Thesis: “Determinants of the splice site selection in protein-coding and long noncoding RNAs”

Author: Zuzana Krchnakova, Institute of molecular genetics, Prague

Supervisor: David Stanek, PhD, Institute of molecular genetics, Prague

Reviewer: Dalibor Blazek, PhD, CEITEC, Masaryk University, Brno

The thesis “Determinants of the splice site selection in protein-coding and long noncoding RNAs” by Zuzana Krchnakova investigates three areas of splicing regulation: 1) interplay between histone modifications, transcription and splicing; 2) determinants of splicing regulation of long non-coding RNAs (lncRNAs) and 3) role of splicing on the function of enhancer-like lncRNAs.

The thesis consists of five major sections: 1) General introduction to the topic; 2) Material and methods; 3) Results; 4) Discussion and 5) References. Result section includes published work as well as unpublished results. Introduction to the topic is generally well-written and provides informative and well-selected overview of the studied field without burdening a reader with unnecessary details. Papers, one first-authored and one co-authored, are published in peer-reviewed journals.

Overall I am very positive about the work presented in the thesis which is without any doubt excellent. It is clear that Zuzana Krchnakova obtained hands-on expertise with current state-of-the-art biochemistry and molecular biology techniques and gained insights into the fields of splicing and transcription. The experiments involve many non-trivial molecular biology and biochemistry techniques including iCLIP-seq. As evident from the thesis she can also summarize scientific text into the form of review. Her experimental work was published in excellent peer-review journal (Nuclear Acids Research) where she is the first author. She is co-author of another paper (Scientific Reports) and likely more paper(s) will be published based on the results presented in the thesis.

In conclusion I can recommend the thesis for successful defense without any reservation.

Dalibor Blazek

Brno 4.3.2019

Student prokázal tvůrčí schopnosti: ANO

Práce splnuje požadavky kladené na PhD disertační práci v daném oboru: ANO

Questions:

1) You show that local changes in histone modification (H3K9me3) can affect constitutive splicing of FOSL1 (Fig. 18). Did you check if recruitment H3K9me3 -specific interactors such as CBX5 was also affected and if total and modified RNAPII levels were changed? If yes, what was the outcome?

2) lncRNAs are poorly spliced and your results suggest that this is the outcome of their sequence composition and consequent weak interactions with SRSFs. This is indication of a passive process. I wonder if splicing of lncRNAs is changed upon any stress cue (such as DNA damage etc), which would be indicative that it can be under certain situations actively regulated? It is surprising that expression of lncRNAs is cell-type specific (it means is actively regulated), but it seems that their splicing is only passive process. Can you comment on this?

3) In connection with the previous question, did you check if there are any genome-wide differences in occupancy of RNAPII (and its modified forms) over lncRNAs and protein-coding genes with similar length/structure?

4) In Fig. 26 you make a conclusion that splicing of ncRNA-a2 is not determined by chromatin elements by using *ncRNA-a2* cloned and transiently expressed from plasmid. I am not sure if you can make such a conclusion since plasmids also get chromatinized. Can you comment on this?