



Přírodovědecká
fakulta
Faculty
of Science

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

STATEMENT OF THE BACHELOR THESIS REVIEWER

Name of the student: Helmut Stanzl

Thesis title: Evaluating the Role of TbTGT2 in the Formation of Queuosine tRNA Modification in the Bloodstream Stage of Trypanosomes

Supervisor: RNDr. Zdeněk Paris, PhD.

Reviewer: Hassan Hashimi

Reviewer's affiliation: Biology Center, AS CR & University of South Bohemia

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	3
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		15
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	2
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Quality of experimental data presentation	0-3	3
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Formal requirements – points in total		26
POINTS IN TOTAL (MAX/AWARDED)	48	41

Comments of the reviewer on the student and the thesis:

The bachelor's thesis by Helmut Stanzl is of high quality in my opinion. First, the written English is quite good considering Helmut is not a native English speaker. Secondly, the clearly stated aims of the thesis have been achieved using quite sophisticated molecular biology techniques. It is an impressive feat by a bachelor's student with limited time. Helmut's hard work and devotion to the project definitely comes through. Helmut also attempts to interpret his results in the context of the background information about Q he describes in his thesis. I really applaud this, but admit he needs to improve this type of synthesis of information.

Mistakes that the candidate should avoid in the future:

The major problems I had with the thesis were related to the writing style, which I consider separately from the use of the English language. Helmut needs to develop a more precise and concise style of writing.

Precise means that words should be used properly to convey to the reader that you understand the topic, and eventually that you are authority on the topic that can be trusted. For example, the title of the thesis is "Evaluating the Role of TbTGT2 in the Formation of Queuosine tRNA Modification in the Bloodstream Stage of Trypanosomes". The word "trypanosome" is a colloquial term for trypanosomatida, an order parasites that include genera such as *Crithidia*, which is monoxenous, and *Phytomonas*, which infects plants. Thus, neither type of trypanosome has a bloodstream stage. I will leave it to Helmut to decide what is a more appropriate term for his title. Other such incidences of imprecise use of words are highlighted in my marked copy of the thesis, which I provide to Helmut in exchange for a fresh copy.

Concise means to use as many words as needed without any redundancies. An example of unconcise writing is this passage from the abstract: "Trypanosoma brucei is a eukaryotic protozoan..." A protist is by definition a unicellular eukaryote, so in the example "eukaryotic" is unnecessary. Again, other numerous examples of such mistakes is in the marked version of the thesis.

Also, I found that while the thesis is well illustrated with figures and tables, these are seldom referred to in the main text, especially in the Introduction. All of these elements in a thesis or scientific paper act as a whole. As such, one should refer to the appropriate part of the text where the reader should look up an appropriate figure or table. Helmut does do this nicely in the Results section, so I am sure he understands this importance. However, this is not done well in the other sections.

I also found section 2.2 about Q to be rather confusingly written. I think it is important that one knows their audience when writing and when you write about a modification such as Q it is important to maybe better describe the differences between q (the quenenine base) and Q (the queuosine nucleoside). In this case, the chances that you would have an opponent that is a nucleotide chemist is small, justifying a better explanation of these nuances. Neither Fig. 2 or 3 from this section really help in clarifying to the reader which is which. Plus, neither Q nor q are included in the abbreviations.

Minor points include not describing the composition of some reagents such as 1X NNB and TAE, vague titles for some sections (e.g. Section 4.4.3.1 called "Method") and some statements I don't think are properly expressed, e.g. "During electroporation, a membrane weakening electric pulse enables the DNA to enter the nucleus." I thought this process created pores. I guess these types of errors come under the category of needing to be more precise.

Suggestions and questions which the student has to address during the defense:

Questions 1: In the thesis, it is stated "most of the research has focused on the bloodstream and procyclic stages, which can be easily cultured in vitro," implying these are the only two stages of the *T. brucei* lifecycle. However, Fig. 1 shows that the lifecycle is more complicated than this statement suggest. Please 1) briefly (1-2 sentences max) describe each stage of the lifecycle (ecological niche, important biological/morphological features); 2) state which of these stages is grown in vitro; 3) reevaluate the statement you made: "After establishment in the fly midgut, trypanosomes reacquire a VSG coat..."

Question 2: This statement about *T. brucei* from the thesis is too vague: "...techniques such as RNA interference (RNAi) and other methods of forward and reverse genetics, are readily available..." I would like Helmut to briefly define forward and reverse genetics. To which category does RNAi belong; from the quoted passage, it is not clear.

Questions 3: Helmut uses a fusion PCR approach to generate knockout constructs, which is depicted schematically in Fig. 4. How do you get from 3 small templates one large, fused PCR product in step 2? Also, how was this final construct sequenced since this detail is mentioned but not explained in the thesis?

Question 4: In section 4.4.1, guanidine extraction is performed to purify RNA from *T. brucei*. If I understand correctly, the RNA was phenol/chloroform extracted and precipitated twice. While I understand that you wanted to get very pure RNA, what was the rationale of doing this protocol in this way instead of a series of phenol extractions, followed by a chloroform extraction and then precipitation once?

Questions 5: This question is a more complex one about the future directions of the project. In the discussion, you speculate that a lack of influence of *T. brucei* fitness upon KO of *TbTGT2* and Q (or q?) starvation may be due to a "...minor Q pathways may exist[ing], which incorporate enough Q into tRNA for the cell to be viable" that is "under [the] detection levels of the Northern blot analysis". While this is certainly a valid interpretation by the author, I somehow do not agree for the following reasons: 1) A vast majority of the tRNA population is demonstrated to lack the Q modification under these conditions; 2) to quote "The omnipresence of Q [throughout the tree of

life] and its location in the anticodon of tRNA suggest that the modification is most likely of a [sic] great importance".

Perhaps as in the other model systems described in the introduction, there needs to be additional stress to reveal any consequence of Q depletion. You mention this interesting link between Q metabolism and tyrosine biosynthesis. Do you plan the experiment of determining whether tyrosine becomes essential for $\Delta TbTGT2$ or Q-starved *T. brucei*? What about determining if these cells are more sensitive to cadmium treatment, as you said is the case for Q-depleted fruit flies?

Did you consider growing the $\Delta TbTGT2$ *T. brucei* in Q-depleted media to see if there is synthetic lethality or some epistatic effect on parasite fitness? Given that in mice, full Q-depletion takes a "period of one year", perhaps both treatments at once could better deplete Q from the system.

Along this last line of questioning, I do see a very slight and persistent reduction in growth in both Q-starved (Fig. 5) and $\Delta TbTGT2$ (Fig. 9) *T. brucei*. Were these growth curves done in duplicate? If yes, maybe you can calculate the doubling times of these cells with standard deviation to see whether this slight difference is statistically significant. Also, maybe you could extend the growth curves (or do the same type of growth curve after growing the cells and appropriate controls for a longer period) to see whether over a longer time there is an effect on fitness?

Finally, what do you expect the outcome of animal infection with $\Delta TbTGT2$ *T. brucei* to be if you do not see any effect on fitness *in vitro*?

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade 1.5 .²

In České Budějovice date 29.8.2016



signature

² You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted.