The structural-functional analysis of complexes of plasmid replication initiation protein, Rep, and the ssDNA

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Process of DNA replication occurs in all living cells. This phenomenon allows for duplication of double-stranded DNA helix, what ensures survival of every organism. Gram-negative bacteria despite of their own chromosomal DNA, can also possess additional DNA, which are iteron plasmids. Those molecules replicate independently from the chromosomal DNA, using host's replication machinery and only two plasmid-encoded elements: origin of replication and replication initiator (Rep protein). Because steps during DNA replication process in prokaryotic and eukaryotic cells are similar, investigating plasmid replication system might contribute to better understanding also eukaryotic one. Moreover, very important feature of iteron plasmids is that they carry antibiotic resistance genes. Therefore, better understanding mechanism of plasmid replication initiation can result in indication of eventual new targets of antibacterial drugs.

The very first step of the replication initiation process is the origin sequence recognition by replication initiator proteins. Formation such a nucleoprotein complex causes melting of double-stranded DNA (dsDNA) within the origin region of a lowest thermodynamic stability, which is named DNA unwinding element (DUE). Origin opening provides the single-stranded DNA (ssDNA) for helicase, primase and polymerase, so the replisome is assembled and the new DNA molecule can be synthesized. It has been demonstrated, that during the opening of the bacterial chromosomal origin (*oriC*), the chromosomal replication initiator, DnaA protein, binds to the specific sequence of dsDNA (DnaA-boxes) via DBD domain (DNA binding domain) and ssDNA within DUE region via AAA+ domain (ATPases Associated with diverse Activities). Rep proteins, which are replication initiators of iteron plasmids, do not possess neither DBD nor AAA+ domains. Instead, Rep proteins consist Winged Helix domains (WH), which binds to the directed repeats localized at the dsDNA within plasmid origin of replication, called iterons. Moreover, our data showed that despite lack of AAA+ domain, Rep proteins also bind ssDNA within DUE region. However, the structure of Rep-ssDNA complex is unknown, as well as its function for replication initiation process. In my research I focus on three research models, which are Rep proteins from plasmid RK2 (TrfA protein), plasmid mini-F (RepE protein) and plasmid P1 (RepA protein). Studies carried on those three systems allow me to better

understanding of Rep-ssDNA complex formation and its functionality during replication initiation process.

So far, crystallographic data obtained by our team, as well as bioinformatic analysis allowed us to resolve the structure of RepE-ssDNA complex. Using *in silico* methods, it was possible to identify domains and propose amino acid residues of Rep proteins, which may be responsible for the interaction with ssDNA within DUE region. This data was used for the construction and purification of mutated Rep proteins variants, which are incapable for binding the ssDNA. Using different biochemical approaches, I try to define role of Rep proteins interaction with ssDNA DUE region for iteron plasmid DNA replication.