REVIEW ARTICLE

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 metalloregulators, 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 homeostatis; 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 comparion, 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 bdelloid 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 Kulbachinskiy 2015; Timmins and Moe 2016). Compared to radiation-sensitive 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 Kulbachinskiy 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). *Deinococus* 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*.

DEINOCOCCUS RADIODURANS MUTANTS AFFECTED IN RADIATION AND OXIDATIVE STRESS RESISTANCE

More than a hundred radiation- and/or oxidative stress-sensitive mutant strains of *D. radiodurans* have been described in numerous studies (Table S1, Supporting Information). A schematic overview of proteins required for radiation and oxidative stress resistance is shown in Fig. 2. Many of the mutants were obtained after deleting or disrupting a specific gene that was selected because of its expected or possible role in DNA repair, oxidative stress defence and regulation of radiation-resistance-associated genes, or because of its radiation-induced expression. Other mutants were obtained after chemical or transposon mutagenesis followed by screening for increased radiation sensitivity.

Only a few mutant strains were found to be very sensitive to IR, showing a strong decrease in survival after exposure to relatively low doses (< 2 kGy) of IR (Table S1, Supporting Information). These strains are mutated for *recA* (locus tag DR_2340), *recF* (DR_1089), *recO* (DR_0819), *recR* (DR_0198), *polA* (DR_1707), *pprA* (DR_A0346), *pprM* (DR_0907) or *irrE* (DR_0167). The DNA repair proteins RecA, RecF, RecO, RecR and PolA are involved in extended synthesis-dependent strand annealing and recombinational repair (Zahradka *et al.* 2006; Slade *et al.* 2009; Bentchikou *et al.* 2010). PprA is a *Deinococcus*-specific protein required for accurate chromosome segregation and cell division after exposure of the cells to radiation (Devigne *et al.* 2013). IrrE, also called PprI, is a metalloprotease required for induced expression of *recA*, *pprA* and other genes after irradiation (Earl *et al.* 2002a; Hua *et al.* 2003; Ludanyi *et al.* 2014). PprM corresponds to the single cold shock protein homologue in *D. radiodurans* (Ohba *et al.* 2009). Many other mutants showed more than 10-fold reduced survival compared to the wild type only at higher irradiation doses (> 5 kGy), or were found only slightly IR sensitive with less than 10-fold decreased survival compared to the wild type at the highest dose tested.

Several gene mutant strains have been characterised by two or more research teams, and, remarkably, the reported results are sometimes rather different, with mutants found sensitive to IR or other agents in one study but resistant in another study (for details, see legend of Table S1, Supporting Information). Such contrasting results may be due to differences in the bacterial strains used and/or in the experimental procedures.
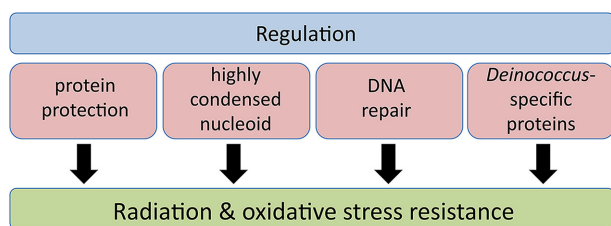

Figure 1. Extreme radiation and oxidative stress resistance in *Deinococcus* involves multiple factors and well-regulated mechanisms.

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 swuensis (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 *recJ* (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; Bentchikou *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 H_2O_2 (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 *DEINOCOCCUS*

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 UvrA (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
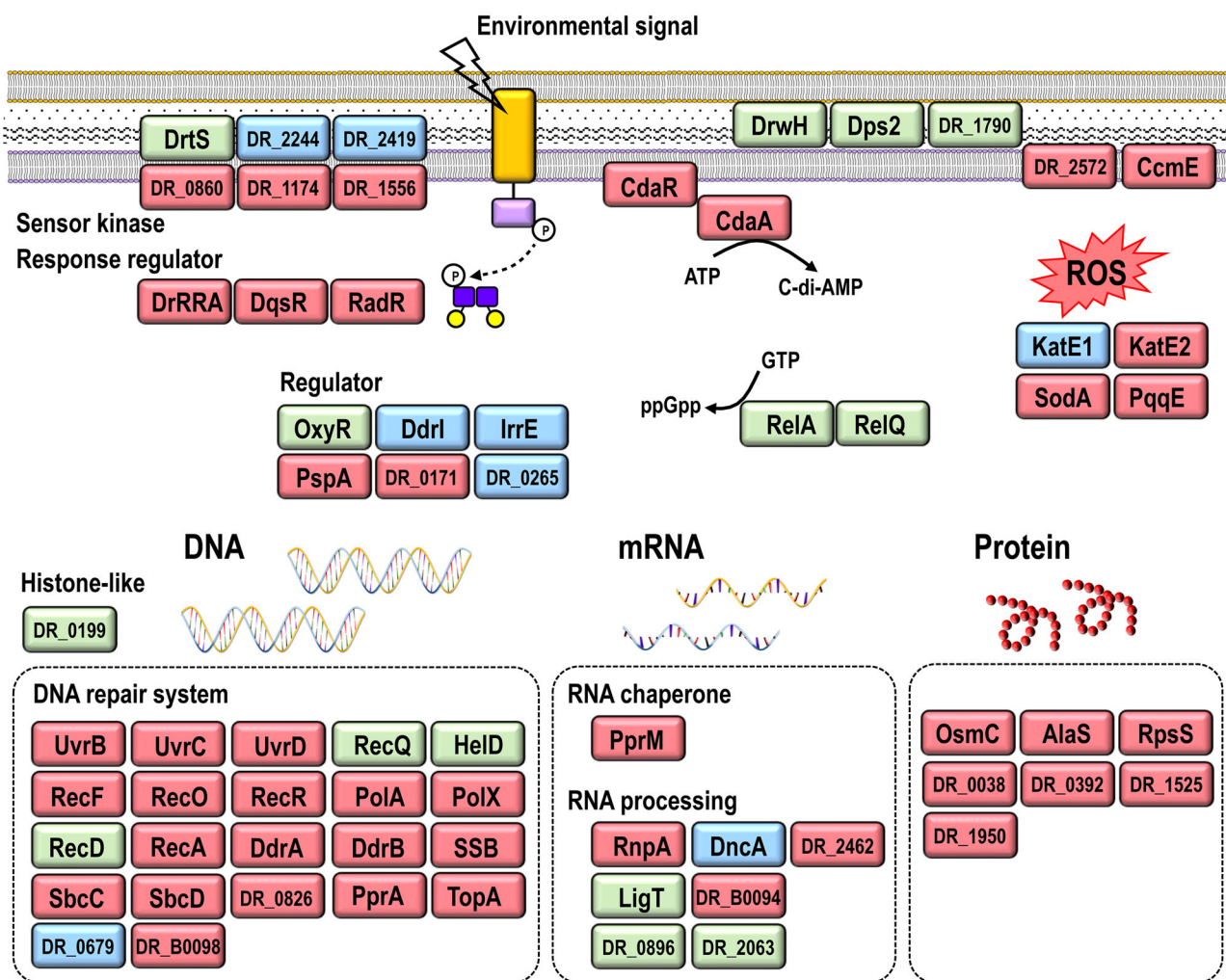
Figure 2. Schematic overview of ionising radiation and oxidative stress resistance-associated proteins in *D. radiodurans*. Many *D. radiodurans* gene deletion or disruption mutants with more than 10-fold increased sensitivity compared to the wild-type strain have been described (Table S1, Supporting 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.

