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Review of PhD thesis of Mahmoud Khaleel Mohammad Shoman

The PhD thesis entitled "**Regulation of mycobacterial transcription by selected small RNAs and proteins**" presents a thorough exploration of bacterial transcription. The research focuses on the identification and characterization of new transcription factors and small RNAs that interact with RNA polymerase (RNAP), primarily within mycobacterial species. In Introduction, the author introduces bacterial species he works with as well as bacterial transcription and its regulation. Introduction is nicely and comprehensively written and it is clear that Mahmoud is more of a molecular biologist than a microbiologist. However, I noticed some text repetitions especially in Chapter 1.2.2.

Results are focused on identification and characterization of RNAs that regulate transcription as well as protein transcription factors. The author applied RNA immunoprecipitation sequencing (RIP-seq) to identify species-specific RNAs interacting with the transcription machinery across different bacterial species. The identification of RNAs such as MTS2823, sigA, sigB, recO, rny, scr0792, and CoRP RNA underscores the diversity and specificity of RNA interactions in bacterial transcription These findings together significantly advance our understanding of RNA-based regulation in bacteria. In addition to regulatory RNAs, Mahmoud also investigated protein factors that affect and regulate bacterial transcription. He specifically analyzed CarD interaction network and identified CrsL as a novel transcription factor in *Mycobacterium smegmatis*. He further combined ChIP-seq and RNA-seq data to identify genes regulated by CarD and CrsL - very impressive piece of work! This approach not only identified the genes regulated by CrsL but also provided insights into its potential role as a transcription enhancer. The results are published in two papers and three mature manuscripts ready to be submitted. Mahmoud is joined first co-author on one publication and the first author on one manuscript.

I was a bit disappointed by Discussion. After comprehensive Introduction and Results, Discussion felt a bit shallow and I was lost in details of individual transcription factors and missed a bigger picture and connection between the specific findings and the larger context of bacterial physiology and adaptation.

Despite minor drawbacks, this PhD thesis makes a significant contribution to the field of bacterial transcription regulation. The detailed characterization of new transcription factors and small RNAs, combined with the innovative use of sequencing technologies, provides valuable insights that enhance our understanding of the regulatory networks governing bacterial gene expression and I fully recommend the thesis to be accepted.

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Here are several questions and comments that need clarification:

• Page 67 - The author was not able to detect CarD in ApeB-gFLAG pulldown while CarD-FLAG clearly interacted with endogenous ApeB (Fig. 17B,C). Any Idea why?

• Page 68 - The author concludes that in CarD-gFLAG strain, the expression of antisense AscarD RNA was disrupted. Was this hypothesis tested experimentally?

• Page 72 (Supplementary Figure 1G). - In gradient centrifugation results, the author focuses on CrsL in the bottom fraction. But the light complex of RNAP sedimenting in fractions 4-5 is also reduced in the stationary phase. Could it be due to lower expression of CarD/CrsL? Any idea about the function of this light RNAP complex?

• Page 77 - The authors states that CrsL associates with promoters and is a transcription factor involved in regulation of gene expression. The CrsL is in operon with cell wall proteins and it was suggested by the author that CrsL might be involved "... in constituting the essential building blocks of the mycobacterial cell wall and its potential role as an osmoprotectant,..." (p. 73). Did you check whether CrsL is preferentially associated with promoters of proteins involved in cell wall building/maintenance and whether CrsL downregulation affects expression of cell wall proteins and cell reaction to osmotic stress?

• Page 116 - I don't fully understand the middle paragraph staring with "As I mentioned in the literature review, 6S RNA...". Does it mean that *C. cresentus* contains both 6S and Ms1 RNAs?

• Page 118 - Again, I don't fully comprehend the middle paragraph starting with " The identification of large number of sRNAs....". You write that only one type of abundant RNA was associating with RNAP in a given specie. Does it mean that if RNAP interacted with e.g. recO fragment in *M. smegmatis* it does not interact with Ms1?

• Page 119 - Does Y RNA (and other RNAs that bind RNAP) have a "bubble" similar to 6S RNA? Or do they interact with RNAP via different module/structure?

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