Posudok dizertační práce Mgr. Denisy Jansové

In her Ph.D. thesis, Mgr. Denisa Jansová studies mRNA localisation and dynamics during mouse oocyte maturation and after fertilization and the regulation of their translation. Her research resulted in two first author publications, one published and one in preparation at the time of thesis submission, one publication as a second author and one review, as well as one oral presentation and four poster presentations at international and Czech conferences.

Denisa shows broad knowledge of the topic of her thesis. She references 185 relevant publications, mostly original manuscripts with minimum of reviews. Information within the literature review is appropriate for the research topics studied. The aims are clear and fulfilled by the presented results. The results, especially those that are part of Denisa's first author publications, are mostly observational and descriptive. Nevertheless, Denisa demonstrates strong expertise in broad range of mRNA and protein visualisation techniques and presents the obtained results in a clear manner. Her results contribute to understanding of mRNA and protein localisation within the complex cell of mouse oocyte. They highlight the importance of specific localisation of particular mRNAs, as well as proteins of translational apparatus, for precisely regulated translation in time and space, crucial for correct oocyte maturation – a topic that only recently started to be uncovered in mammalian oocytes.

The thesis satisfies the formal criteria. Information is correctly referenced, including the figures. Figures are generally well designed with clear and easy-to-understand legends.

I have few comments regarding the text of the introduction:

1. The introduction might benefit from a brief explanation of the specific features of mRNAs with TOP motifs limiting their translation under normal conditions, i.e. why they require phosphorylation of 4EBP1 inactivating its repressing activity on eIF4E, as initiation of translation of other mRNAs also depends on eIF4E.

2. Section 1.2, page 7 – The author states that growing oocytes are so-called NSN type (non-surrounded nucleolus) with decondensed chromatin configuration while fully grown oocytes are SN type (surrounded nucleolus) with condensed chromatin. However, I believe that fully grown oocytes can be both NSN and SN types (this is for example supported by the publication Debey et al. 1993 referenced by the author), therefore, the status of being fully grown in size does not determine the chromatin configuration in the oocyte.

3. Section 1.4, page 10 – This section describes localisation of mRNAs within oocytes of invertebrate and non-mammalian vertebrate species and its important role in embryonic patterning, but does not clearly state that no localisation patterns important for early embryonic development have been found in the mammalian oocytes (and potentially discuss the reports claiming the opposite).

4. Section 1.4, page 10 – The author correctly describes Cdx2 as maternally provided factor essential for correct trophoectoderm specification. Nevertheless, one has to keep in mind that both maternal and zygotic Cdx2 are needed – maternally provided Cdx2 is not sufficient.

In addition, I have few comments regarding the formal side of the thesis pointing out minor issues that could be improved:

1. Some sentences are not clear. For example, last paragraph on page 20 starts with the sentence „mTOR regulates transcript with TOP motif“ – better wording would be for example „mTOR regulates translation initiation of transcripts with TOP motif“. Another example is the first sentence on page 21 „This experiments confirm that downregulation of mTOR-eIF4F translation in the oocyte does not influence the overall translational pattern, but suppress protein synthesis“ – better wording might be for example „These experiments confirm that downregulation of mTOR-eIF4F translation in the oocyte does not influence the overall translational pattern, but suppresses the synthesis of specific proteins.“

2. Some figure legends are below and some above the respective figures – all figure legends should be below the respective figures.

3. Regarding the figures in the manuscript in preparation - statistical significance is correctly denoted by asterisk in graphs. However, the legends always explain only the p-value marked by one asterisk and information about p-values for two or three asterisks is missing (figures 4 and 5).

4. Scientific names of species (in latin) are correctly written with upper-case letter for genus name and lower-case letter for species name. For example, on page 9, *Xenopus laevis* is written with both capital letters.

5. Manuscript by Flemr and Svoboda 2011 is referenced in the text, but is not present in the list of references.

Questions for the author:

1. In the chronologically first publication (Susor et al. 2015) you show that oocyte nucleus retains a pool of mRNAs, and that there is a translational hot-spot at the vicinity of chromosomes post NEBD separated to some extent from the rest of cytoplasm. This suggests that nuclear mRNA pool might be translated in this hotspot after nuclear membrane disappears. Have you ever tried exploring whether the same mRNAs that are inside the nucleus prior to NEBD are then translated at the vicinity of chromosomes post NEBD, for example by visualisation of gene-specific mRNAs before and after NEBD? Would it be feasible?

2. Regarding the Susor et al. 2015 manuscript, have you considered exploring the pool of mRNAs with mTOR-regulated translation post NEBD by high throughput techniques such as RNA-seq of ribosome-bound mRNAs or potentially mass-spectrometry, if sensitive enough, in control vs mTOR-inhibited oocytes?

3. In the third manuscript (Jansova et al. 2017), you demonstrate that 4EBP1 phosphorylation is regulated by mTOR and not by PLK1 in mouse oocytes. There is a recent publication (published after the submission of this thesis) from Severance and Latham in Am J Physiol Cell Physiol (title PLK1 regulates spindle association of phosphorylated eukaryotic translation initiation factor 4E binding protein, and spindle function in mouse oocytes) showing contradictory results that PLK1 regulates 4EBP1 phosphorylation, while they observe no effect of mTOR pathway. Can you comment on these differences between yours and their findings?

4. What would you emphasize as the most interesting or surprising findings in your manuscript in preparation?

5. In the manuscript in preparation, you categorise 4EBP1 as RNA-binding protein, although classically it is known as a repressor binding to eIF4E protein. Is it known that this protein has RNA-binding activity?

6. Why did you choose *Dazl*, *beta-actin* and *Neat1* as example RNAs to be visualised by smRNA FISH in the oocytes and embryos in your manuscript in preparation? Are you planning to visualise more RNAs, or capturing more timepoints during the oocyte maturation?

7. The last section of discussion discussing the future plans and unanswered questions is quite short. Could you provide more suggestions about what remains to be elucidated and how it could potentially be achieved?

I recommend the thesis for defence.

In České Budějovice, 17.8.2017 Mgr. Lenka Gahurová, Ph.D.