

Jury Member Report – Doctor of Philosophy thesis.

Name of Candidate: Marina Kalinina

PhD Program: Life Sciences

Title of Thesis: Long-range complementary interactions in human pre-mRNAs and their implications in splicing

Supervisor: Professor Olga Dontsova

Co-supervisors:

Assistant Professor Dmitri Pervouchine

Dr. Dmitry Skvortsov, Lomonosov Moscow State University

Name of the Reviewer: Eric Westhof

I confirm the absence of any conflict of interest (Alternatively, Reviewer can formulate a possible conflict)	Date: 28-OCT-2021
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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 thesis is clearly written in a very logical fashion. It brings solid evidence towards the main conclusions of the thesis.

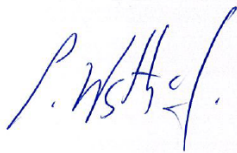
The objectives of the thesis were to analyze quantitatively the effects of long-range base pairing formation on the choice of exons during alternative splicing. Three genes were studied; two were simple and one was highly complex. The potential base pairing formations were disrupted in two ways, first through targeted mutations within the pairing regions and then with the use of antisense oligonucleotides targeted on either strand of the targeted hairpin. Both approaches are accepted for testing base pairing (especially in the absence of covariations due to the high conservation of the genetic elements throughout mammals). The results were analyzed using gel electrophoresis migrations and RT-PCR quantitative measurements. The results were evaluated critically using state-of-the-art statistics. Further, using an inhibitor of the pol II, the authors could show the effects of slowing down the polymerase on the choice of exons.

The data and the conclusions reached are convincing and the main results published in critically reviewed articles (*Nucleic Acids Research* and *Nature Communications*).

At the defense, I will ask the two following questions.

The mutations introduced in the potential segments of the paired regions are rather minimal. Did the author tried other mutations or only those shown? Especially, if experimentally feasible, what would be the effects of a longer stretch mutation?

In the pol II experiments, only the antisense strategy was applied. Did the author test the point mutations to see the effects of pol II slow down?



Nov 2, 2021

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