



OPPONENT'S REVIEW ON DIPLOMA THESIS

Name of the student: Bc. Pavlína Černá

Thesis title: mTOR-regulated translation during first the wave of inner cell generation in mouse preimplantation embryos

Supervisor: doc. Alexander W. Bruce, Ph.D.

Referee: MVDr. Dušan Fabian, DrSc.

Referee's affiliation: Centre of Biosciences, Slovak Academy of Sciences, Šoltésovej 4, Košice, Slovakia

Comments of the reviewer on the student and the thesis:

The thesis presents original research results in the area of developmental biology.

It has been shown that mTOR kinase has important role in maturation of oocytes and 8- to 16-cells stage transition. However, mechanisms of its action are largely unknown. Thus, the aim of the thesis of Pavlína Černá was to elucidate possible mechanisms of mTOR involvement in generation of inner cells in preimplantation embryos. Results have shown that *in vitro* culture of mouse embryos in the presence of the mTORC1 inhibitor Rapamycin during the 8- to 16-cell transition significantly impaired the generation of inner blastomeres. Similar phenotypes were obtained when other mTOR-related pathways (i.e. AMPK, p38-MAPK, AKT, RSK and MEK1/2) were targeted. Moreover, clonal overexpression of a mTORC1 substrate (a recombinant non-phosphorylatable 4EBP1 mutant) also impaired 16-cell stage inner blastomere generation, implicating the phosphorylation of 4EBP1 is an important mechanistic component regulating the generation inner (and outer) blastomeres in the 16-cell stage embryo.

The aims of the thesis were clearly stated and all of them were fulfilled.

The thesis fulfills all the requirements and expectations. The extent is sufficient and separate parts are balanced. Text follows a logical structure. Author demonstrated very good understanding of problematics. The references are recent. I appreciate rich graphics supplementation and clear definition of all tested hypotheses including expected results. This provides high level of readability and understanding even for readers unexperienced in molecular biology. The outcomes of experiments are systematically discussed. Author suggests reasonable further experiments as well.

From the formal point of view, language and stylistics are very good, grammatical or spelling mistakes are only very occasional.

Suggestions and questions, to which the student has to answer during the defense:

1. On p. 8 and 12 author claims that "the salt and pepper pattern" of primitive endoderm (PrE) and epiblast (EPI) progenitors at the mid-blastocyst stage sorts into the distinct late blastocyst tissue layers via a combination of active cell movement and apoptosis of inappropriately positioned cells. Was this hypothesis supported by some experimental studies, e.g. evaluating co-localization of PrE/EPI markers and apoptotic cells?

2. On p. 73 author claims that Rapamycin (unlike Torin1) selectively targets mTOR as part of the mTORC1 and not mTORC2 complexes. On the other hand, on p. 19 refers to previous studies claiming that in oocytes, Rapamycin reduces mTOR expression (both at the mRNA and protein levels), blocks polar body extrusion and reduces mRNA expression of the small GTPases genes (Lee et al., 2012) which is known to be mediated via mTORC2 (Zeng et al., 2007). Can you define inhibitory specificity of Rapamycin and Torin 1 in relation to mTOR, mTORC1 and mTORC2 complexes?

3. Immuno-fluorescence staining showed cytoplasmic and nuclear localization of HA-4EBP1 protein in progeny of mRNA microinjected blastomere of the 2-cell stage embryos (Fig. 22). However, in the case of non-phosphorylatable mutant HA-4EBP1-4Ala, nuclear localization of protein was apparently reduced (Fig. 23). Do you have explanation for this?

Mistakes, which the student should avoid in the future:

- In thesis, time of embryo recovery and embryonic stage are usually described as E1.5, E2.5, E3.0+5 hours, etc. When using such description, embryonic day 1 should be specified in the text. Alternatively, hours post hCG (or hours post supposed ovulation in non-stimulated animals) would be stated. This allows easier comparison of outcomes between similar studies.
- Data in supplementary tables suggest that when 16-cells stage embryos were evaluated, author did not exclude 14, 15, 17 and 18-cells embryos from the evaluation process. Since mouse embryo cleavage is highly asymmetric, such approach is acceptable. However, this fact should be stated in Methods section.
- The main reason for microinjection of the 2-cell embryos instead of pronuclear stage zygotes should be explained in Methods section as well.
- In the thesis, following p-value significance cut-offs were employed: ≥ 0.05 – not significant, <0.05 – significant (*), ≤ 0.005 – very significant (**) and ≤ 0.0005 – extremely significant (***). In similar scientific studies, more usual levels for very significant and extremely significant differences are < 0.01 and < 0.001 . Did author adjust P values via some specific correction (e.g. Bonferroni) or was there other reason for this adjustment?
- When there is a parenthetical phrase within an already parenthetical phrase, it is better to use square brackets for better understanding of the sentence.
- Abbreviation CTCF should be explained in the text.

Conclusion:

In conclusion, I **recommend** the thesis for the defense and I suggest the **grade 1 (excellent)**.

In Košice 12.05.2022

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MVDr. Dušan Fabian, DrSc.