



Natural Transformation in *Deinococcus radiodurans*: A Genetic Analysis Reveals the Major Roles of DprA, DdrB, RecA, RecF, and RecO Proteins

Solenne Ithurbide, Geneviève Coste, Johnny Lisboa, Nicolas Eugénie, Esma Bentchikou, Claire Bouthier de La Tour, Dominique Liger, Fabrice Confalonieri, Suzanne Sommer, Sophie Quevillon-Cheruel, et al.

► To cite this version:

Solenne Ithurbide, Geneviève Coste, Johnny Lisboa, Nicolas Eugénie, Esma Bentchikou, et al.. Natural Transformation in *Deinococcus radiodurans*: A Genetic Analysis Reveals the Major Roles of DprA, DdrB, RecA, RecF, and RecO Proteins. *Frontiers in Microbiology*, 2020, 11, pp.1253. 10.3389/fmicb.2020.01253 . hal-02898103

HAL Id: hal-02898103

<https://hal.science/hal-02898103>

Submitted on 3 Sep 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



OPEN ACCESS

Edited by:

Ludmila Chistoserdova,
University of Washington,
United States

Reviewed by:

Peter Graumann,
University of Marburg, Germany
John R. Battista,
Louisiana State University,
United States

*Correspondence:

Sophie Quevillon-Cheruel
sophie.quevillon-cheruel@
i2bc.paris-saclay.fr
Pascale Servant
pascale.servant@i2bc.paris-saclay.fr

†These authors have contributed
equally to this work

*Present address:

Solenne Ithurbide,
The Ithre Institute, University of
Technology Sydney, Ultimo, NSW,
Australia
Johnny Lisboa,
Group of Fish Immunology &
Vaccinology, IBMC – Institute for
Molecular and Cell Biology, i3S -
Institute for Research and Innovation
in Health, Porto, Portugal

Specialty section:

This article was submitted to
Evolutionary and Genomic
Microbiology,
a section of the journal
Frontiers in Microbiology

Received: 03 May 2019

Accepted: 18 May 2020

Published: 18 June 2020

Citation:

Ithurbide S, Coste G, Lisboa J,
Eugénie N, Bentchikou E, Bouthier de
la Tour C, Liger D, Confalonieri F,
Sommer S, Quevillon-Cheruel S and
Servant P (2020) Natural
Transformation in *Deinococcus*
radiodurans: A Genetic Analysis
Reveals the Major Roles of DprA,
DdrB, RecA, RecF, and RecO
Proteins. Front. Microbiol. 11:1253.
doi: 10.3389/fmicb.2020.01253

Natural Transformation in *Deinococcus radiodurans*: A Genetic Analysis Reveals the Major Roles of DprA, DdrB, RecA, RecF, and RecO Proteins

Solenne Ithurbide^{††}, Geneviève Coste[†], Johnny Lisboa[‡], Nicolas Eugénie,
Esma Bentchikou, Claire Bouthier de la Tour, Dominique Liger, Fabrice Confalonieri,
Suzanne Sommer, Sophie Quevillon-Cheruel* and Pascale Servant*

Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

Horizontal gene transfer is a major driver of bacterial evolution and adaptation to environmental stresses, occurring notably via transformation of naturally competent organisms. The *Deinococcus radiodurans* bacterium, characterized by its extreme radioresistance, is also naturally competent. Here, we investigated the role of *D. radiodurans* players involved in different steps of natural transformation. First, we identified the factors (PilQ, PilD, type IV pilins, PilB, PilT, ComEC-ComEA, and ComF) involved in DNA uptake and DNA translocation across the external and cytoplasmic membranes and showed that the DNA-uptake machinery is similar to that described in the Gram negative bacterium *Vibrio cholerae*. Then, we studied the involvement of recombination and DNA repair proteins, RecA, RecF, RecO, DprA, and DdrB into the DNA processing steps of *D. radiodurans* transformation by plasmid and genomic DNA. The transformation frequency of the cells devoid of DprA, a highly conserved protein among competent species, strongly decreased but was not completely abolished whereas it was completely abolished in $\Delta dprA \Delta recF$, $\Delta dprA \Delta recO$, and $\Delta dprA \Delta ddrB$ double mutants. We propose that RecF and RecO, belonging to the recombination mediator complex, and DdrB, a specific deinococcal DNA binding protein, can replace a function played by DprA, or alternatively, act at a different step of recombination with DprA. We also demonstrated that a $\Delta dprA$ mutant is as resistant as wild type to various doses of γ -irradiation, suggesting that DprA, and potentially transformation, do not play a major role in *D. radiodurans* radioresistance.

Keywords: *Deinococcus radiodurans*, natural transformation, DNA uptake, homologous recombination, DprA, RecA, RecFOR, DdrB

INTRODUCTION

Natural transformation is a mode of horizontal gene transfer which contributes to the acquisition of new properties and thus to genome evolution. Since the discovery of natural transformation of *Streptococcus pneumoniae* (Griffith, 1928), more than 85 species have now been shown to be naturally transformable (Johnston et al., 2014). This mechanism can be divided into three steps:

- (1) External double-stranded DNA (dsDNA) capture followed by the translocation of single-stranded DNA (ssDNA) into the cytosol. In most transformable bacteria (except for *Helicobacter pylori*), dsDNA capture is dependent on type II secretion or type IV pilus system proteins (Kruger and Stingl, 2011; Johnston et al., 2014; Matthey and Blokesch, 2016). The translocation of ssDNA across the cytoplasmic membrane requires three components: the DNA receptor (ComEA) (Seitz et al., 2014), the permease/channel protein (ComEC) (Pimentel and Zhang, 2018), and the ATPase protein (ComF) (Diallo et al., 2017).
- (2) Protection of the translocated ssDNA from degradation. Single-stranded DNA binding proteins (SSB) protect internalized ssDNA from degradation by nucleases and limit the loading of the RecA recombinase. In *S. pneumoniae*, SsbB directly protects internalized ssDNA and maintains a DNA reservoir in the cell (Attaiech et al., 2011). DprA, a member of the recombination mediator proteins (RMP) family dedicated to natural bacterial transformation, interacts with naked and SSB-coated ssDNA and also protects DNA (Mortier-Barriere et al., 2007; Quevillon-Cheruel et al., 2012).
- (3) Integration into the chromosome of the incoming DNA by homologous recombination or a reconstitution of an autonomous plasmid. The integration of the internalized ssDNA into the chromosome is catalyzed by the RecA recombinase. Recombination mediators are required to load RecA on SSB-coated ssDNA. DprA was reported in *S. pneumoniae* as being a key partner of RecA, acting as a specific mediator for its loading on the incoming ssDNA (Mortier-Barriere et al., 2007; Quevillon-Cheruel et al., 2012). The establishment of plasmid DNA requires a single strand annealing activity to pair internalized complementary plasmid DNA fragments in order to reconstitute a circular replicon in naturally transformable bacteria such as *S. pneumoniae* and *Bacillus subtilis* (Saunders and Guild, 1981; Kidane et al., 2009).

The *D. radiodurans* bacterium, characterized by its extreme resistance to the lethal effects of ionizing and ultraviolet radiations, has been shown to be naturally transformable (Moseley and Setlow, 1968), a property that contributed to the development of this species as a model organism. However, discovering the proteins playing an important role in *D. radiodurans* natural transformation has never been a central question in the field. Many studies, focusing on *D. radiodurans* radioresistance, revealed that its extreme resistance to DNA damaging agents is correlated with its ability to reconstruct a functional genome from hundreds of chromosomal fragments, mainly through an extended synthesis-dependent strand annealing (ESDSA) pathway and through homologous recombination, two pathways mediated by RecFOR and RecA (Zahradka et al., 2006; Slade et al., 2009; Bentchikou et al., 2010). A RecA-independent single strand annealing (SSA) process also participates in DNA double strand break (DSB) repair and requires DdrB, a *Deinococcus* single stranded DNA binding protein exhibiting a single strand annealing activity (Xu et al.,

2010; Bouthier de la Tour et al., 2011; Sugiman-Marangos et al., 2016). Remarkably, it was shown that the cells devoid of DdrB were affected in the establishment of plasmid DNA during natural transformation, a process that requires pairing of internalized plasmid single stranded DNA fragments, whereas they were proficient in transformation by a chromosomal DNA marker that integrates into the host chromosome through homologous recombination (Bouthier de la Tour et al., 2011). This study provided the first evidence linking *Deinococcus* specific DNA repair proteins with natural transformation and raising numerous questions regarding the potential specifics of transformation mechanisms in this species.

Here we identified, by genome analysis, candidate genes described in other bacterial species as being involved in different transformation steps. We focused on genes that might be involved in the protection of the incoming ssDNA and its genome integration or plasmid reconstitution. We showed that, as in other bacteria, DprA plays a key role in DNA transformation and we highlighted the ability of the RecFOR complex and the DdrB protein to partially compensate the absence of DprA in the DNA transformation process.

RESULTS

Identification of *D. radiodurans* Genes Involved in DNA Uptake and DNA Translocation During Transformation

Although *D. radiodurans* is classified as a Gram positive bacterium, it was shown that cells contain two membranes (Thompson and Murray, 1981). We identified, by sequence similarity, several *D. radiodurans* genes encoding type IV pilus biogenesis systems, type IV pili, and a fimbrial subunit (Table 1). The *dr0774* gene encodes a protein belonging to the secretin (PilQ) family, which forms, in *V. cholerae*, the entry pore through the outer membrane for the transforming DNA (Seitz and Blokesch, 2014). This protein was found to be one of the most abundant *D. radiodurans* proteins of the inner/outer membrane (Farci et al., 2014). In *D. radiodurans*, the *dr0774* gene is the last gene of an operon encompassing *pilM* (*dr0770*), *pilN* (*dr0771*), *pilO* (*dr0772*), and *pilP* (*dr0773*) genes that encode putative homologs of proteins belonging to an inner membrane alignment subcomplex of type IV pili in *Pseudomonas aeruginosa* and in *V. cholerae* (Burrows, 2012; Tammam et al., 2013). The *dr0548* and *dr1232* genes are homologous to genes encoding type IV pilin in *Deinococcus geothermalis* (Saarimaa et al., 2006). In *Dichelobacter nodosus*, the FimA fimbrial subunit is essential for transformation (Kennan et al., 2001). A homolog of *fimA* was also found in the recent published sequence of *D. radiodurans* R1 (Hua and Hua, 2016). Finally, *dr2065*, *dr1964*, and *dr1963* are *Deinococcus* gene homologs of *pilD*, *pilB*, and *pilT*, encoding the PilD peptidase involved in prepilin processing (Marsh and Taylor, 1998) and two traffic ATPases, PilB and PilT, required for DNA uptake and efficient transformation in *V. cholerae* (Seitz and Blokesch, 2013b), respectively.

We also identified genes encoding the proteins homologous to ComEA (*dr0207* and *dr1855*), ComEC (*dr1854* and *dr0361*),

TABLE 1 | *D. radiodurans* genes putatively involved in natural transformation.

Protein function	Protein	Gene (White et al., 1999)	Gene (Hua and Hua, 2016)
The competence pseudopilus			
Inner membrane subcomplex of type IV pili	PilM	dr0770	A2G07_09655
Inner membrane subcomplex of type IV pili	PilN	dr0771	A2G07_09650
Inner membrane subcomplex of type IV pili	PilO	dr0772	A2G07_09645
Inner membrane subcomplex of type IV pili	PilP	dr0773	A2G07_09640
Secretin	PilQ	dr0774	A2G07_09635
Pilin or pseudopilin (type IV)	PilI	dr0548	none
Pilin or pseudopilin (type IV)	PilJ	dr1232	A2G07_07365
Pilin or pseudopilin (type I)	FimA	None	A2G07_02580
Prepilin/peptidase	PilD	dr2065	A2G07_03280
Pilus retraction	PilT	dr1963	A2G07_03820
Pilus extension	PilB	dr1964	A2G07_03815
DNA translocation machinery			
DNA receptor	ComEA	dr0207	A2G07_12495
	ComEA	dr1855	A2G07_04330
Membrane channel	ComEC	dr1854	A2G07_04335
	ComEC	dr0361	A2G07_11730
ATP-binding protein	ComF	dr1389	A2G07_06600
ATP-binding protein	ComA	dr0847	A2G07_09275
Processing DNA			
DprA	DprA	dr0120	A2G07_12915
Single stranded DNA binding protein	DdrB	dr0070	A2G07_00040
Complex RecFOR	RecF	dr1089	A2G07_08075
Complex RecFOR	RecO	dr0819	A2G07_09415
Complex RecFOR	RecR	dr0198	A2G07_12540
Homologous recombination			
Recombinase	RecA	dr2340	A2G07_01875

ComA (dr0847), and ComF (dr1389) proteins constituting a potential DNA translocation machinery similar to that found in Gram negative bacteria (Table 1). ComEA, a predicted membrane-integral protein, shuttles the dsDNA through the periplasm. The transport of DNA across the cytoplasmic membrane is mediated by ComEC (a cytoplasmic membrane channel), potentially in concert with ComF, to introduce ssDNA into the cytoplasm.

