Abstrakt

Interaction of selected proteins with RNA polymerase from *Bacillus subtilis*

Bacteria are able to adapt to changing enviromental conditions through the regulation of gene expression. Transcription is the most important level of regulation of gene expression, which carries out the multisubunit enzyme RNA polymerase (RNAP). Transcription initiation is a key step of transcription when decisions are made about the future of the cell. This phase is affected by the δ subunit of RNAP in gram-positive bacteria. The physiological role of this protein is not well understood yet. The δ subunit consists of a structured highly conserved N-terminal domain and a flexible and negatively charged C-terminal domain.

This work focuses on the characterization of the δ subunit of RNAP from *Bacillus subitilis*.

In *Streptococcus pneumoniae*, an interaction between δ and protein SP_2234 was found using yeast two-hybrid system. We identified homologs of SP22_34 in *B. subtilis* – YxbF and YwcC as interaction partners of δ. The genes *ywcC* and *yxbF* were cloned and expressed in *E. coli*. The proteins were purified via affinity chromatography. Protein YxbF was purified in a soluble form. We have not succeeded in preparation of YwcC; further experiments were performed with YxbF. Radioactively labeled protein δ was used to study interactions between δ and YxbF using polyacrylamide electrophoresis. It was found, that YxbF did not bind to the δ subunit of RNAP. YxbF also did not help δ interact with RNAP. Further we studied effects of YxbF on transcription and no effects were detected. Contrary to the interaction of SP22-34 with δ in *S. pneumoniae* no interactions between YxbF and δ of *B. subtilis* were detected. However, the interaction between δ subunit of RNAP and the protein SP22_34 in *S. pneumoniae* may be physiologicaly significant.

To clarify the function of each domain of the δ subunit of RNAP, truncated versions of the gene were cloned and expressed in *E. coli*. Protein fragments of δ were prepared by affinity chromatography for further experiments.

Finally, we performed a bioinformatical analysis of the amino acid sequence of δ. In a number of eukaryotic proteins, such as transcriptions factors, we found extended negatively charged regions reminiscent of the C-terminal domain of delta. This domain mimics nucleic acids and increases the specificity of RNAP for promoter DNA. These negatively charged regions could play a similar role in eukaryotic proteins. It would represent a novel general type of a protein domain.