Evaluation of Master thesis:

Functional analysis of novel F_1-ATPase subunit in *Trypanosoma brucei*

Bc. Hana Váchová

Referee: Eva Horáková

In the present Master thesis, Hanka studied function of new subunit of complex V called p18 which is unique to order Kinetoplastida in different stages of *T.brucei*. In addition she performed some studies on putative inhibitor of complex V named JK-11 and evaluated its effectiveness and specificity against *T. brucei* ATPase. The thesis is divided into six sections, with the regular arrangement of the work. I think that Hanka together with her colleagues presented solid project, which brings numerous elaborate experiments which are not easy carry out and needed optimalization.

General comennts:

1. Overall the thesis flows quite nicely and I found it easy to read. The Introduction could be more focused. I found several paragraphs which are unnecessarily detailed and not relevant to the project (paragraph 1.1.1., 1.1.3.). Moreover they are heavily inspired by one particular review (Kennedy, 2013) which I found a little lame. On the other hand section about trypanosoma ATPase or p18 subunit may be more elaborate. Similarly more information about dyskinetoplastid trypanosomes and their mitochondrion and respiratory complex V would be more useful for the reader.

2. I have to admit that I later learned more about p18 subunit in the Result and Discussion. I would probably rearrange the paragraphs and by that the rationale of the project will be more apparent.

3. You are overusing the word "importantly" even in the sentences where I find the statement not that important. You may try to use other adverbs or simply not to use it at all.

4. Some figures presented (1.1., 1.5.) have low resolution and are therefore pixelled. Please pay more attention to the quality of your pictures it will make your work look more professional!

Questions to the student:

1. Does the dyskinetoplastid strain used in the study (Dk 164) have some kDNA left? Can you specify the presence/absence of mini/maxicircles and compare it to the other Dks known?

2. What is the scientific evidence/s for p18 not being subunit b of Fo?

3. In 2009 your supervisor identified 14 novel subunits of *T.brucei* complex V. As far as I know 3 of them are characterized to date, including p 18. What was the rationale for choosing them? Can you speculate how many of them are actually the *bona fide* subunits of complex V? Do you think that it is worth to study them deeper?

4. Do you think that high membrane potential is harming the PF cells? How are they coping with that stress?

5. Was the JK-11 compound studied *in vivo*? Do you think that it will be toxic to the trypanosomes in the bloodstream?

6. How would you study the deoxyhypusine formation in trypanosomes? What is known about this process in tryps?

Overall, this thesis undoubtedly meets the criteria for a Master degree and I am looking forward to see Hanka's defence.

21. května 2015 v Českých Budějovicích

Eva Horáková

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A review of master thesis „Functional analysis of novel F_1-ATPase subunit in *Trypanosoma brucei*" by Hana Váchová, BSc.

Formal part

The master thesis by Hana Váchová is a standard piece of work with regular chapters. It has 47 pages in total, it is written in English and supervised by Dr. Panicucci Zíková. The author could have performed one final spell check to avoid numerous typing errors; also, some expressions bear a mark of Czech-English translation without further stylistic polishing.

The Introduction chapter misses a referred figure 1.2C that I believe is figure 1.3. In Materials and Methods, I was surprised by a familiar word "eppi", an abbreviation for eppendorf tube. In the list of used buffers, I do not understand the buffer between CytoMix and Digitonin. Could you please specify it? In Results, there are multiple growth curves. For an unknown reason, the y-axes are not unified making any cross-comparison rather difficult if not impossible. Moreover, the axes seem to be in linear and not logarithmic scale, a rather unusual situation in field of trypanosomatid research. Did you have any specific reason for this representation (different linear scales)? The text refers to Gahura, manuscript in preparation several times. I would omit is citation as, in my opinion, a reader has no way how to get this source of information. Instead, I would include the relevant information in the thesis itself. Finally, the list of references misses some of the citations from text, e. g. Alberts et al., 2008; some citations have different year in the list and in the text, e. g. Kieft et al., 2001 and 2010. Surprisingly, the references are listed in random styles.