To determine whether these proteins may be involved in *D. radiodurans* transformation, we constructed deletion mutants of dr0774 (pilQ), dr0548 (pilIV), dr1232 (pilJ), fimA, dr2065 (pilD), dr1963 (pilT), dr1964 (pilB), dr0207 (comEA), dr1855 (comEA), dr1854 (comEC), dr0361 (comEA), dr1389 (comF), and dr0847 (comA) genes. Since *D. radiodurans* contains from 4 to 10 genome equivalents, the transformants were purified on selective medium in order to obtain homogenotes containing the deleted allele on all copies of the genome. Homogenotes of these deletion

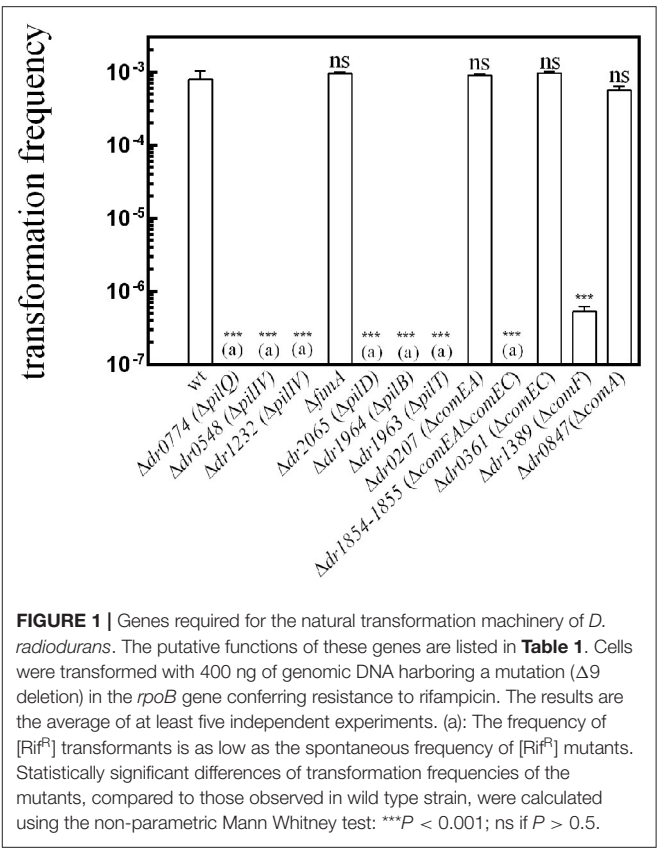


FIGURE 1 | Genes required for the natural transformation machinery of *D. radiodurans*. The putative functions of these genes are listed in Table 1. Cells were transformed with 400 ng of genomic DNA harboring a mutation ($\Delta 9$ deletion) in the *rpoB* gene conferring resistance to rifampicin. The results are the average of at least five independent experiments. (a): The frequency of [Rif^R] transformants is as low as the spontaneous frequency of [Rif^R] mutants. Statistically significant differences of transformation frequencies of the mutants, compared to those observed in wild type strain, were calculated using the non-parametric Mann Whitney test: ***P < 0.001; ns if P > 0.5.

mutants were easily obtained after two cycles of purification on selective medium, and the purity of the strains was verified by PCR. These deletion mutants did not exhibit any significant effect on *D. radiodurans* growth in optimal growth conditions (Table S1), indicating that these genes are not essential for cell viability.

We show here that natural transformation is totally abolished in $\Delta dr0774$ (pilQ), $\Delta dr0548$ (pilIV), $\Delta dr1232$ (pilJ), $\Delta dr2065$ (pilD), $\Delta dr1963$ (pilT), $\Delta dr1964$ (pilB), and $\Delta dr1854\Delta dr1855$ (comEC comEA) mutant bacteria (Figure 1). The transformation frequency was reduced by a factor of $\sim 2,000$ in $\Delta dr1389$ bacteria devoid of the ComF protein (Figure 1). In contrast, $\Delta fimA$, $\Delta dr0207$ (comEA), $\Delta dr0361$ (comEC), and $\Delta dr0847$ (comA) mutant bacteria remained proficient for transformation.

To verify that PilD, type IV pilins, PilB, PilT, and ComEC-ComEA are essential for natural transformation and that ComF plays a major role in the transformation process, we performed complementation assays in the mutant strains. We found that expression of the corresponding wild type pilD (dr0548), comEC-comEA (dr1854-dr1855), and comF (dr1389) genes from a non-essential ectopic chromosomal locus [amyE (dr1472)] in the strains devoid of these proteins restored wild-type transformation frequency (Figure S1). Unfortunately, we were not able to obtain strains expressing, in trans, dr1232, and dr0774.

RecA Is Essential for Chromosomal Transformation but Not for Plasmid Establishment

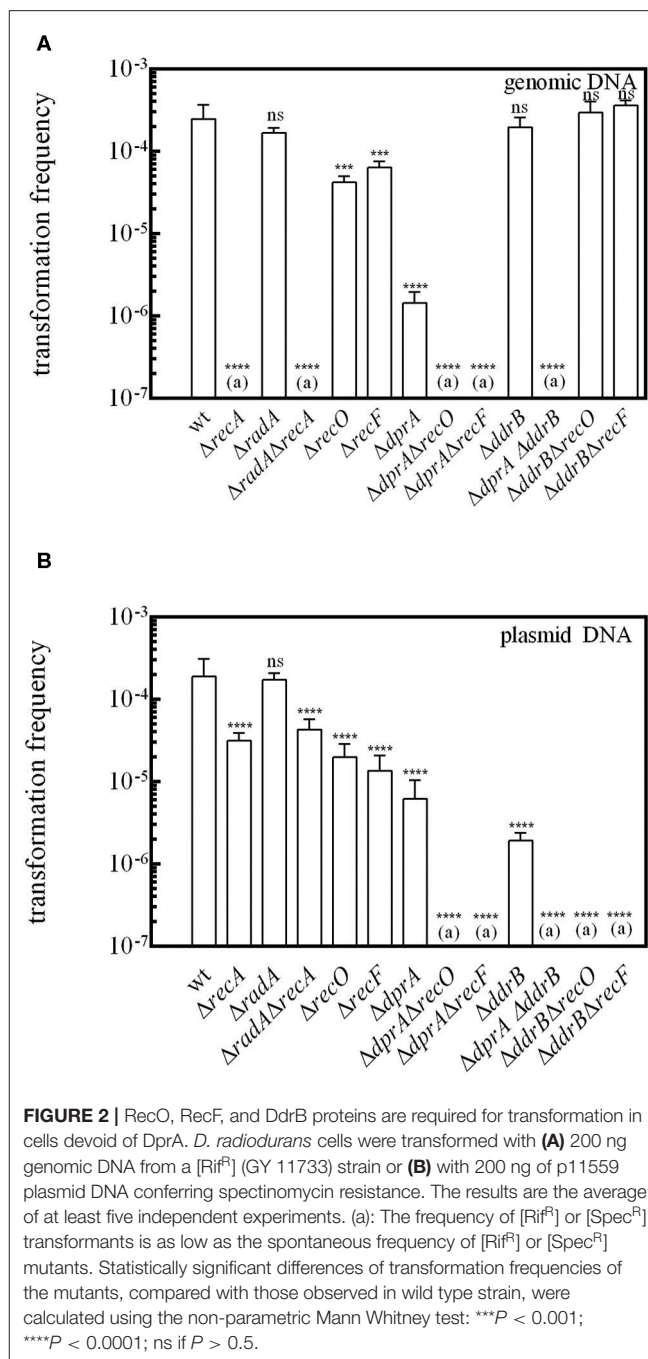
Transformation of bacteria by genomic DNA requires integration of the incoming DNA into the host chromosome by homologous recombination. Here, we show that, as in other species, the transformation of *D. radiodurans* by genomic DNA is a mechanism totally dependent on the RecA protein (**Figure 2A**), whereas cells devoid of the RecA protein were only slightly affected in the frequency of transformation by plasmid DNA (5-fold reduction) (**Figure 2B**). We also measured the frequency of transformation of *D. radiodurans* bacteria devoid of the RadA protein, a conserved homologous recombination effector acting with RecA recombinase to promote ssDNA integration in *S. pneumoniae* (Marie et al., 2017). The $\Delta radA$ mutant bacteria exhibited the same transformation frequency by genomic and plasmid DNA as the wild type cells (**Figure 2**), indicating that RadA is not involved in *D. radiodurans* transformation. Moreover, $\Delta radA \Delta recA$ bacteria exhibited the same transformation frequency as $\Delta recA$ mutant bacteria.

The *Deinococcal* RecF, RecO, and RecR recombination mediator proteins, by their ability to load RecA onto its single-stranded DNA substrate, play a crucial role in DNA double strand break repair via ESDSA and recombinational repair pathways (Bentchikou et al., 2010). We show here that the absence of the RecF and RecO proteins reduces the efficiency of transformation by genomic DNA by a factor of 3.6 and 5.4, respectively (**Figure 2A**), suggesting that other protein(s) are able to promote the loading of RecA on the internalized single-stranded DNA. Expression *in trans* of the *recO*, *recF*, and *recA* genes from the *amyE* ectopic chromosomal locus in the strains devoid of these proteins fully restored a wild type transformation frequency for genomic DNA (**Figure S2**). These results confirm the involvement of these three proteins in the transformation process of genomic DNA.

DprA Plays a Major Role in DNA Transformation

The recombination mediator DprA protein plays a central role in *B. subtilis* and *S. pneumoniae* transformation by facilitating the loading of RecA protein on single-stranded DNA covered with SSB (Morrison et al., 2007; Quevillon-Cheruel et al., 2012; Lisboa et al., 2014). A putative *D. radiodurans* homolog of the *dprA* gene (*dr0120*) (**Table 1**) encodes a protein harboring the 3 characteristic domains of the DprA-family: an N-terminal domain [Sterile Alpha Motif (SAM fold)], a central dimeric domain (Rossmann Fold) and a C-terminal domain [Winged Helix (WH fold)] (**Figure S3**). Using the SWISS MODEL software (Waterhouse et al., 2018), the high sequence identity of 39.47% between DrDprA and *Rhodospseudomonas palustris* DprA (PDB ID: 3MAJ) allowed us to build a convincing 3D structural model (**Figure 3A**). The model cover is almost complete and highly reliable, with a coverage from residues Ala9 to Arg370 (the end of DrDprA) and a QMEAN of -0.91 .

The transformation frequency of cells devoid of DprA by genomic and plasmid DNA dropped 160- and 21- fold, respectively (**Figures 2A,B**), contrasting with the minor effect of



recO and *recF* deletions. In the $\Delta dprA$ mutant, the expression of *dprA* from the ectopic locus *amyE* restored a wild-type level of transformation frequency (**Figure S2**). These results suggest a major role of DprA in DNA transformation, as reported in other bacteria, probably by facilitating the loading of RecA protein onto ssDNA.

Loading of RecA onto the invading ssDNA requires an interaction between SpDprA and SpRecA in *S. pneumoniae* (Mortier-Barriere et al., 2007). Our 3D structural model of DrDprA harbors the electronegative patch found at the surface of SpDprA involved in the interaction with SpRecA (**Figure 3B**,

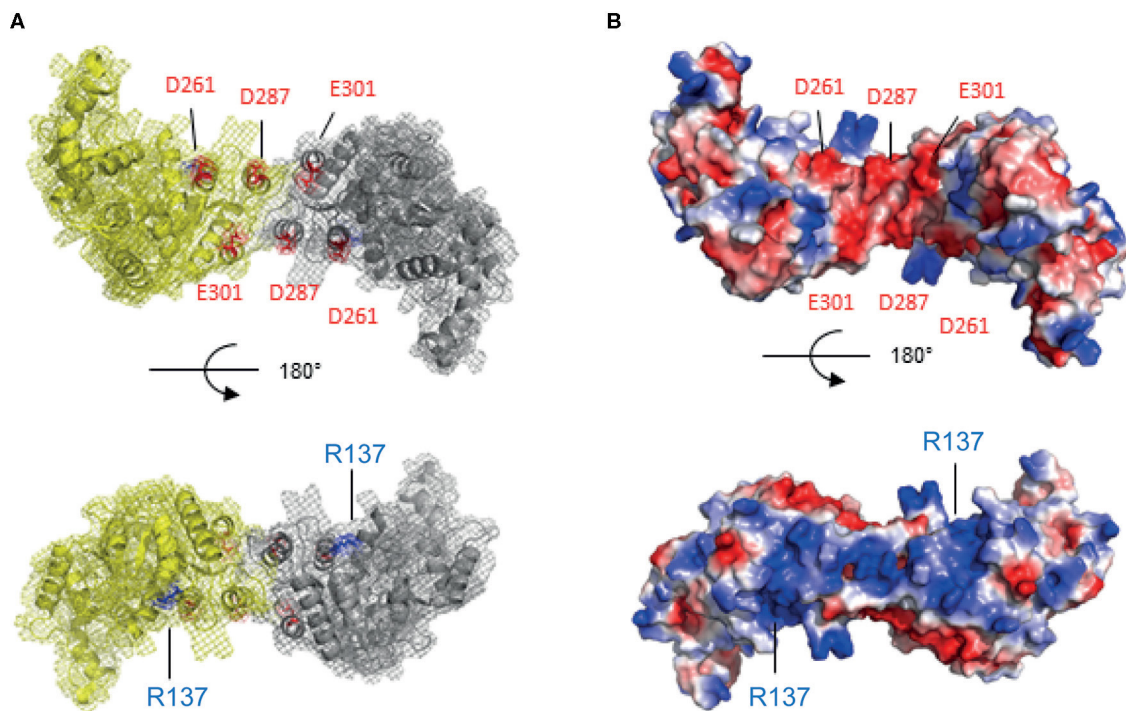


FIGURE 3 | Calculated structural model of DprA dimer from *D. radiodurans*. The WH CTD is missing for clarity. **(A)** Two views as ribbon schematic presentations and surface mesh. **(B)** Same two views of the electrostatic surface of the protein. The knowledge of Sp and Hp DprAs, the electronegative face (in red) in the interaction with RecA via the acidic residues D261, D287, and E301 (top), and the electropositive face (in blue), could thus be involved in the DNA interaction via the R137 residue (bottom).

top). Therefore, we hypothesized that the interplay between DrDprA and DrRecA might use the same strategy, based on electrostatic interactions between electronegative Asp and Glu residues of DprA and electropositive Arg and Lys residues of RecA. Indeed, we observed, using the yeast two-hybrid system, that DrDprA and DrRecA can efficiently interact in an intracellular context (**Figure 4**). As for the *S. pneumoniae* system (Lisboa et al., 2014), the first 27 amino acids of RecA, involved in its polymerization, have to be removed to reveal the interaction between DrDprA and DrRecA (**Figure 4**). Together, these results strongly suggest that DrDprA can function as a recombination mediator through RecA loading onto ssDNA *in vivo*, as described in other bacteria.

