Přírodovědecká fakulta
Faculty of Science

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

OPPONENT'S REVIEW ON DIPLOMA THESIS

Name of the student: **Bc. Monika Švábová**

Thesis title: The role of Q tRNA modification in codon biased translation in *Leishmania mexicana*

Supervisor: RNDr. Zdeněk Paris, PhD; Sneha Kulkarni, PhD

Referee: Michaela Fencková, PhD
Referee`s affiliation: Faculty of Science, University of South Bohemia in České Budějovice

	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	2
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	1
Quality of the annotation	0-3	1
Language and stylistics, complying with the valid terminology	0-3	1
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	0
Formal requirements – points in total		9
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	2

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

Completeness of the description of the used techniques	0-3	1
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	0
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	1
Practical requirements – points in total		13
POINTS IN TOTAL (MAX/AWARDED)	**48**	**(22)**[2]

Comments of the reviewer on the student and the thesis:

The master thesis of Monika Švábová describes optimization of polyribosome extraction and profiling in *Leishmania mexicana* (*L. mexicana*) and analysis of the effect of lack of queosin tRNA modification on the parasite's growth and protein synthesis.

The thesis is well structured with **Introduction** describing *L. mexicana* life cycle, tRNA and its modifications, role of queosin tRNA modification in physiological processes across organisms including another parasitic species *Trypanosoma brucei*. However the tRNA description is relatively broad and lengthy and not completely relevant to the subject of the thesis. Thus the introduction does not clearly converge on the rationale and goals of the project.

The **aims** are clear but they should be more specific. Aims 2-4 speak about the elucidation/studying of the role of queosine but specifically, the candidate is studying the lack of queosine tRNA modification. **Rationale** of the project describes the reasoning for performing of these aims only partially. It is not clear why ribosome profiling has to be standardized. And what role for queosin modification does the candidate expect in *L. Mexicana*. The candidate writes: *„Considering the results obtained in the T. brucei experiments, which were described in the previous chapter, we hypothesized that in closely related organism such as Leishmania, the queosine modification might also play a significant role.“* The previous chapter is 1,5 page long and there are number of effects listed. Some of them are for example specific to mitochondrial translation, which is not a subject of this thesis. The candidate mentions here for the first time an "add-back line" without describing what this add-back line is and, most importantly why is she going to use it. The chapter is not well organized either. Only in the last paragraph, the reader learns that there are limitations in ribosome profiling in *T. brucei* and that this is the reason (or one of the reasons) for utilization of *L. mexicana*. This should be already mentioned at the start and precede the description of advantages of *L. mexicana* as model system.

Methods are described satisfactorily but the description of the add-back line is missing. The polysome and ribosome profiling methods could profit from more consistent description. There are various modifications described throughout the text without description of the rationale for testing of these modifications. In principle, this is not necessary in the Methods and can be specified in the Results part. Unfortunately, the Results part does not address these specifications either.

Result chapters 4.6, 4.7 and 4.8 (the last three chapters) are very well written. The description of the results has clear structure - stating what was done, why was it done, what was

[2] Enter the number of points awarded.

the result and what was concluded from the result. Figures are clearly described with labels and fully understandable figure legends.

Chapters 4.1 – 4.5 (the first five chapters) are less clear and they would profit from substantial improvement. The main limitations are (1) missing explanation of the purpose of the experiment, (2) the used method (specifically if it is necessary for understanding of the result) and (3) poor description of the result. The candidate is often using "phenotype", "significant phenotype" or "rescue of the phenotype" without specifying what the phenotype is.

Experiments performed in Chapters 4.2 (effect of loss of Q modification on parasite growth represented by growth curves) and 4.3 (effect on cell cycle represented by DNA histogram contents of propidium iodide stained cells) are missing statistical evaluation despite they were done in three independent biological replicates. Specifically, in chapter 4.3, the candidate concludes that TGT2 knockout cells, as well as AB cells show increase in G1 fraction and that this increase is meaningful. On the representative histogram, this increase is only by 5.3 (I assume % because units are not stated here either) for the TGT2 KO line. But in the next experiment, a decrease of 7.5 in TGT2 KO line is considered a "slight phenotype" not affecting cell growth. But either of these differences could be pure variation. It is of course sufficient to show one representative histogram but it is also necessary to report data from all biological replicates together with statistical analysis. The applicant concludes that in the first experiment, an increase in G1 fraction is confounded by presence of antibiotics in the media of TGT2 KO and AB lines that were not present in the medium of WT line. This is the reason why she repeats this experiment in more consistent conditions with hygromycin present in all media. But she does not describe what antibiotics were used. Was it hygromycin or something else? And what was the reason for this initial inconsistency of the media composition. The reader only learns the composition of the antibiotics in the Discussion. But it should be reported in the Results.

Ribosome profile graphs in Figures 15, 16 and 17, despite being the main readout of Chapter 4.5.1, are not at all explained. What is position (in mm) and why is OD_{260} (on y axis) different in each graph? What is the indicator of the quality of "polysome profile"? Which peaks indicate polyribosomes? Without this explanation, reader that is not familiar with ribosome profile data cannot understand the conclusions. The applicant is describing optimization with three different detergents without providing information on what is the main difference between them. Similarly with addition of CHX. The candidate optimizes concentration of CHX in Chapter 4.5.1 without explaining what the role of CHX in the ribosome profiling is, not even earlier in the Methods. Only in the next chapter (4.5.2), we learn that it is used to inhibit translational elongation. And also, only in this chapter (4.5.2), in figure 18, after having seen 11 ribosome profile graphs without explanation, the candidate describes what do the peaks mean and how where the samples for polysome profiling prepared and analyzed. This explanation is clear, short and sufficient but it should have come way more earlier in the Results section. In chapter 4.5.3, the candidate concludes that there is a "significant decrease" of polysome profile in the TGT2 KO line, while there is no statistical analysis. It is not per se required to perform statistical analysis for all experiments but without statistical analysis, the findings should not be reported as significant.

