FtsK, a DNA Motor Protein, Coordinates the Genome Segregation and Early Cell Division Processes in *Deinococcus radiodurans*

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**ABSTRACT** Filament temperature-sensitive mutant K (FtsK)/SpoIIIE family proteins are DNA translocases known as the fastest DNA motor proteins that use ATP for their movement on DNA. Most of the studies in single chromosome-containing bacteria have established the role of FtsK in chromosome dimer resolution (CDR), connecting the bacterial chromosome segregation process with cell division. Only limited reports, however, are available on the interdependent regulation of genome segregation and cell division in multipartite genome harboring (MGH) bacteria. In this study, for the first time, we report the characterization of FtsK from the radioresistant MGH bacterium *Deinococcus radiodurans* R1 (drFtsK). drFtsK shows the activity characteristics of a typical FtsK/SpoIIIE/Tra family. It stimulates the site-specific recombination catalyzed by *Escherichia coli* tyrosine recombinases. drFtsK interacts with various cell division and genome segregation proteins of *D. radiodurans*. Microscopic examination of different domain deletion mutants of this protein reveals alterations in cellular membrane architecture and nucleoid morphology. *In vivo* localization studies of drFtsK-RFP show that it forms multiple foci on nucleoid as well as on the membrane with maximum density on the septum. drFtsK coordinates its movement with nucleoid separation. The alignment of its foci shifts from old to new septum indicating its cellular dynamics with the FtsZ ring during the cell division process. Nearly, similar positional dynamicity of FtsK was observed in cells recovering from gamma radiation exposure. These results suggest that FtsK forms a part of chromosome segregation, cell envelope, and cell division machinery in *D. radiodurans*.

**IMPORTANCE** *Deinococcus radiodurans* show extraordinary resistance to gamma radiation. It is polyploid and harbors a multipartite genome comprised of 2 chromosomes and 2 plasmids, packaged in a doughnut-shaped toroidal nucleoid. Very little is known about how the tightly packed genome is accurately segregated and the next divisional plane is determined. Filament temperature-sensitive mutant K (FtsK), a multifunctional protein, helps in pumping the septum-trapped DNA in several bacteria. Here, we characterized FtsK of *D. radiodurans* R1 (drFtsK) for the first time and showed it to be an active protein. The absence of drFtsK causes many defects in morphology at both cellular and nucleoid levels. The compact packaging of the deinococcal genome and cell membrane formation is hindered in *ftsK* mutants. *In vivo* drFtsK is dynamic, forms foci on both nucleoid and septum, and coordinates with FtsZ for the next cell division. Thus, drFtsK role in maintaining the normal genome phenotype and cell division in *D. radiodurans* is suggested.

**KEYWORDS** *Deinococcus radiodurans*, bacterial cell division, FtsK, DNA translocation
in a variety of bacteria like *Escherichia coli*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, etc. (1–3), which ensures an accurate cell constriction, septal peptidoglycan (PG) synthesis, and, finally, cell separation (4). One of these proteins, filament-temperature-sensitive mutant K (FtsK), was first documented in a temperature sensitive mutant of *E. coli* TOE44 that was impaired in cell division. It was observed that, at 42°C, its mutant forms highly indented filaments suggesting blockage at a very late stage of division, and was named filament temperature-sensitive mutant K TOE44 (AB2497 ftsK44) (5).

Later, cellular localization studies revealed that FtsK foci form on the septum of *E. coli* (6), and this localization of FtsK, along with FtsA, recruits other cell division proteins like FtsL, FtsQ, and FtsI, which are required for the stabilization of divisome formed at the mid-cell position (7). FtsK was characterized as a multifunctional DNA translocase belonging to the additional strand conserved E (ASCE) family P-loop NTPases (8), involved, not only in the stabilization of divisional septa and DNA translocation, but also in membrane synthesis and cell envelope remodeling by interacting with various proteins like septal peptidoglycan binding protein rare lipoprotein A (RlpA) and proteins involved in peptidoglycan synthesis like FtsI (9, 10). The protein is divided into 3 domains - the N-terminal domain of around 200 amino acids (NTD), the linker region (FtsKL), and the 500 amino acids C-terminal domain (CTD) (11, 12). In *E. coli*, the NTD is responsible for the attachment to the cell membrane by transmembrane (TM) segments and the interaction with other cell division and cell envelope proteins (13). The FtsKL is not conserved and varies in length and composition between species (14, 15). The CTD is highly conserved and comprises 3 separate subdomains called α, β, and γ (16). This region is known for the motor functions of FtsK and helps in DNA translocation. The γ domain (FtsKy) is attached to the β-domain via a flexible linker and recognizes 8-bp sequences called FtsK Orienting Polar Sequence (KOPS)(GGGNAGGG) in *E. coli* and SpoIIIE recognition sequence (SRS) (GAGAAGGG) in *Bacillus subtilis*. KOPS/SRS sequences are asymmetrically distributed on the chromosome arms, and direct the translocation of FtsK/SpoIIIE/Tra family proteins toward the deletion-induced filamentation (dif) site in the “ter” region (17, 18). In this region, 2 ‘dif’ sites are brought closer by FtsK which thereby recruits and activates tyrosine recombinases XerCD to form the synaptic complex XerCD-dif (19). This XerCD-dif complex is involved in site-specific recombination (SSR) to resolve the chromosome dimers (chromosome dimer resolution- CDR) (20, 21). In this way, FtsK helps in sorting duplicated chromosome copies at the site of cell division (22).

*Deinococcus radiodurans* R1 is a coccus-shaped Gram-positive bacterium that shows extreme resistance to multiple abiotic stresses like gamma radiation, desiccation, and oxidative stress (23, 24). These phenotypes are attributed to its highly efficient DNA double-strand break repair and a strong anti-oxidant mechanism (25, 26). This bacterium exists in tetrads and consists of a multipartite genome system (MGS) with 2 chromosomes (Chr I, Chr II) and 2 plasmids (Mega plasmid [MP] and small plasmid [SP]). It has a polyplod multipartite genome, which is highly catenated and intertwined, making the nucleoid tightly arranged in the form of a very distinct doughnut-shaped toroidal structure (27). How this genome arrangement is maintained and segregated during cell division is still unknown. The *D. radiodurans* genome encodes putative FtsK/SpoIIIE family proteins (28). In what way FtsK might act to help segregate multiple, complex chromosomes is an outstanding question of great interest. Here, we report the characterization of FtsK of *D. radiodurans* (drFtsK) and its potential role in genome separation, septum formation, and cell division. We demonstrated that the purified drFtsK showed concentration-dependent ATPase activity, sequence-specific interaction with KOPS DNA of *E. coli*, and could stimulate SSR by *E. coli* XerCD in vitro. Protein-protein interaction studies indicated that drFtsK interacts with various genome segregation and cell division proteins. Further, different domain deletion mutants of drftsK exhibited a significant change in the growth rate under normal conditions as well as after irradiation during the post-irradiation recovery (PIR) period. Morphological studies indicated that the deletion of different domains of drFtsK affected the nucleoid arrangement and
cellular architecture in this bacterium resulting in some abnormal phenotypes. The localization studies of FtsK-RFP expressed under native promoter showed the appearance of dispersed fluorescent foci on the nucleoid, septal, and peripheral membranes of the cells and a coordinated movement with FtsZ during cell growth. Together, these results suggest that drFtsK helps in cross-talks between cellular and molecular events like genome segregation, cell envelope remodeling, and septum formation in \textit{D. radiodurans}.

