Review of PhD thesis

"Regulation of mycobacterial transcription by selected small RNAs and proteins"
(Author: Mahmoud Khaleel Mohammad Shoman)

The PhD thesis of Mahmoud Shoman entitled "Regulation of mycobacterial transcription by selected small RNAs and proteins" presents results describing various aspects of regulation of transcription in bacteria, mainly in *Mycobacterium smegmatis*. Main aims of the submitted PhD thesis are identification and characterization of novel transcription factors (both proteins and small uncoding RNAs) interacting with RNA polymerase (RNAP) and revealing mechanisms of these interactions and their effects on transcription processes.

The PhD thesis of Mahoud Shoman has been submitted as so called „short form", containing attached publications of the author instead of detailed Materials and Methods and Results sections. Nevertheless, the submitted PhD thesis contains detailed description of materials and methods used (16 pages) and results (45 pages) obtained directly by the author and presented in the Manuscripts II and III. In my opinion, such extensive and detailed decriptions of the most important results obtained would be sufficient for submitting the "classical" form of PhD theses. As concerning the included publications, two of them were published in highly impacted journals (Nucleic Acids Research and Frontiers in Microbiology), which confirms the quality of the results obtained and three manuscripts were ready to be submitted in the time of submitting the PhD thesis. The participation of Mahmoud Shoman in the individual publications is quantified satisfactorily. He is a joined first co-author in NAR publication and the first author of one manuscript, which is presented in detail.

The chapter Literature Review (32 pages, 17 figures, 2 tables) presents briefly the published data on bacteria used and on various aspects of transcription in bacteria with special emphasis to protein and RNA trancriptional regulators interacting with RNAP. The chapter is clearly written and proves good theoretical background of the author. The chapter Detailed Materials and Methods (17 pages including tables) describes material and methods used for obtaining the results presented in the Manusripts II and III and described in detail in the Results section. Use of combinations of various methods of molecular biology, immunology, biochemistry, structural biology and bioinformatics enabled the author to obtain unique results of a high quality.

The main results obtained by Mahmoud Shoman are those published in the publication "RIP-seq reveals RNAs that interact with RNA polymerase and primary sigma factors in bacteria, *Nucl. Acids Res.* **52:**4604–4626 (2024)" and those presented in the manuscript II named "Expanding the CarD interaction network: CrsL is a novel transcription factor in *Mycobacterium smegmatis*" which are described in detail in the PhD thesis. In the published paper, novel uncoding RNAs interacting with transcription machinery of various Actinobacteria were identified by using RNA immunoprecipitation sequencing (RIP-seq) method. These results brings significant advance to understanding RNA-based transcriptional regulation in bacteria. Discovering and characterization of a novel transcription factor of *Mycobacterium smegmatis*, a small unstructured protein designated CrsL, is the main result presented in the manuscript II. Combining the ChIP and RNA sequencing methods enabled to identify genes regulated by transcription factors CarD, RbpA and CrsL interacting with RNAP. Further results described in detail concern the relationships between mycobacterial transcriptional factors HelD, CarD and RbpA in mycobacteria. These results are included in the manucript III named "Mycobacterial HelD is a global transcription regulator and is associated with increased rifampicin resistance in clinical isolates".

The chapter Discussion (15 pages) is divided into ten parts discussing the indvidual results obtained. The Discussion is clearly written and it documents good orientation of the author in the relevant literature. However, adding a more general view on connections between the individual results would be useful. The list of References consists of 394 cited publications which again proves very good theoretical background of the author.

The PhD thesis is carefully written having only minimum of typing errors. I have only a few minor, mainly formal, remarks to the text of the submitted thesis and two mainly general questions.

Specific remarks:

1) P. 63, lines 21-22 – The sentence „MoaB2 has the potential to modulate SigA-dependent but not SigA-dependent transcription" is not clear.
2) P. 67, lines 6 and 8 – There should be „Figure 17A" instead of „Figure 18A".
3) P. 7, lines 25-26 – The sentence „In exponential phase, CrsL, CarD and RNAP proteins were pulled down together in CrsL-FLAG and CarD-FLAG immunoprecipitates (Figure 17E, 17F)" is not clear in the context of the reference to the Figures 17E and 17F.

Questions:

1) How can be explained normal growth of *M. smegmatis* strain with knockdown of the *sigA* gene (Supplementary Fig. 6B) if the sigma factor SigA is considered to be essential in mycobacteria ?
2) What is the present status of the manuscripts I, II and III presented in the PhD thesis ?

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 Mahmoud Shoman to be accepted for defence.

Praha, 31. 8. 2024 RNDr. Jan Nešvera, CSc.