Poster Presentation

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Structural insights into DNA replication initiation in Helicobacter pylori

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In Gram-negative bacteria, opening of DNA double strand during replication is performed by the replicative helicase DnaB. This protein allows for replication fork elongation by unwinding DNA and interacting with DnaG primase. DnaB is composed of two domains: an N-terminal domain (NTD) and a C-terminal domain (CTD) connected by a flexible linker. The protein forms two-tiered hexamers composed of a NTD-ring and a CTD-ring. In Escherichia coli, the initiator protein DnaA binds to the origin of replication oriC and induces the opening of a AT-rich region. The replicative helicase DnaB is then loaded onto single stranded DNA by interacting with DnaA and with the AAA+ helicase loader DnaC. However, AAA+ loaders are absent in 80% of the bacterial genome, raising the question of how helicases are loaded in these bacteria [1]. In the genome of human pathogen Helicobacter pylori, no AAA+ loader has been identified. Moreover H. pylori DnaB (HpDnaB) has the ability to support replication of an otherwise unviable E. coli strain that bears a defective copy of DnaC by complementation [2]. In order to better understand the properties of HpDnaB we have first shown that HpDnaB forms double hexamers by negative stain electron microscopy [3]. Then, we have then solved the crystal structure of HpDnaB at a resolution of 6.7Å by X-ray crystallography with Rfree/Rfactor of 0.29/0.25. The structure reveals that the protein adopts a new dodecameric arrangement generated by crystallographic three fold symmetry. When compared to hexameric DnaBs, the hexamer of HpDnaB displays an original combination of NTD-ring and CTD-ring symmetries, intermediate between apo and ADP-bound structure. Biochemistry studies of HpDnaB interaction with HpDnaG-CTD and ssDNA provides mechanistic insights into the initial steps of DNA replication in H. pylori. Our results offer an alternative solution of helicase loading and DNA replication initiation in H. pylori and possibly other bacteria that do not employ helicase loaders.

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