

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Andrey Krivoy

PhD Program: Life Sciences

Title of Thesis: Primed CRISPR-Cas adaptation in type I-E system of Escherichia coli: use of single-molecule and biochemical assays to verify models of the phenomenon at molecular level

Supervisor: Prof. Konstantin Severinov

Chair of PhD defense Jury: Prof. Konstantin Lukyanov

Email: k.lukyanov@skoltech.ru

Date of Thesis Defense: November 30, 2018

Name of the Reviewer:

<p>I confirm the absence of any conflict of interest</p> <p>(Alternatively, Reviewer can formulate a possible conflict)</p>	<p>Signature:</p>  <p>Date: 11-14-2018</p>
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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

The studies described in this thesis represent pioneering and technically challenging work aimed at gaining a deep mechanistic understanding of a specific mode of CRISPR spacer acquisition (first discovered by the Severinov group) called “primed adaptation” by the I-E CRISPR system of *E. coli*. This specialized anti-viral defense pathway enables host CRISPR systems to overcome viral escape via inevitable point mutations in the PAM and seed sequences of viral target DNA through triggering directed spacer acquisition against the previously encountered and memorized virus.

The candidate’s work has revealed important insight into the primed adaptation mechanism and thus the arms race between bacterial hosts and viruses/phages that infect them. Specifically, original work was executed revealing how various mechanistic steps (e.g. crRNP recognition and binding to target DNA, kinetic analyses of R-loop formation and stability, Cas3 recruitment and nuclease action, etc.) differed for different target DNA mutants or crRNA sizes that were selected for study due to their ability to support (or not) the priming reaction *in vivo*. The *in vitro* findings were related to *in vivo* effects on the rate of CRISPR spacer primed acquisition. The primary conclusion drawn was that the priming reaction is primarily influenced by kinetic effects of the various molecular steps rather than being specifically triggered by conformational changes in the crRNP effector complex. Noble attempts to reconstitute the entire priming reaction *in vitro* were made but did not succeed.

The work is of high quality and rigor. A powerful combination of *in vivo* and *in vitro* analyses (including single molecule (magnetic tweaser) approaches and biochemical assays with reconstituted crRNPs) was employed to gain insight into a relatively unexplored and important area of biology. The findings disclosed in this thesis were published in a relatively high impact journal (Nucleic acid research) and a chapter in a highly respected technical series (Methods in enzymology). The findings were also presented at two international conferences and locally. A major strength of this body of work is how it comprehensively explores diverse aspects of CRISPR function. Moreover, the optimization and refinement of the single-molecule (magnetic tweaser) studies performed to study priming have future potential to provide unique insight into priming as well as other DNA-mediated reactions important for gene expression, etc.

The candidate has displayed a robust grasp of the field and powerfully synthesized the available information into coherent and interesting concepts and summaries of the pertinent information. All written sections of the thesis were easy to follow. Furthermore, the ideas were presented in an relatively economical and thoughtful manner.

Overall, the thesis describes significant achievements in our first understanding of the regulation and mechanism of action of primed adaptation. This thesis should most definitely be accepted in partial fulfillment of the requirement for a degree of Doctor of Philosophy.

Some minor issues to consider correcting or including:

- Pages 12 and 17, use of the term “prove” and “proved” is an overstatement. Alter to a more precise term or phrase (e.g. “allowed to prove” could be changed to “provided evidence”).

- Page 17, “AT-reach” to “AT-rich”.

- Page 84 Remove the question marks that appeared for reference 33 in place of the the letter “i” in numerous locations in this specific citation.

- The reference 82 needs to be updated from BioRxiv to Cell (this study was very recently published in Cell). Moreover, as this is a highly relevant single-molecule study of priming, the specific findings perhaps deserve more description and discussion with regard to the key differences in conclusions reached with the study and the work of this thesis.

- Page 34. Define phrase “ballast shoulders”. (I know it only as a term relating to railroad train tracks and not for DNA molecules...).

- Fig 5a: is “Cas2” a typo? (Cas2 is not a Cascade subunit as described).

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