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. Hana Pejšová
Thesis title: Subgenomic flaviviral RNA and its role in host cells
Supervisor: RNDr. Martin Selinger, Ph.D.
Referee: Mgr. Jaroslava Lieskovská, Ph.D.
Referee`s affiliation: Department of Medical Biology, Faculty of Sciences, 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	2
Quality of the annotation	0–3	3
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	2.5
Formal requirements - points in total		19.5
(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	2.5
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
Practical requirements - points in total		26.5
POINTS IN TOTAL (MAX/AWARDED)	48	46

Comments of the reviewer on the student and the thesis:

The thesis deals with a role of subgenomic flaviviral RNA in host cells. This uncoding region at 3' end of viral genom is believed to have a regulatory role during viral infection. The thesis extends the finding recently published about the tranlation shut-off induced by tick-borne encephalitis virus (TBEV) in neuroblastome cells DAOY HTB-186 and works with hypothesis that sfRNA is responsible for those effects. The major goal was to determine the role sfRNA of two TBEV strain of distinct virulence and 2 mosquito-borne flaviviruses (zika virus and dengue virus) on *de novo* synthesis of host protein and *de novo* production of 45-47 pre rRNA transcript using Click-on chemistry. Author found that *de novo* synthesis of the protein is decreased by sfRNA from all tested flaviviruses while the production of of rRNA stayed unaffected. Third part of the thesis comprises the thorough optimization of FISH method to detect viral sfRNA.
The topic of the thsis is very interesting and of high importance since in general the role of sfRNA seems to be criticly invoved in determination of virulence and pathogenicity. The thesis is compact, elaborative and written by good english. In literature overview the author describes flaviviruses first in general, then with focus on the function of sfRNA. This part of thesis covers studied topic in sufficient extend and detail. Throughout the thesis each experiment is explained why and how was done which gives overal good impression about author's analytic thinking and capability to chose what is important. Numerous technics of molecular and cellular biology were used to achieve set goals, methods were precisily and clearly described. Obtained results are rigid and correctly presented. In addition I would like to emphasize that the load of experimental work was high as well. In spite of few small errors/misspellings present, it is probably one of the best written thesis I ever reviewed.

Comments:

1. It has been concluded from results in Figure 15 that sfRNA from TBEV has no efect on *de novo* rRNA. I am not sure that the quality of shown image is sufficient to make this conclusion.
2. On page 22, 5x concentrated reducing buffer without DTT was used. I would recommend not to use the term „reducing" unless the buffer is really reducing (containing reducing agent as e.g. DTT).
3. When the percentage in numbers is written, the space should be between.
4. On page 54, the reference to the ribosome shunting is missing.
5. The formula for relative quantification is not precise, minus is missing ($2^{-\Delta}$ delta-delta c_t).

Question:

1. There is not quite precize statement on page 18 about what pattern recognition receptors recognize. Correct the statement and state which virus- relevant PRR are present in DAYO cells.
2. As you use and compare sfRNA from four flaviviruses, it would be useful to show the image with the structures of this region for comparison. Could you tell us what diferences are between Hypr and Neudoerfl in 3'UTR?
3. On page 22, a secretory pathway for flaviviruses is described. Is Golgi pathway the only secretory pathway utilized by flaviviruses?
4. You presented the relative quantification of sfRNA upon transfection as a log10 fold-change in Figure 10. It is not clear to me how exactly was this quantification performed. What sample/value was used to set up a reference value to 1? In addition there are significant differences in the amount o sfRNA upon transfection between samples. Do you have some theory to expain it? Have you ever tested whether/how the amount of sfRNA detected upon transfecion corresponds to the amount of 3'UTR region of viral gRNA detected in virus infected cells?
5. You mentioned that the samples (Figure 10) were adjusted according their viability. How was the viability of cells affected by introduced sfRNA?
6. Figure 12 presents successful click-on-membrane assay of HPG labelled *de novo* synthetised proteins. What was used as positive control in this experiment? There are 4 clear bands detected. Do you have some idea what kind of proteins they are?
7. In discussion you mention that DENV sfRNA induces specific translation arrest in contrast to TBEV which induces non-specific translational shut-off of the synthesis of host proteins. I think that on Figure 12, the same negative effect of DENV sfRNA as with sfRNA from TBEV on *de novo* protein sythesis can be noticed. This observation deserved to be mentioned in discussion. Please, comment it.
8. It is believed that sfRNA functions as determinant of virulence and pathogenicity. Would you know how to design the experiment to test it?

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 České Budějovice date 14. 1. 2021.