmerman and Battista 2005). Following exposure to IR or desiccation, the expression of many genes and proteins is induced in D. radiodurans, including DNA repair proteins and proteins of yet unknown function (Liu et al. 2003; Tanaka et al. 2004; Lu et al. 2009; Basu and Apte 2012), and several regulator proteins involved in the radiation or oxidative stress response have been described (Agapov and Kubachinskiy 2015).

Deinococcus radiodurans was the first species of the genus Deinococcus that was isolated, and was also the first Deinococcus species for which the genome sequence was thoroughly analysed (White et al. 1999; Makarova et al. 2001). Deinococcus bacteria are ubiquitous in nature and have been isolated from various environments and locations (e.g. hot and cold desert soil, air, high atmosphere, water). At present, more than 50 radiation-resistant Deinococcus species have been described, and for some of these a complete or draft genome sequence has been obtained. Here, we review the reported data about the mechanisms involved in radiation resistance, oxidative stress defence, DNA repair, and in their regulation in D. radiodurans. The conservation of the proteins involved in these processes was investigated in the 10 other radiation-resistant Deinococcus species for which a complete and assembled genome sequence was available. The 11 analysed Deinococcus species have been isolated from various locations worldwide (Table 1). This comparison showed a remarkable diversity of the radiation resistance-associated proteins among deinococci. Furthermore, sequence analysis improved the characterisation of several of these proteins. Throughout this article we discuss our findings regarding protein functions and resistance-associated mechanisms in the genus Deinococcus.

**Figure 1.** Extreme radiation and oxidative stress resistance in Deinococcus involves multiple factors and well-regulated mechanisms.
Table 1. Information of complete genomes of Deinococcus species.

| Species                        | Identified in                        | Total genome size (Mb) | Replicons (sizes in kb) | Proteins | References                          |
|--------------------------------|--------------------------------------|------------------------|-------------------------|----------|-------------------------------------|
| Deinococcus radiodurans (Drad) | Canned meat, USA                     | 3.28                   | 4 (2649, 412, 177, 46)  | 3167     | Anderson et al. (1956); Brooks and Murray (1981); White et al. (1999) |
| Deinococcus geothermalis (Dgeo) | Hot spring, Italy                    | 3.25                   | 3 (2467, 574, 206)      | 3003     | Ferreira et al. (1997); Makarova et al. (2007) |
| Deinococcus deserti (Ddes)     | Sahara Desert sand, Morocco/Tunisia  | 3.86                   | 4 (2820, 325, 314, 396) | 3503     | de Groot et al. (2005, 2009)        |
| Deinococcus maricopensis (Dmar) | Sonoran Desert soil, USA             | 3.5                    | 1 (3499)                | 3242     | Rainey et al. (2005); Pukall et al. (2011) |
| Deinococcus gobiensis (Dgob)   | Gobi Desert sand, China              | 4.41                   | 7 (3137, 433, 425, 232, 72, 55, 53) | 4140     | Yuan et al. (2009, 2012)            |
| Deinococcus proteolyticus (Dpro)| Lama glama feces, Japan              | 2.89                   | 5 (2147, 315, 196, 132, 97) | 2645     | Kobatake, Tanabe and Hasegawa (1973); Brooks and Murray (1981); Copeland et al. (2012) |
| Deinococcus peraridilitoris (Dper)| Coastal desert soil, Chile           | 4.51                   | 3 (3882, 557, 75)       | 4223     | Rainey et al. (2007)                |
| Deinococcus suuwensis (Dswu)   | Mountain soil, South Korea           | 3.53                   | 1 (3531)                | 3217     | Lee et al. (2013)                   |
| Deinococcus soli (Dsol)        | Rice field soil, South Korea         | 3.24                   | 1 (3237)                | 3055     | Cha et al. (2014); Joo et al. (2015) |
| Deinococcus actinosclerus (Dact)| Rocky hillside soil, South Korea     | 3.26                   | 1 (3264)                | 3073     | Joo et al. (2016); Kim et al. (2016) |
| Deinococcus puniceus (Dpun)    | Mountain soil, South Korea           | 2.97                   | 1 (2972)                | 2681     | Lee et al. (2015)                   |

The species name is followed by an abbreviation that is used in Tables 2 to 6.

Different results have also been reported with respect to obtaining mutant strains: it appeared to be impossible to obtain a recA (DR_1126, single-stranded-DNA-specific exonuclease) or gyrA (DR_1913, DNA gyrase subunit A) null mutant in one or two studies (Nguyen et al. 2009; Bennichou et al. 2010; Cao, Mueller and Julin 2010), suggesting that these genes are essential for viability, whereas others successfully obtained null mutants for these genes (Jiao et al. 2012; Kota, Charaka and Misra 2014).

Besides for the naturally transformable D. radiodurans, genetic tools allowing construction of mutant strains have also been developed for D. deserti and D. geothermalis. Like in D. radiodurans, a D. deserti irrE mutant is highly sensitive to gamma and UV radiation (Vujicic-Zagar et al. 2009). Deletion of the chromosomal recA gene in D. deserti, the third and last gene of an operon equivalent to that in D. radiodurans, did not lead to radiation sensitivity due to the presence of two additional recA genes located on large plasmids (Dulermo et al. 2009). A D. geothermalis cystine ABC transporter mutant showed increased sensitivity to H2O2 (Kim et al. 2017).

One might expect that genes important for radiation resistance in D. radiodurans be conserved in other radiation-resistant species within the genus Deinococcus. To investigate this, not only homologues of the gene products listed in Table S1 (Supporting Information) but also other proteins involved in radiation resistance-associated processes such as DNA repair and oxidative stress defence (Tables S2–S6, Supporting Information) were searched in 10 other complete and assembled Deinococcus genome sequences (Table 1). Besides showing presence or absence of protein homologues, we included a comparative analysis of domain composition in multidomain proteins and of functionally important residues in proteins. The results are described in the following sections.

DNA REPAIR IN DEINOCCUS

Deinococcus radiodurans DNA repair proteins and comparison with E. coli

The genome sequence of D. radiodurans revealed the presence of homologues of most well-known prokaryotic DNA repair proteins involved in base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and recombinational repair, suggesting that the DNA repair machinery of D. radiodurans is globally similar to that of other bacteria (Makarova et al. 2001), but that it functions more efficiently than in radiation-sensitive species because of better protection of the (DNA repair) proteins against oxidative damage (Daly 2012). Indeed, at least some DNA repair proteins of E. coli, namely PolA (Gutman, Fuchs and Minton 1994), RadA (Zhou et al. 2006) and UvR(A) (Agostini, Carroll and Minton 1996), can functionally substitute for their counterparts in D. radiodurans.

However, genetic, biochemical and structural studies have shown that several other ‘classical’ DNA repair proteins from D. radiodurans have characteristics different from their E. coli counterparts. Concerning recombinational repair, E. coli recA (Schlesinger 2007) and recO (Xu et al. 2008) only partially complement the corresponding gene deletion in D. radiodurans. In contrast to E. coli RecA, purified D. radiodurans RecA preferentially binds to double-stranded DNA when also single-stranded DNA is present in the solution, and initiates DNA-strand exchange primarily from the double-stranded DNA (Kim and Cox 2002; Kim
et al. 2002). Such inverse DNA-strand exchange pathway has also been observed for D. geothermalis RecA in one biochemical study (Sghaier et al. 2010) but not in another (Wanarska et al. 2011), possibly because of different experimental conditions. More recent studies have indicated that D. radiodurans RecA forms more frequent but shorter filaments compared to E. coli RecA, and that the specific properties of D. radiodurans RecA contribute to efficient repair of hundreds of double-stranded DNA breaks (Hsu et al. 2011; Ngo et al. 2013; Pobegalo et al. 2015; Warfel and LiCata 2015). Processing of double-stranded DNA ends by the RecFOR pathway requires RecQ helicase in E. coli, but characterisation of mutant strains suggests that D. radiodurans might use UvrD helicase rather than its RecQ protein for this process (Bentchikou et al. 2010). In addition, unlike its E. coli counterpart, UvrD of D. radiodurans is a bipolar DNA helicase that can unwind both 3’- and 5’-tailed double-stranded DNA in vitro (Stelter et al. 2013). Deinococcus radiodurans RecF also interacts with DR_1088, a DNA-binding protein that is encoded by the recF-DR_1088 operon but which is absent in E. coli (Cheng et al. 2017). Levels of single-stranded DNA-binding protein (SSB) are higher in D. radiodurans than in E. coli (Bernstein et al. 2004). Concerning BER, mismatch-specific uracil DNA glycosylase DR_0715 (MUG) has a modified and broadened substrate specificity compared with MUG from E. coli (Moe et al. 2006), DR_0689 uracil DNA glycosylase (Ung, COG0692) of D. radiodurans has high catalytic activity attributed to high substrate affinity (Timmins and Moe 2016), and DNA-3-methyladenine glycosylase 2 family protein DR_2584 (AlkA, COG0122) has altered substrate specificity and a wider DNA-binding cleft compared with E. coli AlkA (Moe et al. 2012). Furthermore, D. radiodurans MutS has higher affinity for mismatched DNA than E. coli MutS (Banasik et al. 2017), and DnaE polymerase of D. radiodurans, but not that of E. coli, features RecA-dependent DNA polymerase activity (Randi et al. 2016). Thus, besides increased protein protection, DNA repair systems may also have evolved to perform better under stress conditions that generate massive DNA damage. This is supported by experiments with E. coli, for which radiation-resistant strains surviving 3 kGy were obtained after repeated exposure to IR (Byrne et al. 2014). In these strains, mutations in recA are prominent and contribute to the acquired radiation resistance (Piechura et al. 2015).

Deinococcus radiodurans also encodes more than one variant of several DNA repair proteins (e.g. multiple uracil DNA glycosylases and endonuclease III proteins), and these variants may have specialised roles that improve the DNA repair repertoire (Sandigursky et al. 2004; Timmins and Moe 2016). Moreover, for various novel proteins more specific to Deinococcus it has been

Figure 2. Schematic overview of ionising radiation and oxidative stress resistance-associated proteins in D. radiodurans. Many D. radiodurans gene deletion or disruption mutants with more than 10-fold increased sensitivity compared to the wild-type strain have been described (Table S1, Supporting Information), and the corresponding proteins are indicated in the figure. Red box, ionising radiation sensitive; green box, oxidative stress sensitive; blue box, ionising radiation and oxidative stress sensitive.
demonstrated or proposed that they contribute to DNA repair or genome preservation (e.g. DdrA to DdrD, PrpA, DR.A0282) (Selvam et al. 2013; Agapov and Kulpachinskii 2015; Bouthier de la Tour et al. 2017).

Analysis of the genome sequence also revealed that D. radiodurans lacks homologues of several well-known DNA repair proteins, indicating that it does not use some repair mechanisms or that it uses alternative mechanisms. Initiation of homologous recombination in E. coli involves either the RecBCD complex, its major pathway for double-strand break repair, or the RecFOR pathway (Rocha, Cornet and Michel 2005). However, recB and recC are absent in D. radiodurans and it uses the RecFOR pathway for processing of double-stranded DNA ends (Bentchikou et al. 2010). In E. coli, the RecFOR pathway is inhibited by SbcB (Exodeoxyribonuclease I) (Kowalczykowski et al. 1994), and D. radiodurans lacks SbcB. Overexpression of E. coli RecBC (Khairnar, Kamble and Misra 2008) or SbcB (Misra et al. 2006) in D. radiodurans leads to reduced resistance to IR and interferes with DNA double-strand break repair. In addition to homologous recombination, several bacteria use non-homologous end joining (NHEJ) to repair DNA double-strand breaks, but there is no evidence that this generally error-prone repair system exists in D. radiodurans (Slade and Radman 2011). Deinococcus radiodurans also misses specialised translesion synthesis (TLS) DNA polymerases such as UmuCD that, in E. coli, are involved in mutagenic lesion bypass. Thus, the absence of certain DNA repair proteins may be important for efficient and error-free repair of massive DNA damage in D. radiodurans.

DNA repair proteins in 11 Deinococcus species: overview

To get more insight in the DNA repair repertoire in the genus Deinococcus, the DNA repair genes in 10 other radiation-resistant Deinococcus species were searched and compared with that of the well-studied D. radiodurans. The conservation of novel proteins possibly involved in DNA repair is described in the sections ‘The Ddr and Ppr proteins’ and ‘Miscellaneous proteins involved in resistance to radiation and other DNA-damaging agents in Deinococcus’. Most genes for important DNA repair mechanisms are highly conserved, whereas homologues of several DNA repair genes, such as recC and sscB and genes for the NHEJ proteins LigD and Ku, are absent in each analysed Deinococcus species. Interestingly, we also observed many differences regarding protein presence/absence, domain composition or numbers of protein variants (see Table 2 for the main differences among the 11 Deinococcus species, and Table S2 (Supporting Information) for accession numbers of all DNA repair proteins). Homologues of several D. radiodurans DNA repair proteins are absent in some of the other species, whereas some other proteins lacking in D. radiodurans are present in others. Intriguingly, the latter include three proteins that, compared to D. radiodurans and E. coli, contain novel combinations of two domains within a single protein: AdaA-AlkA, PhrB-Ung and Nth-Dcm (Fig. 3). The conservation or diversity across the Deinococcus species of DNA repair proteins for different DNA repair pathways is described in detail in the following sections. Here, as an overview, the presence or absence of the DNA repair proteins in the Deinococcus species compared with D. radiodurans is as follows:

(i) Present at least once in each species are AlkA, Mpg, MutY, Mug, Ung (fused or not to PhrB), Fpg, Nth, XthA, Mfd, UvrA1, UvrB, UvrC, UvrD, UvsE, MutL, MutS1, MutS2, XseA, XseB, Ati1 (Yba2), RdgB (YggV), RecA, RecD, RecF, RecF-interacting DR_1088 homologue, RecG, RecJ, RecN, RecO, RecQ (or absent in D. geothermalis), HRC domain protein, RecX, RadA, RuvA, RuvB, RuvC, SscB, SscD, SSB, LigA, Gyra, Gyrb, TopA (topoisomerase 1), Top1 (topoisomerase IB), PolA, PolX, RarA.

(ii) Absent in each are Tag, Nfo, Cho, MutH, AlkB, RecC, RecE, RecT, SbcB, RadC, LigD, Ku, TopB, UmuCD.

(iii) Present in D. radiodurans but not in each of the other species are Udg4, putative Udg DR_0022, Nfl, Uvra2, Sssl2 DNA or RNA helicase, HelD (DNA helicase IV), HepA (SNF2 family helicase), Dj-1 family deglycase, nuclease-related domain (NERD) protein.

(iv) Absent in D. radiodurans but present in one or more of the other Deinococcus species are AlkD, family 5 Udg, Dam, Dcm, Vsr, Ada, PhrB, SpLb, Dut, Dcd, RecB/AddA, RusA, Exo, Xni, NucS, PolB, DnaE2, ImuY, DinP, and the two-domain proteins AdaA-AlkA, PhrB-Ung and Nth-Dcm.

BER, MMR, direct reversal and novel two-domain proteins

Deinococcus radiodurans and the other Deinococcus species encode multiple DNA glycosylases. Each of these species contains one or two genes for 3-methyladenine DNA glycosylase Mpg (COG2094), one gene (two in D. peraridilitoris) encoding DNA-3-methyladenine glycosylase 2 (AlkA, COG0122) and one (two in D. geothermalis) encoding mismatch-specific uracil DNA glycosylase (Mug, COG3663) (Table 2 and Table S2, Supporting Information). Interestingly, D. punicus additionally encodes a 3-methyladenine DNA glycosylase AlkD (COG4912), and three other species have a second AlkA in which the AlkA domain is fused to the AdaA domain (see also below). The single Mpg of D. radiodurans is very similar (more than 70% identity) to an Mpg in eight of the other species, but less similar to others (Fig. 4) (e.g. the single Mpg of D. peraridilitoris shares only 30% identity with D. radiodurans Mpg). The novel catalytic residue (Asp93 in DR_0715) identified in D. radiodurans Mug (Moe et al. 2006) is also present in the other Mug proteins except for the second, less-conserved homologue in D. geothermalis. Besides Mug, D. radiodurans possesses three other predicted uracil DNA glycosylases: DR_0689 (Ung, COG0692), DR_1751 (Udg4, COG1573) and DR_0022 (no COG). In vitro uracil DNA glycosylase activity was demonstrated for DR_0689 and DR_1751, but not detected for DR_0022, and the majority of the in vivo uracil DNA glycosylase activity seemed to result from DR_0689 expression (Sandigursky et al. 2004). Remarkably, a DR_0689 homologue of similar size was not found in D. proteolyticus, D. actinosclerus and D. soli and D. suwensii. However, these four species, as well as D. gobiensis, do contain a protein in which the Ung domain is fused to a photolyase domain (PhrB, COG0415) (Fig. 3). BLASTP analysis revealed that the PhrB-Ung fusion is unique to Deinococcus species. Besides missing a standalone Ung, D. actinosclerus and D. soli also lack Udg4 uracil DNA glycosylase, indicating that uracil repair in these two organisms depends on Mug and the PhrB-Ung fusion protein. DR_1751 (Udg4) homologues are present in seven other species, and D. peraridilitoris in addition encodes a family 5 Udg. No homologue of the putative uracil DNA glycosylase DR_0022 was found in the other Deinococcus species.

