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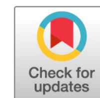
MINIREVIEW



Replication Restart in Bacteria

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ABSTRACT In bacteria, replication forks assembled at a replication origin travel to the terminus, often a few megabases away. They may encounter obstacles that trigger replisome disassembly, rendering replication restart from abandoned forks crucial for cell viability. During the past 25 years, the genes that encode replication restart proteins have been identified and genetically characterized. In parallel, the enzymes were purified and analyzed *in vitro*, where they can catalyze replication initiation in a sequence-independent manner from fork-like DNA structures. This work also revealed a close link between replication and homologous recombination, as replication restart from recombination intermediates is an essential step of DNA double-strand break repair in bacteria and, conversely, arrested replication forks can be acted upon by recombination proteins and converted into various recombination substrates. In this review, we summarize this intense period of research that led to the characterization of the ubiquitous replication restart protein PriA and its partners, to the definition of several replication restart pathways *in vivo*, and to the description of tight links between replication and homologous recombination, responsible for the importance of replication restart in the maintenance of genome stability.

KEYWORDS DNA replication, homologous recombination

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Replication of a circular bacterial chromosome normally initiates at a unique origin. For bidirectional DNA replication, two replication forks are established that replicate the DNA in opposite directions until they meet in the terminus region. Replication fork progression involves the coordinated action of two complexes, the primosome to open the DNA duplex and synthesize primers and the replisome to catalyze the concerted DNA synthesis of both DNA strands. The two DNA strands at the replication fork are antiparallel, and DNA synthesis occurs only in the 5′-to-3′ direction. Therefore, to synthesize both strands in a concerted and semiconservative fashion, one strand is synthesized mainly continuously (the leading strand) and the other is synthesized discontinuously (the lagging strand). The fragments generated on the discontinuous strand are 1 to 2 kb in length and are called Okazaki fragments (OF). In *Escherichia coli*, the primosome is composed of the DnaB helicase that opens the parental strands and the DnaG primase that interacts transiently with DnaB and synthesizes the RNA primers at the onset of each OF synthesis. DnaB is a hexameric helicase that encircles single-stranded DNA (ssDNA) and translocates on the lagging-strand template in a 5′-to-3′ direction. The DNA polymerase III holoenzyme (Pol III HE) synthesizes both nascent DNA strands, and its action is stimulated by interactions with DnaB and with the ssDNA binding protein (SSB) that covers the lagging-strand template (1).

The first committed step in the assembly of a replication fork is DnaB loading. In bacteria, two pathways for DnaB loading have been described, a sequence-specific, DnaA-dependent pathway at the chromosome origin (*oriC*) (2) and a sequence-independent pathway that rescues inactivated or broken and repaired replication forks requiring the replication restart proteins PriA, PriB, PriC, DnaT, and possibly Rep. In both

processes, after identification of the proper DNA sequence or structure, exposure of a region of ssDNA by displacement of DNA-bound SSB allows DnaC to load the DnaB helicase onto the DNA to initiate replisome assembly. Depending on where DnaB is loaded, DnaC will require interactions either between DnaA and DnaB or between combinations of the replication restart proteins to facilitate the process.

Because of the remarkable stability of replisomes assembled from purified components on DNA *in vitro*, it was long thought that *in vivo* replication forks assembled at the origin could progress toward the terminus unimpeded. However, in the 1990s, the preprimosomal proteins were identified as proteins that promote replication initiation independently of DnaA. Originally identified as proteins required for the conversion of phage ssDNA into a duplex and called n (or factor Y), n′, n″, and i replication proteins, the preprimosomal proteins were rebaptized PriA, PriB, PriC, and DnaT when it was realized that they play a role in chromosome replication (but are not required for replication initiation at *oriC*) (3, 4). Their action required the recognition of a specific ssDNA region of the circular ϕX174 genome that is able to adopt a special secondary structure (hairpin) called the primosome assembly site (PAS) (5). The order of assembly and the stoichiometries of final "preprimosome" were determined as PriA (two or three monomers), PriB (two dimers), DnaT (one trimer), PriC (one monomer), and the DnaB helicase loaded by DnaC (one hexamer) (6–8). The preprimosome becomes the primosome in the presence of the primase DnaG. PriC is the only nonessential protein for preprimosome assembly. Its presence stimulated preprimosome assembly, and PriC was considered to be a stability factor in these reports (7, 8). The observation that PriA promoted *oriC*-independent replication *in vivo* (9) and that cells lacking the PriA protein suffered severe growth defects (10) suggested a role for primosome assembly in *E. coli* chromosome replication. Work in several laboratories led to the conclusion that the replisome may need to be reloaded in a PriA-dependent way during chromosome replication and that failure to do so has severe consequences (11, 12).

