Evaluation report from Dr. Zdeněk Paris, Institute of Parasitology, Biology Centre ASC, České Budějovice

Bc Thesis: "The role of Erv1 in the mitochondrial import machinery and iron sulphur cluster export machinery in *Trypanosoma brucei*" by **Michala Boudová**, University of South Bohemia, Faculty of Science, České Budějovice, 2016

The aim of the thesis was to determine the subcellular localization of the protein Erv1 and to test for its involvement in the cytosolic iron sulphur cluster assembly. In addition, author performed a co-immunoprecipitation followed by mass spec analysis to identify potential binding partners of this mitochondrial protein.

The experimental work is preceded by an introductory chapter, which provides the reader with essential up to date and detailed information about iron sulphur cluster biogenesis in different cellular compartments in several model organisms including protozoan parasite *Trypanosoma brucei*.

Based on the plethora of experimental methods used, which led to obtain interesting results, Miss Boudová most certainly has acquired the experimental skills required to continue her future career in natural Sciences.

The thesis is clearly written and I appreciate that it was elaborated in English, although there are a few typos and also several expressions, which are not common in scientific language (see minor points).

Questions:

1. Page 23, Chapter 3.3, second paragraph: explain sentence "....including short hairpin (sh) RNAs capable of DNA integration." Is this correct? Which plasmid did you use for RNAi of TbErv1 and how does it work?
2. As you mention in the text, aconitase is a known iron-sulphur cluster protein of two forms having distinct roles in the cytosol and mitochondrion respectively. In the genome of trypanosomes, aconitase is encoded by a single gene while the protein has a dual localization. How is the dual localization achieved? Do you know any other example?
3. Did you perform immunofluorescent localization of the Erv-V5?
4. Page 40, Chapter 4.5, first paragraph: Since there was no other proteins detected using silver stain gel except TbErv1, how can you lower stringency of your purification condition in order to co-purify proteins with weak or transient interactions?
5. Page 44, Chapter 5.4, first and third paragraph: explain this ".....conditions chosen are in principle suitable for the detection of IMS localized as well as membrane bound proteins....." while you wrote few sentences bellow: "...none of the identified proteins is localized to the IMS of mitochondria."

Minor points:

Page 1: trypanosamiasis should read trypanosomiasis

Page 23, Chapter 3.3.1, last paragraph: How did you calculate the growth rate of cells?

Page 26, Chapter 3.4, first paragraph: explain this sentence "....to detect protein in its native position in fixed cells."

Page 28, first paragraph: Image manipulation.... should read Image processing....

Page 36, chapter 4.2.3, first paragraph: "....measurement of the mitochondrial membrane potential *in situ.*" Should read "....measurement of the mitochondrial membrane potential *in vivo.*"

Non-scientific expressions (underlined)

Page 21, second paragraph: "....were analysed by agarose gel electrophoresis to check success of the reaction."

Page 30, Chapter 3.4.4, first paragraph: .... moved from within the gel..... Should read transferred from the gel.....

Page 31, Chapter 3.5, first paragraph: "After washing high pH buffer is usually applied....."

Page 31, Chapter 3.5, first paragraph: "Effectiveness of the process....."

In conclusion, the thesis is of very high standard and my recommendation is therefore to grant Michala Boudová the Bc degree, which is subject to the quality of the presentation, replies to the questions and discussion with the committee members during the defence.

In České Budějovice, 19.5.2016

RNDr. Zdeněk Paris, PhD.

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