Review

Thiol Reductases in *Deinococcus* Bacteria and Roles in Stress Tolerance

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Abstract: *Deinococcus* species possess remarkable tolerance to extreme environmental conditions that generate oxidative damage to macromolecules. Among enzymes fulfilling key functions in metabolism regulation and stress responses, thiol reductases (TRs) harbour catalytic cysteines modulating the redox status of Cys and Met in partner proteins. We present here a detailed description of *Deinococcus* TRs regarding gene occurrence, sequence features, and physiological functions that remain poorly characterised in this genus. Two NADPH-dependent thiol-based systems are present in *Deinococcus*. One involves thioredoxins, disulfide reductases providing electrons to protein partners involved notably in peroxide scavenging or in preserving protein redox status. The other is based on bacillithiol, a low-molecular-weight redox molecule, and bacilliredoxin, which together protect Cys residues against overoxidation. *Deinococcus* species possess various types of thiol peroxidases whose electron supply depends either on NADPH via thioredoxins or on NADH via lipoylated proteins. Recent data gained on deletion mutants confirmed the importance of TRs in *Deinococcus* tolerance to oxidative treatments, but additional investigations are needed to delineate the redox network in which they operate, and their precise physiological roles. The large palette of *Deinococcus* TR representatives very likely constitutes an asset for the maintenance of redox homeostasis in harsh stress conditions.

Keywords: *Deinococcus*; thiol; reductase; peroxidase; cysteine; oxidative stress; thioredoxin; bacillithiol; protein redox status

1. Introduction

Bacteria belonging to the genus *Deinococcus* are extremely tolerant to radiation, desiccation, and other conditions that generate oxidative damage to biomolecules, including DNA and proteins. The first described member of this genus, *Deinococcus radiodurans*, isolated as a contaminant from canned meat that was supposedly sterilised by a high dose of ionising radiation, has been studied most extensively [1,2]. In the last decades, more than 90 other *Deinococcus* species have been isolated worldwide from different places and environments (e.g., air, water, desert soils). Many genes and several mechanisms contribute to this tolerance [3]. *Deinococcus* bacteria are able to repair massive DNA damage generated by radiation or desiccation [4,5], indicating efficient limitation or repair of damage to other biomolecules, such as DNA repair proteins and other enzymes, to preserve their function under these conditions. Indeed, proteins in *Deinococcus* were found to be much better protected against oxidative damage than those in radiation-sensitive species, such as *Escherichia coli* [6–9]. Both nonenzymatic (e.g., carotenoids, a high intracellular Mn$^{2+}$/Fe$^{2+}$ ratio, and antioxidant complexes containing Mn$^{2+}$, phosphate, and peptides) and enzymatic (e.g., superoxide dismutases, catalases, peroxidases, thioredoxins) antioxidant systems are present in *Deinococcus* [3].
Thiol-dependent antioxidant enzymes, namely, thiol reductases (TRs), are ubiquitous enzymes harbouring catalytic cysteines that modulate the redox status of Cys and Met in partner proteins. Living organisms possess various thiol-dependent antioxidant systems displaying specific features and electron sources [10]. Among them, the thioredoxin (Trx) and glutathione (GSH) systems are the most represented and well characterised [11–13]. The Trx pathway is composed of NADPH, NADPH-dependent Trx reductase (TrxR), and Trx, a disulfide reductase supplying electrons to many types of partners and targets, including peroxiredoxins (Prxs) and methionine sulfoxide reductases (Msrs), which are involved in the scavenging of peroxides and preservation of protein redox status, respectively. In the GSH system, electrons are provided by NADPH and transferred via NADPH-dependent GSH reductase to GSH, a low-molecular-weight (LMW) compound having direct antioxidant activity. GSH is also able to transfer reducing power to glutaredoxins (Grxs), proteins related to Trxs, and fulfilling a similar function in the control of Cys redox status in partner proteins. Trx and GSH systems play critical roles in the maintenance of protein redox homeostasis, particularly in responses to environmental constraints, upon diseases or during aging [10,14–16]. Indeed, these reducing systems participate in the prevention and repair of oxidative damage and in the control of redox post-translational modifications occurring in their partner proteins. This type of modification is associated with changes in enzyme activity, protein conformation or structure, and consequently with redox-based signal transduction pathways. While the Trx system is ubiquitous to all domains of life, the GSH system is absent in many bacteria, including Bacillus species and pathogenic bacteria, such as Mycobacterium tuberculosis or Staphylococcus aureus, in which thiol-based responses are crucial against host oxidative defences [17]. In these species, systems based on other LMW thiol compounds, such as mycothiol (MSH) or bacillithiol (BSH), have been identified and fulfil roles similar to those of GSH [18–20].

The extreme environmental conditions that Deinococcus is able to tolerate strongly affect among others cell redox homeostasis and thiol status. Thus, TRs are very likely part of the mechanisms underlying this outstanding feature [3]. Consistently, it was reported that a Deinococcus geothermalis mutant lacking the cystine importer system, which is a source of thiols, was more sensitive to an H\textsubscript{2}O\textsubscript{2} treatment [21]. Some studies based on biochemical, pharmacological, or genetic approaches characterised the Trx system in Deinococcus [22–24] and indicated that it likely plays an essential role in responses to oxidative stress [25,26]. In a previous review describing Deinococcus antioxidant systems [3], the main TR families were listed, revealing that this bacterial genus possesses a complete Trx system, including a relatively high number of TRs related to Trxs and Prxs. Of note, Deinococcus does not possess GSH, but BSH and the relevant reducing system, including NADPH–BSH reductase (Bdr) and bacilliredoxin (Brx) [27].

In this review, we aim to carry out a more detailed and exhaustive description of TRs and related proteins among a representative set of various Deinococcus species, the genomes of which have been integrated into the MicroScope platform for microbial comparative genome analysis and functional annotation [28]. These species have been isolated from locations in North America, South America, Europe, Africa, and Asia (Table 1). The TRs and related proteins (locus tags) of these bacteria are listed in Table 2. The presence of most of these proteins has been reported in D. radiodurans and Deinococcus deserti, for which several shotgun proteomics studies have been performed [5,29–33]. We analyse the proteins notably with regard to their sequences, to the Cys distribution, and to the tridimensional structures and compare them with TRs from main model species (generally E. coli and/or Bacillus subtilis). We highlight the specificities of Deinococcus TRs and discuss based on available expression and phenotype data their physiological functions in line with the remarkable tolerance of this bacterial genus.
Table 1. Information of complete genomes of *Deinococcus* species.

| Species (Abbreviation) | Identified in                                      | Genome Size (Mb) | Replicons (Kb) | Proteins | References |
|------------------------|---------------------------------------------------|------------------|----------------|----------|------------|
| *D. radiodurans* (DR)  | Canned meat, USA                                  | 3.28             | 4 (2649, 417, 177, 46) | 3167     | [34–36]    |
| *D. deserti* (Deide)  | Sahara Desert sand, Morocco/Tunisia               | 3.86             | 4 (2820, 325, 314, 396) | 3503     | [5,37]     |
| *D. geothermales* (Dgeo) | Hot spring, Italy                               | 3.25             | 3 (2467, 574, 206) | 3003     | [38,39]    |
| *D. gobiensis* (DGo)   | Gobi Desert sand, China                          | 4.41             | 7 (3137, 433, 425, 232, 72, 55, 53) | 4140     | [40,41]    |
| *D. maricopensis* (Deima) | Sonoran Desert soil, USA                      | 3.5              | 1 (3499) | 3242     | [42,43]    |
| *D. peraridilitoris* (Deipe) | Coastal desert soil, Chile                    | 4.51             | 3 (3882, 557, 75) | 4223     | [44]       |
| *D. proteolyticus* (Deipr) | Lama glama faeces, Japan                  | 2.89             | 5 (2147, 315, 196, 132, 97) | 2645     | [35,45,46] |

Table 2. Locus tags of genes encoding thiol reductases and related proteins from seven *Deinococcus* species.

| Name/Description | DR | Deide | Dgeo | DGo | Deima | Deipe | Deipr |
|------------------|----|-------|------|-----|-------|-------|-------|
| **Thioredoxin reductase, thioredoxins, and thioredoxin-like proteins** | | | | | | | |
| TrxR  | _1982 _05800 _1576 _2772 CA2339 _1454 _0175 _3902 _0873 | | | | | | |
| TrxA (Trx1) | _0944 _18600 _1837 CA0861 _2910 _3068 _3901 _0424 | | | | | | |
| TrxC (Trx2) | _A0164 _01140 _2518 | | | | | | |
| Trx-like | _2085 _06390 _1508 CA2073 _1013 _0695 _0576 | | | | | | |
| Trx-like | _0057 _13741 _0729 CA0407 _1186 _0565 _1451 | | | | | | |
| Trx-like | _A0072 _2583 PC0201 _2424 _2732 | | | | | | |
| Trx-like | _B0110 _2876 PA0204 _2190 | | | | | | |
| Trx-like | _0948 _06780 _1960 CA2541 _2994 _1792 | | | | | | |
| **Other thiol-based disulfide oxidoreductases, (predicted) cytoplasmic** | | | | | | | |
| FrnE  | _0659 _00690 _2073 CA0380 _0892 _2202 _1901 | | | | | | |
| FrnE-like | _3p01230 _2335 _22890 _CA0030 _0620 _1019 | | | | | | |
| **Other thiol-based disulfide oxidoreductases, (predicted) periplasmic or cytoplasmic membrane** | | | | | | | |
| DbhA family protein | _2019 _0560 _06420 _0747 CA1399 _1134 _0943 | | | | | | |
| DbhA family protein | _0753 _12740 _0692 CA1008 _1749 | | | | | | |
| DbhB family | _0754 _12730 _0691 CA1007 _1748 | | | | | | |
| DbhD family; CcdA | _1300 _08350 _1241 CA1639 _1155 _0794 | | | | | | |
| TlpA-like family; DsbE/CcmG | _0345 _0189 _08290 _1248 CA2017 _0801 _4366 _0892 | | | | | | |
| DsbD family; CcdA | _2p004030 _2p004020 | | | | | | |
| **Thioredoxin-dependent methionine sulfoxide reductases, cytoplasmic** | | | | | | | |
| MsrA | _1849 _10980 _0843 CA1541 _1788 _3499 _1412 | | | | | | |
| MsrB | _1378 _04050 _2072 CA0919 _1441 _4299 _1900 | | | | | | |
| **Potential Mo-dependent methionine sulfoxide reductases, predicted cytoplasmic** | | | | | | | |
| Molybdopterin oxidoreductase family protein | _0397 _18410 _0402 CA0112 _0538 _1423 _1886 | | | | | | |
| Sulfite oxidase family, molybdopterin-binding domain | _0716 _17540 _1719 CA1115 _0813 _2833 _0695 | | | | | | |
Table 2. Cont.

| Name/Description | DR   | Deide | Dgeo | DGo  | Deima | Deipe | Deipr |
|------------------|------|-------|------|------|-------|-------|-------|
| Mo-dependent methionine sulfoxide reductase system, periplasmic |      |       |      |      |       |       |
| MsrP             | _2536_ | _20380 | _0877 | _CA2733 | _3114 | _2978 | _1129 |
| (frame-shift)    |      |       |      |      |       |       |       |
| MsrQ             | _2537_ | _20370 | _0878 | _CA2734 | _3115 | _2977 | _1128 |
| Thioredoxin-dependent peroxidases |      |       |      |      |       |       |
| PRX_BCP          | _0846_ | _10900 | _DgeoAM_1323 | _CA1364 | _2368 | _0259 | _0703 |
| PRX_BCP          | _1209_ | _1208_ | _09051 | _0990 | _2729 | _CA1403 | _1714 | _0169 | _3580 | _3178 | _1557 |
| PRX_BCP          |       |       | _23291 | (partial?) |       | _CA0314 |
| Alkyl hydroperoxide reductases |      |       |      |      |       |       |
| AhpE (PRX_AhpE_like) | _2242_ | _02430 | _0122 | _CA2657 | _0618 | _1016 | _0175 |
| AhpD-like        | _1765_ | _13030 | _1p00700 | _1446 | _CA1027 | _0298 | _3296 | _4199 | _3903 | _3878 | _3900 | _2741 |
| OsmC/Ohr/YhfA family proteins |      |       |      |      |       |       |
| OsmC             | _1538_ | _16090 | _0526 | _CA1241 | _0667 | _3743 |
| Ohr              | _1857_ |       | _0446 | _CA0901 | _1828 | _2331 | _0137 | _0225 | _0815 | _0816 |
| YhfA             | _1177_ | _10790 | _21170 | _1268 | _CA1763 | _2343 | _0234 | _0648 | _0697 |
| Bacillithiol disulfide reductase, bacilliredoxin |      |       |      |      |       |       |
| Bdr              | _2623_ | _23360 | _2331 | _CA0078 | _0670 | _2475 | _1732 |
| Brx (AbxC)       | _1832_ | _14700 | _1464 | _CA1021 | _1446 | _3166 | _0555 |

*Not initially predicted; corresponds to protein WP_041221145.1.

2. The TrxR/Trx System in Deinococcus

The two main components of the Trx system, TrxR, an NADPH-dependent flavoenzyme, and Trx, a small disulfide reductase, harbour conserved motifs, including two catalytic cysteines, allowing the transfer of reducing power via mixed-disulfide intermediates (Figure 1). This thiol-based system was initially discovered in E. coli through its role in DNA synthesis as an electron donor for ribonucleotide reductase [47]. Since then, this ubiquitous redox system, which regulates protein dithiol/disulfide balance using electrons provided by NADPH, was described as participating in a wide array of signalling and regulation pathways, and also in protective and repair mechanisms [10,15]. E. coli strains deleted for trx genes exhibit increased resistance to H2O2, probably because the more oxidising redox potential causes increased expression of catalase and alkyl hydroperoxidase in these mutants, but is more sensitive to diamide, a compound inducing disulfide bridging [48]. The key roles of the Trx system in the preservation of redox homeostasis and in responses to oxidative stress (Figure 1c) have been extensively documented in many prokaryotes and eukaryotes [10,11,15]. In Deinococcus, the Trx system has been more recently characterised, and in the next sections, we will describe the characteristics of TrxR and Trx families in this genus and what is currently known about their functions.
TrxRs since a threonine is present in the Helicobacter pylori E. coli TrxRs are characterised by a specific fold found in several types of enzymes that catalyse diverse chemical reactions [50]. The electron transfer from NADPH via FAD to the active site disulfide, located in the NADPH-binding domain, leads to a large conformational change [51]. Bacterial TrxR-active sites contain two redox-active Cys within CATC or CAVC motifs in the genomes of representative E. coli strains [52]. Using recombinant proteins, they showed that the reductase provides electrons to the active cysteines remains to be investigated. The overall homology with TrxR of B. subtilis and D. radiodurans is very similar to that of E. coli, they share more overall homology with TrxR of B. subtilis. Thus, D. radiodurans TrxR (locus tag DR_1982) has 43% and 47% identity with E. coli and B. subtilis counterparts, respectively. Deinococcus and B. subtilis TrxRs do not possess extra Cys residues, such as the E. coli enzyme. Of note, Deinococcus TrxRs are characterised by the presence of a threonine just before the active site, this residue being an alanine or a tyrosine in TrxRs from E. coli or B. subtilis, respectively. There is some variability regarding the identity of this residue among bacterial TrxRs since a threonine is present in the Helicobacter pylori representative and a serine in the M. tuberculosis representative. Whether these residues modulate the activity of redox-active cysteines remains to be investigated.

In a pioneer work regarding the characterisation of the Trx system in Deinococcus, Obiero et al. [22] investigated the biochemical and structural features of TrxR of D. radiodurans. Using recombinant proteins, they showed that the reductase provides electrons to D.
radiodurans Trx2 (DR_A0164) at the expense of NADPH, and also to *E. coli* Trx1, but with a lower affinity. The overall structure of *D. radiodurans* TrxR is very similar to that of TrxRs from other bacteria, with both NADPH- and FAD-binding domains containing variants of the canonical Rossmann nucleotide-binding fold. However, when compared with *E. coli* TrxR, some differences in the shape and charge distribution in the Trx-binding pockets were noticed and presumed to underlie the species-specific affinity previously mentioned [22]. This group further analysed the residues involved in TrxR–Trx interaction using computational alanine mutagenesis and by accurate analysis of the interface [23]. They concluded that four residues (M84, K137, F148, and F149) in *D. radiodurans* TrxR mainly account for the stability of the interface and the preferential binding affinity towards *D. radiodurans* Trx2. There is currently no genetic study unveiling the physiological function of TrxR in *Deinococcus*. In *D. radiodurans*, increased levels of the *trxR* transcript and TrxR protein have been detected after exposure to cadmium and UV/vacuum, respectively [32,52]. Using inhibitors of TrxR, Maqbool et al. [26] reported that *D. radiodurans* cells treated with auranofin or ebselen showed increased H$_2$O$_2$ sensitivity and increased levels of intracellular reactive oxygen species (ROS) and protein carbonyls compared with cells treated with H$_2$O$_2$ alone, indicating altered redox homeostasis when TrxR is inhibited.

