# The role of domains I and III of the DnaA protein in the assembly of the *Helicobacter pylori* replication initiation complex


## ABSTRACT

*Helicobacter pylori* is a microaerophilic, Gram-negative bacterium inhabiting the human digestive tract, being the important risk factor for the development of peptic ulcer and gastric cancer. So far, studies on the initiation of the chromosome replication of this bacterium focused on the *oriC* region (*origin of chromosomal replication*) - identified as the first bipartite origin in Gram-negative bacteria. There are still limited biochemical data for *H. pylori* DnaA – a second important factor of the initiation of chromosome replication. The best characterized domain of *H. pylori* DnaA, domain IV, is responsible for recognition and binding to specific DNA sequences, DnaA-boxes, localized in *oriC*. Data about other domains of the protein are mostly based on comparative *in silico* analyses with *E. coli* DnaA. High homology between *E. coli* and *H. pylori* proteins suggests similar characteristic of *H. pylori* DnaA.

The main goal of this work was to characterize the roles of domains I and III in the formation of *H. pylori* initiation complex. The first step of studies focused on purification of recombinant DnaA proteins composed of different domains and variants carrying mutations in domain III. The interactions between protein molecules and protein binding to DNA were studied using *in vitro* DNA unwinding test, protein crosslinking assay, SPR (*Plasmon Surface Resonance*), EMSA (*Electrophoretic Mobility Shift Assay*) and electron microscopy techniques. Results allowed to designate how domain composition influences DnaA interactions. It also allowed to characterize interactions mediated by domains I and III. It was proved that domain I of *H. pylori* DnaA self-interacts and mediates long-distance interactions between suboligomers formed at origin subregions. It was also shown that domain III is responsible for DNA unwinding at DUE (*DNA unwinding element*).

Last part of research was dedicated to *in vitro* studies on the influence of DnaA *H. pylori* mutation in AAA+ motif on protein-protein and protein-DNA interactions. Moreover it was analyzed whether additional copy of mutated DnaA proteins affects *H. pylori* growth. Results suggest high importance of mutated residues in examined interactions and on bacteria growth - additional DnaA mutated copies inhibited bacterial growth.

Presented results allowed to characterize the role of domains I and III of the *H. pylori* DnaA protein in the assembly of the *Helicobacter pylori* replication initiation complex and expanded our knowledge about the initiator protein DnaA.