The essential role of PriA for the recombinational repair of DNA double-strand breaks (DSBs) indicated that homologous recombination triggers replication restart (10, 13–15). Conversely, arrested replication forks were shown to be targeted by recombination enzymes and cause chromosome rearrangements (16, 17). Another demonstration of the close relationship between replication restart and recombination is that the poorly partitioned nucleoids observed in a subpopulation of *priA* mutant cells is caused by the homologous recombination machinery (18). The discovery of links between replication and recombination gave rise to the notion that replication fork restart plays an important role in genome stability, which started an era of strong interest and intense studies.

The viability defects caused by the inactivation of homologous recombination functions are far less dramatic than the inactivation of replication restart. Therefore, it is clear from the genetic data that the replication restart proteins are mainly required to reload a replisome at abandoned replication forks. Accordingly, PriA is required for the viability of cells in which replication arrest is increased but does not trigger fork breakage, for example, in gyrase and topoisomerase IV mutants where forks are arrested by the accumulation of positive supercoils (19, 20). An early estimate of replication restart frequency was based on the percentage of partially replicated chromosomes in a *dnaC2*(Ts) mutant as determined by flow cytometry. That study suggested that the range could be around 18 to 25% of the *E. coli* cells in a population (21). In contrast, direct measurements of helicase stability in the same *dnaC2*(Ts) mutant by single-molecule microscopy showed that both helicase complexes disassembled within 20 min in 86% of the cells studied (22). The reasons for the discrepancy between these population and single-cell studies are unknown. In the same single-molecule microscopy study, measurements of the lifetimes of DNA complexes suggest that restart could be as frequent as five times per generation in *Bacillus subtilis*, as in *E. coli* (22). This result highlights the importance of replication restart and shows clearly that replication restart most often does not involve homologous recombination, as this frequency would then be incompatible with the viability of recombination mutants. In

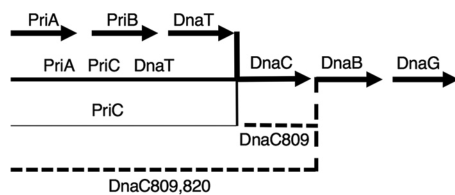

FIG 1 Replication restart pathways. Schematic representation of pathways for replication restart as deduced from genetic evidence. The starting substrates are abandoned forks or D loops formed by homologous recombination at dsDNA ends. The product of the reaction is a DNA replication fork with DnaB and DnaG loaded and ready to interact with the DNA Pol III HE. The PriA-PriB-DnaT pathway is the major pathway *in vivo* and the only one fully reconstituted *in vitro*. The existence of a PriA-PriC-DnaT pathway is deduced from the weak viability of the *priA* and *dnaT* mutants, in contrast to the full viability of a *priB* mutant and the nonviability of a *priB priC* double mutant. This pathway could not be reconstituted *in vitro*, where PriC alone is sufficient for primosome assembly. This model, however, is the only one that accounts for the phenotypes listed above. The PriC pathway was deduced from the colethality of a *priC* mutation with each of the *priA* and *priB* mutations. It was originally proposed to involve Rep because of the lethality of *rep priA* and *rep priB*, but this observation may rather result from replication blockage by DNA-bound RNA polymerases in the *rep* mutant and a need for PriA and PriB in mutants where forks are frequently arrested. Nevertheless, replication restart can be catalyzed by PriC and Rep *in vitro* by using substrates where DnaB loading is blocked by a 5′ lagging-strand end (20). Finally, DnaC809,820 suppresses all replication restart defects, suggesting that it allows DnaB loading at forks on its own *in vivo*. In contrast, DnaC809 is unable to restore *priA* viability in the *rep* or *holD* context, where replication fork arrest is frequent, or in a *priC* context. The latter observation suggested a role for PriC in the DnaC809 pathway. However, *in vivo* and *in vitro* results were contradictory, as DnaC809 could load DnaB on an SSB-covered substrate without the help of any other protein. Some unknown protein might prevent PriC and DnaC809 from acting alone *in vivo*, leading to the existence of the PriA-PriC-DnaT and PriC-DnaC809 pathways. Solid lines represent pathways available in wild-type cells (their thickness represents the relative amount of use), and dashed lines represents suppressor pathways.

this review, we will describe next the PriA-dependent replication restart process and PriA partners *in vivo* and *in vitro* and then various reactions that eventually take place prior to PriA-dependent replication restart.

