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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: Petr Sergiev

I confirm the absence of any conflict of interest	**Signature:**  **Date: 02-05-2018**

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 "Functional and structural analysis of a non-canonical multisubunit RNA polymerase encoded by giant bacteriophage AR9" by Maria Sokolova is devoted to the study of highly unusual RNA polymerase of the phage. While some phages code for their own RNA polymerases, these are mainly single subunit enzymes. Multisubunit RNA polymerases are more common for cellular genomes. In fact, all cellular organisms code for similarly constructed RNA polymerases, which share the general architecture. The AR9 nvRNA polymerase, which is responsible for the transcription of the late genes of the phage has a very unusual composition if compared with the composition of cellular multisubunit RNA polymerases. It has four core subunits that have similarities with N and C terminal parts of β and β' subunits of bacterial RNA polymerase, while completely lacking α and ω subunits. Careful biochemical purification scheme developed by Maria allowed separation of both core enzyme and putative holoenzyme which contained additional gp226 subunit which lacks clear homology with bacterial RNA polymerase subunits. Maria studied enzymatic properties of the AR9 nvRNAP in vitro. It appeared that the polymerase has unique properties related to the mechanism of transcription initiation. Remarkably, AR9 phage genome contains uridine residues completely substituting thymine. This substitution appeared to be critical for the transcription initiation. Maria demonstrated that while "normal" DNA is not recognized by the phage RNAP as a substrate, uridine-containing DNA is transcribed. Mapping of each promoter residue with the help of mutagenesis and thymine to uridine (and back) substitution allowed to reveal the residues recognized by the polymerase. Utilization of hybrid templates and several other experimental tricks allowed Maria to make several groundbreaking conclusions related to the mechanism of initiation by nvRNAP of AR9. It appeared that template DNA strand of a promoter is recognized and conserved uridine residues of the promoter interact directly with RNAP. An accessory subunit gp226 is not needed for the elongation but is necessary for promoter recognition. Non-essentiality of the non-template strand of the promoter DNA for transcription resulted in a revolutionary phenomenon of single-stranded DNA transcription by nvRNAP AR9. Spectacular biochemical study was followed by structural studies aimed in nvRNAP structure determination. It appeared that the task was very complicated and several methods applied lead to a limited success yet, although the work is in progress, as it comes from the text.

Overall impression is that Maria made a titanic work and obtained spectacular results, highly demanded by the molecular biologists. The work is published in 3 international journals with high impact factors. Maria is the first author in one of those. The methods applied are of top level in the field. Thus, there are no doubts that Maria deserves PhD degree.

The only criticism I have is related to in vivo relevance of the single stranded DNA transcription. The trick with lowering DNA concentration allowed to see some amount of RNA transcript that is not RNase H sensitive, but still, the predominant majority of RNA is present apparently in a form of a hybrid. No proof of in vivo relevance for such form of transcription is presented. This small criticism is absolutely not crucial for the very good impression of this titanic work

Provisional Recommendation

V *I recommend that the candidate should defend the thesis by means of a formal thesis defense*