Abstract

RNA polymerase (RNAP) is a key multi-subunit enzyme of gene expression that, together with the σ factor, forms a holoenzyme and transcribes genetic information from DNA to RNA. RNAP from Bacillus subtilis and its primary factor σ^A were studied in this thesis. The σA factor determines the specificity for the promoters to which the holoenzyme binds. Part of its structure is domain 1.1, which is likely to prevent binding of σ^A to the promoter by itself (unless it is part of the holoenzyme) by binding to domains 2 and 4.

The first part of the thesis verifies the hypothesis that domain 1.1 binds domains 2 and 4 and thus prevents binding of σA to the promoter. To this end, various domain constructs have been created and their interactions have been tested. Domain interaction was tested by Nitrocellulose Filter Binding Assay, EMSA, and in vitro transcription. The results did not show significant interaction between domains.

The second part of the thesis deals with the creation of a tool for the study of the enzymatology of RNAP from B. subtilis - recombinant RNAP (rRNAP). First, a plasmid construct for expression of rRNAP in Escherichia coli was constructed by a series of cloning steps, followed by protein isolation and characterization. Isolation was achieved without contamination by σ factors (this contamination is common during isolation of RNAP from B. subtilis). However, the presence of ATP (not GTP) was detected and the binding of this molecule could have biological relevance to the enzyme activity. After isolation, the enzymatic activity of the isolated rRNAP was demonstrated.

In summary, the main outcome of this thesis is the creation of a new, highly effective tool for the study of RNAP from a model soil bacterium B. subtilis.

Keywords: RNAP, sigma factor, interaction, function, expression system