

Filling the Gaps in Replication Restart Pathways

Recent work by [Heller and Marians \(2005\)](#) clarifies two mechanisms of replication fork assembly in *E. coli*: in vitro, the presence of a leading nascent strand dictates a PriA/B DnaT-dependent mechanism, whereas a gapped fork specifies PriC-mediated restart.

Cells regulate replication at the level of initiation. In *Escherichia coli*, replication begins by the loading of the DnaB helicase at the cell's single origin of replication, accomplished by an *oriC*-specific recognition protein, DnaA, and a molecular escort protein, DnaC. However, replication forks frequently arrest, disassemble, or are broken, which necessitates mechanisms to reestablish a fork after its repair is completed. Clues to the factors involved in this mechanism in *E. coli* came from biochemical studies of bacteriophage replication. The novel factors PriA, PriB, PriC, and DnaT, in addition to DnaC, were required for DnaB loading on the ϕ x174 bacteriophage single-strand genome. At the time, it was puzzling why such factors were not required for *E. coli* replication. Later, and deduced from the phenotypic characterization of *priA* mutants, it was hypothesized that these components comprise a replication “restart” pathway that loads DnaB subsequent to repair of damaged forks (reviewed in [Sandler and Marians \[2000\]](#)), which certain plasmids and bacteriophage have co-opted to initiate replication.

E. coli priA mutants are alive, but a subpopulation cannot form colonies and are induced for the “SOS response,” a sign of constitutive DNA damage that presumably results from failed replication forks. Mutants in *priA* are also sensitive to a number of DNA damaging agents and are defective in recombination, especially that involving double-strand ends, implying the involvement of PriA-mediated replication restart in double-strand break repair. Biochemical studies showed that PriA binds to special branched DNA structures such as “D loops,” which we infer are formed during recombinational repair of broken replication forks ([Liu et al., 1999](#); [McGlynn et al., 1997](#)). From such structures, PriA recruits DnaC/B and reestablishes the fork. In agreement with this role, *priA* suppressor mutations can be isolated in the DnaC escort protein, which, in vitro, delivers DnaB to D loops in the absence of PriA and in vivo relieves the cell of a requirement for PriA for robust viability, recombination, and survival to DNA damage ([Liu et al., 1999](#); [Sandler et al., 1999](#)).

The genetics of the remaining primosome assembly factors, however, revealed a more complicated picture than a single mechanism for replication restart. For example, mutants in *priB* or *priC* are much more mildly affected than the *priA* mutant and, perhaps even more surprising, the double *priB priC* mutant is much worse ([Sandler et al., 1999](#)). From these and other synthetic genetic interactions, Steve Sandler proposed several

pathways for primosome assembly, including one dependent on PriC but independent of PriA ([Sandler, 2000](#)). The results of Ryan Heller and Ken Marians ([Heller and Marians, 2005](#)) provide biochemical validation of this PriC pathway and suggest that it may be specialized for replication restart in forks containing single-strand DNA gaps on the leading strand.

[Heller and Marians \(2005\)](#) have devised an in vitro assay for DnaB-mediated unwinding of synthetic forked DNA structures coated with the single-strand DNA binding protein SSB. They show that purified PriA protein, along with PriB, DnaT, and DnaC, effectively recruits DnaB only when a 3' end, equivalent to a nascent leading strand end or the invading strand of a D loop, is nearby. In contrast, PriC, with DnaC, delivers DnaB best when there is a single-strand gap at the fork. Therefore the structure of an arrested fork could dictate which of two alternative recruitment factor complexes, PriA/PriB/DnaT or PriC, will be used to reassemble DnaB onto the fork.

[Heller and Marians \(2005\)](#) also confirm substrate specialization of the two restart systems using an in vitro replication assay, employing DNA polymerase III holoenzyme, DnaG primase, and labeled deoxynucleotides, on longer DNA substrates to which oligonucleotide forks of various structures have been ligated. Again, the PriA/B DnaT/C system can assemble DnaB to mediate both leading and lagging strand synthesis most effectively if a 3' leading strand is present at the fork. The PriC/DnaC system promotes replication of both strands efficiently when the leading strand template is gapped. An intriguing aspect of their data is the partial requirement for DnaG primase to promote leading strand replication in the PriC-assembled fork. This may mean that PriC can restart replication and promote repriming of the leading strand in addition to the lagging strand. If true, this has important implications; contrary to dogma, the leading strand could also be somewhat discontinuous because of this repriming.