**RESULTS**

The \textit{D. radiodurans} genome encodes an FtsK homologue. Upon BLAST search, we found that the putative deinococcal FtsK (drFtsK) was wrongly annotated in the genome of \textit{D. radiodurans}. Indeed, the coding sequence of putative drFtsK spans upon 2 Open Reading Frames (ORF) i.e., Dr\_0400 and Dr\_0401 in chromosome I. Dr\_0401 contains the N-terminal region (229 amino acids) and Dr\_0400 contains the remaining region (980 aa) (28). Recently, this has been corrected and the entire coding sequence of FtsK is annotated with a locus tag- E5E91\_RS02025 (29). Multiple sequence alignment using the PROMALS3D tool showed that drFtsK has \(\sim\)25-30\% identity with FtsK/SpoIIIE members of other species (\textit{E. coli} [FtsK\_Ec], \textit{V. cholerae} [FtsK\_Vc], \textit{P. aeruginosa} [FtsK\_Pa], \textit{L. lactis} [FtsK\_Ll], \textit{S. aureus} [FtsK\_Sa], \textit{B. subtilis} [SpoIIIE\_Bs]). The overall protein homology is less because the N-terminal and linker region is quite variable, but the C-terminal region is highly conserved (Fig. 1A and data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). Consensus sequences for ATP binding P-loop motif (Walker A motif), Walker B motif, and winged helix-turn-helix (wHTH) DNA binding region were compared with the drFtsK sequence. The results showed that the C-terminal domain of drFtsK contains a consensus ATP binding motif and DNA binding motif indicating it is an FtsK homologue (Fig. 1A). Further, the phylogenetic analysis revealed that drFtsK forms a separate clade (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). Domain prediction exhibited that drFtsK contains the canonical FtsK domains- FTSK\_4TM (FtsKN), FtsK\_alpha, FtsK\_SpoIIIE (\(\alpha\beta\) motor pump) and FtsK\_gamma domain (drFtsK\_g) (Fig. 1B). Modeled structures of the drFtsK gamma and motor domains were aligned with the template structures of gamma (2j5p) and motor (2ius) domains in \textit{E. coli} FtsK, respectively. The corresponding aligned models showed RMSD values of 0.21 (TM score 0.938) and 0.34 (TM score 0.962), respectively, indicating that these structures are highly similar (Fig. 1C). This information led us to hypothesize that drFtsK may perform similar functions as that of characterized FtsK/SpoIIIE proteins from other bacteria, provided that this bacterium has the functional homologs of tyrosine recombinases.

We also searched for putative tyrosine recombinases taking \textit{E. coli} XerCD-specific domains for analysis. Six putative ORFs, in chromosome I- E5E91\_RS02620 (old locus tag- Dr\_0513); in chromosome II- E5E91\_RS13705 (old locus tag- Dr\_A0075), E5E91\_RS14115 (old locus tag- Dr\_A0155) and E5E91\_RS14250 (old locus tag- Dr\_A0182); in megaplasmid- E5E91\_RS15640 (old locus tag- Dr\_B0104) and in small plasmid- E5E91\_RS15930 (old locus tag- Dr\_C0018) were identified (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). Which combination of these ORFs would function as tyrosine recombinases and is significant in genome segregation in this bacterium is worth understanding and will be addressed separately.

**drFtsK shows ATPase activity.** The N-terminal truncated drFtsK contains all functional domains like ATP and DNA interacting motifs, and gamma domain and lacks transmembrane segments that would be required for localization and \textit{in vivo} function. So, N-terminal truncated drFtsK (drFtsK\_N) could be used for biochemical characterization \textit{in vitro}. Therefore, we checked the ATPase activity and DNA binding activity of purified drFtsK\_N. Circular dichroism (CD) analysis showed that purified drFtsK\_N contains folded protein and the protein has majorly \(\alpha\)-helical conformation (Fig. 2A), which was supported by \textit{in-silico} secondary structure prediction by PROMALS3D (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). Further, the oligomeric status of the protein was checked by dynamic light scattering. Results showed that the protein exists in...
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**FIG 1** Deinococcal FtsK protein sequence alignment and modeling. (A) Multiple sequence alignment (MSA) of drFtsK with known FtsK/SpoIIE family proteins. The amino acid sequences of FtsK from Deinococcus radiodurans (FtsK_Dr), SpoIIE from Bacillus subtilis (SpoIIE Bs), FtsK of Escherichia coli (FtsK_Ec), Pseudomonas aeruginosa (FtsK_Pa), Lactococcus lactis (FtsK_Ll), Vibrio cholerae (FtsK_Vc), and Staphylococcus aureus (FtsK_Sa) are collected from NCBI and the homology between sequences is checked by PROMALS3D webserver. MSA of the C-terminal region is depicted here. Boundaries of the conserved motifs in the C-terminal are marked as a black box for the walker A domain (ATP binding P-loop motif), a purple box for the Walker B motif, and a red box for the DNA binding motif. Predicted secondary structures are displayed below the sequences. (B) Different domains present in drFtsK are represented as FTSK_4TM (FtsK_N-terminal region), FtsK_alpha, FtsL_SpoIIIE (ab motor pump) and FtsK_gamma domain (drFtsK gamma). (C) Modeled structures of drFtsK and motor domain were aligned with the template structures of *E. coli* FtsK gamma domain (2j5p) and *E. coli* FtsK motor domain (2ius), respectively.

hexameric form even in the absence of DNA (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). This indicates that drFtsK binds to DNA in a pre-formed hexameric form as shown previously for SpoIIE in *B. subtilis* (30). The ATPase activity of purified drFtsKΔN was determined using \[^{32}P\]a-ATP hydrolysis (Fig. 2B) as well as a colorimetric malachite green ATPase activity assay (Fig. 2C). Results showed that drFtsKΔN is an ATPase that could convert ATP to ADP and inorganic phosphate. An increase in the release of inorganic phosphate was seen with the increase in protein concentration. However, the specific activity of the enzyme remained unaltered. Further, the presence of *E. coli* KOPS containing DNA could marginally stimulate the ATPase activity of purified drFtsKΔN (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf).

drFtsK binds to *E. coli* KOPS and activates site-specific recombination catalyzed by tyrosine recombinases - XerCD. It has been known that the FtsK protein of *E. coli* (ecFtsK) binds to KOPS and the orientation of these motifs on the chromosome is highly skewed so that they direct the FtsK translocase toward the terminal replichore region for
decatenation of the duplicated circular chromosome. Several key residues implicated in KOPS recognition have been identified in the ecFtsK gamma domain especially S766, Q769, and N777 (31). We found that these residues are not identical across FtsK/SpoIIIE family proteins, which could lead to differences in the FtsK recognition sites in different organisms. Therefore, we analyzed the distribution of a few KOPS octamers in the deinococcal genome using DistAMo online tool. The results indicated that there is a high frequency of GGGNAGGG motif family and *E. coli* KOPS-GGGCAGGG on deinococcal chromosomes I with high density existing near the ‘ter’ region. Compared to chromosome I, chromosome II has less density (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). Thus, over- or under-representation of these motifs suggests that the GGCGAGGG motif might function as KOPS in *D. radiodurans*. Compared to *E. coli*, however, the overall frequency of the GGCGAGGG motif is much less in the *D. radiodurans* genome. However, we monitored the DNA binding activity of drFtsK by EMSA using radiolabeled dsDNA sequences containing *E. coli* dif + KOPS. The protein showed a sequence-specific binding with dsDNA containing the GGCGAGGG motif (Fig. 3B). The size of the nucleoprotein complex increased gradually (reflected as slower mobility) with the rise in protein concentration suggesting more FtsK molecules are binding to KOPS. Earlier, it was shown that in general, 3 FtsKγ domains bind to 8 bp KOPS DNA (31). The presence of ATP did not affect drFtsKγ binding with KOPS sequences. This shift is not observed in the case of dsDNA of relatively shorter length, containing only the *E. coli* dif site without KOPS (Fig. 3A), suggesting that drFtsK interaction is specific to GGCGAGGG-KOPS that could act as the loading site for drFtsK on the genome in *D. radiodurans*.

