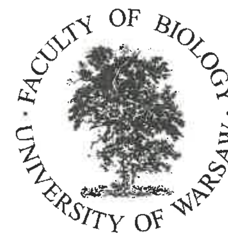
UNIVERSITY
OF WARSAW

Faculty of Biology
Institute of Developmental Biology and Biomedical Sciences
Anna Ajduk, PhD, DSc



Warsaw, January 11th, 2021

Evaluation of Miriama Peklanska's MSc Thesis

The main goal of Miriama Peklanska's MSc thesis was to utilize CRISPR/Cas9 system to create mouse embryos expressing fluorescently tagged MAPK13, MAPK14 and WWC2 proteins, and to examine the localization of these proteins throughout early embryonic development. The task was laborious and ambitious, so it is praiseworthy that Ms Peklanska managed to execute most of it. It is very likely that, if not for the pandemic situation, she would be able to complete all her scientific goals.

The thesis is divided into 6 main chapters: Introduction, Aim, Materials and Methods, Results, Discussion and Conclusion. Those chapters are accompanied by a very useful abbreviation glossary, a list of references, and an appendix with plasmid maps. They are also richly illustrated with figures and tables. The thesis is concise and well written. I have spotted few editorial errors and typos, but overall it is also very carefully edited. My comments on the main thesis chapters are listed below.

1) Comments on Introduction.

The introduction describes, in most cases in a sufficiently detailed way, preimplantation development of mouse embryos and the general mechanism utilized in CRISPR/Cas9 genome editing system. It introduces all terms and processes necessary to understand the subsequent parts of the thesis. I especially appreciate that Ms Peklanska clearly summarized our knowledge regarding the potential developmental role of MAPK13, MAPK 14 and WWC2 proteins, and thus convincingly supported the goal of her thesis. The introduction is illustrated with numerous figures, and I only regret that Ms Peklanska decided to 'copy-paste' figures from other published papers (she clearly indicated the source in the figure legends) instead of preparing them herself. I do realize that this is a common practice among students, but if the figures were prepared specifically to this

Ilji Miecznikowa 1, 02-096 Warsaw, POLAND
tel.: +48 22 55 41 212, fax: +48 22 55 41 210
e-mail: aajduk@biol.uw.edu.pl
http://www.biol.uw.edu.pl

thesis, they would fit the text much better and the author would avoid their redundancy (e.g. Fig 2 content is repeated in Fig. 3).

My detailed criticism/questions:

- p. 3 - Description of oogenesis is a bit too short and general. The author omits the growth phase of oocytes, crucial for their further development.
- p. 3 - Ms Peklanska wrote 'Such maternal factors exclusively control and drive zygote development until the end of the 1-cell stage after which, the zygotic genome becomes transcriptionally activated (ZGA).' This sentence suggests that the ZGA takes place after the 1st embryonic division, which is not entirely true. Minor ZGA occurs at the G2 stage of the 1-cell stage embryo.
- p. 4 – When the author describes different mechanisms leading to the formation of an inner and outer pool of blastomeres, she omits some recent studies regarding the role of cell contractility and cortical tension that would be good to mention, as they nicely describe the mechanism of apolar blastomere internalization (e.g. Samarage et al., 2015, DOI: 10.1016/j.devcel.2015.07.004; Maitre et al., 2016, DOI: 10.1038/nature18958).
- p. 6-7 – Ms Peklanska discusses here the regulative development of mammalian embryos. Unfortunately, the issue is not as simple and unequivocal, as she wrote. According to our current knowledge, 2-cell stage blastomeres are not equivalent (see Casser et al., 2019 – DOI: 10.1093/molehr/gaz051 and papers cited therein) and although some of them may be indeed totipotent, most likely not all of them are (in the sense that not all of them are able to form on their own the whole new organism). There are also numerous data showing that 4-cell stage blastomeres are already different from each other and may have different developmental potency (e.g. White et al., 2016, DOI: 10.1016/j.cell.2016.02.032; Plachta et al., 2011, DOI: 10.1038/ncb2154; Torres-Padilla et al., 2007, DOI: 10.1038/nature05458; Goolam et al., 2016, DOI: 10.1016/j.cell.2016.01.047). Therefore the claim that 8-cell stage blastomeres are totipotent and functionally equivalent is not supported by our current knowledge. Finally, the experiments with blastomeres' disaggregation and re-aggregation have their limitations – see paper by Piotrowska-Nitsche et al., 2005 (DOI: 10.1242/dev.01602).

