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Structural analyses of the bacterial primosomal protein DnaB reveal that it is a tetramer and forms a complex with a primosomal re-initiation protein

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The DnaB primosomal protein from Gram-positive bacteria plays a key role in DNA replication and restart as a loader protein for the recruitment of replisome cascade proteins. Previous investigations have established that DnaB is composed of an N-terminal domain, a middle domain, and a C-terminal domain. However, structural evidence for how DnaB functions at the atomic level is lacking. Here, we report the crystal structure of DnaB, encompassing the N-terminal and middle domains (residues 1–300), from *Geobacillus stearothermophilus* (*Gst*DnaB$_{1-300}$) at 2.8 Å resolution. Our structure revealed that *Gst*DnaB$_{1-300}$ forms a tetramer with two basket-like architectures, a finding consistent with those from solution studies using analytical ultracentrifugation. Furthermore, our results from both GST pulldown assays and analytical ultracentrifugation show that *Gst*DnaB$_{1-300}$ is sufficient to form a complex with PriA, the primosomal reinitiation protein. Moreover, with the aid of small angle X-ray scattering experiments, we also determined the structural envelope of full-length DnaB (*Gst*DnaB$_{FL}$) in solution. These small angle X-ray scattering studies indicated that *Gst*DnaB$_{FL}$ has an elongated conformation and that the protruding density envelopes originating from *Gst*DnaB$_{1-300}$ could completely accommodate the *Gst*DnaB C-terminal domain (residues 301–461). Taken together with biochemical assays, our results suggest that *Gst*DnaB uses different domains to distinguish the PriA interaction and single-stranded DNA binding. These findings can further extend our understanding of primosomal assembly in replication restart.

Accurate and timely DNA replication is essential for cell proliferation in all organisms, which relies on a number of inter-protein interactions (1–3). Sequential assembly of the primosome complex is one the most important processes that drives DNA replication in all bacterial species. In bacteria, DNA replication is initiated when DnaA recognizes and binds to the origin of replication (*oriC*) (4, 5) where all the DNA unwinding machinery is assembled. Subsequently, the hexameric helicase-loader complex is recruited followed by joining of the primase DnaG to complete primosome and replisome assembly (6–8).

During the elongation phase of DNA replication, replication forks are frequently challenged by mutations, nicks, base modifications, and other factors that could result in fork arrest or collapse (3, 9, 10). Once the DNA replication process is stalled, it undergoes a reinitiation process, and the primosome re-initiation protein PriA is recruited at the respective sites (11). The proteins DnaA, PriA, and the replicative helicase are well-conserved in both Gram-negative bacteria (such as *Escherichia coli*) and Gram-positive bacteria (such as *Bacillus subtilis*) (12–14). However, DnaA and PriA in *E. coli* and *B. subtilis* differ in their recruitment of subsequent loader proteins before recruitment of the replicative helicase. During the normal replication process in Gram-negative *E. coli*, DnaA recognizes *oriC* sites and recruits DnaB helicase with the help of the helicase loader protein DnaC and promotes the association of the primase DnaG (2, 15–18). In instances of abnormal DNA repair and restart, PriA recognizes the stalled forks and sequentially recruits three other primosomal reinitiation proteins (PriB, PriC, and DnaT) and a loader protein DnaC to trigger re-loading of hexameric replicative helicase as a helicase-loader complex DnaB-DnaC to restart the replication process (13). These two distinct primosome reinitiation processes share the same helicase (DnaB) and primase (DnaG) in the replisome system.

However, in Gram-positive bacteria homologues of PriB, PriC, and DnaT are absent. Instead, only the primosomal loader proteins DnaB and DnaD are found, which are mediated by both DnaA and PriA to initiate and restart the replication process at *oriC* and stalled forks, respectively (12, 19, 20). Assembly of DnaB and DnaD preloader proteins at replication sites promotes loading of the helicase DnaC (the replicative helicase homologous to *E. coli* DnaB) in complex with the loader protein DnaI (the helicase loader homologous to *E. coli* DnaC) in an ATP-dependent manner (6, 21) to unwind the parental DNA template.

Previous studies suggested that Gram-positive bacterial loader protein DnaB is a multifunctional protein. Its interaction with DnaD on single-stranded DNA-binding protein-coated DNA has been demonstrated (22), and its cooperative role with DnaI in acting as a "co-loader" for the recruitment of DnaC helicase has also been shown (23). Furthermore, the cell mem-

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This article contains supplemental Table S1 and Figs. S1–S9.

The atomic coordinates and structure factors (code 5WTN) have been deposited in the Protein Data Bank (http://wwpdb.org/).

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