2.2. *Deinococcus* Thioredoxins

TrxR supplies electrons to Trx, which is a small heat-stable disulfide reductase of ~10–12 kDa. Canonical Trxs carry a WCGPC-active site motif and share a conserved fold, the basic Trx-fold being composed of three α-helices surrounding four β-strands. The N-terminal catalytic Cys is present mainly as a thiolate, and thus can initiate a nucleophilic attack on oxidised Cys residues present in substrate proteins. This leads to the formation of mixed-disulfide intermediates and triggers deprotonation of the second Cys of the Trx-active site, named resolving Cys, which will catalyse the reduction of the heterodimer and the release of reduced substrate and oxidised Trx (Figure 1b). The *E. coli* genome, like many other bacterial species, contains two *trx* genes, *trxA* and *trxC*, which encode Trx1 and Trx2, respectively. In *E. coli*, a substantially higher level of expression of *trxA* has been reported compared with that of *trxC*. *E. coli* strains lacking both *trx* genes are still viable [48], very likely due to partial overlapping functions with the GSH-dependent system [13]. However, in other bacterial species, such as *B. subtilis*, *Rhodobacter sphaeroides*, and *Synechocystis* sp. PCC 6803, deletion of the *trxA* gene leads to loss of viability [53].

The characterisation of the *E. coli* Trx1 interactome in vivo has led to the identification of more than 250 proteins likely to be redox-regulated at the level of their Cys residues and participating in a wide diversity of cellular processes [54]. Similar conclusions, about the broad spectrum of Trx partners, have been drawn from the comprehensive characterisation of Trx interactome in other organisms, such as mammalian or photosynthetic cells [55,56]. Altogether, these findings clearly validate the central role of Trx in the control of most, if not all, cellular processes in living organisms and highlight its importance in redox biology [15].

The Trx1 isoform represents the most typical Trx form containing around 110 residues without any sequence extension or additional domain. Six of the analysed *Deinococcus* species possess a single *trxA* gene (Figure S2). Surprisingly, three *trxA* genes are present in *D. peraridilitoris*, two being located on a plasmid and encoding proteins more similar to each other (68% identity) than to the chromosome-encoded Trx1 protein. One of these plasmid genes, Deipe._3901, is located directly next to the second *trxR* gene (Deipe._3902) (Figure S3). *D. radiodurans* Trx1 (DR_0944) shares between 76% and 92% identity with Trx1 from other *Deinococcus* species (55% and 60% with the extra Trx1 from *D. peraridilitoris*) and 44% and 49% with Trx1 from *E. coli* and *B. subtilis*, respectively, revealing a closer relationship with the latter, as found for TrxR (Figure S1). Some specificity in *Deinococcus* Trx1 sequences can be noticed in the proximity of the active site motifs. Indeed, whereas the residues preceding the WCGPC-active site are well conserved in bacterial Trx1 proteins, the two subsequent ones differ in chromosome-encoded *Deinococcus* Trx1s (generally RI) compared with those (KM) observed in *E. coli* and *B. subtilis* Trx1 (Figure S2). This specificity...
might confer modified catalytic efficiency or altered access of the active site in *Deinococcus* proteins, but this needs to be further investigated by comparing the biochemical features of mutated recombinant proteins.

The *trxC* gene, encoding Trx2, is present only in three *Deinococcus* species (*D. radiodurans*, *D. deserti*, and *D. geothermalis*) analysed here. Trx2 is characterised by the presence of an additional N-terminal domain of 30 to 35 residues, including 2 other CXXC motifs [57] involved in zinc coordination [58]. This extension has been proposed to modulate Trx activity. Furthermore, if conditions of oxidative stress would affect zinc binding, the released zinc might contribute to a redox signalling pathway [59]. The three Trx2 isoforms from *Deinococcus* share between 51% and 66% identity and around 42% with *E. coli* Trx2 (Figure S2). Some differences are observed regarding the residue just preceding the WCGPC active site motif (T in *D. geothermalis* instead of P in other species) and in the one following this motif (V in *Deinococcus* Trx2 proteins instead of N in *E. coli* Trx2). Again, these specific features might be associated with changes in catalytic properties.

Similar to *E. coli*, a substantially higher expression of Trx1 compared with Trx2 has been observed in *D. deserti* under standard conditions, at both the mRNA and protein levels [31]. Trx1 is also more abundant than Trx2 in *D. radiodurans* [30,33], and both *trxA* and *trxC* showed enhanced expression following treatment with H$_2$O$_2$ [60]. Currently, there are only little data available regarding the biochemical properties and physiological functions of Trxs in *Deinococcus*. In their pioneer work on the Trx system in *D. radiodurans*, Obiero et al. [22] cloned the *trx* gene encoding the Trx2 protein (but improperly named Trx1 since it includes the sequence encoding the N-terminal extension). The recombinant protein catalyses reduction of insulin disulfide bridges, a typical feature of most Trx enzymes, and is reduced by *D. radiodurans* TrxR. Very recently, Kim et al. [60] provided data regarding the two Trx isoforms from *D. radiodurans*. First, they characterised their biochemical properties and showed that both display insulin and DTNB (5,5'-dithiobis(2-nitrobenzoic acid), a small-molecule disulfide) reduction activities in the presence of NADPH and TrxR. These activities were completely lost when active site Cys residues in Trx were replaced by Ser. Consistent with the regulatory role of the additional zinc-binding CXXC motifs in Trx2, substitution of Cys by Ser in these motifs impaired its reduction capacity. Of particular interest regarding Trx2, Kim et al. [60] determined its crystal structure and noticed that the orientation of the N-terminal Zn finger domain within the overall structure and with respect to the Trx-fold was clearly different in *D. radiodurans* from that observed in other bacterial Trx2 isoforms. They proposed that this unique feature could modulate protein–protein interactions, and be involved in the recognition of specific targets in this species. Finally, these authors also constructed *D. radiodurans* strains deleted for *trxA* or *trxC*, and showed that both mutants exhibit strongly reduced survival rates when treated with 60 mM H$_2$O$_2$. Interestingly, the mutant lacking Trx2 appeared to be more sensitive to this reactive oxygen species compared with that impaired for Trx1. Therefore, even though Trx2 is not common to radiation-tolerant *Deinococcus* species, it seems to contribute to stress tolerance in the species possessing this protein. These data, combined with those of Maqbool et al. [26] on TrxR, clearly show the key role of the TrxR/Trx system in the tolerance of *Deinococcus* to oxidative stress. No data about mutants lacking either *trxR* or both *trxA* and *trxC* have been reported, perhaps because such mutants are not viable.

### 2.3. Trx-Like Proteins in *Deinococcus*

The analysis of the *D. radiodurans* genome revealed the presence of several other genes (DR_2085, DR_A0072, DR_0057, DR_B0110, and DR_0948) encoding small proteins harbouring CXXC motifs, such a CPDC, CHLC, and CPGC (Table 3), and predicted to display a Trx-fold (Figure S4). These proteins are present in most *Deinococcus* species analysed here and divided in several subclasses (Table 2; Figure S5e) [3,5]. Based on sequence homology, some of these proteins were first classified as glutaredoxin-like proteins. However, since Grxs generally display a CPYC active site motif and, most importantly, get their reducing power from glutathione, which is absent in *Deinococcus*, these reductases...
should rather be considered as Trx-like proteins. Consistently, DR_2085-type proteins contain a specific structural motif (Figure 2a), SGFRP, which is found in E. coli NrdH, a disulfide reductase possessing a canonical C(M/V)QC active site, participating in the reduction of NrdE, a ribonucleotide reductase, and supplied with electrons by TrxR [61]. A deep analysis of the genomes of various Deinococcus species revealed variability in the number of genes encoding such Trx-like proteins: for instance, three in D. deserti and six in D. gobiensis. The DR_2085 and DR_0057 types, which have CPDC and CHLC active sites, respectively, are present and well conserved in all species selected (Figure 2a; Figure S5a). For D. radiodurans and D. deserti, proteomics indicated low levels of expression of these proteins under standard growth conditions, but their detection might have been limited because of their small size. In the next parts, we will mainly focus on these two Trx-like types, notably in relation to the knowledge gained on related reductases in other organisms.

**Table 3.** Active site motifs in Deinococcus Trx and Trx-like proteins.

| Trx1/Trx2 | DR_2085-Type (TRP14-Like) | DR_A0072-Type | DR_B0110-Type | DR_0057-Type (MGP12-Like) | DR_0948-Type |
|-----------|--------------------------|---------------|---------------|---------------------------|-------------|
| DR        | WCGPC                    | WCPDC         | DCPDC         | ACPGC                     | GCHLC       |
| Deide     | WCGPC                    | WCPDC         | NCSSC         | NCPAC                     | GCHLC       |
| Dgeo      | WCGPC                    | WCPDC         | QCADC GCASC   | ACPDC                     | GCHLC       |
| DO        | WCGPC                    | WCPDC         | DCADC TCPDC   | NCPNC                     | SCKLC       |
| Deima     | WCGPC                    | WCPDC         |               |                           | ECAGC       |
| Deipe     | WCGPC                    | WCPDC         |               |                           | GCHLC       |
| Deipr     | WCGPC                    | WCPDC         |               |                           | ECAGC       |

### 2.3.1. Trx-Like Proteins Carrying a CPDC Active Site Motif

Thioredoxin-Related Proteins of 14 kDa

Among Trx-like proteins present in Deinococcus, the DR_2085 type is present in all species selected for this study and includes well-conserved members sharing between 74% and 92% identity with DR_2085 (Figure 2a). This Trx type is characterised by the presence of a WCPDC active site. Interestingly, this motif is typical of a specific family of Trx-like proteins named TRP14 for Trx-related protein of 14 kDa or Clot in plants [62]. TRP14, first characterised in mammalian cells [63], is present and well conserved throughout the animal kingdom as well as in plants [64–66]. TRP14-related proteins are also present in yeast, but are less represented in bacteria. One ortholog, encoded by locus HP_1458, was identified in H. pylori [67] and shown to be important for maintaining DNA and protein integrity, and for survival under oxidative stress conditions [68]. The WCPDC motif is also present in the thioredoxin-like proteins YdpP and YtpP from B. subtilis, and in YtpP (BA_4945) from Bacillus anthracis [69]. Compared with TRP14s from other organisms, Deinococcus TRP14s are shorter, since they do not display an N-terminal extension (Figure 2b). Furthermore, except for the WCPDC motif, sequence similarity is low between these proteins. Alignment of the sequences of the two TRP14-related proteins from D. radiodurans and H. pylori revealed 15% identity and 30% similarity, while 13% identity and 25% similarity exist between DR_2085 and human TRP14 (Figure 2b). Notably, in addition to the conserved WCPDC active site motif, these proteins share a (V/I)PTL(V/I/L) motif in their C-terminal part.

Mammalian TRP14 has been shown to get electrons from TrxR and displays very poor ability to reduce insulin and typical Trx substrates, such as ribonucleotide reductases, peroxiredoxins, or methionine sulfoxide reductases [63]. Consistently, B. anthracis TRP14 could reduce DTNB, but not insulin in the presence of NADPH and B. anthracis TrxR [69]. Note that H. pylori TRP14 exhibits disulfide reductase activity in both the DTNB and insulin assays [67]. Most interestingly, human TRP14 can efficiently reduce L-cystine, a source of cysteine [70], and also nitrosylated or persulfidated cysteines in proteins [70,71]. Based on these biochemical properties, TRP14 reductases are considered as main modulators...
in redox signalling pathways and could be involved in protection against metal stress or pathogenic viruses [66]. Whether the DR_2085-type proteins fulfil similar activities and physiological functions in stress responses of Deinococcus remains to be investigated. Induced expression of the DR_2085 gene and its Deide_06390 homolog has been observed after irradiation [31,72,73] and upregulation of DR_2085 upon cadmium exposure [52].

Table 3. Active site motifs in Deinococcus Trx and Trx-like proteins.

| Protein     | Motif Description       |
|-------------|-------------------------|
| DR_2085     | XXXXXXXXXX              |

Figure 2. Multiple sequence alignments of TRX-like proteins from Deinococcus. (a) Alignment of Deinococcus DR_2085-type sequences. (b) Alignment of Deinococcus DR_2085 sequence with Homo sapiens (Hs) and Helicobacter pylori (Hp) homologous TRP14 Trxs. (c) Alignment of Deinococcus DR_0057 with Arabidopsis thaliana (At), Mus musculus (Mm), Saccharomyces cerevisiae (Sc), Halobacterium salinarum (Ha) and Chlorobium ferrooxidans (Cf) homologous proteins. Trx active site sequences are highlighted in yellow. An asterisk (*) indicates positions which have a single, fully conserved residue; colon (:) and period (.) indicate conservation between groups of strongly and weakly similar properties, respectively . Abbreviations of Deinococcus species names are as specified in Table 1. Alignments were generated using UniProt ClustalO (https://www.uniprot.org/align/; accessed on 14 February 2022).
DR_A0072-Related Proteins

Another CPDC-containing Trx-like protein (DR_A0072) has been identified in *D. radiodurans*, with an Asp preceding this motif, that is present neither in Trx-like such as TRP14 nor in canonical Trxs. Homologs of DR_A0072 sharing between 61% and 70% identity are present only in three of the analysed *Deinococcus* species (*D. geothermalis*, *D. gobiensis*, and *D. proteolyticus*). Note that these homologs do not harbour a CPDC motif. Such a motif is present in another plasmid-encoded *D. proteolyticus* homolog (Deipr_2732), displaying less identity (41%) with DR_A0072 (Figure S5b). Interestingly, the DR_A0072 gene is located between a gene encoding a heavy metal translocating P-type ATPase and a gene encoding an ArsR family regulator. This is similar in the three other *Deinococcus* species, suggesting a possible role of this Trx-like protein in the maintenance of metal homeostasis. Consistently, the DR_A0072 gene is upregulated after exposure of *D. radiodurans* to cadmium [52].

DR_B0110-Related Proteins

A CPDC motif is also present in DGo_PA0204 from *D. gobiensis*. However, this motif is not entirely conserved. Instead, a CPXC motif is rather present in homologs found only in three other *Deinococcus* species, including DR_B0110. The latter has 48% to 53% identity with Dgeo_1776, DGo_PA0204, and Deipr_2190 (Figure S5c). Interestingly, in the four species, the DR_B0110 gene homologs are preceded by and probably in operon with *nrldEF* gene homologs, suggesting that these Trx-like proteins could participate in the modulation of ribonucleotide reductase activity, thus being functional equivalents of NrdH proteins. Expression of the DR_B0110 gene was induced after exposure to gamma radiation [72,74].

2.3.2. Trx-Like Proteins Carrying Other Active Site Motifs

DR_0057-Related Proteins

Another type of Trx-like proteins represented by DR_0057 in *D. radiodurans* is present in the seven *Deinococcus* species selected. DR_0057 shares between 41% and 65% identity with the six homologs. These proteins are 81 to 106 amino acids long (Figure S5a) and possess a low similarity both with NrdH-redoxin family proteins and proteins possessing a glutaredoxin-like domain (DUF836). In the absence of the C-terminal SGFRP motif typical of NrdH-redoxins, they cannot be classified as such. Instead, they share a dicysteinic CxLC motif that resembles the one present in DUF836 members. With the notable exception of the *D. proteolyticus* representative (SCKLCQ motif), other members of the *Deinococcus* genus analysed exhibit a more conserved GCHLCE motif.

Little information is known among the few members of this class. The tridimensional structure of a related protein from *Mus musculus* has been deposited in the protein databank (pdb code 1WJK), but never published. The yeast protein was referred to as MGP12 (mitochondrial glutaredoxin-like protein of 12 kDa) because it was identified in a study focusing on the mitochondrial proteome of this organism [75]. However, this name obviously does not fit with the widespread presence in prokaryote organisms. Indeed, in addition to their presence in eukaryotes including fungi, plants, and metazoans, homologs are present in some bacterial and archaeal genera. The sequences are highly divergent, and for example, only 17% identity and 17% similarity exist between DR_0057 and *Arabidopsis* MGP12 (Figure 2c). In fact, only the cis-proline that is present in all Trx superfamily members and the CxLC motif are strictly conserved.

DR_0948-Related Proteins

Finally, another type of Trx-like protein is present in six out of the seven *Deinococcus* species analysed (no homolog in *D. peraridilitoris*), the corresponding protein in *D. radiodurans* being DR_0948. The sequence is well conserved, with DR_0948 sharing 64–86% identity with the proteins from the five other species (Figure S5d). They also exhibit longer sequence (ca 150 residues) than that of canonical Trxs, but differ in the second residue of the active site motif (CSCG, CPGC, or CAGC). In *D. deserti*, the expression of the Deide_06780 gene is induced by radiation [31].
2.4. FrnE Oxidoreductase

FrnE subfamily proteins are predicted cytoplasmic thiol oxidoreductases belonging to the DsbA family (domain cd03024 in the Conserved Domain Database) [76]. They contain a CXXC motif in a DsbA-like thioredoxin domain. FrnE homologs are present in many bacteria, and many possess an additional conserved CXXXXC motif at the C-terminus. The name frnE was given for the first time to a gene cluster (frnA to frnU) associated with the biosynthesis of the polyketide antibiotic frenolicin B in Streptomyces roseofulvus (GenBank accession AF058302.1). An frnE gene homolog is present in Deinococcus species but not located in an frn gene cluster as in S. roseofulvus. FrnE (DR_0659, drFrnE) from D. radiodurans has been studied. The expression of DR_0659 is upregulated after exposure of D. radiodurans to gamma radiation and desiccation [74], cadmium [52], and UV/vacuum [32]. Compared with the wild-type strain, a DR_0659 disruption mutant showed increased sensitivity in particular to cadmium and diamide, and less markedly to gamma rays and H$_2$O$_2$ [77]. Recombinant drFrnE showed disulfide isomerase and insulin reduction activities in vitro. In addition to the 22-CXXC-25 active site motif located near the N-terminus, drFrnE has a C-terminal tail harbouring a 239-CXXXXC-244 motif. Four different drFrnE crystal structures have been solved, with the crystals grown in the absence or presence of different reducing agents [78]. A disulfide bond between Cys22 and Cys25 was observed in the absence of a reducing agent. In all structures, the C-terminal tail of one molecule interacts with the active site of another drFrnE molecule, with the C-terminal CXXXXC located close to the active site CXXC. In crystals grown in the presence of DTT, an intermolecular disulfide bond was observed between Cys244 and Cys22. The C-terminal cysteines are not required for insulin reduction activity in the presence of DTT. The drFrnE protein could also reduce DTNB in the presence of NADPH and E. coli TrxR, but this activity was decreased when the C-terminal cysteines were mutated or deleted. Based on structural and biochemical data, the authors proposed that drFrnE is a cytoplasmic disulfide reductase and that the C-terminal CXXXXC is functionally important with a role in the regeneration of the oxidised form is different for these homologs.