GENETICS OF PriA AND ITS PARTNERS

Three pathways of replication restart were originally proposed on the basis of the patterns of synthetic lethality between pairs of null mutants. The three pathways are outlined in Fig. 1. They are referred to as PriA-PriB-DnaT, PriA-PriC-DnaT, and PriC. (As described below, we now feel that a more parsimonious interpretation of the current evidence would remove the Rep helicase from the PriA-independent pathway, where it has been previously placed [15], and call this pathway just PriC, as in Fig. 1.) *priA* and *dnaT* null mutants are deficient for both PriA-PriB-DnaT and PriA-PriC-DnaT pathways and rely solely on the PriC pathway. They have the most extreme phenotypes, showing poor cell growth/viability, high basal levels of SOS expression, defects in nucleoid morphology (a partitioning-defective phenotype), sensitivity to UV irradiation, and recombination deficiency (23–26) (Fig. 2). The *priA dnaT* double mutant is viable and has phenotypes similar to those of the single mutants (24). This shows that PriA and DnaT are not required for the PriC pathway and that PriA and DnaT are often needed, but not at each replication round, or the mutants would not be viable. *priB* and *priC* null mutants individually have little effect on cellular physiology, while the *priBC* double mutant is inviable (27). This result led to the proposals of a PriA-independent PriC pathway (28) and that the PriAB-DnaT and PriAC-DnaT pathways are formally equal. Experimentally, however, one can detect differences between the two PriA-dependent pathways. Inactivation of *priB* or *priC* does not have the same consequences in strains that have an additional mutation that increases the frequency of replication arrest. For example, inactivation of *priB* is five times more deleterious than inactivation of *priC* in a *holD* mutant (defective for a polymerase III subunit) and leads to rich medium sensitivity in a *gyrB*(Ts) mutant at semipermissive temperature (defective for gyrase), whereas inactivation of *priC* has no effect. These results led to the proposal that the PriA-PriB-DnaT pathway is the major PriA pathway *in vivo* (20, 29).

Mutations in *priA* causing defects in the PriA-PriB-DnaT and the PriA-PriC-DnaT

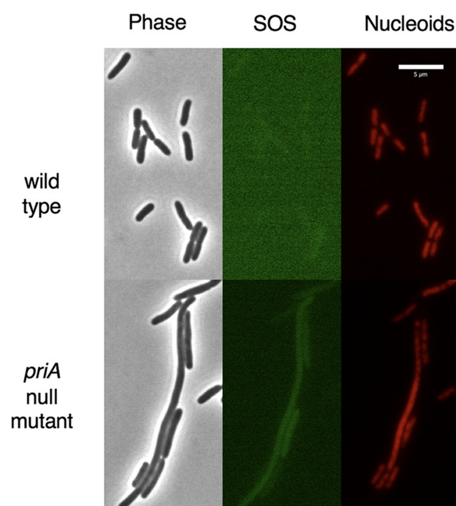

FIG 2 Micrographs showing the growth of two strains of bacteria on an agarose pad made of minimal medium. The two strains contain a *hupA*-mCherry protein fusion to visualize the nucleoids and the *sulAp*-green fluorescent protein promoter fusion to visualize the amount of SOS expression (106, 107). The *priA* null mutation is *priA2::kan*. The lighter green color of the wild-type SOS image is due to increased contrast enhancement to show what little, if any, SOS expression there is in those cells. The *priA* null mutant shows an example of a long cell with high levels of SOS expression and a poorly partitioning nucleoid and examples of middle-sized cells having both high and low levels of SOS expression and nucleoids that look wild type or Par$^-$. Such cells represent 16% of the total cells in a *priA* mutant culture in minimal medium. Also shown are examples of short, wild-type-size cells having very low SOS expression and wild-type nucleoids, which make up the remaining 84%.

pathways can be suppressed by different missense mutations in *dnaC* (27, 28). *dnaC809* is an example of one type of suppressor. It suppresses the *priA* mutant growth defects in an otherwise wild-type context. However, it does not suppress the lethality of the *priA priC*, *priA rep*, and *priA holD* double mutations (28, 29). The first two observations were originally interpreted as replication restart occurring in a *priA dnaC809* mutant via a PriC-Rep replication restart pathway. It was later proposed that the efficiency of replication restart by DnaC809 could be too low for the viability of *priA rep* and *priA holD* mutants because mutations in *rep* and *holD* increase the frequency of replication fork arrest (29). In contrast, the *priC* mutation does not increase replication fork arrest and the lack of suppression by *dnaC809* supports the idea that all replication restart pathways are inactivated in the *priA priC* and *priB priC* double mutants (Fig. 1). A second type of suppressor was selected in a *priBC dnaC809* triple mutant. This suppressor is a second mutation in *dnaC809*, called *dnaC809,820*, and it makes the suppression of *priA* mutants independent of PriC, Rep, and HolD. This suggested that *dnaC809,820* may be a more efficient *dnaC* suppressor mutation than *dnaC809* (28, 29). A third type was found in a *priB rep* double mutant; it was called *dnaC824*, and it only partially suppressed *priA* null mutant phenotypes (30).

