Divisome and segrosome components of *Deinococcus radiodurans* interact through cell division regulatory proteins

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The *Deinococcus radiodurans* genome encodes many of the known components of divisome as well as four sets of genome partitioning proteins, ParA and ParB on its multipartite genome. Interdependent regulation of cell division and genome segregation is not understood. *In vivo* interactions of *D. radiodurans'*'s divisome, segrosome and other cell division regulatory proteins expressed on multicopy plasmids were studied in *Escherichia coli* using a bacterial two-hybrid system and confirmed by co-immunoprecipitation with the proteins made in *E. coli*. Many of these showed interactions both with the self and with other proteins. For example, DrFtsA, DrFtsZ, DrMinD, DrMinC, DrDivIVA and all four ParB proteins individually formed at least homodimers, while DrFtsA interacted with DrFtsZ, DrFtsW, DrFtsE, DrFtsK and DrMinD. DrMinD also showed interaction with DrFtsW, DrFtsE and DrMinC. Interestingly, septum site determining protein, DrDivIVA showed interactions with secondary genome ParAs as well as ParB1, ParB3 and ParB4 while DrMinC interacted with ParB1 and ParB3. PprA, a pleiotropic protein recently implicated in cell division regulation, neither interacted with divisome proteins nor ParBs but interacted at different levels with all four ParAs. These results suggest the formation of independent multiprotein complexes of 'DrFts' proteins, segrosome proteins and cell division regulatory proteins, and these complexes could interact with each other through DrMinC and DrDivIVA, and PprA in *D. radiodurans*.

INTRODUCTION

Cellular processes including chromosome duplication, segregation and cell division occur through the coordinated actions of various proteins that form macromolecular complexes. These processes are tightly regulated both temporally and spatially. In prokaryotes, chromosome duplication followed by segregation is a pre-requisite for cell division. In a few rod-shaped bacteria like *Escherichia coli* and *Bacillus subtilis*, the functional interactions of these processes have been shown and the involvement of some common proteins in both these processes has been demonstrated (Thanbichler, 2010). Cell division in these bacteria occurs through interactions among a dozen proteins forming a higher order structure called the divisome (Vicente & Rico, 2006). FtsZ is the main component of divisome and various proteins regulate its activity both temporally and spatially. Using classical genetics, a bacterial two-hybrid system, fluorescence microscopy, etc., it has been shown that FtsZ localization at the mid cell position is spatially regulated by the 'Min' system comprising MinC, MinD and MinE mostly in Gram-negative bacteria and DivIVA instead of MinE in Gram-positive bacteria (Lutkenhaus, 2007; Conti et al., 2015). Subsequently, FtsA tethers FtsZ to the membrane followed by assembly of other divisome components in a sequential manner as shown in *E. coli* (Di Lallo et al., 2003; Vicente & Rico, 2006). This helps in the progression of FtsZ polymerization into the Z-ring and its subsequent contraction. This process is stalled if any anomalies caused by unresolved duplicated DNA are encountered and does not progress further till duplicated DNA is resolved. A number of regulatory proteins are known to regulate the resolution of duplicated intertwined circular DNA and consequently FtsZ ring dynamics (Goehring et al., 2006; Cho et al., 2011; Tonthat et al., 2011; Bailey et al., 2014). These include DNA topoisomerases, nucleoid occlusion (NOC) proteins and the FtsK-XerCD system. FtsK is a multifunctional DNA translocase and divisome component. It is recruited by FtsA and plays a role in coordination of chromosome segregation (Ip et al., 2003; Massey et al., 2006; Bigot et al., 2007).
Bacterial genome segregation involves mostly a tripartite partitioning system (segrosome) consisting of (i) a centromere-like sequence or cis-element, (ii) a centromere-binding protein (Schumacher et al., 2010; Vecchiarelli et al., 2010) called ParB or its homologues and (iii) an NTPase called ParA or ParA-like proteins. The dynamics created during separation/depolymerization of ParA provide the force for the separation of duplicated plasmids or chromosomes in opposite directions (Ebersbach & Gerdes, 2001; Fogel & Waldor, 2006; Salje et al., 2010; Shebelut et al., 2010; Gerdes et al., 2010). Recently, it has been shown that an appropriate ratio of ParA to ParB within the cell is required for Z-ring formation and cell division in *Caulobacter crescentus*. Depletion of ParB and/or increase in ParA could inhibit Z-ring formation and cell division in *C. crescentus* (Mohl et al., 2001) and *Mycobacterium smegmatis* (Ginda et al., 2013). Similar observation has also been reported in *D. radiodurans* where the role of ParA of chromosome II (ParA2) in the regulation of cell division, in the context of its stoichiometric balance with ParB2, was observed (Charaka & Misra, 2012). DivIVA, a tropomyosin-like coiled-coil protein appears as a bifunctional protein with distinct roles in division-site selection as well as during chromosome segregation at least in *B. subtilis* (Thomaides et al., 2001). *In vivo* interaction of cell division proteins (Di Lallo et al., 2003; Ginda et al., 2013) and proteins involved in genome maintenance (Donovan et al., 2012; Ringgaard et al., 2011) has been demonstrated in other bacteria using a bacterial two-hybrid system.