Because *priA priC* mutants are inviable, virtually all replication forks in *E. coli* must fail and require restart from either of the two restart pathways. The PriA pathway seems to be best suited for reestablishment of forks after breakage and restoration by recombination or after simple mechanical failure and disassembly of the replication complex. The PriC pathway, on the other hand, may be recruited for restart after arrest of the leading strand ([Figure 1](#)). In vivo, blocks to leading strand polymerization do not impede lagging strand synthesis, and the fork continues to move, yielding a large gap on the leading strand ([Pages and Fuchs, 2003](#))—just the structure for a PriC restart pathway. Mutants in *priA*, in which only the PriC restart system is operant, require the RecFOR gap-filling recombination proteins in addition to PriC for viability ([Grompone et al., 2004](#)), consistent with an association of the PriC restart pathway with gapped forks.

As is often the case, the in vivo situation appears more complex (and untidy) than one might expect from

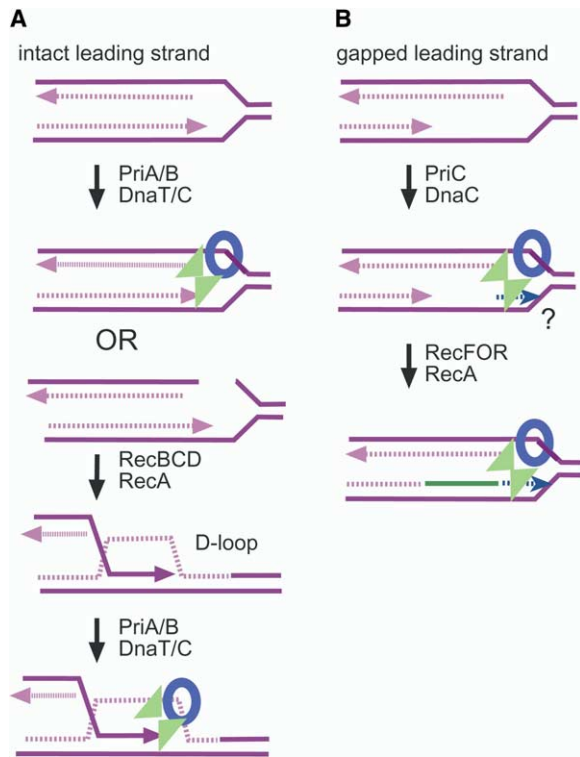


Figure 1. Alternate Replication Restart Pathways

(A) In the presence of an intact leading strand, PriA/B/DnaT/C can reassemble DnaB helicase (blue ovals) and DNA polymerase III holoenzyme (green triangles) on the fork. Alternatively, after fork breakage, recombination via RecABC can form a D loop intermediate, a substrate for PriA/B/DnaT/C restart, with an intact 3' leading strand.

(B) In a gapped fork, PriC/DnaC can reload DnaB (blue ovals) and DNA polymerase II holoenzyme (green triangles) and possibly reprime the leading strand. RecA/FOR recombination is required to fill replication gaps.

the biochemistry. Contrary to expectations from genetic analysis (Sandler, 2000), Heller and Marians (2005) did not observe any requirement for the Rep helicase protein in the PriC-dependent in vitro restart pathway. Perhaps in vivo, Rep is necessary to unwind the lagging strand or dislodge proteins to reveal a DnaB loading site, not an issue in the in vitro reactions. Furthermore, there appear to be more than these two systems for DnaB loading, including PriA/PriC, PriA/PriB/PriC, and even DnaA/PriC-dependent pathways (Hinds and Sandler, 2004; Sandler, 2005; Sandler et al., 1999). This suggests that all four proteins, DnaA, PriA, PriB, and PriC, can be used to provide specificity in a combinatorial fashion, dependent on the initiation substrate.

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