In addition to FrnE, two other predicted cytoplasmic proteins with DsbA-like domains are present in several Deinococcus species. One of these, belonging to the FrnE-like subfamily of the DsbA family (cd03025), is encoded by the genomes of D. deserti and D. proteolyticus. These two proteins (Deide_3p01230 and Deipr_1559) share 37% identity and have a CGWC or CIWC motif but no other Cys residues (Figure S6b). Another protein with a DsbA-like thioredoxin domain, but with only a single conserved Cys residue, is found in five of the analysed species (e.g., DR_2335 in D. radiodurans) (Figure S6c). To our knowledge, no experimental data concerning the function or expression have been reported for these proteins.

2.5. Periplasmic Disulfide Oxidoreductases

Dsb (disulfide bond) family proteins such as DsbA to DsbE and DsbG from E. coli are involved in the formation or reduction of disulfide bonds, and in the repair of oxidised Cys residues in extracytoplasmic proteins [14]. They have redox-active cysteines, including CXXC motifs. DsbA, DsbC, and DsbG are periplasmic proteins synthesised as precursors with an N-terminal signal peptide. DsbA catalyses the formation of disulfide bonds in proteins exported to the periplasm. DsbA is reoxidised by the cytoplasmic membrane protein DsbB. The DsbA and DsbB homologs in B. subtilis are called BdbD and BdbC, respectively, with BdbD attached to the membrane by an N-terminal transmembrane segment [79]. DsbC and DsbG are part of a periplasmic reducing system that repairs oxidised Cys of single-Cys-containing extracytoplasmic proteins [80]. DsbC also catalyses the isomerisation of incorrect disulfide bonds that are often introduced by DsbA, resulting in
proper disulfide bonds and protein folding. DsbE, also called CcmG, reduces the disulfide bond that is formed in apocytochrome c in the periplasm to allow heme ligation. DsbE belongs to the TlpA-like family (domain cd02966 in the Conserved Domain Database) [76], also including TlpA (e.g., from *Bradyrhizobium japonicum*), ResA (*B. subtilis*), and similar proteins with a CXXC motif-containing Trx domain in the periplasm. DsbC, DsbG, and DsbE are maintained in the reduced state by the cytoplasmic membrane protein DsbD that catalyses the transfer of electrons from the cytoplasmic NADPH–TrxR–Trx system across the membrane. Many bacteria, such as *B. subtilis* and *H. pylori*, possess a shorter DsbD analog called CcdA [14,81].

*Deinococcus* species have several predicted Dsb proteins, but there is variation in the presence/absence and number of these proteins across the different species (Table 2). To our knowledge, these proteins have not been studied experimentally. The *B. subtilis* bdbD and bdbC genes are present in an operon (bdbDC). Similarly, two adjacent genes encoding DsbA and DsbB family proteins showing sequence similarity to *B. subtilis* BbdD and BbdC are present in six of the analysed *Deinococcus* genomes (Figure S7). Like *B. subtilis* BbdD, these DsbA family proteins (e.g., DR_0753) have a predicted N-terminal transmembrane segment and a KCPXCK active site motif (Figure S8a). Unlike *B. subtilis* BbdD, but similar to *E. coli* DsbC, two additional conserved Cys residues are present, which might form a disulfide bond as in DsbC. *D. proteolyticus* has an additional more distant homolog (Deipr_2421) encoded by a gene on a plasmid and not adjacent to a bdbC-like gene. The BdbC (DsbB family) homologs (e.g., DR_0754) have a PCXLCW motif and two other conserved Cys residues (Figure S8b). The same six *Deinococcus* species possess another (two in *D. radiodurans*: DR_2019 and DR_0560) DsbA-related protein having a predicted signal peptide, a QCPYC motif, and two other conserved Cys residues (Table 2; Figure S8c). Experimental work is needed to establish whether these deinococcal DsbA family proteins have a role, like *E. coli* DsbA, in generating disulfide bonds in extracytoplasmic proteins or are functionally similar to DsbC or DsbG. Remarkably, DsbA and DsbB family proteins are absent in *D. peraridilitoris* (Table 2).

As reported previously [82], bdbDC homologs in several *Deinococcus* species are directly preceded, and possibly in operon [31], by a gene encoding a *Deinococcus*-specific membrane protein designated dCSP-1 and the gene encoding the DNA repair protein UvrA (Figure S7). It has been proposed that BbdD (DsbA) and, on the cytoplasmic side, UvrA may interact with dCSP-1, and that UvrA may receive electrons from BbdC (DsbB) to reduce oxidatively damaged Cys residues in CXXC motifs of UvrA, restoring its nonoxidised functional state [82]. However, as mentioned above, *D. peraridilitoris* does not have DsbA and DsbB proteins, but possesses UvrA and dCSP-1 (Figure S7).

Homologs of *E. coli* DsbD were not found in *Deinococcus*. However, at least one homolog of the DsbD analog CcdA is present in this bacterial genus. Except for the ccdA gene (DR_1300) in *D. radiodurans*, ccdA homologs are located in gene clusters encoding cytochrome c-type biogenesis proteins CcmA to CcmH in the other species, albeit with some variation in the genetic organisation (Figure S9). A *ccmG* (dsbE) gene is also present in these gene clusters. *D. deserti* and *D. peraridilitoris* have an additional two-gene cluster encoding homologs of CcdA and CcmG (DsbE) (Figure S9). The CcdA proteins have two conserved Cys surrounded by other conserved residues (Figure S8d). The CcmG (DsbE) proteins contain a WXXWCXXC motif as found in TlpA-like family proteins from other bacteria, such as *E. coli* DsbE and *B. subtilis* ResA (Figure S8e). The CXXC motif of the DsbE homolog present in each *Deinococcus* contains a Pro (CXPC) as in ResA and in the uncharacterised YneN of *B. subtilis*. The additional DsbE homologs from *D. deserti* (Deide_2p00420) and *D. peraridilitoris* (Deipe_0660) have a divergent CLVC motif. Deinococcal CcdA and DsbE (CcmG) proteins may be required only for cytochrome c maturation (reduction of disulfide bond in apocytochrome), but could also participate in the reduction of other substrates in the periplasm, and have a role in defence against oxidative stress, as reported for such proteins in other bacteria [83–86]. Alternatively, and in particular for additional proteins in *D. deserti* and *D. peraridilitoris*, DsbE might function as an oxidant instead of a reductant, as
observed for DsbE from *M. tuberculosis* [87], and be involved in the formation of disulfide bonds since, as described above, no DsbA family proteins were found in *D. peraridilitoris*.

3. Main Trx Partners in *Deinococcus*

In addition to their roles in the modification of the structure or activity of numerous partners via redox post-translational modifications, Trxs supply electrons to several types of thiol reductases and peroxidases that have critical roles in the maintenance of redox homeostasis. Among them, two main types of enzymes, methionine sulfoxide reductases (Msrs) and peroxiredoxins (Prxs), control the redox status of proteins and scavenges peroxides, respectively (Figure 1c). Both enzyme types fulfil their biochemical function thanks to catalytic redox-active cysteines.

3.1. Methionine Sulfoxide Reductases

Oxidation of Met results in the formation of Met sulfoxide (MetO) and Met sulfone (MetO₂), the latter modification being irreversible. MetO proportion can reach up to 40% of total Met during acute oxidative stress conditions [88]. MetO is mainly reduced back to Met by Msrs [89]. Oxidation of Met generates *S*- and *R*-MetO diastereoisomers, and two Msr types, A and B, specifically reduce these isomers, respectively. Although sharing a very similar thiol- and sulfenic acid-based redox chemistry, they do not exhibit any sequence similarity [90,91]. Of note, MsrA is able to reduce both peptide-bound and free MetO forms, in contrast to MsrB, which reduces the peptide-bound form in a more efficient way [92]. Most Msrs achieve their activity thanks to two redox-active Cys and a three-step catalytic mechanism following MetO reduction: (i) formation of a Cys sulfenic acid intermediate, (ii) formation of an intramolecular disulfide bond, and (iii) disulfide reduction generally by Trx and regeneration of activity [91,93]. Note that the regeneration of some Msrs having only one catalytic Cys is ensured by other thiol-containing compounds, such as Grxs [93,94].

Thiol-utilising Msrs are present in most living organisms, except in a few eubacteria and in one group of archaea [95]. They fulfil essential functions in stress tolerance and during ageing through modulation of the Met redox status in proteins [96,97]. In pathogenic bacteria, Msrs are considered essential determinants underlying their virulence and capacity to counteract host defences [98]. Thus, modification of the expression of *msr* genes leads to impaired responses to oxidative stress notably due to defective protein repair [99]. Consistently, Henry et al. [100] reported that the ubiquitous recombinase RecA, which promotes DNA recombination and induction of the SOS repair system, is a substrate of both Msr types. Indeed, oxidation of two Met residues in *E. coli* RecA is associated with dramatic changes in DNA recombination activity and SOS induction level, and the Msr system allows preserving the pool of functional RecA. Besides a repair function, these enzymes have also been proposed to play a direct antioxidant function by eliminating ROS via cyclic oxidation of Met in proteins [101]. Most importantly, a lot of evidence highlighted that the control of Met redox status is involved in the transduction of redox signals [102–104]. For instance, exposure to HOCl of *E. coli* cells results in activation via Met oxidation of the HypT transcription factor and enhanced expression of genes involved in protective mechanisms against this harmful agent [105].

In *Deinococcus*, the knowledge regarding the maintenance of Met redox status has only recently been explored. When submitting *E. coli* and *D. radiodurans* cells to ionising radiation (1000 Gy), Bruckbauer et al. [106] observed substantially greater oxidative modifications in *E. coli* proteome. Of note, methionine sulfoxide formation was the most frequent modification in *E. coli*, for which 137 instances were counted, while only one was detected in *D. radiodurans*. In another proteomic-based analysis, Chang et al. [107] exposed these two bacteria to 6700 Gy, a radiation level lethal for *E. coli* and causing damage in *D. radiodurans*. A much lower level of carbonylation was measured in the latter. These authors investigated whether protein-intrinsic properties could underlie this differential tolerance and noticed that in permissive conditions Met was less prevalent in *D. radiodurans* than in *E. coli*. In contrast, following irradiation, Met prevalence decreased in the latter, but strongly
increased in the former, suggesting the occurrence of a more efficient Msr system under oxidative stress in *D. radiodurans* [107]. Consistently, using structure- and sequence-based models, *E. coli* MsrB was predicted to be intrinsically more sensitive to carbonylation than its *Deinococcus* counterpart. These data corroborate the likely importance of Msr proteins in the tolerance of *Deinococcus* species to severe stress conditions.

### 3.1.1. MsrA Proteins in *Deinococcus*

Similar to most non-photosynthetic species, *Deinococcus* has only one *msrA* gene. MsrA proteins from the seven species analysed here share 58% to 77% identity and about 45% identity with those of *E. coli* and *B. subtilis* (Figure S10a). They all display four conserved Cys, which are at positions 25, 28, 60, and 170 in DR_1849, the Cys25 in the conserved motif A(I/L/F)GGCFWCT being the catalytic one. This motif is close to the consensus sequence present in most MsrAs [91,108]. Of note, this motif displays some specificities in *Deinococcus*, which are not observed in *E. coli* and many other organisms. For instance, it includes a second Cys at position 28, whereas Gly or Ser is generally present at this position. Interestingly, this second Cys is also present in *B. subtilis* MsrA. In *E. coli* MsrA, Cys198 has been identified as the resolving Cys allowing activity regeneration of the enzyme [109]. Based on sequence alignment, we presume that the resolving Cys in *Deinococcus* reductases is the one corresponding to Cys170 in DR_1849. Upregulation of DR_1849 gene expression or protein abundance has been observed after exposure of *D. radiodurans* to radiation [32,74] or cadmium [32].

### 3.1.2. MsrB Proteins in *Deinococcus*

Like in most prokaryote genomes [110], one *msrB* gene is present in *Deinococcus*. Among the seven species selected for this study, six MsrB proteins display high homology between them (72% to 82% identity) (Figure S10b). MsrB (Deipe_4299) from *D. peraridilitoris* is more divergent since it exhibits only 51% to 55% identity with MsrBs from other species. *Deinococcus* MsrBs (except Deipe_4299) are closer to the one of *B. subtilis* (ca. 62% identity, against ca. 48% when compared with *E. coli* MsrB). Interestingly, *D. peraridilitoris* MsrB possesses two CXXC motifs like homologs from *E. coli* and eukaryotes [108,111]. These motifs are presumed to be involved in Zn fixation and structural stabilisation of the protein, and are absent in the reductases of the six other *Deinococcus* species analysed here and of *B. subtilis*. In other respects, all *Deinococcus* MsrBs share two well-conserved Cys, at positions 71 and 126 in DR_1378, corresponding to Cys63 and Cys117, respectively, in *E. coli* MsrB. Analysis of the MsrB catalytic mechanism [112] revealed that the latter, which is included in the well-conserved active site motif RYC(I/V)N, corresponds to the catalytic Cys, while the former named resolving Cys is present in the motif GCGWP. Based on these sequence features, we conclude that *Deinococcus* MsrBs belong to the 2-Cys type, which is regenerated by Trx following the reduction of an intramolecular disulfide bridge [113]. Induced expression of the DR_1378 gene was observed after exposure of *D. radiodurans* to cadmium [32], while its gene homolog Deide_04050 in *D. deserti* was induced after gamma radiation [31].

### 3.1.3. Non-Thiol-Based Periplasmic and Possible Cytoplasmic Msr Systems

As described in Section 2.5, the maintenance of Cys redox status in periplasmic proteins in bacteria is ensured by the thiol-dependent Dsb system thanks to reducing power provided by cytoplasmic Trx. In contrast, non-thiol-dependent systems control the redox status of Met in periplasm [114], except in some bacteria, such as *Neisseria* or *Helicobacter*, in which Msrs A and B are present in this compartment as fusion proteins [115,116]. In most other bacteria, various types of molybdenum-containing enzymes exist to reduce MetO [114]. The MsrPQ (YedY/YedZ) system is present in a large subset of bacteria including *E. coli* and *Pseudomonas aeruginosa* [117]. In this system, electrons are provided by the respiratory chain via MsrQ, an inner membrane heme b-containing protein, to MsrP, which reduces MetO diastereoisomers thanks to a molybdopterin cofactor. Both MsrP and
MsrQ are present and well conserved in all *Deinococcus* species analysed here (Table 2), the *D. radiodurans* representatives being DR_2536 and DR_2537, respectively. For DR_2536, upregulated gene expression has been observed following exposure to cadmium [52].

Other molybdenum-containing enzymes exhibiting MetO reductase activity are the cytoplasmic BisC and periplasmic TorZ and DmsA proteins present in *E. coli* and other bacteria. These are oxido-reductases containing the COG0243 or BisC domain [114]. Periplasmic homologs of this enzyme type are not present in *Deinococcus*. However, a predicted cytoplasmic protein (DR_0397 in *D. radiodurans*; Table 2) with only low levels of similarity (less than 30% identity) with BisC is conserved in *Deinococcus*. Furthermore, another predicted cytoplasmic molybdenum-containing enzyme belonging to the sulfite oxidase family and containing the COG2041 (YedY) domain also present in MsrP is highly conserved in *Deinococcus* (DR_0716 in *D. radiodurans*; Table 2). Whether these uncharacterised proteins have MetO reductase activity or another function remains to be established.

### 3.2. Thioredoxin-Dependent Peroxidases/Peroxiredoxins

Peroxiredoxins are ubiquitous enzymes reducing peroxides using a conserved cysteine, named peroxidatic cysteine [118,119]. These enzymes, which are generally highly abundant in cells, function without any cofactor and exhibit a remarkable efficiency in scavenging peroxides and peroxynitrites [118]. They are recognised as key actors in the maintenance of cell redox homeostasis via several functions, including elimination of peroxides, signalling due to hyperoxidation or via redox modulation of protein partners, and also a chaperone role in relation to their redox status and oligomerisation level [119–121]. Since their discovery in yeast [122], Prxs have been the subject of extensive work in all organisms. They are critical actors in responses to environmental constraints, upon diseases such as cancer, or during the course of ageing [118,119]. They also constitute essential virulence factors in pathogenic microorganisms [123].