*D. radiodurans* R1, a Gram-positive bacterium, shows extraordinary tolerance to DNA-damaging agents including radiation and desiccation (Minton, 1994; Slade & Radman, 2011; Misra et al., 2013). Apart from these features, it shows ploidy of its four genomic elements, chromosome I (2.65 Mb), chromosome II (412 kb), a megaplasmid (177 kb) and a small plasmid (46 kb) (White et al., 2011; Misra et al., 2011) has been demonstrated in other bacteria using a bacterial two-hybrid system.

Here we have used BACTH (Bacterial Adenylate Cyclase-based Two Hybrid) system (Karimova et al., 1998) and monitored protein–protein interactions *in vivo*. We produced fusions of all annotated divisome and segrosome proteins in BACTH vectors, confirmed expression of tagged fusion proteins by immunoblotting using antibodies to the respective tags’ antibodies and subsequently demonstrated their *in vivo* interactions that were demonstrated in the *cyaA* deficient *E. coli* strain BTH101. Some of these interactions were confirmed by co-immunoprecipitation (co-IP) using representative examples of qualitatively defined strong interactions, weak interactions and no interactions. We observed *in vivo* interaction among ‘Fts’ proteins and segrosome proteins (hereafter referred to as ‘Par’). Cell division spatial regulatory proteins like DrMinC, DrMinD and DrDivIVA also interacted selectively with certain components of both these macromolecular complexes, thus suggesting their possible involvement in the recruitment and regulation of these two important functions in *D. radiodurans*.

**METHODS**

**Bacterial strains, plasmids and materials.** *D. radiodurans* R1 (ATCC13939), a kind gift from Professor J. Ortner, Germany (Schäfer et al., 2000) was grown in TGY [Bacto tryptone (1 %), glucose (0.1 %) and yeast extract (0.5 %)] medium with shaking at 180 rpm at 32°C. *E. coli* strains DH5α and Novablu were grown at 37°C, while *E. coli* BTH101 (*cyaA∗) was grown at 30°C with shaking at 180 rpm in Luria-Bertani (LB) broth. *E. coli* strain DH5α and Novablu were used for cloning and maintenance of all the plasmids. BACTH vectors like pUT18 and pUT18C expressing ‘T18’ domain and pKT25 and pKNT25 expressing ‘T25’ domain of adenylate cyclase will be referred to as T18 and T25, respectively, throughout the paper. *E. coli* strain BTH101 (hereafter referred as BTH101) was used for co-expression of these proteins on BACTH plasmids for *in vivo* protein–protein interactions. Recombinant *E. coli* harbouring different BACTH plasmid derivatives were grown in the presence of both ampicillin (100 µg ml⁻¹) and kanamycin (50 µg ml⁻¹) as required. Standard protocols for all recombinant DNA techniques were used as described in Green & Sambrook (2012). Antibodies against T18 (SC-33620) and T25 (SC-13582) domains of CyA of *Bordetella pertussis*, respectively, were procured commercially (Santa Cruz Biotechnology, Inc.). Molecular biology grade chemicals and enzymes were procured from Sigma Chemicals Company, USA, Roche Biochemicals, Mannheim, Germany, New England Biolabs, USA and Merck India, Mumbai.

**Construction of recombinant plasmids.** The genomic DNA of *E. coli* MG1655 and *D. radiodurans* R1 were isolated as described in Battista et al.(2001). Details of the primers used for the cloning of various components of divisome and segrosome are given in Table S1 (available in the online Supplementary Material). BACTH vectors were used for making fusions with target proteins as described earlier (Karimova et al., 1998). In brief the coding sequences of divisome proteins (FtsA, FtsE, FtsK, FtsW, FtsQ, FtsZ, MinC, MinD and DivIVA) and genome partitioning proteins ParA1 (DR_0013), ParA2 (DR_0001), ParA3 (DR_B0001) and ParA4 (DR_B0031) as well as ParB1 (DR_0012), ParB2 (DR_A0002) and ParB4 (DR_B0032) of *D. radiodurans* were PCR amplified from genomic DNA using appropriate primers. The required restriction enzymes sites were incorporated at the 5’ end of these primers. PCR products were digested with appropriate restriction enzymes and ligated at compatible ends in BACTH plasmids as detailed in Table S2. For the reader’s convenience, all the proteins of *D. radiodurans* have been named with a prefix ‘Dr’ before their standard...
names (e.g. DrFtsZ) and will be referred to accordingly throughout the manuscript. Likewise, proteins expressed on constructs made using pUT18 and pKT25, which would have T18/T25 at the C-terminus of the target proteins are denoted by a prefix of N18/N25, respectively (e.g. N18-DrFtsZ/C18/DrFtsZ-C25), and those made using pUT18C and pKT25 plasmids, which would have had T18 and T25 at the N-terminus of the protein are denoted by a prefix of N18/N25, respectively (e.g. N18-DrFtsZ/N25-DrFtsZ). Similarly, the coding sequences of FtsA and FtsZ of *E. coli* were also PCR amplified and cloned for use as positive control and will be referred as EcFtsA and EcFtsZ, respectively.

