Structural and functional analysis of the interactions of selected Rep proteins with DUE region of DNA replication origin

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DNA replication occurs in every living cell. The process of DNA replication allows for the multiplication of genetic material and its transfer to daughter cells. One of the key stages of this phenomenon is the initiation of DNA replication. In the bacterial origin of replication, the replication initiation process begins with the binding of specific double-stranded DNA (dsDNA) sequences by bacterial replication initiators. This interaction causes destabilization of dsDNA in the AT-rich region, (DUE). This provides to dsDNA melting. In consequence appearing single-stranded DNA (ssDNA) fragments serves as a scaffold for the other replication proteins, which form a pre-replication complex and enable beginning of a new DNA molecule synthesis. Literature data indicated that bacterial replication initiators, such as DnaA or RctB proteins, not only bind dsDNA but also interact with ssDNA formed after melting of the DUE region. At the time of this project and my research, the structural and functional nature of the nucleoprotein complexes of bacterial initiators with DNA still remained elusive. Various models describing mechanism of replication initiation process have been proposed. Two models: the "two-state model" and the "loop-back model" had the strongest support by the experimental evidence.

Iteron plasmids are extrachromosomal DNA molecules found in Gram-negative bacteria. They can replicate independently of the bacterial chromosome. Iteron plasmids also have their own replication initiators, Rep proteins. During plasmid DNA replication initiation, Rep proteins bind both, repeated sequences on dsDNA, called iterons, and ssDNA within DUE region. In some cases, for the replication initiation process, iteron plasmids also engage bacterial initiator, DnaA protein.

In this work, I described the structural and functional significance of the interactions of Rep proteins (i.e. TrfA, RepE and RepA) with ssDNA during the DNA replication initiation of selected iteron plasmids. Performed *in vitro* analyses of Rep-ssDNA interaction showed that it is crucial at the *origin* opening step and for the open complex formation and maintenance. Disruption of Rep-ssDNA complex formation inhibits the process of plasmid DNA *origin* opening and inhibits further steps of replication initiation, such as: helicase protein recruitment and activation, prereplication complex formation and synthesis of a new DNA molecule. Moreover, in this work, we defined the amino acid residues of Rep proteins, TrfA and RepE, responsible for such a nucleoprotein complexes formation. These amino acids were selected based on the: analysis of the crystallographic structure of RepE-ssDNA complex, analysis of Rep protein cross-linked with ssDNA fragments, combined with mass spectrometry (MS),

comparison of the secondary structures of the Rep proteins, and biochemical analyses. An important aspect of this project was solution of crystallographic structure tripartite nucleoprotein complex of ssDNA-RepE-dsDNA. Structure analysis proves that Rep proteins can interact both up together with ssDNA and dsDNA. Obtained structural data strongly supports the validity of the "back-loop" model proposed as a mechanism of replication initiation process of bacterial DNA.