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Opponent review of Bachelor thesis of Andrea Eder – Investigating the Regulation of Notch Signalling in Drosophila by Trxr-1 gene

Andrea tried to test a potential influence of Notch pathway by Drosophila thioredoxin reductase *Trxr-1* based on a similar expression pattern of this gene with region of a high Notch activity. Her bachelor thesis is written on 30 pages with good English and clear structure. The pictures are in general of high quality and the thesis fulfills all formal requirements.

I was surprised by the amount of work and the range of used methodology; students in this program usually have relatively short time to finish their theses. Andrea clearly adopted many techniques from genetics, molecular biology and developmental biology and she applied them successfully in her work. I was very pleased by that. This fact also demonstrated that Andrea worked on her thesis in excellent group of Alena Krejčí.

Although the presented work is of high quality, there are still some weaker points in the text (results and methodology) which confuse the reader, sometimes I had to jump from one part to another and it was little harder to get the message. For example:

Figure 7 - *"We selected the third instar females from this cross (as males would not contain the arm-lacZ reporter and FRT19A site for recombination) and analyzed the effect of missing Trxr-1 on the expression of cut and wingless genes by immunostaining (Figure 7)."* I do not think that this is on Fig. 7. There is no lacZ reporter on Fig. 7, there is a recombination between FRT and trxr mutant.

Figure 8B - *"After we confirmed that the stock is correct we crossed the females to males bearing ubx-FLP (a flippase under the control of the ubx promoter which is specifically active only in the wing discs) and an arm-lacZ reporter to generate mitotic clones of Trxr alleles (Figure 8B)."* I do not think that this is on Fig 8B. I did not find any information about the lacZ reporter and the chromosome carrying it or cross for mitotic recombination.

Figure 17 – is *cut* or *wingless* shown on this figure?

In general legends for the figures are too simplified without important details.

Questions:

1. Based on the aims the author seemed to hypothesize that Trxr somehow influences the expression of the Notch target genes. What is this hypothesis based on? Could Trxr be influenced by the Notch signaling instead, i.e. to be a Notch target gene?

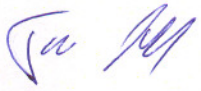
2. The work is based on the expression pattern. On page 24 the author states: *"The CPTI line showed a stripe of enhanced GFP expression at the dorso/ventral boundary of the disc which overlapped with the cut staining."*
Enhanced GFP expression compared to what? I do not really see any enhancement – could the difference in intensity be due to the morphology of the disc? How exactly was the Figure 9 produced (confocal microscopy? technical details …)

3. Fig 11 and 12 – Cut and Wg normal staining in wild-type discs should be shown for a comparison even though there was no effect; in this way the presented result is not complete. Area of En-Gal4 expression in discs could be marked. There seems to be some effect on Wg staining in Fig 12 Trxr-1 MITO (left side of the discs – En-Gal4 part?) – can the author explain that?

4. Page 28 – although the Real-Time PCR confirmed quite clearly the presence of the deletion on the recombined chromosome and it was kind of "cool" molecular technique to do so, wasn't this approach too complicated for this particular purpose? In case when the deletions were molecularly well defined, was it possible to design primers in different way to confirm the deletion by a presence of the PCR product instead of the absence and do it by one simple PCR reaction for each deletion? If yes, how would the author design such primers?

In summary, this is very good bachelor thesis which I strongly recommend for a successful defense with excellent minus or excellent grade.

In České Budějovice, June 7, 2012



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