The PriC pathway is a minor pathway of replication restart in *E. coli*. It was proposed on the basis of the lethality of the *priA priC* and *priB priC* mutations. As explained above, it was also originally proposed to include Rep because of the lethality of each *priC* and *rep* mutation in a *priA dnaC809* context (28). However, Rep possesses another function in *E. coli*, which is to remove DNA-bound proteins from the path of replication forks. Consequently, *rep* inactivation increases the frequency of replication fork arrest, which can account for the lethality of the *rep priA* mutation and the lack of rescue by the *dnaC809* suppressor mutation (31–33). It should be noted that the *priC* mutant does not show any of the *rep* mutant phenotypes besides its colethality with *priA* (28, 34). Conversely, the only other phenotype of the *priC* mutant is colethality with certain *dnaA*(Ts) (*dnaA46* and *dnaA508*) mutations at permissive temperatures. This colethality is not observed in the *rep* mutant (and *dnaA46*) and suggests a role for PriC

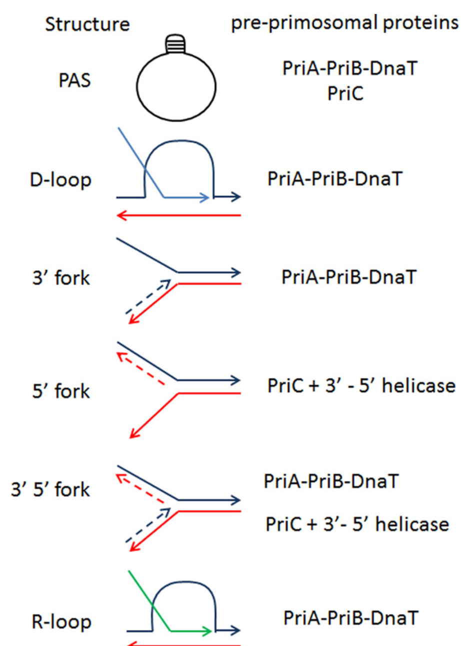

FIG 3 Schematic representation of the DNA molecules recognized and acted upon by preprimosomal proteins *in vitro* and presumably *in vivo*. From top to bottom, ϕX174 PAS, D loop resulting from the RecA-mediated invasion of a parental molecule (dark blue and red lines) by a homologous DNA RecA filament (light blue line), Y structure mimicking a replication fork with a fully synthesized leading-strand end and a gap in the lagging strand, Y structure mimicking a replication fork with a fully synthesized lagging-strand end and a gap in the leading strand, Y structure mimicking a replication fork with fully synthesized leading- and lagging-strand ends (in Y structures, parental strands are shown as full lines and newly synthesized strands are shown as dashed lines), and R loop (the RNA molecule is drawn as a green line). The preprimosomal proteins shown to assemble a primosome in these structures are indicated on the right. PriC action on PAS was deduced from the increased activity of the PriA-PriB-DnaT proteins in its presence. The 3′-5′ helicase function of PriA is required for its action on Y molecules with a dsDNA lagging strand.

during replication initiation, assuming that DnaA does not play a role in replication restart (35, 36).

BIOCHEMISTRY OF PriA AND ITS PARTNERS

Several studies have explored the biochemical details of the restart reaction. The PriA protein was shown to have two activities, a 3′-5′ helicase activity that is not required for primosome assembly *in vitro* or for replication restart *in vivo* and a primosome assembly activity (37). Using PAS as the substrate for loading, it was revealed that PriA would bind first. This binding was then stabilized by the addition of PriB, and the addition of DnaT allows for the loading of DnaB by DnaC (8). High levels of DnaT can render this reaction PriB independent (6). PriA was then shown to recognize and promote primosome assembly at displacement (D) loops, an early recombination intermediate made by RecA (Fig. 3) (38, 39). Finally, the binding of preprimosomal proteins was tested on three types of Y structures resembling a replication fork with only a 3′ leading-strand end at the point of the fork, only a 5′ lagging-strand end, or both (Fig. 3). The structure with a 3′ leading end is an isomer of a D loop. PriA was shown to preferentially recognize forks with such a nascent 3′ end, while PriC recognizes Y structures with a gap in the leading-strand template (40). Surprisingly, the recognition of different structures by these two proteins *in vitro* is at odds with a redundant function *in vivo*, unless each of these structures can be derived from the other one. PriA recognition of DNA 3′ ends at forks is mediated by its N-terminal domain (41). PriA binds PriB in the helicase domain, while PriB has overlapping binding sites for DnaT and ssDNA (42). Weak interactions and competing binding sites trigger a dynamic process for the formation of the PriA-PriB-DnaT complex (42).

