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Skolkovo Institute of Science and Technology

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: Professor Konstantin Lukyanov

I confirm the absence of any conflict of interest	 **Date: 13-11-2021**

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

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

Dissertation by Marina Kalinina studies functional significance of long-range complementary interactions within pre-mRNAs. The main idea of this work is quite straightforward: complementary fragments of a pre-mRNA molecule can form duplex, which potentially affects splicing of nearby exons. To verify this hypothesis, author selected a number of genes potentially involving target sequences and tested them experimentally. Three most interesting cases were then studied in detail. Two complementary experimental approaches were used, namely mutagenesis of artificial minigene constructs and antisense oligonucleotide against endogenous transcripts. This simple and elegant strategy unambiguously demonstrated a key role of long-range complementary interactions for output of splicing of all three target human genes under investigation; the most interesting observation was an ultra-long-range RNA structure interactions at 30 kb distance. In addition, a global effect of RNAPII slowdown on alternative splicing was studied by RNA-Seq. It was found that RNAPII slowdown (induced in different ways) affects splicing differently for short and long introns as well as for introns with and without long-range RNA structures. Overall, the present work provides a significant contribution to understanding of regulation of alternative splicing by long-range RNA hairpins.

The main results of dissertation work were published in the top-level scientific journals from the Nature Journal Index list: Nucleic Acids Research (IF=17; the first author) and Nature Communications (IF=15).

In my opinion, the thesis is almost ready for the formal thesis defense; I have only a few minor remarks:

Figure 2-1: In the current drawing, lines for main chain and base pairs are the same. This is a bit misleading and could be changed (e.g., thin lines for base pairing).

P. 24: “SREs tend to be located close to splice sites tend to have the strongest effect on splicing”. Please reformulate to avoid repetition of “tend to”.

P. 33: “If a length of an MXE is not a multiple of 3, the inclusion of more than one such MXE would lead to a frameshift and create a premature stop codon.” In fact, the inclusion of one such MXE would also cause a frame shift; but the inclusion of three such MXEs would even restore the open reading frame. Please clarify.

P. 49: “These regions were least 10-nt-long …”. It should be “at least”.

Table 5.1 and related text is not very clear. Is it your experimental results or taken from the literature? What means an “opposite” effect? Also, there are some misprints in the legend.

In Figure 5-1, inverted repeat regions are shaded in pink (by the way, it is not described in the legend). Twelve nucleotides are highlighted: CCCAAATAGCAG and complementary CTGTTATTTGGG. However, the sequence shows two additional complementary (and evolutionary conserved) bases CA/TG, so the entire complementary regions are in fact 14-b long: CCCAAATAGCAGCA / TGCTGTTATTTGGG. If so, please modify the Figure 5-1 (and possibly also Fig. 5-2B).

In Figures 5-2 and 5-5, the “fwr” forward primer is shown under “pCMV” region that looks like a promoter. Please modify to avoid misunderstanding.

Parameters of box-and-whisker diagrams used in many figures should be described (I found such a description in Figure 6-5 legend only).

Figures 5-3 and 5-6: It would be helpful to show a panel on quantification of gels (e.g., as standard dose-dependence curves).

P. 58: “Mutated minigenes with disrupted basepairing (which are called mut1 and mut2) generate more transcripts with exon 19 included compared to the WT”. In the following text and in the figure, the mutants are called m1 and m2, not mut1 and mut2.

Figure 6-5: Which introns were considered “short” and “long”? In the legend, reference to the panel B is missed (“The difference between the inclusion rate …” -> “B. The difference between the inclusion rate …”).

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*