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Skolkovo Institute of Science and Technology

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. Ed Bolt

I confirm the absence of any conflict of interest I have no conflict of interest. (Alternatively, Reviewer can formulate a possible conflict)	**Date: 19-08-2023**

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

Overview Comments:

The PhD thesis comprises three data chapters from which the candidate has contributed as named author to three original research data publications, in one of these as first/principal author. The quality of these publications is sound, being all in excellent international journals with rigorous peer review. An outstanding outcome of the candidate's first author publication is that Primed adaptation may rely on DNA translocase activity of RecBCD and nuclease activity of SbcCD – see the specific comment about this below. There is some overlap in the thesis data with data carried out by other members of the team, which is acceptable for a PhD thesis in multi-person collaborative work and is clearly identified in the text of the thesis. The body of work presented by the candidate readily exceeds the minimum requirement for award of PhD, by having generated a robust body of new knowledge about CRISPR biology.

The overall layout of the thesis is standard/good, satisfying expectations. There is very limited use of Figures, especially in the Literature Review section. This needs improvement and is currently a weakness in a thesis that is otherwise very strong, for data and quality of contribution to knowledge internationally – see specific comments for specific Figures as below. With those modifications the use of Figures would become suitable for a PhD thesis.

Below is a summary of specific issues to be addressed before/during the thesis defense:

In the list below, items 4, 5, 6, 8 and 11 should be addressed in a revised thesis before the panel meeting in one month time. I have checked the "Provisional Recommendation" box 2 to reflect this.

Items 1,2,3 7, 9, 10 and 12 in the list below could be revised in the thesis, if the candidate can do so, but can instead be discussed at panel.

1. **Page 16, final paragraph** – It is not clear from the way this paragraph is written if the candidate comprehends/understands the molecular mechanism(s) driving evolution of primed adaptation apparently developed by bacteria as they 'learned to fight' escape mutations. There seems to be detail missing. This section would benefit from a slightly longer and more detailed description about what the candidate is thinking.

2. **Naturally occurring CRISPRi-like functions** for some CRISPR Types (ie for natural gene regulation) are absent from the Introduction. They should be included and linked in narrative to the development and use of biotechnology CRISPRi.

3. The PhD thesis is themed especially on links between genome stability and CRISPR adaptation, but there is **no mention in the Literature Review of these links in archaea**, which seem to be more prolific users of CRISPR systems than bacteria. This should be rectified, being directly relevant to the PhD thesis body of work, especially archaeal Cas3, Cas1 and Cas2, even though the candidate's experimental work was in bacteria. I suggest incorporating the works of Uri Gophna and Anita Marchfelder, as starting points/examples.

4. Page 24, Figure 1: **The figure legend needs to be improved,** though the figure itself is acceptable – it requires additional detail explaining every visual aspect of the Figure without the reader having to find explanation in the main text. For example, why are some genes black, others grey and others white? What is CRISPR I specifically, not simply CRISPR? This is *E. coli* work and as such although some readers may be familiar with the specific paper cited for this Figure (ref 125) many others will not be, or at least would benefit from more explanation.

5. Page 27, Figure 2: **The figure should be improved** by including useful detail such as N- and C-termini of proteins, why different colors are used, and especially, highlighting where the Cas1 active site residues are located, in relation to the PAM(s). Overall, this Figure like others, requires more detail.

6. **Figure 3 on page 29 is incorrect and needs to be re-written and replaced.** The evidence is that the mechanism of integration has the 1st nucleophilic attack at the Leader/repeat junction not where it is shown from this reference (Ref 149). **The candidate needs to read, include, and cite Rollie et al eLife 2015 to correct this**.

7. Page 30-31 section 1.5. **Homologous recombination** is not adequately introduced or is pretty-much omitted entirely, including the interplay of DNA recombination and DNA replication, which is critical for generating CRISR immunity. This section will require substantial re-writing and/or defense at the panel.

8. **Page 39, Project Objectives** do not all match well with the content of the thesis. For example, Chapter 4 contains work that is not at all mentioned in the list of Objectives. Suggest this list be modified, or some statement added about how the initial objectives were evolved.

9. **Figure 15 and associated data** have some aspects that will need to be modified and/or discussed. Including the assertion that the Figure 15 data shows SOS response, the reproducibility of Figure 15 data, presentation of the data (fonts). Some re-arrangement of text is needed. **It will be useful to discuss how the *E. coli* SOS response can be confirmed using a 'gold standard' molecular method** that is not shown in the thesis.

10. The candidate should discuss to what degree of certainty there is that Primed adaptation outcomes associated with RecBCD and SbcCD are directly caused by molecular processes of RecBCD/SbcCd (i.e. functional coupling, even if not physical coupling) rather than the loss/gain of each changing more global DNA replication physiology (esp. that at *Ter* sites) that has an indirect effect 'knock-on' effect on adaptation. The latter may be, for example, by changing DNA substrates available to Cas1-Cas2/Cascade-Cas3.

11. **The Literature review and RecBCD Chapter data need to cite and briefly comment** on prior work linking RecBCD and CRISPR, including data showing the RecBCD helicase activity is not required for adaptation (Radovcic et al 2018, Nucleic Acids Research) and questions re: Chi sites and CRISPR (Subramaniam and Smith, 2022 Adv. In Genetics).

12. The candidate should discuss how it can be reconciled that ssDNA fragments formed by the Cas3 translocase-nuclease can be returned into flayed duplexes suitable for DNA capture and integration by Cas1-Cas2. Or, alternative ideas about this.

Glitches/Typos:

Throughout: Cas3 is called a helicase. But its major function is ssDNA translocation and a 'threshing' mechanism to degrade ssDNA. This is not strictly 'helicase' at all, even though it can (in vitro behave as such). I suggest changing helicase to DNA translocase throughout.

page 13 top line: 'systemc'

page 14 line 2: insert word endonuclease?

Page 15: definition of Cas does not need the word genes.

Page 40, Section 3.1 Bacterial strains – there is no mention of how strains were validated/verified other than (presumably) that the P1 had generated the appropriate antibiotic resistance. Were they sequenced? Were they phenotyped to ensure an absence of suppressor mutations?

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*