Discussion is relatively short and limits mainly to the recapitulation of the results acquired in this thesis with limited discussion and without putting them in the relevance with other published literature. In chapter 5.1, the candidate discusses issues with detection of $tRNA^{Asn}$ but detection of $tRNA^{Asn}$ is not even mentioned in the Results section. If she attempted to detect this tRNA, it should be stated in the Results. It is not clear whether the finding about Q effect on cellular growth, life cycle and membrane potential is in agreement or disagreement with the previously published findings. The text reads as… "*According to previous results from our lab (Boudová, 2018), we have some indications that Q modification plays some role in L. mexicana life cycle. However, these results had to be verified as they were not repeated. Thus, to confirm these*

results, we examined the effect of Q modification on cell growth of WT, TGT2 KO and AB strains. The resulting growth curve confirmed that Q did not affect the cell growth in culture, neither in TGT2 KO, nor in AB cell line compared to WT." So what was the expected effect? Was this master thesis performed JUST to confirm that there is no effect? There are statements in the Discussion that need more explanation. For example… "*There was some different phenotype in cell cycle of TGT2 KO as well as AB cell line*"… What was the phenotype? "*However because the similar phenotype appeared also in the AB line, it gave us the idea to test cells without the presence of mentioned antibiotics*." … The reader does not learn about the reasons why the phenotype was not expected in the AB line, and that it was not expected. An example of missing discussion is this statement …"*In order to test the detergents, we used three different chemicals – IGEPAL, NP40, and Triton X-100. The comparison of all three results revealed that the best detergent is IGEPAL."…* What is the difference between these detergents? What was the effect of the detergents on polysome yield? Why was IGEPAL the best? Or …"*the samples were ultracentrifuged for 3 hours, which did not give us a positive result.*" … What result was expected? "

I attempted to score the most important aspects of the thesis with points, which are shown in the table above. This scoring is just informative and it was not used to calculate the suggested grade. The suggested grade reflects the here mentioned comments and it is based on overall evaluation.

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

Some chapters of the thesis are hard to read. This is mainly due to lengthy descriptions, providing information that is too broad and not to the point and not good organization of some chapters, such as the second part of the Introduction and Rationale. While some parts of the thesis are written with excellent English, other parts suffer from grammatical and sentence structure mistakes, which also contributes to the difficulty with reading and understanding of the text. The thesis showed overall 16% similarity/overlap to another, previously published thesis. This overlap was not concentrated to specific parts and neither a comparison with published literature showed considerable overlap. Because the chapters that are written with very good English also correspond with good organization and flow of the text, the discrepancy in the text quality shall probably be attributed to different level of input from the supervisor during the writing process. The thesis would profit from language corrections and the parts outlined previously by the comments of the reviewer should be improved substantially to meet the criteria for excellent thesis.

Here I mention further suggestions to improve the thesis and mistakes that should be avoided in the thesis writing. Polysome and ribosome profiling modifications in the Methods section could be written in a tabular or bullet point style for better overview of which modifications were done, which steps of the protocol were optimized and which remained unchanged. All leishmania lines should be specified in the methods, including the AB line. There should be consistency in reporting volumes and units. In some places, the candidate writes, for example, "100 ul CHX was added". In another place "100 of CHX was added." In chapter 4.5.1, simultaneously 100 ug/ml vs 1x CHX and 200 ug/ml vs 2x CHX is used. And not explained. This is confusing. Whenever the candidate discusses the results in the Discussion section, she should also refer to the relevant figures. Statistical analysis should be performed for all quantitative experiments with replicates. In the Discussion, the standardization of polysome and ribosome profiling should be separated from the findings that were made with the standardized protocol. This will make the result less chaotic. It will be clear what protocol was eventually used and which modification was the most relevant. When speaking about "phenotype", first describe what the

phenotype is and then discuss it. In the Discussion section, this was often done the other way around, which makes the Discussion hard to read.

Questions to the candidate:

1. While the candidate describes the disease symptoms and severity caused by other New world leishmania, there is little information about pathogenicity of *L. mexicana*. Which type of disease is it causing and how prevalent and severe are *L. mexicana* infections in comparison to other leishmaniasis?
2. In chapter 4.5.3 (Figure 19), the candidate concludes that TGT2 KO line shows a decrease of in the polysome profile. What does this indicate?
3. In chapter 4.5.4 (Figure 20), the candidate observes a significant difference in monosome density. What does it indicate?
4. Please describe clearly the previous findings published in Boudová (2018) and discuss whether your results are in agreement of disagreement with these findings.
5. In chapter 5.3, the candidate hypothesizes about the presence of codon usage bias in *L. mexicana* and refers to previously published work on ribosome profiling. How does no difference between translation of preferred synonymous codons and non-preferred codons relate to codon bias usage?
6. The candidate states that the results from polysome profiling have to be verified by ribosome profiling. Can she specify which results?
7. RNA isolated from monosome and polysome fractions was sent to RNA sequencing. Why was it done and what should the RNA sequencing show?
8. After ribosome isolation, the candidate describes that the isolated RNA was reverse transcribed and used for preparation of cDNA libraries. She also describes that cDNA will be circularized, PCR amplified and sequenced. This procedure does not precede RNA sequencing analysis from polysomes. Can the applicant explain why do the procedures differ?

Conclusion:

In conclusion, I

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the thesis for the defense and I suggest the grade 2-3 .[3]

In **České Budějovice** date 22/05/2022

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signature

[3] 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. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).