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: Thomas Stehrer

Thesis title: Testing knockdown of nucleotidases and the effect on e-Ado production during immune response in *Drosophila melanogaster* larvae
Supervisor: doc. Mgr. Tomáš Doležal, Ph.D.

Reviewer: Mgr. Lenka Bittová, Ph.D.
Reviewer`s affiliation: Biology Centre CAS, Institute of Entomology

	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	2.5
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2.5
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2.5
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		18.5

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

(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	2.5
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	2.5
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2.5
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	2
Practical requirements – points in total		24

POINTS IN TOTAL (MAX/AWARDED)	48	42.5

Comments of the reviewer on the student and the thesis:

The author presents a well-written bachelor thesis investigating mechanisms of adenosine signaling in immune response, a very relevant and timely area of research. The scope of the conducted project is reasonable for a bachelor thesis, the aims are clearly stated, experiments well described and carried out in a logical order. The author demonstrates successful acquisition of experimental techniques necessary for work with *D. melanogaster* and an ability to utilize an established method for extracellular adenosine measurement. Despite the inconclusive outcomes, this work can be build up upon in further research.

Suggestions and questions, to which the student has to answer during the defense:

1. Your work suggests that permanent double knockdown of *D. melanogaster* nucleotidase genes *CG11883* and *NT5E-2* arrests the development prior reaching larval stages. Would it be possible to trigger RNAi after embryonic development?
2. Would it be possible to manipulate adenosine signaling via SAM pathway?

3. What happens to inosine generated by ADA reaction (Figure 1)?
4. How is ATP transported out of the cell (Figure 1)?
5. Would it be possible/beneficial to perform metabolomics analysis on isolated hemocytes instead of lymph gland? Is it possible to collect and culture hemocytes from larvae?
6. Fluorescence-based assays for assessment of adenosine levels in human plasma and urine are commercially available (Abcam, BioVision, etc.). Do you think that they could be adapted to *D. melanogaster* research?
7. According to your discussion, extracellular adenosine in your samples might be degraded by adenosine deaminase. Does it mean that the added ADA inhibitor is ineffective? Is there a way to check that?
8. Would a knockdown of one nucleotidase be sufficient to generate a phenotype?
9. Could be the spectroscopic method for e-Ado measurement affected by a possible DNA contamination from the dissected tissue?
10. Are there any known adenosine receptor antagonist in Drosophila (such as caffeine for human AdoR)? Could they be employed to study adenosine signaling?

Mistakes, which the student should avoid in the future:

1. Use of abbreviations: Title of the thesis should not contain any abbreviation. Every time an abbreviation is first introduced in the text, it should be preceded by a full name (e.g. SAM in the Abstract, etc.). Your full term for RT-qPCR abbreviation does not explain the "q" for quantitative.
2. The Table of Content (not "contents") - Annex could list the items included.
3. The introduction would be improved by a better explanation of aerobic glycolysis and its comparison to oxidative phosphorylation. A diagram depicting the relevant metabolic pathways and their relationship would certainly help to orient a reader.
4. The name Thomas Hunt Morgan is misspelled.
5. The section's 1.1.1 title could be: "Immune response in *D. melanogaster*" to narrow it down.
6. The terms *in vivo, ex vivo, in vitro* should be italicized.
7. Chapter 1.4 could contain a formula for adenosine and perhaps the enzymatic reaction catalyzed by the nucleotidases that are being knocked down as well as adenosine deaminase reaction as it is discussed in 1.4.1.
8. It is not clear (in the section 1.4.1) if the *ADGF* genes directly encode the

adenosine deaminase or just regulate its expression.

9. Text in the section 1.4.2. contains an extra caption of Figure 1 that doesn't belong there.
10. Figure 1 needs a better caption explaining all the abbreviations and perhaps highlighting the enzymes knocked down in your work.
11. The end of the 1.4.2.1 section is unclear – it needs rephrasing.
12. The annotations of fly genotypes might need an explanation for a non-expert What does lat-, FM7 GFP, TM3 Ser GFP and Cyo mean?
13. What is the composition of TRIzol buffer and TRI reagent mentioned in section 3.5.1?
14. The title of 3.5.3 section should include the word "Quantitative" or "Real-time" PCR for a better description.
15. The title of 4.1 could be "The initial extracellular adenosine measurements"
16. Figure 2 presents a box plot for the first time. Please explain the box plot features – vertical and horizontal lines, quartiles, median, whiskers, small circles etc.
17. Figures 3 and 4 need more descriptive graph titles, not "Primer for..."
18. Some of the references are incomplete (missing page numbers in Chiang et al, "(February)" in Kavi et al., "(63)" in Small et al). Strasser, P. (2016) reference is not cited properly. It is not obvious that it refers to a bachelor thesis.

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 8.6.2018

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signature