Deinococcus radiodurans possesses three endonuclease III proteins (Nth; DR_0289, DR_2438, DR_0928). The nth single, double and triple mutants are as resistant to IR and H2O2 as the wild type, but each single mutant shows slightly elevated levels of spontaneous mutation (Hua et al. 2012). In vitro, enzymatic activity has been detected for DR_0289 and
Table 2. Main differences regarding DNA repair-related proteins in Deinococcus species.

| Protein                                      | Drad | Dgeo | Ddes | Dmar | Dgob | Dpro | Dper | Dswu | Dsol | Dact | Dpun |
|----------------------------------------------|------|------|------|------|------|------|------|------|------|------|------|
| BER, MMR, direct reversal and novel two-domain proteins |      |      |      |      |      |      |      |      |      |      |      |
| Mpg                                          | 1    | 1    | 1    | 2    | 2    | 1    | 1    | 2    | 2    | 1    |
| AlkD                                         | 1    | 1    | 1    | 1    | 1    | 1    | fr   |      |      |      |      |
| Ung                                          | 1    | 1    | 1    | 1    | 1    | 1    |      |      |      |      |      |
| Udg4                                         | 1    | 1    | 1    | 1    | 1    | 2    | 1    |      |      |      |      |
| Udg 4 DR                                     |      |      |      |      |      |      |      |      |      |      |      |
| N6                                           | 1    | 1    | 1    | 1    | 1    |      |      |      |      |      |      |
| XthA                                         | 2    | 2    | 1    | 1    | 1    | 2    | 1    | 1    | 1    | 1    |
| Dam                                          | 1    | 1    |      |      |      |      |      |      |      |      |      |
| Dcm                                          |      |      |      |      |      |      |      |      |      |      |      |
| DR 4_020                                     | 1    |      |      |      |      |      |      |      |      |      |      |
| Vsr                                          | 1    | 1    | 1    | 1    |      |      |      |      |      |      |      |
| Ada                                          | 1    |      |      |      |      |      |      |      |      |      |      |
| PhrB                                         |      |      |      |      |      |      |      |      |      |      |      |
| SpLB                                         | 1    | 1    | 1    |      |      |      |      |      |      |      |      |
| AdaA-AlkA                                     | 1    |      |      |      |      |      |      |      |      |      |      |
| PhrB-Ung                                     | 1    | 1    | 1    | 1    |      |      |      |      |      |      |      |
| Nth-Dcm                                      | 1    |      |      |      |      |      |      |      |      |      |      |
| Dut                                          |      |      |      |      |      |      |      |      |      |      |      |
| Dcd                                          | 1    | 1    | 1    | 1    | 1    | 1    | 1    |      |      |      |      |
| Deglycase                                    | 2    | 1    | 1    | 1    | 1    | 2    | 1    | 1    | 2    | 2    |      |
| Nucleotide excision repair                   |      |      |      |      |      |      |      |      |      |      |      |
| UvrA2                                        | 1    | 1    | 2    | 1    | 1    | 1    |      |      |      |      |      |
| SSL2 helicase                                | 1    |      |      |      |      |      |      |      |      |      |      |
| RecA                                         | 1    | 2    | 1    | 1    | 2    | 1    | 1    | 1    | 1    |      |      |
| RecB/AddA                                    |      |      |      |      |      |      |      |      |      |      |      |
| RecQ                                         |      | fr  | 1    | 1    | 2    | 1    | 1    | 1    | 1    | 1    | 1    |
| RusA (YbcP)                                  |      |      |      |      |      |      |      |      |      |      |      |
| SS1                                          | 1    | 4    | 1    | 1    | 3    | 4    | 1    | 1    | 1    | 1    | 1    |
| Ligases and adjacent genes                   |      |      |      |      |      |      |      |      |      |      |      |
| LigA                                         | 1    | 1    | 2    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |      |
| DR 4_0100                                    | 1    |      |      |      |      |      |      |      |      |      |      |
| DR 4_0099                                    |      |      |      |      |      |      |      |      |      |      |      |
| DR 4_0098                                    |      |      |      |      |      |      |      |      |      |      |      |
| DR 4_0094                                    |      |      |      |      |      |      |      |      |      |      |      |
| DR 4_0095                                   | fr  |      |      |      |      |      |      |      |      |      |      |
| Other DNA repair proteins                    |      |      |      |      |      |      |      |      |      |      |      |
| TopA                                         | 1    | 1    | 2    | 3    | 2    | 2    |      |      |      |      |      |
| Exo (Xni)                                    |      |      |      |      |      |      |      |      |      |      |      |
| NERD domain                                  | 1    |      |      |      |      |      |      |      |      |      |      |
| NucS (EndoS)                                 | 1    | 2    | 1    | fr   |      |      |      |      |      |      |      |
| PolB                                         | 1    | 1    | 1    |      |      |      |      |      |      |      |      |
| DNAE2                                        |      |      |      |      |      |      |      |      |      |      |      |
| ImuY                                         |      |      |      |      |      |      |      |      |      |      |      |
| DinP                                         |      |      |      |      |      |      |      |      |      |      |      |
| HeliD                                        |      |      |      |      |      |      |      |      |      |      |      |
| HepA                                         | 2    | 2    | 2    | 1    | 1    | 1    |      |      |      |      |      |

fr, frameshift. Names in **bold** indicate gene inactivation leading to increased sensitivity of *D. radiodurans* to radiation or oxidative stress in at least one study.

DR 2438, but so far not for DR 0928 (Sarre et al. 2015). Homologues of these three Nth proteins are present in the other analysed Deinococcus species, except for *D. peraridilitoris* that lacks a DR 0928 homologue. *Deinococcus suesens* has in addition a protein in which the Nth domain is combined with a DNA-cytosine methylase domain (Dcm, COG0270) (Fig. 3). BLASTP analysis revealed only a few Nth-Dcm fusion proteins in other genera (e.g. protein AYO40_02595 of Planctomycetaceae bacterium).

Compared to *D. radiodurans*, the presence of additional Mpg, AlkA, AlkD, Mug, Udg and two-domain proteins AdaA-AlkA, PhrB-Ung and Nth-Dcm in several species further increases the diversity of DNA glycosylases in Deinococcus. It will be of particular interest to elucidate the precise function(s) of the three novel two-domain proteins.

Besides the Nth-Dcm fusion in *D. suesens*, some Deinococcus species possess genes encoding homologues of Dcm and/or DNA-adenine methylase
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Figure 3. Novel two-domain proteins. The canonical DNA repair proteins AlkA, PhrB, Ung, Nth and Dcm and carotenoid biosynthesis proteins CrtY and CruF are standalone proteins. Genes encoding fusions of two of these proteins were identified in several Deinococcus species. The total number of amino acid residues (aa) of the novel two-domain proteins is indicated at the right.

(Dam, COG0338). However, a homologue of DR_C0020 from D. radiodurans, encoding another DNA methylase (COG0863) and whose inactivation results in reduced IR resistance (Table S1, Supporting Information), is not present in the other Deinococcus species.

The bifunctional transcriptional activator/DNA repair enzyme Ada of E. coli is composed of an N-terminal domain AdaA (COG2169, Methylphosphotriester-DNA–protein–cysteine methyltransferase) and a C-terminal domain AdaB (COG0350, O6-methylguanine-DNA–protein–cysteine methyltransferase). D. maricopensis encodes a similar Ada protein. D. gobiensis, D. actinosclerus and D. soli possess the aforementioned novel two-domain protein in which the AdaA domain is not fused with AdaB but with AlkA (Fig. 3). Such AdaA-AlkA fusion is also found in species from various other genera (e.g. protein Rv1317c of Mycobacterium tuberculosis). Other proteins for direct reversal of damage, and which are absent in D. radiodurans but found in others, include homologues of deoxyribodipyrimidine photolyase PhrB (not fused to Ung), photoproduct lyase family protein (SpIB, COG1533) and deoxycytidine triphosphate deaminase (Dcd) (Table 2).

Recently, a novel important nucleotide repair system, named guanine glycation repair, has been described (Richarme et al. 2017). For this system, it has been shown that the parkinsonism-associated protein DJ-1 and its E. coli homologues Hsp31 (HchA), YhbO and YajL can repair methylglyoxal- and glyoxal-glycated nucleotides, RNA and DNA. These DJ-1/PfpI family proteins, containing domain COG0693 (Thl, putative intracellular protease/amidase), are also protein deglycases that can repair methylglyoxal- and glyoxal-glycated proteins. Homologues are present in most Deinococcus species, but remarkably not in D. punicus. The YhbO homologue DR_1199 of D. radiodurans has been studied previously. Although initially annotated as protease I, no proteolytic or chaperone activity was detected for DR_1199 (Fioravanti et al. 2008), in line with the more recently identified deglycase activity of such proteins.

Nucleotide excision repair

Deinococcus radiodurans possesses two NER pathways for repair of UV-induced DNA damage, the UvrA- and UvsE-dependent pathway, and both are conserved in the other Deinococcus species. A D. radiodurans uvrA1 uvsE mutant is very sensitive to UV (Earl et al. 2002b; Tanaka et al. 2005). Except for D. geothermatis, the other Deinococcus species have one or two additional UvrA-related proteins, UvrA2. Deinococcus radiodurans UvrA2 has structural similarity with UvrA1 (Timmins et al. 2009), but UvrA2 does not contribute to UV resistance in D. radiodurans (Tanaka et al. 2005).

Recombinational DNA repair

The proteins involved in recombinational DNA repair in D. radiodurans, such as Rec, Ruv and SSB proteins, are highly conserved in the other Deinococcus species. Nevertheless, there is some interesting diversity among the bacteria. Concerning the genetic

Figure 4. Three groups of 3-methyladenine DNA glycosylase (MPG) proteins identified in 11 Deinococcus species. The phylogenetic analysis was carried out based on protein sequence alignment of 16 deinococcal MPG proteins (Table S2, Supporting Information) made with Clustal omega. GenBank accession numbers in parentheses follow the species name. The phylogenetic tree was developed using the neighbour-joining algorithm in MEGA 6.0. The scale indicates the number of amino acid substitutions per site, and the node numbers are bootstrap values based on 1000 replications.
organisation of the recA gene, each Deinococcus species contains a cia-ligT-recA gene cluster (probably operon in each), except D. proteolyticus that misses ciaA and has a ligT-recA operon (cia codes for competence/damage-inducible protein A; ligT encodes LigT-like RNA 2′,3′-cyclic phosphodiesterase, originally identified as 2′-5′ RNA ligase). Whereas the majority of the bacteria possess only one RecA, D. deserti and D. peraridilitoris have two different RecA proteins, encoded by three and two different genes, respectively (Table S2, Supporting Information) (see also the section ‘DNA repair proteins lacking in D. radiodurans but present in other deinococci’). The extra recA genes are not within an operon. Like the cia-ligT-recA operon, the additional recA genes in D. deserti (de Groot et al. 2014), and probably also in D. peraridilitoris (Blanchard et al. 2017), are radiation-induced. BLASTP analysis revealed that the extra RecA (RecA2) from both D. deserti and D. peraridilitoris are most similar to RecA proteins from Deinococcus species (e.g. D. radiodurans), suggesting that these RecA2 are of deinococcal origin. However, the two RecA2 proteins do not form a subgroup in a phylogenetic tree (Fig. S1, Supporting Information).

Unlike the other Deinococcus species, D. suwensis codes for a helicase and exonuclease domain-containing protein that is similar to E. coli RecB and Bacillus subtilis AddA, albeit with about 29% identity only. The heterodimer AddAB of B. subtilis, encoded by the addA operon, is a functional homologue of the E. coli RecBCD enzyme (Kooistra, Hajjema and Venema 1993). The RecB/AddA-like protein of D. suwensis shares more than 60% identity with protein fragments from a D. deserti pseudogene that contains two internal stop codons (de Groot et al. 2009). Interestingly, both the D. deserti pseudogene and the recB/addA-like gene of D. suwensis are preceded by a gene coding for a protein that is weakly similar to AddB and that includes a nuclease domain. It will be interesting to investigate if this gene pair from D. suwensis encodes a helicase–nuclease complex with a function similar to AddAB in processing of double-stranded DNA ends.

DNA helicase RecQ is important for genome maintenance and DNA repair in a variety of organisms, including E. coli and humans. RecQ contains a catalytic core for ATP-dependent helicase activity and an HRDC (Helicase-and-RNase-D C-terminal) domain involved in DNA binding. Whereas most RecQ proteins have only one HRDC domain, D. radiodurans RecQ has three HRDC domains at its C-terminal region, and in vitro and in vivo studies have shown that all three are involved in RecQ function (Killoran and Keck 2006; Huang et al. 2007). In vivo studies have also shown that a D. radiodurans recQ mutant is slightly sensitive to IR and very sensitive to UV, mitomycin C (MMC) and H₂O₂. (Huang et al. 2007). However, another study has demonstrated that RecQ is not required for IR resistance and for repair of double-strand DNA breaks in D. radiodurans (Bentchikou et al. 2010). Therefore, the exact role(s) of RecQ in D. radiodurans is unclear. RecQ-encoding sequences are present in all other Deinococcus species. However, the recQ sequence of D. geothermalis contains a frameshift at one position and an internal stop codon at another position. If these are not DNA sequencing errors, D. geothermalis may not produce an intact RecQ protein. Furthermore, only D. radiodurans RecQ contains three HRDC domains, while one or two HRDC domains are present in the RecQ homologues from the other species (Fig. S2, Supporting Information). Deinococcus peraridilitoris RecQ has in addition a C-terminal helix-turn-helix domain. Another HRDC-domain containing protein (DR_2444 in D. radiodurans), in which the HRDC domain is not associated with a helicase domain, is conserved in Deinococcus, but its function is unknown. RecQ-like proteins containing helicase but not HRDC domains are also present in several Deinococcus species (Fig. S2, Supporting Information).

Deinococcus radiodurans and several bacteria from other genera possess recD although recB and recC are absent. The RecD helicases in these species have an N-terminal extension of about 200 residues compared to E. coli RecD, and have been called RecD2. As for RecQ, conflicting results have been published regarding radiation resistance of a D. radiodurans recD mutant (Table S1, Supporting Information). Although the requirement of RecD for radiation resistance is not clear, it probably has an important in vivo role in Deinococcus species because the protein, including the N-terminal extension, is highly conserved. Other DNA helicases such as UvrD and RecG are also highly conserved. Several Deinococcus species encode additional but less conserved variants of some helicases, including UvrD/REP- and RecD-like proteins (Table S2, Supporting Information).

**Ligases**

Like in other bacteria, a gene encoding NAD-dependent DNA ligase (LigA) is present in each Deinococcus. Deinococcus deserti expresses two different LigA proteins that share 57% identity (de Groot et al. 2009). Deinococcus radiodurans also contains a gene (DR_0510, also known as ligB or ddrP) predicted to encode an ATP-dependent DNA ligase (Liu et al. 2003). DR_0510 is the first gene of a radiation-inducible operon also encoding DR_0509 (poly ADP-ribose glycohydrolase) and DR_0508 (nucleotide kinase) (Blasius et al. 2007; Slade et al. 2011). A DR_0510 mutant is IR resistant as the wild type according to one study (Makarova et al. 2007), but sensitive according to another (Kota et al. 2010). The latter study has also reported that functional complementation of the DR_0510 deletion requires expression in trans of the entire operon, and that in vitro DNA ligase activity by DR_0510 requires the presence of DR_0509 as well as another radiation-induced protein, PprA (pleiotropic protein promoting DNA repair; see also section ‘The Ddr and Ppr proteins’). Recently, it has also been described that DR_0509 is required for IR resistance, and that the DR_0509 mutant is equally IR sensitive as the mutant lacking the entire operon (Schmier and Shuman 2018). Homologues of DR_0510, DR_0509 and/or DR_0508 are present in only a few other Deinococcus (Table 2), with D. gobiensis, D. actinoclaserus and D. soli possessing a putative operon composed of DR_0510 and DR_0508 homologues (Fig. S3, Supporting Information). In D. maricopensis, homologues of all three genes are present, but at different locations on its chromosome. Not far downstream of the ligB operon in D. radiodurans is another gene with a reported role in radiation resistance. This gene, DR_0504 (rnl), encodes a nick-sealing RNA ligase. Recent results indicate that Rnl is involved in DNA repair. Inactivation of rnl sensitises D. radiodurans to radiation and also results in a delay of genome reconstitution following exposure to IR (Schmier et al. 2017). However, a DR_0504 homologue is present in only two of the other Deinococcus species, D. punicus and D. gobiensis. In the latter, rnl is located adjacent to ligB. D. radiodurans rnl is the first gene of a probable operon also containing DR_0504. Inactivation of DR_0505 also results in increased sensitivity to IR (Schmier et al. 2017). Sequence analysis suggests that DR_0505 contains a frameshift, and that the entire gene is predicted to encode an exonuclease (homologue in D. maricopensis).

**Multiple variants of a DNA repair protein**

For a dozen DNA repair proteins with only one variant in D. radiodurans, more than one variant exists in several other Deinococcus
species. Besides some of multiple variants mentioned above (e.g. for RecA, RecD, Mpg), another example is DNA topoisomerase I (Escherichia coli DNA topoisomerase I (TopA, 865 amino acids) contains the conserved domains COG0550 (TopA, DNA topoisomerase IA) and COG0551 (YrdD, ssDNA-binding Zn-finger and Zn-ribbon domains of topoisomerase 1) at the N- and C-terminal region, respectively. Deinococcus radiodurans DNA topoisomerase I (DR_1374) is conserved in the other Deinococcus species, but in these deinococcal proteins (of about 1000 residues) the COG0550 domain is not followed by COG0551 but by COG1754 (uncharacterised C-terminal domain of topoisomerase IA). Four Deinococcus species encode one or two additional topoisomerase I proteins (of about 670 amino acids) that contain only the COG0550 domain (Table S2, Supporting Information). Two of these additional topoisomerase I genes, DGoPC0276 in D. gobiensis and Deipr_2353 in D. proteolyticus, are directly followed by a gene encoding a UvrD/REP-like helicase, indicating a possible functional link.

