KATEDRA MOLEKULÁRNÍ BIOLOGIE A GENETIKY
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Reviewer's comments on the bachelor thesis of Klara Kopicova

Klara Kopicova thesis aimed to solve a crucial question in the field of adenosin signalling within the context of the Drsoophila selfish immune system: Could elevated levels of adenosin during an immune challenge directly stimulate the development of lamellocytes via adenosin receptors present on the surface of immune cells? Although this is a simple question the way to answer it is not straightforward. Due to the lack of functional RNAi lines, the only way to specifically knock-down adenosin receptor in immune cells was to use FLP/FRP system to create clones of cells homozygous for the adenosin receptor mutant allele. Although the use of clonal analysis in other Drosophila tissues has a long tradition nobody so far attempted to apply this approach for immune cells. Therefore, Klara not only set up an interesting, important question, but she also used new technique that required to be established and involved relatively complicated genetic crosses and verification of the transgenic elements present. Also, the infection experiment itself is not an easy method and although well established in Tomas Dolezal's lab it requires a lot of patience, skill and time. From this respect Klara did a great job. She succeeded in creating the new genetic tools and she used them to answer the question she asked at the beginning. She concluded an important result that will move the adenosin field a step further and she created tools that will be used by Tomas Dolezal's lab and most probably also by other people who study the Drosophila immune system.

The only downside of the project is the bachelor thesis on its own. It is obvious that the thesis was written up in a hurry and therefore it contains a lot of inaccuracies and gaps that make it difficult to convey the message to the reader. I do appreciate that Klara decided to write her bachelor thesis in English, despite the fact the she did not have to. Therefore I do not mind that some of the sentences, especiall in the introduction, were rather difficult to understand. Nevertheless, the text throughout the thesis contained some wrong statements (like p.1 insulin is a steroid hormone), the method section had many important details missing or wrongly stated and the results were not described with sufficient detail. I will list some of these problems, in the order they appeared in the

thesis. These do not need to be answered during the defence but I list them so as Klara can think about them and perhaps take some inspiration for a future writing.

- the introduction lacks any pictures. As the text itself is quite brief I think that a few pictures would help a lot the readers outside the filed to better understand the scientific background. For example, picture of the current model of the adenosin signalling within the context of the selfish immune system, the picture explaining the principles of the creation of mitotic clones or a scheme of the FLP/FRT and UAS/Gal4 systems.
- Chapter 2 should not be called a 'Summary' but rather something like 'Aims of the thesis'
- instead of using the simple label 'FRT' in the genetic schemes in Fig.1-5 it should be correctly written as 'FRT82B'
- the schemes for genetic crosses in Fig. 1-3 imply the use of TM3/TM3 or TM6/TM6 balancers. Are you sure these are really the flies you used?
- how did you recognize the double recombinant in Fig.2 cross C2 and the triple recombinant in cross C4 from the flies that did not recombine - what are the genetic markers for each elements? Why is it important to set the C4 crosses from a single fly?
- the purpose of the crosses in Fig.5 remain a mystery for me. If this should be the overview of the crosses used for the actual infection experiment then it is wrong. You did not cross to UAS-FLP/UAS-FLP but to a line that contained FRT82B, UAS-GFP and UAS-FLP elements. You also probably forgot to list this line in your Table 1. The scheme in Fig.5 is very important for the understnading of the whole next experimental part and having it wrong confuses the reader. It is not until page 24 in the title of chapter 4.3.1 when the reader finally gets the exact description of the experimental cross. And yet, the description of the other cross is again not correct in the following section 4.3.2.
- PCR reaction described on p.18 certainy did not contain 10uM primer in its final concentration; the 10uM was the stock you used to pipette into the PCR mixture. Same with the amount of DNA in the PCR reaction. As you squashed a single fly you did not quantify the DNA present and you did not know hom much DNA you put in the reaction.
- the legend to the figures often lack the necessary detail needed for understanding. For example, for the description of the gel pictures in Fig.7-9 the reader has to go back to the main text. Also the numbers that should describe the size of the bands in the DNA ladder are completely misaligned. Same wiht the description of all the other figures in the result section that is incomplete and does not allow the reader to understand how the experiment was actually done and what the result shows. The legend to the figures should be self-explanatory, so as the reader does not have to fish the information in the main text.
- it would be better to present the data in Fig.17 as the ratio of gree/non-green lamellocytes rather than the raw numbers of green and non-green lamellocytes.

These are three specific questions that I would like Klara to comment in her defence:

1) The genetic scheme in Fig.1 leads to the creatin of the Srp-gal4;;FRT82B line in 5 generations of crosses. How would you simplify the work by using a double balancer line (a line containing balancers for X. and III. chromosomes, for example UAS-Gal80ts/FM7;;TM3/TM6 flies)?

2) You proved that the number of lamellocytes differentiating after an immune challenge does not change in wildtype versus AdoR mutant clones. This does not exclude the possibility that AdoR has, for example, has a role in the proliferation/maintenance of the hemocyte precursor or plasmatocyte pool. Did you count the non-lamellocyte cells? Have you noticed if the larvae have roughly a similar survival rate after the infection?

3) You excluded the role of AdoR present in the immune cells on the formation of lamellocytes during infection. What are the alternative models to explain the adenosin effects during the immune challenge and which future experiments would you suggest to distinguish between these models?

It is obvious that Klara did a lot of good work in the lab and that is what we should appreciate the most. If she payed more attention while writing up and presenting her results, the thesis would be perfect. It is a steep learning curve for a bachelor student to become a scientist and Klara certainly has the potential. I am looking forward to her master thesis!

Alena Krejci