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; Pobegalov *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

demonstrated or proposed that they contribute to DNA repair or genome preservation (e.g. DdrA to DdrD, PprA, DR_A0282) (Selvam *et al.* 2013; Agapov and Kulbachinskiy 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 *sbcB* 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, Atl1 (YbaZ), RdgB (YggV), RecA, RecD, RecF, RecF-interacting DR_1088 homologue, RecG, RecJ, RecN, RecO, RecQ (or ab-

sent in *D. geothermalis*), HRDC domain protein, RecX, RadA, RuvA, RuvB, RuvC, SbcC, SbcD, 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, Nfi, UvrA2, SSL2 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. puniceus* 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*, *D. soli* and *D. swuensis*. 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 H_2O_2 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	2	1
AlkD											1
Ung	1	1	1	1	1		1				1
Udg4	1	1		1	1	1	2	1			1
Udg DR_0022	1										
Nfi	1			1	1			1			1
XthA	1	2	2	1	1	1	2	1	1	1	1
Dam				1		1					
Dcm				1	1		4	2		2	
DR_C0020	1										
Vsr				1	1		1	1			
Ada				1							
PhrB						1			2	2	1
SplB			1	1	1						
AdaA-AlkA						1			1	1	
PhrB-Ung						1	1	1	1	1	
Nth-Dcm								1			
Dut							1	1			
Dcd		1	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	1	2	2	1
SSL2 helicase	1	1	1	1	2	4	1				
Recombinational repair											
RecA	1	1	2	1	1	1	2	1	1	1	1
RecB/AddA								1			
RecQ	1	fr	1	1	2	1	1	1	1	1	1
RusA (YbcP)									1	2	
SSB	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	1
DR_B0100	1			1	1				1	1	
DR_B0099	1			1							
DR_B0098	1			1	1				1	1	
DR_B0094	1				1						1
DR_B0095	fr			1							
Other DNA repair proteins											
TopA	1	1	1	2	3	2	2	1	1	1	1
Exo (Xni)		1					1				
NERD domain	1				1	1			1		
NucS (EndoMS)				1	2	1	fr	1	1	1	
PolB			1		1		1	1			
DnaE2			1				1				
ImuY			1				1				
DinP				1	1	1	1	1	1	1	
HelD	1		1	1	2	1	1	1	1	1	1
HepA	2	2	2		1	1	1	2	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 swuensis* 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. swuensis*, and unlike *D. radiodurans*, some *Deinococcus* species possess genes encoding homologues of Dcm and/or DNA-adenine methylase
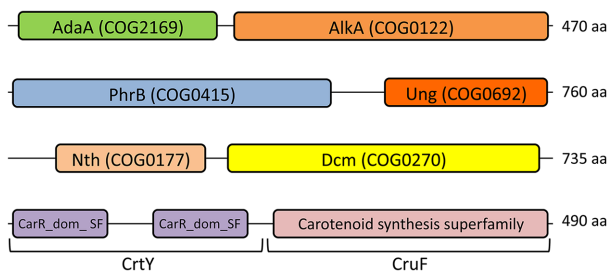
Figure 3. Novel two-domain proteins. The canonical DNA repair proteins AlkA, PhrB, Ung, Nth and Dcm and carotenoid biosynthesis proteins CrtY and CruF are standalone proteins. Genes encoding fusions of two of these proteins were identified in several *Deinococcus* species. The total number of amino acid 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 (SplB,

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 (ThiJ, 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. puniceus*. 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 UvrA1- 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. geothermalis*, 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
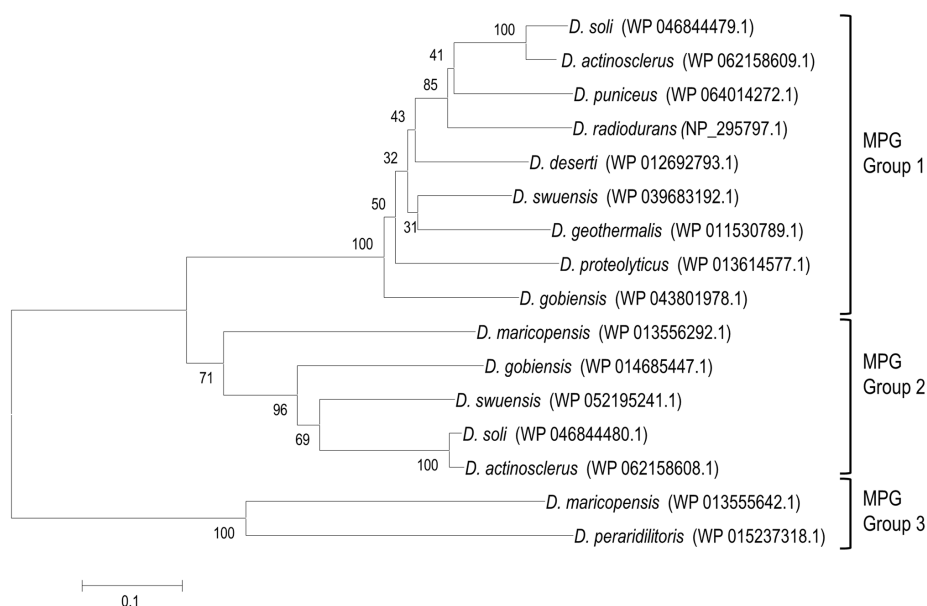
Figure 4. Three groups of 3-methyladenine DNA glycosylase (MPG) proteins identified in 11 *Deinococcus* species. The phylogenetic analysis was carried out based on protein sequence alignment of 16 deinococcal MPG proteins (Table S2, Supporting Information) made with Clustal omega. GenBank accession numbers 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 *cinA-ligT-recA* gene cluster (probably operon in each), except *D. proteolyticus* that misses *cinA* and has a *ligT-recA* operon (*cinA* 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 *cinA-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. swuensis* 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 *addBA* operon, is a functional homologue of the *E. coli* RecBCD enzyme (Kooistra, Haijema and Venema 1993). The RecB/AddA-like protein of *D. swuensis* 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. swuensis* 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. swuensis* 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). The *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 H_2O_2 (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_B0100, also known as *ligB* or *ddrP*) predicted to encode an ATP-dependent DNA ligase (Liu *et al.* 2003). DR_B0100 is the first gene of a radiation-induced operon also encoding DR_B0099 (poly ADP-ribose glycohydrolase) and DR_B0098 (polynucleotide kinase) (Blasius *et al.* 2007; Slade *et al.* 2011). A DR_B0100 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_B0100 deletion requires expression *in trans* of the entire operon, and that *in vitro* DNA ligase activity by DR_B0100 requires the presence of DR_B0098 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_B0098 is required for IR resistance, and that the DR_B0098 mutant is equally IR sensitive as the mutant lacking the entire operon (Schmier and Shuman 2018). Homologues of DR_B0100, DR_B0099 and/or DR_B0098 are present in only a few other *Deinococcus* (Table 2), with *D. gobiensis*, *D. actinosclerus* and *D. soli* possessing a putative operon composed of DR_B0100 and DR_B0098 homologues (Fig. S3, Supporting Informaton). 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_B0094 (*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_B0094 homologue is present in only two of the other *Deinococcus* species, *D. puniceus* 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_B0095. Inactivation of DR_B0095 also results in increased sensitivity to IR (Schmier *et al.* 2017). Sequence analysis suggests that DR_B0095 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, *DGo_PC0276* 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 trancripts 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 MutSL-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 *DEINOCOCCUS*