As drFtsKΔN showed binding with KOPS, we tested the ability of drFtsKΔN and drFtsKγ to activate XerCD site-specific recombination (SSR). For this, the well-characterized *E. coli* recombination system components were utilized and an *in vitro* recombination assay was carried out using purified ecXerC (∼34kDa) and ecXerD (∼34kDa) proteins (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf) as described previously (31) and represented in Fig. 3C. Results showed that drFtsKΔN and drFtsKγ can stimulate ecXerCD mediated recombination between 2 dsDNA substrates- one containing only dif sites and the other containing dif + KOPS (Fig. 3C). Both drFtsKΔN and drFtsKγ showed a nearly similar pattern of recombination products in the presence and absence of ATP. The recombination at dif sites in the presence of drFtsKΔN and drFtsKγ proteins could be possible only when the γ domain in these proteins interacts with the ecXerCD and stimulates recombination activity. Upon quantification of the percent recombination product, it was noticed that the recombination reaction has occurred at low efficiency in the presence of drFtsKΔN. With drFtsKγ, it was found to be nearly similar to that observed with ecFtsKγ (recombinant protein containing *E. coli* FtsK gamma domain) which was used as a positive

**FIG 2** The ATPase activity of drFtsK. (A) Polyhistidine tagged fusion protein of N-terminal truncated protein (FtsKΔN) was purified and the purified protein was checked for proper refolding by circular dichroism as described in the methods. (B) ATPase activity of recombinant purified FtsKΔN protein was checked using radiolabeled ATP (32P)-αATP. Different concentrations of the protein were incubated with radiolabeled nucleotide and reaction products were separated on PEI-Cellulose F TLC. The autoradiogram shows the hydrolysis of 32P-αATP to 32P-αADP. (C) Quantitative analysis of ATPase activity was done by colorimetric malachite green reagent using increasing protein concentration. Data shown here are the mean ± SD (n = 3) plotted in GraphPad Prizm6. Statistical significance was obtained by Student’s t test. The P-values attained at 95% confidence intervals are depicted as (***), for <0.001 and (*) for <0.05.
control (Fig. 3D). These results together suggested that drFtsK\textsubscript{D}
and drFtsK\textsubscript{g} proteins are functional at least in vitro, and exhibit ATPase,
E. coli KOPS binding, and stimulate tyrosine recombination by E. coli XerCD at
E. coli dif sites.

FtsK deletion affects growth rate and morphology in D. radiodurans. To under-
stand the in vivo roles of drFtsK, we created chromosomal deletions of the different
regions in the coding sequences of this protein, and the effects of these deletions on
growth and morphology were examined. We analyzed the growth curve data where
the entire growth period was divided with 2 knots giving the time intervals- T1 (0 to
5 h), T2 (5 to 10 h), T3 (10 to 18 h) corresponding to lag, log, and stationary phases of
growth, respectively, as defined in the methods. The D. radiodurans cells containing
the deletion of full-length FtsK (\textit{D}ftsK\textsubscript{A}), middle and C-terminal domain (\textit{D}ftsKMC)
and N-terminal domain (\textit{D}ftsKN) (see the data at https://barc.gov.in/publications/mbio/dna
_mp/mBio01742-22R1.pdf) showed a differential effect on growth under un-
irradiated (UI) conditions (Fig. 4). For instance, under UI, \textit{D}ftsKN showed a significant change in
the growth compared to wild type (WT) during T2 (Fig. 4A and 4D). On the other hand,
\textit{D}ftsKMC and \textit{D}ftsK mutants showed extended lag period (Fig. 4B, C, and 4D) but
showed faster growth during T3 (Fig. 4B, C, and 4D). This can be understood as the
mutants might have not reached the stationary phase because of the slow growth rate
and the extended lag phase during T1 and T2 (Fig. 4B and 4C). So, we compared the
growth rate of mutants in T3 to that of the WT in T2. The growth rate of the mutants in
the T3 (0.075 ± 0.01-\textit{D}ftsKN, 0.093 ± 0.006-\textit{D}ftsKMC, 0.094 ± 0.009-\textit{D}ftsK) was still

\textbf{FIG 3} DNA binding activity and activation of \textit{E. coli} tyrosine recombinases by deinococcal FtsK\textsubscript{D}N protein. Purified recombinant FtsK\textsubscript{D}N protein was
checked for DNA binding activity with [\gamma-32P] ATP labeled dsDNA containing \textit{E. coli} dif- 40 bp (A), \textit{E. coli} dif and KOPS- 72 bp (B) in the presence/absence
of ATP. Autoradiograms show EMSA gels where no interaction of drFtsK\textsubscript{D}N is seen with only \textit{E. coli} dif sequence but interaction with \textit{E. coli} dif and KOPS is
seen. A schematic representation of the recombination reaction substrate (short radiolabeled dif containing dsDNA sequence) and product (long dif +
KOPS containing dsDNA sequence) as described earlier is given (31) (C). Autoradiogram showing site-specific recombination reaction by \textit{E. coli} tyrosine recombinases XerC and XerD (ecXerCD). Recombination products were obtained in those reactions where FtsK (drFtsK\textsubscript{D}N/drFtsK\textsubscript{g}/ecFtsK\textsubscript{g}) was present with EcXerCD. Band intensities obtained by densitometric analysis of the autoradiogram were used to calculate % recombination product in each reaction (D). Data shown here are the mean±SD (n = 3) and statistical significance was found using the Student’s \textit{t} test. The \textit{P}-values attained at 95% confidence intervals are depicted as (*) for <0.05 and (**) for 0.05-0.001.
lower than that of WT in T2 (0.142 ± 0.008). During the post-irradiation recovery period (IRR), the effect of drFtsK domains deletion on the growth rate is different from unirradiated conditions. The growth of ΔftsKMC and ΔftsK mutants is significantly slower than the WT (R1) and ΔftsKN during T1. The decreased growth rate was observed particularly during the lag phase under the post-irradiation recovery phase and normal growth conditions, where cellular processes like DNA damage repair, genome duplication, segregation, and cell division are actively occurring. This would have increased the generation time of the mutants (Fig. 4E). The slow growth rate in mutants indicated that FtsK may play some critical role in the normal cellular processes like genome segregation and cell division in this bacterium.

