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Skolkovo Institute of Science and Technology

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: Petr Sergiev

I confirm the absence of any conflict of interest	**Date: 07-19-2022**

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

In natural environment bacteria are challenged by a number of dangers, such as antibiotics, toxins and phages. A continuous arms race between bacteria and phages which lasts for billions of years resulted in the evolution of numerous anti-phage systems. Some of those systems are pretty well known, such as restriction-modification, which is widely applied in molecular genetics. However, we only started to understand an enormous diversity of bacterial defense systems.

In this thesis, Julia Gordeeva, who was working under a supervision of professor Konstantin Severinov, advanced our understanding of BREX systems functioning. BREX are anti-phage systems which were discovered relatively recently. In some aspects these systems resemble restriction-modification systems, since they commonly encode a specific DNA methyltransferase which is used to mark self DNA. Unmethylated phage DNA is distinguished by this system and, as was shown in this work, rapidly degraded.

In the literature review of this thesis Julia Gordeeva reviewed different anti-phage systems. The review is well designed, informative and up-to-date. I really enjoyed reading it.

The main text describes the study of two representative types of BREX systems, type I possessed by *E. coli* HS and type V of *Haloarcula hispanica*. Both systems has been cloned into expression vectors and checked for protection against bacterial and archaeal phages. BREX^{Ec} protected E. coli from phage lambda. At the same time, it doesn't prevent lambda to be activated form its lysogenic form. The resulting phages were active in infection of bacteria. It was demonstrated, that methylation of GGTAAG sites protect the phage form the inhibition by the BREX system. Mutational analysis of the BREX operon revealed several genes that are responsible for methylation of DNA and defense against the phage invasion.

While overall, the results are clear and easy to understand, there are several findings that seems mysterious. For example, deletion of brxL from the inducible form of the operon resulted in the formation of two types of colonies, large normally looking ones, which were not able to grow in the liquid culture and small clear colonies that retained such an ability. I'm wondering, whether the phenotype remained after re-streaking of the colonies and whether the plasmids after transformation remained identical or rather accumulated some mutations? This gene is really a mystery. The following experiment demonstrated that overexpression of *brxL* is highly toxic. This effect could be abolished by mutations in the putative catalytic center of the enzyme. This result make sense and it might be hypothesized that proteolytic activity might somehow be implemented for anti-phage defense, e.g. via abortive infection. However, to a surprise inactivation of the proteolytic activity of *brxL* had no influence on anti-phage activity. Crucial question is the target of this mysterious protein. The statement "purified BrxL protein did not show any proteolytic activity" is somewhat unsatisfactory. It is interesting to know what was an assay used? The activity might be very narrow, on some particular protein within the cell of phage proteome. What type of substrates have been checked?

Another mystery is lack of self-toxicity of the BREX system upon inactivation of the methyltransferase gene. It is a really interesting result, which deserve special investigation in the following studies.

In the last part of the thesis, Julia describes experiments on the functioning of type V system of archaea. This system differs from *E. coli* one by a couple of peculiarities. First of all, it looks like it contains two modification systems. Both of those contribute to the defense proportionally to the number of modification sites in the phage. However, unfortunately, the statement that there are two independent modifications is only a hypothesis. It might be relatively easy to check modification status of the DNA in methyltransferase knockouts, so it's a pity this has not been done.

Archaeal type V system is also special in having a putative nuclease gene. Also, quite mysterious is that nuclease gene knockout does not influence anti-phage defense.

As a summary, I have to state that the work of Julia Gordeeva which was described in her thesis is a significant advancement of our understanding of BREX systems. The results were published in two papers in NAR, which is one of the most reputable journals in the field. While, the understanding of BREX systems functioning is still incomplete, it is absolutely clear that Julia Gordeeva should defend the thesis by means of a formal thesis defense.

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*