Přírodovědecká fakulta
Faculty of Science

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

OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Sascha Gratzl

Thesis title: Post-transcriptional regulation of TbIF1 in life cycle of Trypanosoma brucei

Supervisor: Mgr. Ondřej Gahura, PhD

Referee: Sneha Kulkarni, MSc.
Referee`s affiliation: Institute of Parasitology, Biology Centre CAS and Faculty of science, 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	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	3
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		19
(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 the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3

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

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2
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
Practical requirements – points in total		26
POINTS IN TOTAL (MAX/AWARDED)	48	45

Comments of the reviewer on the student and the thesis:

The thesis aims to study the role of various Rbp binding motifs in the 3' UTR of TbIF1 in regulating the stability and translation efficiency of the mRNA. Appropriate techniques are used to answer this question. Polysome profiling combined with RT-qPCR has been used in *Trypanosoma brucei* to study translation efficiency of mRNAs. However, it is applied here for the first time to study translation regulation and therefore could be beneficial for other researchers in this field.

The thesis is generally well written, regarding the language and the grammar, and well-organized. The aims of the thesis have been fulfilled. Some of the results could have been explained in more details. However, separate discussions of experimental troubleshooting and that of results have increased the clarity. The student shows a good grasp of techniques used and also suggests ways to improve wherever possible.

Overall, I am satisfied with the thesis and only point out some mistakes to avoid and a few questions about the scientific part of the project.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

I have included a couple of instances which adversely affect the reader's understanding of the concept and hence could be improved:

1. The abbreviation qPCR is expanded as qualitative PCR. It should have been quantitative. Also, try to use a uniform abbreviation. RT-PCR is expanded as 'real-time' or 'reverse-transcription' PCR in different places. Generally, RT-PCR stands for reverse-transcription PCR.
2. In figure 10, three of the cell lines are mis-labeled.

Questions:

1. You use RT-qPCR to determine mRNA stability. Can you suggest one other technique to analyze stability and half-life of an mRNA?
2. Why did you opt for constitutive expression of CAT over inducible expression? Also, you briefly discuss the doubling times of different cell lines. Could you elaborate on it? Does the doubling time somehow co-relate with CAT expression levels?
3. On page 31, section 4.5.2, you mention that the peak on the left shoulder of the monosome peak is the small subunit of ribosome and should be avoided to reduce the contamination of 18S rRNA. In that case, could you point out the large subunit of

ribosome on your polysome profile? And what would be its rRNA composition?

Conclusion:

In conclusion, I

r e c o m m e n d*

the thesis for the defense and I suggest the grade 1 .

In Č. BUDĚJOVICE date 23/07/20

signature