DNA repair proteins lacking in D. radiodurans but present in other deinococci

About 20 DNA repair-related genes are present in one or several Deinococcus species but absent in D. radiodurans (Table 2). These include genes for error-prone DNA polymerases PolB, DinP, ImuY and DnaE2. For D. deserti it has been shown that an operon containing lexA-imuY-dnaE2 is involved in UV-induced mutagenesis (Dulermo et al. 2009). This operon, which is similar to a RecA/LexA-controlled mutagenesis cassette identified in various bacteria (Erill et al. 2006), is also present in desert isolate D. peraridilitoris. If induced mutations are advantageous, for example by changing characteristics of a protein or by generating transcripts encoding small peptides (see section ‘Oxidative stress defence in Deinococcus’), they may contribute to adaptation to harsh environments such as deserts. Therefore, unlike believed earlier (Sale 2007), absence of error-prone TLS DNA polymerases is not crucial for extreme radiation resistance.

Endonuclease NucS (COG1637) is another protein absent in D. radiodurans but found in seven of the other analysed Deinococcus species (although nucS of D. peraridilitoris has a frameshift) (Table 2). NucS has initially been described in the archaeon Pyrococcus abyssi and identified as a novel, DNA structure-dependent endonuclease for single-stranded DNA (Ren et al. 2009). However, more recently it has been demonstrated that NucS is a mismatch-specific endonuclease acting on double-stranded DNA containing mismatched bases, and that it is required for a non-canonical MMR pathway in prokaryotes as an alternative to the canonical MutS/L-based MMR (Ishino et al. 2016; Castaneda-Garcia et al. 2017). Therefore, NucS has also been named EndoMS (mismatch-specific Endonuclease) (Ishino et al. 2016). Scanning of 3942 reference proteomes has revealed the presence of NucS in 60 archaeal and 310 bacterial species, with the majority of NucS-containing bacterial species (303) belonging to the phylum Actinobacteria and the remaining to the phylum Deinococcus-Thermus (Castaneda-Garcia et al. 2017). Most NucS-encoding species lack MutS and MutL. The presence of both NucS and MutS-MutL has been found in only 28 species (Castaneda-Garcia et al. 2017), and these include the seven nucS-containing Deinococcus species in Table 2. In the archaeon Halobacterium salinarum, which encodes both NucS and MutS-MutL, inactivation of mutS or mutL produced no hypermutability (Busch and DiRuggiero 2010), suggesting redundancy of the two MMR pathways, which may also be the case in the Deinococcus bacteria possessing both systems. Interestingly, the Deinococcus species with nucS are the same as those possessing a dinP gene encoding error-prone DNA polymerase IV (Table 2), suggesting the possibility that NucS might counteract potential replication errors introduced by PolIV.

In summary, the comparison of the 11 genomes shows a remarkable diversity regarding DNA repair genes among Deinococcus species. As has been proposed for the unusually high number of DNA glycosylases in D. radiodurans, additional DNA repair proteins or variants in other Deinococcus species may contribute to efficient error-free DNA repair and stress survival in these organisms. Interestingly, the presence of different error-prone DNA polymerases indicates that there is also diversity in mechanisms generating genetic variability in Deinococcus species.

OXIDATIVE STRESS DEFENCE IN DEINOCCUS

IR induces DNA damage in cells either via direct (energy deposition in the deoxyribose moiety) or indirect (water radiolysis generating ROS) action. Since ROS including superoxide (O2·−), hydrogen peroxide (H2O2) and hydroxyl radicals (OH·) can damage not only DNA but also other macromolecules such as proteins, radiation-resistant organisms should develop efficient anti-oxidative system to cope with oxidative stress. The model organism D. radiodurans has some metabolic configurations that suppress endogenous ROS production, such as the relatively low number of respiratory chain enzymes, the import of peptides and amino acids, and the induction of the glyoxylate bypass of the tricarboxylic acid cycle following IR (Ghosal et al. 2005; Slade and Radman 2011). In addition, D. radiodurans is well equipped with enzymatic and non-enzymatic systems to curb ROS levels (Slade and Radman 2011). Here, we compared proteins involved in these direct anti-oxidative systems across 11 Deinococcus species. The differences regarding oxidative stress defence-related proteins between these species are shown in Table 3. Besides these differences, each of the analysed Deinococcus genomes encodes one homologue of SodA, MsrA, MsrB, HslO (Hsp33), DR_80067 extracellular nuclease, FmE (truncated in D. maricopensis), Dps1, MsrP, MsrQ, Pkp1 (frameshift in D. maricopensis), Pkp2, Ppx, Crate, -B, -I, -D, -O, BshA, -B, -C, YpdA, Ybtx (Table S3, Supporting Information). Genes that are absent in each of the 11 genomes include sodB, katG, tpx, grxB, grxD, ahpC and ahpF.

Enzymatic systems for oxidative stress defence

Superoxide dismutases

Superoxide dismutases (SODs) are metalloenzymes that catalyse the disproportionation of O2− to give H2O2 and O2 using a redox-active metal. SOD is classified according to metal cofactor into the manganese-containing SOD (MnSOD), the iron-containing SOD (FeSOD) and the copper/zinc-cofactored type (CuZnSOD) (Imlay 2013). The accepted nomenclature for bacterial SODs is SodA, SodB and SodC for the Mn-, Fe- and Cu/Zn-SODs, respectively (Broxton and Culotta 2016). Escherichia coli contains three SODs: two cytoplasmic SODs, SodA and SodB, and the periplasmic SOD (Imlay 2013). Deinococcus radiodurans has one cytoplasmic SodA (DR_1279) and two periplasmic SodCs (DR_1546 and DR_4020). FeSOD (SodB) is not present in D. radiodurans or any of the other Deinococcus species analysed. SodA is present constitutively at high levels in D. radiodurans (Basu and Apte 2012) and is well conserved across all Deinococcus species analysed, suggesting that SodA plays an important role in superoxide detoxification in Deinococcus. However, a sodA mutant strain of D. radiodurans is only slightly sensitive to very high...
Table 3. Differences regarding oxidative stress defence-related proteins in Deinococcus species.

| Protein                                      | Drad | Dgeo | Ddes | Dmar | Dgob | Dpro | Dper | Dswu | Dsol | Dact | Dpun |
|----------------------------------------------|------|------|------|------|------|------|------|------|------|------|------|
| Superoxide dismutases and catalases          |      |      |      |      |      |      |      |      |      |      |      |
| SodC (DR_1546)                               | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| SodC (DR_A0202)                              | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| KatE (clade 1)                               | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| KatE (clade 2)                               |      |      |      |      |      |      |      |      |      |      |      |
| KatE (clade 3)                               | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| MnCat                                        |      |      |      |      |      |      |      |      |      |      |      |
| DR_A0146 (kat-like)                          |      |      |      |      |      |      |      |      |      |      |      |
| Peroxiredoxins, Prx-related proteins, and other peroxidases |      |      |      |      |      |      |      |      |      |      |      |
| BCP                                          | 3    | 3    | 2    | 3    | 3    | 2    | 3    | 3    | 4    | 4    | 4    |
| AhpE                                         | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 2    | 1    | 1    | 1    |
| AhpD                                         | 1    | 1    | 2    | 1    | 1    | 1    | 5    | 1    | 1    | 1    | 1    |
| DR_A0145 (EfeB)                              |      |      |      |      |      |      |      |      |      |      |      |
| OsmC                                         | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| Ohr                                          | 1    | 1    | 2    | 2    | 2    | 1    | 1    | 1    | 1    | 1    | 1    |
| YhfA                                         | 1    | 1    | 2    | 1    | 1    | 1    | 2    | 1    | 1    | 1    | 1    |
| Thioredoxins, Trx reductase and glutaredoxin-like proteins |      |      |      |      |      |      |      |      |      |      |      |
| TrxA                                         | 1    | 1    | 1    | 1    | 1    | 1    | 3    | 1    | 1    | 1    | 1    |
| TrxC                                         | 1    | 1    | 1    |      |      |      |      |      |      |      |      |
| TrxR                                         |      |      |      |      |      |      |      |      |      |      |      |
| Grx/NrdH-like                                | 4    | 4    | 2    | 2    | 5    | 4    | 2    | 4    | 4    | 3    | 3    |
| Carotenoid                                   |      |      |      |      |      |      |      |      |      |      |      |
| CrtLm                                        | 1    | 1    | 1    | 1    | 1    | 1    |      | 1    | 1    | fr   | 1    |
| CrtY-CruF                                    |      |      |      |      |      |      |      |      |      |      |      |
| CruF                                         | 1    | 1    | 1    | 1    | 1    |      | 1    | 1    | 1    | 1    | 1    |
| CYP287A1                                     |      |      |      |      |      |      |      |      |      |      |      |
| Manganese transport                          |      |      |      |      |      |      |      |      |      |      |      |
| MnTH                                         | 1    | 1    | 1    | 1    | 1    |    | 3    | 1    | 1    | 1    | 1    |
| MntA                                         | 1    | 1    | 3    | 1    | 1    | 1    | 2    | 1    | 1    | 1    | 2    |
| MntB                                         | 1    | 1    | 5    | 1    | 1    | 1    | 3    | 1    | 1    | 1    | 1    |
| MntC                                         | 1    | 1    | 3    | 1    | 1    | 1    | 2    | 1    | 1    | 1    | 1    |
| MntE                                         | 1    | 1    | 1    | 2    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| Others                                       |      |      |      |      |      |      |      |      |      |      |      |
| Dps2                                         | 1    |      |      |      |      |      |      |      |      |      |      |
| FrnE                                         | 1    | 1    | 1    | 1    | 1*   | 1    | 1    | 1    | 1    | 1    | 1    |
| DsbA-like (DR_2335)                          | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| PqqE                                         |      |      |      |      |      |      |      |      |      |      |      |

*FrnE from D. maricopensis lacks an extended C-terminal tail.
See further the legend to Table 2.

doses of IR (Table S1, Supporting Information). Escherichia coli SodA and D. radiodurans SodA share a positively charged region located at the dimer interface providing DNA-anchoring loops (Dennis et al. 2006; Smolik et al. 2014). It has been proposed that interaction of SodA with DNA would be helpful in protecting a genome against radiation or oxidative attack in bacterial cells (Dennis et al. 2006; Smolik et al. 2014). Because $O_2^{-}$ cannot cross membranes (Imlay 2013), the extracytoplasmic location of the two D. radiodurans SodCs (Farci et al. 2014) implies that these enzymes may play a role in defending bacteria against oxidative stress from the surrounding environment. However, D. soli, D. peraridilitoris and D. geothermalis do not contain SodC. Besides its N-terminal SodC domain, the amino acid sequence of the DR_A0202-type SodC predicts a beta-propeller fold in the C-terminal domain (Fig. S4, Supporting Information). Moreover, DR_A0202 and the homologue in each of the other Deinococcus species are directly preceded, and probably forming an operon, by a gene encoding a predicted exported glucose/arabinose dehydrogenase, which also contains a beta-propeller fold. This conserved gene organisation suggests that their gene products may functionally interact.

**Catalases**

Catalase is a metalloenzyme that converts $H_2O_2$ to water and $O_2$. Catalases are divided into three families, namely typical (monofunctional) heme catalases (KatEs), (bifunctional) heme catalase-peroxidases (KatGs) and (non-heme) manganese catalases (MnCats) (Zamocky et al. 2012). Although D. radiodurans encodes two KatE-type catalases, DR_1998 (KatE1) and DR_A0259 (KatE2), and a eukaryotic-type catalase, DR_A0146, it was recently reported that DR_A0146 does not have catalase activity and that the highly expressed DR_1998 has a more critical role in detoxifying $H_2O_2$ than DR_A0259 (Jeong et al. 2016a). Most, but not all, of the Deinococcus species encode catalase, but there is remarkable diversity in the number and type of catalases across these species (Table 3). KatEs are subdivided into clades 1, 2 and 3 (Zamocky et al. 2012). As reported previously (Jeong et al. 2016a), clade 1, which includes DR_1998, is the most common catalase
in Deinococcus, but KatE catalases of clade 2 (e.g. DR_A0259) and clade 3 are also present in some Deinococcus species. The KatG-type catalase was not found in Deinococcus, but MnCat was found in D. proteolytica, D. peraridilitoris, D. maricopensis and D. gobiensis.

Interestingly, neither heme catalase (KatE and KatG) nor non-heme catalase (MnCat) was found in D. puniceus even though the radiation resistance of this species was comparable to that of D. radiodurans (Lee et al. 2015), indicating that catalase is not crucial for IR resistance in Deinococcus. Indeed, another study showed that there is no significant correlation between levels of catalase activity and IR resistance within seven Deinococcus species (Shashidhar et al. 2010). Nevertheless, moderately increased sensitivity, albeit at high doses only, has been reported for D. radiodurans mutants in which either DR_1998 or DR_A0259 was disrupted (Table S1, Supporting Information). To our knowledge, a strain lacking both DR_1998 and DR_A0259 has not been characterised. In contrast to the moderate effect at most on resistance to acute high IR dose, disruption of DR_1998 strongly sensitises D. radiodurans to high H2O2 concentrations (> 20 mM) (Jeong et al. 2016a). Interestingly, very recent data have indicated that DR_1998 is required for resistance of D. radiodurans to chronic IR (36 Gy/h) (Shuryak et al. 2017).

Peroxiredoxins

Peroxiredoxins (Prxs) represent a family of thiol-dependent peroxidases catalysing the reduction of H2O2 or organic hydroperoxides (Meyer et al. 2009), and include bacterioferritin comigratory protein (BCP), thiol peroxidase (Tpx) and alkyl hydroperoxide reductase (AhpC) (Mishra and Imlay 2012). RPS-BLAST was used to search the Deinococcus genomes for members of the PRX family (cd02971). Compared to E. coli encoding BCP, Tpx and AhpC (Meyer et al. 2009), Tpx and AhpC are absent in the 11 Deinococcus species. Besides BCP, however, the Deinococcus species analysed encode AhpE (DR_2242 in D. radiodurans), which is an atypical type of AhpC (Fig. S5, Supporting Information). AhpC is classified as 2-Cys Prx with conserved N-terminal peroxidatic CysP and C-terminal resolving CysS, but AhpE, which has been characterised in M. tuberculosis, contains no CysS (Perkins et al. 2015). Ahp is a two-component (AhpC/AhpF) peroxidase, and AhpF restores the disulphide in AhpC to its reduced form (Mishra and Imlay 2012). However, there is no AhpF homologue in Deinococcus species. In M. tuberculosis encoding AhpE, AhpD is known to substitute for AhpF (Lu and Holmgren 2014). Deinococcus radiodurans possesses the AhpD homologue (DR_1765), which is alkyl hydroperoxide D-like protein (COG2128, YciW), and this protein is observed in all other Deinococcus species. Surprisingly, D. peraridilitoris encodes five AhpD homologues. A comparison of AhpDs from M. tuberculosis and Pseudomonas aeruginosa, whose crystal structures have been determined (Koshkin et al. 2003; Clarke et al. 2011), shows that the important residues involved in the proton relay system, Glu118, Cys130, Cys133 and His137 (numbering based on AhpD from M. tuberculosis), are invariant for deinococcal AhpDs (except for the less conserved second AhpD-like protein of D. deserti that lacks the equivalent Glu and His) (Fig. S6, Supporting Information). These data suggest that AhpD may play a role as a reducing partner of AhpE in Deinococcus as shown in M. tuberculosis (Lu and Holmgren 2014).

BCP is a 1-Cys Prx, and its peroxidatic CysP is contained within a universally conserved PxxxT(S)xSxC motif (Lu and Holmgren 2014). Deinococcal BCPs can be divided into two groups (Fig. S5, Supporting Information), with the proteins in group 2 showing more sequence variation of the PxxxT(S)xSxC motif including a less conserved T(S). Group 1 including D. radiodurans DR_0846 is closer to the typical BCP from E. coli or B. subtilis than group 2, which includes DR_1208 and DR_1209. All Deinococcus species analysed encode one or two BCPs from each group (Table 3 and Fig. S5, Supporting Information).

Other peroxidases

In terms of antioxidant defences, a few peroxidases, such as catalase and Prx, play important roles because they have a primary purpose of reducing peroxides. In contrast, for a second group of peroxidases the primary purpose is to use the peroxide as an oxidising agent to oxidise a second molecule (Karplus 2015). Cytochrome c peroxidases (CCP) are heme enzymes that catalyse the two-electron reduction of H2O2 to water by accepting electrons from a soluble cytochrome c. CCP displays peroxidase activity in vitro but its physiological function seems to enable H2O2 to serve as an alternative respiratory electron acceptor in the absence of oxygen (Mishra and Imlay 2012). Only two Deinococcus species, D. radiodurans and D. proteolyticus, possess CCP (DR_A0301 in D. radiodurans). The D. radiodurans DR_A0145 protein, which was predicted to be an iron-dependent peroxidase (Slade and Radman 2011) and which is encoded by a gene located next to the catalase-like gene DR_A0146, is assigned to the COG2837 category for periplasmic deferoxochelatase/peroxidase EfeB. In E. coli, EfeB showed a deferrochelation activity, releasing iron from heme leaving the tetrapyrrolic intact (Letoffe et al. 2009). Homologues of DR_A0145 were not found in other Deinococcus species.

