Brno, 26.11. 2018

Evaluation report on the PhD thesis of Sravya

I have critically and thoroughly read the Ph.D, thesis “Long Non-Coding RNAs in Oocyte-to-Embryo Transition", submitted by Ms. Sravya Ganesh and supervised by Prof. Petr Svoboda at the Institute of Molecular Genetics of the Czech Academy of Sciences.

The RNA field has witnessed a dramatic boom in great part thanks to the technical advancements that allowed identification of completely new classes of RNAs. Among those, long noncoding RNAs (lncRNAs) became a "hot topic" mainly thanks to the identification of their regulatory potential at the epitranscriptome level. Many groups have tackled the question of specific functions of lncRNAs in different tissues, diseases etc. Sravya Ganesh PhD project had yet another rather ambitious goal. She aimed to identify, annotate and functionally analyze lncRNAs during oocyte-early embryo transition. And she was successful in most of her aims outlined at the beginning of the thesis.

The thesis is very well and clearly written and I enjoyed reading all its parts. It consists of 20 pages long relevant Introduction, three pages of Methods, 28 pages of Results, followed by Discussion, Conclusions, References, Supplementary Material and Published works. She included the following published works; an invited first-author review on retrotransposon-associated lncRNAs published in Pflugers Archives, a shared first-author paper published in DNA Research and second-author paper published in Genome Research. Already this list of works is sufficient to promote the applicant for the PhD title. Eventhough, Sravya could have just used the text from her review, she wrote a brand new text for the introduction, where she very competently and reader friendly described the history and current knowledge about oocyte to zygote transition and the role of small and long noncoding RNAs and retrotransposons in this process. The results are split to four chapters based on the initial aims of the thesis. They mostly cover the findings that were published in the two other above mentioned research papers. Therefore they underwent a strict review process already.

The thesis is written in a very good English. Since the thesis has been assembled as a text independent on the publications, I would welcome more detailed description of the methods, which would be helpful for the next members of the Svoboda group. For instance, not many readers are familiar with the induction of superovulation, or monitoring whether the collected oocytes are useful for subsequent experiments.It would have been informative to mention the RNA yields obtained from the collected oocytes, that were then used for RT-PCR analyses. The description of the bioinformatics approaches

are completely missing. They are described to some extent in the Results part, but at least a workflow in the Methods with appropriate references to the software used or scripts links would have been useful. In addition, Sravya could mention during her thesis defense what was her particular contribution to the two included published works.

I have got few questions.

1. How do you define transcription start sites for lncRNAs? Did you validate the presence of Pol II promoter elements upstream of annotated 5' lncRNA ends? Is there any difference compare to coding gene transcription start sites?
2. During the library preparation, what was the frequency of enrichment of RNAs with internal polyA stretches?
3. Sravya found, that oocyte-expressed lncRNAs were also identified in sequencing datasets from testis. Does this imply, that they are expressed in spermatocytes? Could these lncRNAs be linked to meiosis?
4. In a group of lncRNAs, they identify the cytoplasmic polyadenylation element. Can Sravya elude a little bit more on the possible role(s) of readenylation of lncRNAs?
5. In the Part 4 of the Results, it is mentioned that expression of the two well-studied lncRNAs MALAT1 and NEAT1 was detected only in the samples after ZGA. However, both lncRNAs undergo an atypical 3' end processing. MALAT1 contains encoded nonhomogeneous poly(A) 3' end. Is this sufficient to be enriched by the protocol used to prepare the libraries? Similarly for NEAT1-1 long isoform. From the Figure 20B, it is not possible to read whether the reads end upstream of the MASC RNA region (the downstream cleavage product of RNAseP MALAT1 processing). Can Sravya discuss the analysis of MALAT1 and NEAT a bit more during the discussion after the thesis defense?

I have had several opportunities to see Sravya presenting and communicating her research and I was since the first encounter very impressed by the maturity and dedication with which she is approaching her work. The submitted thesis is of a high quality. Therefore, I strongly support Sravya for the defense of her PhD thesis.

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