TABLE 1 Genes that encode replication restart proteins and activities, functions, and interactions of their products

Gene	Protein activity *in vitro*	Protein function(s) *in vivo*	Interaction partner(s)
dnaA	Site-specific DNA binding protein at *oriC*	Targets replication initiation to *oriC*	DnaB, DnaC
dnaB	5′-3′ helicase	Replicative helicase	Pol III clamp loader, DnaC, DnaG, Rep
dnaC	DnaB helicase loader	DnaB helicase loader during chromosome replication	DnaB, DnaA
dnaG	Primase	Primer synthesis during chromosome replication	DnaB
dnaT	Part of PriA-PriB-DnaT preprimosome complex	Essential for PriA-PriB-DnaT and PriA-PriC-DnaT replication restart pathways	PriA, PriB
priA	Structure-specific DNA binding protein (PAS, D loop, Y structure), 3′-5′ helicase	Targets replication restart to PAS, inactivated forks, and recombination intermediates	PriB, DnaT, SSB
priB	Part of PriA-PriB-DnaT preprimosome complex	Essential for the PriA-PriB-DnaT replication restart pathway	PriA, DnaT
priC	Preprimosome protein that promotes DnaB loading by itself	Preprimosome protein of PriC and PriA-PriC-DnaT replication restart pathways	SSB DnaB
rep	3′-5′ helicase, removes proteins from DNA, can act together with PriC at a 5′ fork	Removes proteins from path of replication forks	ϕX174 protein A, M13 gpII, DnaB, PriC
ssb	ssDNA binding protein	Protects ssDNA, targets proteins to forks	PriA, PriC, Pol III, several other proteins

This provides a molecular handle for DnaC to recognize the proper substrate to load DnaB (42, 43). PriC alone provides a platform to load DnaB onto a substrate *in vitro* (40, 42, 44). Although no helicase function is required for primosome assembly *in vitro* when the lagging-strand template is single stranded, the presence of a 5′ DNA end at the tip of the fork renders a 3′-5′ helicase activity necessary for the reaction. This activity can be provided *in vitro* by PriA or Rep, whose role in the reaction may thus be to unwind a region of lagging-strand duplex so that DnaC can have a region of ssDNA to load DnaB (45). Note that, *in vivo*, a Y structure with a gap in both strands is not possible since the cDNA strands would anneal. The study of physical interactions between the replication restart proteins and their multisubunit complexes with DNA offers more in-depth insight into these sophisticated reactions (Table 1). To this end, several groups have reported functional and/or physical interactions between *E. coli* PriA and PriB (6, 42, 43), PriA and DnaT (6), PriB and DnaT (42), PriC and DnaB (46), PriC and Rep (45), and Rep and DnaB (47) and between *Klebsiella pneumoniae* PriC and DnaT (48).

It is clear that all substrates considered to be important in the restart process have regions of ssDNA that are likely to be coated with SSB *in vivo. In vitro* study has shown that DnaC809 can load DnaB on a DNA substrate bound with SSB, whereas DnaC cannot, suggesting that an important role of the preprimosomal proteins is to overcome the SSB barrier to DnaB loading (49). It has been shown that SSB can interact with many different replication and recombination proteins through its C terminus, including PriA and PriC (44, 50–52). These interactions were proposed to be important for SSB to recruit these proteins to replication forks and to play a role in primosome assembly. It is also known that SSB can bind ssDNA in two modes, one SSB tetramer binding either 35 (SSB_{35}) or 65 (SSB_{65}) nucleotides. SSB_{35} is associated with DNA replication, and SSB_{65} is associated with DNA repair. Single-molecule experiments have shown that both PriA and PriC promote shifting of the SSB binding mode from SSB_{65} to SSB_{35} (44, 53). It was proposed that PriA and PriC might thus release a small region of ssDNA where DnaC can load DnaB.

Other studies have revealed structures of PriA, PriB, and PriC by themselves and in the absence of DNA. A full-length structure of PriA from *K. pneumoniae* with 88% identity with *E. coli* PriA and the ability to complement PriA mutants of *E. coli* revealed a structure with six tightly clustered domains (53). The first domain has been crystallized before from the *E. coli* protein and shown to be able to bind the 3′-OH group of ssDNA (41). The next is a winged helix domain that is capable of binding double-stranded DNA (dsDNA). Then come two domains that comprise the ATPase activity. Encoded between these two larger domains is a cysteine-rich region (CRR) that binds two Zn^{2+} atoms. The CRR domain is thought to be important for both helicase activity

and protein-protein interactions (6, 54). Lastly, the C terminus stabilizes the N-terminal DNA binding domain and the ATPase domains. It also has the ability to bind ssDNA. A model has been put forth that has PriA interacting with the DNA at the point of a replication fork with the 3′ end of the newly synthesized strand of DNA, with a small amount of duplex DNA ahead of the fork (yet to be unwound), and with a region of ssDNA on the lagging-strand template where DnaB is to be loaded. The CRR domain associated with helicase activity is poised to peel away the 5′ end of the strand of DNA annealed to the lagging-strand template. An X-ray crystal structure of *E. coli* PriB revealed an OB fold that is strikingly similar to SSB, although these two proteins bind ssDNA in different ways (55). This study also revealed residues important for the binding of PriA and ssDNA. A nuclear magnetic resonance structure of PriC protein from *Cronobacter sakazakii* (41% identical to *E. coli* PriC) revealed a compact alpha-helical structure that brings together residues identified as involved with the binding of ssDNA, SSB, and possibly DnaB (46).