The OsmC (osmotically inducible protein C) family is divided into three subgroups: Ohr (organic hydroperoxide resistance protein), OsmC and YhfA (Shin et al. 2004). Among them, Ohr and OsmC have been identified as a new family of 2-Cys peroxidases (Zhang and Baseman 2014) because two additional residues (an Arg and a Glu) required for the peroxidatic activity are absent in YhfA (Shin et al. 2014). Deinococcus radiodurans encodes OsmC family proteins: DR_1857, DR_1538 and DR_1177 are homologues of Ohr, OsmC and YhfA, respectively. A sequence alignment of the deinococcal proteins belonging to the OsmC family revealed that the catalytic Arg and Glu residues are conserved in Ohr and OsmC but not in YhfA proteins (Fig. S7, Supporting Information). Although the conserved Arg residue is present at different positions in the sequences of Ohr and OsmC proteins, in the tertiary structures they occupy a similar orientation between the conserved Glu and Cys (Meireles et al. 2017). Ohr protein exhibits peroxidatic activity that is much more substantial against organic peroxides than against H2O2 itself (Mishra and Imlay 2012). In constrast, H2O2 appears to be a good substrate for OsmC (Zhang and Baseman 2014). The OsmC protein is conserved in Deinococcus species except for D. proteolyticus, while the Ohr homologue is absent in D. soli, D. deserti and D. actinosclerus. Two members of the Ohr subfamily are present in D. proteolyticus, D. maricopensis and D. gobiensis. The conservation of OsmC and the activity of OsmC towards H2O2 suggest that it plays a more important role in the resistance of Deinococcus to oxidative stress compared to Ohr as well as to CCP and DR_A0145.

Thioredoxins

The thioredoxin system, comprising NADPH, thioredoxin reductase (TrxR) and thioredoxin (Trx), is a major disulphide reductase system, which can provide electrons to a large range of enzymes, and is found to be critical for DNA synthesis and defence against oxidative stress in diverse organisms (Lu and Holmgren 2014). CDD search with RPS-BLAST showed that D. radiodurans contains two Trxs (cd02947) with classic active site motif CGPC, DR_0944 and DR_A0164, which are similar to thioredoxin 1 (trxA)
Ribonucleotide reductases (RNRs) are essential enzymes catalysing conversion of the four ribonucleotide triphosphates (NTPs) into their corresponding dNTPs necessary for DNA replication and repair (Torrents 2014). Until now, three different RNR classes have been described (I, II and III), and class I is further subdivided into la (NrdA/B) and lb (NrdEF) (Torrents 2014). Class lb genes are organised as an nrdHIEF operon in E. coli and Mycobacterium, but in Bacillus and Staphylococcus the class Ib-specific nrdH gene is located elsewhere on the chromosome (Rabinovitch et al. 2010). Deinococcus radiodurans (genes DR_B0107-DR_B0109) and six other Deinococcus species also have an nrdIEF gene cluster. In each of these Deinococcus species, nrdIEF is followed, probably in the same operon, by a gene encoding a small protein (86 to 100 residues) containing a characteristic CPXC redox motif (Fig. 5). These small proteins (DR_B0110 in D. radiodurans), which have been annotated as Trx, Trx-related or hypothetical proteins, presumably function as electron donor for the NrdIEF RNR encoded by the same gene cluster. Class II RNRs encoded by a single nrdl gene are represented not only in the four species lacking NrdIEF (i.e. D. deserti, D. maricopensis, D. peraridilitoris and D. punicus), but also in five Deinococcus species (D. actinomycetemcomitans, D. geothermalis, D. radiodurans, D. soli and D. suwensii) having NrdIEF (Table S7, Supporting Information). Class II RNRs are also reduced by Trx (Jordan et al. 1997; Torres et al. 2014). Although NrdH can act as a hydrogen donor for the class Ib NrdEF RNR (Jordan et al. 1997), it has been supposed to be one of the candidates in the antioxidant system of bacteria lacking GSH. In Corynebacterium glutamicum, overexpression of NrdH increased the resistance to oxidative stress by reducing ROS accumulation (Si et al. 2014). Together, Deinococcus species encode two to five Grx/NrdH-like proteins (Table 3). Further research is needed to investigate the physiological function of these proteins in Deinococcus.

Other proteins involved in ROS protection

In Gram-negative bacteria, disulphide bond formation occurs in the periplasm and is catalysed by Deb proteins. In Gram-positive bacteria, however, disulphide bond formation is not fully understood due to lack of periplasmic space (Reardon-Robinson and Ton-That 2015). Deinococcus radiodurans DR_0659 encodes a DebA-like protein (COG2761) designated FrnE, which belongs to the Trx superfamily of proteins, and the fnrE mutant strain shows reduced tolerance to IR and H2O2 (Table S1, Supporting Information). It has been recently reported that DR_0659 represents a novel cytoplasmic thiol-disulphide oxidoreductase system that could be functional in eubacteria under conditions where Trx/Grx systems are inhibited or absent (Bihani et al. 2018). This protein contains a canonical 22-CPCWC-25 active site motif embedded in the Trx fold and an additional, functionally important 239-CxxxxC-244 motif in a unique extended C-terminal tail. A protein homologous to DR_0659 is observed in all of the Deinococcus species analysed. However, the FrnE proteins from D. proteolyticus and D. maricopensis have a CPFC motif instead of CPWC and, moreover, the CxxxxC motif is absent in FrnE from D. maricopensis, which is shorter (about 20 residues) than the other FrnE proteins (Fig. S10, Supporting Information). Some Deinococcus species encode an additional, predicted cytoplasmic DebA-like protein (DR_2335 in D. radiodurans) (Table 3), but the characteristic motifs (both CPWC/CPFC and CxxxxC) are not present in DR_2335 and its homologues. Instead, they contain a single conserved Cys residue.

In D. radiodurans, deletion of DR_B0067, which encodes an extracellular nuclease (COG2374), might slightly decrease the survival ability after H2O2 or IR treatment (Li et al. 2013). The degradation of extracellular DNA into dNMPs (especially, dGMP) by extracellular nuclease DR_B0067 might enhance D. radiodurans tolerance to oxidative stress (Li et al. 2013). Homologues of DR_B0067 are found in all other Deinococcus species. However, their C-terminal regions have different combinations and arrangements of a few domains (Fig. S11, Supporting Information).

Iron is essential for the life processes of all living organisms, but the element is toxic when in excess of that needed for cellular homeostasis. The primary role of ferritin family proteins...
is to sequester iron to protect cells from the damage caused by Fenton reaction, where free ferrous ions react with H₂O₂ to produce ·OH (Smith 2004). Three subfamilies of proteins representing the ferritin fold are observed in bacteria: ferritin, bacterioferritin (Bfr) and the ferritin-like Dps (DNA-binding proteins during stationary phase). Among them, Dps plays an important role in the detoxification of ROS, in iron scavenging and in the mechanical protection of DNA (Zeth 2012). Whereas E. coli produces the three ferritin family proteins (Smith 2004), the Deinococcus species analysed contain only Dps. Deinococcus radiodurans encodes two Dps proteins, Dps1 (DR_2263) and Dps2 (DR_B0092). Dps1 is well conserved across all of the Deinococcus species, but Dps2 is present only in D. radiodurans and D. gobiensis. Dps1 has a longer N-terminal extension (54 amino acids) before the ferritin fold compared to other Dps, which is essential for stabilising the protein–DNA complex (Santos et al. 2017). The N-terminal extension region is observed in all of the Dps1 homologues (Fig. S12, Supporting Information). Although there is...
some variation of sequences and amino acid composition, the N-terminal extension is rich in positively charged residues, in particular lysine and has been proposed to be involved in the association with DNA (Santos et al. 2017). Similar N-terminal lysine-rich extensions are present in nucleoid-associated HU proteins of Deinococcus (Bouthier de la Tour et al. 2015). Remarkably, the Dps2 proteins of D. radiodurans and D. gobiensis possess a predicted N-terminal signal peptide, indicating translocation of Dps2 across the cytoplasmic membrane. Experimental evidence for the extracytoplasmic localisation of D. radiodurans Dps2 has indeed been obtained, suggesting that Dps2 may have an iron-sequestering role outside the cytoplasm and, like SodC, may protect against exogenously derived ROS (Reon et al. 2012). Reduced resistance to H₂O₂ has been reported for a D. radiodurans dps2 mutant, whereas resistance to IR appeared unaffected for dps1 and dps2 single and double mutants (Table S1, Supporting Information). Interestingly, both in D. radiodurans and D. gobiensis the dps2 gene is directly adjacent to genes encoding the two-component signal transduction system (TCS) Rads/RadR (Fig. S13, Supporting Information), which, like Dps2, is only found in these two species (see section ‘Radiation and oxidative stress resistance-associated regulatory proteins’).

While non-cytoplasmic SodC and, in particular, Dps2 are not strictly conserved across the Deinococcus species, Mrsp and Mrsq homologues of the periplasmic MsrPQ system for repair of methionine sulfoxide damage in bacterial cell envelope proteins (Ezraty et al. 2017) are present in each Deinococcus (Table S3, Supporting Information).

Non-enzymatic systems for oxidative stress defence

Carotenoid

Carotenoids are widespread natural pigments and act as ROS scavengers in non-phototrophic bacteria for cellular protection. One important group of non-phototrophic bacteria that produce carotenoids is the phylum Deinococcus–Thermus (Tian and Hua 2010). The major carotenoid in D. radiodurans is deinoxanthin, a unique ketocarotenoid, which gives the bacterium its characteristic red color. Deinoxanthin shows higher scavenging activity on H₂O₂ than carotenoids (lycopene and β-carotene) and xanthophylls (zeaxanthin and lutein) and has a protective effect in vitro on DNA and protein (Tian et al. 2007, 2009). In vivo, decreased resistance to radiation or H₂O₂ has been observed for D. radiodurans crt gene mutants lacking deinoxanthin (Table S1, Supporting Information). The biosynthetic pathway for deinoxanthin includes the reactions catalysed by geranylgeranyl diphosphate synthase (CrtE, DR_1395), phytoene synthase (CrtB, DR_0862), phytoene desaturase (CrtI, DR_0861), lycopene cyclase (CrtLm, DR_0801), carotenoid 3′,4′-desaturase (CrtD, DR_2250), carotenoid 1,2-hydration (CrtU, DR_0091), carotenoid ketolase (CrtO, DR_0093) and carotenoid 2-β-hydroxylase (cytochrome P450 CYP287A1, DR_2473) (Zhou et al. 2015). Most of these proteins are well conserved in the Deinococcus species analysed except for CrtLm and CYP287A1 (Table 3). Following synthesis of lycopene, carotenoid biosynthesis is diversified into acyclic or cyclic carotenoids. The cyclisation of lycopene on one or both Ψ-ends of lycopene is usually catalysed by CrtL- or CrtY-type lycopene β-cyclase (Tian and Hua 2010). An asymmetrically acting lycopene β-cyclase (CrtLm), which catalyses the production of monocylic carotenoids, is encoded by eight of the analysed Deinococcus species, although the gene in D. actinocolus contains a frameshift. For the three species that lack CrtLm (i.e. D. proteolyticus, D. peraridilitoris and D. deserti), genes encoding CrtY-CruF fusion proteins were detected (Fig. 3). A single gene encoding a bifunctional enzyme with lycopene cyclase (CrtY) and phytoene synthase (CrtB) activities in fungi has been reported previously (Velayos, Esilava and Iturriaga 2000; Guo, Tang and Zhang 2014). BLASTP analysis revealed that the CrtY-CruF fusion protein is unique to the class Deinococc. Deinoxanthin is a unique C2-hydroxylated monocyclic ketocarotenoid, and the 2-β-hydroxyxylase CYP287A1 catalyses β-ring hydroxylation at the C2 position of 2-deoxydeinoxanthin in D. radiodurans (Zhou et al. 2015). The 2-β-hydroxylase, which completes the biosynthetic pathway of deinoxanthin, was not found in D. deserti and D. geothermalis. Taken together, these results suggest that D. proteolyticus, D. peraridilitoris, D. deserti and D. geothermalis are likely to produce different kinds of carotenoid distinct from deinoxanthin. This may explain the observed colony colours of these four Deinococcus species, which are orange-red, light pink, whitish/light pink and orange, respectively (Brooks and Murray 1981; Ferreira et al. 1997; de Groot et al. 2005; Rainey et al. 2007).

Bacillithiol

Bacillithiol (BSH), the α-anomeric glycoside of l-cysteinyl-d-glucosamine with l-malic acid, is a low-molecular-weight thiol analogous to GSH and is found in several Firmicutes (e.g. Bacillus, Staphylococcus) and in D. radiodurans, which lack GSH (Newton et al. 2009; Perera, Newton and Pogliano 2015). Some other bacteria produce the low-molecular-weight thiol mycocothiol (MSH) instead of GSH or BSH (Rosario-Cruz and Boyd 2016). The biosynthesis and roles of BSH have been studied in Bacillus and Staphylococcus species. Similar to GSH, BSH protects against H₂O₂, hypochlorite and thiol/disulphide stress. BSH also plays a role in metal ion buffering and thereby protects cells from metal ion intoxication (Rosario-Cruz and Boyd 2016; Chandranugsa et al. 2018). BSH synthesis initiates with a glycosyltransferase (BshA) that couples N-acetylglucosamine (GlcNAc) and l-malate. The BshB deacetylase hydrolyses the acetyl group from GlcNAc-Mal to generate GlcN-Mal. Subsequent addition of cysteine, catalysed by BshC, generates the final product, BSH (Gaballa et al. 2010). Homologues of these three enzymes are encoded within the genomes of each of the analysed Deinococcus species (Table S3, Supporting Information). In D. radiodurans, the levels of bsh gene expression and BSH were slightly reduced during irradiation and increased again in the recovery period (Luan et al. 2014).

In analogous systems, in which enzymes function with GSH as cofactor, Grx is reduced by the oxidation of GSH, and the oxidised GSH is then regenerated by GSH reductase. Phylogenomic profiling identified a putative BSH reductase, YpdA, which is related to Trx reductase (24% identity with B. subtilis TrxB), and three putative bacilliredoxin (Brx) proteins (i.e. YqiW, YphP and Ytxj) in B. subtilis (Gaballa et al. 2010). Bacilliredoxin activity has been demonstrated for YphP (renamed BrxA) and YqiW (BrxB) (Gaballa et al. 2014). Homologues of YpdA and Ytxj (but not BrxA and BrxB) were found in the analysed Deinococcus species. However, a putative monothiol active site (TCPIS) observed in Bacillus Ytxj is replaced with TCHKT in all Deinococcus Ytxj homologues, and the deinococcal Ytxjs (more than 200 residues) are longer than B. subtilis Ytxj (108 residues). Under oxidising conditions, BSH has been shown to form mixed disulphides with the Cys residues of several proteins, termed S-bacillithioliation, which is a widespread thiol protection and redox-regulatory mechanism in Firmicutes (Loi, Rossius and Antelmann 2015). The organic hydroperoxide regulator OhrR is a redox-sensing transcriptional repressor that is bacillithiolated by BSH in Bacillus, but the 11 Deinococcus species lack this protein. BSH transferases (Bst) are enzymes that catalyse the transfer of BSH to target substrates. In B. subtilis, the YfT (BstA) protein, which is a member of the
oxidative stress. Consistent with these characteristics, the
manganese transporters MntC and solute-binding component MntA, respectively) involved in the
FUNCTIONAL ELEMENTS MntC and MntA. 

Groot et al. (2007), and the heterozygous mntH mutant shows
The MnH homologues sharing 42–77% identity are found in
only seven of the other Deinococcus species analysed. Deinococcus
peraridilitoris and D. puneicus lack the MnH homologue. The
MntH homologue from D. deserti and two of the three MnH homologues from D. proteolyticus share only 26% identity with D. radiodurans. 

Fe2+ is highly reactive OH-
function with alternative proteins that do not require iron. This
adaptive response to oxidative stress and Fe limitation is well understood in E. coli. In this organism, FeSod (SodB) is replaced by MnSod (SodA) during periods of Fe starvation. At the same time, E. coli also replaces the Fe-dependent RNR NrdAB with its Mn-dependent isozyme, NrdEF, which is crucial for survival under conditions of H2O2 stress (Chandransu, Rensing and Helmann 2017). Neither SodB nor NrdAB is found in the Deinococcus species analysed here. The constitutively high intracellular Mn/Fe ratio as an adaptation to harsh environmental conditions might drive the loss of such iron-dependent proteins during evolution. Notably, the radiation-sensitive bacterium Shewanella oneidensis (Mn/Fe ratio < 0.001) encodes 65% more proteins containing Fe-S clusters than D. radiodurans (Ghosal et al. 2005).

Other antioxidants: pyrroloquinoline quinone and polyphosphate
Pyrroloquinoline quinone (PQQ) was initially characterised as a redox cofactor for membrane-bound dehydrogenases in bacteria. Subsequently, PQQ was shown to be an antioxidant protecting cells from oxidative damage (Misra, Rajpurohit and Khairnar 2008). Moreover, E. coli mutant was reported to be more resistant to H2O2, UV and IR, supporting the involvement of Mn in the radiation resistance of D. radiodurans (Sun et al. 2010). All the other Deinococcus species encode one MntE homologue except for D. maricopensis encoding an additional homologue. MntH is essential in D. radiodurans (Makarova et al. 2007), and the heterozygous mntH mutant shows increased sensitivity to IR (Dulermo et al. 2015). However, D. radiodurans MntH homologues sharing 42–77% identity are found in only seven of the other Deinococcus species analysed. Deinococcus peraridilitoris and D. punicicus lack the MntH homologue. The MntH homologue from D. deserti and two of the three MnH homologues from D. proteolyticus share only 26% identity with D. radiodurans. MnH transports Mn into the cytoplasmic proteins of Mn2+ transporter encoded by a mntACB gene cluster or by separate mntA and mntB genes. Deinococcus peraridilitoris and D. deserti additionally possess respectively one and two operones containing four ABC-type Mn2+ transporter genes encoding MntA, MntC and two different MntB homologues (Fig. S14, Supporting Information), which may improve their Mn2+ import capacity or compensate for the absence of MntH.