Further, we compared the cell morphology of ftsK deletion mutants with WT under non-irradiated (normal) conditions at 14 h post subculturing. The FtsK mutants, ΔftsKN, ΔftsKMC, and ΔftsK showed statistically significant changes in their cellular parameters like percent of tetrads and diads in a population, cell diameter, nucleoid compaction (as determined by nucleoid diameter), and nucleoid intensity (as determined by measuring DAPI fluorescence intensity). These phenotypes were quantified and compared to the wildtype (Fig. 5C, D, E, and 5F). The mutants showed a significantly higher number of diads than tetrads compared to the WT. Also, the mutant cells showed bigger cell and nucleoid diameter, and higher DAPI fluorescence intensity compared to the

**FIG 4** Effect of deinococcal FtsK deletion on the growth of *D. radiodurans*. Cell survival studies of different deletion mutants of ftsK-ΔftsKN (A), ΔftsKMC (B), and ΔftsK (C) were monitored under un-irradiated conditions (normal) and after gamma irradiation treatment (6kGy). The growth data (circles) were fitted by a linear spline regression model (lines). The dashed vertical lines show the knots dividing the spline modeled growth curve into three intervals- 0 to 5 h (T1), 5 to 10 h (T2), and 10 to 18 h (T3). The difference in the growth rates of wild-type (R1) and different domain deletion mutants under normal and post-irradiation recovery conditions at each time interval was calculated by estimating the slopes of each line segment from the spline regression (D). The generation time (doubling time) of wildtype and ftsK mutants under normal and irradiated conditions was calculated as described in the methodology and plotted (E). Data shown here are the mean ± SD (n = 9) and the statistical significance of the differences was found using the Student’s *t* test. The *P*-values attained at 95% confidence intervals are depicted as (*) for <0.05 and (**) for 0.05-0.001.
WT cells (Fig. 5A and 5B). Besides these, around ~10% cell population of the mutants showed atypical cellular morphologies like abnormal tetrad arrangements (ABN), bent septum (BS), and anucleated cells within the tetrad (AC-T). An increase in the nuclear parameters like diameter and DAPI intensity suggests a putative role of drFtsK in genome segregation and chromosome organization, while changes in the cellular parameters in mutants, like cell diameter, indicate growth/cell division impairment. To understand these phenotypes further, we expressed FtsZ, a major cell division protein, as FtsZ-GFP (pVHZGFP) in ΔftsK mutant, and cells were monitored for changes, if any, in FtsZ functions. We observed that a significant population (~10%) in ΔftsK deletion mutant got affected in the positioning of the FtsZ ring in *D. radiodurans*, as seen by FtsZ-GFP ring misplacement (Fig. 5G). Earlier, it was shown that deletion of the C-terminal region of DivIVA protein in *D. radiodurans* generates the bent septum (32). So, the presence of bent septum in ΔftsK mutant may be attributed to its probable role in the positioning of the pole determining protein and divisome components (like FtsZ) in this bacterium. In addition to bent septum and defective FtsZ ring formation, we also found that the FtsK mutants formed membrane bulges and showed changes in membrane staining properties at exponential phase time points of 6 and 9 h of subculturing. As shown at the 6 h time point, all the mutants showed membrane bulging in a significant number of cells. Notably, the ΔftsKN mutant cell was able to mostly recover from this phenotype at 9 h, but the other 2 mutants could not recover, indicating the role of drFtsK in membrane biology in *D. radiodurans* (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). These phenomena also coincide...
with the extended lag period and increased growth rate during initial growth periods, further suggesting the drftsK role in bacterial cell cycle processes. Further, the survival of cells upon ftsK deletion, even after significant phenotypic changes, might argue its functional redundancy in this bacterium.

FtsK localizes on nucleoid and septum in *D. radiodurans*. The translational fusion of drftsK-RFP was expressed under a native promoter in *D. radiodurans*, and the cellular localization of drftsK was observed microscopically. Interestingly, FtsK-RFP produced foci on the membrane (septal and peripheral) and the nucleoid (Fig. 6A). When we quantitated the number of foci in the cell, most of the foci were found to be on the septal membrane (SM) in comparison with the peripheral membrane (PM), and nucleoid (N), both in the diad and tetrad population (Fig. 6B). This result became more apparent when the cells’ growth rate was reduced by growing on agar plates and observing them under the microscope (Fig. 6C). Under these slow growth rate conditions, maximum FtsK-RFP foci density was seen on the septum. Around 200 cells were analyzed to determine the fraction of cells showing localization at the old or new septum, dispersed, and both on the new and old septum making a cross pattern (Fig. 6D). The formation of the new septum was determined based on the vancomycin staining, or the appearance of septum constriction as observed from the DIC pictures. Based on these observations, it can be speculated that FtsK protein localization is dynamic in *D. radiodurans*, showing on both nucleoid and septum depending on the growth stage of the cells.

Time-lapse microscopy shows the movement of FtsK-RFP along the new septum in *D. radiodurans*. We observed that FtsK-RFP localization on the nucleoid and the septum is heterogeneous, possibly because of the non-synchronous population of the cells. This prompted us to do time-lapse microscopy to observe the FtsK dynamics in dividing cells of *D. radiodurans*. Time-lapse microscopy was performed under both normal growth and the post-irradiation recovery phase (Fig. 7). Under normal
conditions, at time point \( t = 0 \), maximum FtsK-RFP foci were aligned at the septum. With progression in the cell division, the alignment of the foci shifted to the newly forming septum perpendicular to the old septum \( (t = 2) \). This alignment was seen when the nucleoid separated into 2, and the septum constriction was just initiated. By \( t = 4 \), FtsK-RFP foci were seen to be moving to align along the next probable plane of division (Fig. 7A). In gamma radiation treated cells, the transition in the alignment of FtsK foci from the old septum to the new septum as the nucleoid divides was more

**FIG 7** Cellular dynamics of deinococcal FtsK-RFP in normal and post-irradiation conditions. Time-lapse confocal microscopic images show FtsK-RFP (red foci) expressing cells stained with SYTO 9 green dye (green) during the normal condition (A) and during post-irradiation recovery (PIR) (B). The top panels indicate the cell in plan view at different time points \((t = \text{hrs})\) in the DIC channel, the middle panels indicate the genome arrangement in the FITC channel and the bottom panels show the same cell with TRITC and FITC channel merged to see FtsK-RFP foci dynamics at different \( t' \) (scale bar-1 \( \mu \text{m} \)). The dynamics of FtsK-RFP foci is schematically represented under normal condition. During PIR, line scan analysis (LSA) shows the increase in the intensity of FtsK-RFP along the emerging septum.
apparent (Fig. 7B). At t = 0, FtsK-RFP foci were arranged along the membrane, mostly with prominently high foci density at the initiation sites of the new pole on septal and peripheral membranes. As time proceeded, most of FtsK-RFP foci positioned along the new division septum, perpendicular to the old one. Notably, line scan analysis (LSA) showed that the progression of FtsK alignment with the nucleoid separation process was very well synchronized (Fig. 7B). Thus, FtsK-RFP dynamically switched from old septa to new septa as the cell division progressed under both normal and irradiated conditions. Orientation of FtsK at the septum would be functionally essential to activate CDR so that the genome can be separated properly and segregated correctly into daughter cells. It was observed that the nucleoid divides into 2 daughter toroidal structures simultaneously with the FtsK dynamics from the old septum to the new septum. In E. coli, FtsK is known to bind to KOPS sequences on the genome and translocates toward the dif site. It also acts as a translocase by pumping out the septum-trapped DNA during chromosome separation and cell division (12). Our results obtained from the localization studies of drFtsK-RFP suggest a similar type of function in D. radiodurans also. Interestingly, there is an evident increase in the level of FtsK protein expression after irradiation (33). This could be explained by the transcriptome sequencing analysis where an increase in the ftsK transcript levels was seen after irradiation (33). D. radiodurans contains 6 to 8 copies of the genome which are damaged when treated with 6kGy gamma radiation. So, the participation of translocases like FtsK during the repair and resolution of the multipartite genome cannot be ruled out. There may be an increase in nuclear entanglement and chromosome dimers formation at the time of extensive synthesis and recombination of DNA during post-irradiation recovery. These cells may require the participation of FtsK and other proteins for activation of CDR. Thus, the results so far suggested that FtsK is dynamic in the cells under both normal and post-irradiation recovery conditions, and may be actively involved in the coordination of genome segregation and early cell division processes in this bacterium.

