Dr Berenika Plusa
Manchester Fellow
Michael Smith Building
Faculty of Biology, Medicine and Health
Oxford Road
MANCHESTER
M13 9PT
Tel: 0161 275 1563
Email: berenika.plusa@manchester.ac.uk

PhD viva report for Vasanth Thamodaran thesis: **The role of p38-Mapk14/11 as an enabler of primitive endoderm (PrE) maturation and as a sensor of metabolism during mouse preimplantation embryo development.**

The PhD candidate Vasanth Thamodaran undertook a challenging project, aiming to establish the role of p38-mitogen activated protein kinase (p38-Mapk) in the specification of two lineages within inner cell mass (ICM) of mouse blastocyst - the primitive endoderm (PrE) and the epiblast (EPI). Although the role of p38-Mapk in early mammalian development was previously studied, previous investigations focused solely on the first developmental event, namely the specification of the trophectoderm (TE) vs. ICM. Later developmental events during preimplantation development have not been investigated thus far in this regards. The PhD candidate took advantage of an existing small chemical compound inhibitor (SB220025) to specifically target the kinase activity of p38α/Mapk14 and p38β/Mapk11 during ICM lineage specification (E3.5 till E4.5 day of development). Vasanth discovered that p38Mapk14/11 inhibition severely affects ICM lineage specification. Specifically, the number of Gata4-positive PrE cells was greatly reduced, alongside with the significant increase in the number of uncommitted ICM cells, expressing markers of both PrE and EPI lineage. The PhD candidate also established that p38-Mapk acts downstream of Fgf signalling and Bmp-ligand activated kinase, Tak1. In addition, Vasanth also investigated whether the effect of p38Mapk14/11 inhibition may be modulated by the presence or absence of exogenously supplemented amino acids in the culture media. Part of the data presented in the thesis contributed to the published manuscript, of which Vasanth Thamodaran is the first author.

The PhD thesis of Vasanth Thamodaran is that of a good quality and represents a significant contribution to the early mammalian development field. The thesis contains all the necessary parts including Summary, Introduction, Material and Methods, the Results section and general Discussion at the end. In majority of thext, the transitions between different parts of the PhD candidate's thesis are good and logical.

Information included in the Introduction is relevant to the topic, accurate, well interpreted and in most of the cases correctly referenced. Aims are introduced and presented in a clear way.

The Material and Methods section contains a description of all experimental techniques as well as the mouse and cell lines used in experiments described in the thesis.

Experiments described in the Result section were well planned and performed, included very meticulous control experiments and were then carefully discussed in the Discussion section. The PhD candidate's own opinions are clearly stated and well presented within the text. Vasanth made a good use of diagrams and tables.

In summary the thesis meets all the requirements from the Faculty of Science, University of South Bohemia.

Although I believe that the submitted thesis is sufficient to grant PhD and no major changes to the thesis are necessary, there are several issues that need to be clarified during examination. Below, I list my comments and problems that need clarification.

1. Are ICM cells totipotent or pluripotent? How can we test the potency of ICM cells?
2. What is Nanog and Gata6 expression pattern? How Gata+/Nanog+ cells arise in embryo?

3. What is known about the differences in metabolic processes/requirements between naïve and prime pluripotent stem cells?
4. Was any experiment performed to confirm that the Fgf addition affects pERK1/2 expression (confirmed by immunostaining)? How many embryos were analysed in Fig 12?
5. In your opinion, is p38Mapk14/11 inhibition more affecting the initiation of PrE or of EPI cell fate? Your figure 16th seems to suggest the latter, whereas figure 10 points towards the PrE.
6. How many embryos were used in experiments with p38Mapk14/11 inhibition followed by pNanog immunostaining?

Berenika Plusa 4 December 2016

Institute of Animal Physiology and Genetics CAS, v. v. i.
Rumburská 89, 277 21 Liběchov, Czech Republic

tel.: +420 315 639 532; fax: +420 315 639 510
E-mail: uzfg@iapg.cas.cz; URL http://www.iapg.cas.cz

The role of p38-Mapk14/11 as an enabler of primitive endoderm (PrE) maturation and as a sensor of metabolism during mouse preimplantation embryo development

Author of the thesis Vasanth Thamodaran studied the role of the Mitogen activated protein kinase 14, in the preimplantation development of mouse embryo. Moreover, PhD candidate documented the influence of the exogenously supplemented Amino acids on development of primitive endoderm cell-fate.
During five years of post gradual study, the PhD candidate generated data published in two peer reviewed international journals. In his first paper published in the Scientific Reports journal he performed experiments with the first author of the study. Recently, he published a comprehensive first author paper in the Open Biology journal, where the candidate performed experiments and together with the supervisor designed the experiments, participated in data analysis and drafted the manuscript. In addition, he generated data, which I believe will be soon published. All presented data are narrowly focused to the topic of the thesis.
The thesis is well written, data were obtained by scientifically appropriate methods, command of relevant literature and interpreting biologically relevant conclusions. Overall, the thesis meets all requirements from the Faculty of Science, University of South Bohemia.

A few aspects of the thesis do leave some scope for improvement. These issues primarily relate to:

- Despite that the study fulfills the requirements of the scientific journal I am missing the validation of used inhibitors. We know that inhibitors might be variable in quality from batch to batch. To see, if inhibitor influences MAPK14 activity, or its well-defined substrate would be beneficial for the study.
- The thesis also presents unpublished data, unfortunately the PhD candidate doesn't mention future direction/experiments or when the manuscript will be submitted.

I have few questions related to the study:

1) How early the fate of the blastomere becomes predetermined in the mammalian embryo?
2) The author mentions problematic 2-cell embryo stage in the hamster. Why this stage of early embryogenesis is problematic?
3) It is known that mTOR is responsible for sensing AA availability for the cell. Do you see any link between AA influence on MAPK14 function in the early embryo and recently published induced developmental arrest of the mouse blastocyst (*Bulut-Karslioglu et al. 2016, Inhibition of mTOR induces a paused pluripotent state*)?
4) The study of the PhD candidate is also focused on the effect of media quality on Mapk14 related functions during preimplantation embryo development, in respect to blastocyst ICM cell-fate maturation. Do the suboptimal *in vitro* culture conditions significantly affect the future individual?

Overall, my comments outlined above do not call for major changes, and I recommend that the permission for a public defense of the dissertation is granted.

In Libechov 29.11.2016

Andrej Susor, PhD