REPLICATION RESTART IN BACTERIA OTHER THAN *E. COLI*

PriA is a ubiquitous protein in bacteria with a conserved action, even though it acts with different partner proteins in different bacteria (56–58). In *B. subtilis*, the three proteins required for PriA-dependent replication restart, DnaB, DnaI, and DnaD, also act at the replication origin (56–59). The helicase loader is a two-protein complex, DnaB-DnaI, and interestingly, helicase loading is catalyzed in *E. coli* and *B. subtilis* by two different molecular mechanisms. In *E. coli*, the hexameric helicase is preassembled in solution as a ring that is opened and reclosed around ssDNA during loading, while in *B. subtilis*, monomers are directly assembled onto ssDNA to form the hexameric ring structure (56–58). The third protein, DnaD, may play a structural role in the process (60). In *Helicobacter pylori*, the only replication restart protein identified is PriA (reviewed in reference 61). Like all bacteria that lack homologues of the *E. coli* DnaC and *B. subtilis* DnaI helicase loaders, *H. pylori* encodes a protein called DciA, which represents a third class of helicase loader (62). Replication restart proteins have been characterized genetically and biochemically in several bacterial species. For example, PriA and PriB were studied in *Neisseria gonorrhoeae*, a bacterium that lacks PriC (63, 64). PriB from *K. pneumoniae* was shown to be very similar to the *E. coli* enzyme, in contrast to *N. gonorrhoeae* PriB (65). The *Deinococcus radiodurans* PriA protein lacks the helicase activity (66). The function and structure of Gram-positive DnaD protein have been explored in *Staphylococcus aureus* (67 and references therein). The *priA* gene is absent from certain intracellular symbionts, and this sometimes coincides with the absence of the *recA* gene (68).

REACTIONS THAT CAN TAKE PLACE PRIOR TO PriA-DEPENDENT RESTART

Replication restart from a D loop made by RecBCD- and RecA-dependent strand invasion. Several conditions where PriA is required for viability are linked to the occurrence of chromosomal DSBs and the coupling of DSB repair with PriA-dependent replication restart. Replication restart from a D loop formed by homologous recombination involves the same PriA partners as replication restart from replication fork structures (Fig. 3). PriA and PriB, together with DnaT, target the D loop and allow DnaC-catalyzed loading of DnaB on the strand that will become the lagging-strand template, which triggers the assembly of a replisome and replication initiation (69). As for replication initiation *in vitro* from other structures, PriC had only a minor stimulatory effect on the reaction. Break-induced DNA replication occurs in bacteria, phages, and eukaryotic cells (70). The coupling of DSB repair and replication restart ensures that replication is restarted after a broken or reversed fork is reintegrated into the chromosome by homologous recombination (Fig. 4, one-ended recombinational repair). On the other hand, owing to the potent exonuclease V action of RecBCD, DSB repair in bacteria is accompanied by extensive DNA degradation. Consequently, two D loops are independently formed at the two dsDNA ends of a DSB, and DSB repair in bacteria involves the merging of two PriA-dependent replication forks assembled at these D loops (71,

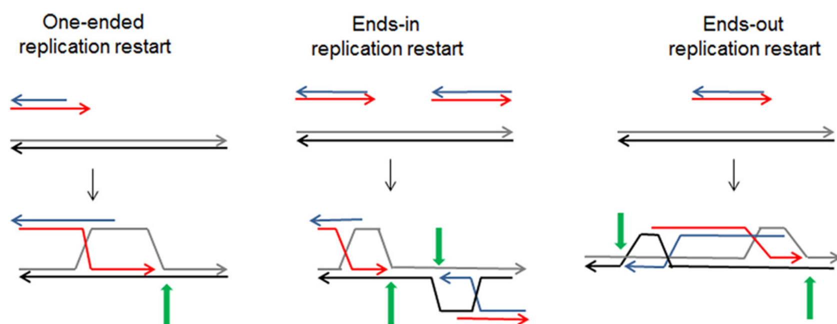

FIG 4 Replication restart following the repair of different substrates by RecBCD, RecA-mediated homologous recombination. On the left, one-ended homologous recombination at a broken replication fork restores unidirectional replication. In the middle, homologous recombination at a dsDNA break installs two converging replication forks. On the right, homologous recombination at the two ends of a linear molecule installs two diverging replication forks. Lines are DNA strands. Green arrows indicate the sites of replication restart in D loops. Adapted from reference 73.

72) (Fig. 4, ends-in replication). Finally, insertion of a linear DNA creates oppositely oriented replication forks that can eventually copy the entire molecule (Fig. 4, ends-out replication) (73, 74).