**RESULTS**

**Translational fusions of divisome and segrosome proteins with BACTH partners**

The coding sequences of cell division proteins like DrFtsZ, DrFtsA, DrFtsK, DrFtsE, DrFtsW, DrFtsQ, DrMinC, DrMinD and DrDivIVA and genome partitioning proteins like ParA1, ParB1, ParA2, ParB2, ParA3, ParB3, ParA4 and ParB4 were cloned in BACTH plasmids. The recombinant plasmids generated in this study and the expected size of the corresponding target proteins fused with either T18 or T25 of BACTH system are given in Table S2. The plasmids were transformed into BTH101 and expression of fusion proteins was confirmed by immunoblotting using antibodies against T18 and T25. Immunodetection using these antibodies showed expression of fusion proteins of the expected sizes in all the recombinant strains. These antibodies cross-reacted with a protein from the cells expressing anticipated protein, and immunosignals of the expected size of fusion proteins were detected (Fig. 1). The majority of the constructs tested for expression contained C-terminal tags and some were N-terminal fusions with T25. We observed that all these proteins express as fusion with T25 on respective BACTH plasmids. Interestingly, we noticed that the signal intensities of N25-DrFtsA-C18 (Fig. 1g) and DrDivIVA-C18 (Fig. 1f) were consistently lower than DrFtsE-C25 and DrDivIVA-C25, respectively. This confirmed the expression of divisome and genome partitioning proteins of *D. radiodurans* as translational fusion with T18/T25, on BACTH plasmids in BTH101. Since, T25 antibodies were polyclonal and were suspected for cross-reactivity with T18, hybridization conditions were standardized so that only specific hybridization of T18 (Fig. 1c) and T25 (Fig. 1d) antibodies was detected. These results ascertained the expression of target proteins with respective tags on recombinant BACTH plasmids in BTH101.

**Cell division proteins (Fts) interact in vivo**

It was shown earlier that when the T18 and T25 domains of CyaA is fused separately with two interacting proteins, the activity of CyaA is restored when the domains come together leading to the dimerization of T18 and T25 domains. Reconstitution of activity of CyaA is monitored as the expression of β-galactosidase in BTH101 (cyaA) cells. Therefore, BTH101 was co-transformed with constructs expressing ‘Fts’ proteins fused with either T18 or T25 in different combinations, and the expression of β-galactosidase was monitored as an indication of the interaction of two proteins in vivo. We observed that only certain combinations showed the expression of β-galactosidase in the spot assay.
as detected by blue colour colonies as well as β-galactosidase activity in solution (Fig. 2). For example, DrFtsA-C25 co-expression with DrFtsZ-C25 produced blue colour colonies and high levels of β-galactosidase expression. Similarly, the cells co-expressing DrFtsZ-C25 with DrFtsA-C18 (Fig. 2)/N18-DrFtsA (Table S3) showed β-galactosidase expression. However, in a majority of the cases, co-expression of target proteins including DrFtsZ with DrFtsQ, when fused with either tags did not induce reporter gene expression significantly (Table S3). These results indicated that DrFtsZ and DrFtsA proteins form at least homodimers as well as interacting with each other. Since, the position of T18/T25 tags at N- or C-termini in the proteins is known to affect their possible interactions, the possibility of the tag position affecting target proteins’ interaction and, therefore, resulting in no induction of β-galactosidase expression in some combinations would be worth investigating. To address this, both T18 and T25 tags were fused at the N- as well as the C-terminus in the majority of the ‘DrFts’ proteins. These were co-expressed in different combinations and development of blue colour colonies and the inducible expression of β-galactosidase were monitored.

DrFtsA-C18 interacted with DrFtsE-C25, N25-DrFtsK and DrFtsW-C25 (Fig. 3) as well as with N25-DrFtsW (Table S3). However, when T25 was placed at the N-terminus of DrFtsE (N25-DrFtsE) and C-terminus of DrFtsK (DrFtsK-C25), the co-expression of proteins with DrFtsA-C18 did not induce β-galactosidase expression in BTH101 background (Fig. 3). Similarly, DrFtsE-C18 showed interaction with N25-DrFtsW (Fig. 3), while DrFtsE-C18 was not observed to interact with DrFtsW-C25, N18-DrFtsE interacted poorly with DrFtsW having T25 at either end (Table S3). But the co-expression of N25-DrFtsZ with DrFtsW-C18 did not induce β-galactosidase expression at least in BTH101 background (Table S3). Interaction of DrFtsQ with DrFtsA was
also checked by co-expressing these two proteins in all possible combinations. DrFtsQ having T25/T18 at the C-terminus when co-expressed with DrFtsA-C18 did not induce expression of β-galactosidase (Table S3). These results suggested that many of the cell division proteins of D. radiodurans interact with each other as assayed in E. coli. However, some expected interactions were not observed in BACTH analysis. Since, BTH is known to give false positive and false negative results it could explain the lack of some of the expected interactions.