However, transformation of a $\Delta dprA$ mutant by genomic DNA was not completely abolished (**Figure 2A**). We postulated that the RecFOR pathway could be involved in these residual events. In order to test this hypothesis, we constructed $\Delta dprA \Delta recF$ and $\Delta dprA \Delta recO$ double mutants. The transformation frequency of genomic DNA was completely abolished in these double mutants (**Figure 2A**) suggesting that, in the absence of DprA, the RecFOR complex may favor the loading of RecA protein on the internalized ssDNA.

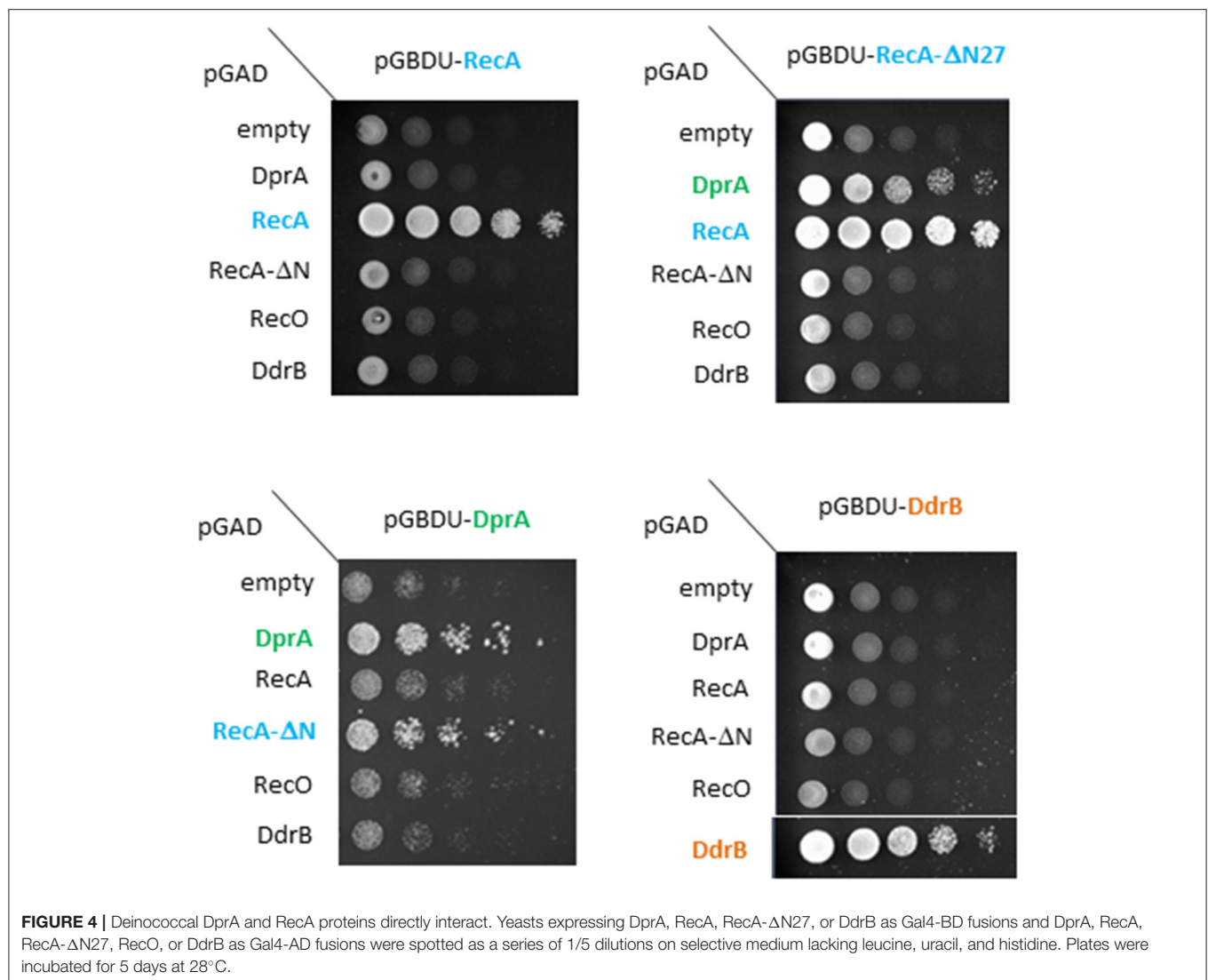
Interestingly, we also observed, in the absence of DprA, a 21-fold reduction of the transformation frequency by plasmid DNA (**Figure 2B**), even though these transformation events do not involve homologous recombination between plasmid and host genome DNA. This result suggests that DprA has a role

in plasmid transformation different from its requirement for RecA loading.

DprA from *B. subtilis* and *S. pneumoniae* are involved, in addition to SSB, in the protection of the internalized ssDNA from degradation. Using a purified recombinant DrDprA protein, we first demonstrated its ability to bind ssDNA *in vitro*. The affinity of the protein increased with the length and the GC enrichment of the ssDNA substrate (**Figure 5**). The affinity for the ssDNA is of the same order of magnitude as the other studied DprAs (Lisboa et al., 2014; Dwivedi et al., 2015). One arginine residue has been shown to be important by biochemical and structural studies in *S. pneumoniae* and *H. pylori* DprA (R115 and R52, respectively). Because this residue is strictly conserved in all known DprA proteins (**Figure S3**), we unambiguously designated the R137 residue of DrDprA as the major determinant for the interaction of the electropositive face of DprA with ssDNA (**Figure 3B**, bottom). These results indicate that DrDprA has the same properties as the other studied DprA and might be involved in ssDNA protection when binding to the ssDNA and therefore protect the incoming ssDNA in plasmid transformation as well.

The N-Terminal SAM Domain of DprA May Be Implicated in the Expression or the Stability of the Protein

As mentioned earlier, DrDprA is a modular protein containing 3 domains, as described in *R. palustris* (**Figure S3**): a central

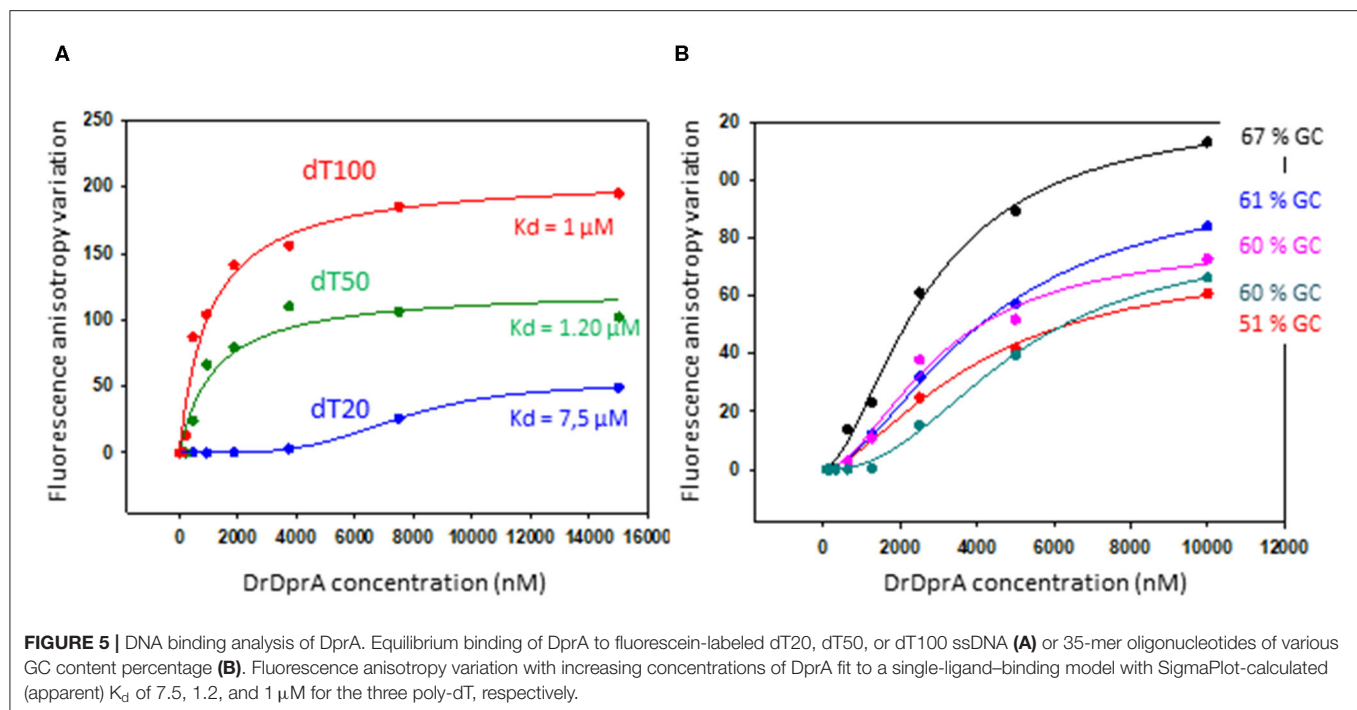


Rossmann Fold domain, an N-terminal SAM domain and, a C-terminal WH domain (Dwivedi et al., 2015; Lisboa et al., 2019). However, the organization around the central Rossmann Fold domain of DprA from different species is variable, as one of the two C-terminal and N-terminal domains may be absent.

Thus, we investigated whether the deletion of the N-terminal ($\Delta 80_DprA$) or the C-terminal ($DprA_ \Delta 63$) residues of the *D. radiodurans* DprA protein affected transformation frequency. For this purpose, we constructed *D. radiodurans* mutants expressing the truncated forms of DprA by allelic replacement of the wild type *dprA* gene. The absence of the C-terminal domain only decreased the transformation frequency of genomic DNA 10-fold (Figure 6A) and did not affect plasmid transformation (Figure 6B). On the other hand, in the absence of the DprA N-terminal domain, the transformation frequency of genomic DNA and plasmid DNA (Figure 6) is reduced by 190- and 40-fold, respectively, conferring a phenotype very similar to those of a $\Delta dprA$ strain (Figure 2).

Since the expression and the stability of the truncated proteins can be modified with respect to that of the wild-type protein, strains expressing the intact and the truncated proteins fused to an HA tag were constructed to verify protein expression by Western Blot analysis. As shown in Figure 6, the transformation frequency obtained in the strains expressing the DprA::HA protein and the truncated proteins fused to a HA-tag were the same as those obtained in strains expressing the proteins without the HA-tag (Figure 6), indicating that this tag did not alter the functionality of these proteins. While DprA::HA (41 kDa) and DprA Δ Cter::HA (35 kDa) were detected by Western Blot in similar amounts, Δ Nter DprA::HA protein (33 kDa) was not detected (Figure 7). Therefore, the lower transformation frequency of Δ Nter *dprA* mutant strain, which is comparable to that of $\Delta dprA$ mutant, is likely due to a reduced stability of this truncated protein or a lower expression of the Δ Nter *dprA* gene.

Taken together, these results suggest that the C-terminal domain of DrDprA is not essential for DNA protection of the



internalized DNA, but rather plays a role in the interaction with the RecA protein and the loading of RecA on the internalized genomic ssDNA, whereas the N-terminal domain is required for the correct folding of the protein or for its stability.

DprA Is Not Implicated in the Radioresistance of *D. radiodurans*

Because of its ability to protect ssDNA against degradation and its interaction with the RecA protein, we also investigated if DprA might participate in *D. radiodurans* radioresistance. Survival after exposure to γ -irradiation, at doses ranging from 0 to 15 kGy, was measured (Figure 8). The $\Delta dprA$ mutant showed the same resistance to γ -radiation as the wild type strain, suggesting that DprA was not required to protect ssDNA generated after irradiation during maturation of the DNA double strand breaks and in the ESDSA repair pathway. The interaction of DprA with the RecA protein is not crucial for ESDSA or recombinational repair of the DNA double strand breaks, confirming the major role of the RecFOR proteins in these repair pathways (Bentchikou et al., 2010).