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 ($O_2{}^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH\cdot$) 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_B0067 extracellular nuclease, FrnE (truncated in *D. maricopensis*), Dps1, MsrP, MsrQ, Ppk1 (frameshift in *D. maricopensis*), Ppk2, Ppx, CrtE, -B, -I, -D, -O, BshA, -B, -C, YpdA, YtxJ (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 $O_2{}^{\cdot-}$ to give H_2O_2 and O_2 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 SodC (Imlay 2013). *Deinococcus radiodurans* has one cytoplasmic SodA (DR_1279) and two periplasmic SodCs (DR_1546 and DR_A0202). 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					
SodC (DR_A0202)	1		1	1	1	1		1		1	1
KatE (clade 1)	1	1	1	1	1	1	1	1			
KatE (clade 2)	1					1					
KatE (clade 3)					1				1	1	
MnCat				1	1	1	1				
DR_A0146 (kat-like)	1				2					1	
Peroxiredoxins, Prx-related proteins, and other peroxidases											
BCP	3	3	2	3	3	2	3	3	4	3	4
AhpE	1	1	1	1	1	1	2	1	1	1	1
AhpD	1	1	2	1	1	1	5	1	1	1	1
DR_A0145 (EfeB)	1										
CCP	1					1					
OsmC	1	1	1	1	1			1	1	1	1
Ohr	1	1		2	2	2	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	1	2	1	1	1	1	2	1	1	1	1
Grx/NrdH-like	4	4	2	2	5	4	2	4	3	3	2
Carotenoid											
CrtLm	1	1		1	1			1	1	fr	1
CrtY-CruF		1				1	1				
CruF	1	1		1	1			1	1	1	1
CYP287A1	1			1	1	1	1	1	1	1	1
Manganese transport											
MntH	1	1	1	1	1	3		1	1	1	
MntA	1	1	3	1	1	1	2	2	1	1	2
MntB	1	1	5	1	1	1	3	1	1	1	1
MntC	1	1	3	1	1	1	2	2	1	1	1
MntE	1	1	1	2	1	1	1	1	1	1	1
Others											
Dps2	1					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
PqqE	1										

*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 (Smolik *et al.* 2014). Because $O_2^{\cdot-}$ 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 organi-

sation 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. proteolyticus*, *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 H_2O_2 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 H_2O_2 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 Cys_P and C-terminal resolving Cys_R, but AhpE, which has been characterised in *M. tuberculosis*, contains no Cys_R (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 hydroperoxidase 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 Cys_P is contained within a universally conserved PxxxT(S)xxC 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)xxC 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 H_2O_2 to water by accepting electrons from a soluble cytochrome c. CCP displays peroxidase activity *in vitro* but its physiological function seems to enable H_2O_2 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 deferrochelatase/peroxidase EfeB. In *E. coli*, EfeB showed a deferrochelation activity, releasing iron from heme leaving the tetrapyrrol 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.* 2004). *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_P (Meireles *et al.* 2017). Ohr protein exhibits peroxidatic activity that is much more substantial against organic peroxides than against H_2O_2 itself (Mishra and Imlay 2012). In contrast, H_2O_2 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 H_2O_2 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*)

and 2 (*trxC*) of *E. coli*, respectively. TrxC contains two additional CXXC motifs that are involved in zinc ion binding (Collet *et al.* 2003), and these motifs are conserved in DR_A0164. The DR_0944-type Trx is present in each of the other *Deinococcus* species (three homologues in *D. peraridilitoris*), but the DR_A0164-type in only two other species (Table 3). The antioxidant activity of the Trx system is manifested when electrons are transferred to antioxidative proteins such as BCP. In addition, AhpE in *M. tuberculosis* can be reduced by the Trx system (Lu and Holmgren 2014). This suggests that deinococcal AhpE can obtain electrons from AhpD and/or Trx. The methionine sulfoxide reductase enzymes MsrA and MsrB catalyse the reduction of protein-bound methionine sulfoxide to methionine in the presence of Trx and have been shown to have an important role in protecting organisms against oxidative damage (Cabreiro *et al.* 2006). Hsp33 (heat shock protein 33) is a chaperone specialised for oxidative stress protection (Dahl, Gray and Jakob 2015). Hsp33 is rapidly activated through formation of a homodimer in response to severe oxidative stress to prevent aggregation of unfolding proteins, and oxidised Hsp33 dimers are converted into reduced Hsp33 dimers by the Trx system (Meyer *et al.* 2009; Mayer 2012). MsrA, MsrB and Hsp33 are well conserved across all *Deinococcus* species analysed. Thioredoxin reductase TrxR is known to reduce oxidised Trx in the presence of NADPH. DR_1982 of *D. radiodurans* is a homologue of *E. coli* TrxR (Fig. S8, Supporting Information), and is able to revert DR_A0164 (TrxC) to its reduced form (Obiero *et al.* 2010). Each *Deinococcus* species contains one or two DR_1982 homologues (Table 3).

Glutaredoxin-like proteins

The glutathione (GSH) system, which, besides GSH, comprises GSH peroxidase, GSH reductase and glutaredoxin (Grx), is one of the major antioxidant systems, but is lacking in some bacteria such as *Helicobacter pylori*, *M. tuberculosis* and *B. subtilis* (Lu and Holmgren 2014). It has been previously reported that *D. radiodurans* has three small (80 to 100 residues) CXXC motif-containing Grx-like proteins (i.e. DR_2085, DR_A0072 and DR_0057) (de Groot *et al.* 2009; Yuan *et al.* 2012), while GSH, GSH reductase and GSH peroxidase are absent (Slade and Radman 2011). Of these three Grx-like proteins, the DR_2085- and DR_0057-type proteins are present in all other *Deinococcus* species, but the DR_A0072-type in only 5 of the 11 species (Fig. 5 and Table S3, Supporting Information). A multiple-sequence alignment of the deinococcal Grx-like proteins shows that 'CPDC' and 'CHLC' motifs are well conserved in the DR_2085-type and DR_0057-type proteins, respectively (Fig. 5), whereas canonical Grx contains a CPYC redox-active motif. Interestingly, the C-terminal regions of the DR_2085-type proteins contain the SGFRP structural motif that is present in canonical NrdH proteins (Rabinovitch *et al.* 2010) (Fig. S9, Supporting Information). NrdH is a Grx-like protein disulphide oxidoreductase of the Trx superfamily, typically containing the redox-active C(M/V)QC motif and functioning primarily as the electron donor for the NrdEF ribonucleotide reductase, and is reduced by the Trx system but not by GSH (Rabinovitch *et al.* 2010).

