

## **Title: Analysis of species – specific interactions of mycobacterial chromosome segregation proteins**

### **Abstract**

The actinobacteria that belong to *Mycobacterium* genus are either slow-growing pathogens e.g. *M. tuberculosis*, or fast-growing saprophytes e.g. *M. smegmatis*. Mycobacterial cells elongate at the poles and their growth is controlled by polar DivIVA protein. However, the characteristic feature of mycobacteria is the asymmetric elongation of the cell, accompanied by asymmetric positioning and segregation of the chromosome. In mycobacteria, as in other bacterial species, chromosomes are segregated by ParA and ParB proteins. ParB binds to specific sites in the chromosomes, forming nucleoprotein complexes. ParB complexes are actively moved by ParA protein ATPase that nonspecifically binds to DNA. Earlier analysis revealed that mycobacterial ParA protein interacts with DivIVA and this interaction is unique to this bacterial genus. However, the biological role of this interaction remained unknown until now.

The aim of the PhD project was to elucidate the role of the ParA-DivIVA interaction in *M. smegmatis* cells. Application of the set of ParA mutants allowed to determine that inhibition and enhanced ATP binding diminished ParA – DivIVA interaction but disruption of ParA-DNA binding enhanced the interaction between the studied proteins. Identification of a mutation in the N-terminal threonine in ParA which inhibited the interaction with DivIVA demonstrated that abolished interaction only slightly impaired segrosomes separation and modestly affected ParA localisation. The further analysis of the *M. smegmatis* strains with ParA mutations showed that both, inhibition and enhanced ParA-DivIVA interaction accelerated cell elongation. These results suggest that ParA affects DivIVA activity. It was also shown that the elimination of ParA-DivIVA interaction impaired recovery from stress conditions such as drying, starvation or antibiotic treatment. The role of ParA-DivIVA interaction may be important in *M. tuberculosis* cells, in which N-terminal ParA fragment was also confirmed to be engaged in the interaction with DivIVA.

Thus, the obtained results revealed that the ParA-DivIVA interaction coordinates chromosome segregation with cell elongation and that this coordination is critical for surviving unfavourable conditions in *M. smegmatis* cells.