Apart from those issues, I believe the thesis fulfils all formalities.

Scientific part

Introduction is sub-divided into parts describing the model organism *Trypanosoma brucei*, mitochondria, F_0F_1-ATPase and finally 4-oxopiperidine-3,5-dicarboxylates. The chapter covers all areas required for understanding of the work with only few sentences being redundant or contradictory; I believe a protozoan parasite is always unicellular as well as for me the disease is either distributed worldwide or limited to sub-Saharan Africa, not both. A description of life cycle misses an existence of short-stumpy bloodstream forms of trypanosomes.

I appreciate a part on disease treatment, however, the part about the most recent promising drug candidate being followed by a sentence describing its toxicity and ultimately abandonment of the drug is confusing.

Regarding the specificities of *T. brucei*, I miss a deeper description of complex V in this organism, e. g. a description of p18, the protein being analyzed. It is mentioned in Aims section for the first time and then introduced in Results section. This is also true for ATPaseTb2, apparently a specific subunit of complex V in *T. brucei*, yet it appears without any further description in Result section as a part of F_oF_1 assembly.

Results describe all observed phenotypes and they are divided to two parts: p18 and JK-11 analyses. Regarding p18, RNAi cell lines were prepared and cellular growth, protein abundance and complex V stability and mitochondrial membrane potential were assessed. Regarding this part, I have several questions to be answered. Apart from those issues, I did not find the Hsp70 loading control in Fig. 4.11.

The second part describes analyses of JK-11 impact on mitochondrial features. EC50 value was calculated for each cell line (PF/BF/Dk) finding only a minor variation among them. Upon this, effects on mitochondrial membrane potential, isolated F1-subcomplex, ATPase and ATP synthase activities were assessed.

The discussion part summarizes and comments the results; again, it is split and p18 is discussed independently of JK-11. The conclusions are logical and fit nicely the observed data; I really enjoyed reading it as this chapter asks and answers the questions I had in my head during reading of the Results section.

I consider the master thesis by Hana Váchová to fulfil all requirements for such a work. I believe that upon answering questions she should be considered eligible for MSc. title.

In Prague, 22/05/2015

RNDr. Zdeněk Verner, Ph. D.

Questions:

The part describing complex V and its function is the best part of introduction. Regarding the function of the complex, I have the following question:

You describe the function as the reversible one being dependent on the mitochondrial proton gradient. Is the direction of the c-ring/gamma-subunit rotation connected to synthesis/hydrolysis of ATP or not? Is there any model connecting the proton movement (in/out of matrix) with the direction or synthesis/hydrolysis of ATP or its change?

At the Result section, you described observed phenotypes in p18 KDs. Here, I would like to ask three questions:

Firstly, the structural integrity of the complex V was evaluated using western blot following a native gel; meanwhile, the amount of proteins was assessed on a separate gel. Why did you choose this approach instead of re-probing the original membrane? Fig. 4.5, left panel shows a dramatic decrease of all signals. How can you be sure that this is not merely due to an unefficient protein transfer?

Secondly, you described Dk cells as not pumping protons but instead using difference in charges of ATP/ADP for generation of electrochemical potential across the inner mitochondrial membrane. If this is true, why did you use FCCP? FCCP is described as a protonophore dissipating proton gradient. Why did not you use atractyloside, azide and oligomycine as controls in this experiment?

Thirdly, upon RNAi in Dk cells, the amount of remaining protein is rather high. Did you check the level of corresponding mRNA? If not, why?

Following p18 analyses, you focused on mode of function of JK-11. Regarding this part, I would like to ask two questions:

I am surprised by the high concentration used in the tests following EC50 calculation. What was the reason to go to such high concentrations?

You concluded that JK-11 most-likely influences other cellular processes than only complex V. Have you considered the component(s) involved in transport of electrons downstream of complex II could be target(s) of the compound? Did you test whether this compound affect respiratory complexes other than ATP synthase?