Bacteria often replace some proteins that have Fe-dependent functions with alternative proteins that do not require iron. This adaptive response to oxidative stress and Fe limitation is well understood in E. coli. In this organism, FeSod (SodB) is replaced by MnSod (SodA) during periods of Fe starvation. At the same time, E. coli also replaces the Fe-dependent RNR NrdAB with its Mn-dependent isozyme, NrdEF, which is crucial for survival under conditions of H2O2 stress (Chandransu, Rensing and Helmann 2017). Neither SodB nor NrdAB is found in the Deinococcus species analysed here. The constitutively high intracellular Mn/Fe ratio as an adaptation to harsh environmental conditions might drive the loss of such iron-dependent proteins during evolution. Notably, the radiation-sensitive bacterium Shewanella oneidensis (Mn/Fe ratio < 0.001) encodes 65% more proteins containing Fe-S clusters than D. radiodurans (Ghosal et al. 2005).
expressing DR_C0034 showed an increased tolerance to oxidative stress, possibly by scavenging of ROS by PQQ and/or by stimulation, through an unknown mechanism, of catalase and SOD activities (Khairnar, Misra and Apte 2003). Disruption of pqqE in D. radiodurans decreases the resistance not only to oxidative stress (H₂O₂) but also to the DNA-damaging agents MMC and IR (Rajpurohit, Gopalakrishnan and Misra 2008). Recently, the involvement of PQQ in signal transduction mechanisms involved in radiation resistance and DNA double-strand break repair has also been reported (see the section ‘Radiation and oxidative stress resistance-associated regulatory proteins’). However, a homologue of PqqE is not found in the other Deinococcus species analysed.

Inorganic polyphosphate (Poly P) found as unbranched chains up to 1000 residues long in cells is a universally conserved biopolymer. Poly P functions as a protein-protective chaperone that is able to protect a broad spectrum of proteins from aggregation, so its accumulation significantly increases bacterial oxidative stress resistance (Dahl, Gray and Jakob 2015; Gray and Jakob 2015). Deinococcus radiodurans exponential-phase cells contain electron-dense granules for the likely storage of Poly P (Slade and Radman 2011). Bacterial polyphosphate kinases (PPK) reversibly catalyse the generation of Poly P directly from ATP, whereas exopolyphosphatases (PPX) can degrade Poly P into Pi molecules (Dahl, Gray and Jakob 2015). PPKs are subdivided into two families: PPK1 is responsible for Poly P synthesis and PPK2 preferentially catalyses the reverse reaction (utilisation of Poly P) (Rao, Gomez-Garcia and Kornberg 2009). Deinococcus radiodurans has PPK1 and 2 homologues (Zhang, Ishige and Kornberg 2002), and its PPK2 belongs to the class III subfamily that is characterised by being able to catalyse both ADP and AMP phosphorylation, enabling synthesis of ATP from AMP and Poly P by a single enzyme (Motomura et al. 2014). PPK1, PPK2 and PPX are well conserved across the 11 Deinococcus species (Table S3, Supporting Information), although the PPK1 gene of D. maricopensis, downstream of gene Deima_3207, has a frameshift. Hydrolysis of Poly P by PPX could generate orthophosphate that may be used for the generation of Mn²⁺-phosphate complexes (Slade and Radman 2011).

**THE DDR AND PPR PROTEINS**

Transcriptomics experiments with D. radiodurans revealed the induced expression of various novel genes of unknown function in cells recovering from IR and desiccation (Tanaka et al. 2004). These DNA damage response genes have been named ddrA to ddrP. An additional radiation- and desiccation-induced gene, pprA (pleiotropic protein promoting DNA repair), has also been identified in another study (Narumi et al. 2004). The name ppr has been given to two other D. radiodurans genes that are required for radiation resistance, but which are not radiation induced. One has been designated ppr1 (inducer of pleiotropic proteins promoting DNA repair), has also been identified in another study (Earl et al. 2002a). The other has been named pprM (a modulator of the Ppr1-dependent DNA damage response), although it encodes a homologue of cold shock protein usually designated Csp in bacteria (Ohba et al. 2009). The five genes most highly induced in response to each stress were ddrA, ddrB, ddrC, ddrD and pprA (Tanaka et al. 2004). More recently, additional radiation-induced genes of unknown function were identified in D. deserti and designated ddrQ to ddrX, with ddrT to ddrX organised in an operon (Blanchard et al. 2017). It has also been reported that the genuine DdrC proteins of D. radiodurans and D. geothermalis, and the real DdrH protein of D. radiodurans, are encoded by the DNA strand opposite to the initially annotated gene, which is of course crucial for characterisation of these proteins (de Groot et al. 2009). Together, 23 ddr and ppr loci have been described (Table 4).

The (highly) radiation-induced expression of pprA and ddr genes in D. radiodurans (Tanaka et al. 2004), and also in D. deserti (de Groot et al. 2014), suggests a role of these genes in radiation resistance. This has been confirmed for the genes that have been studied in more detail (i.e. ddrA to ddrD, ddrI, ddrO, ddrP, ddrR, pprA). DdrI, DdrO and PprF (IrrE) are involved in transcriptional regulation and are described in the section ‘Radiation and oxidative stress resistance-associated regulatory proteins’. DdrP (DR_B0100) corresponds to the putative DNA ligase LigB and is described in section ‘DNA repair in Deinococcus’. Strongly or moderately decreased resistance of D. radiodurans to radiation, MMC and/or oxidative stress has been observed in one or more studies after deletion of ddrA (DR_0423), ddrB (DR_0070), ddrR (DR_0053), pprA (DR_A0346) or pprM (DR_0907) (Table S1, Supporting Information). Mutant strains lacking either ddrC (reversed DR_0003) or ddrD (DR_0326) were found as resistant as the wild type to IR, UV and MMC in earlier studies (Tanaka et al. 2004; Selvam et al. 2013), but more recent work reported slight sensitivity of a ddrC mutant to high doses of UV (Bouthier de la Tour et al. 2017). Larger effects on resistance have been observed when the ddrC or ddrD deletion is combined with another gene deletion (Tanaka et al. 2004; Selvam et al. 2013; Bouthier de la Tour et al. 2017). However, the results are rather complex and depend on the genetic background and on the applied stress. For example, whereas ΔddrC ΔddrB and ΔddrD ΔddrB double mutant strains are more sensitive to MMC than the ΔddrB strain, the ΔddrC ΔpprA and ΔddrD ΔpppA strains appear less sensitive to gamma rays than the ΔpprA strain (Tanaka et al. 2004; Selvam et al. 2013). Compared to ΔddrC and ΔddrD single deletion mutants, the ΔddrC ΔddrD double mutant is slightly more sensitive to IR and MMC, but not to UV (Tanaka et al. 2004; Selvam et al. 2013). Remarkably, unlike the ΔddrC and ΔddrD single deletion mutants, the ΔddrC ΔddrD double mutant is very slow growing, indicating that the presence of either ddrC or ddrD is required for normal growth. However, the functions of DdrC and DdrD do not seem redundant because deletion of ddrD, but not of ddrC, dramatically increases UV sensitivity of a ΔpprA strain (Selvam et al. 2013).

Massive DNA damage is generated after exposure to high doses of radiation, and extensive DNA degradation and formation of potentially lethal DNA repair intermediates must be avoided. DdrA, DdrB, DdrC and PprA proteins are able to bind DNA in vitro, and may contribute to repair of heavily damaged DNA and/or to preserving genome integrity by preventing DNA degradation. DdrA is a Rad52 family protein, but unlike eukaryotic Rad52, DdrA does not display DNA-strand annealing activity (Harris et al. 2004). DdrA forms ringlike oligomers (Gutsche et al. 2008), and it binds to 3’ single-stranded DNA ends and protects those ends from nuclelease degradation (Harris et al. 2004). Deletion of ddrA in a D. radiodurans strain expressing a low constitutive level of RecA dramatically decreases resistance to IR, supporting the proposed role of DdrA in preserving DNA ends for recombinational repair (Jolivet et al. 2006). DdrB forms pentameric rings that bind single-stranded DNA but not double-stranded DNA (Norais et al. 2009; Sugiman-Marangos and Junop 2010). DdrB promotes high-fidelity DNA annealing and is involved in an early step of DNA double-strand break repair (Xu et al. 2010; Bouthier de la Tour et al. 2011; Sugiman-Marangos, Weiss and Junop 2016). DdrC binds to DNA with a preference for single-stranded DNA, protects DNA from nucleases and exhibits...
single-strand annealing activity (Bouthier de la Tour et al. 2017). It also promotes circularisation but not ligation of linear plasmid DNA. The DdrD protein has not been characterised in vitro yet, but its recruitment to the nucleoid and dynamics of localisation comparable to that of RecA after irradiation have been observed, suggesting a possible functional interaction between DdrD and RecA (Bouthier de la Tour et al. 2013). Regulation of ddrR (DR_0053) has been investigated and the DR_0053 protein has been purified, but its specific function is still unknown (Appukuttan et al. 2015). DdrR belongs to the DinB superfamily of predicted metalloenzymes, of which the protein encoded by the DNA damage-inducible dinB gene of B. subtilis is the founding member (the DinB superfamily proteins are not to be confused with DNA polymerase IV encoded by E. coli gene dinB, also called dinP). The DinB superfamily includes bacillithiol transferases that can detoxify toxic molecules such as reactive electrophiles (Chandransu et al. 2018). Besides homologues of DdrR, Deinococcus species contain several additional genes encoding DinB superfamily proteins. Further research is needed to determine if DdrR and other deinococcal DinB superfamily proteins are bacillithiol transferases, and if so, to identify their target compounds in vivo.

According to the literature, PprA is a protein with various functions and able to stimulate activity of several, diverse enzymes. In vitro, PprA preferentially binds to double-stranded DNA with strand breaks, inhibits exonuclease activity and stimulates DNA end-joining by T4 DNA ligase and E. coli DNA ligase, which has led to the suggestion that PprA plays a role in a possible NHEJ-like DNA repair mechanism in vivo (Narumi et al. 2004). Moreover, PprA is required for the in vivo activity of the putative ATP-dependent DNA ligase LigB (DdrP) from D. radiodurans (Kota et al. 2016). PprA also interacts with D. radiodurans topoisomerase IB in a bacterial two-hybrid system, and enhances the topoisomerase IB-mediated relaxation activity of supercoiled DNA in vitro (Kota et al. 2014).

Cold shock proteins (Csp) are small (less than 100 amino acid residues), single-strand nucleic acid-binding proteins widely conserved in bacteria. CspS are known for being induced during cold shock, and they are thought to prevent the formation of secondary structures in mRNA at low temperature. However, CspS are not only produced during cold stress. Experimental data have indicated that CspS are also required under standard growth conditions and for adaptation to stresses other than cold, during which CspS may influence transcription or translation of many genes (Keto-Timonen et al. 2016). Many bacteria encode several CspS (e.g. nine CspS in E. coli) with at least some of these having overlapping functions. Deinococcus radiodurans has only one csp gene (pprM), whereas two or three csp genes are present in some other Deinococcus species (Table 4 and Fig. 6). Compared to Csp from other bacteria, CspS from Deinococcus contain a C-terminal extension of 10–20 residues of unknown function (Keto-Timonen et al. 2016). Many bacteria encode several CspS (e.g. nine CspS in E. coli) with at least some of these having overlapping functions. Deinococcus radiodurans has only one csp gene (pprM), whereas two or three csp genes are present in some other Deinococcus species (Table 4 and Fig. 6). Compared to Csp from other bacteria, CspS from Deinococcus contain a C-terminal extension of 10–20 residues of unknown function (Fig. 6). The PprA protein is able to stimulate activity of E. coli catalase in vivo and in vitro (Kota and Misra 2006). More recent results indicate that PprA plays a major role in chromosome segregation via its physical and functional interaction with DNA gyrase in D. radiodurans after IR exposure and DNA repair (Devigné et al. 2013, 2016). In vitro, PprA stimulates the decatenation activity of DNA gyrase (Devigné et al. 2016). PprA also interacts with D. radiodurans topoisomerase IB in a bacterial two-hybrid system, and enhances the topoisomerase IB-mediated relaxation activity of supercoiled DNA in vitro (Kota et al. 2014).

Table 4. The ddr and ppr genes in Deinococcus species.

| Gene | Drad | Dgeo | Ddes | Dmar | Dgob | Dpro | Dper | Dswu | Dsol | Dact | Dpun |
|------|------|------|------|------|------|------|------|------|------|------|------|
| ddrA | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 2    | 1    | fr   | 1    |
| ddrB | 1    | 1    | 1    | 1    | 3    | 2    | 2    | 1    | 1    | 1    | 1    |
| ddrC | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| ddrD | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| ddrE | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| ddrF | 1    |      |      |      |      |      |      |      |      |      |      |
| ddrG | 1    |      |      |      |      |      |      |      |      |      |      |
| ddrH | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| ddrI | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| ddrJ | 1    |      |      |      |      |      |      |      |      |      |      |
| ddrK | 1    |      |      |      |      |      |      |      |      |      |      |
| ddrL | 1    |      |      |      |      |      |      |      |      |      |      |
| ddrM | 1    |      |      |      |      |      |      |      |      |      |      |
| ddrN | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| ddrO | 1    | 1    | 2    | 1    | 3    | 1    | 2    | 1    | 1    | 1    | 1    |
| ddrP (ligB) | 1 |      |      |      |      |      |      |      |      |      |      |
| ddrQ | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| ddrR | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| ddrS | 1    | 1    |      |      |      |      |      |      |      |      |      |
| ddrTUVWX | 1 |      |      |      |      |      |      |      |      |      |      |
| pprM (csp) | 1 | 2    | 3    | 2    | 2    | 1    | 1    | 3    | 3    | 3    | 2    |

See the legend to Table 2.
Figure 6. Phylogenetic relationship between two types of cold-shock proteins (Csp) identified in 11 Deinococcus species. The phylogenetic analysis was carried out based on protein sequence alignment of 19 deinococcal Csp homologues (Table S4, Supporting Information) made with Clustal omega. Two Csps of *D. soli* are identical to Csps of *D. actinosclerus*. See further the legend to Figure 4.

The phylogenetic analysis was carried out based on protein sequence alignment of 19 deinococcal Csp homologues (Table S4, Supporting Information) made with Clustal omega. Two Csps of *D. soli* are identical to Csps of *D. actinosclerus*. See further the legend to Figure 4.

served in a D. radiodurans pprM mutant (Jeong et al. 2016b). PprM also confers higher oxidative stress tolerance to E. coli (Park et al. 2017). Further studies are needed to elucidate how PprM and other deinococcal Csps influence expression of various genes and proteins.

Of the 23 ddr and ppr loci (Table 4 and Table S4, Supporting Information), only 8 are conserved in each of the 11 analysed *Deinococcus* genomes: ddrB, ddrC, ddrH, ddrI, ddrO, pprJ (itrE) and pprM (csp). The other 15 loci are absent in one, several or even most of the *Deinococcus* species. Remarkably, these non-conserved genes include ddrA, ddrD, ddrP (ligB), ddrR and pprA, for which an important or moderate contribution to radiation and oxidative stress resistance in *D. radiodurans* has been reported.

**MISCELLANEOUS PROTEINS INVOLVED IN RESISTANCE TO RADIATION AND OTHER DNA-DAMAGING AGENTS**

**Widely conserved proteins**

In addition to the genes described in the preceding sections, many other genes appear to be required for full resistance of *D. radiodurans* to radiation and other DNA-damaging agents (Table S1, Supporting Information). These include genes widespread in other bacterial genera and that are also present in each of the analysed *Deinococcus* species (Table S5, Supporting Information), for example homologues of *DR*0199 (encoding nucleoid-associated protein, YbaB/EbfC family), *DR*0382 (YgjD/TsaD), *DR*0756 (YeaZ/TsaB), *DR*1321 (signal peptidase I), *DR*1525 (fructokinase RbsK), *DR*1972 (ClpP, ATP-dependent Clp protease proteolytic subunit), *DR*1973 (ClpX, ATP-dependent Clp protease ATP-binding subunit), *DR*2417 (DncA/RNase J), *DR*2462 (Rnase Y). Homologues of *DR*0342 (Rieske-like Fe-S protein) and *DR*1471 (chromosome partition protein Smc) are also present in each *Deinococcus*, although these have a frameshift in *D. gobiensis* and *D. actinosclerus*, respectively (Table 5). The smc mutant of *D. radiodurans* is not affected in growth and IR resistance, but has an increased sensitivity to gyrase inhibitors (Bouthier de la Tour et al. 2009).

The yeaZ and ygjD mutants of *D. radiodurans* are very sensitive to MMC, but not or only marginally sensitive to radiation and H2O2, and therefore a role of YeaZ and YgjD in repair of DNA cross-links has been proposed (Onodera et al. 2013). However, other studies have reported that YeaZ and YgjD are required for an essential and universal modification of ANN-decoding tRNAs (Deutsch et al. 2012), and therefore these proteins have been renamed TsaB (tRNA threonylcarbamoyladenosine biosynthesis protein TsaB) and TsaD (tRNA N6-adenosine threonylcarbamoyltransferase), respectively. Remarkably, *yeaZ* and *ygjD* are not essential for viability in *D. radiodurans*, unlike in other species (e.g. *E. coli*) (Onodera et al. 2013). It remains to be established how YeaZ and YgjD are specifically required for MMC resistance in *D. radiodurans*.

*DR*2417 corresponds to RNase J that belongs to the β-CASP family of nucleases. A *DR*2417 (rnj) null mutant could not be obtained, and cells having reduced copy number of *DR*2417 are affected in growth and radiation resistance (Das and Misra 2012). Remarkably, one study has reported that *DR*2417 has strong DNAse but poor RNase activity, and therefore the protein has also been called DncA (Das and Misra 2012), whereas in another
study a clear preference for RNA has been observed (Zhao et al. 2015).