DdrB Is Required for Transformation of Cells Devoid of DprA

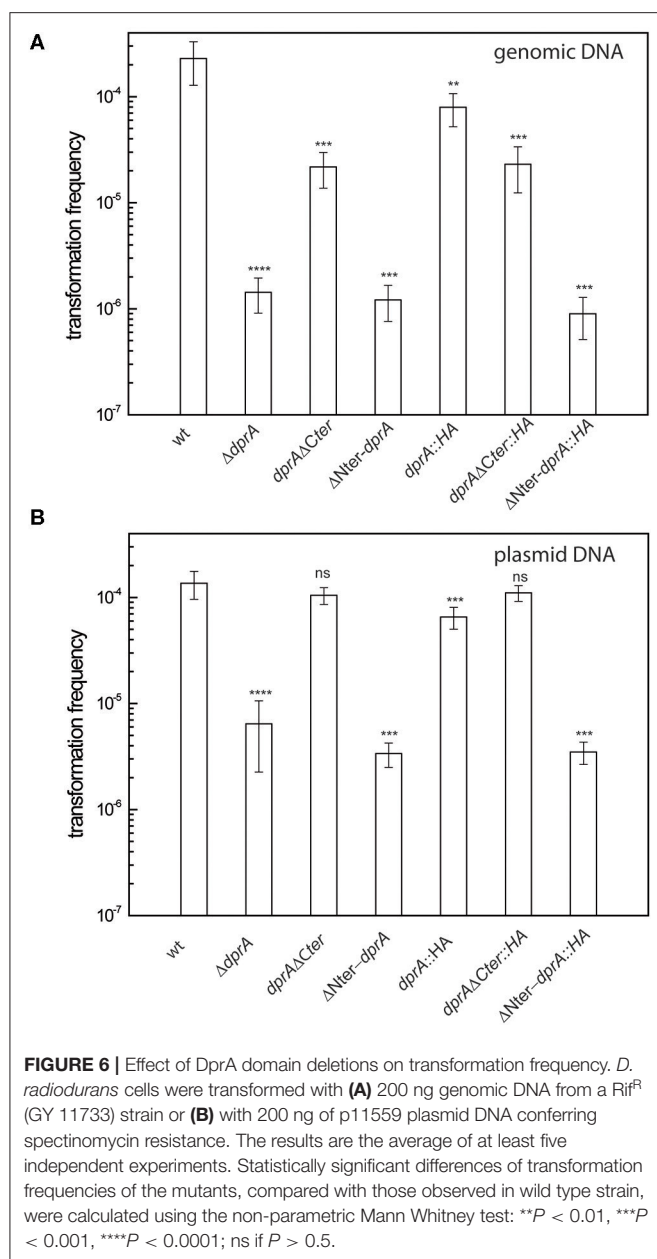
DdrB, a protein specific to *Deinococcaceae*, binds to ssDNA and exhibits some biochemical properties similar to those of the *E. coli* SSB protein (Norais et al., 2009). Whereas the absence of DdrB had no influence on the transformation efficiency of genomic DNA, it strongly affected the frequency of transformation by plasmid DNA (Bouthier de la Tour et al., 2011). We have previously proposed that DdrB likely participates to the plasmid establishment through its single strand annealing activity (Bouthier de la Tour et al., 2011).

Here, we obtained the same result (Figure 2) and showed that expression *in trans* of the *ddrB* gene in a $\Delta ddrB$ mutant restored a wild-type level of transformation frequency with plasmid DNA (Figure S2B).

However, plasmid transformation is not fully abolished in a $\Delta ddrB$ mutant, suggesting that other proteins may be able to compensate for its role in plasmid transformation. The frequency of plasmid transformation was completely abolished in $\Delta ddrB \Delta recO$ and $\Delta ddrB \Delta recF$ double mutants (Figure 2B). These results are difficult to interpret if we take into account the lethal sectoring of the $\Delta ddrB \Delta recO$ (Ithurbide et al., 2015) and $\Delta ddrB \Delta recF$ double mutants and the reduced efficiency of plasmid transformation when cells are devoid of the DdrB protein. In contrast, the frequencies of genomic transformation of $\Delta ddrB$, $\Delta ddrB \Delta recO$, and $\Delta ddrB \Delta recF$ bacteria were the same as those measured in wild type bacteria (Figure 2A).

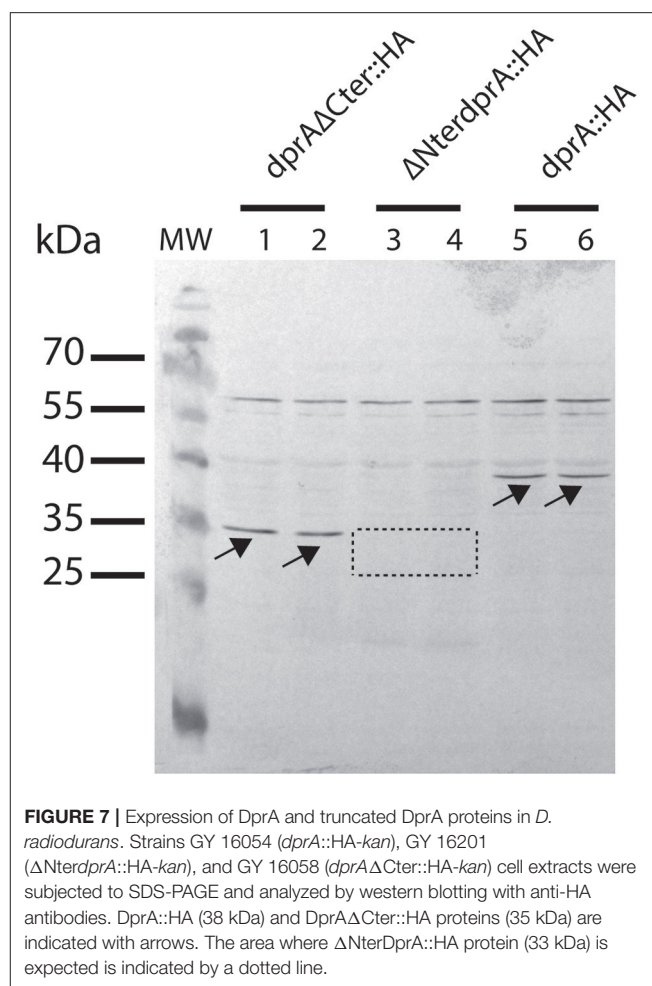
As the ssDNA enters the cell, this becomes a substrate for several single stranded DNA binding proteins, either SSB, DdrB, DprA, RecO, and RecA and thus they all compete at one point with one or several of the others for binding to the ssDNA. Thus, we tested whether some of these proteins could interact among each other using the yeast two-hybrid system. As mentioned earlier, DprA and RecA are able to form homodimers and this interaction was used as a positive control for interaction in these studies (Figure 4). No interaction was detected between DdrB/DprA, DprA/RecO nor DdrB/RecO (Figure 4).

Interestingly, we also showed that transformation by plasmid DNA, and also by genomic DNA, was completely abolished in a $\Delta dprA \Delta ddrB$ double mutant (Figure 2), suggesting that, in the absence of DprA, DdrB might participate in the protection of internalized ssDNA during transformation.



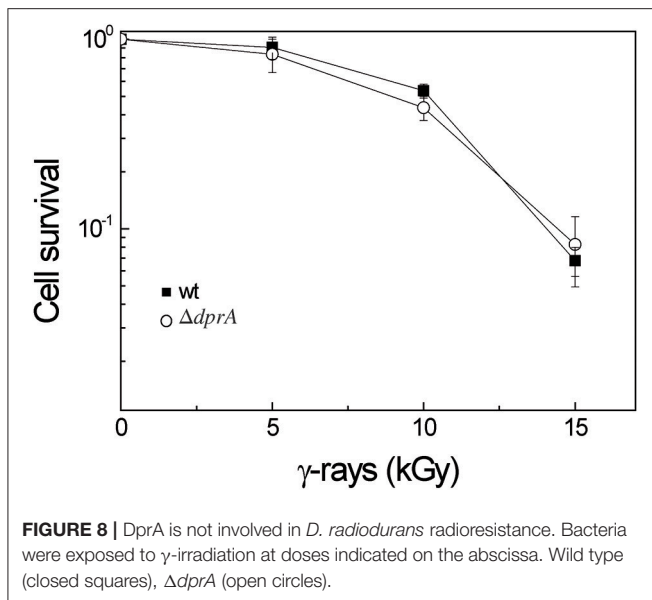
DISCUSSION

D. radiodurans is the object of major interest due to its exceptional capacity to resist high levels of radiation and to reconstruct a functional genome from hundreds of radiation-induced chromosomal fragments. Homologous recombination is a mechanism for repairing DNA double-strand breaks and contributes to preserve the integrity of genomes. It also plays an important role in horizontal gene transfer and, more particularly, in bacterial transformation and thus in the evolution of genomes. Although *D. radiodurans* natural transformation allowed its use as a major model organism to study radioresistance, little is known about the proteins and mechanisms involved in its



transformation. Here, we investigate the involvement of different *Deinococcus* proteins in natural transformation and propose a model showing their role at the three stages of the process: DNA internalization, DNA protection and DNA establishment into the host cell (Figure 9).

Initial stages of DNA transformation involve the internalization of external DNA into the cytosol and therefore the passage of the DNA through the different layers of the cell envelope, a step that represents different challenges in monoderm or diderm bacteria. Although *D. radiodurans* stains Gram-positive in a manner similar to monoderm bacteria, *D. radiodurans* possesses an outer membrane (Thompson and Murray, 1981). However, *D. radiodurans* lacks the lipopolysaccharides (LPS) considered as a defining characteristic of the Gram-negative group (Gupta, 2011). Due to this cell envelope characteristic, *D. radiodurans*, and more generally the *Deinococcus-Thermus* phylum, is proposed to be an intermediate lineage in the monoderm-diderm transition in bacterial evolution and that the LPS appeared later in evolution (Gupta, 2011). Due to this difference in cell envelope structure, Gram-negative and Gram-positive bacteria have different DNA uptake apparatus (Mell and Redfield, 2014). Where most of



the competent species from both groups share the presence of ComeEA (Seitz et al., 2014), a DNA receptor, and ComeEC, a conserved membrane pore mediating the transfer of one DNA strand through the inner membrane (Pimentel and Zhang, 2018), Gram-negative bacteria possess another pore located in the outer membrane. This pore is formed by PilQ (Seitz et al., 2014) and the external DNA is pulled through by a type IV derived competence pilus. Here, after a genomic survey for putative DNA uptake genes, we report for the first time the major proteins involved in the competence pseudo-pilus and the DNA translocation machinery in *D. radiodurans*. We showed that the DNA translocating machinery of *D. radiodurans* is related to type IV pili with the predicted PilQ (DR0774) protein, the pilins DR0548 and DR1232, the PilD (DR2065) peptidase subunit and the PilB (DR1964), PilT (DR1963) ATPases being essential for transformation. In addition, *D. radiodurans* homologs of ComeEA (DR1855) and ComeEC (DR1854), generally found in competent bacteria, are similarly essential for transformation. The presence in the cells of a second homolog of ComeEA (DR0207) and ComeEC (DR0361) was not sufficient to compensate the absence of DR1855 and DR1854. The results obtained in this study suggest that the model of the DNA-uptake machinery in *D. radiodurans* is similar to that extensively described in the Gram-negative bacterium *V. cholerae* (Seitz and Blokesch, 2013b) (Figure 9, I). Further and in depth studies of the DNA uptake apparatus in *D. radiodurans* could potentially provide insights into the evolution of the Type 4 apparatus in relation to the evolution of the outer layers and competence in the transition from monoderm to diderm bacteria.

In bacteria, competence development is often tightly regulated in response to specific environmental signals and is usually limited to a small time-frame window during the growth cycle [for review, see (Seitz and Blokesch, 2013a)]. Different signals, such as genotoxic stresses causing DNA damage, including UV light or some antibiotic treatments (Dorer et al., 2011; Corbinais

et al., 2016), quorum sensing (Havarstein et al., 1996; Claverys et al., 2006; Boudes et al., 2014), or starvation for carbon sources (Redfield, 1991) are needed to trigger the physiological state of competence. Competence regulatory circuits are not universal and differ according to the species. In *B. subtilis*, the transcriptional regulator ComA regulates the development of genetic competence (Comella and Grossman, 2005) and is part of, along with ComP, a two component system. An analog of ComP was not described in *D. radiodurans*. *D. radiodurans*, as are *Neisseria gonorrhoeae* and *H. pylori*, is constitutively competent throughout its growth phases (Tirgari and Moseley, 1980; Aas et al., 2002; Dorer et al., 2011). In *D. radiodurans*, in the absence of the DR0847 protein, annotated as being a member of the ComA family, the transformation frequency is the same as the wild type strain, suggesting that DR0847 is not involved in the regulation of genetic competence in this bacterium.