**Coordinated dynamics of FtsZ and FtsK in D. radiodurans.** The septum localization of FtsK-RFP and the presence of bent septum in the deletion mutant of ftsK led us to study the dynamics of FtsK with another cell division protein-FtsZ concurrently in D. radiodurans. For this, the cells expressing FtsK-RFP under the native promoter were expressed with FtsZ-GFP on the pHZGFP plasmid. During time-lapse microscopy, ~75 cells were monitored for studying FtsZ and FtsK dynamics and analyzed. A single cell is shown in Fig. 8A. At the start of imaging (t = 0), the FtsZ ring was not complete, but as the cell growth proceeded, the FtsZ ring was formed in ~3 h (t = 3). The majority of

![FIG 8 Co-ordinated cellular dynamics of cell division proteins FtsK and FtsZ in D. radiodurans. Time-lapse fluorescence microscopic images of cells expressing both FtsK-RFP and FtsZ-GFP show the dynamics of both the proteins in dividing cells under normal conditions. The top panel indicates the cell in planar view at different time points (t = hr) in the DIC+TRITC, the middle panels indicate the FITC+TRITC channel and merged images, and the bottom-most panel shows the same cell in voxel view with white foci showing the co-localization of FtsZ and FtsK (PCC > 0.7) (scale bar=1μm). The majority of FtsK-RFP (red) can be seen aligned at the division septa in t = 0.5 h and then moving to position themselves along the almost complete FtsZ ring at t = 3h (A). The total number of FtsK foci and FtsK-FtsZ co-occurring foci in a population of ~75 cells at time points of 0, 0.5 h, 1.5 h, 2 h, and 3 h were calculated and plotted (B).](image-url)
FtsK-RFP could be seen aligning around the division septa at $t = 0.5$, and then moving to position itself along the growing FtsZ ring at $t = 3$ (Fig. 8B). This suggests that drFtsK localizes to the newly forming divisional septum after the FtsZ ring formation is completed. These microscopic results showed that FtsK aligns along the developing septum acting in coordination with FtsZ. Further, co-localization analysis of FtsZ and FtsK proteins suggest that both the proteins significantly co-localize in some places. Our microscopic results are the first reports showing coordinated dynamics of the important cell division proteins i.e., FtsZ and FtsK in *D. radiodurans*.

FtsK interacts with divisome and segrosome components of *D. radiodurans*. Results obtained so far suggest that FtsK is dynamic during different stages of bacterial growth, and plays an important role in both genome segregation and early cell division. The molecular basis of its dynamicity mainly relating to cell division and genome segregation would be worth understanding. Further, FtsK in other bacteria is known to interact with segrosome and divisome proteins (14). Therefore, the interaction of drFtsK with cell division and chromosome segregation proteins of *D. radiodurans* was checked by co-immunoprecipitation. It was observed that drFtsK interacts with deinococcal genome segregation proteins ParB2, ParB3, ParB4, and TopoIB; cell division proteins FtsZ, FtsA, and pole determining protein- DivIVA (Fig. 9). Its interaction with DivIVA and FtsZ could explain the reason behind the bent septum phenotype observed in *ftsK* mutants. Surprisingly, it does not interact with some of the deinococcal proteins like ParAs, GyrA, FtsW, and FtsE which are involved in nucleoid segregation and cell division. These results suggest that drFtsK, along with other genome segregation and cell division proteins, may be involved in macromolecular complex formation for its overall functions, and the possibility of this driving the dynamicity of FtsK independently of FtsZ cannot be ruled out in *D. radiodurans*.

**DISCUSSION**

*D. radiodurans* R1 is a multipartite genome harboring (MGH) bacterium having 2 chromosomes and 2 plasmids of 8 to 10 copies each per cell during the exponential growth phase (34, 35). The genome exists in a highly compacted nucleoid form which has been recently shown to dynamically change its shape during the cell division cycle (36). *D. radiodurans* exists in tetrads and grows by alternate planes of division where new cell division occurs in a plane perpendicular to the previous one. FtsK/SpoIIIE DNA translocases in bacteria are known to be involved in chromosome dimer resolution and pumping the trapped DNA through the newly formed septum during cell division or sporulation. Despite the critical role of FtsK, it is reported to be dispensable for division under certain conditions (37, 38). Most of these studies are done in single chromosome containing bacteria. Studies on the role of FtsK in MGH bacteria would be fascinating due to the complexities of multiple genome components present in the cell and among MGH, the work on FtsK have been
reported only in *V. cholerae* (39, 40). Our genome analysis revealed that *D. radiodurans* encodes putative FtsK. Unlike *V. cholerae*, where both the chromosomes exist separately in the cells, the entire genetic material in *D. radiodurans* is packaged in the form of a doughnut-shaped toroidal nucleoid. Therefore, the possibility of FtsK functioning differently in maintaining the genome integrity of *D. radiodurans* cannot be ruled out. Here, we have brought forth some proof to indicate that drFtsK is active and is involved in both genome segregation and early cell division processes (Fig. 10). We demonstrated that recombinant FtsK is an ATPase with *E. coli* KOPS binding activity and could activate *E. coli* tyrosine recombinases XerCD in vitro, suggesting that drFtsK is functional in stimulating SSR. Fluorescence microscopy of cells expressing drFtsK-RFP showed the formation of multiple foci on the nucleoid as well as on the membrane. Multiple foci on the nucleoid could be attributed to the presence of KOPS on the genome, while foci formation on the membrane and/or septum could be attributed to FtsK transmembrane domain interacting with divisome components. A similar distribution of FtsK foci (~30 to 100 molecules of FtsK per cell) was reported in *E. coli* (41). Further, the localization pattern of FtsK in *D. radiodurans* seems to be cell cycle-specific where maximum protein intensity was observed on the septum in the dividing cells.