Several other miscellaneous proteins required for radiation or oxidative stress resistance in D. radiodurans are not conserved in other Deinococcus species (Table 5). DR_0679 encodes a small putative nucleotidyld transferase (COG1669). DR_0392 contains a BON (bacterial OsmY and nodulation) domain that is found in a family of osmotic shock protection proteins. DR_1262 (rsr) codes for a 60 kDa SS-A/Ro ribonucleoprotein homologue. DR_1838 (conserved in the other Deinococcus species) and DR_1631 are homologues of RelA and RelQ, respectively, and are predicted to be involved in the synthesis and hydrolysis of the stringent response signal molecule (p)ppGpp in D. radiodurans (Wang et al. 2016a).

Table 5. Differences regarding miscellaneous proteins involved in resistance to radiation and other DNA-damaging agents in Deinococcus species.

| Protein            | Drad | Dgeo | Ddes | Dmar | Dgob | Dpro | Dper | Dswu | Dsol | Dact | Dpun |
|--------------------|------|------|------|------|------|------|------|------|------|------|------|
| DR_0342            | 1    | 1    | 1    | 1    | 1    | fr   | 1    | 1    | 1    | 1    | 1    |
| DR_1471 (fr) Smc   | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| DR_0679            | 1    |      |      |      |      |      |      |      |      |      |      |
| DR_0392            | 1    | 1    |      | 2    | 1    | 1    |      |      |      |      |      |
| DR_1262 Rsr        | 1    |      |      | 1    |      |      |      |      |      |      |      |
| DR_1631 RelQ       | 1    | 1    | 1    |      | 1    | 1    | 1    | 1    |      |      |      |
| DR_1790            | 1    |      |      |      |      |      |      |      |      |      |      |
| DR_2310            | 1    | 1    | 1    |      |      |      | 1    | 1    | 1    |      |      |
| DR_A0018 (fr)      | 1    | 1    | 1    | 1    | 1    |      |      |      |      |      |      |
| DR_A0281-DR_A0282 (fr) | 2  | 1    | 1    | 1    | 1    |      |      |      | 1    |      |      |
| DR_A0283           | 2    | 1    | 1    | 1    | 1    | 1    |      |      |      |      | 2    |
| DR_B0118           | 1    | 2    | 2    | 4    | 2    | 2    | 2    | 2    | 2    |      |      |
| DR_1172 / DR_0105  | 2    | 1    | 1    |      | 1    | 1    |      |      |      |      |      |
| Deide,15148        |      | 1    | 1    |      | 1    |      |      |      |      | 1    |      |

*Bold,* gene inactivation leading to increased sensitivity of D. radiodurans to radiation, desiccation or oxidative stress in at least one study. fr, frameshift.

Signal-peptide containing proteins

Interestingly, some proteins with a reported role in radiation and/or oxidative stress resistance contain a predicted N-terminal signal peptide, although the presence of this signal peptide and its role (translocation of the protein across the cytoplasmic membrane) has not always been realised or considered. These proteins include Dps2 (as described above), DR_1790, DR_2310, DR_A0018, DR_A0282 and DR_A0283 (Fig. S16, Supporting Information), which have homologues in some but not all of the other Deinococcus species (Table 5). Except for the hydrophobic region in the signal peptide, which is cleaved off by signal peptidase I, or by signal peptidase II in case of a lipoprotein, none of these proteins possess a predicted transmembrane helix in their mature region. These proteins are thus expected to have their function in the cell wall/periplasm or extracellularly. Some of these proteins have indeed been identified in the cell wall, for example DR_A0282 and DR_A0283, and also (see below) DR_0505 (Farci et al. 2014).

DR_1790 is known as yellow-related protein and belongs to the major royal jelly protein family. In addition to increased sensitivity to H<sub>2</sub>O<sub>2</sub> and IR, decreased growth rate of the DR_1790 mutant compared to the wild type has been reported (Cheng et al. 2015). The molecular mechanism by which DR_1790 contributes to growth and resistance to radiation and oxidative stress in D. radiodurans has not been elucidated.

DR_2310 is a reported serralysin metalloprotease that is secreted (Basu and Apte 2008). Our sequence and comparative analysis strongly suggest a re-annotation of the start codon position of DR_2310, resulting in a protein that, like its homologues, has an N-terminal lipoprotein signal peptide. Together with other extracellular proteases, DR_2310 probably has a role in amino acid nutrition. The DR_2310 mutant and wild-type strains grow equally well in rich medium; nevertheless, the mutant shows a marginal sensitivity to radiation when irradiated in rich medium (Basu and Apte 2008).

The protein encoded by the corrected DR_A0018 gene, which showed a frameshift in the first genome sequence but not after resequencing (Hua and Hua 2016), is a 5′-nucleotidase family protein (COG0737). DR_0505, which is conserved in the other Deinococcus species, is another 5′-nucleotidase family protein. No radiation sensitivity was observed for the DR_0505 mutant (Kota, Kumar and Misra 2010) unlike for the DR_A0018 mutant (Lu et al. 2009). A role for DR_0505 in DNA double-strand break processing and repair has been suggested (Kota, Kumar and Misra 2010). However, DR_A0018 and DR_0505, as well as their homologues in the other Deinococcus species, possess a predicted N-terminal signal peptide or lipoprotein signal peptide, respectively.

DR_A0282 is another protein with a suggested role in DNA repair; recombinant DR_A0282 was found to bind DNA and to protect it from exonuclease digestion (Das and Misra 2011). Homologues of DR_A0282 are present in some other Deinococcus species and also in D. radiodurans itself (DR_B0068). However, resequencing of the D. radiodurans genome and our protein comparisons and sequence analyses show that the original DR_A0282 gene contains a frameshift and that the N-terminal region of the actual gene product corresponds to the protein that was annotated as DR_A0281 in the first genome sequence. This corrected DR_A0281/0282 protein (Fig. S16, Supporting Information), as well as each homologue, contains an N-terminal lipoprotein signal peptide. Conserved domain analysis indicates a carboxypeptidase regulatory-like domain in these proteins. The DR_A0281/0282 gene is directly followed by DR_A0283 encoding a probable subtilase-type serine protease. Interestingly, each DR_A0281/0282 homologue is followed by a DR_A0283 homologue, suggesting a functional link of this conserved gene pair. Also DR_A0283 and homologues contain a lipoprotein signal peptide.

Although these various signal-peptide containing proteins may have a role in radiation or oxidative stress resistance, a direct role in DNA repair, as suggested for DR_0505 and DR_A0282,
**Table 6. Radiation and oxidative stress resistance-associated regulator proteins in Deinococcus species.**

| Protein | Drad | Dgeo | Ddes | Dmar | Dgob | Dpro | Dper | Dswu | Dsol | Dact | Dpun |
|---------|------|------|------|------|------|------|------|------|------|------|------|
| **DNA repair** |      |      |      |      |      |      |      |      |      |      |      |
| DdrO    | 1    | 1    | 2    | 1    | 3    | 1    | 2    | 1    | 1    | 1    | 1    |
| IrrE (PprI) | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| LexA-ArsR | 1    |      | 1    | 1    |      | 1    |      |      |      |      |      |
| LexA-XRE  | 1    | 1    |      |      | 1    | 1    |      |      |      |      |      |
| RqkA    | 1    | 1    | 1    | 1    |      | fr   | 1    | 1    | 1    | 1    | 1    |
| **Oxidative stress response and Mn/Fe homeostasis** |      |      |      |      |      |      |      |      |      |      |      |
| OxyR1-like (LysR) | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| OxyR2-like (LysR) | 1    |      |      |      |      |      |      |      |      |      |      |
| Mur-like (FUR 1) | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| PerR-like (FUR 2) | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| Irr-like (FUR 3) | 1    | 1    |      |      |      |      |      |      |      |      |      |
| DtxR    | 1    | 1    | 2    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| MntR    |      |      |      |      |      |      |      |      |      |      |      |
| SoxR    |      |      |      |      |      |      |      |      |      |      |      |
| **Two-component systems** |      |      |      |      |      |      |      |      |      |      |      |
| DrRRA   | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| DR_2419 |      |      |      |      |      |      |      |      |      |      |      |
| RadS    | 1    |      |      |      |      |      |      |      |      |      |      |
| RadR    |      |      |      |      |      |      |      |      |      |      |      |
| DrtS    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| DrtR    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| DR_1556 | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| DR_A0205 | 1    | 1    |      |      |      |      |      |      |      |      |      |
| **Quorum sensing** |      |      |      |      |      |      |      |      |      |      |      |
| Dqls-l-1 | 1    | 2    | 1    | 1    | 1    | 1    | 1    | 2    | 1    | 1    | 1    |
| Dqls-l-2 | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| DqslR   | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| LuxS    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| LsrB-like | 1    |      |      |      |      |      |      |      |      |      |      |
| **Others** |      |      |      |      |      |      |      |      |      |      |      |
| DdrI    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    | 1    |
| DR_0171 |      |      |      |      |      |      |      |      |      |      |      |
| DR_0265 | 1    | 2    | 2    | 1    | 1    | 1    | 2    | 1    | 1    | 1    | 1    |

See the legend to Table 2.

Radiation resistance is probably a consequence of adaptation to natural stress conditions such as desiccation. Various *D. radiodurans* mutants that are sensitive to IR are also sensitive to desiccation (Mattimore and Battista 1996). A common pool of genes is induced after exposure of *D. radiodurans* to either IR or desiccation (Tanaka et al. 2004). Both radiation and desiccation generate oxidative stress, DNA damage and, especially in radiation/desiccation-sensitive species, protein damage (Fredrickson et al. 2008).

Genes encoding proteins that may specifically contribute to desiccation tolerance have been identified in *D. radiodurans*, and include homologues of plant desiccation resistance-associated proteins (Makarova et al. 2001) and proteins containing large unstructured low-complexity regions (Krisko et al. 2010). Four of these genes have been studied for their role in desiccation tolerance: *DR_1172*, *DR_1372*, *DR_1769*, *DR_B0118* (Table S1, Supporting Information). Inactivation of *DR_1172* and *DR_B0118* sensitises *D. radiodurans* to desiccation, but not to IR. The *DR_1769* mutant is sensitive to desiccation and also slightly sensitive to IR. The *DR_1372* (*druH*) mutant, however, has increased sensitivity to oxidative (H₂O₂) stress, but not to desiccation. In *D. deserti*, *Deide_15148* encodes a protein almost entirely consisting of a low complexity region, and it may contribute to protein and membrane protection and prevention of protein aggregation during desiccation (de Groot et al. 2014). Homologues of these various proteins are present in all (*DR_1372, DR_1769*) or most other *Deinococcus* species (Table 5). Some of these proteins (e.g. *DR_1372, DR_B0118*) possess an N-terminal signal peptide, indicating an extracytoplasmic location.

**Desiccation tolerance-associated proteins**

Radiation resistance is probably a consequence of adaptation to natural stress conditions such as desiccation. Various *D. radiodurans* mutants that are sensitive to IR are also sensitive to desiccation (Mattimore and Battista 1996). A common pool of genes is induced after exposure of *D. radiodurans* to either IR or desiccation (Tanaka et al. 2004). Both radiation and desiccation generate oxidative stress, DNA damage and, especially in radiation/desiccation-sensitive species, protein damage (Fredrickson et al. 2008).

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**RADIATION AND OXIDATIVE STRESS RESISTANCE-ASSOCIATED REGULATORY PROTEINS**

Like other bacteria, *Deinococcus* species encode many proteins with a predicted role in signal transduction and gene regulation, and a requirement for radiation and oxidative stress resistance in *D. radiodurans* has been reported for several of these proteins (Table 6 and Table S6, Supporting Information). Expression or activity of DNA repair and other stress response proteins may be controlled by more than one regulatory protein.
Regulators of DNA repair system

The metallopeptidase/repressor pair IrrE/DdrO

Based on the currently available data, the IrrE/DdrO pair appears to be the most important regarding gene regulation in response to radiation and DNA-damaging agents. Indeed, *D. radiodurans* and *D. deserti* *irrE* deletion mutants are very sensitive to radiation (Earl et al. 2002a; Hua et al. 2003; Vujicic-Zagar et al. 2009), and the most highly induced genes in *D. radiodurans* and *D. deserti* following exposure to IR are regulated by IrrE/DdrO (Tanaka et al. 2004; Makarova et al. 2007; Blanchard et al. 2017). The crystal structure of *D. deserti* IrrE showed structural similarity with zinc metallopeptidases (Vujicic-Zagar et al. 2009). IrrE (PprI) is indeed a metallopeptidase (COG2856) that, after exposure of the cells to radiation, cleaves and inactivates DdrO, the XRE family transcriptional repressor of *pprA* and several *ddr* and DNA repair genes (e.g. *recA*) (Fig. 7) (Ludanyi et al. 2014; Devigne et al. 2015; Wang et al. 2015; Blanchard et al. 2017). This proteolytic activity of IrrE is essential for radiation resistance because strains expressing IrrE with point mutations in the active site that abolish its protease activity, or a strain expressing uncleavable DdrO, are as sensitive to radiation as *irrE* deletion mutants (Vujicic-Zagar et al. 2009; Ludanyi et al. 2014; Wang et al. 2015). The molecular mechanism by which radiation triggers DdrO cleavage has not been elucidated, but may be related to oxidative stress and metal ion availability for IrrE (Blanchard et al. 2017). The genes regulated by IrrE/DdrO are preceded by a 17-bp palindromic motif named RDRM (radiation/desiccation-response motif) (Makarova et al. 2007), which overlaps with or is located very close to the promoter of these genes (de Groot et al. 2014). Binding of repressor protein DdrO to RDRM-containing fragments has been demonstrated in vitro (Wang et al. 2015; Blanchard et al. 2017), and constitutive, derepressed expression has been observed in vivo for genes carrying point mutations in their preceding RDRM (Devigne et al. 2015; Anaganti et al. 2017). Cleaved DdrO does not bind the RDRM (Blanchard et al. 2017). IrrE, DdrO and the RDRM are highly conserved in Deinococcus species, but there is diversity in the RDR regulon composition (Blanchard et al. 2017). Demonstrated or predicted RDR regulon members in Deinococcus bacteria include *ddrA, ddrB, ddrC, ddrD, ddrF, ddrO, ddrQ, ddrR, ddrS, ddrTUWX, pprA, recA operon, extra recA, ssb, gyrA, gyrB, recD, recO, ruvB, urvA, urvB and urvD*, but some of these genes are not present in each Deinococcus species (Table 4). The RDR regulon includes *ddrO* itself, allowing DdrO to re-accumulate and repress the RDR regulon when the stress is alleviated. Interestingly, DdrO is essential for viability in at least *D. deserti* (Ludanyi et al. 2014) and *D. radiodurans* (Devigne et al. 2015), and its prolonged depletion in *D. radiodurans* results in apoptotic-like cell death (Fig. 7) (Devigne et al. 2015). The genes/proteins that provoke this apoptotic-like death are currently unknown. It is also noteworthy that three Deinococcus species encode one or two additional DdrO homologues (Table 6). Protein sequence comparisons suggest that these extra DdrO proteins can be cleaved and inactivated by IrrE (Fig. S17, Supporting Information), and in vivo evidence for this has indeed been obtained for the second DdrO in *D. deserti* (Ludanyi et al. 2014). Either one or the other *ddrO* gene is required for viability in *D. deserti*, suggesting that its two DdrO proteins have at least partially overlapping function regarding the genes they regulate. The different DdrO proteins in *D. gobsiens* and *D. peraridilitoris* may also have overlapping functions or, alternatively, regulate different genes.

Potential metallopeptidase/repressor pairs related to IrrE/DdrO have been identified in other bacterial genera, for example in *Meiothermus* spp. and some other species belonging to the phylum Deinococcos-Thermus and also in bacteria more distant to Deinococcus, indicating that a similar regulatory mechanism involving cleavage of an XRE family repressor by a specific COG2856 protease may also occur in these species (Ludanyi et al. 2014).

**RecA/LexA**

Induction of DNA repair genes in *Deinococcus* after cleavage of repressor DdrO by the separate protease IrrE is different from the well-known SOS response in *E. coli* and many other bacteria, in which *recA* and other DNA repair genes are induced after RecA-stimulated autocleavage of repressor LexA (Butala et al. 2011). Nevertheless, *D. radiodurans* encodes two LexA-related proteins, LexA1 (DR_A0344) and LexA2 (DR_A0074). Moreover, RecA-dependent cleavage of both these proteins has been demonstrated in vitro and in vivo (Narumi et al. 2001; Sheng et al. 2004; Satoh et al. 2006), strongly suggesting that expression of some currently unknown genes is under direct control of these LexA proteins, and induced following DNA damage. Indeed, the lexA1 mutant forms cell aggregates (Bonaccossa de Almeida et al. 2002). However, LexA1 and LexA2 are not involved in *recA* induction and at least LexA1 does not play a major role in radiation resistance in *D. radiodurans* (Jolivet et al. 2006). A lexA2 mutant has increased IR resistance according to one study (Satoh et al. 2006), but not according to another (Sheng et al. 2004). One or two LexA-related proteins are encoded by most, but not all, *Deinococcus* bacteria (Table 6 and Fig. 8). However, the similarities between these LexA proteins are rather low, and therefore the LexA-regulated genes might be different in these species. For example, the best hit in BLASTP analysis of *D. radiodurans* LexA1 against the other 10 *Deinococcus* species is Deide_1p01870 from *D. deserti* with only 50% identity. BLASTP of *D. radiodurans* LexA2 against these 10 other species does not give any hit at all. For comparison, *D. radiodurans* DdrO has 86 to 97% identity with a DdrO from each of these other species. The deinococcal LexA-related proteins contain either an ArsR-type (e.g. DR_A0344 and
Deide₁p01870) or an XRE-type (e.g. DR₂518) DNA-binding domain (Fig. S1B, Supporting Information).

