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Skolkovo Institute of Science and Technology

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Maria Sokolova

PhD Program: Life Sciences

Title of Thesis: Functional and Structural Analysis of a Non-Canonical RNA Polymerase Encoded by Giant Bacteriophage AR9

Supervisor: Prof. Konstantin Severinov

Chair of PhD defense Jury: Prof. Yuri Kotelevtsev ***Email:*** *y.kotelevtsev@skoltech.ru*

Date of Thesis Defense: 15 June 2018

Name of the Reviewer: Dr. Andrey Kulbachinskiy

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	**Signature:** **Date: 15-05-2018**

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 thesis of Maria Sokolova describes biochemical and structural characterization of a noncanonical multisubunit RNA polymerase (RNAP) encoded by phage AR9. Noncanonical double-psi-beta-barrel polymerases are an exciting object of recent research as they are often considered a primordial form of all cellular polymerases. Alternatively, they might have originated from the cellular transcription machinery, followed by fast viral evolution, loss of protein segments, domains and subunits. In any case, studies of these highly divergent group of enzymes may provide novel insights into the mechanisms of one of the most universal processes in life (i.e. transcription) and reveal the basic principles of structural organization of all RNA polymerases. In addition, analysis of regulatory strategies of bacteriophages always yields some unexpected and delightful mechanisms of genetic regulation, which is also true for the current study.

The thesis is perfectly written and structured, well-illustrated and brings all the parts of this work together. It contains all necessary chapters, including Literature Review, Materials and Methods, two chapters of Results and Discussion, Conclusions and Appendixes. Most importantly, it is shown that:

1) promoter recognition by the AR9 RNAP depends on the presence of uracil in phage DNA;

2) uniquely, the RNAP recognizes the template promoter strand and can initiate transcription from ssDNA - with the aid of a phage factor that has very limited homology with bacterial sigma factors;

3) for the first time, a partial structural model for a noncanonical multisubunit RNAP is obtained; while the overall structure of the AR9 RNAP is similar to cellular RNAPs, it reveals important variations which are probably related to its function and evolution.

The crystallization and structure determination was a technical *tour de force* and although no complete RNAP/TEC model has been obtained to date, the great progress already made by the author gives hopes that such model will be built in the future.

The study opens new directions of further research that, besides structural analysis of transcription complexes of the AR9 polymerase, include mutagenesis of its functional elements, study of the mechanisms of RNA/DNA separation and promoter melting by this RNAP (it seems to be more active on single-stranded than on double-stranded DNA), and possible analysis of its homologues from other phages.

I have several minor points, which can be addressed at the time of defense.

1) The active site of RNAP contains two Me^{2+} ions, not just one (p. 15).

2) According to recent reports, transcriptional pausing is not accompanied by clamp opening but depends on other conformational changes in the elongation complex (Guo et al., Kang et al., Mol Cell 2018) (p. 21).

3) The description of sigma dissociation and the role of sigma finger in this process does not include all necessary references (pp. 33-34).

4) Some minor details are missing from Materials and Methods (such as the step of DNA precipitation after piperidine treatment in $KMnO_4$ footprinting).

5) The chapter 'Conclusions' would have benefited from a more succinct and concentrated formulation of the main findings.

The results of the study have been published in three high-quality papers in good international journals (Nucleic Acids Research, Virology, MBio) and it is obvious that more publications will follow when the study is continued.

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*