**Cell division spatial regulatory proteins (Min) interact selectively**

Likewise, interaction of DrMinC, DrMinD and DrDivIVA among each other was also monitored using the BACTH system. Results showed self-interaction of DrMinC, DrMinD and DrDivIVA fused with T18 and T25 tags, respectively, as well as DrMinC-C18 interaction with DrMinD-C25 (Fig. 4). However, the levels of interactions vary depending upon the tag’s position in these proteins (Fig. 3). Interestingly, DrMinC-C18 co-expression with DrDivIVA in all four combinations could induce β-galactosidase expression, which was not observed when DrMinD was co-expressed with DrDivIVA (Table S4). This suggested that DrMinC, DrMinD and DrDivIVA at least form homodimers in addition to DrMinC forming heterodimers with DrMinD. Interaction of DrMinC with other two ‘Min’ regulatory proteins (DrMinD and DrDivIVA) may be required to bring these proteins together for their possible coordinated functions. Subsequently, these constructs of DrMinC, DrMinD and DrDivIVA were used for monitoring the interaction of these proteins with other divisome and segrosome proteins in different combinations. DrMinD tagged at different termini showed interaction with DrFtsE-C18, DrFtsA-C25 and N25-DrFtsW (Fig. 4). However, BTH101 co-expressing DrMinD-C25 with N18-DrFtsE or DrFtsE-C18, N25-DrMinD with DrFtsW tagged with T18 at either terminus as well as DrDivIVA tagged with T18 at either terminus as well as DrMinD-C18 with DrFtsW-C25 did not induce β-galactosidase expression (Table S5). In addition, many of these proteins co-expressed with other proteins in different combinations did not show β-galactosidase expression (Tables S4 and S5). This suggested that interactions of DrMinD with DrFtsE and DrFtsA and DrFtsW interaction with DrMinD were affected by the position of the tag in these proteins. Strikingly, DrMinC having T18 or T25 at its C-terminus interacted with some proteins but did not induce β-galactosidase expression with the majority of

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**Fig. 2. In vivo interaction of divisome components of D. radiodurans.** E. coli BTH101 cells co-expressing ‘Fts’ proteins tagged with T18/T25 at either terminals as required, in different combinations were checked for the expression of β-galactosidase activity both by spot assay and in liquid culture. Cells co-expressing T18 or T25 tag on vectors were used as negative control while E. coli FtsA (EcFtsA-C18) with E. coli FtsZ (EcFtsZ-C25) as well as chemotaxis protein (CheA-C18 with CheA-C25) were used as positive control. β-Galactosidase activity (units/mg protein) is shown here as mean±SD (n=9) and the significance of the possible difference was analysed using Student’s t-test, and P values, obtained at 95% confidence intervals, are shown as (*) for <0.05, (**) for <0.01 and (***) for <0.001. Total results obtained in several other combinations are provided in supplementary materials (Table S3).
divisome components tagged with either T18/T25 (Table S5). This indicated that DrMinC does not interact directly with ‘DrFts’ proteins, at least those monitored here, while DrMinD interacts with DrFtsE, DrFtsA and DrFtsW. Absence of interaction between DrMinC and DrFtsZ monitored as the expression of β-galactosidase is again a surprising result because MinC interaction with both FtsZ and MinD has been unambiguously reported in other microbes, albeit mostly rod-shaped bacteria (Shen & Lutkenhaus, 2011; Ghosal et al., 2014). Since DrFtsZ does not interact with either DrMinD or DrMinC but shows interaction with other ‘DrFts’ proteins, the possibility of DrFtsZ interacting with the DrMinCD copolymer through other DrFts proteins cannot be ruled out.

Interactions of DivIVA with ‘Fts’ proteins have not been studied in much detail, perhaps because DivIVA is not found universally in all bacteria. Here, we monitored DrDivIVA interaction with all the ‘Fts’ proteins in different possible combinations. Though all the fusions of DrDivIVA interacted with at least one other protein of D. radiodurans, DrDivIVA co-expression with any of the ‘DrFts’ proteins did not induce β-galactosidase expression (Table S5). This might suggest that DrDivIVA does not interact directly with ‘Fts’ proteins of D. radiodurans. However, D. radiodurans also contains MinE, which appears to be truncated as compared to Min E of other bacteria (White et al., 1999) but the possibility of involvement of this protein in in vivo interaction of the ‘Min’ system cannot be ruled out. Molecular mechanism(s) pertaining to roles of MinC, MinD and DivIVA roles in FtsZ localization and divisome assembly have largely been studied in rod-shaped bacteria. Further, the role of DivIVA in bacterial shape determination and genome segregation has also been shown recently (Thomaides et al., 2001; Vicente & Garcia-Ovalle, 2007; Lenarcic et al., 2009). Therefore, the possibility of DrDivIVA regulating cell division through the interaction of genome maintenance proteins would be worth speculating on.

