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Report on the PhD thesis of Sravya GANESH entitled
"Long Non-Coding RNAs in Oocyte-to-Embryo Transition"

The mammalian oocyte-to-embryo transition (OET) is one of the most complex developmental processes that requires precise coordination. While the mechanisms of OET have been extensively studied, the regulation of OET by noncoding RNAs remains elusive. Expression of a large number of long noncoding RNAs (lncRNAs) in the germline raises the possibility that lncRNAs may contribute to OET regulation.

The main goal of the study, carried out by Sravya Ganesh in the Svoboda laboratory, was to define the expression and potential biological functions of lncRNAs during mouse oocyte development and oocyte-to-embryo transition (OET). As virtually nothing was known at the beginning of the project about lncRNA content, expression or functions in the mammalian oocyte development, Sravya and colleagues first identified and annotated lncRNAs using RNA-seq data sets from three different mouse OET transition stages. Sravya contributed to optimization of the pipeline for lncRNA identification and annotation and manually curated around 2000 lncRNAs that helped to overcome the shortcoming of the pipeline and further improved its accuracy. Based on these data, 1600 non-redundant lncRNA clusters were identified. The analysis of these so-called OET lncRNAs revealed around 1200 lncRNA clusters that are specific to OET stages. Their further characterization revealed the temporal and tissue-specific expression of lncRNA loci dividing OET lncRNAs into one of three categories: maternal (the majority of lncRNAs), zygotic and embryonic (the least represented category). Consistent with findings on other vertebrate lncRNAs, OET lncRNAs showed overall low expression levels, which may reflect their non-essential biological functions. In addition, several new and very intriguing findings were made by Sravya and colleagues by comparative analyses of OET lncRNAs: (1) the identification of the presence of cytoplasmic polyadenylation

elements (CPE) in OET lncRNAs (resembling dormant meternal RNAs) and the potential implication of the cytoplasmic polyadenylation mechanisms in the regulation of maternal lncRNAs, and (2) the identification of siRNA-producing maternally-expressed lncRNA transcripts that might play a role during OET. Sravya identified antisense sequences produced from pseudogene-carrying lncRNA transcripts (over 100 lncRNAs that carry "processed" pseudogene sequences were identified in this study). These lncRNAs serve as a unique class of maternal RNAs that are substrates for the endo-siRNA machinery.

Another novel and exciting point of the study was the evolutionary analysis of OET lncRNAs. Sravya found that retrotransposons contribute to generation of novel OET lncRNAs as 1/3 of 5' exons of novel lncRNAs came from LTR transposons. Interestingly different types of transposons contributed differentially to lncRNA sequences: while SINEs and LINEs contribute to mature lncRNA sequences, LTR transposons make a strong contribution to lncRNA promoters and TSSs. Based on these analyses of lncRNA evolutions, Sravya concluded that transposons are important contributors to lncRNA sequence evolution and turnover.

Finally, one of the most challenging and exciting part of the study are the functional analyses of a selected set of OET lncRNAs performed by Sravya. Based on the expression, promoter sequence conservation and synteny, Sravya selected a set of maternal lncRNAs for detailed functional interrogations. By deleting lncRNA promoter along with the first exon with CRISPR-Cas9, Sravya generated three lncRNA knock-out mouse alleles, which she analyzed in detail. Interestingly, none of the lncRNA null alleles had an effect on mouse viability or fertility. However, detailed analyses of two lncRNA mutants revealed novel molecular mechanisms lncRNA may contribute to. One of the lncRNAs is a maternal transcript that provides substrate to the endo-RNAi machinery in mouse oocyte. The other lncRNA harbors a functional CPE sequence, the exact role of which will be further elucidated in the future.

In summary, the thesis represents a large body of work with carefully planned and performed experiments. It is written in a logical way and thus is easy and interesting to read and follow the progress of the projects. I believe the projects undertaken by the candidate were very promising and yielded exciting results that lead to a co-first author publication for Sravya published in the major journal "*DNA Research*". Moreover, Sravya is a second author on another paper published in "*Genome Research*", as well as a co-author of a review article. In addition, another manuscript on the functional analyses of one of the lncRNA mutants, which was driven by Sravya, is in preparation. Taken together, as this thesis is ready to be defended, I strongly support Sravya Ganesh to defend her PhD thesis for obtaining a doctoral degree.

Yours sincerely,
Alena Shkumatava