Review of PhD thesis

Bacillus subtilis senses the antibiotic rifampicin through convergent promoters that control expression of genes providing protective mechanism

(Author: Tamara Knežová Balgová)

The PhD thesis of Tamara Knežová Balgová entitled "*Bacillus subtilis* senses the antibiotic rifampicin through convergent promoters that control expression of genes providing protective mechanisms" is focused on studying the role of transcriptional factor HelD in resistance to antibiotic rifampicin in *Bacillus subtilis* and on detailed analysis of molecular mechanisms involved in resistence to this antibiotic. Main aims of the submitted PhD thesis were to investigate whether *B. subtilis* HelD is involved in rifampicin resistance. to identify the part of the HelD molecule that is responsible for rifampicin resistance. to determine which part of the *helD* gene upstream region is responsible for regulation of its expression by rifampicin and to elucidate mechanisms of expression control of the *helD* gene by rifampicin.

The PhD thesis of Tamara Knežová Balgová has been submitted as so called „short form“, containing two attached publications of the author instead of detailed Materials and Methods and Results sections.. The basic methods used by the author are only very briefly listed in a general way. Presentation of more information on the specific types of some of the methods used would be useful. The most important results from the attached publications, obtained by the author, are summarized briefly in the chapter Summary of publications (4 pages), including the instructive figure „Model of regulation of *helD* gene expression by rifampicin“. The participation of Tamara Knežová Balgová in the individual publications is clearly described and quantified satisfactorily. He is a joined first co-author in one publication and the second author in the second publication. As concerning the included publications, they were published in journals of high quality (the principal publication in Nucleic Acids Research and the second one in Current Opinion in Microbiology), which confirms quality of the results obtained.

The chapter Literature Review (21 pages, 12 figures) summarizes the published data on various aspects of transcription in bacteria with special emphasis to function of inhibitors of bacterial transcription, mainly rifampicin.

The main results obtained by Tamara Knežová Balgová are those presented in the publication **„**Bacteria sense the antibiotic rifampicin through a widespread dual-promoter based alarm system**.** *Nucl. Acids Res.* 54, gkaf1407 (2026)". It was proved for the first time that Class I HelD trancription regulator, present in Firmicutes, protects RNA polymerase against rifampicin, similarly as perviously characterized Class II HelD occuring in Actinobacteria, and

that its N-terminal domain is essential for this function. Furthermore, level of the *helD* gene expression in *B. subtilis* was found to be positively controlled by rifampicin due to its inhibitory effect on transcriptional interference based on the existence of two convergent promoters, the stronger antisense promoter P*anti-helD* and the weaker promoter P*helD*. The same transcriptional regulation was found also for expression of the *B. subtilis pps* gene which encodes the rifampicin inactivating enzyme, rifampicin phosphotransferase. Bioinformatic analysis revealed presence of similar arrangement of convergent promoters upstream of various genes of different microorganisms, which suggests that some of these genes may be involved in resistence to rifampicin.

The chapter Discussion (11 pages) is divided into eight parts discussing the obtained results, mainly mechanisms of resistence to rifampicin mediated by HelD, as well as other known mechanisms of resistance to rifampicin and characteristics of other inhibitors of RNA polymerase. It contains five instructive figures. The Discussion is clearly written and it documents good orientation of the author in the relevant literature. The list of References consists of 149 cited publications which again proves good theoretical background of the author.

The PhD thesis is carefully written having only minimum of typing errors. I have only three minor remarks to the text of the submitted thesis and two questions.

Specific remarks:

1) P. 33, line 3 – The term „ribosomal transcription“ is not clear.
2) P. 43, lines 11-13 – Sentence „Phenotypic experiments revealed growth defects in the Δ*helD* strain in both liquid (prolonged lag-phase) and solid (slow growth) medium, suggesting a link between HelD and RIF resistance.“ is not clear.
3) No data describing antisense RNA transcribed from the P*anti-helD* promoter are presented in the text of the PhD thesis.

Questions:

1) What are the length and suggested structure of antisense regulatory RNA transcripts transcribed from the P*anti-helD* promoter.

2) Is there any progress in studies mentioned in the part of the Discussion section called Future direction (e.g. studies on potential role of HelD in resistence to Fidaxomicin or experiments using the *helD* upstream region fused with GFP) ?

The minor critical remarks do not lower high scientific level of the submitted thesis, which clearly shows the capacity of the author to carry out independent research of high quality. Therefore, I fully recommend the PhD thesis of Tamara Knežová Balgová to be accepted for defence.

Praha, 1. 6. 2026 RNDr. Jan Nešvera, CSc.