FtsK/SpoIIIE DNA translocases are multidomain and multifunctional proteins that are part of bacterial cell divisome machinery (42). In *D. radiodurans*, our earlier work showed the involvement of ParB6, TopoI6 in genome segregation, and FtsZ, FtsA, and DivIVA in cell division (43–46). The co-immunoprecipitation results showed that drFtsK interacts with these proteins indicating its role in both of these processes. When different domain-specific drftsK deletion mutants were generated, the growth rate of mutants was significantly reduced during the early and exponential phases. Remarkably, the mutants delay growth

**FIG 10** Schematic model of the dynamics of FtsK in *D. radiodurans* during cell division. Based on the biochemical assays and cellular localization studies, the probable role and dynamics of FtsK protein in exponentially growing *D. radiodurans* were proposed. FtsK forms foci on the nucleoid as well as on the membrane. The movement of FtsK can be linked with the different growth phases (P) as described. Initially, a maximum number of FtsK foci are aligned at the septum (P1). As the cell grows and the genome duplicates, FtsK foci move in the cell (P2). With the formation of the FtsZ ring and subsequent initiation of the new septa, the alignment of FtsK starts shifting perpendicularly from the old one to the new one (P3). When a new septum is partially formed, FtsK foci alignment is found on the old as well as new septum (P4). With complete new septum formation, FtsK moves entirely to the new septa before cell wall constriction starts (P5). The whole process repeats in the next cell division. The ATPase activity, sequence-specific DNA binding activity, and cellular interaction of FtsK with other segrosome and divisome proteins may aid in the smooth and coordinated progression of different cellular processes to maintain the genome stability in *D. radiodurans*. 
and extend the growth phase to a region where the WT has already reached the stationary phase. Microscopy data showed improper membrane staining and membrane bulges in ftsK mutants. Membrane bulges were also observed earlier in S. aureus ΔftsKΔspoIIIE mutants (47). Recently, the roles of FtsK/SpoIIIE have been suggested in envelope remodeling in bacteria (48). All of this indicates the role of drFtsK in envelope reorganization during cell division and in mutants, where the lack of this function might have caused the delay in the growth rate (38). Previously, the movement of DNA through the tetrad compartments during PIR was shown to be necessary for the radiation resistance phenotype (27). The reduced growth rate was observed only during the initial time points (T1 period) in mutants exposed to gamma radiation, which could also be attributed to the delayed DNA translocation through the septum which may be required for accurate genome reassembly. Further, under normal growing conditions, the fast recovery of the mutants at the later phase can be speculated to be due to the bypass of FtsK, as seen earlier in E. coli by FtsA and FtsN interaction (49).

The genome segregation process in E. coli progresses from the ori-to-ter loci, and FtsK protein was actively involved in the “ter” locus positioning at the mid-cell region (50). In V. cholerae with 2 divergent difs, chromosome resolution sites were identified for 2 chromosomes, and both are activated by FtsK (39). In D. radiodurans also, genome sequence analysis has revealed putative “dif” sites and 6 putative tyrosine recombinases on both primary and secondary chromosomes, which show around 25 to 30% similarity to E. coli tyrosine recombinases (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). An independent study will be conducted to check the functional interacting partners of drFtsK among these recombinases and their role in chromosome dimer resolution at “dif” sites.

Recent electron cryo-microscopy (cryo-EM) structure studies of dsDNA bound FtsK<sub>ΔN</sub> from P. aeruginosa revealed changes in different conformational states of ATPase domains in homo-hexameric rings that generate the translocation movement on DNA (51). drFtsK also forms homo-hexamers and the time-lapse microscopy results showed that FtsK-RFP is dynamic, orientating its translocating activity along the new septum from the old septum in dividing cells. Based on the observed results, FtsK movement can be linked to the different growth phases (P), as described earlier in exponentially growing D. radiodurans (36), where the active role of FtsK protein in the smooth coordination of genome segregation and cell division processes can be thought of (Fig. 10). As shown in the pictorial form, during the exponential growth phase, when a cell is divided into 2 forming the diad, most of the FtsK aligns on the old septum (P1) (Fig. 10). During the subsequent cell growth and genome duplication and elongation, FtsK disperses on the genome (P2) (Fig. 10). At the early cell division stage, when FtsZ ring formation and initial constriction occur, FtsK alignment shifts from the old to the new septum (P3) (Fig. 10). Foci alignment on the new septum is more prominent at the later stages of genome segregation and cell septum formation (P4 and P5) (Fig. 10). Thus, FtsK may be involved in marking the new septum, and each cell divides into 2, forming a tetrad after complete septum formation and cytokinesis (P6) (Fig. 10).

All the results suggest that the dynamic multiprotein interactions coordinate accurate segregation of duplicated intertwined circular genome elements, as well as the next plane of cell division in this coccus. In conclusion, the drFtsK characterized in this report is a multifunctional protein having ATPase and DNA binding activities, and has a role in the determination of cell architecture, in general, and a faithful inheritance of its multipartite genome system.

MATERIALS AND METHODS

**Bacterial strains, growth conditions, plasmids, and materials.** All bacterial strains and plasmids used in this study are listed at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf. D. radiodurans R1 (ATCC13939) was a kind gift from Professor J. Ortner, Germany (52). This bacterium was grown in Tryptone (0.5%), Yeast extract (0.3%), and Glucose (0.1%) (TYG) medium at 32°C. E. coli strains were grown in LB broth (1% tryptone, 0.5% yeast extract, and 1% sodium chloride) at 37°C. E. coli strain DH5α was used for cloning purposes and maintenance of all the plasmids. For the expression of recombinant proteins, E. coli strain BL21(DE3) pLysS was used. Cells harboring PET28a (+) derivatives or Bacterial Two-Hybrid System (BACTH) vectors and their derivatives were maintained in the presence of the respective antibiotic selection pressure. Shuttle expression vector pVHSM (53) and its derivatives.
were maintained in the presence of spectromycin in *E. coli* and *D. radiodurans* cells (70 μg/mL and 40 μg/mL, respectively). Deinococcal cells harboring a suicide vector Dr- pNOOKOUT (S4) derivatives were grown in the presence of kanamycin (8 μg/mL). All the enzymes and molecular biology grade chemicals were purchased from Sigma Chemical Company, and Merck India Pvt. Ltd. Isopropyl-β-D-1-thiogalactopyranoside (IPTG), DAPI, Nile red, and vancomycin hydrochloride (unlabeled) were purchased from Merck Inc. Vancomycin BODIPY FL conjugate (labeled) was purchased from Invitrogen. SYTO 9 green fluorescent nucleic acid stain was purchased from Sigma-Aldrich. Antibody against T18 (SC-33620) domain of CyaA of * Bordetella pertussis*, was obtained commercially from Santa Cruz Biotechnology, Inc. Antibody against polyhistidine tag was procured from Sigma. Labelled nucleotides were acquired from the Board of Radiation and Isotope Technology, Department of Atomic Energy (DAE), India (BRTI, India).

**Biological analysis and molecular modeling.** The *D. radiodurans* genome encodes a putative FtsK (locus tag E5E91_RS02025, old locus tags Dr_0400 and Dr_0401) on chromosome I, annotated as DNA translocase FtsK (hereafter called drFtsK). The full-length FtsK sequence of *D. radiodurans* and its other characterized homologs in *B. subtilis* (SpoIIIE, Ec), *E. coli* (FtsK, Ec), *P. aeruginosa* (FtsK, Pa), *S. aureus* (FtsK, Sa), and *V. cholerae* (FtsK, Vc) were retrieved from the NCBI genome database. Multiple sequence alignment and secondary structure prediction were performed with the Promals3D online server (55). The neighbor-joining phylogenetic tree (without distance corrections) between deinococcal FtsK and known FtsK family proteins was constructed using the PHYLIP program. After alignment, the Walker box (walker A and walker B) ATP binding motif and DNA binding motif were searched. Structural models of drFtsK protein domains (motor domain and gamma domain) were constructed by the I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER). The models were validated by the Swiss model workspace. Templates used for the modeling of drFtsK domain structures were derived from the known structures of respective *E. coli* FtsK motor domain (PDB ID: 2ius) and gamma domain (PDB ID: 2jsp). The modeled structures of the deinococcal FtsK domains were superimposed with *E. coli* FtsK domain structures (2ius and 2jsp) using PyMOL software and RMSD, and TM score were analyzed which indicated high similarity between the models. KOPS motifs were searched across the *D. radiodurans* genome by DistAMo online tool, shown at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf (56).