We also studied the implication of several recombination and DNA repair proteins during natural transformation of *D. radiodurans*. During internalization, the exogenous ssDNA is taken in by numerous proteins allowing its protection from nuclease attack and integration into the genome (Figure 9, II and III). These steps mainly involve single-strand DNA binding proteins, homologous recombination mediator proteins and RecA recombinase in the different bacterial species studied so far [for review (Kidane et al., 2012; Johnston et al., 2014)]. The transformation frequency of genomic DNA that requires integration of the DNA into the genome is completely abolished in cells devoid of the RecA protein. In *S. pneumoniae*, the incoming DNA is immediately degraded in the absence of RecA, demonstrating that the internalized ssDNA requires protection prior to the search for homology and that RecA is needed for this protection (Berge et al., 2003). The reduction of plasmid DNA transformation frequency in the absence of the RecA protein in *D. radiodurans* suggests that, as in *S. pneumoniae*, RecA has a role in protecting incoming ssDNA during the first step of the natural transformation process, corresponding to DNA internalization, although it might not be the key player, as the reduction is not drastic. In *S. pneumoniae* and *B. subtilis*, RadA is involved in the transformation by genomic DNA (Kruger et al., 1997; Carrasco et al., 2002; Burghout et al., 2007). Here, we showed that RadA is not involved in *D. radiodurans* transformation.

Numerous studies about homologous recombination in DNA repair have shown that RecA requires Recombination Mediator Protein (RMP) for its loading onto single stranded DNA. In *D. radiodurans*, we observed a 160-fold reduction of genomic DNA transformation in the absence of DrDprA. Therefore, we propose that, as previously demonstrated in *S. pneumoniae* (Quevillon-Cheruel et al., 2012), DrDprA facilitates the loading of RecA protein on ssDNA (Figure 9, IIIa), a hypothesis supported by the positive results of the yeast two-hybrid test between DrDprA and DrRecA. Interestingly, genomic transformation is not completely abolished in the absence of DprA. Although deletion of *recO* or *recR*, encoding proteins of the RecFOR RMP complex, had only a slight effect on genomic DNA transformation efficiency, genomic DNA transformation was completely abolished in $\Delta dprA \Delta recF$ and $\Delta dprA \Delta recO$ double mutants. We propose that the RecFOR complex could partially compensate for the role of DprA as a

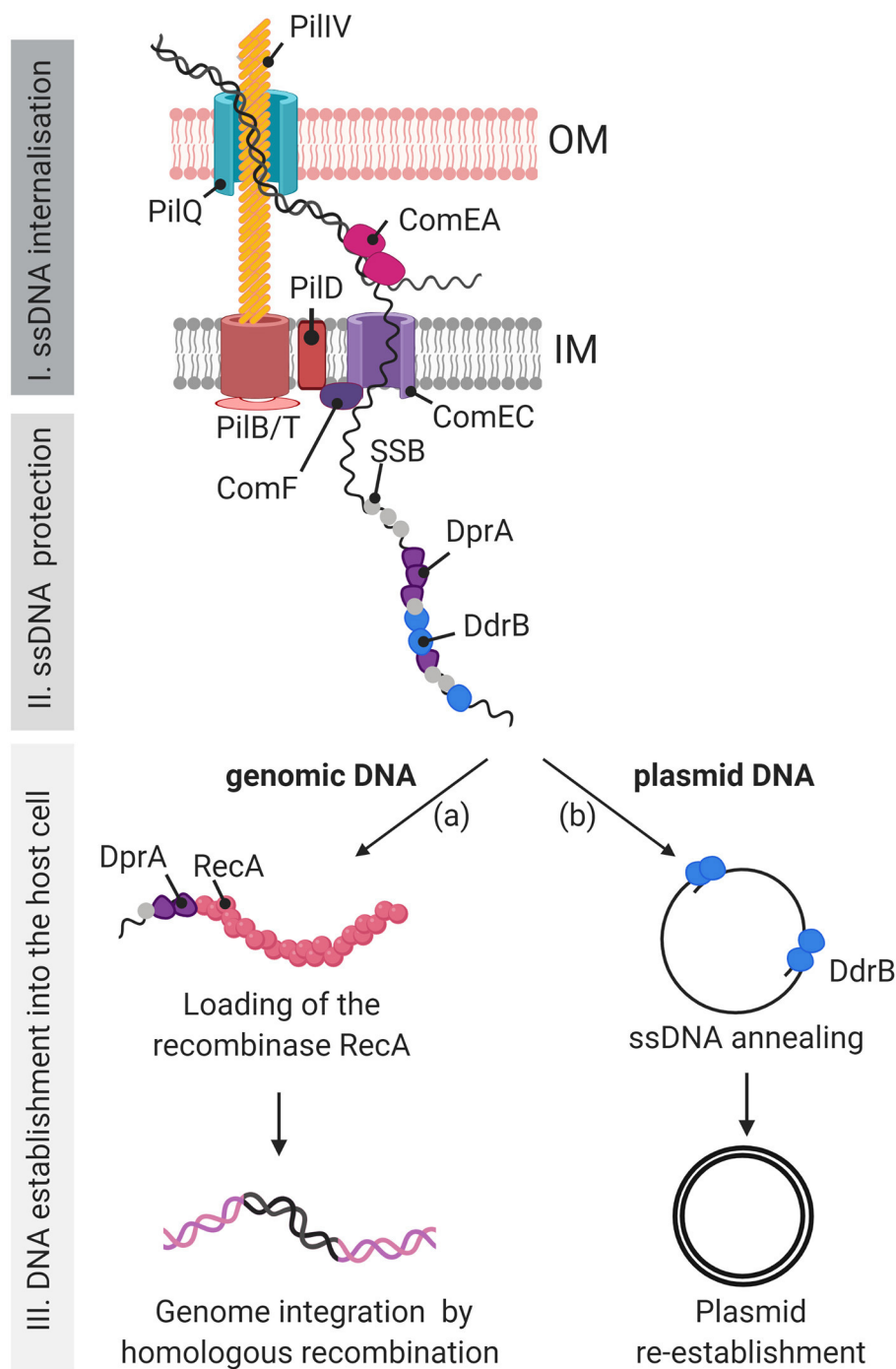


FIGURE 9 | Model of the DNA uptake apparatus and DNA processing during natural transformation in *D. radiodurans*. Our study suggests that, during natural transformation, the exogenous DNA is internalized into the *D. radiodurans* cytosol via a type IV-like DNA uptake machinery, similar to that of *V. cholerae*, which is composed of homologues of the pilins PilIV (DR0548 and DR1232), the outer membrane (OM) channel PilQ (DR0774), the ATPases PilB (DR1964) and PilT (DR1963), and the pre-pilin peptidase PilD (DR2065). We present here a hypothetical inner membrane channel which could be similar to PilC in *V. cholerae* (Matthey and Blokesch, 2016). (I) The external DNA passes through the OM via the PilQ channel and the DNA binding protein ComEA (DR1855) pulls the DNA into the periplasm. Only one DNA strand is translocated into the cytoplasm through the ComEC (DR1854) inner membrane (IM) channel, probably assisted by ComF (DR1389). (II) The ssDNA is protected after its entry in the cell by ssDNA binding proteins such as SSB, DdrB and DprA (DR0120). (III) Plasmid and genomic DNA are differentially processed. (IIIa) DprA, bound to genomic DNA, will favor the loading of RecA onto the genomic DNA to initiate homologous recombination with the genome of the host cell. (IIIb) In a RecA independent manner, DdrB, via its single strand annealing (SSA) activity, favors the annealing of plasmid DNA fragments to allow DNA replication, reconstitution of a circular double-stranded DNA molecule, and establishment of the plasmid in the host cell. The RecO protein is proposed to play a major role as a back-up transformation protein for both the genomic and plasmid DNA processing pathways either by replacing DdrB for the annealing of the plasmid DNA fragments, or by replacing DprA for the loading of RecA onto genomic DNA prior to recombination through the RecFOR pathway (not presented here).

recombination mediator protein to load RecA on the internalized ssDNA or, alternatively, the RecFOR complex and the DprA protein act at a different step of the recombination process, i.e., they are epistatic.

Although plasmid transformation does not require genome integration of the incoming DNA by homologous recombination, we observed in *D. radiodurans* a 21-fold reduction of plasmid transformation frequency in the absence of the DprA protein. This suggests that DprA may also be involved in the protection of the internalized plasmid DNA fragments by coating the ssDNA, as shown in *S. pneumoniae* (Mortier-Barriere et al., 2007; Quevillon-Cheruel et al., 2012). By extension, we propose that DrDprA might also protect the incoming genomic DNA in addition to favoring the loading of RecA on ssDNA (Figure 9, II). Moreover, Yadav et al. (2013) have shown that *B. subtilis* DprA anneals complementary strands coated by SsbB to reconstitute a dsDNA circular plasmid molecule (Yadav et al., 2013), another role that can be played by DrDprA in plasmid transformation.

In *B. subtilis*, RecO plays a major role during plasmid transformation, whereas it plays only a minor role during chromosomal transformation (Yadav et al., 2013). It was shown that RecO anneals complementary ssDNA complexed with SsbA proteins (Kidane et al., 2009). This activity is required to reconstitute an intact plasmid from ssDNA fragments. In *D. radiodurans*, the RecO protein seems to play only a minor role in plasmid transformation when DdrB is present in the cells, consistent with the low *in vitro* DNA single strand pairing activity of the Deinococcal RecO protein (Makharashvili et al., 2004) and the single strand annealing activity of the DdrB protein (Xu et al., 2010). In contrast, RecF plays a minor role in *B. subtilis* as well as in *D. radiodurans* for plasmid and chromosomal transformation [(Kidane et al., 2009), this work]. The $\Delta recO$ and $\Delta recF$ single mutants showed lethal sectoring, and this phenotype is increased in $\Delta recO \Delta ddrB$ and $\Delta recF \Delta ddrB$ double mutants (Ithurbide et al., 2015). The limit of detection of transformants by plasmid DNA is therefore reached and makes the interpretation difficult. Previously, we have shown that the *Deinococcus* specific protein, DdrB, is involved in a large number of cellular processes: (i) DdrB participates in the genetic instability of repeated sequences via SSA (Ithurbide et al., 2015), (ii) it is probably required to support blocked replication forks if recombination proteins are absent (Ithurbide et al., 2015), (iii) it participates in early genome repair by SSA after exposure to high doses of ionizing radiation (Xu et al., 2010; Bouthier de la Tour et al., 2011), (iv) it favors reconstitution by single strand annealing of plasmid DNA (Bouthier de la Tour et al., 2011) during plasmid transformation (Figure 9, IIb).

Whereas the frequency of plasmid transformation of the single $\Delta ddrB$ mutant by plasmid DNA was reduced (Bouthier de la Tour et al., 2011), transformation was completely abolished in a $\Delta dprA \Delta ddrB$ double mutant. We also showed that genomic transformation was completely abolished in cells devoid of DprA if the DdrB protein was not present. In the absence of DprA, DdrB might be required during transformation for the protection of internalized ssDNA, a role that might not be played alone by the essential *Deinococcus* SSB protein (DR0100). Thus, in the absence of DprA, DdrB might be involved at two levels in the

transformation by plasmid DNA: (i) protection of the ssDNA against degradation due to its SSB-like properties (Figure 9, II) (ii) reconstitution of the plasmid by its SSA activity (Figure 9, IIb) (Xu et al., 2010; Bouthier de la Tour et al., 2011). Therefore, the DdrB protein, whose expression is strongly induced after irradiation, exhibits pleiotropic roles in the cell that go beyond its role in DNA repair (Tanaka et al., 2004; Bouthier de la Tour et al., 2011).

As for SsbB from *S. pneumoniae*, DdrB loaded on the transforming ssDNA might be a barrier for the recruitment of RecA, requiring the action of a recombination mediator protein. In the absence of DprA, we propose that the RecFOR complex plays this role and alleviates the DdrB barrier to facilitate the loading of RecA by displacing the DdrB protein, as supported by (i) the complete abolition of transformation in absence of both DprA and RecO and (ii) the restoration of the wild type frequency of transformation in the $\Delta recO \Delta ddrB$ and $\Delta recF \Delta ddrB$ double mutants.

Here, we also demonstrated that DprA is not involved in *D. radiodurans* radioresistance, suggesting that the DprA protein is not required for the loading of RecA or ssDNA protection during recombinational repair and ESDSA repair of DNA double strand breaks, RecFOR being the RMP essential in these processes (Bentchikou et al., 2010). Thus, a high transformation frequency observed in *D. radiodurans* is not required for efficient DNA repair of DNA double strand breaks after irradiation.

In conclusion, as in other naturally competent bacteria, the DprA protein plays a major role in *D. radiodurans* natural transformation, whereas the RecFOR complex and the Deinococcal-specific protein DdrB might partially compensate for the absence of DprA, possibly by favoring the loading of RecA on internalized ssDNA and by protecting it from nuclease attack, respectively.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, Oligonucleotides, Media

Bacterial strains and plasmids are listed in Tables 2, 3, respectively. The *E. coli* strain DH5 α was used as the general cloning host and strain SCS110 was used to propagate plasmids prior to introduction into *D. radiodurans* via transformation (Meima et al., 2001). All *D. radiodurans* strains were derivatives of the wild-type strain R1 ATCC 13939. The deletion mutants were constructed by the tripartite ligation method. An antibiotic cassette (kanamycin or chloramphenicol resistance gene) and two 500 bp genomic fragments from upstream and downstream of the coding region of target gene were amplified by PCR using primer pairs that introduced *Bam*HI and *Xba*I restriction sites (to construct *pilT* and *pilB* mutant, we used *Xho*I and *Xba*I restriction sites). The three fragments were ligated together in molecular ratio 1/1/1 (100 ng of 500 bp fragments) so that the antibiotic cassette was flanked by the two genomic fragments. The constructs were then introduced into *D. radiodurans* by genetic transformation selecting for antibiotic resistance. This led to the replacement of the wild-type allele by the mutant

TABLE 2 | Bacterial strains used in this study.

