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CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

UNIVERSITE PAUL SABATIER TOULOUSE III

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Michele CROZATIER 's report on Milena NOVAKOVA's PhD defence .

During her PhD work, Milena NOVAKOVA worked on the regulation of the extracellular adenosine level by ADGF-A in *Drosophila melanogaster*. Adenosine exists as a cellular metabolite and as an extracellular form that serves as a signaling molecule. Abnormal regulation of adenosine leads to several pathologies including immunodeficiency in humans. Given the high conservation of genes and signaling pathways during evolution and *Drosophila melanogaster* being a very powerful genetic model, Milena NOVAKOVA decided to investigate the regulation of adenosine levels by studing adenosine deaminase activity in Drosophila. Adenosine deaminases (ADAs) are required for adenosine degradation. The adenosine deaminase called ADGF-A is the main source of ADA activity during Drosophila development. During her PhD work Milena NOVAKOVA studied ADGF-A expression through the construction and characterization of a ADGF-A reporter line.

In the first part of her project, a reporter line for ADGF-A was constructed via the ends-in homologous recombination method, a technique recently described. The ADGF-A coding sequence was replaced with the coding sequence of a destabilized version of GFP now expressed under the control of ADGF-A regulatory sequences.

In the second part of the PhD project, the ADGF-A reporter line expression profile was analyzed during Drosophila development. Whereas both ADGF-A and ADGF-AGFP reporter mRNAs are expressed in most Drosophila tissues, the GFP was not detectable in normal conditions. These data reveal a posttranscriptional regulation of ADGF-A by a yet unknown mechanism. However, ADGF-A GFP reporter line expression was strongly increased in adhering hemocytes (which correspond to immune cells) during immune responses. Various mutants inducing melanotic tumors and natural immune challenges such as wasp parasitism, induced GFP expression in hemocytes present around melanotic tumors. This indicates that the acute expression of ADGF-A protein is not induced by one specific signaling cascade but is associated with the behavior of hemocytes during the inflammatory response .

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This work gave rise to a publication in a high impact factor journal where Milena NOVAKOVA is the first author. The PhD work performed by Milena NOVAKOVA under the direction of Tomas DOLEZAL is very rigorous. The successful adaptation of a technique recently developed in Drosophila to perform homologous recombination is remarkable. The data obtained lead to novel questions that open large perspectives regarding the way ADAs are involved in controlling adenosine levels. Milena NOVAKOVA has shown her capacity to run a research project and contribute to opening novel research axes.

Based on these conclusions I strongly support Milena NOVAKOVA's work in order to obtain her PhD degree.

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Review of Ph.D. thesis written by Mgr. Milena Nováková: Regulation of extracellular adenosine level by ADGF-A in *Drosophila melanogaster*

Mgr. Milena Nováková's Ph.D. thesis describes the role of adenosine deaminase-related growth factor A (ADGF-A) in regulation of extracellular adenosine in *Drosophila melanogaster*. For this purpose new application of ends-in homologous recombination method was used to create an expression reporter system with dGFP, which allows detection of changes in adenosine deaminase activity *in vivo*. Results show low level of ADGF-A in control flies, but its increase after immune stimulation in locally adhering haemocytes, where probably the behaviour of haemocytes leads to higher ADGF-A level. The ADGF-A protein expression is suggested as an important factor for glucose release in the site of immune reaction. In addition, the results provide an interesting comparative approach showing evolutionary ancient regulation of inflammatory response.

The thesis (57 pages) consists of introduction part, methodological part describing development of GFP reporter system (Part I), and published article (Part II). The Introduction provides the background about adenosine, adenosine receptors, nucleoside transporters, ectonucleotidases, and adenosine deaminases. The information is explained in human first and than homologs in flies are added. Part one is structured in a form of a scientific paper and also presents unpublished data. A detailed description of demanding selection of *adgf-a* mutants documents the amount of time-consuming work with flies and necessary knowledge of molecular methods needed for experiments. Developed GFP reporter system is included in published article (Part II). Mgr. Milena Nováková published the results as first author of scientific paper in an international, peer-reviewed, open-access journal Plos One (IF = 4,351) which is considered as high quality journal in the fields of molecular biology, entomology or genetics. The peer-review process in the journal redaction is also a guarantee of results quality. The thesis is written in English, the text is understandable, results are clearly documented and discussed with correct using of references (39 in Introduction + 22 in Part I).

Comments:

- The aim of the study should be clearly stated at the end of Introduction.
- Page 3: "*Drosophila* and probably insects in general contain only a single adenosine receptor homolog (AdoR,)." To which human adenosine receptor is the most functionally related Ado-R in *Drosophila*?
- Page 8: "during evolution ADA lost its enzymatic activity due to accumulated mutations and it seems likely that the members of AGDFs family replaced its function." According to previous information, can we find ADGF-A only in *Drosophila* or we can enlarge it to Diptera, all insects or even invertebrates?
- Page 11: "Oxidative stress preserve ADA activity in humans." It would be interesting to study AGDFs also in insects with induced oxidative stress.
- Encapsulation of parasitic wasp eggs was tested and during this reaction melanotic capsules were determined. Is there any idea what will happen after block of phenoloxidase cascade? How it will affect the flies (and ADGF-A expression) after wasp parazitation?
- If the ADGF-A is related to glucose release, is there any connection also with insulin signalling pathway (insulin receptor as a potent regulator of cell growth)?

Minor correction:

- Fig. 1. – poor quality of the figure.
- Drosophila x *Drosophila* is not uniform in the thesis and literature cited
- List of abbreviations used could be useful for the readers.
- Page 19, last sentence – wrong reference order.

Evaluation:

To produce highly valuable results suitable for publication in reputed scientific journal Mgr. Milena Nováková needed large experimental up to date background in molecular biology and *Drosophila* techniques. Except published article, she attended two foreign stays and is co-author of four international conference presentations. Ph.D. thesis is well written and meets the requirements, thus **I suggest to accept the thesis for defence as one part of Ph.D. degree**.

Yours sincerely,

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