University of Cyprus
Department of Biological Sciences

## Ph.D. Thesis Defense

# Student Presentation

**Thursday, 18 May 2023 at 11:30**

***This seminar is open to the public via Zoom***

**CTF02 Building, class 011 (ΧΩΔ02- αίθουσα 011)**

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## Anastasia Ignatiou

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# "Investigating the role of the genetic background and Chitin Binding Domain proteins in intestinal homeostasis and regeneration in *Drosophila*"


The fruit fly, *Drosophila melanogaster*, is an ideal model to study intestinal homeostasis, regeneration and tumorigenesis because its midgut exhibits molecular, cellular and functional similarities with the mammalian gut. In this thesis, I assessed the role of the genetic background in intestinal homeostasis and infection-induced regeneration, and I also characterized novel intestinal homeostasis and tumorigenesis regulators. To test the phenotypic variation of complex traits, we screened the *Drosophila* Genetics Reference Panel collection of inbred isogenic strains for six physiological and fitness traits. We found that female fecundity, survival and intestinal mitosis upon oral infection, defecation rate and fecal pH upon oral infection, and terminal tracheal cell branching in hypoxia exhibited continuous variation. Furthermore, we assessed the effects of commonly used *UAS-RNAi* transgenic strains of the Vienna Drosophila Resource Center and their isogenized counterparts in the same traits, plus intestinal mitosis without infection and tracheal branching in normoxia. The randomly selected 20 non-isogenic *UAS-RNAi* strains and their isogenic equivalents were tested without Gal4 induction. Survival upon infection and female fecundity exhibited differences in 50% and 40% of the tested isogenic vs. nonisogenic pairs, intestinal mitosis with and without infection varied by 25% and all other traits were affected in only 10-20% of the cases. Furthermore, when a single *UAS-RNAi* line was crossed with the same Gal4 driver

inserted in different genetic backgrounds, the quantitative variations observed were unpredictable on the basis of pure line performance. Thus, irrespective of the trait of interest, the genetic background of commonly used transgenic strains needs to be considered carefully during experimentation. To identify novel regulators of intestinal tumorigenesis, we used reverse genetics to test the role of 55 genes with differential expression in tumorous fly intestines identified through transcriptomics. Each candidate gene was individually silenced in *Ras ** tumorous midguts and intestinal mitosis was monitored as a tumor growth proxy. We selected *CG13309, CG7298* and *CG10154*, which encode chitin-binding domain (CBD) proteins, and significantly reduced Ras tumorigenesis when silenced, for further analysis. We found that, in non-tumorous midguts, *CG13309* and *CG10154* were necessary in ISCs for damage induced ISC-mediated midgut regeneration. Furthermore, *CG13309* and *CG10154* ISC specific overexpression was sufficient to drive midgut mitosis in the absence of damage. *CG7298* was shown to have only a tumor-specific role. To further understand CBD gene function, we raised an antibody against CG13309. We found that in wild-type midguts, the CG13309 protein was closely associated with intestinal progenitors and its punctate localization was extracellular. Through mosaic clonal analysis and marker co-expression upon *CG13309* silencing, we showed that *CG13309* was necessary for ISC mitosis but not maintenance or differentiation. We also tested gut physiology in *CG13309*-silenced midguts. We found no effect on fly survival upon infection, but contradictory results regarding intestinal barrier permeability that need further investigation. Moreover, to identify effectors of *CG13309* function in the adult intestine, we performed RT-qPCR in control and *CG13309*-silenced midguts both in tumorigenic and under homeostatic and regeneration conditions, which revealed changes in expression of ISC mitosis regulators including ligands of the Notch, EGFR, Toll, and Jak-Stat pathways. Last, to identify novel effectors of CBD genes, we performed mRNA-Seq transcriptomics of control vs. ISC-specific *CG13309*- and *CG10154*-silenced midguts in baseline and infection conditions. Preliminary gene expression enrichment analysis identified a common signature of genes with lysozyme activity and transmembrane domains upon CBD silencing, that require further experimentation. Since the fly CBD genes encode small secreted peptides associating with tumors with sequence similarity to human chitin lectins, our future research can provide mechanistic insights into the action of human chitin lectins, which are upregulated in colon cancer and inflammation.