Role of HupB protein in the organization of *Mycobacterium smegmatis* chromosome

Bacterial chromosome undergoes constant spatial rearrangements and topological modifications during the cell cycle. Among many proteins responsible for chromosome organization the most abundant is HU protein (Heat-Unstable protein). Its homologue from *Mycobacterium* is composed of N-terminal domain that shares homology with *E. coli* HU, and the C-terminal domain occurring exclusively in *Actinobacteria*. Interestingly, the C-terminal domain of HupB contains PAKK motifs, which are characteristic of eukaryotic linker histones. Thus far, very little is known about the involvement of the HupB protein in mycobacterial chromosome organization during the cell cycle and the biological function of its C-terminal domain remains unknown. To address the above topics, *M. smegmatis* fluorescent reporter strains producing either the wild-type HupB or its truncated version, (lacking the C-terminal domain) fused with fluorescent protein (instead of the native HupB) were constructed. Fluorescence microscopy analysis revealed that the HupB protein occupies whole *M. smegmatis* chromosome, while the truncated HupB version does not bind DNA effectively. Those observations were then confirmed using high-resolution PALM microscopy (Photoactivated Localization Microscopy). Additionally, ChIP-Seq experiments (Chromatin Immunoprecipitaion Sequencing) showed that the highest number of HupB binding sites occurs in *oriC* (origin of chromosomal replication) proximal region and decreasing towards *ter* (chromosome terminus). That may suggest the role of HupB in organization newly replicated *oriC* regions. To elucidate the influence of HupB on replication dynamics, there were constructed strains with *hupB* gene deletion and producing DnaN fused with EGFP (allowing to track the replication progression). Time-lapse fluorescence microscopy analysis showed that strains lacking HupB exhibit delay in initiation of the new round of replication, which is consistent with the ChIP-Seq results. In further studies, HupB fused with fluorescent protein was used as a chromosomal marker to analyze the localization of *M. smegmatis* chromosome in real-time at the single cell level. Results of the presented studies contribute to better understanding of the HupB role in chromosome organization and expand the knowledge in a field of bacterial chromosome structure.