Školitelský posudek/Supervisor's evaluation

on Sravya Ganesh's Dissertation Thesis: *Long Non-Coding RNAs in Oocyte-to-Embryo Transition*

I'm pleased to provide this evaluation of Sravya Ganesh and her thesis work.

Sravya joined my group at the beginning of 2014 when she was selected from over 70 applicants for a PhD position in the Marie Curie Initial Training Network RNATRAIN, a consortium formed mainly of research laboratories studying long non-coding RNAs. She subsequently joined the PhD program in Developmental and Cell Biology at the Charles University, where she started her PhD studies in Fall of the Academic year 2014/2015. At the same time, Sravya was one of the pioneers building the international PhD community at the Institute of Molecular Genetics of the ASCR, where she's currently one of the two elected student representatives.

Sravya has been a curious and determined student who can work independently while being able to discuss and justify her experimental design and decisions which directions to pursue. She came well trained in a number of methods and quickly adopted necessary background and skills for her PhD project, which aimed at annotating and analyzing long non-coding RNAs in mouse oocytes and early embryos. This was a major task, considering that there was essentially no annotation of this class of RNA and the starting material was high-throughput RNA sequencing in a form of 65 nucleotide paired-end reads from Fugaku Aoki, our collaborator from Japan. Transcriptome assembly and lncRNA annotation was assisted by four collaborating bioinformaticians who were scripting and executing the assembly and annotation pipeline. In this work, Sravya made a major contribution in (1) deciding, which transcript model assembly algorithm was finally chosen and (2) testing countless transcript models in order to identify artifacts, which would be eliminated from ty annotation by adding and optimizing specific filtering steps in annotation pipeline. This work yielded a comprehensive publication *Long non-coding RNA exchange during the oocyte-to-embryo transition in mice* in DNA Research, 2017, in which is Sravya the first shared author. Sravya's contribution in this work went beyond working with bioinformaticians on transcript annotation and bioinformatic characterization of their structure and expression. She also produced and characterized two lncRNA knock-outs in mice using CRISPR/Cas9 technology. The annotation and initial characterization brought several significant discoveries, which laid foundations for subsequent research, which included production of five different mouse models using CRISPR/Cas9 technology. For the later work were important three key discoveries made during lncRNA annotation:

1) Long Terminal Repeats (LTRs) from a rodent class of LTR retrotransposons contribute frequently promoters and first exons to lncRNA, hence could be a major driving force in lncRNA evolution. Consequently, analysis of lncRNA evolution and the role of LTRs then became a part of a large study on rodent LTR retrotransposons *Long terminal repeats power evolution of genes and gene expression programs in mammalian oocytes and zygotes*, which was published in Genome Research in 2017.

2) Some maternal lncRNAs carry antisense pseudogene sequences and serve as substrates for biogenesis of siRNAs in the endogenous RNAi pathway in mouse oocytes. Existence of such transcripts was predicted based on previous small RNA analysis. Sravya's work identified structure and evolution of such transcripts. For two of them, she prepared mouse knock-out models and demonstrated that these lncRNA indeed function in RNAi.

3) Some maternal lncRNAs apparently undergo cytoplasmic polyadenylation. One of such lncRNAs was selected for further analysis and was confirmed to be a dormant maternal lncRNA. This result is significant because this type of regulation of maternal RNAs has been exclusively associated with translational control of maternal mRNAs. Sravya's work shows that lncRNAs may adopt such post-transcriptional regulations despite they lack protein coding capacity.

Sravya presented her work at a number of conferences at posters or by talks and, notably, she received a poster award at the EMBO Mobile Genome conference in 2017. She now works towards completing remaining analyses and publishing her functional analyses of two remaining lncRNAs, which will hopefully take place in 2019 before she moves at a postdoctoral position abroad.

From methodological perspective, she learned how to use CRISPR/Cas9 technology and work with knock-out models in mice and cell culture. She is learned methods of molecular cloning and gene expression analysis, including production of libraries for RNA-seq from mouse oocytes. Her understanding of bioinformatic analysis of RNA-seq data is sufficient for collaborating with bioinformaticians on data analysis and data mining. She can work with embryonic stem cells and is familiar with FACS and confocal microscopy.

Sravya wrote her thesis under my supervision but alone. I'll leave picking up errors to reviewers. Here, I can't help but add a note that her writing process was within the framework of other frantically finished theses where the hectic work grew as an inverse function of the time remaining to the deadline. While Sravya did not reach the bar set up by legendary Radek Jankele, she could consider herself a worthy contender. In any case, I think the thesis is well-written and the outcome is nicely compact and coherent and could serve as an example of how a good thesis could look like.

Conclusions

The presented dissertation thesis fulfills requirements for its defense. Sravya Ganesh demonstrated that she has mastered research methods and acquired necessary theoretical knowledge for her future research. Therefore, I recommend hereby that she would be allowed to defend her dissertation thesis and, if successful, she would receive the PhD. degree.

Student demonstrated creative skills – YES

The work fulfills requirements for dissertation thesis in the respective field - YES

Závěr

Předložená disertační práce splňuje podmínky stanovené pro její obhajobu. Sravya Ganesh prokázala, že ovládá vědecké metody a má i potřebné teoretické znalosti pro budoucí vědeckou práci v příslušném vědním oboru. Z výše uvedených důvodů tedy doporučuji, aby byla jmenovaná připuštěna k obhajobě své doktorandské disertační práce a byl jí, v případě jejího kladného výsledku, udělen titul PhD.

Studentka prokázala tvůrčí schopnosti – ANO

Práce splňuje požadavky kladené na disertační práci v daném oboru – ANO

V Praze, dne 4.12. 2018

Petr Svoboda, školitel