



Přirodovědecká
fakulta
Faculty
of Science

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

STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student: Dajana Tanasić

Thesis title: The role of CG18446 gene in immune response in *Drosophila melanogaster*

Supervisor: RNDr. Alena Krejčí Bruce, Ph.D.

Reviewer: Mgr. Adam Bajgar Ph.D.

Reviewer's affiliation: University of South Bohemia, Department of Molecular Biology and Genetics

	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	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	3
Formal requirements – points in total		21
(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 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
Experimental difficulty of the thesis, independence in experimental work	0-3	3

* Choose one

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
Formal requirements – points in total		27

POINTS IN TOTAL (MAX/AWARDED)

48

(0-48)²

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

Q1 – Data presented in the work suggest that the gene CG18446 plays role in several organs. What genetic tools can be used for tissue specific analysis of gene function in Drosophila and why didn't you try such possibilities?

Q2 – Hemolymph was used as a marker of matured immune cells, is there something known about the homologue of this gene in mammals and regulation of its expression?

Q3 – There is strong difference between females and males in stress resistance experiments, how would you explain these observations?

SUGGESTION – the role of the CG18446 gene in stress resistance was shown mainly on adult flies, therefore I would be very careful about connecting of these observations with the data from larva – there is possibility that genes important in regulation of larval hematopoiesis play completely different role in adults where the hematopoiesis is already finished.

Eventual mistakes, which the students should avoid in the future:

Even though I tried really hard, I was not able to find any mistake to be mentioned.

Eventual additional comments of the reviewer on the student and the thesis:

The whole work is on very high level. The particular parts are logically divided and excellent in explanations.

Introduction gives us very good understanding of the problematics, ideas of the author, and reasoning why these particular aims were selected.

Materials and methods are described in details necessary for repeating of the experiments without boring unwanted details.

In result we are guided through the data with emphasis to experimental logic which gave us very comprehensible text. All questions raised during reading of the text are answered in the next few sentences.

The real strength of the author is fully exposed in the discussion, usually the most problematic part. In this case we can read an excellent section describing the connection between obtained data, published and unpublished results, their interpretation (often very open minded) together with suggestion of necessary experiments to clarify emerging hypothesis. Readers of this part must be completely convinced that author is already very well developed in scientific kind of thinking and able to write very good scientific publication.

It is also important to mention that work contains a big amount of data in supplement. These data are not used just as simple appendix but are comprised into the discussion and are involved in formation of the overall picture of CG18446 role.

At the end of the review I would like to congratulate to Alena Krejci and Zorana Mihajlović, because their role in the education of such student is much better achievement with bigger impact

² Enter the number of points awarded.

than publication in the best journals.

Conclusion:

**In conclusion, I strongly
r e c o m m e n d**

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

In České Budějovice date 3.1.2017

A handwritten signature in blue ink, reading "Aden Bajger", written over a horizontal dotted line.

signature



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STATEMENT OF THE DIPLOMA THESIS REVIEWER

Name of the student: Bc. Dajana Tanasić

Thesis title: The role of CG18446 gene in immune response in *Drosophila melanogaster*

Supervisor: RNDr. Alena Krejčí, Ph.D.

Reviewer: Mgr. Hana Sehadová, Ph.D.

Reviewer's affiliation: Institute of Entomology, Biology Center Czech Academy of Sciences,
Branišovská 31, 370 05 České Budějovice, Czech Republic

The presented diploma project is aimed to investigate the role of CG18446 gene that is proposed to be involved in the Notch signaling pathway of cell to cell communication. Based on the preliminary results that localized the expression of CG18446 gene in the lymph gland of *Drosophila melanogaster*, the involvement of this gene in the *Drosophila* hematopoiesis was investigated. The students mastered in several techniques of the molecular biology. An anti-GFP antibody was used to immunohistochemically examined the expression of CG18446 gene in CG18446-GFP transgenic flies. The localization of GFP signal in the lymph gland was compared to the distribution of different type of blood cells. The X-gal staining in several larval and adult tissues of CG18446-lacZ flies was performed and influence of the CG18446 on the number and the class of hemocytes was analyzed.

The master thesis has 75 pages. It contains introduction, material and methods, results, discussion, conclusion and 12 pages of the supplementary data. Together 90 references of scientific papers used in this thesis reflect ability of the author to work independently with scientific English literature. Detailed description of *Drosophila* immune system and hematopoiesis together with high quality results followed by well organized discussion exceed requirements of a master thesis.

I have only several comments and suggestions:

- 1) There is no specification of the percentage of Triton X-100 and BSA in the PBS buffer. What is the function of Triton X-100 and BSA in the immunohistochemical procedure?
- 2) All secondary antibodies are not characterized (i.e. donor animal, antigen, fluorophore, company), in some cases also the information about the working dilution is missing).
- 3) For mounting medium CityFlour please specify the company.
- 4) It would be better to remove the microscopy from paragraph "Statistics and microscopy" and in "Immunohistochemistry" and "X-gal staining" paragraphs specify the type of the microscopes and the company. Which type of the confocal and bright-field microscope did you use?
- 5) In legend to the Figures 10, 11, 14, 15 there is no note about the DAPI staining.
- 6) Did you perform some negative control to verify that the GFP staining in the lymph gland is not due to unspecific binding of anti-GFP antibody you used in your study?

- 7) Please specify how did you measure the intensity of GFP signal (the expression of CG18446) in the Figure 13? What are unites on the Y axis? Did you made a background subtraction?
- 8) Did you considered the possible role of CG18446 in the vitellogenesis? The follicular cells in the ovary are involved in the vitellin synthesis and in the transduction of vitellin from the fat body. Less volume of the yolk can caused a reduction in number of laying eggs.
- 9) Since quality and quantity of the signal of the reporter gene can vary depending on different insertion site. Do you think about production of anti-CG18446 antibody and/or performing *in situ* hybridization experiments?
- 10) Neither in the text nor in the legend to Figure S2, you did not specify if the signal is due to the fluorescence of tagged reporters or if you used antibodies against the reporter.
- 11) Based on your funding about the possible role of CG18446 gene, did you think about the suitable name for this gene?

The thesis solved the determined aims and partially revealed the function of CG18446 in immune response. Also the possible roles of CG18446 in stress response and in oogenesis were discussed. Author eruditely answers all questions that come out. Also the formal part of the thesis fulfill all essential requirements of a diploma thesis.

I recommend the thesis for the defense and I suggest the grade excellent.

In České Budějovice date 13.1.2017

Hana Sehadová



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