Two lysine posttranslational modifications, acetylation and pupylation and their role in the regulation of *Streptomyces* chromosome topology

Protein posttranslational modifications **(PTMs) in bacteria, contrary to the prior notions, are a common, dynamic and reversible strategy to control intracellular activity of bacterial proteins** by the attachment of different chemical groups. Among several bacterial PTMs, lysine modifications seem to be the most abundant and their role in crucial cellular processes i.e. DNA replication, transcription and metabolism regulation has been recently reported. **In our project, we plan to investigate the poorly explored regulation of DNA organizing proteins by their posttranslational modifications.**

In our studies we focus on *Streptomyces*, soil Gram-positive bacteria, known as producers of many secondary metabolites used broadly in medicine and industry. *Streptomyces* undergo complex development and encompasses formation of branched and multigenomic hyphae as well as production of unigenomic spores. During *Streptomyces* life cycle their chromosome topology changes dramatically. Whereas vegetative hyphal cells contain multiple copies of the linear chromosomes which are uncondensed and uniformly distributed along cells, during sporulation they become separated and highly compacted. **Changes in the chromosome topology are tightly controlled by the activity of DNA topoisomerases, including the TopA protein as well as by the coordinated activity of nucleoid associated proteins (NAPs) and condensins**. Among NAPs, the most conserved and abundant in bacteria are HU proteins. Whereas topoisomerases regulate globally DNA supercoiling, HU proteins promote local organization of DNA into supercoiling independent microdomains.

Recent studies of other groups as well as our observations suggest that TopA and HU homologs (HupA and HupS) in *Streptomyces* are posttranslationally modified by the acetylation and/or pupylation of their lysine residues. Whereas the reversible acetylation is common and well described lysine modification carried by acetyltransferases, protein pupylation is still poorly described modification strictly limited to *Actinobacteria*. The protein pupylation constitutes the attachment of small Pup protein (prokaryotic ubiquitin-like protein), which, similarly to eukaryotic ubiquitination, marks proteins for their subsequent degradation in bacterial proteasomes

The aim of the project is to characterize the role of the reversible lysine modifications of DNA organizing proteins in the regulation of chromosome topology in *Streptomyces*. Moreover, in our studies we will also determine how PTMs are changed during *Streptomyces* growth, differentiation and stress response. We plan to perform a set of biochemical studies on posttranslationally modified proteins as well as, using advanced microscopy techniques, we will investigate the changes of chromosome organization in the pupylation or acetylation defective mutants.