Strains	Description	Source or reference
<i>E. coli</i>		
DH5 α	<i>supE44</i> Δ <i>lacU</i> (Φ 80 <i>lacZ</i> Δ M15) <i>hsdR17</i> <i>recA1</i> <i>endA1</i> <i>gyrA96</i> <i>thi-1</i> <i>relA1</i>	Laboratory stock
SCS110	<i>endA</i> <i>dam</i> <i>dcm</i> <i>supE44</i> Δ (<i>lac-proAB</i>) (<i>F'</i> <i>traD36 proAB lacP'</i> Δ M15)	Laboratory stock
BL21(DE3)Gold	<i>F</i> ⁻ <i>ompT</i> <i>hsdS</i> (<i>rB</i> ⁻ <i>mB</i> ⁻) <i>dcm</i> ⁺ <i>Tet</i> ^r <i>gal</i> λ (DE3) <i>endA</i> <i>Hte</i>	Laboratory stock
<i>D. radiodurans</i>		
R1/GY9613	ATCC 13939	Laboratory stock
GY 11733	<i>rif</i> Δ 9	Mennecier et al., 2004
GY 12835	Δ <i>ddrB</i> Ω <i>kan</i>	Bouthier de la Tour et al., 2011
GY 12958	Δ <i>radA</i> Ω <i>cat</i>	Ithurbide et al., 2015
GY 12965	Δ <i>recF</i> Ω <i>cat</i>	Bentchikou et al., 2010
GY 12966	Δ <i>recO</i> Ω <i>hph</i>	Bentchikou et al., 2010
GY 12968	Δ <i>recA</i> Ω <i>kan</i>	Bentchikou et al., 2010
GY 12970	Δ <i>radA</i> Ω <i>cat</i> Δ <i>recA</i> Ω <i>kan</i>	GY12958 $\times$ GY12968 DNA
GY 13915	Δ <i>ddrB</i> Ω <i>cat</i>	Ithurbide et al., 2015
GY 15121	Δ <i>dprA</i> Ω <i>kan</i>	This work ^a
GY 16052	Δ <i>recO</i> Ω <i>cat</i>	This work ^a
GY 16054	<i>dprA</i> :: <i>HA</i> Ω <i>kan</i>	This work ^a
GY 16056	Δ <i>Nter dprA</i> Ω <i>cat</i>	This work ^a
GY 16058	<i>dprA</i> Δ <i>Cter</i> Ω <i>cat</i>	This work ^a
GY 16084	Δ <i>dprA</i> Ω <i>kan</i> Δ <i>recF</i> Ω <i>cat</i>	GY12965 $\times$ GY15121 DNA
GY 16086	Δ <i>dprA</i> Ω <i>kan</i> Δ <i>recO</i> Ω <i>cat</i>	GY16052 $\times$ GY15121 DNA
GY 16683	Δ <i>dr1854-dr1855</i> Ω (<i>comEC/comEA</i>) Ω <i>kan</i>	This work ^a
GY16201	Δ <i>Nter dprA</i> :: <i>HA</i> Ω <i>kan</i>	This work ^a
GY 16203	Δ <i>ddrB</i> Ω <i>cat</i> Δ <i>dprA</i> Ω <i>kan</i>	GY13915 $\times$ GY15121 DNA
GY 16205	<i>dprA</i> Δ <i>Cter</i> :: <i>HA</i> Ω <i>kan</i>	This work ^a
GY 16956	Δ <i>recF</i> Ω <i>cat</i> Δ <i>ddrB</i> Ω <i>kan</i>	GY12965 $\times$ GY12835 DNA
GY 16964	Δ <i>amyE</i> :: <i>P</i> _{<i>dr1963</i>} <i>dr1963</i> Ω <i>kan</i> Δ <i>dr1963</i> Ω <i>cat</i>	This work ^a
GY 16966	Δ <i>amyE</i> :: <i>P</i> _{<i>dr1963</i>} <i>dr1963</i> Ω <i>kan</i> Δ <i>dr1963</i> Ω <i>cat</i>	GY16964 $\times$ GY17790 DNA
GY 17018	Δ <i>dr0774</i> (<i>pilQ</i>) Ω <i>cat</i>	This work ^a
GY 17019	Δ <i>dr0207</i> (<i>comEA</i>) Ω <i>cat</i>	This work ^a
GY 17020	Δ <i>dr2065</i> (<i>pilD</i>) Ω <i>cat</i>	This work ^a
GY 17021	Δ <i>fimA</i> Ω <i>cat</i>	This work ^a
GY 17052	Δ <i>ddrB</i> Ω <i>cat</i> Δ <i>recO</i> Ω <i>hph</i>	GY13915 $\times$ GY12966 DNA
GY 17130	Δ <i>amyE</i> Ω <i>cat</i>	This work ^a
GY 17132	Δ <i>amyE</i> :: <i>P</i> _{<i>dr0548</i>} <i>dr0548</i> Ω <i>cat</i>	This work ^a
GY 17134	Δ <i>amyE</i> :: <i>P</i> _{<i>dr1854</i>} <i>dr1854-dr1855</i> Ω <i>cat</i>	This work ^a
GY 17136	Δ <i>amyE</i> :: <i>P</i> _{<i>dprA</i>} <i>dprA</i> Ω <i>cat</i>	This work ^a
GY 17138	Δ <i>amyE</i> :: <i>P</i> _{<i>dr1389</i>} <i>dr1389</i> Ω <i>cat</i>	This work ^a
GY 17140	Δ <i>amyE</i> Ω <i>kan</i>	This work ^a
GY 17144	Δ <i>amyE</i> :: <i>P</i> _{<i>dr2065</i>} <i>dr2065</i> Ω <i>kan</i>	This work ^a
GY 17148	Δ <i>amyE</i> :: <i>P</i> _{<i>recO</i>} <i>recO</i> Ω <i>kan</i>	This work ^a
GY 17152	Δ <i>amyE</i> :: <i>P</i> _{<i>dr1964</i>} <i>dr1964</i> Ω <i>kan</i>	This work ^a
GY 17154	Δ <i>amyE</i> :: <i>P</i> _{<i>spac</i>} <i>recA</i> Ω <i>cat</i>	This work ^a

(Continued)

TABLE 2 | Continued

Strains	Description	Source or reference
GY 17156	Δ <i>amyE</i> :: <i>P</i> _{<i>dr0548</i>} <i>dr0548</i> Ω <i>cat</i> Δ <i>dr0548</i> Ω <i>kan</i>	GY17133 $\times$ GY17784 DNA
GY 17158	Δ <i>amyE</i> :: <i>P</i> _{<i>dr1389</i>} <i>dr1389</i> Ω <i>cat</i> Δ <i>dr1389</i> Ω <i>kan</i>	GY17138 $\times$ GY17788 DNA
GY 17160	Δ <i>amyE</i> :: <i>P</i> _{<i>dr2065</i>} <i>dr2065</i> Ω <i>kan</i> Δ <i>dr2065</i> Ω <i>cat</i>	GY17144 $\times$ GY17020 DNA
GY 17162	Δ <i>amyE</i> :: <i>P</i> _{<i>recO</i>} <i>recO</i> Ω <i>kan</i> Δ <i>recO</i> Ω <i>cat</i>	GY17148 $\times$ GY16052 DNA
GY 17166	Δ <i>amyE</i> :: <i>P</i> _{<i>dr1854</i>} <i>dr1854-dr1855</i> Ω <i>cat</i> Δ <i>dr1854-dr1855</i> Ω <i>kan</i>	GY17134 $\times$ GY16683 DNA
GY 17169	Δ <i>amyE</i> :: <i>P</i> _{<i>dprA</i>} <i>dprA</i> Ω <i>cat</i> Δ <i>dprA</i> Ω <i>kan</i>	GY17136 $\times$ GY15121 DNA
GY 17170	Δ <i>amyE</i> :: <i>P</i> _{<i>spac</i>} <i>recA</i> Ω <i>cat</i> Δ <i>recA</i> Ω <i>kan</i>	GY17154 $\times$ GY12968 DNA
GY 17175	Δ <i>amyE</i> :: <i>P</i> _{<i>dr1964</i>} <i>dr1964</i> Ω <i>kan</i> Δ <i>dr1964</i> Ω <i>cat</i>	GY17152 $\times$ GY17792 DNA
GY 18223	Δ <i>amyE</i> :: <i>P</i> _{<i>ddrB</i>} <i>ddrB</i> Ω <i>kan</i> Δ <i>ddrB</i> Ω <i>cat</i>	GY18099 $\times$ GY13915 DNA
GY 17782	Δ <i>dr1232</i> (<i>pilV</i>) Ω <i>cat</i>	This work ^a
GY 17784	Δ <i>dr0548</i> (<i>pilV</i>) Ω <i>kan</i>	This work ^a
GY 17786	Δ <i>dr0847</i> (<i>comA</i>) Ω <i>cat</i>	This work ^a
GY 17788	Δ <i>dr1389</i> (<i>comF</i>) Ω <i>kan</i>	This work ^a
GY 17790	Δ <i>dr1963</i> (<i>pilT</i>) Ω <i>cat</i>	This work ^a
GY 17792	Δ <i>dr1964</i> (<i>pilB</i>) Ω <i>cat</i>	This work ^a
GY 17796	Δ <i>dr0361</i> (<i>comEC</i>) Ω <i>kan</i>	This work ^a
GY 18091	Δ <i>amyE</i> :: <i>P</i> _{<i>recF</i>} <i>recF</i> Ω <i>kan</i>	This work ^a
GY 18097	Δ <i>amyE</i> :: <i>P</i> _{<i>recF</i>} <i>recF</i> Ω <i>kan</i> Δ <i>recF</i> Ω <i>cat</i>	GY18091 $\times$ GY12965 DNA
GY 18099	Δ <i>amyE</i> :: <i>P</i> _{<i>ddrB</i>} <i>ddrB</i> Ω <i>kan</i>	This work ^a

^astrains were constructed by the tripartite ligation method.

TABLE 3 | Plasmid used in this study.

Plasmids	Description	Source or reference
pPS6	Source of chloramphenicol cassette	Passot et al., 2015
p11086	Source of kanamycin cassette	Laboratory stock
p11559	Shuttle vector <i>E. coli</i> / <i>D. radiodurans</i> , Spec ^R	Laboratory stock
p11562	p11559 with a fragment encoding RecA, <i>P</i> _{<i>spac</i>} :: <i>recA</i>	Jolivet et al., 2006
P12764	Source of HA-tag kanamycine cassette	Touelle et al., 2012
pET29	Expression vector	Novagen

counterpart via homologous recombination. *D. radiodurans* is multigenomic, with cells containing from 4 to 10 genome equivalents. Homogenotes of the deletion allele were obtained after two or three cycles of purification on selective medium. The genetic structure and purity of the mutants were verified by PCR.

We used the same strategy (tripartite ligation procedure) to construct strains expressing ectopically (in an *amyE* locus)

genes to perform complementation assays in the corresponding deletion background. The gene of interest (promoter + coding sequence), a 550 bp genomic fragment located upstream *amyE*, and a fragment containing an antibiotic cassette and the downstream region of *amyE* (using genomic DNA of GY 17130 or GY 17140 strains as template) were amplified by PCR using primer pairs that introduced restriction sites. After enzymatic digestion, the three fragments were ligated and the ligation mixture was used to transform the wild type *D. radiodurans* strain. The genetic structure and purity of the mutants were verified by PCR and sequencing. These strains were transformed with genomic DNA of a strain containing a deletion of the corresponding gene to obtain the complemented strains (see **Table 2**). The *recA* gene, which is the third gene of an operon, is expressed from the *Pspac* promoter and was amplified using p11562 plasmid DNA as template.

The sequence of oligonucleotides used for strain construction and diagnostic PCR are listed in **Table S2**. Chromosomal DNA of *D. radiodurans* was extracted as previously described (Norais et al., 2013). PCR amplification of DNA fragments, using plasmid or genomic DNA as a template, was performed using Phusion DNA polymerase (Thermo Scientific) or GoTaq Flexi G2 (Promega).

D. radiodurans strains were grown at 30°C in TGY2X (1% tryptone, 0.2% dextrose, 0.6% yeast extract), or plated on TGY1X containing 1.5% agar, and *E. coli* strains were grown at 37°C in LB (Lysogeny Broth). When necessary, media were supplemented with the appropriate antibiotics used at the following final concentrations: kanamycin, 6 µg/mL; chloramphenicol, 3 µg/mL; rifampicin, 25 µg/mL; spectinomycin, 80 µg/mL, hygromycin 50 µg/mL for *D. radiodurans*, and 40 µg/mL for *E. coli*.

