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Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Julia Gordeeva

PhD Program: Life Sciences

Title of Thesis: Recognition strategies of Type I and Type V BREX systems

Supervisor: Professor Konstantin Severinov

Name of the Reviewer: Konstantin Lukyanov

I confirm the absence of any conflict of interest	**Date:** 10-08-2022

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

BREX is a family of antivirus defense systems of bacteria and archaea. Molecular mechanisms of BREX defense are poorly studied and represent undoubted fundamental interest. Besides, similarly to restriction-modification and CRISPR-Cas defense systems, BREX could find its practical uses in the future. The present work, Julia Gordeeva studied BREX system type I from *Escherichia coli* and BREX system type V from archaeon *Haloarcula hispanica*. She established convenient and efficient model systems where BREX genes are expressed from plasmid(s). Using various experimental strategies, such as deletion and site-directed mutagenesis, expression of individual BREX genes and their combinations, Julia provided intriguing insights into molecular mechanisms of BREX functioning. In particular, specific asymmetric recognition sites for these BREX systems were determined; their methylation was demonstrated to protect DNA from BREX action. Thus, main mechanisms of distinguishing between host and viral genomic DNA by BREX were revealed.

Literature review describes molecular mechanisms of diverse pathways that defend prokaryotes from viruses. It is written in a clear and concise manner and cover many different defense systems as well as corresponding viral strategies to overcome them. It is a bit pity that all ten Figures in the literature review chapter were taken from some papers (with permission), and no single one was designed by the author.

The results were published in two papers in Nucleic Acids Research (impact factor 19.16) – one of the top journal in the field (Julia is the first author is one of them). Also, Julia attended two international conferences. Overall, this is a high quality impactful experimental work.

Questions and comments.

1. Section 3.3 describes the finding that BREX^{Ec} recognizes GGTAAG site, and methylation of this site at the fifth adenosine makes the phage and bacterial DNA resistant to BREX action. In particular: “The results showed that all 18 GGTAAG sites present in the phage λ genome were methylated at the fifth adenosine residue (Fig. 15c, d). No such modification was present in DNA of phages induced from BREX- cells. Sequencing of BREX+ cells DNA showed that 94% of the 1708 genomic GGTAAG sites were also modified”. Is it true that BREX- cells (E. coli BW25113) lacks methylated GGTAAG sites in the genome? If so, why does the plasmid-expressed BREX system (pBREXAL) not attack bacterial genomic DNA just after transformation? At the same time, unmodified phage DNA is efficiently removed. It might be helpful to discuss this in the text.
2. Page 19, a paragraph on Type IV R-M systems. Please extend it with a brief explanation of how bacteria avoid cleavage of their own genomic DNA.
3. Section “3.4 The role of individual brx genes in phage protection and DNA modification”: Some introductory phrase is missing in the beginning (for example, “To reveal roles of components of BREX system, we used deletion analysis, site-directed mutagenesis, and expression of individual brx genes.”). Also, this section is too rich in emotional introductory words such as “curiously” and “interestingly”.
4. Page 63: “Substitution of a serine in the serine-lysine proteolytic diad …”. Mentioning the serine number (S597) would be helpful here.
5. Page 64, Figure 19 (“BrxL inhibits FtsZ-ring formation and induces SOS-response”): In the panel c, I can not see any significant differences between -ara and +ara plates in terms of yellow/red coloration of the corresponding streaks. Please explain or highlight the differences. In the text, the author noted “Unlike the Lon protease, the Lon-like BrxL induced SOS-response even when arabinose is not added.”

If the SOS response is independent of BrxL expression, can we conclude that the BrxL protein actually induces the SOS response? Did you check the arabinose induction procedure by protein quantification before and after induction?

6. The scheme in Fig. 19b is a bit misleading. It looks like “Lactose hydrolysis” leads to “SOS-response”. If you move “No SOS-response” and “SOS-response” signs from the bottom to the top of the scheme, it would be clearer.
7. Page 66 (section 3.5): “Moreover, we calculated the fractions of linear and circular forms to track a translocation process because phage lambda DNA is circularized upon the injection (Fig. 20c) (101). A proportion of the circular form increases in BREX+ cells proving that the injection stage proceeded successfully (Fig. 20d)”. Please describe briefly how you made the calculation. Figure 20d contains no y-scale.
8. Page 71, Table 3: What do dashes mean, 0% or absence of data?
9. Page 76: “We showed that the modification module of E. coli BREX system functions by methylating a specific asymmetric site in phage DNA (Fig. 24)”. It should be Fig. 25.
10. Incomplete or incorrect formatting of some references. For example: no pages in Refs. 1, 11, 67, 84, 85, 102, 107; “Nat.” for “Nature” and fused volumes and numbers in Refs. 88 and 90; Ref. 101 is a book, which should be cited accordingly. Please check the list and make sure all formatting is consistent.

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*