



OPPONENT'S REVIEW ON DIPLOMA THESIS

Name of the student: Helmuth Stanzl

Thesis title: Localization and functional characterization of a mealy bug rRNA methyltransferase of bacterial origin.

Supervisor: Zdeněk Paris (Filip Husník, Hana Sehadová)

Referee: Pavel Doležal

Referee's affiliation: Dept. of Parasitology, Faculty of Science, Charles University

	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	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	3
Formal requirements – points in total		18
(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	2
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	2
Quality of experimental data presentation	0-3	2
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	3
Practical requirements – points in total		23
POINTS IN TOTAL (MAX/AWARDED)	48	41

Comments of the reviewer on the student and the thesis:

The topic of the diploma thesis by Helmut Stanzl is clear, well defined and exciting. The scientific interest, as explained in the introduction, concerns two linked fundamental aspects of the eukaryotic cells: Metabolic adaptation of the serial endosymbiosis within insect cells and the evolutionary boundaries between an endosymbiont and the actual organelle(s). As a whole, the submitted thesis presents exciting story which follows a structure of a short publication and which has, in most places, corresponding quality. On the experimental side, Helmut succeeded in demonstrating that the studied rRNA methyltransferase is present in the bacterial endosymbiont. This was achieved by the expression and purification of the recombinant protein, by raising the specific polyclonal antibody and its use in the immunofluorescence analysis.

After critical reading I have several comments to the scientific and also the formal part of the thesis.

The overall quality of English is excellent, my main concerns relate to some of the expressions, which are sometimes too strong, unfounded or without necessary context.

Introduction

1. *“Many organisms require intracellular bacteria for survival. The oldest and best-known examples are organelles in eukaryotes, most importantly mitochondria and chloroplasts [1].”* – connecting intracellular bacteria to mitochondria and chloroplast makes sense in general but sounds inappropriate here as they are not intracellular bacteria....
2. Are we sure that an α -proteobacterium was the ancestor of mitochondrion?
3. *„More than a decade ago the sequencing of the complete genome of a recently evolved photosynthetic body in Paulinella chromatophora raised questions on the distinction between ‘endosymbiont’ and ‘organelle’”* Too strong expression, I dont think anybody doubts that ER, peroxisomes etc are organelles.
4. What about membrane-less organelles? Do they exist?
5. Should be membrane-bounded not *membrane-bound*
6. What do you mean by *„gram-negative cell wall without peptidoglycan?”* Is there just double membrane or some other polysaccharide?
7. What do you mean by *“that horizontally transferred gene (HTG) products from the insect genome are targeted and functional in the endosymbiont.”*? DNA, RNA or proteins?
8. Why do you think there is no documented gene transfer from the endosymbiont to the host?
9. *„If Rlml would have been a standard eukaryotic protein, its name would be in all capitals “RLMI”;* is it true for the nomenclature of all eukaryotic organisms?
10. Not clear what is meant by *„Interestingly, the sequence of rlml is more closely related to known 5-methyluridin methyltransferases than to the two other methyltransferases in E. coli [60].”*
11. Methyluridine not methyluridin
12. *„In bacteria, the 23S rRNA forms together with the 5S rRNA the 50S rRNA also called large*

ribosomal subunit, which assembles together with the 16S rRNA and additional ribosome proteins to the complete ribosomes“ What do you mean by additional proteins?

13. The expression „the prediction of targeting signals achieved a high score for some HTGs“ sounds awkward.
14. “the sequences completely align” should be rephrased
15. „we hypothesized that the insect-encoded HTG *rlmI* is responsible“ no need to stress out that it is an HTG.

Methods

16. *Moranella. Endobia* – should be *Moranella endobia*
17. *Western blot* should be western blot
18. Avoid using „to get familiar“
19. I could not find the arrow in figure 11A☺
20. Did you do the western blot on the cell lysate, which would show the correct size of the protein? and
21. Which antibody was eventually used for the localization study?

Discussion

22. „one of those genes, *murF*, was localized inside *M.endobia* [15].“ Do you mean MurF protein?
23. Signal peptide encoded by a separate exon indicate the presence of two forms mRNA (alternate splicing). Do you think there is a secreted and non-secreted version of the protein?
24. How would you experimentally check that SP is removed upon the translocation to the endosymbiont?
25. Could you think of an experimental approach (imagine you have unlimited budget), which enable biochemically identify the proteins secreted from the insect cells to the endosymbiont cells?

References

26. Please be careful and always double check the format of the reference generated by the used software. The organism names are not in italics, some reference contain capitalised words some not....

I would appreciate the answers to the highlighted questions/comments.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future (see above☺)

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade 1 .²

PRAGUE
In date 21.1.2020 Paul Jole

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