„Phosphorylation of the DnaA initiator protein in *Streptomyces coelicolor* - molecular mechanism and biological function" - abstract

Streptomyces are Gram-positive, mycelium forming, mainly soil-dwelling bacteria. These bacteria are characterized by distinctive features and the complex life cycle that resemble the development cycle of eukaryotic filamentous fungi. *Streptomyces* hyphae consist of long, multinucleoid cells (compartments) that undergo morphological differentiation during the life cycle. In addition to the unique lifestyle, one of the remarkable features of *Streptomyces* is the apical growth that occurs by peptidoglycan incorporation at the tips of growing hyphae. *Streptomyces* chromosome also exhibit distinctive features among the bacteria - it is a linear, large (ca. 8-10 Mbp) and GC rich (up to 80%) molecule. Replication of the chromosomal DNA initiates asynchronously in *Streptomyces*, not every chromosome localized within a single cellular compartment of the hyphae undergoes replication.

Chromosome replication is the key step of the cell cycle that has to be tightly and precisely controlled, mainly at the initiation step. In bacteria, replication begins at a strictly defined chromosomal locus, *oriC*, which harbours unique nucleotide motifs (DnaA boxes) that are bound by the initiator protein, DnaA. To date, most of the knowledge regarding the molecular mechanisms that control the bacterial initiation of chromosome replication comes from studies in *Escherichia coli*, *Bacillus subtilis* and *Caulobacter crescentus*. Not much is known regarding these mechanisms in *Streptomyces*.

Global phosphoproteomic analysis of *Streptomyces coelicolor* A(3)2 revealed that DnaA might be phosphorylated at threonine 486 residue. This apparently unique in bacteria post-translational modification of the initiator protein may represent a novel mechanism for controlling replication initiation. This work provides insight into the biological function of DnaA phosphorylation.

Results obtained during *in vivo* experiments confirm that DnaA can be phosphorylated in *S. coelicolor* and further show that this phenomenon occurs during growth stages in which chromosomal DNA replication is active.

Results of *in silico* molecular modelling suggested that phosphorylation may trigger conformational changes in DnaA protein structure and consequently affect the key activities of the analyzed protein. The conducted *in vitro* experiments confirmed that phosphorylation of DnaA elevates its ATP-ase activity and weakens its affinity towards the *oriC* region. These findings lead to hypothesis that phosphorylation of DnaA may generate an initiation-inactive pool of the initiator protein.

Further studies lead to identification of AfsK kinase as an enzyme responsible for DnaA post-translational modification. As it was shown earlier, AfsK is a negative regulator of polar growth and branching of *S. coelicolor* hyphae that localizes at the hyphal tips and functions primarily in response to inhibition of cell wall synthesis. Experiments performed in this work show that upon overproduction of AfsK, replication machinery localizes further from the hyphal tips.

To date, orthologs of AfsK kinase are found only in mycelial actinomycetes related to *Streptomyces*. Results obtained in this work lead to suggestion that in streptomycetes, phosphorylation of DnaA initiator protein (catalyzed by AfsK kinase) prevents the initiation of chromosome replication within growth-inhibited apical cellular compartments.