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Skolkovo Institute of Science and Technology

Thesis Changes Log

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

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Dr. Dmitry Skvortsov, Lomonosov Moscow State University

The thesis document includes the following changes in answer to the external review process.

I would like to thank all jury members for their reviews and comments. As a result of their feedback, I have made several changes reported in detail below.

Professor Konstantin Lukyanov

1. 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).

   Modification to the thesis:
   Figure 2-1 has been changed according to the recommendation.

2. 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”.

   Modification to the thesis:
   “SREs tend to be located close to splice sites tend to have the strongest effect on splicing” -> “SREs that are located close to splice sites tend to have the strongest effect on splicing”.

3. 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.

   Modification to the thesis:
   “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.” -> “If the lengths of both MXEs are not multiples of 3 nt and the rest of the transcript complements them to maintain the reading frame, then the inclusion of both MXEs, or skipping of both of them would lead to a frame shift and consequent introduction of a premature stop codon”.

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

   Modification to the thesis:
   “These regions were least 10-nt-long ...” -> “These regions were at least 10-nt-long ...”

5. 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.

   Modification to the thesis:
   The legend to the table 5.1 has been changed.
   “Table 5-1. A short summary of all experimentally tested targets by AONs and/or minigenes. Columns are (left to right): NCBI gene name; cell line; id of PCCR from [2] or location of the alternative splicing event; AON sequences; AON effect classified by size (no effect, small, large), predictability (the opposite effect identifies effect different from that predicted for the secondary structure regulation), and reproducibility (non-reproducible effects); mutagenesis effect classified by size (no effect, large) and problems (aberrant denotes problems with minigene splicing pattern, contradicting effect describes problems with the explanation of mutagenesis results). N/A – there were no such experiments with the particular target”.

6. 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).

   Modification to the thesis:
   Fig 5-1 and 5-2B were modified (the length of complementary regions was changed to 14 nucleotides). The descriptive sentence “Stem-forming sequences are highlighted in orange” was added to the legend in Figure 5-1 (and to the legend in Figure 5-4). The descriptive sentence “The mutated nucleotides are highlighted in blue” was added to the legend in Figure 5-2 (and to the legend in Figure 5-5).

7. 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.

   Modification to the thesis:
   Figures 5-2 and 5-5 have been modified the following way: pCMV and SV40pA are shown in boxes, and arrows for primers are shown above the scheme.

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

   Modification to the thesis:
   “In all figures, we used standard notation for boxplots including the median, upper and lower quartiles and upper and lower fences without outliers” was added in the 4.9 section in Materials and methods. Additionally, “Asterisks indicate the range of P-values: $0.05 \leq P < 0.1$ (*); $0.01 \leq P < 0.05$ (**); $P < 0.01$ (***); not significant (NS)” was added to the legends to all figures with boxplots.

9. 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).

   Modification to the thesis:
   Panels of gels quantifications were added to figures 5-3 and 5-6.

10. P. 58: “Mutated minigenes with disrupted base pairing (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.

    Modification to the thesis:
    Mut 1 and mut 2 on the p. 58 were changed to m1 and m2.

11. 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 ...”).

    Modification to the thesis:
    “All introns shorter than a median value (925 nt) were considered ‘short’, all introns longer than the median were considered ‘long’”.

    Reference to the panel B is restored.

<u>Dr. Timofei Zatsepin</u>

1. p.28 “There are already two drugs for SMN2 splicing correction approved by U.S. FDA (antisense oligonucleotide nusinersen in December 2016 and small molecule risdiplam in August 2020)”. I suggest to add Zolgensma to the list of SMA therapy

    Modification to the thesis:

    There are already two drugs for SMN2 splicing correction approved by the U.S. FDA (antisense oligonucleotide nusinersen in December 2016 and small molecule risdiplam in August 2020), but other therapeutic solutions may also soon be available in clinical practice, such as Zolgensma, a gene therapy approved by the U.S. FDA in May 2019.

2. p.37 “physico-chemical methods” I would dissect them according to common practice – into physical and chemical

    Modification to the thesis:
    “..using physico-chemical methods” -> “..using physical and chemical methods”.