LABORATORY OF REGULATION
OF GENE EXPRESSION

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Evaluation Report for the Ph.D. award

Name of the Student: **MSc. Edgar del Llano**

Title of Thesis**: Age-related differences in the translational landscape of mammalian oocytes**

Name of the Supervisor: **Ing. Michael Kubelka, CSc.**

Dear chair, dear committee members,

Here I submit to your hands the evaluation report on the dissertation work of MSc. Edgar del Llano for the award of Degree of Doctor of Philosophy (Ph.D.).

MSc. del Llano's work is based on three accepted papers and one manuscript that is currently under review in Aging Cell. Out of these studies, only the latter features Edgar as the first author. The most recently accepted paper (2020) in Int. J. Mol. Sci. features Edgar as the second author with a significant contribution, whereas his contribution to the preceding two articles seems to be relatively minor but still counts, indeed.

As the title states, Edgar's work deals with several aspects of the development of oocytes and their aging from the perspective of translational control and claims that post the NEBD stage, transcriptional silent oocytes from young *vs.* aged mouse females start to manifest protein expression differences in the gene-specific manner that could contribute to the increasing rate of aneuploidy known to be on the rise in aged animals. Altogether three aims with several sub-aims are listed that are, as far as I can tell, all diligently fulfilled.

This thesis is written in a shortened format in good English and provides the reader with a broad overview of the entire problematic, as well as with an easy-to-follow synopsis of all relevant publications. Perhaps, the Introduction part is too much focused on the oocyte development and could have provided a broader overview of the translational control mechanisms. The placement of the chapter 1.4 on Polysome profile analysis is unfortunate as it breaks the flow – it should have been at the end. The discussion is very thorough, no objections there. I noticed that some references are missing in the list (like Flemr et al. 2020) but it is certainly not a big issue.

I tried to focus my review mainly on the paper under review bearing the same title as this thesis since it obviously forms the basis of Edgar's thesis. My specific questions are shown below:

- Page 13; the author claims that during meiotic progression, after NEBD, eIF4E-BP1 becomes hyperphosphorylated, which should unblock cap-dependent translation but, "surprisingly", it does not. My understanding has always been that cessation of cap-dependent translation accompanies mitosis/meiosis and other forms of cap-independent mechanisms take over to fulfill the cell's needs for protein expression. These often allow synthesis of only a specific subset of proteins (but I admit that I have not followed this particular field for years, so my knowledge might be outdated). Nonetheless, since the author further claims that this ostensible paradox most likely means that only specific mRNAs are made via otherwise attenuated cap-dependent translation during meiosis, I was wondering if he has investigated whether other means of cap-independent translation could have been involved too (or instead), as my hazed memory recalls.

- As for the SSP-profiling, it is certainly a great achievement, however, I think that a more fine separation of the gradients should be aimed for; ideally all fractions should be always analyzed with qRT-PCR-based quantification of rRNAs to obtain a real profile. This would be a lot more informative than just showing pooled NP and P fractions, like those shown in Fig. 1A of the unpublished story. The author claims that there are no real differences between YF and AF but I do not think that he is entitled to make such a claim. What if one group (the one with faster meiosis – AF) has a higher proportion of heavy polysomes compared to YF, where light polysomes would be enriched? This would indicate a less severe block of general translation in AF vs. YF, which could correlate with a faster progression of meiosis in AF. Therefore, at the minimum, the P fraction should be divided into light and heavy polysomes (LP vs. HP). The changes in distribution between LP vs. HP would be, in my opinion, more relevant to what he wishes to study because 1) the source of the monosome peak (featuring in your NP) is always unclear and 2) an mRNA association with polysomes does not necessarily tell that it is actively translated. The most reliable indicator of a real change in the expression of a particular mRNA is, in my opinion, a shift from LP to HP or vice versa. What do

you think? Do you think this modification if applied would change the outcome of your analysis in YF vs. AF samples shown in Fig. 1B-D?

- Fig. 1B, on a similar note, I would not call these as differently translated (page 2, beginning of the last paragraph of the unpublished story) but differently ribosome-occupied, unless you provide more compelling evidence as hinted above. Can you think of any other way how to achieve this distinction on a translatome-wide scale?

- As for the author's manipulations with protein levels/activity of two of his candidates (CASTOR1 and SGK1), it is impressive, yet I would be more reserved with implicating them in age-related chromosomal aneuploidy. The author's analysis suggests that it is a very complex process involving age-specific sets of numerous proteins acting together as whole either in YF or AF. Changing expression/activity of one of them to mimic the expression/activity in the other age-specific set may lead to various non-specific artefacts. A lot more candidates should be tested with plentiful of negative controls (for example genes that are expressed during meiosis but should not affect chromosome segregation) in order to identify the key players in this problem. Can you design a complex study that could provide a more definitive answer to this issue? What are you future plans, anyway?

Taken together, this thesis represents a rather large amount of the quality work of this PhD candidate and clearly demonstrates his experimental, as well as intellectual skills that are required to obtain a Ph.D. degree. Unfortunately, the regulations of the Branch Board of the Developmental and Cellular Biology dictate that at least one first-authored publication must be accepted by some journal for the applicant to comply formally with all obligations that are required to obtain a Ph.D. degree, which is not the case at the time when this review is being written. To conclude, I gladly recommend acceptance of this PhD. work but only under the condition specified above.

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