Abstract

Bacteria inhabit various environmental niches and compete for survival. One of the survival strategies of some bacteria is to produce antibiotics. The selection pressure of antibiotics on other bacterial species has led to the evolution of antibiotic resistance mechanisms. Many of these mechanisms are still unknown.

Adaptation to inhospitable conditions can be achieved most rapidly at the transcriptional level. The central enzyme of bacterial transcription is RNA polymerase (RNAP), whose activity is precisely regulated by various factors. Its essentiality for bacterial cells makes it a perfect target for antimicrobial compounds. One of these antimicrobial compounds is rifampicin (RIF), which prevents the synthesis of RNA beyond two to three nucleotides and forms stalled RNAP complexes on DNA, thereby inhibiting transcription. Bacteria possess various transcription factors (TFs), that help release or restart stalled RNAP complexes. One of these TFs is HelD.

HelD interacts with RNAP and binds to it in both the primary and secondary channels. This leads to the release of RNAP from the DNA and helps restart transcription. There are at least two different classes of HelD, which are structurally different: Class I (*e.g.* Firmicutes) and Class II (*e.g.* Actinobacteria). Previous studies have shown that Class II HelD mediates RIF resistance. However, no such effect has been observed for Class I HelD.

This thesis focuses on the role of Class I HelD in RIF resistance and its expression control in response to this antibiotic. We show that Class I HelD in *Bacillus subtilis* plays a role in RIF resistance and that its N-terminal domain is essential for this function. Furthermore, the regulation occurs already at the transcriptional level. Induction of the *helD* gene expression by RIF is controlled by two convergent promoters employing transcriptional interference where transcription from the stronger promoter (P*anti-helD*, antisense) interferes with transcription from the weaker promoter (P*helD*, sense). In the presence of RIF, transcription from P*anti-helD* is more likely inhibited due to the "higher flux" of RNAP through this promoter, allowing transcription from P*helD* to proceed and leading to the production of the HelD protein. Subsequently, the newly synthesized HelD facilitates the release of stalled RNAP complexes. We identify the same regulation also for the *pps* gene expression; this gene encodes a RIF inactivating enzyme. This dual-promoter regulatory arrangement is present also upstream of various genes in different organisms as identified by bioinformatic analysis. These genes encode potential RIF resistance mediating proteins.

In summary, this thesis reveals a role for Class I HelD in RIF resistance, describes a novel mechanism of gene expression stimulation at the transcriptional level by an inhibitor of transcription, and identifies new genes potentially linked to RIF resistance in various bacteria.