

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Elena Kurilovich

PhD Program: Life Sciences

Title of Thesis: The role of genome maintenance proteins in primed CRISPR adaptation by the type I-E CRISPR-Cas system

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Prof. Peter Fineran

I confirm the absence of any conflict of interest I have no conflict of interest.	Date: 19-08-2023
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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.
- The relevance of the topic of dissertation work to its actual content
- The relevance of the methods used in the dissertation
- The scientific significance of the results obtained and their compliance with the international level and current state of the art
- The relevance of the obtained results to applications (if applicable)
- The quality of publications

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

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.

Abstract. The abstract was well written and a nice succinct summary of the main findings of the thesis. If a suggestion were to be made, it would be about including some description of the work presented in chapter 4 around the role of bacteriostatic antibiotics affecting CRISPR-Cas adaptation.

Chapter 1 Introduction: The introduction was well written with only a few grammatical or typographical issues. It provided an excellent introduction into the topic. Other defence systems were discussed, but fairly briefly to set the broader context, without overwhelming the reader. Coverage of CRISPR-Cas was very good. The complex topic of DNA repair and genome maintenance was well covered. However, it is my opinion that – due to the complexity – this section would benefit from a few more figures/models of how these work and even showing some of the (hypothetical) models for how they are involved in CRISPR-Cas adaptation.

Chapter 2 Project objectives: This brief 1 page provided an appropriate overview of the I-E work in the thesis. However, similar to the abstract, I thought that the work in Chapter 4 should have been mentioned here in the objectives.

Chapter 3 Methods: The methods chapter provided sufficient detail to be able to reproduce the experiments.

Chapter 4.1 Antibiotic effect on CRISPR adaptation: As mentioned above, it would have been good to provide more context for this chapter in abstract, introduction and objectives sections, since it is slightly tangential to the main goals of the project on host factors for I-E primed adaptation. This chapter provided further support for the evidence that increasing the time that DNA substrates are available (by slowing growth in this case) can increase the probability of spacer acquisition. It would have been good for the contribution of the candidate to be more explicit – e.g. state precisely which figures were contributed to.

Chapter 4.2 Genome maintenance proteins and CRISPR adaptation: The candidate investigated the effects of recBCD, recJ, sbcD and sbcB on primed adaptation. This revealed that RecJ and SbcB influenced the frequency (abundance) of spacer acquisition. In contrast, RecBCD and SbcD influenced the distribution of spacers. Significant effects on PAM selection were also detected in these high-throughput sequencing assays. RecJ affected the frequency of flipped spacers and those containing internal AAG motifs. Overall, this chapter

revealed the importance of these components, while leaving the mechanism currently unclear.

Chapter 4.3 Host nucleases generate pre-spacers for CRISPR adaptation: The candidate investigated the effects of recBCD, recJ, sbcD and sbcB on primed adaptation. This revealed that RecJ and SbcB influenced the frequency (abundance) of spacer acquisition. In contrast, RecBCD and SbcD influenced the distribution of spacers. Significant effects on PAM selection were also detected in these high-throughput sequencing assays. RecJ affected the frequency of flipped spacers and those containing internal AAG motifs. Further work using a combination of deep sequencing and in vitro assays led to a convincing model for the role of the host nucleases and helicases during pre-spacer formation. I would have liked to see a schematic of the model to help aid the reader in understanding the proposed mechanism. I actually think that bringing Figure 36 earlier into the thesis and using it to help frame the questions and explain the data as it arises could be useful.

Chapter 4.4 Detection of half integrated pre-spacers: A newly developed assay in the lab enabled either half-site integration to be sequenced and quantified in the various mutant backgrounds. This provided data that supported the model proposed in 4.3. It would be useful to provide a schematic outlining the principle of this methods since is has not been published previously.

Chapter 5 Discussion: The candidate clearly summarized the results of the thesis and provided a nice graphical representation of the proposed mechanism. It would have been nice to have some zoomed in regions in this figure showing some of the mechanism in a little more detail.

Chapter 6 Conclusions: Well summarized with clear bullet points.

Referencing: I would prefer to see full author lists in the references. This helps work out which groups/teams have contributed to the work discussed.

- 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

Methods: The methods used were entirely appropriate for the topic of the thesis and the candidate demonstrated a highly-proficient grasp of these – theoretically and practically.

- 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 of pre-spacer generation by host nucleases and helicases during adaptation by the type I-E CRISPR-Cas system. 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 3 manuscripts, one of these as first author and two as co-author. The papers are all of a solid quality and have provided information that has advanced the field of priming in type I CRISPR-Cas systems.

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

It would be good to hear from the candidate what they view as the limitations of the study (this was not covered particularly in the thesis).

It would also be interesting to hear their opinion about the next big questions that the work has raised and what approaches they would take to tackle these.

Provisional Recommendation

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