RecA-mediated cleavage has also been demonstrated for Deide₁p01870, which controls expression of an operon that is radiation-induced in a RecA-dependent but IrrE-independent manner and that encodes Deide₁p01870 itself and the TLS DNA polymerases ImuY and DnaE2 (Dulermo et al. 2009). This operon is also found in D. peraridilitoris (see the section 'DNA repair in Deinococcus'), strongly suggesting that both D. deserti and D. peraridilitoris possess RecA/LexA-dependent regulation of TLS DNA polymerases besides the IrrE/DdrO-dependent regulation of other DNA repair genes (Fig. 9). Interestingly, both D. deserti and D. peraridilitoris encode two different RecA, and for D. deserti it has been shown that only one of its two RecA proteins mediates induction of the lexA-imuY-dnaE2 operon.

Serine/threonine-protein kinase RqkA

It has been reported that transgenic E. coli cells expressing D. radiodurans pqqE (DR₁C0034), encoding coenzyme PQQ-synthesis protein E, showed an improved tolerance to DNA-damaging agents (UV, IR and MMC) (Khairnar et al. 2007), and that the D. radiodurans pqqE mutant lacking PQQ was sensitive to IR and MMC (but not to UV) (Rajpurohit, Gopalakrishnan and Misra 2008). The higher radiation tolerance possibly mediated by PQQ in E. coli was suggested to require YfgL, which contains PQQ-binding motifs and a Ser/Thr protein kinase domain (Khairnar et al. 2007). To identify one or more proteins through which PQQ could contribute to IR resistance in D. radiodurans, five genes predicted to encode PQQ-binding proteins were inactivated. Among the five mutants, the strain lacking DR₂518 showed a similar phenotype as the pqqE mutant, that is, increased radiation sensitivity and impaired DNA double-strand break repair (Rajpurohit and Misra 2010). Increased sensitivity to DNA-damaging agents upon DR₂518 inactivation was also found independently in another study (Dulermo et al. 2015). DR₂518 encodes a protein containing a eukaryotic-type Ser/Thr protein kinase domain in addition to the multiple PQQ-binding motifs, suggesting that, like in E. coli, the PQQ contribution to radiation tolerance is functionally linked to a protein kinase. Moreover, PQQ-stimulated kinase activity of DR₂518 in vitro, and IR exposure induced autophosphorylation of DR₂518 in vivo (Rajpurohit and Misra 2010), and therefore DR₂518 was designated RqkA, radiation and PQQ-inducible protein kinase (Rajpurohit and Misra 2013). Remarkably, only D. radiodurans possesses both pqqE and rqkA, whereas most other IR-resistant Deinococcus species have only rqkA (Tables 3 and 6), suggesting that RqkA may play a role in IR

Figure 8. Phylogenetic relationship of LexA homologues identified in Deinococcus species. The phylogenetic analysis was carried out based on protein sequence alignment of 12 deinococcal LexA homologues (Table S6, Supporting Information) with some representative proteins taken from Uniprot: LexA from B. subtilis (Uniprot Number P31080) and E. coli (P0A7C2). See further the legend to Figure 4.

Figure 9. IrrE/DdrO- and RecA/LexA-regulated expression of DNA repair genes within the same bacterium. The cinA-ligT-recA operon (ligT-cinA in D. proteolyticus) and genes for IrrE and DdrO are present in each Deinococcus species. Additional RecA (RecA-2) and the lexA-imuY-dnaE2 operon are found in D. deserti and D. peraridilitoris. Experimental data, obtained for D. deserti only, have shown that both recA genes are radiation-induced in an IrrE-dependent way, and that the presence of either recA-1 or recA-2 is sufficient for radiation resistance. Radiation exposure also induces expression of the lexA-imuY-dnaE2 operon leading to induced mutagenesis mediated by the translesion polymerases ImuY and DnaE2, and this induction requires recA-1 but not recA-2. The RecA-2 product is thus functional for recombinational repair but not for induction of mutagenic lesion bypass. The lexA-imuY-dnaE2 operon is also radiation induced in the irrE mutant, indicating that basal level of RecA-1 is sufficient for this induction. The red symbols indicate transcriptional repression by DdrO or LexA, or repressor inactivation by IrrE- or RecA-mediated cleavage. HP, hypothetical protein.
resistance without PQO. However, *D. proteolyticus* lacks *rqrA* and the homologous sequence in *D. gobiensis* contains a frameshift. To find potential target proteins for *rqrA*, conserved phosphorylation motifs have been identified using bioinformatics in many *D. radiodurans* proteins, including *pprA* and *recA* (Rajpurohit and Misra 2013). Subsequently, in vitro phosphorylation of *D. radiodurans* *pprA* (the majority at residue T72, and also at S112 and T144) and *D. radiodurans* *recA* (at Y77 and T318) by *rqrA* has been reported, although at other residues than predicted (Rajpurohit and Misra 2013; Rajpurohit et al. 2016). However, T72 of *pprA* and T318 of *recA* are not conserved among deinococcal homologues, and understanding the role of phosphorylation of these proteins by *rqrA* requires further investigation.

Regulators of oxidative stress response and Mn/Fe homeostasis

Several studies have revealed that when bacteria are exposed to *H₂O₂* and other ROS, they not only induce ROS detoxification enzymes such as catalases and SOD, but also adapt to the oxidative stress by modifications of metal ion homeostasis with the net effect of reducing the damage caused by reactive ferrous iron (Faulkner and Helmann 2011). Generally, bacteria reduce levels of free Fe²⁺ in the cell, for example by sequestration in storage proteins (e.g. Dps) and repression of Fe²⁺ uptake, and elevate cytosolic levels of Mn²⁺, which can replace Fe²⁺ from sensitive sites in enzymes, by inducing Mn²⁺ import. For *D. radiodurans*, which has a high Mn/Fe ratio under standard laboratory conditions, five regulators with a proposed role in oxidative stress response and Mn/Fe homeostasis have been described. These are two *lysR* family regulators (proposed to be 1-Cys-OxyR proteins), two *fur* family regulators (possible *perR* and *mur* proteins) and a DtxR-like regulator (see below for more details). Of these, the possible Mur seems the most important under standard conditions, because its inactivation results in a growth defect (Ul Hussain Shah et al. 2014), while such defect was not observed or reported for mutant strains lacking one of the other four regulator proteins. Except for the mutant strain lacking the *perR*-like protein, transcriptomics experiments have been performed to analyse global gene expression in these *D. radiodurans* regulator mutants compared to the wild-type strain, which revealed differential expression (more than 2-fold up- or downregulation) of dozens to hundreds of genes. However, more work is needed to determine which genes are regulated directly by these regulators and to decipher if and how these regulators respond to oxidative stress or levels of specific metals.

Hydrogen peroxide sensor OxyR

*Escherichia coli* encodes dozens of *lysR*-type transcriptional regulators. One of these has been characterised as the *H₂O₂*-sensing regulator OxyR. *Escherichia coli* OxyR contains two Cys residues that are essential for *H₂O₂* sensing: upon oxidation by *H₂O₂* of the sensing cysteine C199 followed by intramolecular disulphide bond formation with the resolving cysteine C208, OxyR is locked in a conformation that activates the protein as a transcription factor (Dubbs and Mongkolsuk 2012; Imlay 2013, 2015). Only two *lysR*-type regulators, *DrO615* and *DrA0336*, are found in *D. radiodurans*. The e. coli *lysR*-type proteins most similar to both *DrO615* and *DrA0336* are *YnhL* and *HcaR* (34–38% identity), while e. coli OxyR is about 30% identical to *DrO615* and *DrA0336*. Nevertheless, *DrO615* and *DrA0336*, which share 38% identity, have been described as OxyR1 and OxyR2, respectively, each containing only one cysteine residue that was reported to be essential for protein function in vivo (Chen et al. 2008; Yin et al. 2010). The single Cys residue (C210) of *DrO615* could be oxidised to sulfenic acid in vitro (Chen et al. 2008).

The OxyR regulon of *E. coli* is comprised of more than 20 genes, including genes involved in *H₂O₂* scavenging (*katG*, *ahpCF*), Fe-S cluster assembly (*sufABCDE*), iron scavenging (*dps*), Mn import (*mntH* and disulphide reduction (*trxC*, *grxA*, etc.) (Imlay 2015). The genes directly regulated by *DrA0336* (OxyR2) are unknown, and *DrA0336* has clear homologues (60% identity) only in two of the analysed Deinococcus species (Table 6). Under standard conditions, hundreds of genes showed either increased or decreased expression in the *DrO615* (OxyR1) deletion mutant compared to the wild-type strain (Chen et al. 2008). *DrO615* may directly regulate the genes *DrR0125* (iron transporter), *katE1*, *mntH* and *dps1*, because binding of *DrO615* to DNA regions upstream of these genes has been observed in vitro. The same DNA-binding pattern was observed with oxidised *DrO615*, reduced *DrO615* or *DrO615* with the C210A mutation. However, the precise mechanism by which *DrO615* may regulate genes either positively or negatively upon *H₂O₂* exposure has not been elucidated. The *katE1* expression was induced 4-fold by *H₂O₂* in wild type, but an approximately 3-fold induction was still observed in *DrO615* mutants. In addition, although *DrO615* is proposed to be a negative regulator of *mntH* and *dps1*, *mntH* expression is reduced, whereas *dps1* is highly induced by *H₂O₂* stress in *DrO615* mutants (Chen et al. 2008). *DrO615* is highly similar (74–80%) to one protein in each of the other 10 Deinococcus species (Fig. S19, Supporting Information), indicating a crucial conserved role. Importantly, however, the single and proposed sensing Cys of *DrO615* is not strictly conserved. The homologues from *D. peraridilitoris* and *D. deserti* lack Cys, and therefore at least these Cys-less *DrO615* homologues cannot function as canonical or 1-Cys-OxyR. Clearly, further research is necessary to elucidate the precise role and mode of function of *DrO615* and homologues.

**Fur family regulators**

In most bacteria, proteins of the Fur (ferric uptake regulator) family act as metal sensors that regulate genes connected to metal homeostasis and response to oxidative stress. Although the iron-responsive regulator Fur is a well-known member of the Fur family, there is diversity in metal selectivity and biological function within the Fur family which includes manganese uptake regulator Mur (responding to Mn²⁺), Zur (responding to Zn²⁺), Nur (responding to Ni²⁺), PerR (responding to peroxide stress) and Irr (heme-dependent iron responsive) (Lee and Helmann 2007; Fillat 2014). Initially, only one gene encoding a Fur family protein was predicted in *D. radiodurans* (*DrO865*, possible *mur*). Later, a second gene was identified (de Groot et al. 2009) and proposed to encode PerR (Liu et al. 2014).

The Fur family protein *DrO865* of *D. radiodurans* was proposed to correspond to Mur (Ul Hussain Shah et al. 2014). To date, Mur proteins have been found in some α-proteobacteria, such as *Rhizobium* and *Sinorhizobium*, and the only known target for Mur is the ABC-type Mn²⁺ transporter (Johnston et al. 2007; Fillat 2014). Of the five Mn²⁺-transport-related genes of *D. radiodurans*, the expression of *mntA*, *mntB* and *mntH* increased, while *mntE* was repressed in the *DrO865* mutant strain compared to the wild-type strain under standard laboratory conditions. Moreover, expression of *mntA* and *mntE* in the *DrO865* mutant under Mn²⁺ stress was significantly higher and lower, respectively, compared to the Mn²⁺-stressed wild type (Ul Hussain Shah et al. 2014). Differential gene expression in Mn²⁺-stressed cells versus non-stressed cells was not reported.
Figure 10. Three groups of FUR family proteins identified in 11 Deinococcus species. The phylogenetic analysis was carried out based on protein sequence alignment of 26 deinococcal FUR family proteins (Table S6, Supporting Information) with some representative proteins taken from Uniprot: Fur proteins from *Vibrio cholerae* (Uniprot Number P0C6C8), *E. coli* (P0A9A9), *P. aeruginosa* (Q03456), *Campylobacter jejuni* (P0C631) and *H. pylori* (O25671); Mur from *Rhizobium leguminosarum* (Q1MMB4); Zur from *Streptomyces coelicolor* (Q9L2H5), *M. tuberculosis* (P9WN85), *B. subtilis* (P54479), and *E. coli* (P0ACS5); Nur from *S. coelicolor* (Q9K4F8); PerR from *B. subtilis* (P71986) and *Streptococcus pyogenes* (Q1J1US); Irr from *R. leguminosarum* (I9X7E3) and *Bradyrhizobium japonicum* (O85719). GenBank accession numbers in parentheses follow the species name. The phylogenetic consensus tree was developed using the neighbour-joining algorithm in MEGA 6.0. The node numbers are bootstrap values based on 1000 replications.

DR_0865 was found to bind to DNA fragments containing the promoters of MntABC transporter genes in vitro but not to the mntf promotor (Sun et al. 2012). However, there is a controversy about mutant phenotypes: the DR_0865 mutant strain was reported to be sensitive to Mn$^{2+}$ and H$_2$O$_2$ (Ul Hussain Shah et al. 2014), but other studies mentioned that the mutant strain exhibited greater resistance to H$_2$O$_2$ than wild type (Chen et al. 2008) and showed that its Mn$^{2+}$ resistance was comparable that of wild type (Sun et al. 2012). Because the Mur and Fur proteins have similar sequences, and Murs from Rhizobiales can complement *E. coli* fur mutants (Johnston et al. 2007; Hohle and O’Brian 2016), further research is needed to define the exact role of DR_0865, especially in terms of metal homeostasis. Moreover, phylogenetic analysis indicates that DR_0865 and homologues (FUR group 1), present in each Deinococcus, are more related to some of the Zur proteins than to the Mur of Rhizobium (Fig. 10).
PerR is a global regulator that responds primarily to H$_2$O$_2$, and substitutes for OxyR in many Gram-positive bacteria, although it may also coexist with OxyR (Dubbs and Mongkol suk 2012). In, for example, B. subtilis, PerR is inactivated by H$_2$O$_2$ stress, leading to derepression of the PerR regulon, including the genes for Pnr, catalase and the ferritin-like proteins (Hillion and Antelmann 2015). In D. radiodurans, disruption of the putative perR gene increased katE1 and dps1 expression and resistance to H$_2$O$_2$ stress (Liu et al. 2014). However, it is necessary to investigate if the derepression of these genes, especially dps1, occurs via inactivation of the PerR-like protein under oxidative (H$_2$O$_2$) stress condition, because the dps1 gene expression is not induced by H$_2$O$_2$ (Liu et al. 2014). Homologues of the putative PerR of D. radiodurans are present in all other Deinococcus species (FUR group 2) (Fig. 10). PerR is a metal-dependent transcriptional regulator, and the Fe$^{2+}$-containing form is thought to be responsible for H$_2$O$_2$ sensing, in which Fe$^{2+}$ bound at the regulatory site is coordinated by three His (H37, H91, H93) and two Asp (D104, D85) residues in B. subtilis (Dubbs and Mongkol suk 2012). Exposure to H$_2$O$_2$ leads to oxidation of Fe$^{2+}$ in the regulatory site by a Fen- ton reaction, which causes oxidation of H37 and H91 to 2-oxohistidine and inactivation of PerR (Hillion and Antelmann 2015). The five amino acid residues (H23, H79, H81, D71 and D92 in the PerR-like protein from D. radiodurans) are strictly conserved in all FUR group 2 proteins (Fig. S20, Supporting Information). Phylogenetic analysis also indicates that these deinococcal proteins are related to PerR (Fig. 10).

An additional, third FUR family protein (FUR group 3) is found in four of the analysed Deinococcus species (Table 6), increasing the diversity of FUR metalloregulators compared to D. radiodurans. Like the deinococcal PerR-like proteins, these proteins contain the three His and two Asp residues that are involved in metal ion binding at the regulatory site of B. subtilis PerR (Fig. S20, Supporting Information). However, phylogenetic analysis shows that these proteins of FUR group 3 are more closely related to the heme-dependent iron responsive regulator Irr (Fig. 10). Irr binds heme at a HXH motif, conserved in most FUR family proteins, and acts as both positive and negative regulator of gene expression modulating a number of genes related to iron metabolism in Rhizobiales (Fillat 2014). However, unlike Irr proteins but similar to many other FUR family proteins (including the deinococcal FUR group 1 and 2, PerR, Zur and some Fur proteins), this third group of deinococcal FUR proteins contain two CXXC motifs. The Cys residues in these motifs may be required for activity and protein stability, and probably coordinate a structural zinc ion (Lee and Helmann 2007; Fillat 2014).