### Genome partitioning proteins interact in vivo

The D. radiodurans genome encodes four ParAs and 4 ParBs on different genome elements (White et al., 1999). The C-terminal fusion of ParA proteins of D. radiodurans with T25 was made and expressed (Fig. 1). Similarly, all four ParB proteins were tagged at the C-terminus with T18 (-C18), and at the N-terminus with either T18 (N18-) and T25 (N25-) tags (Table S2). These were transformed into

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**Fig. 3.** Effect of BACTH tag position on protein–protein interaction. E. coli BTH101 cells co-expressing all the proteins tagged with T18/T25 at either terminus in different combinations were checked for the expression of β-galactosidase activity both by spot assay (a) and in liquid culture (b). Cells co-expressing T18 or T25 tag on vectors were used as negative control and E. coli FtsA (EcFtsA-C18) with E. coli FtsZ (EcFtsZ-C25) was used as positive control. β-Galactosidase activity (units/mg protein) is shown here as mean±SD (n=9) and the significance of the possible difference was analysed using Student’s t-test and P values obtained at 95 % confidence intervals are shown as (*) for <0.05, (**) for <0.01 and (***) for <0.001. Total results obtained in several other combinations are provided in supplementary materials (Table S4).
BTH101 in different combinations for their co-expression and their interaction, if any, was monitored as the expression of β-galactosidase. Results showed that all four deinococcal ParBs dimerize and interestingly, ParB3 also dimerizes with other ParBs except ParB2 at least in *E. coli* (Fig. 5). Interactions of ParAs with ParBs were checked using C-terminal fusion of T25 tag with all four ParAs. ParA1-C25 showed interaction with N18-ParB1 and ParB4-C18 (additional ParB on megaplasmid). Similarly, ParA2-C25 and ParA4-C25 interacted with both the ParBs (ParB3 and ParB4) of megaplasmid, albeit having tags at different termini (Fig. 6). None of the other combinations of ParAs and ParBs could induce β-galactosidase expression in BTH101 (Table S6). Interactions of the megaplasmid-encoded ParB4 with both ParA1 and ParA2 chromosomal ParAs are notable. Earlier, it was shown that interaction of deinococcal ParA1 and ParB1 is required for stable maintenance of unstable *mini-F* plasmid carrying *cis* elements of *D. radiodurans* in *E. coli* (Charaka & Misra, 2012). These results suggest that all four ParBs undergo dimerization in vivo and ParB4 could interact with ParA1, ParA2 and ParA4 while ParB3 interacted with ParA4 alone.

**Min proteins talk with Par proteins**

The genome duplication followed by segregation is a prerequisite for productive cytokinesis (except in mini cell formation) and both occur in tandem. Therefore, the interdependent regulation of these processes can be partly understood if we understand the interaction of divisome proteins with genome partitioning proteins. The fusions of DrFts and DrMin proteins with T18 or T25, which had previously shown interaction with other proteins, were co-expressed with ‘Par’ proteins tagged with T18/T25 at their C-terminus, respectively. BTH101 cells co-expressing deinococcal ParAs and ParBs with different ‘DrFts’ proteins on BTH plasmids did not induce β-galactosidase expression (data not shown). However, *in vivo* interactions of certain ‘Min’ proteins with a few ‘Par’ proteins were observed (Fig. 7). For example, N25-DrMinC showed interaction with ParB1 and ParB3. Similarly, DrDivIVA having T18 or T25 tags on different termini interacted with ParA2, ParA3 and ParA4 as well as with ParB1, ParB3 and ParB4 (Fig. 6). However, the co-expression of DrMinC with ParB2, ParB4 and all four ParAs, DrDivIVA with ParA1 and ParB3 and of DrMinD with all the segrosome proteins in different combinations did not induce β-galactosidase expression (Table S7). This indicated that DrMinC and DrDivIVA talk to some of the ‘Par’ proteins of this bacterium while DrMinD does not do so with any of them. Further, DrMinD interaction with some of the ‘DrFts’ proteins as well as DrMinC and DrDivIVA interaction with ‘Par’ proteins together indicated the possible crosstalk between divisome and genome segregation complexes in the regulation of cell division in *D. radiodurans*.

**PprA interacts with ParA but not cell division proteins**

PprA, a pleiotropic protein associated with radiation resistance in *D. radiodurans* is found to be unique to the members of the Deinococcaceae family. The role of PprA in cell division and genome segregation has been reported in *D. radiodurans* (Devigne et al., 2013; Kota et al., 2014a). In *E. coli* FtsA (EcFtsA-C18) with *E. coli* FtsZ (EcFtsZ-C25) were used as positive control. β-Galactosidase activity (units/mg protein) is shown here as mean±s.d (n=9) and the significance of the possible difference was analysed using Student’s t-test, and P values obtained at 95 % confidence intervals are shown as (*) for <0.05, (**) for <0.01 and (***) for <0.001. Total results obtained in several other combinations are provided in supplementary materials (Table S5).