**Cloning, expression, and purification of recombinant proteins.** A list of all the primers used for constructing recombinant plasmids and generating deletion/insertion mutants are mentioned at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf. The open reading frames (ORF) of drFtsK (Dr_0400/0401), drFtsKΔN (Dr_0400), drFtsKΔ C, drFtsKΔN, and ecFtsKΔN were PCR amplified from *D. radiodurans* or *E. coli* genome (as applicable) using sequence-specific primers. PCR products were purified and ligated at NdeI and BamHI sites in pET28a (+) to produce the respective plasmids (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). These plasmids were transformed into *E. coli* BL21 (DE3) pLyS. Induction of expression and preparation of cell extract was performed as reported earlier (57). Purification of proteins was done by nickel affinity chromatography. For that, the cell extract was loaded onto NiCl3-charged fast-flow-chelating-Sepharose column (GE Healthcare) which was pre-equilibrated with buffer (20 mM Tris-HCl, 300 mM NaCl, 10% glycerol) containing 10 mM imidazole. Extensive washing of column was done with 20 volumes of buffer A having 50 mM imidazole. Stepwise elution of recombinant proteins was done using 100 mM, 200 mM, and 250 mM imidazole in buffer A, and analysis of each fraction was carried out on 10% SDS-PAGE. Fractions containing the desired proteins at the expected size were pooled and dialyzed in buffer A containing 100 mM NaCl, and proceeded for HitTrap Heparin HP column purification. drFtsKΔN protein quality was examined by Circular dichroism (CD) spectroscopy in buffer (20 mM Tris-HCl, pH 7.6, 100 mM NaCl) using JASCO, 815 with ~0.2 mg/mL, as described earlier (43). The oligomeric status of the protein was checked by Dynamic Light Scattering (DLS) in the same buffer as mentioned before, using the Malvern Panalytical Zetasizer nano range instrument with/without KOPS DNA (100 nM) and ATP (0.5 mM).

**ATPase activity assay.** ATPase activity detection was done by thin-layer chromatography method to track the release of ([32P]〜)ADP from ([32P]〜)ATP and by malachite green assay to quantify the amount of inorganic phosphate (Pi) released as described in (43). Briefly, different concentrations of drFtsKΔN were added with 30 mM [32P]〜 ATP in a buffer containing 20 mM Tris (pH 7.6), 75 mM KCl, 2 mM MgCl2, and incubated at 37°C for 0.5 h. The reaction was discontinued using 10 mM EDTA, and 1 μL of each reaction mixture was spotted on the FEI-Cellulose F+ TLC sheet. Components were separated on solid support after being air-dried in a buffer containing 0.75M KH2PO4/H3PO4 (pH 3.5), and an autoradiogram was developed. For the colorimetric quantitative assay, ATPase/GTPase activity assay kit (Sigma-Aldrich) was used with/without KOPS DNA (100 nM) and μmole Pi was released per min per μL was calculated. Data were plotted using the GraphPad Prism 6 software.

**Protein-DNA interaction study.** The DNA binding activity of drFtsKΔN was checked by electrophoretic mobility shift assay (EMSA) as described in (58). In brief, ssDNA containing *E. coli* dif A or KOPS (72mer) or *E. coli* dif (40mer) sequence were radiolabeled with [32P]〜ATP using T4 polynucleotide kinase (31). Forward strands were annealed with the complementary strands to yield radiolabeled dsDNA (dif and dif-KOPS). Approximately 30 nM radiolabeled dsDNA was incubated with different concentrations of drFtsKΔN (0 to 2 μM) in a buffer containing 50 mM Tris-Cl (pH 8.0), 75 mM NaCl, 5 mM MgSO4, and 0.1 mM DTT for 30 min at 37°C with/without 2 mM ATP. The reaction mixture was loaded on 8% native PAGE gel, the gel was dried, and the autoradiogram was developed.

**Recombination assay.** An in vitro recombination reaction was performed as reported by (31). A total of 1 nM radiolabeled dif fragment and 10 nM dif-KOPS fragment was incubated with 150 mM ecXerC, 30 nM ecXerD in buffer containing 10 mM MgCl2, 20 mM Tris/HCl pH 7.6. To this, 40 nM FtsK variants (drFtsKΔN, drFtsKΔC, and ecFtsKΔN) were added separately and incubated for 2 min. The reaction was monitored in the presence/absence of 2 mM ATP for 30 min at 37°C. The reaction was stopped by adding

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**DNA Motor Protein FtsK Shows Dynamics in *D. radiodurans***

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0.1% SDS and 0.1 mg/mL protease K, and the mixture was run through a 7% denaturing PAGE gel containing 0.1% SDS in 1X TBE buffer. The gel was dried, and an autoradiogram was developed. Band intensities on the autoradiogram were estimated densitometrically by ImageJ 2.0 software and % recombination product was calculated.

**Generation of drftsK deletion mutants and ftsK-rfp knock-in mutant.** For creating deletion mutants of the different domains of FtsK in *D. radiodurans*, the respective regions of the coding sequence of ftsK (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf) were replaced by nptII as described earlier (54). In brief, ~1 kb upstream and ~1 kb downstream sequences to the target region were PCR amplified from *D. radiodurans* genomic DNA using sequence-specific primers (as given at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). The upstream fragment was cloned at Apal and EcoRI sites while the downstream fragment was cloned at BamHI and Xbal sites into a suicide pNOKOUT vector (54) to produce pNKFKUD, pNFKL_UUD, and pNFKL_UD plasmids (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). The recombinant plasmids were linearized with XmaII and transformed into *D. radiodurans* separately. Transformants obtained were plated on TGY plates containing kanamycin (8 µg/mL) and grown for many generations under necessary antibiotic pressure to obtain homozigous insertion of nptII and replacement of the target portions of ftsK in the whole deinococcal genome. This was confirmed by PCR amplification using ftsK domain-specific primers as well as antibiotic (nptII) cassette-specific primers (see the data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf).

The homozigous replacement of the target genes with the nptII cassette was accomplished, and these cells with genotype ftsK::nptII, ftsKNC::nptII were denoted as ΔftsK, ΔftsKN, ΔftsKMC respectively. The ftsKNC::nptII mutant has a deletion of N-terminal 1 to 163 amino acids of drFtsK (M1-L163), ftsK::ftsK-rfp mutant has a deletion of 295 to 1209 amino acids of drFtsK (D295-K1209), and ftsK::nptII has a deletion of full-length drftsK (M1-K1209).

