In Prague May 12th, 2022

Review on the PhD thesis of Mgr. Aleshkina Daria „Non-coding RNAs in oocyte and early embryo "

PhD thesis is based on three shortly commented publications accompanied with the introduction part, description of the thesis aims and discussion. Fourth output is an accepted manuscript, however out of the focus of PhD thesis. In total, the thesis is written on 40 pages and the layout is consistent with the general rules required by the Developmental and Cell Biology Board.

Introduction part is well written and fully covers the problematics of the structure and function of non-coding (ncRNA) and long-non-coding (lncRNAs) in the transcription, posttranscriptional control, and translation in somatic cells and mainly in mammalian oocyte and early embryos. The thesis focuses on three basic aims: 1) study of the regulatory function of neuronal BC1 ncRNA in the mammalian oocyte translation, 2) study of the spatial and temporal distribution of novel unannotated lncRNA Rose during oogenesis and early embryonic development and 3) introduction and verification of simplified scoring technique of RNA FISH (Fluorescent In Situ Hybridization) signals in the mammalian oocytes using only one equatorial Z stack instead of whole oocyte volume and detection of active translation sites of specific mRNA through the RNA-puro-PLA (proximity ligation assay) system.

In the first publication „ncRNA BC1 influences translation in the oocyte" printed in the journal RNA Biology (IF = 4,652) the author determined a novel function of BC1 ncRNA previously described as a translational repressor in dendrites in mammalian oocytes. Overexpression of BC1 ncRNA resulted in the inhibition of selected mRNA translation through the interplay with FMRP protein. The second publication "Oocyte specific lncRNA variant Rose influences oocyte and embryo development" published in Non-coding RNA Research (IF=5.978) was aimed on the role of previously unannotated lncRNA Rose in oocyte and early embryos. Interestingly, author observed alternatively spliced variants and their specific function in the germ cells and blastomeres. dsRNA approach to downregulate the lncRNA Rose transcription revealed the importance of this non-coding RNA in the regulation of protein synthesis from polysomic fraction. In the third paper" Single Molecule RNA Localization and Translation in the Mammalian Oocyte and Embryo" published in Journal of Molecular Biology (IF = 5.469) author described the introduction of improved RNA FISH technique coupled with the detection of active translation sites able to fluorescently detect even single molecule of mRNAs or ncRNAs in the mammalian oocyte and early embryos. In addition, simplified evaluation of the amount of RNA molecules in the oocyte from only equatorial Z stack will help to save the microscopic time and accelerate the scientific research in this dynamic and biomedicinally important field.

Discussion briefly and clearly summarizes these parts in submitted publications. Following chapter Conclusions concentrates the PhD thesis outputs in seven points. Finally, I would like to sum up that PhD thesis is written concisely and freshly without dispensable details which really help to understand the complex nomenclature and function of ncRNA

and lncRNA by a broader readership groups. I fully recommend the PhD thesis as a basis for awarding the PhD degree.

Questions:

1) Are there differences (if any) between the translational suppression mechanism of selected mRNAs in somatic cells (neurons) and germ cells (oocyte)?

2) How the "large" oocyte ensures the specific translational inhibition regarding a low concentration of inhibited mRNA and ncRNA? What is the usual concentration ratio of inhibited mRNA and ncRNA?

3) Is it possible to extrapolate your improved scoring approach based on only one Z stack to all ncRNAs? What is generally known about the uniformity of ncRNA distribution in mammalian oocytes?

Assoc.prof. RNDr. Ing. Vladimír Krylov, PhD.