STATEMENT OF THE BACHELOR THESIS REVIEWER

"Elucidating the subunit composition of tRNA-guanine transglycosylase in *Trypanosoma brucei*"

Name of the student: Bc. Veronika Běhálková
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The bachelor's thesis of Bc. Veronika Běhálková contains theoretical overview of the selected topic and the experimental part. The thesis is distributed into 43 pages, having a classical structure.
The aim of the thesis was to elucidate the relationship and/or interaction between two tRNA-guanine transglycosylase paralogs (putative subunits of the TGT enzyme) identified in *Trypanosoma brucei*. The overexpression of these proteins was tested in a rescue experiment in yeast *Schizosaccharomyces pombe* experimental system.
The experimental work is preceded by an introductory chapter, which provides the reader with essential up to date and detailed information about the life cycle of the parasite *T. brucei*, tRNA maturation and post-transcriptional modifications with the stress on the studied queuosine modification and tRNA-guanine transglycosylase (TGT) enzymes.
The thesis is clearly written and I appreciate that it was elaborated in English, moreover of high quality.
Despite the fact that the main aim of this thesis was not reached, I believe that the used methodology will serve as a good basis to the author to continue her future career in Natural Sciences.

Questions and comments:

1. Page 7, Chapter 1.4 -Biosynthesis of queuosine:
Is it known why some yeasts (like *S. cerevisiae* or *C. albicans*) dont have or dont need queuosine (Q) modifications while the others (like your model *Schizosaccharomyces pombe*) do? Is it known whether the yeasts lacking Q modification do not acquire queuine (queuosine precursor) from the environment or they acquire it, but they just do not use it? Is it know how is queuine transported to the cell -does it need any protein transporter?

2. Page 10, Chapter 1.6
How looks like the situation in *Trypanosoma brucei*? Do you have already any indications what happens in case of the cells that lack TGT enzymes (they lack the queuosine modification)? Do they show any phenotype? Do you plan to prepare knock-outs of TGTs in *T. brucei*? And is it known whether any trypanosomatids lack TGTs?

3. Is there any severe phenotype caused by lacking TGTs in some other organisms?

4. Page 13, Chapter 3.1
Could you please explain why did you choose to study the interaction of TGT proteins in *S. pombe*? Actually, I was missing the alignments of TGTs of *T. brucei* and *S. pombe* in the thesis, could you please show it during the defence? I noticed that there is a big difference in the size of TGT2 in *T. brucei* and its paralog in *S. pombe* (40.4 kDa x 73.2kDa).

5. What was the reason you choose N-terminal tagging (and not C- terminal) of the TGTs protein in *S. pombe*?

6. Page 29-36, Chapter 4 (Results)
The author successfully created 3 different *S. pombe* cell lines that should overexpress TGT1, TGT2 or both proteins. However the expression of these proteins was not proven neither by Western blot nor by Northern blot. The Nothern blot results were inconclusive because it was not possible to see the signal even in the *T. brucei* control. What should be the size of the signal?
To sum up, it is not possible to say why exactly the overexpression didnt work, but the author tried to explain all the possibilities in the clear discussion.

7. Do you plan to further continue on this project? If yes, my suggestion would be to use another *T. brucei* protein and try to express it in the same vectors as a control.

Minor points (I didnt find any typos in the text):
*Page 17, Table 7 – in the reaction there should be 3.5ul of 10x Cut smart buffer and 0.5ul of water
*Page 18, Table 8 - see table X, should be: see table 2
*How did you centrifuge the *S. pombe* cells? - on p.17 you write 3000rpm, 10 min, while on p.18 - 6000 rpm, 5 min (which is quite a difference).
*How did you cryopreserve *S. pombe* cells?

In conclusion, the thesis is of very high standard and my recommendation is therefore to grant **Veronika Běhálková** 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, 17.5.2017

RNDr. Drahomíra Faktorová, PhD.