![Fig. 4. Interaction of DrMin proteins with cell division proteins of *D. radiodurans*. The T18 or T25 tags fused to DrMinC (DrMin-C18/DrMin-C25), DrMinD (DrMin-D18/DrMin-D-C25/N25-DrMinD) and DrDivIVA (DrDivIVA-C18/N25-DrDivIVA) regulatory proteins and cell division proteins DrFtsA (DrFtsA-C25), DrFtsE (DrFtsE-C18) and DrFtsW (N25-DrFtsW) were co-expressed in *E. coli* BTH101 in different combinations. Expression of β-galactosidase activity was measured both by spot assay (a) and in liquid culture (b). Cells co-expressing T18 or T25 tag on vectors were used as negative control, while *E. coli* FtsA (EcFtsA-C18) with *E. coli* FtsZ (EcFtsZ-C25) were used as positive control. β-Galactosidase activity (units/mg protein) is shown here as mean±s.d (n=9) and the significance of the possible difference was analysed using Student’s t-test, and P values obtained at 95 % confidence intervals are shown as (*) for <0.05, (**) for <0.01 and (***) for <0.001. Total results obtained in several other combinations are provided in supplementary materials (Table S5).](http://mic.microbiologyresearch.org)
order to get mechanistic insights on PprA function(s) in cell division and genome segregation. Interactions of PprA with all the cell division proteins including DrFtsZ and DrFtsA and ‘Par’ proteins were monitored using the BACTH system. Cells co-expressing PprA-C18 with either ParA1-C25 or ParA4-C25 expressed significantly high levels of β-galactosidase activity (Fig. 8). The levels of β-galactosidase activity in cells co-expressing PprA-C18 with ParA2-C25 or ParA3-C25 were low as compared to ParA1-C25 with ParA4-C25 but significantly more than vector control (Fig. 8). Notably, PprA-C18 did not show interaction with some of the ‘Fts’ and ‘Min’ proteins tested in different combinations (Table S9). However, the expression of β-galactosidase was not observed in cases where PprA-C25/PprA-C18 were co-expressed with any one of the four ParBs (Table S8) as well as in cases where ParBs were co-expressed with Min system proteins or Fts proteins (Table S10). These results suggested that PprA plays a role in genome partitioning and cell division perhaps by directly interacting with ParAs and indirectly with DrDiv-IVA, through ParA.

Co-immunoprecipitation confirmed in vivo interaction of deinococcal proteins

The results obtained from BACTH analyses indicated possible in vivo interaction of different cell division and genome segregation proteins of D. radiodurans. Some of these results were further confirmed by co-IP using antibodies against T25 (fused with one partner) followed by detection of the interacting partner using antibodies against T18 (fused with other partners) as described in Methods. This study reports a huge number of interactions between ‘Fts’, ‘Min’, ‘Par’ and PprA proteins. Although, it is possible to conduct co-IPs for all the tested interactions, we understandably decided to select a few representative samples expressing different levels of β-galactosidase activity. All the samples were qualitatively divided into three categories, i.e. strong, weak and no interactions. A few representatives from each category that were also to be further studied independently were selected. For instance, DrFtsZ-C25 with DrFtsA-C18, ParA2/ParA3/ParB1 with DrDivIVA, PprA with ParA4 represented strong interaction; DrFtsW with
**Fig. 6.** In vivo interaction of different genome partitioning proteins ParAs and ParBs of *D. radiodurans*. *E. coli* BTH101 cells co-expressing T18 or T25 fusion of ParA1 (ParA1-C25), ParA2 (ParA2-C25), ParA3 (ParA3-C25), ParA4 (ParA4-C25), and ParB1, ParB2, ParB3 and ParB4 tagged with T18 and T25 at either terminus in different combinations was checked for the expression of β-galactosidase both by spot assay (a) and in liquid culture (b). Vector expressing either T18 or T25 tag was used as negative control while *E. coli* FtsA (EcFtsA-C18) with *E. coli* FtsZ (EcFtsZ-C25) was used as positive control. β-Galactosidase activity (units/mg protein) is shown here as mean±SD (*n*=9) and the significance of the possible difference was analysed using Student’s *t*-test, and *P* values obtained at 95 % confidence intervals are shown as (*) for <0.05, (**) for <0.01 and (***) for <0.001. Data obtained from other combinations of ParA and ParB proteins are shown in supplementary materials (Table S6).