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 Ia (NrdAB) and Ib (NrdEF) (Torrents 2014). Class Ib 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 NrdEF RNR encoded by the same gene cluster. Class II RNRs encoded by a single *nrdJ* gene are represented not only in the four species lacking NrdIEF (i.e. *D. deserti*, *D. maricopensis*, *D. peraridilitoris* and *D. puniceus*), but also in five *Deinococcus* species (*D. actinosclerus*, *D. geothermalis*, *D. radiodurans*, *D. soli* and *D. swuensis*) having NrdIEF (Table S7, Supporting Information). Class II RNRs are also reduced by Trx (Jordan *et al.* 1997; Torrents 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 Dsb 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 DsbA-like protein (COG2761) designated FrnE, which belongs to the Trx superfamily of proteins, and the *frnE* mutant strain shows reduced tolerance to IR and H_2O_2 (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-CPWC-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 DsbA-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 H_2O_2 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
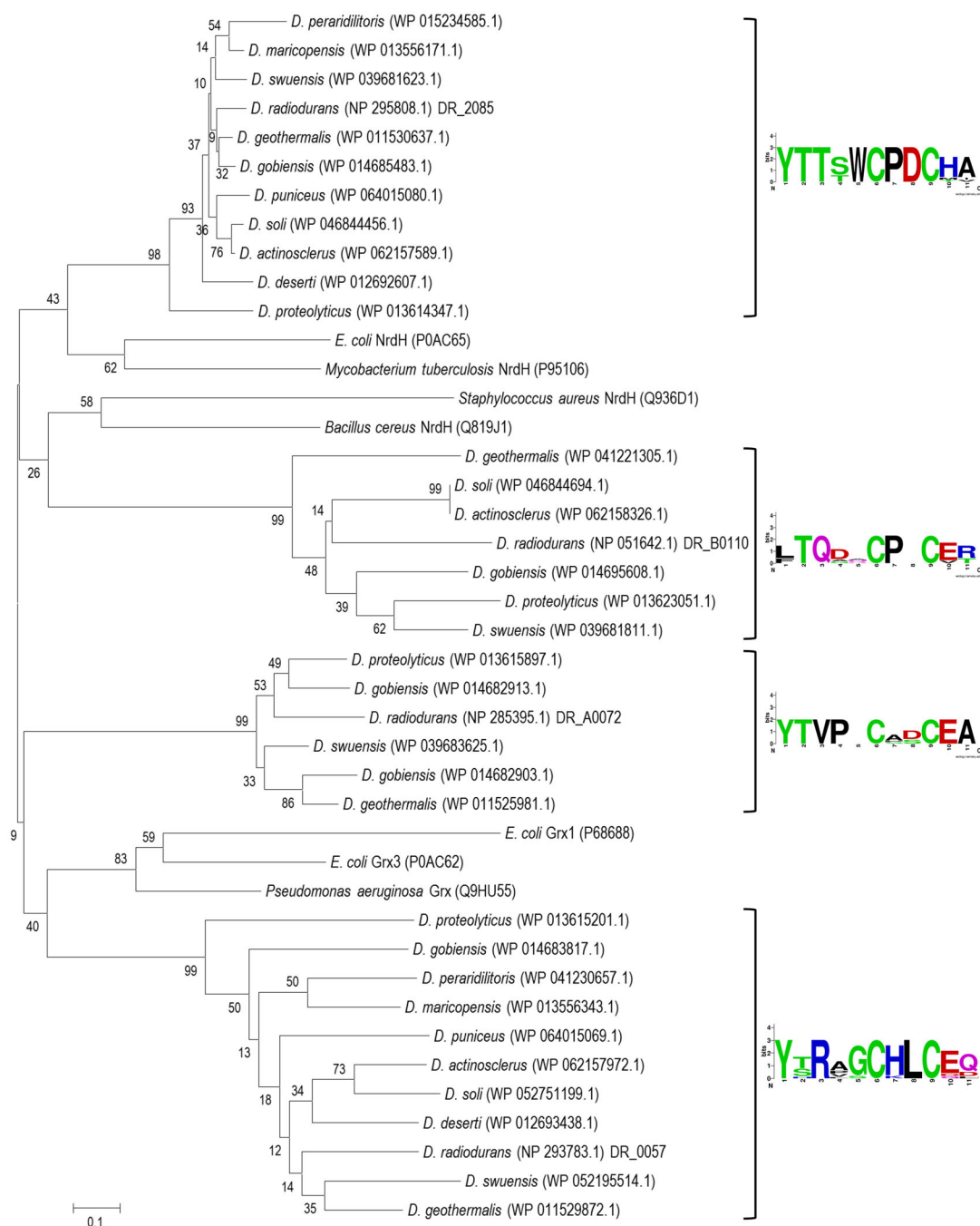
Figure 5. Phylogenetic relationship of glutaredoxin-like proteins identified in 11 *Deinococcus* species. The phylogenetic analysis was carried out based on protein sequence alignment of 35 deinococcal Grx/NrdH-like proteins (Table S3, Supporting Information) with some representative proteins taken from Uniprot: NrdH from *E. coli* (Uniprot Number P0AC65), *M. tuberculosis* (P95106), *S. aureus* (Q936D1) and *B. cereus* (Q819J1), and Grx from *E. coli* (P68688 and P0AC262) and *P. aeruginosa* (Q9HU55). Weblogo plots show sequences around the CxxC motif extracted from deinococcal proteins of each group. See further the legend to Figure 4.

is to sequester iron to protect cells from the damage caused by Fenton reaction, where free ferrous ions react with H_2O_2 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 H_2O_2 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, MsrP and MsrQ 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 H_2O_2 than carotenes (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 H_2O_2 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-hydratase (CruF, 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 monocyclic carotenoids, is encoded by eight of the analysed *Deinococcus* species, although the gene in *D. actinosclerus* 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, Eslava and Iturriaga 2000; Guo, Tang and Zhang 2014). BLASTP analysis revealed that the CrtY-CruF fusion protein is unique to the class *Deinococci*. Deinoxanthin is a unique C2-hydroxylated monocyclic ketocarotenoid, and the 2-β-hydroxylase 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 mycothiol (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 H_2O_2, 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; Chandrangsu *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-bacillithiolation, 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 YfiT (BstA) protein, which is a member of the

DinB superfamily, functions as Bst to conjugate reactive electrophiles with BSH, such as monobromobimane (Newton *et al.* 2011). One of the largest protein expansions observed in *D. radiodurans* is the DinB/YfiT protein family (Makarova *et al.* 2001). Among them, DR_0841 and DR_1024 were found to be close sequence homologues (>40% identity) of *B. subtilis* BstA and belong to the DinB_2 family (PF12867) (Newton *et al.* 2011). One or two homologues of *B. subtilis* BstA are detected in each of the other *Deinococcus* species except for *D. proteolyticus*.

BSH is proposed to have a role in Fe–S cluster biogenesis (Rosario-Cruz and Boyd 2016). Three Fe-S biogenesis systems have been identified and characterised in bacteria, namely Isc (iron-sulphur cluster), Suf (sulphur formation) and Nif (nitrogen fixation) systems (Roche *et al.* 2013). The role of BSH in Fe–S biogenesis has functional overlap with Fe–S cluster carriers in Gram-positive bacteria, such as *Staphylococcus aureus* and *B. subtilis*, in which Suf is the sole Fe–S cluster biosynthetic machinery system (Rosario-Cruz and Boyd 2016). *Deinococcus radiodurans* carries the Suf system constituted by the SufSE complex, which serves as the sulphur donor, the Fe-S scaffold SufBCD and the SufA Fe-S carrier protein. All of the *Deinococcus* species have these Suf proteins, and five of these species contain additionally the alternate Fe-S scaffold SufU, which is an IscU-like protein (Table S3, Supporting Information).