Prxs are classified in six evolutionary distinct subfamilies (Prx1, Prx6, AhpE (alkyl hydroperoxide reductase E), PrxQ/BCP (bacterioferritin comigratory protein), Tpx (thiol peroxidase), and Prx5), which differ by the presence and position of resolving cysteine, the oligomeric state, and the type of electron donor [118]. All are characterised by the presence of a conserved motif, Pxxx(T/S)xxC, containing the peroxidatic Cys, the thiolate form of which attacks peroxides. The resulting Cys sulfenic acid is generally resolved by the formation of an inter- or intramolecular disulfide bond with a resolving Cys, which will be further reduced by Trx in most cases, except for AhpE and AhpC, a member of the Prx1 subclass. AhpC-type Prxs receive electrons from AhpF, a flavoprotein pyridine nucleotide–disulfide oxidoreductase related to, but different from, TrxR [124].

The survey of *Deinococcus* genomes revealed the presence of two types of Prxs, namely, PrxQ/BCP and AhpE (Figure 3), which are present in all analysed species of the genus [3] (Table 2). In this section, we will focus on the Trx-dependent PrxQ/BCP type, first identified and characterised in *E. coli*, and named bacterioferritin comigratory protein in bacteria [125], but PrxQ in plants [126]. In *E. coli*, the *bcp* gene is induced by oxidative stress conditions, and strains lacking it exhibit hypersensitivity to hydrogen and organic peroxides [125]. The first characterised bacterial or plant representatives of this class harboured two Cys, forming an intramolecular disulfide bond following the catalytic act, which was subsequently reduced by Trx [126,127]. However, sequence alignment of BCP proteins from prokaryotes revealed the absence of the resolving Cys in a majority of them and raised the question of the regeneration of their activity [127]. When investigating the catalytic mechanism of 1-Cys BCP from *Burkholderia cenocepacia*, Clarke et al. [128] observed that the formed Cys sulfenic acid could be reduced directly by Trx, or in a more efficient way by the GSH/Grx pathway.
or regeneration pathways with electron donors. An additional, longer and more distant, predicted BCP type Prx is present in B. subtilis. DGo_CA0314 and Deide_23291 may represent inactive BCPs since they lack the PxxxTxxC motif, and a 15-residue insertion is present in the corresponding region.

These sequence features might alter the peroxidase catalytic properties and underlie specific interactions or regeneration pathways with electron donors. An additional, longer and more distant, predicted BCP-type Prx is present in D. gobiensis (Figure S11c). This DGo_CA0314 protein shares 64% identity with D. deserti Deide_23291, but the latter seems truncated. Both DGo_CA0314 and Deide_23291 may represent inactive BCPs since they lack the PxxxTxxC motif, and a 15-residue insertion is present in the corresponding region.

Cho et al. [25] investigated the expression levels of the three bcp genes in D. radiodurans exposed to oxidative stress generated by H₂O₂ or subjected to gamma irradiation. DR_0846 was strongly induced by both treatments, while the expression of DR_1209 was moderately increased by the peroxide, and that of DR_1208 was substantially triggered by irradiation. In proteomics studies, DR_0846 (and its D. deserti homolog Deide_10900) was well detected compared with DR_1209 and DR_1208 (and Deide_09051), suggesting higher expression levels of the DR_0846-type BCP under standard conditions [5,29–33].

The D. radiodurans DR_0846 BCP protein has been further characterised with regard to its activity and physiological function [25]. The recombinant protein exhibits peroxidase activity towards H₂O₂ and holdase chaperone activity when measuring thermal-induced aggregation of a heat-sensitive substrate. Interestingly, a D. radiodurans strain lacking DR_0846 displays similar sensitivity to H₂O₂ and heat stresses compared with WT. Most importantly, a strain lacking both DR_0846 and catalase exhibited increased sensitivity to these stresses compared with the strain lacking only catalase, indicating that the BCPs participate in redox signalling. TrxR, thioredoxin reductase; Trx, thioredoxin; BCP, bacterioferritin comigratory protein; SucB, dihydrolipoamide succinyltransferase; PDHB, pyruvate dehydrogenase beta; Ahp, alkyl hydroperoxide reductase; Ohr, organic hydroperoxide resistance enzyme; OsmC, osmotically induced bacterial protein C. Dashed arrows indicate possible transfer of electrons.

Figure 3. Electron sources for Deinococcus NADPH- and NADH-dependent thiol peroxidases (BCP, AhpE, AhpD, Ohr, OsmC) scavenging hydrogen and organic peroxides and peroxynitrates and participating in redox signalling. TrxR, thioredoxin reductase; Trx, thioredoxin; BCP, bacterioferritin comigratory protein; SucB, dihydrolipoamide succinyltransferase; PDHB, pyruvate dehydrogenase beta; Ahp, alkyl hydroperoxide reductase; Ohr, organic hydroperoxide resistance enzyme; OsmC, osmotically induced bacterial protein C. Dashed arrows indicate possible transfer of electrons.
peroxidase activity in *Deinococcus* may represent a second line of defence towards oxidative stress. In other respects, BCP proteins from various bacterial species have been reported to bind DNA in vitro and protect it against thermal and oxidative damage [129,130]. The physiological relevance of such a function remains to be established, but in the case of *Deinococcus*, where DNA damage is repaired in an efficient and rapid manner following extreme stress conditions, we can propose that BCPs, which are over-represented in this species, participate in such protective mechanisms.

4. Thioredoxin-Independent Thiol Peroxidases

Besides Trx-dependent Prxs, other less ubiquitous types of thiol peroxidases have been identified in the last decades. Among them, three are present in *Deinococcus*, namely, AhpE, AhpD, and OsmC/Ohr.

4.1. The AhpF/D-AhpC/E Thiol-Dependent Peroxidase Systems

The alkyl hydroperoxide reductase AhpF-AhpC system has been first identified in *Salmonella typhimurium*, and later in many other bacteria, yeast, and mammals [131–133]. This system, catalysing the reduction of peroxides at the expense of NADH, is composed of AhpF, a FAD-containing protein, which mediates electron transfer to the AhpC thiol peroxidase [124]. As already mentioned, AhpF, like TrxR, belongs to the family of pyridine nucleotide–disulfide oxidoreductases. Both share a domain harbouring two conserved catalytic Cys [131,134]. However, AhpF has a higher molecular mass (57 kDa) due to the presence of an N-terminal extension of 200 residues, including two other Cys ensuring electron transfer from the TrxR-like domain to AhpC [135]. AhpC is a 21-kDa thiol peroxidase belonging to the Prx1 subfamily initially named typical 2-Cys Prx [118]. These highly expressed Prxs function as dimers and form during the catalytic cycle an inter-subunit disulfide bond. Further, as a function of their redox status, they are able to oligomerise and fulfil then a molecular chaperone function [136,137]. The AhpF–AhpC system is widespread in microorganisms, and strains modified for its expression exhibit altered susceptibility to oxidative stress generated by hydrogen or organic peroxides [133,138].

AhpE is an atypical type of AhpC having only one Cys and possessing also both peroxidase and chaperone activities [139]. It has been first characterised in *M. tuberculosis*, where it shares 34% sequence identity with AhpC [140]. Most interestingly, AhpE has been found to display specificity towards long-chain fatty acid hydroperoxides [141]. Interestingly, another type of reductase named AhpD is able to provide electrons to AhpC [142]. AhpD shares little sequence homology but common structural features with AhpF [10]. This reductase is present in some bacteria (*M. tuberculosis*, *Corynebacterium glutamicum*) and cyanobacteria (*Anabaena PCC7120*) [143]. AhpD is reduced by a lipoamide-containing protein, dihydrolipoamide succinyltransferase or lipoamide reductase, using NADH as a source of electrons [142]. AhpD plays essential roles in response to oxidative stress [143,144] that could be carried out by direct peroxidase activity towards organic peroxides [144] or reduction of AhpC/AhpE [10,142]. However, the experimental evidence for the latter biochemical function remains to be firmly established regarding AhpE.

In *Deinococcus* genomes, there are no close homologs of *ahpF* and *ahpC*. However, all species possess one *ahpE* gene (DR_2242 in *D. radiodurans*). DR_2242 shares 53% to 82% identity with the homologs from the other six species and 35% identity with AhpE from *M. tuberculosis* (Figure S12a). In addition to the AhpE homolog Deipe_1016, the *D. peraridilitoris* genome encodes Prx-like protein Deipe_4202, which has less than 30% identity with the deinococcal AhpE homologs. Unlike other AhpE proteins, Deipe_4202 has a CxxC motif and lacks the PxxxT/SxxC motif. In *M. tuberculosis*, which does not possess GSH but another LMW thiol compound referred to as mycolthiol (MSH), the reduction of AhpE is ensured by a MSH- and/or mycoredoxin-dependent mechanism [145]. This raises the question of the reducing system for AhpE in *Deinococcus*, in which the bacillithiol system is present. In other respects, all *Deinococcus* species harbour one *ahpD*-like gene (DR_1765 in *D. radiodurans*), two and five being present in *D. deserti* and *D. peraridilitoris*, respectively.
Four of the *ahpD* genes of *D. peraridilitoris* are located on a plasmid, three being adjacent or close to extra *trxR* and *trx* genes in this species (Figure S3). However, these proteins show substantial variation in their sequences. DR_1765 shares 72–76% identity with Deide_13030, Dgeo_1446, and DGo_CA1027; 54% with Deima_0298; 36% with Deipe_3296; but only 17–25% with the other deinococcal AhpD-like proteins (including the single AhpD-like from *D. proteolyticus*) and 12% with AhpD from *M. tuberculosis* (Figure S12b).

Biochemical and structural investigations of the *D. radiodurans* AhpD-like protein were performed by Zhao et al. [146], who reported that a mutant strain lacking the reductase exhibits increased sensitivity to H$_2$O$_2$. Of note, complementation assays using wild-type AhpD revealed partial restoration of this phenotype, and this level of restoration was much lower when using AhpD variants mutated for the two Cys of the conserved CxxC motif [146]. In other respects, determination of a crystal structure revealed that it adopts a fold representative of the AhpD superfamily [146]. Taken collectively, these data strongly support a role of AhpD and AhpE enzymes in *Deinococcus* responses to oxidative stress via peroxidase activity for both or possibly through electron supply from AhpD to AhpE.

### 4.2. Ohr/OsmC/YhfA Thiol-Dependent Peroxidases

Another class of bacterial thiol peroxidases named Ohr (organic hydroperoxide resistance) has been identified by complementation with *Xanthomonas campestris* genes of *E. coli* strains deficient in the AhpF–AhpC system and susceptible to organic hydroperoxides [147]. This 14-kDa protein shares some sequence homology (around 20% identity) with other stress-induced proteins termed OsmC (Osmotically induced protein C) first identified in *E. coli* [148]. Ohr and OsmC are structurally related enzymes [149]. Atichartpongkul et al. [150] reported that these two subfamilies are widely distributed in Gram-negative and Gram-positive bacterial species. Later, members of the Ohr/OsmC family have been identified in several eukaryotic clades, especially in fungi but also in mosses, such as *Physcomitrella patens* [151].

Ohr/OsmC proteins display specific expression patterns depending on stress nature and on peroxide type. In *P. aeruginosa*, ohr expression is induced only by organic peroxides, and not by other oxidative or stress treatments, while osmC expression is specifically triggered by salt treatment [150]. The phenotype of strains lacking these genes and the presence of two conserved Cys (one positioned in a typical VCPY motif in Ohr) in both protein types indicated that they could fulfil a thiol peroxidase function [150]. This role in metabolising hydrogen and organic peroxides was demonstrated in vitro using recombinant Ohr or OsmC forms and in vivo by generating deletion strains in several bacterial species [152–155]. Moreover, Ohr was shown to participate in response to peroxynitrite in *P. aeruginosa* [152]. Site-directed mutagenesis revealed that the two Cys are critical for the catalytic activity [154], and provided clear evidence that Ohr functions thanks to reactive thiol groups. Of note, these enzymes do not share structural relationship with Prxs, such as BCP, AhpC, or AhpE, as shown by the determination of crystal structures of *P. aeruginosa* Ohr and *E. coli* OsmC [149,154] (Figure S13). Following peroxide reduction, these enzymes form an intramolecular disulfide bridge that Trx- and GSH-based systems are unable to reduce, but require lipoylated proteins as an electron source (Figure 3) [156]. Indeed, *Xylella fastidiosa* Ohr was found to interact with lipoylated proteins (dihydrolipoyl dehydrogenase LpdA, pyruvate dehydrogenase beta PDHB, and dihydrolipoamide succinyltransferase SucB), and its peroxidase activity was supported by PDHB and SucB in the presence of lipoamide or by LpdA alone [156]. Similarly, OsmC is reduced by lipoyl-dependent systems [156]. In *C. glutamicum*, a bacterial species highly resistant to organic hydroperoxides, OsmC activity was preferentially regenerated through a pathway involving the AhpD reductase, another lipoyl-dependent system [157].

Another family of proteins structurally related to Ohr/OsmC has been described by Shin et al. [149]. It is present in *E. coli* (YhfA) but also in other bacterial species, such as *Vibrio cholerae*. YhfA proteins possess the two cysteines that are well conserved in Ohr and OsmC peroxidases and likely to participate in the catalytic mechanism, but they lack...
two residues, one Arg and one Glu, neighbouring the peroxidatic cysteine [149]. The biochemical properties and physiological roles of this class of putative thiol peroxidases remain to be investigated.

Among the Deinococcus species analysed in this review, most possess genes encoding these three types of atypical thiol peroxidases (Ohr, OsmC, and YhfA corresponding to DR_1857, DR_1538, and DR_1177 in D. radiodurans), except D. deserti and D. proteolyticus, which do not have ohr and osmC genes, respectively (Table 2). Note also that the genomes of several species contain two ohr or yhfA genes. Deinococcus OsmC proteins share 54–83% identity, and around 42% with the E. coli homolog (Figure S14a). Concerning Ohr, D. radiodurans shares 55–78% identity with the closest homologs in the five other Deinococcus species, around 40% with the extra Ohr present in three of these species, and around 47% with the two Ohr proteins from B. subtilis (Figure S14b). YfhA homologs are present in the seven Deinococcus species (Figure S14c). They are divided into two subgroups, one containing highly conserved proteins in the seven species sharing 70–92% identity and around 35% with E. coli YhfA, and the second containing the two extra proteins from D. deserti and D. peraridilitoris having 71% identity between them but low-sequence identity with the other YhfA proteins (e.g., 21% between the two from D. deserti).

The knowledge about these atypical thiol peroxidases in Deinococcus remains limited. Atichartpongkul et al. [150] reported that in D. radiodurans, osmC (DR_1538) is induced by ethanol treatment and not by salt, while ohr (DR_1857) expression is enhanced by organic hydroperoxides. Cadmium exposure enhanced the expression of osmC, ohr, and yhfA in D. radiodurans [52]. Radiation-induced expression of D. deserti osmC [31] and D. radiodurans Ohr [158] has also been observed. The crystal structure of D. radiodurans Ohr has been solved [159] (Figure S13). Comparison with the structure of P. aeruginosa Ohr showed that Ohr proteins adopt two different conformations depending on the enzyme redox state. It was postulated that one configuration is consistent with efficient catalysis of the reduction of organic hydroperoxides, whereas the other form is required for enzyme recycling. Compared with wild-type D. radiodurans, a mutant strain deleted for osmC is more sensitive to gamma irradiation, particularly at high doses (10 kGy) [160], indicating the participation of OsmC peroxidase in the radiotolerance of Deinococcus.

5. The Bacillithiol System

Low-molecular-weight thiols are crucial for maintaining an intracellular reducing environment and for protection against ROS and other reactive species. Glutathione is the best-studied LMW thiol present, for example, in E. coli and eukaryotes. Deinococcus bacteria do not produce GSH but BSH, which is the most widespread LMW thiol in bacteria [161]. BSH is also involved in metal homeostasis [18]. The structure of BSH, the α-anomeric glycoside of L-cysteinyl-D-glucosamine with L-malic acid (Figure 4a), was determined after purification from D. radiodurans [162]. However, the BSH system has been studied most extensively in B. subtilis and S. aureus [18]. This system includes NADPH–BSH disulfide reductase (Bdr, previously called YpdA) and three bacilliredoxin (Brx) proteins (Figure 4b) [163–165]. Under oxidising conditions, BSH can form a disulfide (BSSB) and also mixed disulfides with protein thiols, a modification referred to as S-bacillithiolation. Like S-glutathionylation and S-mycothiolation with GSH and MSH, S-bacillithiolation protects protein thiols from overoxidation, and may also function in redox regulation [19]. Brx proteins catalyse protein debacillithiolation, resulting in the formation of Brx-SSB. Oxidised Brx is likely preferentially reactivated by BSH or possibly by another Brx (see below) [18,165]. The regeneration of BSH from BSSB is catalysed by the NADPH-dependent Bdr enzyme [163,166] (Figure 4b). Recently, B. subtilis Bdr was shown to additionally have Brx reductase activity [165].
with the bacterial self-protective colicin-like immunity domain (pfam09204). The function and TCHKT. Moreover, parts in Bacillus possess a redox-active CGC motif, whereas BrxC has only one Cys in a TCPIS motif. Debacillium [52]. A Bdr homolog is also present in all Deinococcus of this domain in located in the N-terminal part of the protein. The C-terminal region has some similarity with BrxC from Bacillus instead of 108 residues for the TCPIS motif found in the latter, the corresponding sequence in deinococcal proteins is low levels of similarity with BrxC from Bacillus DR_1832 has 71% to 82% identity with BrxC from the other analysed species), but displays the same may be true for the Brx reductase activity [165]. In summary, the precise roles of S. aureus Of note, analysis of the crystal structures of Bdr from Bacillus cereus dependent oxidoreductase. While Bacillus cereus related oxidoreductase. While Bacillus cereus motif. Debacillithiolation of Gap, MetE, and OhrR by BrxA and/or BrxB has been demonstrated [168,169]. The three Brx proteins identified in Bacillus subtilis and S. aureus are named BrxA (previously YphP), BrxB (YqiW), and BrxC (YtxJ). BrxA and BrxB are paralogs and possess a redox-active CGC motif, whereas BrxC has only one Cys in a TCPIS motif. Debacillithiolation of Gap, MetE, and OhrR by BrxA and/or BrxB has been demonstrated [165]. BSSB reductase activity was also demonstrated for Bacillus Bdr variant, in which Cys14 was replaced by Ala, suggested that Cys14 is at position 14 shared with Bacillus cereus Bdr (Figure S15). The characterisation of a S. aureus Bdr variant, in which Cys14 was replaced by Ala, suggested that Cys14 is the redox-active Cys as it seems required for BSSB reductase activity [163]. BSSB reductase activity was also demonstrated for Bacillus cereus Bdr [164]. For Bacillus cereus Bdr, which could debacillithiolate BrxB-SSB and less efficiently BrxC-SSB, mutation of any of the three Cys residues led to decreased capacity to reduce both mixed disulfides [165]. A variant mutated for all Cys was essentially inactive. Of note, analysis of the crystal structures of Bdr from S. aureus and Bacillus cereus indicated that Cys14 does not directly participate in the BSSB reductase reaction mechanism [164], and the same may be true for the Brx reductase activity [165]. In summary, the precise roles of these Cys in the BSSB or Brx reductase activities of Bdr remain to be delineated.

