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Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Matvei Kolesnik

PhD Program: Life Sciences

Title of Thesis: RNA targeting defence systems in prokaryotes

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Prof. Peter Fineran

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Date: 11-12-2024**

The purpose of this report is to obtain an independent review from the members of PhD defense Jury before the thesis defense. The members of PhD defense Jury are asked to submit signed copy of the report at least 30 days prior the thesis defense. The Reviewers are asked to bring a copy of the completed report to the thesis defense and to discuss the contents of each report with each other before the thesis defense.

If the reviewers have any queries about the thesis which they wish to raise in advance, please contact the Chair of the Jury.

Reviewer's Report

Reviewers report should contain the following items:

- Brief evaluation of the thesis quality and overall structure of the dissertation.

Overall the thesis was of a high-quality and meets the standards expected internationally for the award of a PhD degree. The candidate has shown the ability to carry out original research and to appropriately present and interpret the data acquired. The candidate has presented a huge volume of work with seven results chapters (publications).

Abstract. The abstract was well written and a nice summary of the main findings of the thesis. It brought together the two different types of RNA -targeting CRISPR-Cas systems nicely.

Introduction: The introduction was well written with only a few grammatical or typographical issues. It provided an excellent introduction into the topics and I found the length and depth of content just right to have the background without excessive detail. Although some of the later chapters appear at immediate viewing a bit disconnected from each other, the candidate has done a good job on drawing these together under the related theme of RNA-targeting defence systems.

Chapter 1. The work provides an excellent examination of the diversity of type III spacers in natural populations of Thermus. The work has been published in a well-respected journal of the Royal Society UK. As stated in the contributions: "M.K. performed PCR amplification of spacers and isolated phiYS40-Isch phage" which demonstrates basic molecular and phage skills.

Chapter 2. This is a really interesting chapter on adaptation in type III systems with direct measurement of this process in the laboratory. Few studies have focused on adaptation in these systems and therefore the results are important and impactful. Hot spots of acquisition are seen from the early region of the virus genome and a strong selection for spacers that are antisense to the mRNA. Interestingly, viruses escape type III targeting via deletions typically rather than point mutations. The candidate performed analysis of high throughput sequencing data of acquired spacers amplicons and conducted an analysis of phiKo phage transcriptome. The work is published in a top specialist journal.

Chapter 3. This chapter elucidated the structure and function of virion RNA polymerase of a crAss-like phage and is published in the top journal Nature. Interestingly this RNAP is most similar to eukaryotic ones that are involved in RNA interference and the authors propose that these may have arisen from distant phage RNAPs. There are a large number of authors on the papers and the candidate analysed the RNA seq data and annotated the phage genomes.

Chapter 4 is a comprehensive first author review about the biology of type III CRISPR-Cas systems. This is an incredibly detailed and thorough review covering an extensive body of literature. Collating and synthesizing this vast literature is a valiant effort!

Chapter 5 investigates that base pairing requirements of type III immunity and how these change depending on the abundance of the transcripts that are being targeted/recognized. This paper reports interesting findings that are not only of biological relevance to how these systems target phages but also with implications to the application of type III systems in diagnostics. The candidate is co-first author on this paper, the results are solid and it is published in a respected journal.

Chapter 6 investigates the mechanism whereby Cas13 triggers cell dormancy. They demonstrate that the protein when activated, cleaves the anti-codons in some tRNAs and that this interferes with translation. In addition, bacterial toxin-antitoxin systems are triggered – providing a back-up route to dormancy. The candidate is co-first author on this paper, the results are highly interesting and it is published in a top journal.

Chapter 7 presents the isolation and characterization of an expanded set of phages that infect Thermus. This analysis extends the known phage infecting this thermophile but also revealed genes of potential further interest such as putative prophage-encoded defence systems. The candidate is first author. The manscript is on biorxiv and is of publishable quality.

Conclusions – provided a good short summary of the presented publications.

- The relevance of the topic of dissertation work to its actual content

The topic addressed by the candidate was suitably covered by the content of the thesis.

- The relevance of the methods used in the dissertation

The methods used were entirely appropriate for the topic of the thesis.

- The scientific significance of the results obtained and their compliance with the international level and current state of the art

The thesis has provided further insight into the mechanism across diverse related topics including type III spacer acquisition, vRNAP and the activity of type VI CRISPR. The work has been published in reputable international journals and meets the standards expected in the field.

- The relevance of the obtained results to applications (if applicable)

Not applicable

- The quality of publications

The candidate has published 6 manuscripts and has one on BioRxiv. He is co-first author on two research articles, first author on a review and the preprint. The papers are all of a solid quality and have provided information that has advanced the field of RNA target CRISPR-Cas systems, RNAPs and viruses of Thermus.

- The summary of issues to be addressed before/during the thesis defense

It would be good to hear from the candidate why they think that adaptation by type III systems has typically been so low in laboratory experiments.

Does the candidate think that the Cas13 proteins have evolved to target the tRNAs or their cleavage sites just match a subset of these by chance?

Provisional Recommendation

X *I recommend that the candidate should defend the thesis by means of a formal thesis defense*

☐ *I recommend that the candidate should defend the thesis by means of a formal thesis defense only after appropriate changes would be introduced in candidate's thesis according to the recommendations of the present report*
☐ *The thesis is not acceptable and I recommend that the candidate be exempt from the formal thesis defense*