Transformation of *D. radiodurans*

To prepare naturally competent cells, exponentially growing bacteria were harvested by centrifugation, re-suspended at a concentration of 5×10^8 cells/mL in TGY2X medium supplemented with 30 mM CaCl₂ and 10% (V/V) glycerol, and immediately stored at −80°C. For transformation, aliquots (100 µL) of competent cells were thawed on ice and mixed with an equal volume of TGY containing 30 mM CaCl₂ before DNA (genomic or plasmid DNA) was added. After 20 min at 0°C and 60 min at 30°C, 800 µL of TGY2X were added and the cells were incubated for a further 5 h to allow expression of rifampicin (transformation by genomic DNA) or spectinomycin (transformation by plasmid DNA) resistance. Diluted samples were plated on TGY plates containing the appropriate antibiotics.

γ-Irradiation Assay

To measure cell survival after exposure to ionizing radiation, exponential phase cultures, grown in TGY2X, were concentrated to an A_{650nm} = 20 in TGY2X media and irradiated on ice with a ⁶⁰Co-irradiation system (Centre d'Energie Atomique, Saclay, France) at a dose rate of 6,000 Gy/h. Following irradiation, diluted samples were plated on TGY plates. Colonies were counted after 3–4 days incubation at 30°C.

Western Blot Analysis

Mutant *dprA::HAΩkan*, ΔNter-*dprA::HAΩkan* and *dprA* ΔCter::HAΩkan bacteria were grown to an A_{650nm} of 0.4–0.6 in TGY2X and 20 mL of culture was centrifuged. The cell pellets were resuspended in 150 µL of SSC 1X buffer and the cells disrupted as described previously (Bouthier de la Tour et al., 2009). After centrifugation, the protein concentration was measured (using the Bio-Rad protein assay dye reagent kit), and 10 µg of proteins were subjected to electrophoresis through a 12% Glycine SDS polyacrylamide gel. The proteins were transferred onto a PVDF (polyvinylidene difluoride) membrane. The membrane was blocked with TBS containing 5% powdered milk and 0.05% Tween 20 before being incubated overnight at 4°C with a 1:5000 dilution of polyclonal rabbit anti-HA antibodies (Life Technologies) in TBS containing 3% powdered milk, 0.05% Tween 20. After extensive washes in TBS-0.05% Tween 20, the membrane was incubated with anti-rabbit IgG alkaline phosphatase conjugate (Promega) used as secondary antibody and revealed by a colorimetric reaction.

DprA Overexpression and Purification

Cloning of the *dprA* coding region was performed using genomic DNA from wild type *D. radiodurans* as a template for PCR. Six histidine codons were added at the 3' end of the construct during the PCR process. The fragments were inserted into the *NdeI-XhoI* sites of the pET29 vector (Novagen). Over-expression of the protein in the BL21(DE3) Gold strain was performed in 800 mL 2xYT erlenmeyer flasks, overnight at 15°C, after induction with 0.5 mM IPTG (Sigma). Cells were harvested by centrifugation and resuspended in buffer A [200 mM NaCl, 20 mM Tris-HCl (pH 7.5)] for all of the constructs. The cells were stored overnight at −20°C. Cell lysates were prepared by sonication using a Branson probe-tip sonicator. After 30 min centrifugation at 20,000 g at 8°C, the His-tagged recombinant proteins were loaded on to a Ni-NTA column (Qiagen Inc.), and eluted with imidazole in buffer A. The protein was then loaded onto a Superdex TM200 column (GE), equilibrated in 100 mM NaCl, 1 mM ATP, 3 mM MgCl₂, 20 mM Tris-HCl (pH 7.5). The protein was concentrated using Vivaspinn 5,000 nominal molecular weight limit cut-off centrifugal concentrators (Vivascience), aliquoted and flash frozen in liquid nitrogen, and stored at −80°C.

DNA-Binding Assays

Fluorescence anisotropy was measured in a CARY Eclipse (Varian) spectrofluorometer, at 20°C, in a final reaction volume of 200 µL buffered with 25 mM NaCl, 20 mM Tris (pH 7.5), 2.5 mM MgCl₂, 2.5% (vol/vol) glycerol, and supplemented with 25 nM of a 20-mers, 50-mers, or 100-mers of a polydT oligonucleotide, or 35-mers oligonucleotides of various GC content percentage, and then 5'-labeled with fluoresceine (GeneCust). The excitation wavelength was set to 490 nm, and emission was observed at 525 nm (10 nm bandwidth). Protein injections were 0.25–1 µL from a 10 mg/mL stock solution. The data were treated with SigmaPlot 12.0 (Systat Software).

Structural Modeling of DprA

A structural model of the full-length protein was made using the Swiss Model server (Schwede et al., 2003) and the crystal structure of DprA from *R. palustris* as a template (PDB ID = 3MAJ).

Cloning and Yeast Two-Hybrid (Y2H) Assays

Yeast strain pJ69-4A was the host for the Y2H experiments, using plasmids pGAD-C1 and pGBDU-C1 as the starting material for generating plasmids encoding Gal4-AD and Gal4-BD fusion proteins, respectively (James et al., 1996). *D. radiodurans* *dprA*, *recA*, *recO*, and *ddrB* genes were subcloned into plasmid pGAD-C1 as well as into plasmid pGBDU-C1 for *dprA*, *recA* and *ddrB* in order to test for cross-interactions using Y2H assays. Two versions, with varying length of the N-terminal α -helix of RecA (full-length FL and Δ N27), were constructed. Pairs of pGAD-C1 and pGBDU-C1 variants were introduced into strain pJ69-4A by co-transformation, and transformants were selected on synthetic complete medium lacking leucine and uracil. Interactions between AD and BD fusions were assayed for selected clones on synthetic complete medium lacking leucine, uracil, and histidine. The clones were spotted as a series of 1/5 dilutions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

REFERENCES

- Aas, F. E., Wolfgang, M., Frye, S., Dunham, S., Lovold, C., and Koomey, M. (2002). Competence for natural transformation in *Neisseria gonorrhoeae*: components of DNA binding and uptake linked to type IV pilus expression. *Mol. Microbiol.* 46, 749–760. doi: 10.1046/j.1365-2958.2002.03193.x
- Attaiach, L., Olivier, A., Mortier-Barriere, I., Soulet, A. L., Granadel, C., Martin, B., et al. (2011). Role of the single-stranded DNA-binding protein SsbB in pneumococcal transformation: maintenance of a reservoir for genetic plasticity. *PLoS Genet.* 7:e1002156. doi: 10.1371/journal.pgen.1002156
- Bentchikou, E., Servant, P., Coste, G., and Sommer, S. (2010). A major role of the RecFOR pathway in DNA double-strand-break repair through ESDSA in *Deinococcus radiodurans*. *PLoS Genet.* 6:e1000774. doi: 10.1371/journal.pgen.1000774
- Berge, M., Mortier-Barriere, I., Martin, B., and Claverys, J. P. (2003). Transformation of *Streptococcus pneumoniae* relies on DprA- and RecA-dependent protection of incoming DNA single strands. *Mol. Microbiol.* 50, 527–536. doi: 10.1046/j.1365-2958.2003.03702.x
- Boudes, M., Sanchez, D., Graille, M., van Tilbeurgh, H., Durand, D., and Quevillon-Cheruel, S. (2014). Structural insights into the dimerization of the response regulator ComE from *Streptococcus pneumoniae*. *Nucleic Acids Res.* 42, 5302–5313. doi: 10.1093/nar/gku110
- Bouthier de la Tour, C., Boissard, S., Norais, C., Touille, M., Bentchikou, E., Vannier, F., et al. (2011). The deinococcal DdrB protein is involved in an early step of DNA double strand break repair and in plasmid transformation through its single-strand annealing activity. *DNA Repair.* 10, 1223–1231. doi: 10.1016/j.dnarep.2011.09.010
- Bouthier de la Tour, C., Touille, M., Jolivet, E., Nguyen, H. H., Servant, P., Vannier, F., et al. (2009). The *Deinococcus radiodurans* SMC protein is dispensable for cell viability yet plays a role in DNA folding. *Extremophiles* 13, 827–837. doi: 10.1007/s00792-009-0270-2
- Burghout, P., Bootsma, H. J., Kloosterman, T. G., Bijlsma, J. J., de Jongh, C. E., Kuipers, O. P., et al. (2007). Search for genes essential for pneumococcal transformation: the RADA DNA repair protein plays a role in genomic recombination of donor DNA. *J. Bacteriol.* 189, 6540–6550. doi: 10.1128/JB.00573-07
- Burrows, L. L. (2012). *Pseudomonas aeruginosa* twitching motility: type IV pili in action. *Annu. Rev. Microbiol.* 66, 493–520. doi: 10.1146/annurev-micro-092611-150055
- Carrasco, B., Fernandez, S., Asai, K., Ogasawara, N., and Alonso, J. C. (2002). Effect of the *recU* suppressors *sms* and *subA* on DNA repair and homologous recombination in *Bacillus subtilis*. *Mol. Genet. Genomics* 266, 899–906. doi: 10.1007/s00438-001-0616-7
- Claverys, J. P., Prudhomme, M., and Martin, B. (2006). Induction of competence regulons as a general response to stress in gram-positive bacteria. *Annu. Rev. Microbiol.* 60, 451–475. doi: 10.1146/annurev.micro.60.080805.142139
- Comella, N., and Grossman, A. D. (2005). Conservation of genes and processes controlled by the quorum response in bacteria: characterization of genes controlled by the quorum-sensing transcription factor ComA in *Bacillus subtilis*. *Mol. Microbiol.* 57, 1159–1174. doi: 10.1111/j.1365-2958.2005.04749.x
- Corbinais, C., Mathieu, A., Kortulewski, T., Radicella, J. P., and Marsin, S. (2016). Following transforming DNA in *Helicobacter pylori* from uptake to expression. *Mol. Microbiol.* 101, 1039–1053. doi: 10.1111/mmi.13440

AUTHOR CONTRIBUTIONS

SI, GC, JL, DL, NE, EB, CB, and PS: Performing the experiments. PS and SQ-C: Conceptualization, supervision, and writing—original draft preparation. SI, SS, GC, PS, JL, and SQ-C: Analyzing data. SS, EB, CB, SQ-C, FC, SI, and PS: Writing—review and editing.

FUNDING

SI, NE, and JL were supported by a Ph.D. fellowship from the French Ministry of Education. This work was supported by the Centre National de la Recherche Scientifique, the University Paris-Sud, the Agence Nationale de la Recherche (ANR Radioresistance-11-BSV3-01701 to SS), and by the French Infrastructure for Integrated Structural Biology (FRISBI) ANR-10-INSB-05-01.

ACKNOWLEDGMENTS

We thank Stéphanie Marsin and Adriana Bailone for valuable discussions and critical reading of the manuscript and Michael DuBow for polishing our English. This work was carried out in compliance with the current laws governing genetic experimentation in France.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.01253/full#supplementary-material>