BSH is expected to be common to all members of the genus Deinococcus because the BSH biosynthesis genes bshA, bshB, and bshC are present in all species [3,27]. However, the other components of the BSH system, in particular Brx, appear to differ from their counterparts in Bacillus and Staphylococcus. Only a BrxC-related protein is present in Deinococcus species. This protein, containing a single Cys, is highly conserved (e.g., D. radiodurans DR_1832 has 71% to 82% identity with BrxC from the other analysed species), but displays low levels of similarity with BrxC from Bacillus and Staphylococcus (Figure S16). Instead of the TCPIS motif found in the latter, the corresponding sequence in deinococcal proteins is TCHKT. Moreover, Deinococcus BrxC proteins are substantially longer (204 to 219 residues instead of 108 residues for Bacillus BrxC). The Trx-like domain in Deinococcus BrxC is located in the N-terminal part of the protein. The C-terminal region has some similarity with the bacterial self-protective colicin-like immunity domain (pfam09204). The function of this domain in Deinococcus BrxC is unknown. Because of the differences with BrxC from Bacillus and Staphylococcus, DR_1832 has been named AbxC (atypical BrxC) [27]. In D. radiodurans, expression of the abxC gene (DR_1832) is induced following exposure to cadmium [52]. A Bdr homolog is also present in all Deinococcus. D. radiodurans Bdr (DR_2623)

Figure 4. The bacillithiol system. (a) Structure of bacillithiol (BSH), glycoside formed of L-cysteinyl-D-glucosamine and malic acid. (b) Electron transfer pathways in the BSH system. Bdr, NADPH–bacillithiol disulfide reductase. Brx, bacilliredoxin; S-Ox, thiol oxidised (bacillithiolated) form; S-Red, thiol reduced form.
has 74% to 89% identity with the homologs of the other six species and 40% with B. subtilis Bdr. DR_2623 contains three Cys residues (Cys22, Cys36, Cys275) that are conserved in deinococcal Bdr proteins, except for Deipe_2475, which lacks the first Cys corresponding to Cys22 of DR_2623 (Figure S15). Whether and how the Cys residues, located at different positions compared with those in Bdrs from Bacillus and Staphylococcus, support the catalytic mechanism of deinococcal Bdr is currently unknown. Upregulated expression of DR_2623 (Bdr) has been observed after exposure of D. radiodurans to cadmium [52], gamma radiation [158,170], and desiccation [171].

Experimental data obtained with D. radiodurans indicated that AbxC (BrxC) contributes to oxidative stress resistance [27]. In vitro, incubation of AbxC with H$_2$O$_2$ resulted in the formation of AbxC dimers through an intermolecular disulfide bond. NADPH consumption was observed when oxidised AbxC was incubated with BSH, NADPH, and Bdr (DR_2623), indicating reduction of the AbxC dimer by BSH and reduction of the formed BSSB by Bdr [27]. S-bacillithiolated proteins in Deinococcus have not been identified yet, and it is currently unknown whether the atypical AbxC can debacillithiolate proteins.

6. Conclusions

The remarkable tolerance of Deinococcus bacteria to extreme environmental conditions relies on a wide array of mechanisms ensuring efficient repair of DNA and limiting damage in proteins, many being present in all species. However, some genes, such as those coding for specific catalase types that confer resistance to oxidative stress, are not present in all species of the genus, revealing diversity in these mechanisms [3]. Regarding thioreductases, most representatives are found in all Deinococcus species analysed here (Table 2), except Trx2, indicating that they are likely essential actors in the stress responses of this genus, as it is now well established in many other organisms. Consistently, deletion mutants for trx, fnxE, bcp, osmC, ahpD-like, and abxC genes show increased susceptibility to oxidative treatments [25,27,60,77,146,160]. Nonetheless, the knowledge about the functions of Deinococcus thioreductases remains limited. Thorough investigations need to be performed to delineate the precise physiological roles of these enzymes, notably by characterising mutants impaired in their expression in conditions of extreme stress, and by identifying their partners and substrates.

In addition to the NADPH-dependent Trx-and BSH-systems, Deinococcus possesses various types of thiol peroxidases using NADH as an electron source. Of note, Yamashiro et al. [172] reported a much greater concentration of NADH compared with that of NADPH in this genus, and a much higher NADH/NADPH ratio compared with E. coli, indicating that NADH is likely an essential actor in metabolism and stress responses in Deinococcus. Most interestingly, this genus displays a large diversity in thiol peroxidase types. On one hand, Trx-dependent BCPs are well represented in all Deinococcus species, since each possesses two or three genes coding for these Prxs, instead of one in E. coli. In addition, all Deinococcus species have two distinct types of NADH-dependent thiol peroxidases (i.e., AhpE/AhpD and Ohr/OsmC). The gene copy number and diversity of thiol peroxidases in bacteria, notably regarding the specialisation of their active sites, are likely associated with their level of stress tolerance [118,157]. Thus, AhpC, Ohr, and OsmC are widely distributed among bacterial species, but only some species simultaneously possess all of them, the others having generally one or two genes. For instance, Ohr is absent in M. tuberculosis and E. coli. The mycobacterium C. glutamicum, which is highly resistant to oxidative stress, possesses AhpD, Ohr, and OsmC homologs [143,157]. Regarding BCPs, Limauro et al. [173] identified three genes in the hyperthermophilic archaeon Sulfolobus solfataricus and proposed that this copy number was associated with the tolerance of this archaea to extreme temperature. The Deinococcus genus appears thus to be remarkably well equipped with a diversity of thiol peroxidases, since most species possess one or several bcp, ahpD, ahpE, osmC, ohr, and yfjA genes. This palette is very likely an asset in the extreme resistance of Deinococcus via efficient scavenging of all types of peroxides and peroxynitrites. Indeed, these thiol peroxidases display in vitro different specificity towards
substrates [144,154,155,174]. For instance, OsmC is more efficient in metabolising organic peroxides than H$_2$O$_2$ [153]. In vivo investigations in P. aeruginosa showed increased susceptibility to artificial and fatty acid hydroperoxides of strains lacking Ohr, this phenotype being not restored by the expression of other types of thiol peroxidases, such as AhpC [152]. Taken collectively, these data strongly suggest that the diversity of thiol peroxidases in Deinococcus is associated with specialisation and efficiency regarding the scavenging of the various peroxide types produced upon stress conditions, and thus contributes to the outstanding tolerance of this genus.

The various electron transfer pathways involving thiol reductases present in Deinococcus are shown in Figure 5. Some pathways need to be precisely delineated, notably regarding the substrates of Trx and Brx proteins, which remain to be identified using biochemical and proteomics approaches. Based on the knowledge gained in other organisms, connections and interplays between these pathways very likely occur in Deinococcus. Indeed, in E. coli, there is functional redundancy between Trx and GSH systems [175], and an interplay between the two systems has been unveiled in plants during development [176]. Consistently, by generating B. subtilis strains impaired in BSH biosynthesis, Gaballa et al. [177] showed that this LMW thiol was not required to maintain the reduced status of protein thiols in the absence of stress, but noticed altered stress sensitivity of these strains. Regarding Deinococcus, it was recently reported that D. deserti and D. radiodurans mutant strains deficient in BSH biosynthesis are viable [27,59], indicating that in the absence of stress conditions the BSH system is not essential and likely compensated by the Trx system. Increased sensitivity to H$_2$O$_2$ has been reported for the D. radiodurans BSH-deficient mutant [27]. The occurrence of such compensation mechanisms and interplays is corroborated by the presence of Trx substrates or of other thiol reductases in bacillithiolated or Brx-interacting proteins. Among the bacillithiolated proteins identified in Bacillus and Staphylococcus, glyceraldehyde-3-phosphate dehydrogenase, a well-known Trx substrate [18,54,165,168], and a predicted 2-Cys Prx (YkuU) [167] have been found. The BSH system in Deinococcus is likely to allow the maintenance of the redox status and/or the regeneration of the activity of Trx-dependent enzymes, as shown for GSH in other bacteria [13]. In Deinococcus, Bihani et al. [78] proposed that in conditions of cadmium stress, which inhibits TrxR, Trx substrates could be supplied with electrons thanks to BSH. In D. radiodurans, 25 proteins potentially interacting with AbxC were isolated after incubation of cell extracts with AbxC-immobilised beads [27]. Among them, Ohr was identified, revealing interplays between the BSH/Brx system and NADH-dependent thiol peroxidases. BSH might also participate in the reduction of AhpE by analogy with the role of mycothiol. Indeed, in M. tuberculosis, reduction of AhpE can be performed via mycoredoxin- or mycothiol-dependent mechanisms [145]. Concerning lipoyl-dependent thiol peroxidases, a link has been unveiled in C. glutamicum between the AhpD system and Ohr/OsmC peroxidases, which in this species can be reduced by AhpD [157]. Taking into consideration all these data, we can thus hypothesise that the various pathways for electron transfer via thiol reductases and peroxidases in Deinococcus give rise to multiple connections and possible redundancy among them. This very likely allows the establishment of protective and repair mechanisms that can substitute or be switched on differentially depending on stress conditions, leading to the implementation of a complex and robust network (Figure 5) maintaining protein redox homeostasis in the extreme conditions that this bacterial genus can tolerate.
Figure 5. Relationships and possible interplays between *Deinococcus* thiol reductase systems. Dashed arrows indicate possible transfer of electrons between Trx-, BSH-, and lipoylated-dependent systems. TrxR, thioredoxin reductase; Trx, thioredoxin; Bdr, NADPH–BSH disulfide reductase; BSH, bacillithiol; Brx, bacilliredoxin; Ahp, alkyl hydroperoxide reductase; Ohr, organic hydroperoxide resistance enzyme; OsmC, osmotically induced bacterial protein C.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox11030561/s1: Figure S1: Multiple sequence alignment of *Deinococcus*, *E. coli*, and *B. subtilis* NADPH thioredoxin reductases. Abbreviations of *Deinococcus* species names (i.e., the first part of the locus tags (gene numbers)) are as specified in Table 1. TRXB_ECOLI and TRXB_BACSU, thioredoxin reductases from *E. coli* and *B. subtilis*, respectively. The alignment was made with ClustalW at NPS@ [178] and ESPript [179]. Figure S2: Multiple sequence alignment of *Deinococcus*, *E. coli*, and *B. subtilis* thioredoxins. (a) Alignment of Trx1 sequences. (b) Alignment of Trx2 sequences. Abbreviations of *Deinococcus* species names are as specified in Table 1. THIO_ECOLI and THIO_BACSU, thioredoxin 1 from *E. coli* and *B. subtilis*, respectively. THIO2_ECOLI, thioredoxin 2 from *E. coli*. Alignment was made as in Figure S1. Figure S3: Gene clusters with additional *trxR*, *trxA*, and *ahpD* genes in *D. peraridilitoris*. The two clusters shown are both located on plasmid pDEIPE01. Locus tags (Deipe_3903 etc.) are indicated. Gene sizes not drawn to scale. Figure S4: Three-dimensional models of Trx and Trx-like proteins of *D. radiodurans*. Models in panels (a) (Trx1) and (c) to (g) (Trx-like) were obtained with AlphaFold2_Advanced [180]. (b) Crystal structure of Trx2 (PDB 7DL6) showing the extra N-terminal zinc binding domain in dark blue [60]. Catalytic and resolving cysteine residues are shown in green and blue, respectively. Three-dimensional structure or model images were generated using PyMOL (PyMOL Molecular Graphics System, Version 2.0, Schrödinger, LLC). Figure S5: Sequence alignments of Trx-like proteins from *Deinococcus*. (a) Alignment of *Deinococcus* DR_0057-type sequences. (b) Alignment of *Deinococcus* DR_A0072-type sequences. (c) Alignment of *Deinococcus* DR_B0010-type sequences. (d) Alignment of *Deinococcus* DR_0948-type sequences. (e) Tree showing sequence relationship between Trx-like proteins shown in Figure 2 and in panels (a–d). (f) Guide tree is calculated based on the pairwise distances. Trx active site sequences are highlighted in yellow; residues highlighted in cyan in Deipr sequences differ in the proximity of the active site. Abbreviations of *Deinococcus* species names are as specified in Table 1. Alignments and tree were obtained with UniProt ClustaO (https://www.uniprot.org/align/) [181]. Figure S6: Multiple sequence alignment of *Deinococcus* FnrE reductases. (a) FnrE proteins (DsbA family, FnrE subfamily). The N-terminal CxxC corresponds to residues 22–25 in *D. radiodurans* FnrE (DR_0699), and the C-terminal CxxxC to residues 239–244. (b) FnrE-like proteins found in two *Deinococcus* species (DsbA family, FnrE-like subfamily). The CxxC motif is highlighted in yellow. (c) Proteins containing a DSBA-like Trx domain. The single Cys is highlighted in yellow. Alignments were made as in Figures S1 and S5. Figure S7: Putative operons encoding DsbA, DsbB, and UvrA. Similar to the *B. subtilis bbdDC* operon encoding DsbA and DsbB homologs, putative operons encoding DsbA and DsbB family proteins are present in most *Deinococcus* species. In five of the seven analysed species, these genes are likely in operon with genes encoding DNA repair protein UvrA and a membrane protein designated dCSP-1 (*Deinococcus*-specific conserved
signature protein-1). In *D. radiodurans* (DR), the *uwrA-dCSP-1* genes are located separate from the *bdbDC* genes. *D. peraridilitoris* (Deipe) lacks the *bdbDC* homologs. Gene numbers (locus tags) for *D. radiodurans*, *D. deserti*, and *D. peraridilitoris* are indicated. Figure S8: Multiple sequence alignments of *Deinococcus* Dsb oxidoreductases. (a) DsbA family proteins with an N-terminal transmembrane helix from *Deinococcus* species aligned with *B. subtilis* BbdD. Except for the second homolog of *D. proteolyticus* (Deipr_2421), the genes are adjacent to a gene encoding the DsbB/BbdC homologs shown in panel (b) (see also Figure S7). Residues highlighted in cyan in Deipr_2421 differ in the proximity of the active site. (c) DsbA family proteins with N-terminal signal peptide. (d) Homologs of the DsbD family protein CcdA from *Deinococcus* aligned with *B. subtilis* CcdA. The deinococcal *ccdA* homologs are in gene clusters also encoding DsbE/CcmG homologs (see Figure S9). (e) DsbE/CcmG homologs from *Deinococcus* encoded by gene clusters also encoding CcdA and by isolated genes aligned with the TlpA-like family proteins ResA and YneN from *B. subtilis* and DsbE/CcmG from *E. coli*. Cys residues and CxxC motifs are highlighted in pink. Guide trees showing sequence relationship between various proteins are included in case more than one homolog is present in the same *Deinococcus* species. Alignments and trees were obtained with UniProt ClustalO. Figure S9: Gene clusters encoding CcdA and DsbE/CcmG homologs. (a) The seven analysed *Deinococcus* species possess *ccdA* (DsbD family) and *dsbE/ccmG* homologs in gene clusters containing other cytochrome c biogenesis genes. Different genetic organisations are present in the different species, as shown. The *ccm* cluster from *E. coli* is also shown for comparison. Gene numbers (locus tags) for the indicated *Deinococcus* species are indicated. (b) Additional two-gene cluster encoding CcdA and DsbE/CcmG found in two *Deinococcus* species. Figure S10: Multiple sequence alignment of *Deinococcus* Msr proteins. (a) Alignment of *Deinococcus, E. coli*, and *B. subtilis* MsrA proteins. MSRA_ECOLI and MSRA_BACSU, MsrAs from *E. coli* and *B. subtilis*, respectively. (b) Alignment of *Deinococcus, E. coli*, and *B. subtilis* MsrB sequences. MSRB_ECOLI and MSRB_BACSU, MsrBs from *E. coli* and *B. subtilis*, respectively. Catalytic and resolving cysteines are highlighted in green and cyan, respectively. Extra cysteines present in some sequences are highlighted in yellow. The black lines indicate the consensus sequences proximal to catalytic cysteines and prevalent in MsrA and MsrB. Figure S11. Multiple sequence alignments of BCP peroxiredoxins from *Deinococcus*. (a) Alignment of *Deinococcus* DR_0846-type, *B. subtilis*, and *E. coli* BCP sequences. (b) Alignment of other *Deinococcus* BCP sequences. (c) Tree showing sequence relationship between BCP proteins shown in panels (a,b) and two more distant proteins from *D. gobiensis* and *D. deserti*. BCP, bacterioferritin comigratory protein. BCP_ECOLI and BCP_BACSU, BCP peroxiredoxins from *E. coli* and *B. subtilis*, respectively. Peroxidatic and resolving cysteines are highlighted in green and cyan, respectively. The black line indicates the sequence proximal to peroxidatic cysteine prevalent in BCPs, PKxxTPGCTxEAC [118]. The two other residues of the catalytic triad are highlighted in grey. Residues highlighted in yellow are highly conserved in bacterial BCPs [128]. Extra cysteines present in some sequences are highlighted in pink. Alignments and tree were obtained with UniProt ClustalO as in Figure S5. Figure S12: Multiple sequence alignment of *Deinococcus* AhpE and AhpD proteins. (a) Alignment of *Deinococcus* and *M. tuberculosis* AhpE sequences. AHPE_MYCTU, AhpE from *M. tuberculosis*. Peroxidatic cysteine (green) and other catalytic triad residues (grey) are highlighted. (b) Alignment of *Deinococcus* and *M. tuberculosis* AhpD sequences. AHPD_MYCTU, AhpD from *M. tuberculosis*. Catalytic and resolving cysteines are highlighted in green and cyan, respectively. The residues highlighted in grey in AhpD sequences are involved in the catalytic mechanism. The guide tree showing sequence relationship between the various AhpD-like proteins is included. Alignments and tree were obtained with UniProt ClustalO. Figure S13: Three-dimensional models of BCPs and OsmC of *D. radiodurans*. (a) The three BCPs having a Trx-fold. (b) Model of OsmC and crystal structure of Ohr (PDB 1USP). Peroxidatic and resolving cysteine residues are shown in green and blue, respectively. The OsmC/Ohr monomer is composed of two distinct subdomains. The N-terminal domain (orange) consists of three β-strands folded into a β-sheet. In the C-terminal domain, helices α1 and α2 (the latter severely kinked) are stacked on a β-sheet formed by strands β4−β6. The active OsmC and Ohr protein is formed by a homodimer [159]. Models and images were obtained as described in Figure S4. Figure S14: Multiple sequence alignment of *Deinococcus* OsmC, Ohr, and YhfA proteins. (a) Alignment of *Deinococcus* and *E. coli* OsmC sequences. OSMC_ECOLI, OsmC from *E. coli*. (b) Alignment of *Deinococcus* and *B. subtilis* Ohr. OHRA_BACSU and OHRB_BACSU, OhrA and OhrB proteins, respectively, from *B. subtilis*. (c) Alignment of *Deinococcus* and *E. coli* YhfA sequences. YHFA_ECOLI, YhfA from *E. coli*. Peroxidatic and resolving cysteines are highlighted in green and cyan, respectively. The two residues highlighted in grey in Ohr and OsmC sequences are involved in the catalytic mechanism [154].
Figure S15: Multiple sequence alignment of *Deinococcus* bacillithiol reductases. Bdr homologs from *Deinococcus* are aligned with Bdr proteins YPDA_BACSU from *B. subtilis*, BC_1495 from *B. cereus*, and SACOL1520 from *S. aureus*. Cysteine residues are highlighted in yellow. Figure S16: Multiple sequence alignment of the *Deinococcus* bacilliredoxins. The BrxC-type bacilliredoxins (also called AbxC for atypical BrxC) from *Deinococcus* are aligned with BrxC proteins YtxJ_BACCE from *B. cereus*, YTXJ_BACSU from *B. subtilis*, and SACOL0804 from *S. aureus*. Active site cysteine is highlighted in yellow.