**DtxR and MntR regulators**

The control of iron metabolism and its coupling with regulation of defences against oxidative stress is carried out by Fur in most prokaryotes, but high-GC Gram positive bacteria, such as Mycobacterium, tend to use the DtxR (diphtheria toxin repressor) family for iron homeostasis (Fillat 2014). In D. radiodurans, DR$_{2539}$ was proposed to be a novel DtxR-like regulator (Chen et al. 2010). Compared to the wild-type strain, the iron transporter genes DR$_{1219}$ and DR$_{B0125}$ were downregulated in the DR$_{2539}$ mutant under standard growth conditions. Under these conditions, the manganese transporter genes mntBC were found (slightly) upregulated in the DR$_{2539}$ mutant, while expression of the manganese transporter gene mntH and of the two dps genes was not affected by the DR$_{2539}$ disruption (Chen et al. 2010). In another study, mntH expression was found to be reduced in wild-type strain, but not in the DR$_{2539}$ mutant, by the addition of Mn$^{2+}$ or Fe$^{3+}$ to the growth medium. In vitro DNA-binding experiments indicated that this Mn$^{2+}$/Fe$^{2+}$-dependent mntH repression occurs through the direct binding of DR$_{2539}$ to the mntH promoter, while binding of DR$_{2539}$ to the promoters of MntABC transporter genes was not observed (Sun et al. 2012). Although DR$_{2539}$ seems to function as a repressor of mntH only upon addition of Mn$^{2+}$/Fe$^{2+}$, the data indicate that DR$_{2539}$ is also functional under standard conditions because expression of dozens of genes is affected in the DR$_{2539}$ mutant in standard growth medium (Chen et al. 2010). The other Deinococcus species produce one DR$_{2539}$ homologue except D. deserti encoding two homologues. Metal-binding site 1 (MBS1) of DR$_{2539}$ from D. radiodurans is composed of His79, Glu83, His98, Arg176 and Pro179, and MBS2 is composed of Asp11, Glu102, Glu105 and His106 (Chen et al. 2010). Sequence alignment analysis shows that most of the residues located in MBS are conserved in the deinococcal DR$_{2539}$ homologues except Arg176 in MBS1 and Glu102 in MBS2 (Fig. S21, Supporting Information). DtxR is composed of two domains: the N-terminal domain is involved in metal binding, dimerisation and DNA recognition, and the C-terminal Src homology 3 (SH3)-like domain is involved in metal ion binding at MBS1, thereby affecting repressor activity (Love, VanderSpek and Murphy 2003). MntR is a DtxR homologue regulated by Mn$^{2+}$. DtxR and MntR share similar structure and metal-binding residues, but MntR lacks the C-terminal SH3-like domain (Stoll et al. 2009). Interestingly, an MntR-like regulator is found in D. deserti and D. peraridilitoris (Fig. S21, Supporting Information), and in both species this regulator is encoded by a gene located directly after and likely in operon with four ABC-type Mn$^{2+}$ transporter genes encoding MntA, MntC and two different MntB homologues.

**SoxR: redox-sensitive transcriptional activator**

Escherichia coli and several other bacterial species encode the MerR family regulator protein SoxR, involved in induction of sodA and other defensive genes upon exposure to superoxide-generating redox-cycling compounds such as phenazines and quinones. SoxR contains a [2Fe-2S] cluster, which involves cysteine residues that are present in the conserved motif CIGCCGxxxxxC located in the C-terminal region of the protein. Oxidation of the iron–sulphur cluster activates SoxR and transcription of target genes. Initially, it was expected that superoxide directly oxidises the [2Fe-2S] clusters, but experiments have indicated that the redox-cycling compounds themselves activate SoxR. In E. coli, SoxR induces a second transcription factor, SoxS, which then induces expression of sodA and other target genes. In non-enterics, however, SoxR directly controls expression of SoxR regulon genes, which are different from those of E. coli and may encode pumps to excrete redox-cycling compounds but generally do not include sodA (Imlay 2013, 2015).

Deinococcus radiodurans does not possess a SoxR gene. However, homologues of E. coli SoxR (54–60% identity), including the CIGCCGxxxxxC motif, are found in D. deserti, D. proteolyticus, D. soli and D. actinomycetemcomitans (although the gene in D. actinomycetemcomitans has a frameshift). In these Deinococcus species, SoxR may regulate currently unknown target genes involved in defence against redox-active compounds.

**Other radiation and oxidative stress resistance-associated regulators**

**Two-component signal transduction systems**

TCSSs, composed of a histidine kinase (HK) and a response regulator (RR), are major means by which bacteria adapt to
changing environments. Typically, the environmental signal triggers HK autophosphorylation at one His residue, followed by phosphoryl transfer from the phospho-His to an Asp residue in the RR, thereby regulating expression of genes and/or modulating activity of proteins (Casino, Rubio and Marina 2010; Agrawal, Sahoo and Saini 2016). DrRRA (DR_2418) was the first RR identified as contributing to the resistance of \( D. \) radiodurans not only to IR and \( H_2O_2 \) but also to desiccation (Wang et al. 2008). Compared to the wild type, the expression of numerous genes, including stress response and DNA repair genes as well as many uncharacterised genes (e.g. katE1, katE2, sodA, sodC, dps1, recA, uvrA, gyrB, \( ddrC \), pprA, \( ddrI \), \( ddrP \)), is lower in the \( drr \) mutant, both under standard growth conditions and after irradiation (Wang et al. 2008). However, it appears that at least several genes that are IR induced in the wild type are still IR induced in the \( drr \) mutant (e.g. recA, pprA, \( ddrC \)). Binding of DrRRA protein to a \( ddrI \) promoter-containing DNA fragment has been observed in vitro (Wang et al. 2008), but the DrRRA–DNA interaction has not been studied in more detail. At the protein level, one study suggested reduction of RecA and PprA levels in the \( drr \) mutant compared to the wild type (Wang et al. 2008), but this was not observed in another study (Wang et al. 2016b). DrRRA is conserved in the other Deinococcus species except for \( D. \) peraridilitoris. Concerning the genetic organisation, \( drr \) in \( D. \) radiodurans is adjacent to the HK gene \( DR_2419 \), suggesting that DrRRA might be the cognate RR for \( DR_2419 \), but the \( DR_2419 \) disruption has a less strong effect on IR resistance than the \( drr \) disruption (Wang et al. 2008; Im et al. 2013). Moreover, several \( drr \)-containing Deinococcus species do not encode the homologue of HK \( DR_2419 \) (Table 6). In addition, \( DR_2420 \) encoding another RR is adjacent to \( DR_2419 \), and the ‘RR-HK-RR’ gene cluster is also found in some other Deinococcus species (Fig. S22, Supporting Information). Further research is needed to identify HKs that can phosphorylate DrRRA.

The \( Rads/RadR \) (\( DR_20090/DR_20091 \)) TCS contributes to radiation resistance in \( D. \) radiodurans (Desai et al. 2011; Im et al. 2013). However, homologues are only present in \( D. \) gobiensis. Both in \( D. \) radiodurans and \( D. \) gobiensis this radSR gene pair is directly adjacent to the divergently oriented gene encoding extracytoplasmic Dps2, indicating a possible functional link (see also the section ‘Other proteins involved in ROS protection’).

In \( D. \) radiodurans, inactivation of either \( DR_2416 \) encoding a HK or \( DR_2415 \) encoding the probable cognate RR of HK \( DR_2416 \) resulted in slightly reduced resistance to radiation, MMC and/or oxidative stress; hence, \( DR_2415 \) and \( DR_2416 \) were designated as DrrR and DrrS (DNA damage response TCS regulator and sensor), respectively (Im et al. 2013). Contrary to DrRRA and RadR/RadS, both DrrR and DrrS are conserved in the other analysed Deinococcus species.

In addition to \( radS \) and \( drrS \), 10 other HK genes have been inactivated separately in \( D. \) radiodurans, resulting in slightly reduced resistance to radiation and/or oxidative stress for each mutant (Im et al. 2013). Except for \( DR_1556 \) and \( DR_20025 \) (Table 6), these HKs are conserved in the other analysed Deinococcus species.

**Quorum-sensing systems**

Quorum sensing (QS) is a cell-to-cell communication process that enables bacteria to behave coordinately and to regulate gene expression in response to changes in the cell density. QS involves the production, release and detection of extracellular signalling molecules called autoinducers (AIs) (Papenfort and Bassler 2016). There are several QS systems used by bacteria: the LuxR/I-type systems, primarily used by Gram-negative bacteria, in which the signaling molecule is an acyl-homoserine lactone (AHL or AI-1); the peptide signaling systems used primarily by Gram-positive bacteria; and the LuxS/furanone metabolites (collectively called AI-2) signaling used for interspecies communication (Reading and Sperandio 2006). A few studies have indicated that QS may also contribute to the resistance phenotype of \( D. \) radiodurans (Lin et al. 2016a,b). Slightly reduced resistance to radiation and/or oxidative stress has been reported for strains carrying disruptions of genes involved in AHL- and AI-2-mediated QS systems: \( DR_2587 \) and \( DR_0090 \) encoding homologues of AHL synthase, designated \( dqsA-1 \) and \( dqsA-2 \) for Deinococcus quorum sensing autoinducer-1 and -2, respectively. \( DR_0987 \) encoding the AHL-responsive regulator DqsR, and \( DR_2387 \) encoding the LuxS enzyme responsible for the synthesis of AI-2. \( H_2O_2 \) treatment was found to induce AHL accumulation in \( D. \) radiodurans. Deinococcus radiodurans also possesses the quorum quenching enzymes AHL-acylase (QqsR, \( DR_2055 \)) and AHL-lactonase (QqlR, \( DR_0172 \)), which are able to inactivate foreign AHLs (Koch et al. 2014), and AHL levels are higher in the qqsR and qqlR mutants (Lin et al. 2016a). The expression of many genes was affected in \( luxS \) and \( dqsR \) mutants compared to the wild-type strain, including stress response-related genes (Lin et al. 2016a,b). DqsR-binding sites have been predicted in the upstream regions of various genes that are downregulated in the \( dqsR \) mutant, and in vitro binding of DqsR to three selected regions has been observed: upstream of \( DR_1436 \) (ABC transporter), \( DR_20158 \) (phosphate ABC transporter) and \( DR_20067 \) (extracellular nuclease) (Lin et al. 2016a).

The \( dqsA-1 \) and \( dqsA-2 \) proteins involved in the AHL-mediated QS system are conserved in the other analysed Deinococcus species, but \( D. \) proteolyticus lacks the AI-2 synthesis protein LuxS. The AI-2 signal molecule is detected by LuxP that functions in conjunction with the two-component sensor kinase LuxQ in Vibrionaceae, or is imported by the LsrABC transporter, in which LsrB acts as an AI-2 receptor, in Enterobacteriaceae (Rezzonico, Smits and Duffy 2012). LuxP homologues were not found in the Deinococcus species. An LsrB-like protein is detected only in \( D. \) deserti and \( D. \) geothermalis. Given that the supernatant of the \( D. \) radiodurans wild-type strain restores the radioresistance phenotype of the \( luxS \) mutant strain (Lin et al. 2016b), it is possible that additional, yet undiscovered, AI-2 receptors exist in \( D. \) radiodurans and other deinococci (Rezzonico, Smits and Duffy 2012).

**Other DNA-binding transcriptional regulators**

The cyclic AMP receptor protein (CRP) is a global regulator that regulates over 490 genes in \( E. \) coli, especially in relation to carbon metabolism, and can indirectly mediate the expression of a large number of stress response proteins (Geng and Jiang 2015). Of the four genes encoding putative CRP family proteins (\( DR_0997 \), \( DR_1646 \), \( DR_2362 \) and \( DR_2834 \)) in \( D. \) radiodurans, \( DR_0997 \) (also referred to as \( ddrI \)) is highly induced by IR (Tanaika et al. 2004), and its disruption, but not that of any of the other predicted CRP genes, results in increased sensitivity to \( H_2O_2 \), MMC, UV and IR (Yang et al. 2016). The transcriptional levels of a series of genes involved in DNA repair, oxidative resistance and other cellular pathways were measured, and expression of several of these genes (e.g. \( katE1 \), \( DR_20020/sodC \), PprA, \( uvrA \), \( uvrC \), \( ruvC \), \( recA \), \( recF \), \( recN \), \( ddrB \), \( ddrC \), \( ddrD \)), Lon protease genes, glycollabolism gene \( glyC \) was found to be lower in the \( ddrI \) mutant than in the wild-type strain under both normal and stress (IR or \( H_2O_2 \)) conditions (Yang et al. 2016). Moreover, the \( ddrI \) deletion mutant grows slower than the wild-type under standard conditions. The upstream region of at least 18 genes in \( D. \) radiodurans contains...
sequences similar to the E. coli CRP-binding site, and binding of the CRP family protein Ddrl to these regions has been demonstrated in vitro (Yang et al. 2016). These 18 genes, including ppRA, uwe and recN, may be regulated directly by Ddrl, whereas the reduced expression of many other genes in the ddrI mutant may be caused indirectly (e.g. katE1, recA, ddrC). A recent study indicated that Ddrl expression is DrRRA dependent in D. radiodurans, and that Ddrl may regulate hundreds of genes involved in various cellular processes, underlining its important role in cell physiology under normal and stress conditions (Meyer et al. 2018). The Ddrl homologue, but not DrRRA, is present in each Deinococcus species (Table 6). The studies on Ddrl and other data (Kamble et al. 2010) suggest that cyclic AMP signalling contributes to expression of stress response and DNA repair genes. Also cyclic di-AMP signalling may contribute in the recovery of D. radiodurans cells from genotoxic stresses, because inactivation of DR_0007, encoding a homologue of B. subtilis CdaA that catalyses cyclic di-AMP synthesis, sensitises D. radiodurans to radiation (Table S1, Supporting Information). Homologues of DR_0007, as well as of the adjacent DR_0008 gene encoding a homologue of CdaR that stimulates CdaA activity in B. subtilis, are present in all of the analysed Deinococcus species (Table S5, Supporting Information).

DR_0171 is a predicted DNA-binding protein that is induced after exposure to high doses of IR (Liu et al. 2003; Lu et al. 2011). Compared to the wild type, a lower expression of various genes, encoding proteins belonging to different functional categories as well as proteins of unknown function, has been reported in the DR_0171 mutant after exposure to IR (Lu et al. 2011). DR_0171 is not conserved in Deinococcus (Table 6), and understanding how it directly or indirectly regulates gene expression in D. radiodurans requires further work.

DR_0265 is another predicted DNA-binding transcription factor (GntR family). Its contribution to radiation resistance has been found after screening transposon mutants (Dulermo et al. 2015). The target genes for DR_0265 are currently unknown. Deinococcus proteolyticus lacks a DR_0265 homologue, while three other Deinococcus species possess two DR_0265 homologues (Table 6).

CONCLUDING REMARKS

Repair of massive DNA damage is not given to everyone. Deinococcus bacteria have this astonishing skill when facing high doses of radiation, desiccation and oxidative stress-generating conditions. To decipher the underlying mechanisms, D. radiodurans appeared to be an excellent model organism. Its thorough characterisation over the last decades has led to many important discoveries, and indicated that its extreme resistance results from a combination of multiple factors and well-regulated mechanisms that limit oxidative protein damage and enable repair of massive DNA damage. At a first glance, the DNA repair machinery of D. radiodurans seems globally similar to the one of other bacteria like E. coli, but detailed studies revealed several specificities that may contribute to radiation resistance (proteins like RecA may have evolved to perform better under stress conditions, multiple variants of some DNA repair proteins such as DNA glycosylases, novel Deinococcus-specific proteins such as DdrB and PPRA). Although the various resistance and repair mechanisms are not fully understood, these pioneering results obtained with D. radiodurans have greatly advanced our understanding of radiation resistance in general, with some common aspects (but also differences) observed in other radiation-resistant organisms that have evolved either naturally (i.e. archaea, small invertebrates) or after repeated irradiation in the laboratory (i.e. E. coli mutants), and may pave the way to better understand radiation resistance in cancer cells emerging after radiotherapy.

Since the description of D. radiodurans, many other radiation-resistant Deinococcus species have been isolated offering potential sources of new discoveries. In this paper, we explored this large biodiversity by analysing and comparing the genomes of 11 Deinococcus species. For this, we have investigated the conservation of more than 250 genes, including genes with a reported contribution to radiation or oxidative stress resistance in D. radiodurans and other genes with an expected role in radiation resistance-associated mechanisms such as DNA repair, oxidative stress defence and their regulation. Conservation was indeed observed for many genes encoding proteins that are also important or even essential in non-Deinococcus bacteria (RecA, SSB, GyrAB, SoDA, etc.). Several proteins with currently unknown precise role are also present in each Deinococcus (e.g. RecR, glutaredoxin-like proteins, DdrC). Striking specificities also emerged, with a huge diversity with respect to the presence/absence or number of variants of radiation resistance-associated proteins. The radiation/desiccation response regulon is partly constituted of Deinococcus-specific proteins, of which only a few are conserved in each Deinococcus, including its regulator pair IrrE/DdR (essential for radiation resistance) and DdrB (single-stranded DNA-binding protein). Several genes encoding DNA repair or oxidative stress response proteins are present in one or several of the more recently sequenced Deinococcus species but not in D. radiodurans (e.g. DNA repair and carotenoid biosynthesis proteins composed of novel two-domain combinations, endonuclease NucS, photolyase, TLS polymerases, SoxR, manganese-containing catalase). Conversely, and remarkably, dozens of genes encoding proteins with a reported contribution to radiation and oxidative stress resistance in D. radiodurans are absent in one, several or even most other Deinococcus species (e.g. the RNA or DNA ligases Rnl and Ligg, PPRA, PqQE, the RadS/RadR two-component system, one or both heme-containing catalases). The high sensitivity to IR of the ppRA mutant in particular has been demonstrated in several independent studies. Why are these genes absent in other Deinococcus species? One could argue that the presence of these genes in D. radiodurans makes this species more radiation resistant than all the other Deinococcus species, but this seems unlikely. Indeed, D. radiodurans, D. geothermalis and D. actinosclerus are equally resistant to IR under identical experimental conditions (Makarova et al. 2007; Joo et al. 2016). The absence of a gene homologue in another Deinococcus species may be compensated by another gene that has little sequence similarity but may encode a protein with similar function, or the gene is not required because the species employs other molecular or regulatory mechanism(s). As some non-conserved genes contribute to radiation resistance in D. radiodurans, it is reasonable to propose that the other Deinococcus species also contain genes that have an important role in radiation resistance but which are absent in D. radiodurans or others. These may be genes of currently unknown function, for example genes under control of the important regulator pair IrrE/DdR, or some of the additional DNA repair or oxidative stress defence genes identified in several of the Deinococcus species.

It is clear from this comprehensive analysis that there is not only one winning combination that leads to radiation resistance even in the Deinococcus genus. These bacteria not only possess common protection and repair systems but also molecular
mechanisms that are different between species, including diversity in DNA repair mechanisms and oxidative stress response. These results open the way to deciphering new protein functions and new mechanisms.

SUPPLEMENTARY DATA
Supplementary data are available at FEMSRE online.

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