The Characterisation of the Interaction between PcrA and RNA Polymerase

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Statement of Originality

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Contents

Abstract

RNA polymerase (RNAP) is the highly conserved multi-subunit enzyme that carries out transcription in all life forms. RNAP in bacterial species carries out all forms of RNA transcription (mRNA, rRNA and tRNA) and requires the interaction of additional factors to produce full length transcripts. The process of transcription is complex and new information is constantly emerging about the protein-protein interactions involved in the RNAP complex and the functional significance of these interactions.

This project aimed to characterise the interaction between the DNA helicase, PcrA and RNAP in the model Gram-positive organism *Bacillus subtilis.* This interaction was originally identified in a genomic library screen performed to identify novel transcription factors. The characterisation of the interaction provided information that allowed potential of roles for PcrA during transcription to be hypothesized. The PcrA-RNAP interaction was shown to be strong, resistant to high salt concentrations. The interaction was shown to be stable and could withstand salt concentrations which disrupted the interaction between RNAP and known transcription factor GreA. Furthermore, the interaction studies showed that there were multiple binding sites on both PcrA and RNAP. Two sites of interaction between PcrA and RNAP were identified in this study. Firstly, the PcrA CTD (amino acid 577-739) binds to the β subunit (amino acids 1-400) and secondly, the PcrA NTD (amino acids 85-310) interacts with the β' subunit (amino acids 1-102 and 228-310). The sites of interaction were investigated using a combination of techniques, including far-Western blotting, mutagenesis and single particle analysis. Extensive work was completed to identify the

regions of interaction in both PcrA and RNAP. This required the use of many different PcrA and RNAP protein fragments, which were created in this study. Additionally, mutagenic analysis was used to identify the specific amino acids involved in the interaction.

Excitingly, the characterisation of the PcrA-RNAP interaction in *B. subtilis* led to the investigation of this interaction in other bacteria. This work confirmed an interaction between PcrA and RNAP in closely related Gram-positive bacterium *Geobacillus stearothermophilus* and also between UvrD and RNAP in the Gram-negative bacterium *Escherichia coli*. The extension of this interaction into other bacterial species increases the significance of the interaction and indicates that the function of helicase binding to RNAP is conserved in both Gram-negative and Gram-positive bacteria.

The identified regions of interaction were used in conjunction with the PcrA-RNAP complex structure, to present a model of the interaction, which confirms PcrA binds to the upstream face of RNAP. The PcrA NTD-β' subunit interacting regions and the PcrA CTD-β subunit interacting regions were docked into the PcrA-RNAP complex structure. From the model of interaction it was hypothesised that PcrA was translocating upstream of RNAP in order to remove stalled RNAP complexes.