For expression of ftsK-rfp under the native promoter of ftsK, the replacement of chromosomal copy of ftsK with ftsK-rfp was carried out using a similar approach as mentioned above. The translational fusion of FtsKy-RFP was generated by cloning the ftsK gamma domain (ftsKγ) in pDsRed vector to yield pDsRedFkγ. Further, ftsKγ-RFP (from pDsRedFkγ) was cloned upstream to nptII and downstream ftsK sequence was cloned downstream to nptII in pNOKOUT to yield pNFKFkγR. This was transformed into *D. radiodurans* to obtain cells with genotype ftsK::ftsK-rfp, which expressed FtsK-RFP under the native promoter.

**Growth studies of different deletion mutants of ftsK.** *D. radiodurans* R1 WT and ftsK mutants were exposed to 6kGy gamma radiation as described in (59). Briefly, overnight grown bacterial cultures (with/without kanamycin: 8 µg/mL) were washed and suspended in sterile phosphate-buffered saline (PBS). These cells were then exposed to 6 kGy gamma radiation at a dose rate of 1.5 kGy/h (Gamma Cell 5000, 60Co, Board of Radiation & Isotopes, Kolkata, India). Isolated numbers of IR and control UI cells were grown in TYG medium (with/without kanamycin: 8 µg/mL) in 96-well microtiter plates after washing with PBS (Nunc, Denmark) (from pDsRedFK). Optical density at 600 nm was measured to examine the growth in replicates at 32°C for 18 h using Synergy H1 Hybrid multi-mode microplate reader. The growth curves were fitted using the spline regression model. Statistical analysis was carried out using the statistical programs R, http://www.r-project.org/, the “spline” package was used to generate the spline regression-based models in R. The model was developed using linear b-splines with 2 knots producing 3 intervals (1,2,3). Fixed time intervals were chosen as 0 or 5 h (T1), 5 to 10 h (T2); and 10 to 18 h (T3). The linear growth rates were computed by the change in absorbance (OD) per interval. UI samples were compared with R1-UI as control, and IR samples were compared with R1-IRR as control. The generation time of wildtype and ftsK mutants were calculated as mentioned in (60).

Graphs of growth rate coefficient v/s strain at each time interval and generation time were plotted using GraphPad Prism 6.0. Significance value (P value) obtained at 95% confidence intervals were considered to be significantly different.

**Microscopy studies of different deletion mutants of ftsK.** Confocal microscopy was done on IX53VR using an Olympus IX83 inverted microscope with the laser beams focused on the back focal plane of a 100 × 1.40 NA oil-immersion apochromatic objective lens (Olympus) as described earlier (44). The time sequence and intensity of laser illumination at samples were adjusted using the installed FLUOVIEW software. For imaging, a series of Z-planes were acquired at every 400 nm using a motorized stage, and then z-stacking was done to create 3D images. In brief, *D. radiodurans* R1 WT cells and different deletion mutants of ftsK in exponential phase at 3 time points, 6 h, 9 h, and 14 h were taken (Fig. 5 and data at https://barc.gov.in/publications/mbio/dna_mp/mBio01742-22R1.pdf). The cells were washed twice with phosphate-buffered saline (PBS) (pH 7.4). These cells were stained with Nile red (1 mg/mL) for membrane and DAPI (40,6-diamino-2-phenylindole, dihydrochloride) (0.2 mg/mL) in 96-well microtiter plates after washing with PBS (Nunc; Sigma-Aldrich). Optical density at 600 nm was measured to examine the growth in replicates at 32°C for 18 h using Synergy H1 Hybrid multi-mode microplate reader. The growth curves were fitted using the spline regression model. Statistical analysis was carried out using the statistical programs R, http://www.r-project.org/, the “spline” package was used to generate the spline regression-based models in R. The model was developed using linear b-splines with 2 knots producing 3 intervals (1,2,3). Fixed time intervals were chosen as 0 to 5 h (T1), 5 to 10 h (T2); and 10 to 18 h (T3). The linear growth rates were computed by the change in absorbance (OD) per interval. UI samples were compared with R1-UI as control, and IR samples were compared with R1-IRR as control. The generation time of wildtype and ftsK mutants were calculated as mentioned in (60).

Graphs of growth rate coefficient v/s strain at each time interval and generation time were plotted using GraphPad Prism 6.0. Significance value (P value) obtained at 95% confidence intervals were considered to be significantly different.
described above. Around 150 diad and 150 tetrad cells were counted separately for the quantification of localization of FtsK-RFP foci on the nucleoid and the peripheral membrane or the septal membrane. Data were plotted as a scatterplot in GraphPad Prism 6 software. For FtsZ localization studies, the recombinant plasmid pHZGFP expressing FtsZ-GFP was transformed in WT and ΔftsK-mutant cells of D. radiodurans. Cells were grown overnight, and cells equivalent to 0.05 to 0.1 OD₆₀₀ were diluted with a fresh medium containing the required antibiotic and induced with 10 mM IPTG overnight.

**Growth phase-dependent studies on FtsK and FtsZ dynamics.** Time-lapse imaging was done for the monitoring of the dynamics of FtsK with respect to genome movement, cell growth, and division. Cells expressing FtsK-RFP were stained with 150 nM SYTO 9 dye and PBS washed cells were placed on an agarose pad made in 2X-TYG and constructed with air holes for oxygenation of cells. Fluorescence emission was grabbed with DM-488/561 dichroic mirror and corresponding single-band emission filters at different time intervals. Images were taken for a period of 4 h at intervals of 1 h using very low laser power (561 nm and 488 nm). For monitoring FtsK localization and dynamics under gamma radiation exposure, the cells were treated with 6kGy radiation and grown for 1 h with constant shaking. Then, the cells were visualized as mentioned above. We did a line scan analysis (LSA) for each time point with CellSens software. In LSA, the represented co-localization patterns showed Pearson’s correlation coefficient (R(ř)) > 0.7-1.0 and Overlap coefficient (R) of 0.9 to 1.0. Co-localization was represented as white foci in the images. A total of 76 cells were analyzed at each time point to determine the number of FtsK foci co-occurring with FtsZ. Values obtained were plotted using GraphPad prism-6.0.

**Protein-protein interaction study.** The possible interaction of drFtsK with other deinococcal proteins of cell division like FtsZ, FtsA, and DivIVA, and genome segregation proteins like TopoIB, ParB2, ParB3, and ParB4 was monitored in surrogate E. coli by using the co-immunoprecipitation method. The recombinant pUT18 plasmids that were already used in the earlier studies (45, 46, 62, 63) were transformed in surrogate E. coli by using the co-immunoprecipitation method. The recombinant pUT18 plasmids were already used in the earlier studies (45, 46, 62, 63) were transformed in E. coli BL21(DE3) pLyS strain expressing histidine-tagged FtsK (pETFtsK). E. coli cells co-expressing histidine-tagged FtsK with T18-tagged protein in separate combinations were obtained at the log phase and cell extracts were prepared. Total proteins were immunoprecipitated using anti-poly-histidine antibodies and the possibility of T18 tagged partners’ presence in immunoprecipitate was checked using monoclonal antibodies against the T18 domain of CyaA as depicted earlier (62, 63).

**Statistical analysis.** All the statistical analysis was done using Student’s t test. Significance value (P value) obtained at 95% confidence intervals are depicted as **** for P value <0.0001, *** for P value <0.001, ** for P value of 0.005-0.001, and * for P value <0.05.

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