Manganese and peptides
In biology, Fe and Mn play important roles in cellular adaptation to oxidative stress. Whereas Fe^{2+} is known as a prooxidant because of its reactivity with peroxide, generating the highly reactive $OH\cdot$ through Fenton reaction, Mn^{2+} is considered an antioxidant (Aguirre and Culotta 2012). In *E. coli*, oxidative stress induces import of Mn^{2+} that can replace Fe^{2+} from sensitive targets in metalloenzymes (Faulkner and Helmann 2011). Manganese ions can also form antioxidant complexes with small metabolites such as orthophosphate, carboxylates, amino acids and small peptides. These manganese antioxidants can scavenge or catalytically remove ROS (Culotta and Daly 2013). *Deinococcus radiodurans* and other radiation-resistant bacteria have a very high intracellular Mn content leading to a high Mn/Fe ratio (0.24 in *D. radiodurans*) compared to that of radiation-sensitive bacteria (<0.01 in *E. coli*) (Daly *et al.* 2004). It was established that protein-free *D. radiodurans* cell extracts prevent protein oxidation at high doses of radiation, and that these extracts are enriched in Mn^{2+}, inorganic phosphate, peptides and nucleosides (Daly *et al.* 2010). When reconstituted *in vitro* in phosphate buffer, peptides or Mn-peptide complexes appeared to be the most protective (Daly *et al.* 2010; Berlett and Levine 2014). The accumulated small peptides in cells may be derived from proteolysis, peptide import and/or peptide-encoding transcripts. Indeed, many novel leaderless transcripts predicted to code for small peptides were discovered in *D. deserti*, a species in which 60% of the mRNAs were found to be leaderless (de Groot *et al.* 2014). Regarding manganese, *D. radiodurans* has three types of predicted Mn transport systems involved in maintenance of the intracellular Mn concentration homeostasis: Mn^{2+} efflux protein MntE (DR_1236), Nramp H-dependent Mn^{2+} transporter MntH (DR_1709) and the ATP-binding cassette (ABC)-type Mn^{2+} transporter (DR_2283, DR2284 and DR_2523 encoding transmembrane subunit/permease component MntB, ATPase component MntC and solute-binding component MntA, respectively) (Sun *et al.* 2010; Ul Hussain Shah *et al.* 2014,). The *D. radiodurans mntE* mutant exhibited higher intracellular Mn concentrations and lower protein oxidation level than wild type under oxidative stress. Consistent with these characteristics, the *mntE*

mutant was reported to be more resistant to H_2O_2, 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. puniceus* lack the MntH homologue. The MntH homologue from *D. deserti* and two of the three MntH homologues from *D. proteolyticus* share only 26% identity with *D. radiodurans* MntH. ABC transporters act in conjunction with extracytoplasmic solute-binding proteins that feed the solute into the transmembrane channel, which is energised by the cytoplasmic ATP-binding cassette protein. Both the transmembrane protein and the ATPase are usually present as dimers, each consisting either of a single gene product, yielding a homodimer, or two gene products, yielding a heterodimer (Lorca *et al.* 2007). Each *Deinococcus* species has a predicted ABC-type Mn^{2+} transporter encoded by an *mntACB* gene cluster or by separate *mntA* and *mntCB* genes. *Deinococcus peraridilitoris* and *D. deserti* additionally possess respectively one and two operons containing four ABC-type Mn^{2+} transporter genes encoding MntA, MntC and two different MntB homologues (Fig. S14, Supporting Information), which may improve their Mn^{2+} 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 H_2O_2 stress (Chandrangsu, 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 2012). PQQ neutralises ROS including $O_2^{\cdot-}$ and $OH\cdot$ *in vitro* without producing reactive molecular products and protects plasmid DNA and proteins *in vitro* from oxidative damage caused by IR (Misra *et al.* 2004). Several prokaryotes are able to produce PQQ, and its biosynthesis involves the gene products of a specific *pqq* operon consisting of five or six genes. For example, expression of the *pqqA-F* operon from *Klebsiella pneumoniae* in the non-PQQ producer *E. coli* led to PQQ synthesis, and genetic studies revealed that four of the six *pqq* gene products (PqqA, PqqC, PqqD and PqqE) are absolutely required for this PQQ production in *E. coli* (Klinman and Bonnot 2014). *Deinococcus radiodurans* has a gene (*DR_C0034*) encoding a homologue of PqqE. It has been reported that *DR_C0034* (*pqqE*) mediates PQQ production in *D. radiodurans* and also in transgenic *E. coli*, despite absence of other *pqq* gene homologues (Khairnar, Misra and Apte 2003; Rajpurohit, Gopalakrishnan and Misra 2008). Moreover, *E. coli*

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 (H_2O_2) 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 Ps (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 Mn^{2+}-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 *pprI* (inducer of pleiotropic proteins promoting DNA repair) (Hua *et al.* 2003), and this gene is identical to *irrE*, identified independently in another study (Earl *et al.* 2002a). The other has been named *pprM* (a modulator of the PprI-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 PprI (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 ΔpprA 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 or 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 nuclease 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

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	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
ddrE	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								1	1	1
ddrK	1										
ddrL	1					1				1	
ddrM	1					1		1	1	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			1	1				1	1	
ddrQ		1	1	1	1	1	1	1	1	1	1
ddrR	1		1	1	1	1	1	1	1	1	2
ddrS		1	1	1		1	1	1	1	1	1
ddrTUVWX			1	1	1		1	1	1	1	
pprA	1	1	1	1	1		1				
pprI (irrE)	1	1	1	1	1	1	1	1	1	1	1
pprM (csp)	1	2	3	2	2	1	1	1	3	3	2

See the legend to Table 2.

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 (Chandrangsu *et al.* 2018) (see also the section 'Bacillithiol'). 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 vitro* activity of the putative ATP-dependent DNA ligase LigB (DdrP) from *D. radiodurans* (Kota *et al.* 2010) (see also section 'DNA repair in *Deinococcus*'). However, some *Deinococcus* species possess PprA but not LigB, or possess LigB but not PprA (Table 4), strongly suggesting that stimulating LigB activity cannot be the major function of PprA. Moreover,