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**References**

1. Battista, J.R. Against All Odds: The Survival Strategies of *Deinococcus radiodurans*. *Annu. Rev. MicroBiol.* 1997, 51, 203–224. [CrossRef] [PubMed]
2. Slade, D.; Radman, M. Oxidative Stress Resistance in *Deinococcus radiodurans*. *MicroBiol. Mol. Biol. Rev.* 2011, 75, 133–191. [CrossRef] [PubMed]
3. Lim, S.; Jung, J.-H.; Blanchard, L.; de Groot, A. Conservation and Diversity of Radiation and Oxidative Stress Resistance Mechanisms in *Deinococcus* Species. *FEMS MicroBiol. Rev.* 2019, 43, 19–52. [CrossRef] [PubMed]
4. Mattimore, V.; Battista, J.R. Radioresistance of *Deinococcus radiodurans*: Functions Necessary to Survive Ionizing Radiation Are Also Necessary to Survive Prolonged Desiccation. *J. Bacteriol.* 1996, 178, 633–637. [CrossRef] [PubMed]
5. de Groot, A.; Dulerro, R.; Orlet, P.; Blanchard, L.; Guerin, P.; Fernandez, B.; Vacherie, B.; Dossat, C.; Jolivet, E.; Siguer, P.; et al. Alliance of Proteomics and Genomics to Unravel the Specificities of Sahara Bacterium *Deinococcus deserti*. *PLoS Genet.* 2009, 5, e1000434. [CrossRef] [PubMed]
6. Daly, M.J.; Gaidamakova, E.K.; Matrosova, V.Y.; Vasilenko, A.; Zhai, M.; Leapman, R.D.; Lai, B.; Ravel, B.; Li, S.M.; Kemner, K.M.; et al. Protein Oxidation Implicated as the Primary Determinant of Bacterial Radioresistance. *PLoS Biol.* 2007, 5, e92. [CrossRef] [PubMed]
7. Fredrickson, J.K.; Li, S.M.; Gaidamakova, E.K.; Matrosova, V.Y.; Zhai, M.; Sulloway, H.M.; Scholten, J.C.; Brown, M.G.; Balkwill, D.L.; Daly, M.J. Protein Oxidation: Key to Bacterial Desiccation Resistance? *ISME J.* 2008, 2, 393–403. [CrossRef] [PubMed]
8. Krisco, A.; Radman, M. Protein Damage and Death by Radiation in *Escherichia coli* and *Deinococcus radiodurans*. *Proc. Natl. Acad. Sci. USA* 2010, 107, 14373–14377. [CrossRef]
9. Daly, M.J. Death by Protein Damage in Irradiated Cells. *DNA Repair* 2012, 11, 12–21. [CrossRef]
10. Lu, J.; Holmgren, A. The Thioredoxin Antioxidant System. *Free Radic. Biol. Med.* 2014, 66, 75–87. [CrossRef]
11. Arnér, E.S.; Holmgren, A. Physiological Functions of Thioredoxin and Thioredoxin Reductase. *Eur. J. Biochem.* 2000, 267, 6102–6109. [CrossRef] [PubMed]
12. Carmel-Harel, O.; Storz, G. Roles of the Glutathione- and Thioredoxin-Dependent Reduction Systems in the *Escherichia coli* and *Saccharomyces cerevisiae* Responses to Oxidative Stress. *Annu. Rev. MicroBiol.* 2000, 54, 439–461. [CrossRef] [PubMed]
13. Toledano, M.B.; Kumar, C.; Le Moan, N.; Spector, D.; Tacnet, F. The System Biology of Thiol Redox. System in *Escherichia coli* and Yeast: Differential Functions in Oxidative Stress, Iron Metabolism and DNA Synthesis. *FEBS Lett.* 2007, 581, 3598–3607. [CrossRef] [PubMed]
14. Ezraty, B.; Gennaris, A.; Barras, F.; Collet, J.F. Oxidative Stress, Protein Damage and Repair in Bacteria. *Nat. Rev. MicroBiol.* 2017, 15, 385–396. [CrossRef] [PubMed]
15. Meyer, Y.; Buchanan, B.B.; Vignols, F.; Reichheld, J.P. Thioredoxins and Glutaredoxins: Unifying Elements in Redox. Biology. *Annu. Rev. Genet.* 2009, 43, 353–367. [CrossRef] [PubMed]
16. Vieira Dos Santos, C.; Rey, P. Plant Thioredoxins Are Key Actors in the Oxidative Stress Response. *Trends Plant. Sci.* 2006, 11, 329–334. [CrossRef]
17. Staerck, C.; Gastebois, A.; Vandeputte, P.; Calenda, A.; Larcher, G.; Gillmann, L.; Papon, N.; Bouchara, J.-P.; Fleury, M.J.J. Microbial Antioxidant Defense Enzymes. *Microb. Pathog.* 2017, 110, 56–65. [CrossRef]

18. Chandrangsu, P.; Loi, V.V.; Antelmann, H.; Helmann, J.D. The Role of Bacillithiol in Gram-Positive Firmicutes. *Antioxid. Redox Signal.* 2018, 28, 445–462. [CrossRef]

19. Imber, M.; Pietrzyk-Brzezinska, A.J.; Antelmann, H. Redox. Regulation by Reversible Protein S-Thiolation in Gram-Positive Bacteria. *Redox. Biol.* 2019, 20, 130–145. [CrossRef]

20. Reyes, A.M.; Pedre, B.; De Armas, M.I.; Tossounian, M.-A.; Radi, R.; Messens, J.; Trujillo, M. Chemistry and Redox. Biology of Mycosthio. *Antioxid. Redox. Signal.* 2018, 28, 487–504. [CrossRef]

21. Choo, K.; Kim, M.; Nansa, S.A.; Bae, M.K.; Lee, C.; Lee, S.-J. Redox. Potential Change by the Cystine Importer Affected on Enzymatic Antioxidant Protection in *Deinococcus geothermalis*. *Antonie Van Leeuwenhoek* 2020, 113, 779–790. [CrossRef] [PubMed]

22. Obiero, J.; Pittet, V.; Bonderoff, S.A.; Sanders, D.A. Thioredoxin System from *Deinococcus radiodurans*. *J. Bacteriol.* 2010, 192, 494–501. [CrossRef] [PubMed]

23. Obiero, J.; Sanders, D.A.R. Design of *Deinococcus radiodurans* Thioredoxin Reductase with Altered Thioredoxin Specificity Using Computational Alanine Mutagenesis. *Protein Sci.* 2011, 20, 1021–1029. [CrossRef] [PubMed]

24. Chen, Z.; Tang, Y.; Hua, Y.; Zhao, Y. Structural Features and Functional Implications of Proteins Enabling the Robustness of *Deinococcus radiodurans*. *Comput. Struct. Biotechnol. J.* 2020, 18, 2810–2817. [CrossRef]

25. Cho, C.; Lee, G.W.; Hong, S.H.; Kaur, S.; Jung, K.-W.; Jung, J.-H.; Lim, S.; Chung, B.Y.; Lee, S.S. Novel Functions of Peroxiredoxin Q from *Deinococcus radiodurans* R1 as a Peroxidase and a Molecular Chaperone. *FEBS Lett.* 2019, 593, 219–229. [CrossRef]

26. Maqbool, I.; Ponniresan, V.K.; Govindasamy, K.; Prasad, N. Understanding the Survival Mechanisms of *Deinococcus radiodurans* against Oxidative Stress by Targeting Thioredoxin Reductase Redox. System. *Arch. Microbiol.* 2020, 202, 2355–2366. [CrossRef]

27. Jeong, S.; Jung, J.-H.; Kim, M.-K.; de Groot, A.; Blanchard, L.; Ryu, S.; Bahn, Y.-S.; Lim, S. Atypical Bacillithiol AbxC Plays a Role in Responding to Oxidative Stress in Radiation-Resistant Bacterium *Deinococcus radiodurans*. *Antioxidants* 2021, 10, 1148. [CrossRef] [PubMed]

28. Vallenet, D.; Calteau, A.; Dubois, M.; Amours, P.; Bazin, A.; Beuvin, M.; Busslet, X.; Fouteau, S.; Gautreau, G.; et al. MicroScope: An Integrated Platform for the Annotation and Exploration of Microbial Gene Functions through Genomic, Pangenomic and Metabolic Comparative Analysis. *Nucleic Acids Res.* 2020, 48, D579–D589. [CrossRef]

29. Lipton, M.S.; Pasa-Tolic, L.; Anderson, G.A.; Anderson, D.J.; Auberry, D.L.; Battista, J.R.; Daly, M.J.; Fredrickson, J.; Hixson, K.K.; Kostandarithes, H.; et al. Global Analysis of the *Deinococcus radiodurans* Proteome by Using Accurate Mass Tags. *Proc. Natl. Acad. Sci. USA* 2002, 99, 11049–11054. [CrossRef]

30. Schmid, A.K.; Lipton, M.S.; Mottaz, H.; Monroe, M.E.; Smith, R.D.; Lidstrom, M.E. Global Whole-Cell FTICR Mass Spectrometric Proteomics Analysis of the Heat Shock Response in the Radioresistant Bacterium *Deinococcus radiodurans*. *J. Proteome Res.* 2005, 4, 709–718. [CrossRef]

31. de Groot, A.; Roche, D.; Fernandez, B.; Ludanyi, M.; Cruveiller, S.; Pignol, D.; Vallenet, D.; Armengaud, J.; Blanchard, L. RNA Sequencing and Proteogenomics Reveal the Importance of Leaderless MRNAs in the Radiation-Tolerant Bacterium *Deinococcus deserti*. *Genome Biol. Evol.* 2014, 6, 932–948. [CrossRef] [PubMed]

32. Ott, E.; Kawaguchi, Y.; Köbl, D.; Chaturvedi, P.; Nakagawa, K.; Yamagishi, A.; Weckwerth, W.; Milojevic, T. Proteometabolic Response of *Deinococcus radiodurans* Exposed to UVC and Vacuum Conditions: Initial Studies Prior to the Tanpopo Space Mission. *PLoS ONE* 2017, 12, e0189381. [CrossRef] [PubMed]

33. Ott, E.; Fuchs, F.M.; Moeller, R.; Hemmersbach, R.; Kawaguchi, Y.; Yamagishi, A.; Weckwerth, W.; Milojevic, T. Molecular Response of *Deinococcus radiodurans* to Simulated Microgravity Explored by Proteometabolic Approach. *Sci. Rep.* 2019, 9, 18462. [CrossRef] [PubMed]

34. Anderson, A.W.; Nordan, H.C.; Cain, R.F.; Parrish, G.; Duggan, D. Studies on a Radio-Resistant Micrococcus. I. Isolation, Morphology, Cultural Characteristics, and Resistance to Gamma Radiation. *Food Technol.* 1956, 10, 575–578.

35. Brooks, B.W.; Murray, R.G.E. Nomenclature for “Micrococcus radiodurans” and Other Radiation-Resistant Cocci: Deinococcaceae Fam. Nov. and *Deinococcus* Gen. Nov., Including Five Species. *Int. J. Syst. Bacteriol.* 1981, 31, 353–360. [CrossRef]

36. White, O.; Eisen, J.A.; Heidelberg, J.F.; Hickey, E.K.; Peterson, J.D.; Dodson, R.J.; Haft, D.H.; Gwinn, M.L.; Nelson, W.C.; Richardson, D.L.; et al. Genome Sequence of the Radioresistant Bacterium *Deinococcus radiodurans* R1. *Science* 1999, 286, 1571–1577. [CrossRef]

37. de Groot, A.; Chapon, V.; Servant, P.; Christen, R.; Fischer-Le Saux, M.; Sommer, S.; Heulin, T. *Deinococcus deserti* Sp. Nov., a Gamma-Radiation-Tolerant Bacterium Isolated from the Sahara Desert. *Int. J. Syst. Evol. Microbiol.* 2005, 55, 2441–2446. [CrossRef]

38. Ferreira, A.C.; Nobre, M.F.; Rainey, F.A.; Silva, M.T.; Wait, R.; Burghardt, J.; Chung, A.P.; da Costa, M.S. *Deinococcus geothermalis* Sp. Nov. and *Deinococcus murrayi* Sp. Nov. Two Extremely Radiation-Resistant and Slightly Thermophilic Species from Hot Springs. *Int. J. Syst. Bacteriol.* 1997, 47, 939–947. [CrossRef]

39. Makarova, K.S.; Omelchenko, M.V.; Gaidamakova, E.K.; Matrosova, V.Y.; Vasilenko, A.; Zhai, M.; Lapidus, A.; Copeland, A.; Kim, E.; Land, M.; et al. *Deinococcus geothermalis*: The Pool of Extreme Radiation Resistance Genes Shrinks. *PLoS ONE* 2007, 2, e955. [CrossRef]

40. Yuan, M.; Zhang, W.; Dai, S.; Wu, J.; Wang, Y.; Tao, T.; Chen, M.; Lin, M. *Deinococcus gobiensis* Sp. Nov., an Extremely Radiation-Resistant Bacterium. *Int. J. Syst. Evol. Microbiol.* 2009, 59, 1513–1517. [CrossRef]
41. Yuan, M.; Chen, M.; Zhang, W.; Lu, W.; Wang, J.; Yang, M.; Zhao, P.; Tang, R.; Li, X.; Hao, Y.; et al. Genome Sequence and Transcriptome Analysis of the Radioreistant Bacterium Deinococcus gobiensis: Insights into the Extreme Environmental Adaptations. *PloS ONE* **2012**, *7*, e34458. [CrossRef] [PubMed]