Replication fork reversal. Although bacteria are fully equipped to restart replication from abandoned replication forks, in some cases, replication forks are processed prior to restart. The reaction of replication fork reversal involves annealing of the newly synthesized leading- and lagging-strand ends at a blocked fork. This forms a dsDNA end adjacent to a Holliday junction (Fig. 5). The dsDNA end can be either degraded by

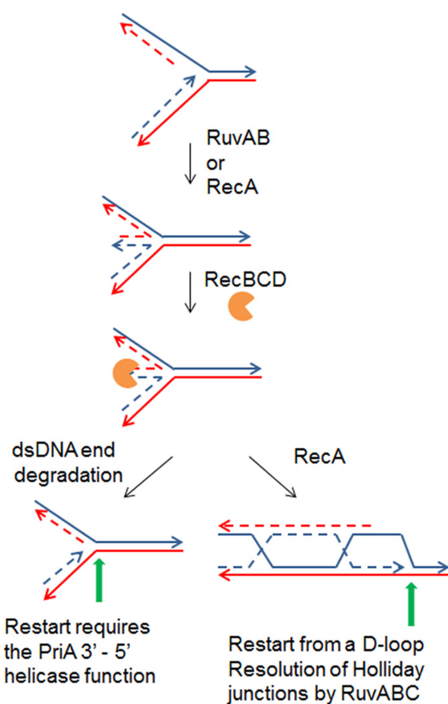

FIG 5 Replication restart after replication fork reversal. In a first step, the arrested fork is reversed by annealing of the leading- and lagging-strand ends, a reaction catalyzed by RuvAB or RecA. A dsDNA end is generated and acted upon by RecBCD. Either it is entirely degraded, resulting in a fork with full leading- and lagging-strand ends, which could account for the requirement for the PriA helicase function to unwind the lagging-strand end for DnaB loading, or the dsDNA end is recombined with the template DNA, creating a D loop that can be restarted by the PriA-PriB-DnaT pathway. Solid lines represent template DNA, dashed lines represent newly synthesized DNA, indented circles represent RecBCD, and green arrows represent sites of PriA binding for replication restart.

RecBCD or recombined by RecBCD and RecA. Both reactions result in the formation of a PriA substrate that allows replication restart. Replication fork reversal was observed (i) in several *E. coli* replication mutants in which different replisome components are affected (75), (ii) in the *rep* mutant that lacks the main helicase responsible for clearing DNA-bound proteins (mainly RNA polymerases) from the path of replication forks (76), (iii) in the *priA* mutant, suggesting that it occurs in cells that fail to restart replication after spontaneous arrest (77), (iv) in HU-treated cells and in a ribonucleotide reductase mutant (*nrdA*) (78, 79), (v) after accumulation of topoisomerase I-DNA covalent complexes (80), (vi) in UV-irradiated cells (81), (vii) after encounter of replication forks with an oppositely oriented highly transcribed sequence (82), and (viii) in bacteria other than *E. coli*, such as in *Pseudomonas syringae* at low temperature (83).

PriA is required for the viability of cells that undergo replication fork reversal (28, 29, 76). After replication fork reversal, the dsDNA end can be either degraded or recombined by RecBCD. As described above, homologous recombination renders all proteins of the PriA-PriB-DnaT pathway essential for restart. Although PriA helicase activity is not required for replication restart after homologous recombination (10), the growth of *rep* and *holD* mutants is impaired by inactivation of this activity (28, 29). This requirement could result from full degradation by RecBCD of the dsDNA end at some reversed forks, producing a fully duplex fork (with full leading- and lagging-strand ends and no gap) and thus rendering 5′ unwinding by the PriA helicase activity essential for primosome assembly (Fig. 5). Finally, PriA is required in UV-irradiated cells for *oriC*-independent replication (9) and for replication fork resumption after UV irradiation (84). The requirement for PriA may relate to the occurrence of replication fork reversal in UV-irradiated cells (81).

Replication restart allows the action of the UvrD helicase at protein-blocked forks. Ter-Tus are physiological replication arrest sites present in the terminus region of the chromosome. They arrest replication forks coming from one direction and are positioned to form a replication fork trap ensuring that replication terminates in the region opposite to the origin (85). Interestingly, homologous recombination proteins and the UvrD helicase were shown to be required for replication restart at forks blocked by ectopic Ter-Tus complexes (86, 87). It was shown that a following round of *oriC*-initiated replication results in "rear ending" at the first blocked forks and the formation of dsDNA ends that need to be recombined for viability. The UvrD helicase was also required for viability and was proposed to dislodge the Tus-Ter complex from DNA. However, the requirement for homologous recombination proteins in a UvrD^{+} context implies that the UvrD helicase cannot directly clear Tus-blocked forks. It was proposed that *in vivo* replication restart from the D loop formed by homologous recombination allows UvrD to gain access to the fork, to clear DNA-bound proteins, and thus allow replication restart (87). It implies that PriA-dependent replication forks initiated from a recombination intermediate may differ from forks initiated at the chromosome origin, in this particular case, by the presence of UvrD. Furthermore, in the *rep* mutant, UvrD is essential for the removal of transcriptional obstacles from the path of replication forks (31, 32, 88). However, the presence of UvrD does not prevent replication fork reversal in this mutant. The data indicate that UvrD cannot clear blocked forks directly but goes in after fork reversal and resetting, acting at PriA-dependent restarting forks. Finally, the same phenomenon was also observed upon replication blockage by an oppositely oriented highly transcribed sequence (inverted *rrn*), where two accessory helicases are required to remove the obstacle, and they also act after replication fork reversal (31, 82).