there is no evidence that NHEJ occurs in *Deinococcus*. It has also been reported that PprA 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 (Devigne *et al.* 2013, 2016). *In vitro*, PprA stimulates the decatenation activity of DNA gyrase (Devigne *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 (Csps) 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 (Fig. S15, Supporting Information), which appears to be unique to the class *Deinococci* (LaGier 2017). Each deinococcal Csp ends with the residues RW, and a subgroup (type A) has a highly conserved C-terminal sequence RD(N)DRW. Under normal growth conditions, increased amount of PprA protein (Ohba *et al.* 2009), decreased levels of catalase KatE1 mRNA and protein, and increased transcription of three proteins (i.e. PerR-like, DtxR-like and RecG) that negatively affect *katE1* expression have been ob-
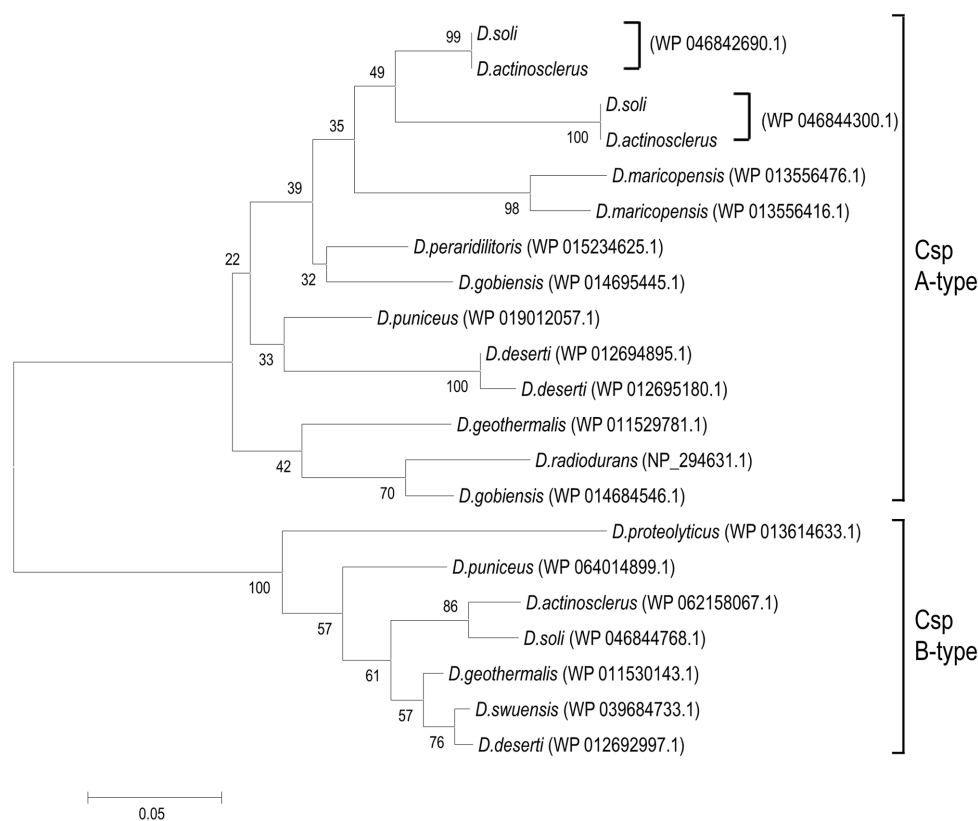
Figure 6. Phylogenetic relationship between two types of cold-shock proteins (Csp) identified in 11 *Deinococcus* species. The phylogenetic analysis was carried out based on protein sequence alignment of 19 deinococcal Csp homologues (Table S4, Supporting Information) made with Clustal omega. Two Csps of *D. soli* are identical to Csps of *D. actinosclerus*. See further the legend to Figure 4.

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 deinococcocal 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*, *ddrN*, *ddrO*, *pprI* (*irrE*) 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 H$_2$O$_2$, 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 threonyl-carbamoyltransferase), 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

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	fr	1	1	1	1	1	1
DR_1471 (fr) Smc	1	1	1	1	1	1	1	1	1	fr	1
DR_0679	1										
DR_0392	1	1		1		2	1	1			
DR_1262 Rsr	1			1							
DR_1631 RelQ	1	1	1	1	1			1	1	1	1
DR_1790	1				1						
DR_2310	1			1	1	1		1	1	1	
DR_A0018 (fr)	1	1	1	1	1			1			
DR_A0281-DR_A0282 (fr)	2		1	1	1	1		1			2
DR_A0283	2		1	1	1	1		1			2
DR_B0118	1	2	2	4	2		2	2	2	2	2
DR_1172 / DR_0105	2	1	1		1	1			1	1	1
Deide_15148		1	1	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.

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 nucleotidyltransferase (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).

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_2O_2 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	1	1			
LexA-XRE	1	1	1					1	1		
RqkA	1	1	1	1	fr		1	1	1	1	1
Oxidative stress reponse and Mn/Fe homeostatis											
OxyR1-like (LysR)	1	1	1	1	1	1	1	1	1	1	1
OxyR2-like (LysR)	1								1	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		1			1			
DtxR	1	1	2	1	1	1	1	1	1	1	1
MntR			1					1			
SoxR			1				1		1	fr	
Two-component systems											
DrRRA	1	1	1	1	1	1		1	1	1	1
DR_2419	1	1						1	1	1	1
RadS	1				1						
RadR	1				1						
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
DR_A0205	1		1		1				1	1	
Quorum sensing											
DqsI-1	1	2	1	1	1	1	1	2	1	1	1
DqsI-2	1	1	1	1	1	1	1	1	1	1	1
DqsR	1	1	1	1	1	1	1	1	1	fr	1
LuxS	1	1	1	1	1		1	1	1	1	1
LsrB-like		1	1								
Others											
DdrI	1	1	1	1	1	1	1	1	1	1	1
DR_0171	1										
DR_0265	1	2	2	1	1		2	1	1	1	1

See the legend to Table 2.

seems unlikely because of their predicted 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).

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* (*drwH*) mutant, however, has increased sensitivity to oxidative (H_2O_2) 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.

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.
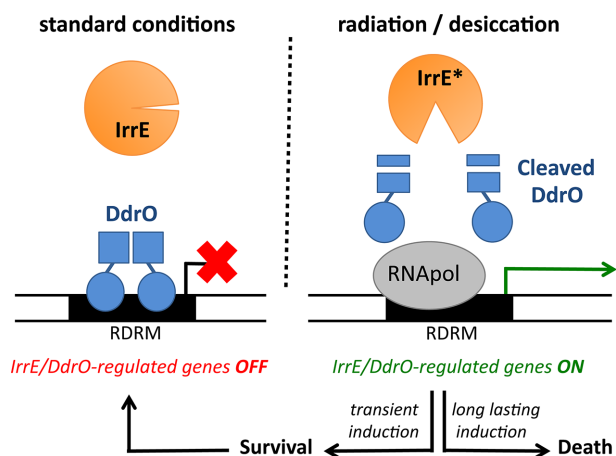
Figure 7. Model for the radiation/desiccation response (RDR) mechanism in *Deinococcus*: induction of the RDR regulon after cleavage of a repressor by a separate and specific metalloprotease. Under standard conditions, DdrO probably binds as a dimer to the two half-sites of the palindromic motif RDRM, thereby inhibiting transcription of IrrE/DdrO-regulated genes. Exposure of *Deinococcus* to radiation or desiccation stimulates cleavage of DdrO by IrrE. Cleaved DdrO may not form dimers and no longer binds the RDRM, allowing rapid induction of RDR regulon genes (e.g. DNA repair genes and *ddrO* itself). Persistence of the signal leading to DdrO cleavage may induce, directly or indirectly, one or more proapoptotic genes.

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 metalloprotease (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*, *ddrTUVWX*, *pprA*, *recA* operon, extra *recA*, *ssb*, *gyrA*, *gyrB*, *recD*, *recQ*, *ruvB*, *uvrA*, *uvrB* and *uvrD*, 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 re-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. gobiensis* and *D. peraridilitoris* may also have overlapping functions or, alternatively, regulate different genes.