42. Rainey, F.A.; Ray, K.; Ferreira, M.; Gatz, B.Z.; Nobre, M.F.; Bagaley, D.; Rash, B.A.; Park, M.J.; Earl, A.M.; Shank, N.C.; et al. Extensive Diversity of Ionizing-Radiation-Resistant Bacteria Recovered from Sonoran Desert Soil and Description of Nine New Species of the Genus *Deinococcus* Obtained from a Single Soil Sample. *Appl. Environ. Microbiol.* **2005**, *71*, 5225–5235. [CrossRef] [PubMed]

43. Pukall, R.; Zeytun, A.; Lucas, S.; Lapidus, A.; Hammon, N.; Deshpande, S.; Nolan, M.; Cheng, J.F.; Pitluck, S.; Liolios, K.; et al. Complete Genome Sequence of *Deinococcus maricopensis* Type Strain (LB-34). *Stand. Genomic Sci.* **2011**, *4*, 163–172. [CrossRef] [PubMed]

44. Argyrou, A.; Blanchard, J.S. Flavoprotein Disulfide Reductases: Advances in Chemistry and Function. *Prog. Nucleic Acid Res. Mol. Biol.* **2004**, *78*, 79–142. [CrossRef] [PubMed]

45. Hammerstad, M.; Hersleth, H.-P. Overview of Structurally Homologous Flavoprotein Oxidoreductases Containing the Low Mr Thioredoxin Reductase-like Fold—A Functionally Diverse Group. *Arch. Biochem. Biophys.* **2021**, *702*, 108826. [CrossRef] [PubMed]

46. Lennon, B.W.; Williams, C.H.; Ludwig, M.L. Twists in Catalysis: Alternating Conformations of *Escherichia coli* Thioredoxin Reductase. *Science* **2000**, *289*, 1190–1194. [CrossRef] [PubMed]

47. Joe, M.-H.; Jung, S.-W.; Im, S.-H.; Lim, S.-Y.; Song, H.-P.; Kwon, O.; Kim, D.-H. Genome-Wide Response of *Drosophila melanogaster* Vivo Highlights the Central Role Played by This Ubiquitous Oxidoreductase in Redox. Control. *Mol. Cell Proteomics* **2016**, *15*, 2125–2140. [CrossRef] [PubMed]

48. Perez-Perez, M.E.; Mauries, A.; Maes, A.; Tousasse, N.J.; Hamon, M.; Lemaire, S.D.; Marchand, C.H. The Deep Thioredoxome in *Chlamydomonas reinhardtii*: New Insights into Redox. Regulation. *Mol. Plant.* **2017**, *10*, 1107–1125. [CrossRef] [PubMed]

49. Zeller, T.; Klug, G. Thioredoxins in Bacteria: Functions in Oxidative Stress Response and Regulation of Thioredoxin Genes. *Naturwissenschaften* **2006**, *93*, 259–266. [CrossRef]

50. Jeong, W.; Yoon, H.W.; Lee, S.-R.; Rhee, S.G. Identification and Characterization of TRP14, a Thioredoxin-Related Protein of 14 KDa. New Insights into the Specificity of Thioredoxin Function. *J. MicroBIol. Biotechnol.* **2012**, *22*, 1107–1125. [CrossRef] [PubMed]

51. Lennon, B.W.; Williams, C.H.; Ludwig, M.L. Twists in Catalysis: Alternating Conformations of *Escherichia coli* Thioredoxin Reductase. *Science* **2000**, *289*, 1190–1194. [CrossRef] [PubMed]

52. Magerand, R.; Rey, P.; Blanchard, L.; de Groot, A. Redox. Signaling through Zinc Activates the Radiation Response in *Deinococcus* Bacteria. *Sci. Rep.* **2021**, *11*, 4528. [CrossRef] [PubMed]

53. Giordano, E.; Peluso, I.; Rendina, R.; Digilio, A.; Furia, M. The Clot Gene of *Drosophila melanogaster* Encodes a Conserved Member of the Thioredoxin-like Protein Superfamily. *Mol. Genet. Genomics* **2003**, *268*, 692–697. [CrossRef]

54. Rainey, F.A.; Ferreira, M.; Nobre, M.F.; Ray, K.; Bagaley, D.; Earl, A.M.; Battista, J.R.; Gomez-Silva, B.; McKay, C.P.; da Costa, M.S. *Deinococcus peraridilitoris* Sp. Nov., Isolated from a Coastal Desert. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 1408–1412. [CrossRef] [PubMed]

55. Rainey, F.A.; Ray, K.; Ferreira, M.; Gatz, B.Z.; Nobre, M.F.; Bagaley, D.; Rash, B.A.; Park, M.J.; Earl, A.M.; Shank, N.C.; et al. Complete Genome Sequence of *Deinococcus maricopensis* Type Strain (LB-34). *Stand. Genomic Sci.* **2011**, *4*, 163–172. [CrossRef] [PubMed]

56. Lennon, B.W.; Williams, C.H.; Ludwig, M.L. Twists in Catalysis: Alternating Conformations of *Escherichia coli* Thioredoxin Reductase. *Science* **2000**, *289*, 1190–1194. [CrossRef] [PubMed]

57. Magerand, R.; Rey, P.; Blanchard, L.; de Groot, A. Redox. Signaling through Zinc Activates the Radiation Response in *Deinococcus* Bacteria. *Sci. Rep.* **2021**, *11*, 4528. [CrossRef] [PubMed]

58. Asgharpour, A.; Orsi, P.; Marzetti, G.; Weng, K.; Denhardt, D.T.; Vibert, J. Identification of the Thioredoxin-Related Domain MrPz of *Deinococcus radiodurans*. *Int. J. Syst. Evol. Microbiol.* **2011**, *61*, 981–986. [CrossRef] [PubMed]

59. Collet, J.F.; D’Souza, J.C.; Jakob, U.; Bardwell, J.C. Thioredoxin 2, an Oxidative Stress-Induced Protein, Contains a High Affinity Zinc Binding Site. *J. Biol. Chem.* **1997**, *272*, 30841–30847. [CrossRef] [PubMed]

60. Argyrou, A.; Blanchard, J.S. Flavoprotein Disulfide Reductases: Advances in Chemistry and Function. *Prog. Nucleic Acid Res. Mol. Biol.* **2004**, *78*, 79–142. [CrossRef] [PubMed]

61. Lennon, B.W.; Williams, C.H.; Ludwig, M.L. Twists in Catalysis: Alternating Conformations of *Escherichia coli* Thioredoxin Reductase. *Science* **2000**, *289*, 1190–1194. [CrossRef] [PubMed]

62. Magerand, R.; Rey, P.; Blanchard, L.; de Groot, A. Redox. Signaling through Zinc Activates the Radiation Response in *Deinococcus* Bacteria. *Sci. Rep.* **2021**, *11*, 4528. [CrossRef] [PubMed]

63. Lennon, B.W.; Williams, C.H.; Ludwig, M.L. Twists in Catalysis: Alternating Conformations of *Escherichia coli* Thioredoxin Reductase. *Science* **2000**, *289*, 1190–1194. [CrossRef] [PubMed]

64. Argyrou, A.; Blanchard, J.S. Flavoprotein Disulfide Reductases: Advances in Chemistry and Function. *Prog. Nucleic Acid Res. Mol. Biol.* **2004**, *78*, 79–142. [CrossRef] [PubMed]

65. Lennon, B.W.; Williams, C.H.; Ludwig, M.L. Twists in Catalysis: Alternating Conformations of *Escherichia coli* Thioredoxin Reductase. *Science* **2000**, *289*, 1190–1194. [CrossRef] [PubMed]
66. Espinosa, B.; Arnér, E.S.J. Thioredoxin-Related Protein of 14 KDa as a Modulator of Redox. Signalling Pathways. Br. J. Pharmacol. 2019, 176, 544–553. [CrossRef]

67. Baker, L.M.; Raudonikiene, A.; Hoffman, P.S.; Poole, L.B. Essential Thioredoxin-Dependent Peroxiredoxin System from Helicobacter pylori: Genetic and Kinetic Characterization. J. Bacteriol. 2001, 183, 1961–1973. [CrossRef]

68. Kuhns, L.G.; Wang, G.; Maier, R.J. Comparative Roles of the Two Helicobacter pylori Thioredoxins in Preventing Macromolecule Damage. Infect. Immun. 2015, 83, 2935–2943. [CrossRef]

69. Gustafsson, T.N.; Sahlin, M.; Lu, J.; Sjöberg, B.-M.; Holmgren, A. Bacillus anthracis Thioredoxin Systems, Characterization and Role as Electron Donors for Ribonucleotide Reductase. J. Biol. Chem. 2012, 287, 39686–39697. [CrossRef]

70. Pader, I.; Sengupta, R.; Cebula, M.; Xu, J.; Lundberg, J.O.; Holmgren, A.; Johansson, K.; Arnér, E.S.J. Thioredoxin-Related Protein of 14 KDa Is an Efficient L-Cystine Reductase and S-Denitrosylase. Proc. Natl. Acad. Sci. USA 2014, 111, 6964–6969. [CrossRef]

71. Díka, E.; Pader, I.; Bíró, A.; Johansson, K.; Cheng, Q.; Ballagó, K.; Prigge, J.R.; Pastor-Flores, D.; Dick, T.P.; Schmidt, E.E.; et al. A Novel Persulfide Detection Method Reveals Protein Persulfide- and Polysulfide-Reducing Functions of Thioredoxin and Glutathione Systems. Sci. Adv. 2016, 2, e1500968. [CrossRef] [PubMed]

72. Liu, Y.; Zhou, J.; Omelchenko, M.V.; Beliaev, A.S.; Venkateswaran, A.; Stair, J.; Wu, L.; Thompson, D.K.; Xu, D.; Rogozin, I.B.; et al. Transcriptome Dynamics of Deinococcus radiodurans Recovering from Ionizing Radiation. Proc. Natl. Acad. Sci. USA 2003, 100, 4191–4196. [CrossRef] [PubMed]

73. Wang, L.; Xu, G.; Chen, H.; Zhao, Y.; Xu, N.; Tian, B.; Hua, Y. DrRRA: A Novel Response Regulator Essential for the Extreme Radiooresistance of Deinococcus radiodurans. Mol. MicroBiol. 2008, 67, 1211–1222. [CrossRef] [PubMed]

74. Tanaka, M.; Earl, A.M.; Howell, H.A.; Park, M.J.; Eisen, J.A.; Peterson, S.N.; Battista, J.R. Analysis of Thioredoxin Radiodurans’s Transcriptional Response to Ionizing Radiation and Desication Reveals Novel Proteins That Contribute to Extreme Radioresistance. Genetics 2004, 168, 21–33. [CrossRef] [PubMed]

75. Morgenstern, M.; Stiller, S.B.; Lübbert, P.; Peikert, C.D.; Dannenmaier, S.; Drepper, F.; Welli, U.; Höß, P.; Feuerstein, R.; Gebert, M.; et al. Definition of a High-Confidence Mitochondrial Proteome at Quantitative Scale. Cell Rep. 2017, 19, 2836–2852. [CrossRef]

76. Lu, S.; Wang, J.; Chitsaz, F.; Derbyshire, M.K.; Geer, R.C.; Gonzales, N.R.; Gwadz, M.; Hurwitz, D.I.; Marchler, G.H.; Song, J.S.; et al. CDD/SPARCLE: The Conserved Domain Database in 2020. Nucleic Acids Res. 2020, 48, D265–D268. [CrossRef]

77. Khairnar, N.P.; Joe, M.H.; Misra, H.S.; Lim, S.Y.; Kim, D.H. FrnE, a Cadmium-Inducible Protein in Deinococcus radiodurans, Is a Reductant for the Copper Metallochaperone ScoI. BMC MicroBiol. 2018, 18, 296–310. [CrossRef] [PubMed]

78. Bihani, S.C.; Panicker, L.; Rajpurohit, Y.S.; Misra, H.S.; Kumar, V. DrFmRRE Represents a Hitherto Unknown Class of Eubacterial Cytoplasmic Disulfide Oxido-Reductases. Antioxid. Redox. Signal. 2017, 28, 296–310. [CrossRef] [PubMed]

79. Crow, A.; Lewin, A.; Hecht, O.; Carlsson Möller, M.; Moore, G.R.; Hederstedt, L.; Le Brun, N.E. Crystal Structure and Biophysical Properties of Bacillus subtilis BdbD. An Oxidizing Thiol:Disulfide Oxidoareductase Containing a Novel Metal Site. J. Biol. Chem. 2009, 284, 23719–23733. [CrossRef] [PubMed]

80. Depuydt, M.; Leonard, S.E.; Vertommen, D.; Denoncin, K.; Morsomme, P.; Wahni, K.; Messens, J.; Carroll, K.S.; Collet, J.F. A Novel Persulfide Detection Method Reveals Protein Persulfide- and Polysulfide-Reducing Functions of Thioredoxin and Glutathione Systems. Sci. Adv. 2017, 3, e1601765. [CrossRef] [PubMed]

81. Bushweller, J.H. Protein Disulfide Oxidoareductase Protects Single Cysteine Residues from Oxidation. J. Biol. Chem. 2004, 279, 25091–25092. [CrossRef] [PubMed]

82. Hassan, F.M.N.; Gupta, R.S. Novel Sequence Features of DNA Repair Genes/Proteins from Deinococcus Species Implicated in Protection from Oxidatively Generated Damage. Genes 2018, 9, 149. [CrossRef] [PubMed]

83. Achard, M.E.S.; Hamilton, A.J.; Dankowski, T.; Heras, B.; Schembri, M.S.; Edwards, J.L.; Jennings, M.P.; McEwan, A.G. A Novel Thioredoxin-like Protein Plays a Role in Defense against Oxidative Stress in Neisseria gonorrhoeae. Infect. Immun. 2009, 77, 4934–4939. [CrossRef] [PubMed]

84. Tanboon, W.; Chuchue, T.; Vattanaviboon, P.; Mongkolsuk, S. Inactivation of Thioredoxin-like Gene Alters Oxidative Stress Resistance and Reduces Cytochrome c Oxidase Activity in Agrobacterium tumefaciens. FEMS MicroBiol. Lett. 2009, 289, 110–116. [CrossRef]

85. Mohorko, E.; Abicht, H.K.; Bühler, D.; Glockshuber, R.; Hennecke, H.; Fischer, H.-M. Thioredoxin-like Protein TlpA from Bradyrhizobium japonicum Is a Reductant for the Copper Metallochaperone Scl. FEMS Lett. 2012, 586, 4094–4099. [CrossRef] [PubMed]

86. Roszczenko, P.; Grzeszcuk, M.; Kobierecka, P.; Wywial, E.; Urbanowicz, P.; Wincek, P.; Nowak, E.; Jagusztyn-Krynicka, E.K. Helicobacter pylori HP0377, a Member of the Dsb Family, Is an Unusual Multifunctional CcmG That Cooperates with Dimeric Thiolsulfide Oxidase HP0371. BMC MicroBiol. 2015, 15, 135. [CrossRef]

87. Goulding, C.W.; Apostol, M.I.; Gleiter, S.; Parseghian, A.; Bardwell, J.; Gennaro, M.; Eisenberg, D. Gram-Positive DsbE Proteins Function Differently from Gram-Negative DsbE Homologs. A Structure to Function Analysis of DsbE from Mycobacterium tuberculosis. J. Biol. Chem. 2004, 279, 3516–3524. [CrossRef] [PubMed]

88. Luo, S.; Levine, R.L. Methionine in Proteins Defends against Oxidative Stress. FASEB J. 2009, 23, 464–472. [CrossRef]

89. Brot, N.; Weissbach, L.; Werth, J.; Weissbach, H. Enzymatic Reduction of Protein-Bound Methionine Sulfoxide. Proc. Natl. Acad. Sci. USA 1981, 78, 2155–2158. [CrossRef]

90. Grimaud, R.; Ezratty, B.; Mitchell, J.K.; Laffitte, D.; Briand, C.; Derrick, P.; Barras, F. Repair of Oxidized Proteins. Identification of a New Methionine Sulfoxide Reductase. J. Biol. Chem. 2001, 276, 48915–48920. [CrossRef]
116. Boschi-Muller, S. Molecular Mechanisms of the Methionine Sulfoxide Reductase System from Neisseria meningitidis. *Antioxidants* 2018, 7, 131. [CrossRef]

117. Gennaris, A.; Ezraty, B.; Henry, C.; Agrebi, R.; Vergnes, A.; Oheix, E.; Bos, J.; Leverrier, P.; Espinosa, L.; Szewczyk, J.; et al. Repairing Oxidized Proteins in the Bacterial Envelope Using Respiratory Chain Electrons. *Nature* 2015, 528, 409–412. [CrossRef]

118. Perkins, A.; Nelson, K.J.; Parsonage, D.; Poole, L.B.; Karplus, P.A. Peroxiredoxins: Guardians against Oxidative Stress and Modulators of Peroxide Signaling. *Trends BioChem. Sci.* 2015, 40, 435–445. [CrossRef]

119. Rhee, S.G.; Woo, H.A. Multiple Functions of Peroxiredoxins: Peroxidases, Sensors and Regulators of the Intracellular Messenger H₂O₂, and Protein Chaperones. *Antioxid. Redox. Signal.* 2011, 15, 781–794. [CrossRef] [PubMed]

120. Day, A.M.; Brown, J.D.; Taylor, S.R.; Rand, J.D.; Morgan, B.A.; Veal, E.A. Inactivation of a Peroxiredoxin by Hydrogen Peroxide Is Critical for Thioredoxin-Mediated Repair of Oxidized Proteins and Cell Survival. *Mol. Cell.* 2012, 45, 398–408. [CrossRef]

