MASAKO ASAHINA-JINDROVA, PH.D
BIOLOGY CENTRE
ACADEMY OF SCIENCES OF THE CZECH REPUBLIC

BRANIŠOVSKÁ 31, 370 05 ČESKÉ BUDĚJOVICE
CZECH REPUBLIC
PHONE&FAX: +420-38-777-5426
E-mail: masako@paru.cas.cz

Bc. Thesis Review

Author: **Tamara Bernadette Aigner**

Title: **Study of Dco role in *Drosophila melanogaster* hematopoiesis**

This thesis is focused on whether RNAi construct targeted for Disc overgrown gene (Dco), the homolog of Casein Kinase I epsilon gene that is involved in human carcinogenesis, can be used to monitor loss of function phenotypes especially in hepatocytes in the fly. Ubiquitous and tissue-specific RNAi were performed utilizing Gal4-UAS system with three independent promoters (actin, engrailed and hemolectin) and phenotypes caused by *dco* RNAi were further analyzed.

The thesis was well structured and was easy to follow. Although some parts of writing style were more like essays (e.g. "The flies seem to be very fragile, but they are not." in page 3) and some wording could be improved (e.g. contamination instead of "plagues", fly-pushers etc.) for scientific writing, I could feel that Tamara was enjoying and astonished with her first experience in developmental biology. Tamara clearly stated the aim of the study and evidently she understood her tasks. She performed the series of genetic crosses carrying those transgenes, select the genotypes of interest, then dissected the larvae to further observe the morphology of tissues such as imaginal discs. The number of hemocytes was also counted. She went through a nice flow of work and achieved to observe defects by Gal4-UAS-driven RNAi.

Here I would like to ask some specific questions.

[1] In Introduction, chapter 1.3.1 describes the advantages of balancer chromosomes, however the purpose or the importance of having balancers is missing. Could you explain why you don't want recombination and why markers are useful when you perform genetic crosses?

[2] When $RNAi^{dco}$ was driven by *actin* promoter, it is noted "the larval period took very long, longer than the larval period of the tubby TM6B larvae". Can you be more specific? Is the difference by hours, days or weeks? Is this phenotype also enhanced with the addition of Dcr?

[3] When extra Dicer was introduced into $RNAi^{dco}$ background, flies are generally sicker and seem to have slow development and partial sterility. I imagine that having more Dcr protein can enhance overall RNAi machinery in the fly. Are those all defects described in the chapter

3.2 specific to *dco* or does UAS-Dcr itself cause some abnormality? What kind of control experiment can be done to test this possibilities?

[4] Fig. 11 shows GFP positive cells. In both Materials and Methods and Results mention *Act* GAL4 without - UAS GFP. The only construct mentioned with GFP was *Hml* Gal4-UAS GFP. Are these cells really from the fly carrying *Act*-GAL4?

[5] Author argues that *Hml* RNAi^{dco} did not show the hemocyte number defect due to too late activity of the promoter. Is there other promoter available to target the earlier stage of haematopoesis? How about the possibility that hemocyte number is regulated non-cell-autonomous manner?

I would like to evaluate here Tamara Bernadette Aigner's Bc. thesis "**Excellent**" and happily recommend to grant Bachelor degree.

České Budějovice 13 June 2011

Masako A-Jindrová, Ph. D