Replication fork restart from R loops and during phage Mu integration. In addition to recombination intermediates and abandoned forks, PriA can initiate replication from R loops *in vivo* (Fig. 3). R loops are formed in the *E. coli* chromosome in cells that lack the enzymes that degrade or unwind them, i.e., *rnh* (RNase H) and *recG* mutants, and are used in these mutants for *oriC*-DnaA-independent replication (9, 89–91). Finally, PriA and DnaT are essential for phage Mu integration into the *E. coli* chromosome by replicative and nonreplicative pathways (92, 93). During *in vitro*

replicative transposition, PriA and its partners act after the transpososome is released by a specific complex. Interestingly, *in vitro*, the choice of the PriA pathway depends on whether the substrate is deproteinized (PriA-PriB) or whether the complex that releases the transpososome is still present (PriA-PriC) (93).

Overreplication in the RecG mutant. The RecG helicase can unwind various DNA structures *in vitro*, leading to the proposal that RecG has multiple functions *in vivo* (reviewed in reference 94). *In vitro* and *in vivo*, in addition to targeting Holliday junctions, RecG acts at R loops, D loops, and replication forks. It is known that DNA replication initiation is increased at these three different types of substrates in a *recG* mutant. This suggests that one role of RecG *in vivo* is to counteract these *oriC*-independent methods of initiation of DNA replication (89, 95, 96). Furthermore, suppressors of the *recG* mutant's hypersensitivity to DNA-damaging agents lie in the *priA* gene and inactivate the helicase activity of PriA. This further suggests that that PriA and RecG have targets in common *in vivo* (97). In early 2000, it was proposed that RecG promotes replication across UV lesions by catalyzing fork reversal. However, when this model was revisited, it was shown that *recG* inactivation either had the opposite effect, enhancing replication rather than decreasing it (95), or no effect on replication restart in UV-irradiated cells (81, 98). It was recently proposed that RecG prevents overreplication initiation by directing PriA binding to blocked forks or to D loops formed by homologous recombination (71, 99, 100). Action of RecG and PriA on the same forks would allow RecG to determine the direction of replication restart by PriA and, conversely, allow PriA to prevent replication fork reversal by RecG (71, 99). Note that RecG has the same preferential DNA target as PriC (Y structure with a 5′ lagging-strand end and a gap in the leading strand), but whether RecG affects PriC action *in vitro*, accounting for the *in vivo* versus *in vitro* discrepancies, has not be explored.

Replication restart-associated genomic instability. Although PriA-dependent replication restart may occur less than once per generation, these events are important because of their consequences for genome stability. A *holD* point mutant, affected in one of the Pol III HE subunits and isolated by screening for hyperrecombination mutants, undergoes replication fork reversal (101, 102). Recombination-dependent replication occurs in stationary phase, where it leads to Pol IV (DinB)-dependent mutagenesis (reviewed in reference 103). Stationary-phase DinB-dependent mutagenesis was largely increased in the *recG* mutant (104), possibly because of abnormal replication initiations from recombination intermediates in the absence of RecG. A *dnaB*(Ts) mutation strongly stimulates tandem repeat recombination, and, as for stationary-phase mutagenesis, in addition to all DSB repair proteins, DinB and RecG play a role in *dnaB*(Ts)-promoted instability, presumably through the stabilization of D loops used for replication restart (105). These few examples illustrate how, because of the action of recombination proteins at blocked forks, replication arrests do not need to be frequent to have dramatic consequences for genome stability.

CONCLUSION

The actors of replication restart in bacteria have now been identified and characterized both genetically and biochemically. The amount of knowledge accumulated to date on their crucial role *in vivo* and on their functioning as molecular machines *in vitro* is impressive. Nevertheless, several questions remain open. For example, why does PriC require either a mutated DnaC protein (such as DnaC809) or PriA and DnaT to function *in vivo*, whereas it acts alone *in vitro*? How can different substrates for PriA and PriC *in vitro* allow redundant functions *in vivo*? Why is transcription identified as the main cause of replication arrest in wild-type cells, although replication and transcription have evolved together and bacteria are equipped to deal with such obstacles? What is the activity altered in some *dnaA*(Ts) mutants that is required for viability in *priC* mutants at the permissive temperature? The *E. coli* chromosome is entirely replicated in about 40 min, which implies that each ~2,320-kb chromosome arm is replicated with an average speed close to 1 kb/s. Is replication restart so rapid that replication arrests have

a negligible effect on the average replication speed, or is replication progression even more rapid than we believe?

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