DNA replication is a process that ensures the duplication of genetic material in every living cell. The initiation of this process is triggered by replication initiation proteins, which bind to specific sequences within origin region. Binding of the initiator results in melting of the double-stranded DNA in a region named DUE (*DNA unwinding element)*. Next, the formed single-stranded DNA (ssDNA) region becomes the place where the replication machinery is assembled and a new DNA molecule is synthesized. The initiators differ in their structure. The replication initiation of bacterial chromosomes is triggered by DnaA protein, which binds dsDNA via a DBD (*DNA binding domain*) domain. Next, DnaA protein via its AAA+ domain forms a complex with the ssDNA within the DUE region. The formation of DnaA-ssDNA DUE nucleoprotein complex is required for bacterial chromosome replication. The initiation of extrachromosomal genetic elements, plasmids, is induced by Rep proteins. These proteins contain WH (*Winged Helix*) domains, which are responsible for dsDNA binding. To date, the AAA+ domains were not identified in any of the known Rep proteins. My research showed, that although the Reps lack the AAA+ domain, they bind specific region of ssDNA of DUE. However, the structure of the nucleoprotein complexes of Reps and the ssDNA DUE is unknown. Neither is known which domain(s) of the Reps bind ssDNA DUE, nor which amino acid residues of the initiator are engaged in this interaction. The aim of the proposed project is the structural-functional analysis of complexes of plasmid replication initiation proteins, Rep, and the ssDNA DUE. Within this project I plan to determine the domains and the amino acid residues of Rep proteins which are responsible for binding of the ssDNA DUE and to investigate the role of ssDNA DUE binding by Reps.
During research presented in this project proposal I plan to analyze several plasmid replication initiators: Rep proteins initiating replication of narrow-host-range (RepE protein of plasmid F and RepA protein of plasmid P1) as well as Rep protein initiating replication of broad-host-range plasmid (TrfA protein of plasmid RK2). First, I plan to use the truncated mutants of Reps to determine the region of initiator involved in interaction with the ssDNA DUE. Simultaneously, I plan to apply mass spectrometry (MS) to identify the amino acids residues of Rep proteins that directly contact the ssDNA DUE. An attempt will also be taken to determine the structure of Rep proteins in complex with the ssDNA with the use of bioinformatic analyses and crystallography. Basing on the obtained data point mutants of Rep proteins, defective in ssDNA DUE binding, will be designed and isolated. Mutants of Rep proteins will be tested *in vivo* and *in vitro* and their phenotype will be analyzed in sequential steps of the process of DNA replication.

The obtained results should allow to acquire structural data on the formation of nucleoprotein complexes by plasmid replication initiators and to broaden our knowledge concerning the process of DNA replication. Therefore they will have an impact on the development of the field. The planned research conducted with plasmid research model might also, in further perspective, have great impact on the development of new antimicrobial strategies.