Ceske Budejovice, 14th June 2010

Review of the bachelor thesis of Radka Hobizalova

The Bachelors thesis of Radka Hobizalova entitled 'Developmental profile of putative ecto-5'-nucleotidase genes of *Drosophila melanogaster*' describes work aimed at characterising the temporal mRNA expression of four candidate genes with potential ecto-5'-nucleotidase activity throughout *Drosophila melanogaster* development. In mammalian systems genes with ecto-5'-nucleotidase activity have been shown to effect extra-cellular adenosine (a potent biologically active metabolite implicated in numerous and varied patho-physiological roles), levels by catalysing the conversion of AMP to adenosine. Whether such activities exist in the *Drosophila melanogaster* model is unknown. As such four potential ecto-5'-nucleotidase homlogs were identified, termed NT1-4, in *Drosophila melanogaster* and PCR primers and conditions were optimized to measure their expression during development by RTPCR. Finally cDNA samples were generated to permit later cloning of these genes and further biochemical analysis informed by the results of the expression assays described above.

In general the thesis was well written and easy to follow. The comprehensive Introduction section was particularly impressive and well written. However there were some problems/ inconsistencies in the reporting of the methods and results that detracted from the overall impression. These are expanded on below:

1) When one writes the methods, they should be in sufficient detail that the reader could repeat your experiments and arrive at the same result. Therefore, in describing the PCR and reverse transcription one should state the concentration of the primers used and the number of enzymatic units etc. In papers this can often be included in a citation but for a thesis it is better to be comprehensive.

2) The references to tables in the main text are not consistent with those in the table legends e.g. page 14 refers to table 2 (PCR program conditions) when the information is contained on table 3. This error continues up to and including table 4 that is actually table 5.

3) It would be useful to modify table 7 to include an extra column that gives a cross-reference (*e.g.* lane number) to figure 7 to enable the reader to know which developmental stage is which more easily. Although as far as I can tell there is no actual reference to this table in the main text!

4) Section 4.1 entitled 'Optimization of primers' would benefit from an extra introductory sentence that makes it explicit that the aim of these experiments are to ascertain the optimal annealing temperature for each primer pair in the PCR assays.

5) In the data presented in Fig. 8 and accompanying main text the author makes a number of observations about low expression and poorly visible bands. I would suggest that inverted images (*i.e.* black bands on a white background) would greatly aid the interpretation. For example, in the data for NT3C I would say from the gel that there is only expression in lanes 2, 5 and 8 but it is reported that is also low expression detected in lanes 4, 6 and 7.

6) Note that there is inconsistency between the main text and the legend in Fig.8 concerning which lanes represent the expression at the adult male and female stages. Please correct this. As a consequence one cannot make the conclusion stated in the Discussion section that *'First of all genes, the genes were more expressed in the male than female adults'* with any confidence.

7) Although I do not require the following to be changed in this thesis, it is always best to show any gel images in their entirety together with the appropriate size markers. This way the reader can see whether there are any non-specific products and more easily ascertain the size of the main product.

I do have a couple of other questions relating to data and students basic understanding of the assays used and underlying biology.

a) The NT1 and NT2 genes appear to be adjacent on chromosome 2R. Can the student speculate about their evolutionary origin?

b) Could the student expand why she thinks that in the optimisation of the PCR conditions for NT2 she observed a spurious band of larger than expected size?

c) Can the student think of a way of confirming the products she detects by RTPCR are not as the result of potential contamination in her samples. *i.e.* an appropriate negative control?

d) In terms of PCR primer specificity, would the student expect that lowering the annealing temperature of primers would result in more or less spurious products being amplified? – *in reference to sentence of page 18 ' PCR at 58oC yielded no band, which can be explained either by the fact that the temperature is too low for the particular genes or it was caused by some PCR mistake'.*

e) Finally given the expression profiles obtained, which of the characterized NT genes would she recommend to be prioritised for biochemical analysis assuming all the cDNA's were available?

In conclusion it is my judgement that this thesis of Radka Hobizalova is more than satisfactory for the Bachelors degree program and that it is passed with an 'excellent' mark for the record.

Alexander W. Bruce, Ph.D.