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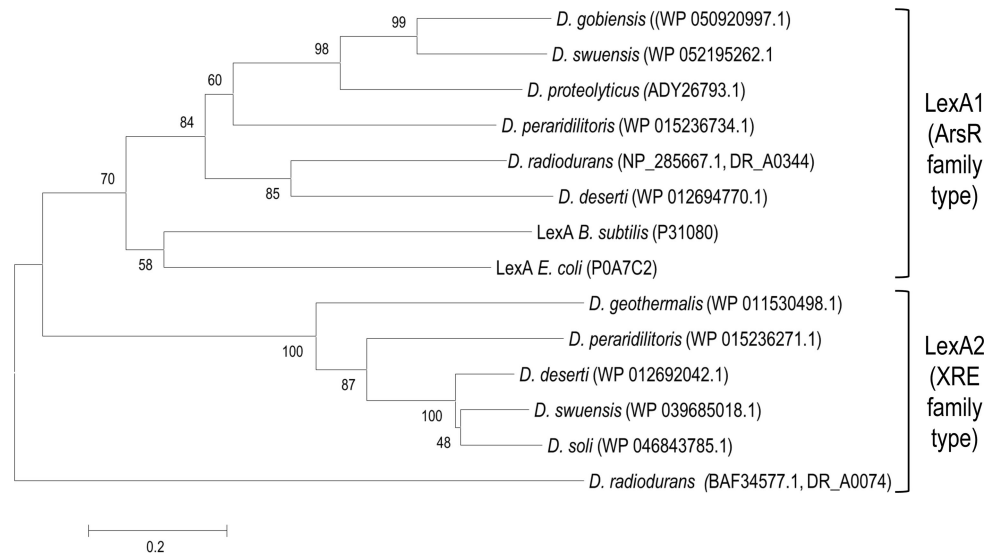
Figure 8. Phylogenetic relationship of LexA homologues identified in *Deinococcus* species. The phylogenetic analysis was carried out based on protein sequence alignment of 12 deinococcal LexA homologues (Table S6, Supporting Information) with some representative proteins taken from Uniprot: LexA from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

Deide_1p01870) or an XRE-type (e.g. DR_A0074) DNA-binding domain (Fig. S18, Supporting Information).

RecA-mediated cleavage has also been demonstrated for Deide_1p01870, which controls expression of an operon that is radiation-induced in a RecA-dependent but IrrE-independent manner and that encodes Deide_1p01870 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* (DR_C0034), 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 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 DR_2518 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 DR_2518 inactivation was also found independently in another study (Dulermo *et al.* 2015). DR_2518 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
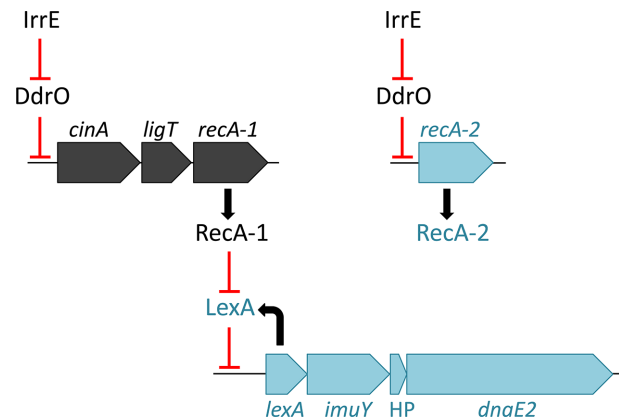
Figure 9. IrrE/DdrO- and RecA/LexA-regulated expression of DNA repair genes within the same bacterium. The *cinA-ligT-recA* operon (*ligT-cinA* in *D. proteolyticus*) and genes for IrrE and DdrO are present in each *Deinococcus* species. Additional RecA (RecA-2) and the *lexA-imuY-dnaE2* operon are found 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.

functionally linked to a protein kinase. Moreover, PQQ stimulated kinase activity of DR_2518 *in vitro*, and IR exposure induced autophosphorylation of DR_2518 *in vivo* (Rajpurohit and Misra 2010), and therefore DR_2518 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

resistance without PQQ. However, *D. proteolyticus* lacks *rqkA* and the homologous sequence in *D. gobiensis* contains a frameshift. To find potential target proteins for RqkA, 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 RqkA 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 RqkA requires further investigation.

Regulators of oxidative stress response and Mn/Fe homeostasis

Several studies have revealed that when bacteria are exposed to H_2O_2 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 Fe^{2+} in the cell, for example by sequestration in storage proteins (e.g. Dps) and repression of Fe^{2+} uptake, and elevate cytosolic levels of Mn^{2+}, which can replace Fe^{2+} from sensitive sites in enzymes, by inducing Mn^{2+} 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 homeostatis 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 H_2O_2-sensing regulator OxyR. *Escherichia coli* OxyR contains two Cys residues that are essential for H_2O_2 sensing: upon oxidation by H_2O_2 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, DR_0615 and DR_A0336, are found in *D. radiodurans*. The *E. coli* LysR-type proteins most similar to both DR_0615 and DR_A0336 are YnfL and HcaR (34–38% identity), while *E. coli* OxyR is about 30% identical to DR_0615 and DR_A0336. Nevertheless, DR_0615 and DR_A0336, which share 38% identity, have been described as OxyR1 and OxyR2, respectively, each containing only one cysteine residue that was re-