**Fig. 7.** In vivo interaction of cell division regulatory proteins with genome partitioning proteins of *D. radiodurans*. The genome partitioning proteins ParB1 (ParB1-C18), ParB3 (ParB3-C18), ParB4 (ParB4-C18), ParA2 (ParA2-C25), ParA3 (ParA3-C25) and ParA4 (ParA4-C25) were co-expressed with DrMinC (DrMinC-C25) and DrDivIVA (DrDivIVA-C18/DrDivIVA-C25/N25-DrDivIVA) in different combinations. Expression of β-galactosidase was monitored both by spot assay (a) and in liquid culture (b). Vector expressing either T18 or T25 tag was used as negative control while *E. coli* FtsA (EcFtsA-C18) with *E. coli* FtsZ (EcFtsZ-C25) was used as positive control. β-Galactosidase activity (units/mg protein) is shown here as mean±SD (*n*=9) and the significance of the possible difference was analysed using Student’s *t*-test, and *P* values obtained at 95 % confidence intervals are shown as (*) for <0.05, (**) for <0.01 and (***) for <0.001. Data obtained from other possible combinations of Par proteins and Min proteins are provided as supplementary materials (Table S7).
DrFtsE, ParA2 with ParB4, DrMinD with DrFtsE represented weak interactions; and DrFtsD with ParB1, PprA with ParB1 and T18 with T25 represented no interaction categories. Cell-free extracts of such cells were incubated with polyclonal antibodies against T25 and immunoblotted using monoclonal antibodies against T18. The polyclonal antibodies against T25, which also showed specific hybridization in Western blotting (Fig. 1), did not immunoprecipitate T18 fused target proteins in the absence of T25 tagged partner or in the absence of interaction. On the other hand, T25 antibodies could immunoprecipitate T18 tagged proteins and the expected size fusion protein bands were detected in samples expressing β-galactosidase (Fig. 9). The signal intensities were also found to be nearly proportional to the levels of β-galactosidase expression. The combinations not supporting reporter gene (lacZ) expression also did not show any hybridization signal with anti-T18 monoclonal antibodies (Fig. 9). Absence of signals on using T18 antibodies in controls expressing only T18 fused proteins and its presence in samples expressing both T25 and T18 fused proteins, which also expressed β-galactosidase activity, further suggested a strong correlation between partners' interaction and β-galactosidase activity levels. These results indicated, though indirectly, that various proteins that showed in vivo interaction of divisome and segrosome proteins of D. radiodurans as demonstrated using BACTH system in BTH101 are true interacting partners, at least in the synthetic E. coli system.

**DISCUSSION**

*D. radiodurans* is a Gram-positive round-shaped radioreistant bacterium, which divides in alternate planes with a doubling time of ~90 min under normal growth conditions in a rich medium (Harris et al., 2004). Earlier, bacterial cell division was studied mostly in rod-shaped/filamentous bacteria, which apparently have well-defined poles. In such cases, the regulation of FtsZ localization in mid cell position and the polarity for genome segregation could be better modelled and explained. In cocci, however, these aspects are not clearly understood, except that the second division is perpendicular to first plane of cell division in *Staphylococcus*like bacteria. Here, we made translational fusions of almost all annotated cell division proteins (‘Fts’ and ‘Min’), ‘Par’ proteins and PprA of D. radiodurans, with T25 and T18 in different combinations. Although this study has ascertainment all the possible interactions of cell division proteins by taking into consideration a majority of the combinations of division proteins, the possibility of some of these interactions being affected by the position and size of the tags as well as the differences in the microenvironment of D. radiodurans and E. coli cannot be ruled out. The poor interaction of DrFtsA with DrFtsZ in E. coli background as reported earlier has been attributed to the differences in the microenvironment between E. coli and D. radiodurans (Modi & Misra 2014). Absence of interactions of some of the D. radiodurans proteins came as a surprise because such interactions are conserved in other bacteria. For example, interaction of FtsA with FtsQ has been found functionally critical in the assembly of FtsK, FtsL and FtsB components in the divisome of other bacteria (Buddelmeijer & Beckwith, 2002; Gonzalez & Beckwith, 2009). The results presented here clearly indicated that DrFtsA, DrFtsZ, DrFtsE, DrFtsK and DrFtsW proteins interact with their respective partners at least in *E. coli*. Several of these interactions amongst the divisome, segrosome and ‘Min’ system

**Fig. 8. In vivo interaction of genome partitioning proteins with PprA of D. radiodurans.** *E. coli* BTH101 cells co-expressing ParA1 (ParA1-C25), ParA2 (ParA2-C25), ParA3 (ParA3-C25) or ParA4 (ParA4-C25) with PprA (PprA-C18) were checked for expression of β-galactosidase both by spot assay (a) and in liquid culture (b). Vector expressing either T18 or T25 tag was used as negative control while *E. coli* FtsA (EcFtsA-C18) with *E. coli* FtsZ (EcFtsZ-C25) was used as positive control. β-Galactosidase activity (units/mg protein) is shown here as mean±SD (n=9) and the significance of the possible difference was analysed using Student’s *t*-test, and *P* values obtained at 95% confidence intervals are shown as (*) for <0.05 and (**) for <0.001. Data obtained from various other possible combinations are shown in supplementary materials (Tables S8, S9 and S10).
components of D. radiodurans have been depicted diagrammatically (Fig. 10).

