

Indispensible Replication Restart Helicase PriA Aids Bacterial Survival



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I. Abstract

Approximately one disruption in DNA replication occurs every cell cycle in bacteria. One possible consequence of this disruption is that the replisome can dissociate from the chromosome. Since unfinished replication can result in genome instability and cell death, bacteria need a mechanism to reload the replication machinery onto the genome. Known as the replication restart primosome (RRP), several proteins function to reload the essential replicative helicase onto the abandoned replication fork, thereby reinitiating DNA replication. PriA, a 3' to 5' DNA helicase, is the most conserved member of the RRP proteins and it initiates the dominant replication restart pathway. PriA remodels collapsed forks and serves as a platform for binding of other primosomal proteins. The Madison West SMART (Students Modeling a Research Topic) Team modeled PriA using 3D printing technology. PriA has six domains: a 3' DNA-binding domain, a winged helix (WH) domain, two helicase lobes, a zinc-binding domain, and a C-terminal domain. PriA's WH domain is attached to the rest of the protein via two extended loops, implying its position could be quite dynamic. PriA Phe16, Asp17, Tyr18, Leu55, and Lys61 recognize 3' hydroxyl groups of DNA, allowing PriA to bind the replicative fork. PriA hydrolyzes ATP; Lys229 coordinates the ATP complex while Asp319 and Lys543 aid in regulating ATP hydrolysis. PriA also binds two zinc ions, coordinated by eight cysteine residues. Since DNA replication restart pathways are essential in preserving genomic integrity and cell viability in bacteria, studies of PriA and its replication restart pathways represent an approach to developing novel antibacterial compounds.

II. Replication Restart Is Essential in Bacteria

All organisms must faithfully duplicate their genomes for survival. Bacterial genomes consist of a double-stranded circular chromosome with a single origin of replication (*oriC*). Replication is initiated at *oriC*, where two sets of the replicative helicase DnaB and the rest of replication machinery bind and move away bi-directionally to duplicate the genome. Frequently, however, DNA replication is halted due to DNA damage or stalled protein complexes, resulting in the replication complex falling off and potential genome instability and/or cell death.

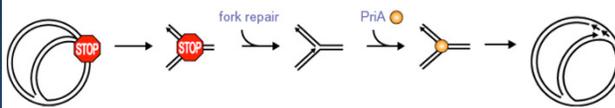


Figure 1 (above). Replication restart in bacteria. Bacteria use the mechanism of replication restart to reload the replicative helicase DnaB onto abandoned replication forks. PriA, the most conserved protein of the replication restart primosome (RRP), mediates mechanisms in the dominant replication restart pathway in bacteria.

III. DNA Binding Capability of PriA

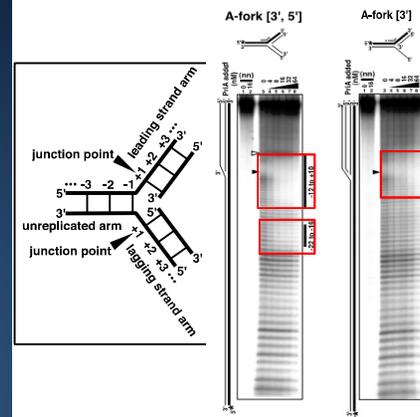


Figure 2 (left) PriA recognizes specific DNA structures. DNase I footprinting is used to highlight how PriA binds to a replication fork. PriA is pre-bound to different sections of diverse DNA substrates. DNase I was added to the solution to digest the components of the DNA that were unbound to PriA. Increased concentrations of PriA cause an absence of an appropriate sized band, showing protection and PriA's ability to bind to DNA (Tanaka, et. al 2007).

IV. Structure of PriA

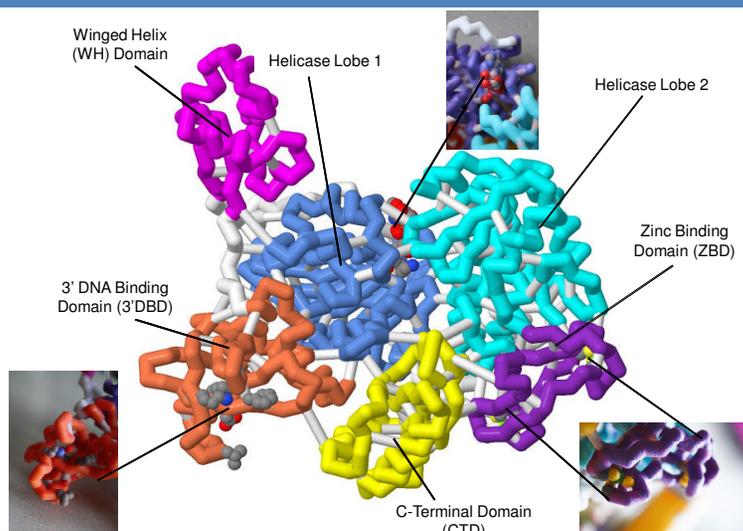


Figure 3 (above). Structure of PriA. In the 3' DBD, Phe16, Asp17, Tyr18, Leu55, and Lys61 recognize the 3' hydroxyl at replication forks, allowing PriA to bind an abandoned fork. In helicase lobe 1, Lys229 coordinates the ATP complex. Asp319 and Lys543 aid ATP binding. Cysteine residues in the ZBD coordinate two zinc ions. Overall, the structure of PriA reveals core helicase domains surrounded by DNA binding elements and hints at a potential mechanism of PriA binding to abandoned replication forks.

V. PriA's Proposed DNA Binding Mechanism

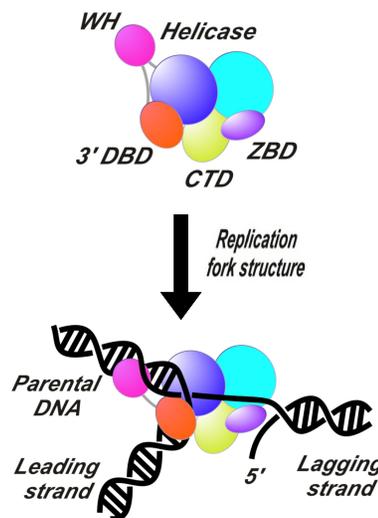


Figure 4 (above). PriA recognizes an abandoned replication fork. Binding of PriA to an abandoned replication fork positions the various domains to bind DNA (3'DBD, WH, and CTD), unwind duplex DNA (helicase and ZBD) and allow for subsequent binding of other RRP proteins, and ultimately, the replication machinery.

VI. Implications and Conclusions

PriA is the first response protein in the replication restart pathway; without it, stalled replication could lead to the eventual death of the bacterium.

PriA's structure consists of several domains which allow the recognition of a stalled replication fork and its subsequent binding to DNA. Its importance is highlighted by the high rate of conservation among several different types of bacteria.

This information suggests a possible mechanism for disabling bacteria by hindering their ability to replicate as well as shedding light on the unnoticeably fragile nature of the bacteria on which we so heavily depend.

VII. References

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