MS05 Nucleic acids and their interaction

MS5-03

DciA, the ancestral replicative helicase loader

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Abstract

DNA replication is a crucial step for the proliferation of all organisms. Multi-subunits complex termed replisome carried out strand synthesis while controlling the replication fidelity. In front of the replisome, the replicative helicase unwinds DNA by its translocation on the lagging strand. One of the essential steps of replication is the recruitment and loading of the helicase at the replication initiation site. In bacteria, the initiation protein DnaA localized at the unique origin of replication *oriC* recruits two helicases DnaB, but the loading depends on a protein loader. DnaB loading has been well described within the *Escherichia coli* model, where DnaC ensures DnaB loading by cracking open the helicase. Yet, DnaC distribution is marginal in the bacterial domain. It was established phylogenetically that the *dnaC* gene is a domesticated phage element that has replaced several times, through the evolution, the ancestral gene *dciA* (1). Despite the preponderance of *dciA* in bacteria, the loading mechanism of DnaB managed by DciA was not yet studied.

Our work focused on the biochemical and structural characterization of DciA and DnaB from *Vibrio cholerae*. Like other bacterial replicative helicases, *Vc*DnaB adopts a toroid-shaped homo-hexameric structure but with a slight opening (2). Performing helicase assays and SPR analyses, we showed that *Vc*DnaB can load itself on DNA and that *Vc*DciA stimulates this function, resulting in an increased DNA unwinding (2). We obtained a crystal structure of the *Vc*DnaB•*Vc*DciA complex, which we compared to the DnaB•DnaC complex from *E. coli*. Surprisingly, despite no structural nor sequence similarity between the two loaders, both target the same binding site. We determined that *Vc*DciA is composed of two structural domains: a globular NTD (structure solved by NMR), which binds to DNA, and an unstructured CTD, which can fold transitory in two small helices (3) and bind to *Vc*DnaB. We combined a multi-disciplinary approach to study the structural and functional interplay between the three partners of the *Vc*DnaB•*Vc*DciA•DNA complex in order to understand the loading mechanism of DnaB by DciA. We expect a completely different mechanism than the one known for DnaC.

References

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