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Statement to Kristina Preuer bachelor thesis:

The bachelor thesis "Study of Ectonucleotidases and Adenosine Deaminases in *Drosophila*" by Kristina has 28 pages and is divided to 5 main sections (Introduction, Materials and Methods, Results and Discussion, Conclusion, and Bibliography with about 35 references) and 2 additional sections (List of Tables, and List of Figures).

The aim of this thesis was to test the possibility of down-regulation of three proteins (ADGF-A, NT5E-1 and NT5E-2) by RNAi in *Drosophila* and additionally to investigate and quantify the effect of over-expression of Dicer 2 on the down-regulation of ADGF-A and NT5E-1 proteins.

First of all Kristina had to cross flies with different genotypes to generate flies carrying the required mutations. Subsequently Kristina wanted to test if the levels of proteins of interest were down-regulated by using confocal microscopy and GFP tagged proteins. Afterwards she used qRT-PCR to test if the levels of RNA of these genes were also down-regulated.

Kristina was successful in generating flies carrying NT5E-1 RNAi mutation and control flies with normal level of NT5E-1 protein. On the other hand and despite the fact that the work was carefully done and I´m sure the methodology of the crosses was chosen properly, Kristina was not able to generate flies with ADGF-A RNAi and NT5E-2 RNAi mutations. Kristina discusses this unsuccessful result very well and she also proposes the solution that she was not able to try because of the time limit.

The results with NT5E-1 mutated flies are very promising. First of all Kristina's results from confocal microscopy support the hypothesis of Míša Fencková namely that NT5E-1 protein is GPI anchored to the membrane. Kristina also proved that the silencing of this gene was successful both on the protein (confocal microscopy) and on the RNA levels (qRT-PCR). Since Kristina succeed only in NT5E-1 experiments, I think she should dedicate more writing space to the results of NT5E-1 experiments. The legends of the figures of NT5E-1 results are too simplified and without important details. For example in fig. 7 what type of cells is on the picture, what does the green and blue color stand for; in figures 8 and 9 what does the blue and green color stand for; in figure 10 what the whiskers and relative mRNA ratio mean. None of the figures of the "Results and Discussion" chapter are mentioned in the text, I consider this a serious mistake.

In addition, I have to point out these formal mistakes in the text:

- Methods:
  - the company producing TriZol Reagent calls INVITROGEN instead of VITROGEN (p.16)
  - diethylpyrocarbonate treated water should be used at first instead of DEPC water, then the abbreviation could be used (p.16)
  - molarity of the primer and dNTP mix used in cDNA synthesis is missing (p.16)
  - tab. 3 is not mentioned in the text (there is Trab. XX instead) (p.17)
  - the length of the qPCR products is missing (p.17)
- Bibliography:
  - 6 references mentioned in the text are missing in the list of references – I consider this a serious mistake. (It is namely Dietzl et al., 2007; Fencková et al., 2011; Morgan, 1910; Radoslaw et al., 2009; Yegutkin, 2008; Zhang, 2008)

Questions:

1. Do you think that there is any possibility to avoid negative result while producing ADGF-A and NT5E-2 mutant using different stock of flies (if you had more time)?
2. What kind of method did you use to calculate the RNA level of *nt5e-1* gene? Was it the comparative method of relative quantification (ddCt)? If yes, did you somehow count the efficiency of the primers you used?
3. Since you didn´t mention how long the PCR products were, I wonder if it was the same for the gene of your interest as for the housekeeping gene. And if not how did you cope with the fact that the intensity of fluorescence will be higher for larger products?
4. Do you know any other methods how to support your NT5E-1 results (down-regulation on both the protein and RNA level)? Do you think you would be able to use them in your laboratory?
5. Why do you think you were so successful in RNAi in comparison to other studies?

In summary, I think that despite the short-term stay in Tomáš Doležal´s laboratory Kristina made a big piece of work. I find this to be a very good bachelor thesis which I strongly recommend for a successful defense with excellent minus or excellent mark.

Olga Bazalová