New insight into the structure and function of the DNA segregation systems of alphaproteobacterial *repABC* plasmids and secondary chromosomes

Many bacterial genomes are divided among multiple replicons, i.e. DNA molecules capable of autonomous replication. Besides the chromosome, the genomes often contain chromids and plasmids. Unlike chromids, plasmids do not typically carry essential genes required by the host. However, these replicons are efficient carriers of adaptive genes, which allow bacteria to adapt better to rapid environmental changes and to survive in the presence of diverse antimicrobial compounds, including antibiotics, xenobiotics and heavy metals.

Plasmids and chromids are very stably maintained in bacterial cells. The maintenance of these low-copy number replicons relies mainly on the partitioning systems (PAR), which ensure active segregation of daughter replicons within dividing bacterial cells. The PAR systems consist usually of two genes encoding partition proteins and a centromere-like *parS* sequence, which is a functional analogue of eukaryotic centromeres.

The PAR systems are usually located in close proximity to the replication systems (REPs). An interesting example of the PAR–REP arrangement are *repABC* replicons, which predominate in *Alphaproteobacteria* [this metabolically diverse group of bacteria include human, animal and plant pathogens (e.g. *Brucella* spp., *Agrobacterium* spp.), nitrogen-assimilating plant symbionts (e.g. *Rhizobium* spp.), as well as numerous saprophytic bacteria commonly found in marine (e.g. *Roseobacter*) and terrestrial environments (e.g. *Paracoccus* spp.)]. The genes involved in DNA segregation (*repA* and *repB*) and replication (*repC*) of the *repABC* replicons are organized into one operon. Active partitioning of newly replicated replicon copies is initiated by the RepB protein, which binds to *parS* (located near the *repABC* genes) to form a nucleoprotein partition complex. Our preliminary studies allowed identification of numerous additional *parS* sites located distantly from the *repABC* operons. Many of these sites are located within protein coding sequences, with an interesting example of *cas* genes of the bacterial defense CRISPR-Cas systems. The main goal of this project is to understand the biological role of these *parS*s. The planned studies will be performed with the use of three *repABC* replicons, representing: plasmids (pcai42C of *Frigidibacter mobilis*), megaplasmids (pAMI4 of *Paracoccus aminophilus*) and chromids (chromosome 2 of *Allorhizobium vitis*, containing *repABC* module of a non-canonical structure).

The *parS* sites of plasmid pcai42C are placed within the *cas3* gene. In this case, we plan to investigate whether binding of the RepB protein to *parS* exerts regulatory effects on the expression of *cas* operon. The *parS* repeats of pAMI4 and chromosome 2 occur within long intergenic regions. In this cases, we plan to investigate (a) the influence of direction of DNA replication on genomic distribution of *parS*s (pAMI4 model) and (b) the role of the additional *parS* sites in the maintenance of an essential replicon (chromosome 2), whose replication (and partition) can be synchronized with replication (and segregation) of the main chromosome.

The mechanisms responsible for stable maintenance of *repABC* replicons in bacterial cells have so far been analyzed only through the prism of the partitioning-replication operons themselves. The implementation of the presented project will provide first holistic insight into the structure and functioning of entire *repABC* replicons. The obtained results may reveal the dualistic role of the *parS* sites – their involvement in both active partitioning of DNA molecules and regulation of the neighboring genes expression. Moreover, analysis of the non-canonical *repABC* operon of *A. vitis* may provide valuable information on the role of the operon in synchronization of the maintenance functions of the main chromosome and chromosome 2 in the *A. vitis* cell cycle.