121. Stöcker, S.; Maurer, M.; Ruppert, T.; Dick, T.P. A Role for 2-Cys Peroxiredoxins in Facilitating Cytosolic Protein Thiol Oxidation. *Nat. Chem. Biol.* 2018, 14, 148–155. [CrossRef] [PubMed]

122. Chae, H.Z.; Kim, I.H.; Kim, K.; Rhee, S.G. Cloning, Sequencing, and Mutation of Thiol-Specific Antioxidant Gene of Saccharomyces cerevisiae. *J. Biol. Chem.* 1993, 268, 16815–16821. [CrossRef]

123. de Oliveira, M.A.; Tairum, C.A.; Netto, L.E.S.; de Oliveira, A.L.P.; Aleixo-Silva, R.L.; Cabrera, V.I.M.; Breyer, C.A.; Dos Santos, M.C. Relevance of Peroxiredoxins in Pathogenic Microorganisms. *Appl. Microbiol. Biotechnol.* 2021, 105, 5701–5717. [CrossRef]

124. Poole, L.B.; Ellis, H.R. Flavin-Dependent Alkyl Hydroperoxide Reductase from Salmonella typhimurium. 1. Purification and Enzymatic Activities of Overexpressed AhpF and AhpC Proteins. *Biochemistry* 1996, 35, 56–64. [CrossRef] [PubMed]

125. Jeong, W.; Cha, M.K.; Kim, I.H. Thioredoxin-Dependent Hydroperoxide Peroxidase Activity of Bacterioferritin Comigratory Protein (BCP) as a New Member of the Thiol-Specific Antioxidant Protein (TSA)/Alkyl Hydroperoxide Peroxidase C (AhpC) Family. *J. Biol. Chem.* 2000, 275, 2924–2930. [CrossRef] [PubMed]

126. Rouhier, N.; Gelhaye, E.; Gualberto, J.M.; Jordy, M.-N.; De Fay, E.; Hirasawa, M.; Duplessis, S.; Lemaire, S.D.; Frey, P.; Martin, F.; et al. Poplar Peroxiredoxin Q. A Thioredoxin-Linked Chloroplast Antioxidant Functional in Pathogen Defense. *Plant Physiol.* 2004, 134, 1027–1038. [CrossRef]

127. Clarke, D.J.; Mackay, C.L.; Campopiano, D.J.; Langridge-Smith, P.; Brown, A.R. Interrogating the Molecular Details of the Peroxiredoxin Activity of the Escherichia coli Bacterioferritin Comigratory Protein Using High-Resolution Mass Spectrometry. *Biochemistry* 2009, 48, 3904–3914. [CrossRef] [PubMed]

128. Clarke, D.J.; Ortega, X.P.; Mackay, C.L.; Valvano, M.A.; Govan, J.R.W.; Campopiano, D.J.; Langridge-Smith, P.; Brown, A.R. Subdivision of the Bacterioferritin Comigratory Protein Family of Bacterial Peroxiredoxins Based on Catalytic Activity. *Biochemistry* 2010, 49, 1319–1330. [CrossRef]

129. Hicks, L.D.; Raghavan, R.; Battisti, J.M.; Minnick, M.F. A DNA-Binding Peroxiredoxin of Coxiea burnetii Is Involved in Countering Oxidative Stress during Exponential-Phase Growth. *J. Bacteriol.* 2010, 192, 2077–2084. [CrossRef] [PubMed]

130. Lee, S.; Chung, J.M.; Yun, H.J.; Won, J.; Jung, H.S. New Insight into Multifunctional Role of Peroxiredoxin Family Protein: Determination of DNA Protection Properties of Bacterioferritin Comigratory Protein under Hyperthermal and Oxidative Stresses. *BioChem. Biophys. Res. Commun.* 2016, 469, 1028–1033. [CrossRef]

131. Chae, H.Z.; Robison, K.; Poole, L.B.; Church, G.; Storz, G.; Rhee, S.G. Cloning and Sequencing of Thiol-Specific Antioxidant from Mammalian Brain: Alkyl Hydroperoxide Reductase and Thiol-Specific Antioxidant Define a Large Family of Antioxidant Enzymes. *Proc. Natl. Acad. Sci. USA* 1994, 91, 7017–7021. [CrossRef] [PubMed]

132. Jacobson, F.S.; Morgan, R.W.; Christman, M.F.; Ames, B.N. An Alkyl Hydroperoxide Reductase from Salmonella typhimurium Involved in the Defense of DNA against Oxidative Damage. Purification and Properties. *J. Biol. Chem.* 1989, 264, 1488–1496. [CrossRef]

133. Storz, G.; Jacobson, F.S.; Tartaglia, L.A.; Morgan, R.W.; Silveira, L.A.; Ames, B.N. An Alkyl Hydroperoxide Reductase Induced by Oxidative Stress in Salmonella typhimurium and Escherichia coli. Characteristic Zation and Cloning of ahp. *J. Bacteriol.* 1989, 171, 2049–2055. [CrossRef]

134. Poole, L.B.; Reynolds, C.M.; Wood, Z.A.; Karplus, P.A.; Ellis, H.R.; Li Calzi, M. AhpF and Other NADH-Peroxidoxin Oxidoreductases, Homologues of Low Mr Thioredoxin Reductase. *Eur. J. Biochem.* 2000, 267, 6126–6133. [CrossRef] [PubMed]

135. Poole, L.B.; Godzik, A.; Nayeem, A.; Schmitt, J.D. AhpC Can Be Dissected into Two Functional Units: Tandem Repeats of Two Protease Detoxification Enzymes. *J. Bacteriol.* 2000, 182, 6845–6849. [CrossRef]

136. Jang, H.H.; Lee, K.O.; Chi, Y.H.; Jung, B.G.; Park, S.K.; Park, J.H.; Lee, J.R.; Lee, S.S.; Moon, J.C.; Yun, J.W.; et al. Two Enzymes in One; Two Yeast Peroxiredoxins Display Oxidative Stress-Dependent Switching from a Peroxidase to a Molecular Chaperone Function. *Cell 2004*, 117, 625–635. [CrossRef] [PubMed]

137. Parsonage, D.; Youngblood, D.S.; Sarma, G.N.; Wood, Z.A.; Karplus, P.A.; Poole, L.B. Analysis of the Link between Enzymatic Activity and Oligomeric State in AhpC, a Bacterial Peroxidoxin. *Biochemistry* 2005, 44, 10583–10592. [CrossRef]

138. Mongkolkus, S.; Whangsuk, W.; Vattanaviboon, P.; Loprasert, S.; Fuangthong, M. A Xanthomonos Alkyl Hydroperoxide Reductase Subunit C (AhpC) Mutant Showed an Altered Peroxide Stress Response and Complex Regulation of the Compensatory Response of Peroxide Detoxification Enzymes. *J. Bacteriol.* 2000, 182, 6845–6849. [CrossRef]

139. Lee, S.; Jeong, H.; Lee, J.H.; Chung, J.M.; Kim, R.; Yun, H.J.; Won, J.; Jung, H.S. Characterisation of Conformational and Functional Features of Alkyl Hydroperoxide Reductase E-Protein. *BioChem. Biophys. Res. Commun.* 2017, 489, 217–222. [CrossRef]
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140. Li, S.; Peterson, N.A.; Kim, M.-Y.; Kim, C.-Y.; Hung, L.-W.; Yu, M.; Lekin, T.; Segelke, B.W.; Lott, J.S.; Baker, E.N. Crystal Structure of AhpE from Mycobacterium tuberculosis, a 1-Cys Peroxiredoxin. J. Biol. Chem. 2005, 280, 1035–1046. [CrossRef] [PubMed]

141. Reyes, A.M.; Hugo, M.; Trostchansky, A.; Capece, L.; Radi, R.; Trujillo, M. Oxidizing Substrate Specificity of Mycobacterium tuberculosis Alkyl Hydroperoxide Reductase E: Kinetics and Mechanisms of Oxidation and Overoxidation. Free Radic. Biol. Med. 2011, 51, 464–473. [CrossRef] [PubMed]

142. Bryk, R.; Lima, C.D.; Erdjument-Bromage, H.; Tempst, P.; Nathan, C. Metabolic Enzymes of Mycobacteria Linked to Antioxidant Defense by a Thioredoxin-like Protein. Science 2002, 295, 1073–1077. [CrossRef] [PubMed]

143. Hong, E.-J.; Jeong, H.; Lee, D.-S.; Kim, Y.; Lee, H.-S. The ahpD Gene of Corynebacterium glutamicum Plays an Important Role in Hydrogen Peroxide-Induced Oxidative Stress Response. J. BioChem. 2019, 165, 197–204. [CrossRef] [PubMed]

144. Hillas, P.J.; del Alba, F.S.; Oyarzabal, J.; Wilks, A.; Ortiz De Montellano, P.R. The AhpC and AhpD Antioxidant Defense System of Mycobacterium tuberculosis. J. Biol. Chem. 2000, 275, 18801–18809. [CrossRef] [PubMed]

145. Kumar, A.; Balakrishna, A.M.; Narrey, W.; Manimekalai, M.S.S.; Grüber, G. Redox. Chemistry of Mycobacterium tuberculosis Alkylhydroperoxide Reductase (AhpE): Structural and Mechanistic Insight into a Mycoredoxin-1 Independent Reductive Pathway of AhpE via Mycothiol. Free Radic. Biol. Med. 2016, 97, 588–601. [CrossRef]

146. Zhao, L.; Jeong, S.; Zhang, J.; Jung, J.-H.; Choi, J.-I.; Lim, S.; Kim, M.-K. Crystal Structure of the AhpD-like Protein DR1765 from Deinococcus radiodurans R1. BioChem. Biophys. Res. Commun. 2020, 529, 444–449. [CrossRef] [PubMed]

147. Mongkolksuk, S.; Prattuan, W.; Loprasert, S.; Fuangthong, M.; Chamnongpol, S. Identification and Characterization of a New Organic Hydroperoxide Resistance (ohr) Gene with a Novel Pattern of Oxidative Stress Regulation from Xanthomonas campestris. PLoS. phaseoli. J. Bacteriol. 1998, 180, 2636–2643. [CrossRef]

148. Gutierrez, C.; Devedjian, J.C. Osmotic Induction of Gene osmC Expression in Escherichia coli K12. J. Mol. Biol. 1991, 220, 959–973. [CrossRef]

149. Shin, D.H.; Choi, I.G.; Busso, D.; Jancarik, J.; Yokota, H.; Kim, R.; Kim, S.H. Structure of OsmC from Escherichia coli: A Salt-Shock-Induced Protein. Acta Crystallogr. D Biol. Crystallogr. 2004, 60, 903–911. [CrossRef] [PubMed]

150. Atichartpongkul, S.; Loprasert, S.; Vattanaviboon, P.; Helmann, J.D.; Mongkolsuk, S. Bacterial Ohr and OsmC Paralogues Define Two Protein Families with Distinct Functions and Patterns of Expression. Microbiology 2001, 147, 1775–1782. [CrossRef] [PubMed]

151. Meireles, D.A.; Domingos, R.M.; Gaiarsa, J.W.; Ragnoni, E.G.; Bannitz-Fernandes, R.; da Silva Neto, J.F.; de Souza, R.F.; Netto, L.E.S. Functional and Evolutionary Characterization of Ohr Proteins in Eukaryotes Reveals Many Active Homologs among Pathogenic Fungi. Redox. Biol. 2017, 12, 600–609. [CrossRef] [PubMed]

152. Alegria, T.G.P.; Meireles, D.A.; Cussiol, J.R.R.; Hugo, M.; Trujillo, M.; de Oliveira, M.A.; Miyamoto, S.; Queiroz, R.F.; Valadares, N.F.; Garratt, R.C.; et al. Ohr Plays a Central Role in Bacterial Responses against Fatty Acid Hydroperoxides and Peroxynitrite. Proc. Natl. Acad. Sci. USA 2017, 114, E132–E141. [CrossRef] [PubMed]

153. Lesnjak, J.; Barton, W.A.; Nikolov, D.B. Structural and Functional Characterization of the Pseudomonas Hydroperoxide Resistance Protein Ohr. EMBO J. 2002, 21, 6649–6659. [CrossRef] [PubMed]

154. Lesnjak, J.; Barton, W.A.; Nikolov, D.B. Structural and Functional Features of the Escherichia coli Hydroperoxide Resistance Protein OsmC. Protein Sci. 2003, 12, 2838–2843. [CrossRef] [PubMed]

155. Zhang, W.; Baseman, J.B. Functional Characterization of Osmotically Inducible Protein C (MG_427) from Mycoplasma genitalium. J. Bacteriol. 2014, 196, 1012–1019. [CrossRef] [PubMed]

156. Cussiol, J.R.R.; Alegria, T.G.P.; Szweda, L.I.; Netto, L.E.S. Ohr (Organic Hydroperoxide Resistance Protein) Possesses a Previously Undescribed Activity, Lipoyl-Dependent Peroxidase. J. Biol. Chem. 2010, 285, 21943–21950. [CrossRef] [PubMed]

157. Si, M.; Su, T.; Chen, C.; Wei, Z.; Gong, Z.; Li, G. OsmC in Corynebacterium glutamicum Was a Thiol-Dependent Organic Hydroperoxide Reductase. Int. J. Mol. Macromol. 2013, 56, 642–652. [CrossRef]

158. Basu, B.; Apte, S.K. Gamma Radiation-Induced Proteome of Deinococcus radiodurans Primarily Targets DNA Repair and Oxidative Stress Alleviation. Mol. Cell Proteomics 2012, 11, M111.01734. [CrossRef] [PubMed]

159. Meunier-Jamin, C.; Kapp, U.; Leonard, G.A.; McSweeney, S. The Structure of the Organic Hydroperoxide Resistance Protein from Deinococcus radiodurans. Do Conformational Changes Facilitate Recycling of the Redox. Disulfide? J. Biol. Chem. 2004, 279, 25830–25837. [CrossRef]

160. Wang, L.; Hu, J.; Liu, M.; Yang, S.; Zhao, Y.; Cheng, K.; Xu, G.; Li, M.; Tian, B.; Hua, Y. Protemic Insights into the Functional Basis for the Response Regulator DrDRA of Deinococcus radiodurans. Int. J. Radiat. Biol. 2016, 92, 273–280. [CrossRef]

161. Hiras, J.; Sharma, S.V.; Raman, V.; Tinson, R.A.J.; Arbach, M.; Rodrigues, D.F.; Norambuena, J.; Hamilton, C.J.; Hanson, T.E. Physiological Studies of Chlorobiaceae Suggest That Bacilli thiochrome Derivatives Are the Most Widespread Thiols in Bacteria. mBio 2018, 9, e01603-18. [CrossRef] [PubMed]

162. Newton, G.L.; Rawat, M.; La Clair, J.J.; Jothivasan, V.K.; Budiarto, T.; Hamilton, C.J.; Claiborne, A.; Helmann, J.D.; Fahey, R.C. Bacillilthiol Is an Antioxidant Thiol Produced in Bacilli. Nat. Chem. Biol. 2009, 5, 625–627. [CrossRef] [PubMed]

163. Linzner, N.; Loi, V.V.; Fritsch, V.N.; Tung, Q.N.; Stenzel, S.; Wirtz, M.; Hell, R.; Hamilton, C.J.; Tedin, K.; Fulde, M.; et al. Staphylococcus aureus Uses the Bacillilthirboxin (BrxAB)/Bacillilthiol Disulfide Reductase (YpdA) Redox. Pathway to Defend against Oxidative Stress under Infections. Front. MicroBiol. 2019, 10, 1355. [CrossRef] [PubMed]
164. Hammerstad, M.; Gudim, I.; Hersleth, H.-P. The Crystal Structures of Bacillithiol Disulfide Reductase Bdr (YpdA) Provide Structural and Functional Insight into a New Type of FAD-Containing NADPH-Dependent Oxidoreductase. *Biochemistry* **2020**, *59*, 4793–4798. [CrossRef]

165. Gaballa, A.; Su, T.T.; Helmann, J.D. The *Bacillus subtilis* Monothiol Bacilliredoxin BrxC (YtxJ) and the Bdr (YpdA) Disulfide Reductase Reduce S-Bacillithiolated Proteins. *Redox. Biol.* **2021**, *42*, 101935. [CrossRef]

166. Linzner, N.; Loi, V.V.; Fritsch, V.N.; Tung, Q.N.; Stenzel, S.; Wirtz, M.; Hell, R.; Hamilton, C.J.; Tedin, K.; Fulde, M.; et al. Highly Accurate Protein Structure Prediction with AlphaFold. *Nature* **2021**, *596*, 583–589. https://doi.org/10.1038/s41586-021-03819-2.

167. Prinz, W.A.; Aslund, F.; Holmgren, A.; Beckwith, J. The Role of the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

168. Zhang, C.; Wei, J.; Zheng, Z.; Ying, N.; Sheng, D.; Hua, Y. Proteomic Analysis of Deinococcus radiouarans Recovering from Gamma-Irradiation. *Proteomics* **2005**, *5*, 138–143. [CrossRef] [PubMed]

169. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

170. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

171. Prinz, W.A.; Aslund, F.; Holmgren, A.; Beckwith, J. The Role of the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

172. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

173. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

174. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

175. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

176. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

177. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

178. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

179. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

180. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]

181. Bashandy, T.; Guilleminot, J.; Vernoux, T.; Caparros-Ruiz, D.; Ljung, K.; Meyer, Y.; Reichheld, J.-P. Interplay between the Thioredoxin and Glutaredoxin Pathways in Reducing Protein Disulfide Bonds in *E. coli*. *FEMS Microb. Lett.* **2008**, *275*, 2067–2077. [CrossRef]