

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: Yuri Kotelevtsev

I confirm the absence of any conflict of interest	
(Alternatively, Reviewer can formulate a possible conflict)	Date: 09-11-2021

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

This study is devoted to the investigation of the fundamental mechanism of alternative exon inclusion. Three different genes were selected for investigation of long range interactions: Phf2011, Cask, and Ate1. Using mutagenesis of minigenes It was demonstrated that RNA helices are responsible for looping out of alternative exon in Phf2011 and Cask. The data was confirmed using steric blocking LNA antisense oligonucleotides. The author managed to regulate the alternative splicing outcome of the endogenous Phf2011 and Cask transcripts.

In the third gene, Ate1, extensive mutagenesis of the minigene in combination with antisense oligonucleotide approach identified structural modules responsible for alternative splicing regulation in the mutually exclusive exons cluster.

Comparative sequence analysis was applied to identified regulatory intronic elements in Ate1 premRNA. Their function was examined in alternative splicing using site-directed mutagenesis and locked nucleic acid (LNA)/DNA mixmers. The study benefited from the clever strategy where in point mutations that disrupt RNA structure when introduced alone, were complemented by different combinations of mutations which restored splicing.

All together the performed experiments allowed to conclude that alternative exon exclusive splicing in the human Ate1 gene is regulated by specific RNA structure composed of two relatively short modules: a competing RNA structure module responsible for including one and only one exon in the mature mRNA. In contrary, the ratio of splice variants was controlled by an ultra-long-range module, which contains base pairs spanning over 30,000 nts. The author demonstrated that alternative splicing can be adjusted using LNA-based antisense oligonucleotides, which underpins emerging therapeutic platforms for targeting alternative splicing of the genes of interest particularly in cancer and neurodegenerative disorders.

The dissertation is well written, has a traditional structure. The methods are well described, the data presented thoroughly and properly evaluated.

- The relevance of the topic of dissertation work to its actual content

Completely relevant

- The relevance of the methods used in the dissertation

The methods are adequate and carefully executed to prove the hypothesis.

- The scientific significance of the results obtained and their compliance with the international level and current state of the art

The results are on the cutting edge of the investigation of alternative splicing which is reflected in the highest level of publicatytions (NAR and Nat.Com)

- The relevance of the obtained results to applications (if applicable)

Non applicable at this stage

- The quality of publications:

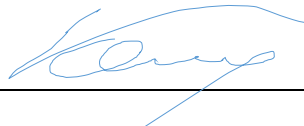
Both publications are relevant to the topic and of highest possible quality.

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



Y.Kotelevtsev 09.11.2021