ported to be essential for protein function *in vivo* (Chen *et al.* 2008; Yin *et al.* 2010). The single Cys residue (C210) of DR_0615 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 H_2O_2 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 DR_A0336 (OxyR2) are unknown, and DR_A0336 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 DR_0615 (OxyR1) deletion mutant compared to the wild-type strain (Chen *et al.* 2008). DR_0615 may directly regulate the genes *DR_B0125* (iron transporter), *katE1*, *mntH* and *dps1*, because binding of DR_0615 to DNA regions upstream of these genes has been observed *in vitro*. The same DNA-binding pattern was observed with oxidised DR_0615, reduced DR_0615 or DR_0615 with the C210A mutation. However, the precise mechanism by which DR_0615 may regulate genes either positively or negatively upon H_2O_2 exposure has not been elucidated. The *katE1* expression was induced 4-fold by H_2O_2 in wild type, but an approximately 3-fold induction was still observed in DR_0615 mutants. In addition, although DR_0615 is proposed to be a negative regulator of *mntH* and *dps1*, *mntH* expression is reduced, whereas *dps1* is highly induced by H_2O_2 stress in DR_0615 mutants (Chen *et al.* 2008). DR_0615 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 DR_0615 is not strictly conserved. The homologues from *D. perarididilitoris* and *D. deserti* lack Cys, and therefore at least these Cys-less DR_0615 homologues cannot function as canonical or 1-Cys-OxyR. Clearly, further research is necessary to elucidate the precise role and mode of function of DR_0615 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 reponse 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 Mn^{2+}), Zur (responding to Zn^{2+}), Nur (responding to Ni^{2+}), 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* (DR_0865, 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 DR_0865 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 Mn^{2+} transporter (Johnston *et al.* 2007; Fillat 2014). Of the five Mn^{2+}-transport-related genes of *D. radiodurans*, the expression of *mntA*, *mntB* and *mntH* increased, while *mntE* was repressed in the DR_0865 mutant strain compared to the wild-type strain under standard laboratory conditions. Moreover, expression of *mntA* and *mntE* in the DR_0865 mutant under Mn^{2+} stress was significantly higher and lower, respectively, compared to the Mn^{2+}-stressed wild type (Ul Hussain Shah *et al.* 2014). Differential gene expression in Mn^{2+}-stressed cells versus non-stressed cells was not reported.
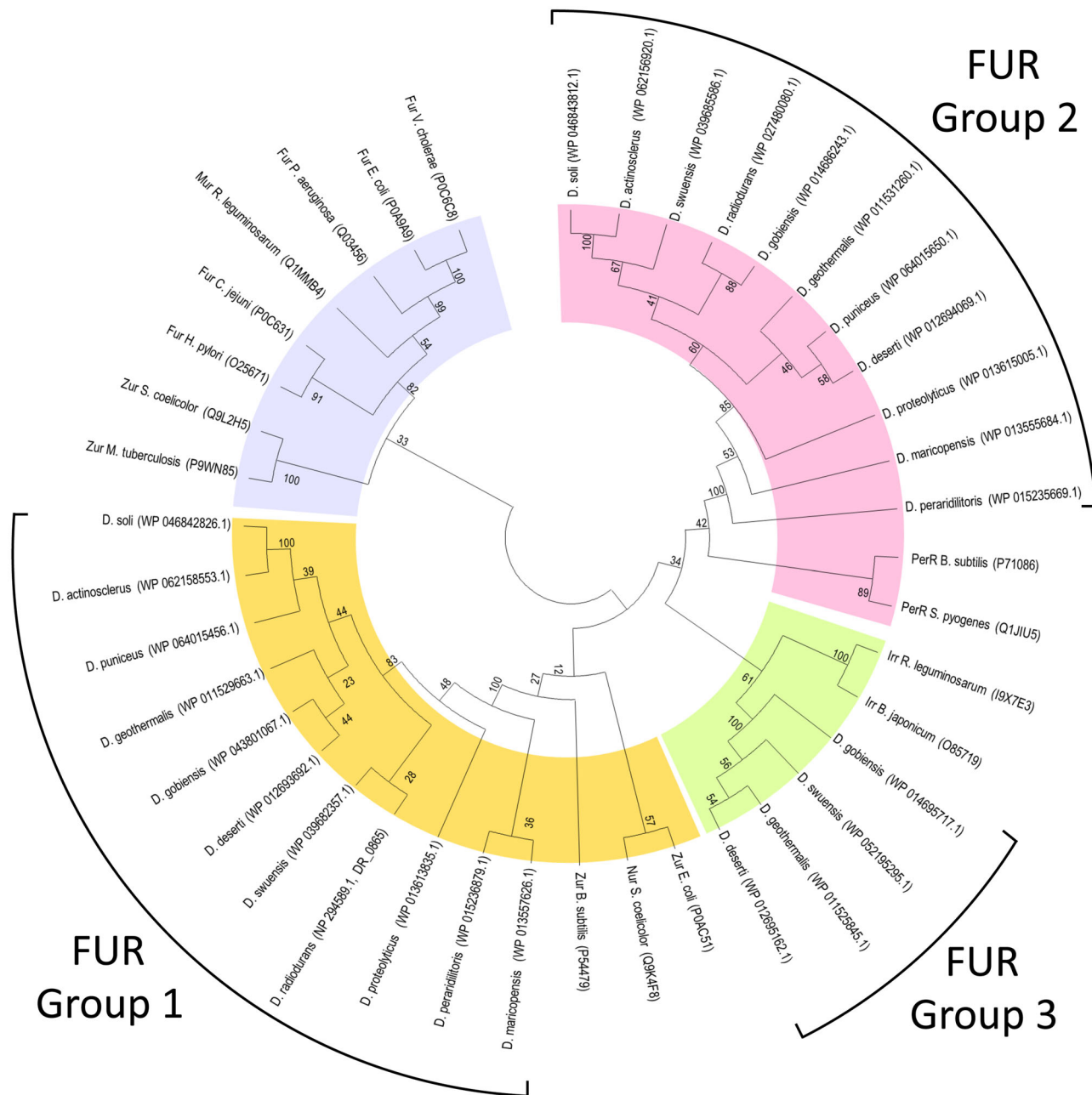
Figure 10. Three groups of FUR family proteins identified in 11 *Deinococcus* species. The phylogenetic analysis was carried out based on protein sequence alignment of 26 deinococcal FUR family proteins (Table S6, Supporting Information) with some representative proteins taken from Uniprot: Fur proteins 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* (P0AC51); Nur from *S. coelicolor* (Q9K4F8); PerR from *B. subtilis* (P71986) and *Streptococcus pyogenes* (Q1JIU5); 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 *mntH* promoter (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_2O_2 (Ul Hussain Shah *et al.* 2014), but other studies mentioned that the mutant strain exhibited greater resistance to H_2O_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_2O_2, and substitutes for OxyR in many Gram-positive bacteria, although it may also coexist with OxyR (Dubbs and Mongkolsuk 2012). In, for example, *B. subtilis*, PerR is inactivated by H_2O_2 stress, leading to derepression of the PerR regulon, including the genes for Prx, 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_2O_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_2O_2) stress condition, because the *dps1* gene expression is not induced by H_2O_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_2O_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 Mongkolsuk 2012). Exposure to H_2O_2 leads to oxidation of Fe^{2+} in the regulatory site by a Fenton reaction, which causes oxidation of H37 and H91 to 2-oxo-histidine 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^{2+} 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 CIGCGCxxxxxC 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 CIGCGCxxxxxC motif, are found in *D. deserti*, *D. proteolyticus*, *D. soli* and *D. actinosclerus* (although the gene in *D. actinosclerus* 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

TCSs, 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 *drRRA* 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 *drRRA* 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 *drRRA* 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. perardilitoris*. Concerning the genetic organisation, *drRRA* 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 *drRRA* disruption (Wang *et al.* 2008; Im *et al.* 2013). Moreover, several DrRRA-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_B0090/DR_B0091) 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 DrtR and DrtS (DNA damage response TCS regulator and sensor), respectively (Im *et al.* 2013). Contrary to DrRRA and RadR/RadS, both DrtR and DrtS are conserved in the other analysed *Deinococcus* species.

In addition to *radS* and *drtS*, 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_A0205 (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 *dqsI-1* and *dqsI-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 (QqaR, DR_A0255) and AHL-lactonase (QqlR, DR_0172), which are able to inactivate foreign AHLs (Koch *et al.* 2014), and AHL levels are higher in the *qqaR* 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_A0158 (phosphate ABC transporter) and DR_B0067 (extracellular nuclease) (Lin *et al.* 2016a).

The DqsI and DqsR 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), however, 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_0834) in *D. radiodurans*, DR_0997 (also referred to as *ddrI*) is highly induced by IR (Tanaka *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_A0202 *sodC*, *pprA*, *uvsE*, *uvrC*, *ruvC*, *recA*, *recF*, *recN*, *ddrB*, *ddrC*, *ddrD*, Lon protease genes, glycometabolism gene *glgC*) 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 DdrI to these regions has been demonstrated *in vitro* (Yang *et al.* 2016). These 18 genes, including *pprA*, *uvsE* and *recN*, may be regulated directly by DdrI, 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 DdrI expression is DrRRA dependent in *D. radiodurans*, and that DdrI 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 DdrI homologue, but not DrRRA, is present in each *Deinococcus* species (Table 6). The studies on DdrI 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 each 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. RecD, 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/DdrO (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 LigB, 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/DdrO, 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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