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Faculty of Science

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University of South Bohemia in České Budějovice

STATEMENT OF THE BACHELOR * THESIS REVIEWER

Name of the student: David Hollaus

Thesis title: Determining the role of FoF1-ATP synthase dimers in *Trypanosoma brucei* mitochondrial biogenesis

Supervisor: RNDr. Alena Panicucci Zíková, Ph.D.
Co-supervisor: Bc. Brian Panicucci

Reviewer: Roman Sobotka, Ph.D.
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This bachelor thesis deals with the role of dimeric ATP synthase complexes in functioning of mitochondria in *T. brucei*. In this organism mitochondria show many oddities including the fact that the inner mitochondrial membrane system is extensively rebuilt during the Trypanosoma life cycle. Spontaneous assembly of ATP synthase dimers is expected to play a crucial role (by bending the membrane) in the formation of mitochondria cristae. A Trypanosoma mutant cell line with a specifically impaired dimerization of ATP synthase would be therefore of a great value for future studies. In his thesis David Hollaus used RNAi approach to deplete 3 selected subunits of ATP synthase, potentially contributing to the dimerization of this enzyme, and characterized obtained lines.

The thesis is written in very good English and it is easy to read. Introduction provides a solid background for readers working on different topics or model organisms. I would nonetheless suggest to a) add a figure of mitochondria highlighting the morphology of cristae including MICOS, OPA1 etc; b) to describe here, or in discussion, what is known about the phenotype of mutants in ATP dimerization for other model organisms. Or no such mutants have been published yet? Method part is very detailed with minimum mistakes; mostly just typos like this one "Since this dye is light sensitive, the agarose first had to be cooled". However, better subtitles could be chosen; I have never heard about 'Steady state western blot analyses and Blue Native western blot analyses'. Western blotting is a method how to transfer proteins on the membrane, the title might sound "Blue-native PAGE followed by immunodetection". The experiments are organized in a relevant order following a logical flow, and lead to a preliminary conclusion that the Tb9 subunit is a candidate for a factor required for the ATP synthase dimerization. Figure 16 is however difficult to understand, I'm not sure I did. Does the value in the graph (e.g. 0.101) means that the level of the particular mRNA dropped, after induction, to 10% of the control? Obtained results are well discussed, author however did not discuss quite strong increase in several ATP synthase subunits in knockdown strains (Table 20; Tb12). Is it this phenomenon common for the ATP synthase mutants?

Questions:

1. Describing RTq-PCR author says: "Since single stranded RNA is not very stable, it needs to be reverse transcribed into complementary DNA (cDNA) before it is analyzed". In fact, the stability of mRNA is not such a big issue and cDNA is not generated because of the mRNA stability. What is the reason the cDNA needs to be prepared for RTq-PCR? Do you know another method to assess gene expression directly using the isolated mRNA (not using cDNA)?

2. I do not understand this point from the method part: "The assay depends on supplying the mitochondria with ADP and a metabolite that is known to generate ATP either through OxPhos (glycerol-3-phosphate) or SubPhos (ketoglutarate). Why ketoglutarate for SubPhos? I would believe ketoglutarate will be quickly converted into succinate and then used for OxPhos?

3. Author says: "The upper band of 1000-1200 kDa is believed to represent oligomers (including dimers) of ATP synthase". How this 'believe' is rationalized? The mass of this putative dimer would be suspiciously small given the migration of the monomeric complex (~ 800 KDa). How the migration of ATP synthase on blue-native gels looks for other organisms?

4. Should be the activity of ATP synthase dimers in an in vitro assay lower than of the monomeric complexes (per one FoF1)?

Conclusion:

In conclusion, I

r e c o m m e nd

the thesis for the defense and I suggest the grade 1

In České Budějovice, date 30.6.2020