Here, we observed that several cell division, genome segregation and regulatory proteins of D. radiodurans interact in synthetic E. coli expressing recombinant proteins on multi-copy plasmids. For example, the absence of interaction among the following proteins: (1) DrDivIVA with DrMinD, (2) DrMinD with ‘Par’ proteins and (3) none of the ‘DrMin’ proteins with DrFtsZ, is intriguing. On the other hand, interaction of DrMinC and DrDivIVA with a few ‘Par’ proteins (Fig. 10d) and interaction of DrMinD directly with DrFtsA, DrFtsW and DrFtsE (Fig. 10c) also suggest the possibility of different mechanism(s) of ‘Min’ proteins regulation in septum site selection in D. radiodurans. Earlier, interaction of MinC with both MinD and DrFtsZ in E. coli (Shen & Lutkenhaus, 2011; Ghosal et al., 2014), interaction of DivIVA with ParB in Corynebacterium glutamicum, Wag31 (DivIVA homologue) of Mycobacterium tuberculosis and DivIVA of Streptomyces coelicolor with its ParB in synthetic E. coli (Donovan et al., 2012) have already been demonstrated. Similarly, in M. smegmatis, Wag31 and ParA co-localization at poles (Ginda et al., 2013) and effect of minD mutation on Soj protein (ParA homologue) and localization at poles in B. subtilis (Aautret & Errington, 2003; Marston et al., 1998) are other examples that suggest interaction of the Min system with the genome partitioning system in bacteria. Therefore, interactions of DrMinC and DrDivIVA with ‘Par’ proteins as demonstrated here indicate the importance of these interactions in the regulation of cell division and genome segregation in this bacterium. The functional significance of the interaction of ‘Min’ proteins with ‘Par’ proteins is not clear yet. However, it has been shown that MinC-MinD dimers localize at the septum site towards the late stage of cell division, and thus, predetermine the poles for the next cycle of cell division in some bacteria (Treuner-Lange & Søgaard-Andersen, 2014). DivI-VAs from several bacteria have been shown to interact with their cognate ParB and RodA and co-localize at the poles in respective hosts (Laloux & Jacobs-Wagner, 2014; Sieger & Bramkamp, 2015). Our results provided evidence of such
interactions in D. radiodurans also. Since the poles in cocci including D. radiodurans are not predefined, the possibility that interactions of DrDivIVA and DrMinC with Par proteins may somehow help this bacterium in determining the next plane of cell division would be worth speculating on.

Since PprA is found to be involved in cell division and genome maintenance in D. radiodurans, its interaction with cell division and genome partitioning proteins was investigated. Interactions of PprA with ‘Fts’ proteins were not observed using the BACTH system (Table S9). However, PprA did show interaction with ParA homologues of D. radiodurans (Fig. 10c). ParAs also showed interaction with ‘DrMin’ proteins (Fig. 10d). Earlier, BACTH analyses have been used for determining cell division and interaction of genome maintenance proteins and the results have been validated by other approaches (Karimova et al., 2005).

Furthermore, we demonstrated by BACTH analyses and co-IP that a large number of D. radiodurans proteins whose homologues have roles in cell division and genome maintenance in other bacteria do not interact in E. coli as host (Tables S3–S9). The reason for this is not clear yet. However, it is important to know that BACTH analysis can give false positive and false negative results, which may have influenced some interactions. Additionally, there are other factors that can also influence interaction of deinococcal proteins in E. coli. These could be (i) the competition by E. coli homologues of many of these proteins, (ii) the effect of phosphorylation by serine/threonine protein kinases (S/TPKs) on the interaction of deinococcal proteins and (iii) the absence of some other deinococcal proteins that are required for interaction of these proteins in E. coli. The D. radiodurans genome encodes a large number of S/TPKs (White et al., 1999). One such S/TPK (RqkA) has been characterized and its role in radiosensitivity and DSB repair has been demonstrated in D. radiodurans (Rajpurohit & Misra, 2010). Recently, it has been shown that RqkA plays a role in radiation resistance through phosphorylation-mediated

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**Fig. 10.** Diagrammatic representation of cell division and genome segregation multiprotein complexes of D. radiodurans. The core components of cell division (a), Min system proteins (b), Par proteins with PprA (c) and Par proteins with PprA and Min system proteins (d) of D. radiodurans in *in vivo* interaction with their partners are shown. Integrating all four independent proteins complexes through some common partners yielded a higher order multiprotein complex of divisome and segrosome (e). Some of the components studied here, which have not shown *in vivo* interaction directly, are actually the STRING partners reported in other bacteria and therefore shown through dotted lines (f).
activity modulation of DNA repair proteins (Rajpurohit & Misra, 2013). We have also shown earlier, that DrFtsZ interaction with DrFtsA is less efficient compared to DrFtsZ interaction with EcFtsA in E. coli as host (Modi & Misra, 2014). The effect of phosphorylation of genome partitioning proteins by S/T protein kinase on functions of these proteins has also been demonstrated in M. tuberculosis (Baronian et al., 2015). Since we know that RqkA could phosphorylate a few other D. radiodurans cell division and genome segregation proteins in vitro (H. S. Misra and colleagues, unpublished data), determining whether any of these factors affect the interaction of D. radiodurans proteins in E. coli would be worth speculating on and investigating independently.

In summary, using the bacterial two-hybrid system and co-IP, we showed that divisome, segresome and cell division regulatory proteins of D. radiodurans when overexpressed from the plasmids interact with each other, which possibly suggests the formation of multiprotein complexes of such deinococcal proteins, at least in E. coli. Such macromolecular complexes appear to interact with each other through cell division regulatory proteins like PprA and ‘Min’ proteins of D. radiodurans and may play important roles in interdependent regulation of cell division and genome segregation in this bacterium.

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