2) Comments on Thesis' Aims.

The aims are clearly specified and well supported by the literature data presented in the introduction. I like the idea of the project flow diagram very much, unfortunately, its last part is lacking – probably due to some editing error.

Ilji Miecznikowa 1, 02-096 Warsaw, POLAND
tel.: +48 22 55 41 212, fax: +48 22 55 41 210
e-mail: aaduk@biol.uw.edu.pl
http://www.biol.uw.edu.pl

3) Comments on Materials and Methods.

The experimental procedures are described meticulously. I have a feeling that I could easily repeat the procedures just following the instructions included in the thesis. I also really like that the author presents all reaction conditions and reaction mixes' compositions in tables. It makes the text more accessible.

My detailed criticism/questions:

- Table legends – µl, µg, etc. – these are well-known unit abbreviations, they do not need explanation in table legends.
- p. 36 – Why was the additional polyadenylation step required? Was the standard polyadenylation acquired during the IVT reaction not sufficient?
- p. 36 – What was glyoxal concentration?
- p. 39 – Has the author tried visualising embryos w/o fixation? Or using an anti-mCherry antibody to intensify the tag signal after fixation? Fixation may sometimes affect the fluorescent signal from proteins.

4) Comments on Results

The results are clearly and convincingly presented and richly illustrated. The amount of work done by Ms Peklanska is admirable. It is a pity that the Covid 19 pandemic did not permit her to finish all the experiments. I also really appreciate that the author clearly indicates which parts of the experiments were conducted by others.

My detailed criticism/questions:

- The gel images are not labelled well enough. The key bands/specific products should be indicated with arrows, asterisks, etc. It is especially important in Fig. 30, where I can see only continuous smears. Also, the size of ladder bands, at least these the most useful for gel interpretation, should be labelled directly in the figure, not – as it is done now – listed in the legend.
- Fig. 11B – It seems to me that the green arrow should indicate lane 1, not 2, as this is the only lane with 1.1kb product.
- p. 53 – I am not an expert here, but I wonder whether biotinylation could be responsible for the failure of the 2nd round of BIO-PCR of *Mapk14* homology arm. Why did the author use BIO-PCR instead of a normal PCR to separate these 2 products?

5) Comments on Discussion

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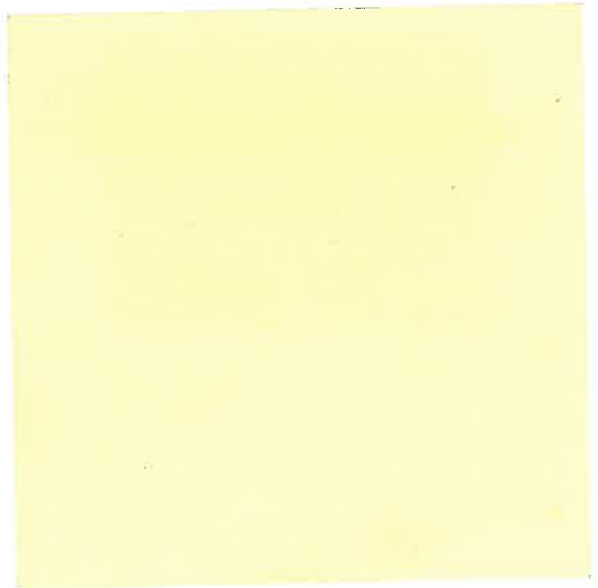
The Discussion is very concise and carried out from an interesting angle. It was a good idea to focus it on the potential usefulness of the CRISPR/Cas9-derived mCherry reporters in the examination of mouse embryonic development. I only lack some explanation of the potential MAPK13 localization. The protein is accumulated in a form of foci. Could they correspond to MTOCs/centrosomes? Are there any data - possibly regarding other cell types - to explain this kind of localization?

6) Comments on Conclusion

Ms Peklanska summarized and concluded her thesis well.

In summary, I really enjoyed reading Ms Peklanska's thesis. I think it is well written and shows an impressive amount of molecular biology work conducted by the student. Despite my criticism listed above – which was generally rather minor – I have an impression that Ms Peklanska understands well both practical and theoretical aspects of her thesis' subject. **I grade this thesis as very good (1).**



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