- Diallo, A., Foster, H. R., Gromek, K. A., Perry, T. N., Dujeancourt, A., Krasteva, P. V., et al. (2017). Bacterial transformation: ComFA is a DNA-dependent ATPase that forms complexes with ComFC and DprA. *Mol. Microbiol.* 105, 741–754. doi: 10.1111/mmi.13732
- Dorer, M. S., Sessler, T. H., and Salama, N. R. (2011). Recombination and DNA repair in *Helicobacter pylori*. *Annu. Rev. Microbiol.* 65, 329–348. doi: 10.1146/annurev-micro-090110-102931
- Dwivedi, G. R., Srikanth, K. D., Anand, P., Naikoo, J., Srilatha, N. S., and Rao, D. N. (2015). Insights into the functional roles of N-terminal and C-terminal domains of *Helicobacter pylori* DprA. *PLoS ONE* 10:e0131116. doi: 10.1371/journal.pone.0131116
- Farci, D., Bowler, M. W., Kirkpatrick, J., McSweeney, S., Tramontano, E., and Piano, D. (2014). New features of the cell wall of the radio-resistant bacterium *Deinococcus radiodurans*. *Biochim. Biophys. Acta* 1838, 1978–1984. doi: 10.1016/j.bbame.2014.02.014
- Griffith, F. (1928). The significance of pneumococcal types. *J. Hyg.* 27, 113–159. doi: 10.1017/S0022172400031879
- Gupta, R. S. (2011). Origin of diderm (Gram-negative) bacteria: antibiotic selection pressure rather than endosymbiosis likely led to the evolution of bacterial cells with two membranes. *Antonie. Van. Leeuwenhoek*. 100, 171–182. doi: 10.1007/s10482-011-9616-8
- Havarstein, L. S., Gaustad, P., Nes, I. F., and Morrison, D. A. (1996). Identification of the streptococcal competence-pheromone receptor. *Mol. Microbiol.* 21, 863–869. doi: 10.1046/j.1365-2958.1996.521416.x
- Hua, X., and Hua, Y. (2016). Improved complete genome sequence of the extremely radioresistant bacterium *Deinococcus radiodurans* R1 obtained using pacbio single-molecule sequencing. *Genome. Announc.* 4:e00886-16. doi: 10.1128/genomeA.00886-16
- Ithurbide, S., Bentschikou, E., Coste, G., Bost, B., Servant, P., and Sommer, S. (2015). Single strand annealing plays a major role in RecA-independent recombination between repeated sequences in the radioresistant *Deinococcus radiodurans* bacterium. *PLoS Genet.* 11:e1005636. doi: 10.1371/journal.pgen.1005636
- James, P., Halladay, J., and Craig, E. A. (1996). Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* 144, 1425–1436.
- Johnston, C., Martin, B., Fichant, G., Polard, P., and Claverys, J. P. (2014). Bacterial transformation: distribution, shared mechanisms and divergent control. *Nat. Rev. Microbiol.* 12, 181–196. doi: 10.1038/nrmicro3199
- Jolivet, E., Lecoite, F., Coste, G., Satoh, K., Narumi, I., Bailone, A., et al. (2006). Limited concentration of RecA delays DNA double-strand break repair in *Deinococcus radiodurans* R1. *Mol. Microbiol.* 59, 338–349. doi: 10.1111/j.1365-2958.2005.04946.x
- Kennan, R. M., Dhungyel, O. P., Whittington, R. J., Egerton, J. R., and Rood, J. I. (2001). The type IV fimbrial subunit gene (*fimA*) of *Dichelobacter nodosus* is essential for virulence, protease secretion, and natural competence. *J. Bacteriol.* 183, 4451–4458. doi: 10.1128/JB.183.15.4451-4458.2001
- Kidane, D., Ayora, S., Sweasy, J. B., Graumann, P. L., and Alonso, J. C. (2012). The cell pole: the site of cross talk between the DNA uptake and genetic recombination machinery. *Crit. Rev. Biochem. Mol. Biol.* 47, 531–555. doi: 10.3109/10409238.2012.729562
- Kidane, D., Carrasco, B., Manfredi, C., Rothmaier, K., Ayora, S., Tadesse, S., et al. (2009). Evidence for different pathways during horizontal gene transfer in competent *Bacillus subtilis* cells. *PLoS Genet.* 5:e1000630. doi: 10.1371/journal.pgen.1000630
- Kruger, E., Msadek, T., Ohlmeier, S., and Hecker, M. (1997). The *Bacillus subtilis* clpC operon encodes DNA repair and competence proteins. *Molecular Biology* 143(Pt. 4), 1309–1316. doi: 10.1099/00221287-143-4-1309
- Kruger, N. J., and Stigl, K. (2011). Two steps away from novelty—principles of bacterial DNA uptake. *Mol. Microbiol.* 80, 860–867. doi: 10.1111/j.1365-2958.2011.07647.x
- Lisboa, J., Andreani, J., Sanchez, D., Boudes, M., Collinet, B., Liger, D., et al. (2014). Molecular determinants of the DprA-RecA interaction for nucleation on ssDNA. *Nucleic Acids Res.* 42, 7395–7408. doi: 10.1093/nar/gku349
- Lisboa, J., Celma, L., Sanchez, D., Marquis, M., Andreani, J., Guérois, R., et al. (2019). The C-terminal domain of HpDprA is a DNA-binding winged helix domain that does not bind double-stranded DNA. *FEBS J.* 286, 1941–1958. doi: 10.2210/pdb6gww/pdb
- Makharashvili, N., Koroleva, O., Bera, S., Grandgenett, D. P., and Korolev, S. (2004). A novel structure of DNA repair protein RecO from *Deinococcus radiodurans*. *Structure* 12, 1881–1889. doi: 10.1016/j.str.2004.08.006
- Marie, L., Rapisarda, C., Morales, V., Berge, M., Perry, T., Soulet, A. L., et al. (2017). Bacterial RadA is a DnaB-type helicase interacting with RecA to promote bidirectional D-loop extension. *Nat. Commun.* 8:15638. doi: 10.1038/ncomms15638
- Marsh, J. W., and Taylor, R. K. (1998). Identification of the *Vibrio cholerae* type 4 prepilin peptidase required for cholera toxin secretion and pilus formation. *Mol. Microbiol.* 29, 1481–1492. doi: 10.1046/j.1365-2958.1998.01031.x
- Matthey, N., and Blokesch, M. (2016). The DNA-uptake process of naturally competent *Vibrio cholerae*. *Trends Microbiol.* 24, 98–110. doi: 10.1016/j.tim.2015.10.008
- Meima, R., Rothfuss, H. M., Gewin, L., and Lidstrom, M. E. (2001). Promoter cloning in the radioresistant bacterium *Deinococcus radiodurans*. *J. Bacteriol.* 183, 3169–3175. doi: 10.1128/JB.183.10.3169-3175.2001
- Mell, J. C., and Redfield, R. J. (2014). Natural competence and the evolution of DNA uptake specificity. *J. Bacteriol.* 196, 1471–1483. doi: 10.1128/JB.01293-13
- Mennecier, S., Coste, G., Servant, P., Bailone, A., and Sommer, S. (2004). Mismatch repair ensures fidelity of replication and recombination in the radioresistant organism *Deinococcus radiodurans*. *Mol. Genet. Genomics* 272, 460–469. doi: 10.1007/s00438-004-1077-6
- Morrison, D. A., Mortier-Barrière, I., Attaiach, L., and Claverys, J. P. (2007). Identification of the major protein component of the pneumococcal eclipse complex. *J. Bacteriol.* 189, 6497–6500. doi: 10.1128/JB.00687-07
- Mortier-Barrière, I., Velten, M., Dupaigne, P., Mirouze, N., Piétrement, O., McGovern, S., et al. (2007). A key presynaptic role in transformation for a widespread bacterial protein: DprA conveys incoming ssDNA to RecA. *Cell* 130, 824–836. doi: 10.1016/j.cell.2007.07.038
- Moseley, B. E., and Setlow, J. K. (1968). Transformation in *Micrococcus radiodurans* and the ultraviolet sensitivity of its transforming DNA. *Proc. Natl. Acad. Sci. U.S.A.* 61, 176–183. doi: 10.1073/pnas.61.1.176
- Norais, C., Servant, P., Bouthier-de-la-Tour, C., Coureux, P. D., Ithurbide, S., Vannier, F., et al. (2013). The *Deinococcus radiodurans* DR1245 protein, a DdrB partner homologous to YbjN proteins and reminiscent of type III secretion system chaperones. *PLoS ONE* 8:e56558. doi: 10.1371/journal.pone.0056558
- Norais, C. A., Chittani-Pattu, S., Wood, E. A., Inman, R. B., and Cox, M. M. (2009). DdrB protein, an alternative *Deinococcus radiodurans* SSB induced by ionizing radiation. *J. Biol. Chem.* 284, 21402–21411. doi: 10.1074/jbc.M109.010454
- Passot, F. M., Nguyen, H. H., Dard-Dascot, C., Thermes, C., Servant, P., Espeli, O., et al. (2015). Nucleoid organization in the radioresistant bacterium *Deinococcus radiodurans*. *Mol. Microbiol.* 97, 759–774. doi: 10.1111/mmi.13064
- Pimentel, Z. T., and Zhang, Y. (2018). Evolution of the natural transformation protein, ComEC, in bacteria. *Front. Microbiol.* 9:2980. doi: 10.3389/fmicb.2018.02980
- Quevillon-Cheruel, S., Campo, N., Mirouze, N., Mortier-Barrière, I., Brooks, M. A., Boudes, M., et al. (2012). Structure-function analysis of pneumococcal DprA protein reveals that dimerization is crucial for loading RecA recombinase onto DNA during transformation. *Proc. Natl. Acad. Sci. U.S.A.* 109, E2466–E2475. doi: 10.1073/pnas.1205638109
- Redfield, R. J. (1991). sxy-1, a *Haemophilus influenzae* mutation causing greatly enhanced spontaneous competence. *J. Bacteriol.* 173, 5612–5618. doi: 10.1128/JB.173.18.5612-5618.1991
- Saarimaa, C., Peltola, M., Raulio, M., Neu, T. R., Salkinoja-Salonen, M. S., and Neubauer, P. (2006). Characterization of adhesion threads of *Deinococcus geothermalis* as type IV pili. *J. Bacteriol.* 188, 7016–7021. doi: 10.1128/JB.00608-06
- Saunders, C. W., and Guild, W. R. (1981). Pathway of plasmid transformation in *Pneumococcus*: open circular and linear molecules are active. *J. Bacteriol.* 146, 517–526. doi: 10.1128/JB.146.2.517-526.1981
- Schwede, T., Kopp, J., Guex, N., and Peitsch, M. C. (2003). SWISS-MODEL: an automated protein homology-modeling server. *Nucleic Acids Res.* 31, 3381–3385. doi: 10.1093/nar/gkg520
- Seitz, P., and Blokesch, M. (2013a). Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. *FEMS Microbiol. Rev.* 37, 336–363. doi: 10.1111/j.1574-6976.2012.00353.x

- Seitz, P., and Blokesch, M. (2013b). DNA-uptake machinery of naturally competent *Vibrio cholerae*. *Proc. Natl. Acad. Sci. U.S.A.* 110, 17987–17992. doi: 10.1073/pnas.1315647110
- Seitz, P., and Blokesch, M. (2014). DNA transport across the outer and inner membranes of naturally transformable *Vibrio cholerae* is spatially but not temporally coupled. *MBio* 5:e01409-14. doi: 10.1128/mBio.01409-14
- Seitz, P., Pezeshgi Modarres, H., Borgeaud, S., Bulushev, R. D., Steinbock, L. J., Radenovic, A., et al. (2014). ComEA is essential for the transfer of external DNA into the periplasm in naturally transformable *Vibrio cholerae* cells. *PLoS Genet.* 10:e1004066. doi: 10.1371/journal.pgen.1004066
- Slade, D., Lindner, A. B., Paul, G., and Radman, M. (2009). Recombination and replication in DNA repair of heavily irradiated *Deinococcus radiodurans*. *Cell* 136, 1044–1055. doi: 10.1016/j.cell.2009.01.018
- Sugiman-Marangos, S. N., Weiss, Y. M., and Junop, M. S. (2016). Mechanism for accurate, protein-assisted DNA annealing by *Deinococcus radiodurans* DdrB. *Proc. Natl. Acad. Sci. U.S.A.* 113, 4308–4313. doi: 10.1073/pnas.1520847113
- Tammam, S., Sampaleanu, L. M., Koo, J., Manoharan, K., Daubaras, M., Burrows, L. L., et al. (2013). PilMNOPQ from the *Pseudomonas aeruginosa* type IV pilus system form a transenvelope protein interaction network that interacts with PilA. *J. Bacteriol.* 195, 2126–2135. doi: 10.1128/JB.00032-13
- Tanaka, M., Earl, A. M., Howell, H. A., Park, M. J., Eisen, J. A., Peterson, S. N., et al. (2004). Analysis of *Deinococcus radiodurans*'s transcriptional response to ionizing radiation and desiccation reveals novel proteins that contribute to extreme radioresistance. *Genetics* 168, 21–33. doi: 10.1534/genetics.104.029249
- Thompson, B. G., and Murray, R. G. (1981). Isolation and characterization of the plasma membrane and the outer membrane of *Deinococcus radiodurans* strain sark. *Can. J. Microbiol.* 27, 729–734. doi: 10.1139/m81-111
- Tirgari, M., and Moseley, B. E. (1980). Transformation in *Micrococcus radiodurans*: measurement of various parameters and evidence for multiple, independently segregating genomes per cell. *J. Gen. Microbiol.* 119, 287–296. doi: 10.1099/00221287-119-2-287
- Touelle, M., Mirabella, B., Guérin, P., Bouthier de la Tour, C., Boissard, S., Nguyen, H. H., et al. (2012). A comparative proteomic approach to better define *Deinococcus* nucleoid specificities. *J. Proteomics* 75, 2588–2600. doi: 10.1016/j.jprot.2012.03.002
- Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., et al. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* 46, W296–W303. doi: 10.1093/nar/gky427
- White, O., Eisen, J. A., Heidelberg, J. F., Hickey, E. K., Peterson, J. D., Dodson, R. J., et al. (1999). Genome sequence of the radioresistant bacterium *Deinococcus radiodurans* R1. *Science* 286, 1571–1577. doi: 10.1126/science.286.5444.1571
- Xu, G., Lu, H., Wang, L., Chen, H., Xu, Z., Hu, Y., et al. (2010). DdrB stimulates single-stranded DNA annealing and facilitates RecA-independent DNA repair in *Deinococcus radiodurans*. *DNA Repair* 9, 805–812. doi: 10.1016/j.dnarep.2010.04.006
- Yadav, T., Carrasco, B., Hejna, J., Suzuki, Y., Takeyasu, K., and Alonso, J. C. (2013). *Bacillus subtilis* DprA recruits RecA onto single-stranded DNA and mediates annealing of complementary strands coated by SsbB and SsbA. *J. Biol. Chem.* 288, 22437–22450. doi: 10.1074/jbc.M113.478347
- Zahradka, K., Slade, D., Bailone, A., Sommer, S., Averbeck, D., Petranovic, M., et al. (2006). Reassembly of shattered chromosomes in *Deinococcus radiodurans*. *Nature* 443, 569–573. doi: 10.1038/nature05160

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Ithurbide, Coste, Lisboa, Eugénie, Bentchikou, Bouthier de la Tour, Liger, Confalonieri, Sommer, Quevillon-Cheruel and Servant. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.