Skoltech
Skolkovo Institute of Science and Technology

Thesis Changes Log

Name of Candidate: Matvei Kolesnik

PhD Program: Life Sciences

Title of Thesis: RNA TARGETING DEFENCE SYSTEMS IN PROKARYOTES

Supervisor: Prof. Konstantin Severinov

The thesis document includes the following changes in answer to the external review process.

Dear jury members,
I am very grateful to you for the reading and reviewing of my thesis. Thank you for your suggestions and useful comments. Here is a list of modifications introduced into the thesis text in response on your comments.

Prof. Uri Gophna did not have any questions.
Prof. Konstantin Miroshnikov did not have any questions.
Prof. Mikhail Gelfand addressed the following issues:

First of all, the copy I've downloaded from the Skoltech site is very difficult to read due to some problem with the fonts. The main font is nice, but barely readable, and it likely lacks italic and boldface variants, as these are set in a completely different (default?) font; the latter is also used in the standard form.

Answer: I apologize for the technical issue, which was caused on my end. The font error occurred due to the compression applied to reduce the file size. In the resubmitted version, the fonts are displayed correctly.

The aims of the study are covered in Chapters I through VI; the topic of Chapter VII (new Thermus thermophilus phages) is not mentioned among the aims.

Answer: I added a paragraph describing the aims of the work provided in Chapter VII.

Similarly, the Conclusions chapter provides an integrated story of the CRISPR-Cas studies, it does not relate them to other presented results.

Answer: I included a paragraph explaining the connection between the work on isolating new *Thermus* bacteriophages (Chapter VII) and the CRISPR-Cas studies. As for the research on phage RNA polymerase (Chapter III), I agree that this project is outside the primary focus of the thesis. As a part of the investigation of Type III (Chapter II) immunity I analysed the transcription of phage genes across the infection. Some of the investigated *Thermus* phages encode their own RNA polymerases, and in a context of the studying of Type III immunity it is important to resolve which

genes are transcribed by cellular or phage RNA polymerases. I joined the project on the investigation of phi14:2 polymerase particularly in order to apply the same approach in my work with *Thermus* phages, but the latter project was not completed due to technical challenges. Meanwhile, the work on the phi14:2 RNA polymerase resulted in exciting discoveries, related to the evolutionary history of eukaryotic defense systems relying on the RNA interference, and in some sence this work is connected with RNA targeting defense systems.

(No. 6) with no bibliographic information in the list (although it has been published).

Answer: The bibliographic information was updated.

The candidate is also the first author of a paper about new T. thermophilus viruses, the project not mentioned in the Abstract.

Answer: Abstract was supplemented with the information of this project.

Overall, the publication requirements are clearly met, and the level of early publications is very high.

Answer: I highly appreciate your evaluation. The level of our recent publication (Chapter VI, in *Science Advances*) is also very high.

The candidate's contribution in each particular chapter is stated rather formally; in most cases just by relating to the relevant section of the paper. Given the diversity of projects, I believe this should have been spelled out in more detail.

Answer: Each chapter was supplemented with the description of the role of the work within my thesis and the detailed description of my contribution.

Prof. Fineran addressed the following questions:

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.

Answer: This question should be placed in context, as I am not sure which experiment it refers to. Speaking on our experiments on the adaptation in Type III systems of *T. thermophilus* I would say that infection of *T. thermophilus* liquid cultures with phage in low MOI with the following incubation did not result in detectable accumulation of cells harboring new spacers (at least, these extended CRISPR arrays could not be detected by PCR). To detect spacer adaptation, it was necessary to perform serial passages of the infected cultures into fresh medium, and the extension of CRISPR arrays could be detected only after few of such passages.
While the precise mechanism governing spacer adaptation in this system remains unclear, I hypothesize that *T. thermophilus* cells can acquire resistance to phage infection through two distinct pathways: CRISPR-dependent (spacer adaptation) or CRISPR-independent mechanisms (such as receptor mutations). The latter is likely a faster process, as cells with receptor mutations presumably preexist in the culture. However, receptor mutations may confer fitness defects, allowing cells that acquire phage resistance through spacer adaptation to outcompete those relying on receptor-based resistance over time. It is important to note that these suggestions have not been tested and remain purely speculative.

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?

Answer: This is an important question, one that (as far as I can see it) was also raised by the reviewers of our paper on *L. shahii* Cas13a. Type VI Cas13 proteins are highly diverse, and we currently do not know whether the targeting of tRNA molecules is a feature specific to a particular group(s) of Cas13 enzymes or the specificity toward tRNA is an ancient trait of Type VI CRISPR-Cas systems. Addressing this question requires determining the *in vivo* specificity of distinct orthologous Cas13 enzymes (as suggested by the reviewers). Currently I am participating in this work and hope to share the results soon.

Prof. Kulbachinskiy addressed the following issues:

1) Although each of the chapters has indubitable scientific interest and was published in reputable (and in some cases top-ranked) journals, their connections with each other are not always clear. Each chapter should be placed in the context of the whole work by adding at least a brief description of how it relates to the rest of the thesis. This analysis should be placed at the beginning of each chapter.

Answer: I supplemented each chapter with the description of how this work is connected to the topic of my thesis.

2) Furthermore, the author should try to put all the parts of the thesis together in the Conclusions section. Currently, it contains several paragraphs separately summarizing the findings of individual chapters, even missing some of them. As stated in the Skoltech's PhD Thesis Defense Policy, the thesis based on the work associated with the peer-reviewed academic papers should be accompanied by appropriate explanatory, connective, and integrative analysis, and should not be a mere compilation of published papers themselves.

Answer: I tried to do my best to put all the parts of the thesis together in the Conclusions section.

3) I also recommend summarizing the main findings of the study as several short bullet points at the end of the thesis, similar to conclusions usually found in traditional 'candidate of science' theses.

Answer: I added such list at the end of the thesis.

4) The author's contributions should be comprehensively outlined at the beginning of each chapter.

Answer: I included the detailed descriptions of my contributions at the beginning of each chapter.

5) Chapter VII contains a BioRxiv preprint version instead of the final article published in Viruses, this should be replaced.

Answer: Chapter VII was updated.