

## RNA polymerase of *Streptococcus pneumoniae*, potential substrate of protein kinase StkP

Protein phosphorylation by protein kinases is a key mechanism that enables both eukaryotic and prokaryotic organisms sense and read environmental signals and convert these signals into changes in gene expression and thus proper biological response. In prokaryotes signal transduction by protein phosphorylation was originally thought to occur primarily by histidine kinases which are integral part of so called two-component signalling pathways. Lately, bacterial homologues of eukaryotic Ser/Thr kinases have been found to regulate wide variety of cellular functions such as growth, differentiation, secondary metabolism and pathogenicity.

The genome of pathogenic bacteria *Streptococcus pneumoniae* contains single Ser/Thr protein kinase StkP. Analysis of phosphoproteome maps of both wild-type and *stkP* null mutant strains labelled *in vivo* revealed a possible substrate of StkP: alpha-subunit of RNA polymerase (RpoA) (Nováková, 2005). DNA-dependent RNA polymerase complex (RNAP) plays the central role in gene transcription. Alpha-subunit of RNAP has an important regulatory role in transcription initiation: it interacts specifically with upstream promoter elements and with DNA-bound transcription activators.

The aim of this thesis was to prove that the alpha-subunit of RNA polymerase is a target substrate of StkP. The *rpoA* gene was cloned, expressed as His-tagged protein in *E. coli* and purified to homogeneity. *In vitro* phosphorylation assay showed that recombinant RpoA is a substrate for StkP. Further we isolated RNA polymerase from *Streptococcus pneumoniae* by affinity chromatography and we found that StkP is not able to phosphorylate native RpoA in the RNA polymerase complex. These results may indicate that either StpK phosphorylates only uncomplexed RpoA or that StpK requires additional components/cofactors to phosphorylate RpoA in native complex.

**Keywords:** RNA polymerase, regulation of gene expression, transcription initiation, *Streptococcus pneumoniae*, phosphorylation, protein kinases, signal transduction

**Klíčová slova:** RNA-polymerasa, regulace genové exprese, iniciace transkripce, *Streptococcus pneumoniae*, fosforylace, proteinkinasy, signalizace