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Michèle CROZATIER's report for the presentation of the master degree of Miss Lucie Jonatova

For her Master degree Lucie Jonatova studied the role of Disc overgrown (Dco) in drosophila hematopoiesis. Dco is a ser/ther protein kinase highly conserved during evolution and is the human orthologue of casein kinase Iδ/ε. Dco is involved in many processes including the control of cell proliferation. Several mutant drosophila alleles have been generated including one lof allele (dco-) and 3 gof alleles (dco3, dco L29Q and dcoL39QS101R). In this study Lucie Jonatova investigated phenotypes of these 4 Dco alleles in drosophila hematopoiesis. The purpose was to define Dco's role in controlling drosophila hematopoiesis.

In a first step, she looked both at the morphology of the larval hematopoietic organ called the lympgh gland (LG) and larval circulating blood cells called hemocytes. Two types of hemocytes are present in healthy larvae: plasmatocytes and crystal cells, which are equivalent to mammalian macrpophages (phagocytosis) and platelet like cells (healing), respectively. She analyzed both the differentiation state and the amount of circulating hemocytes in the 4 dco mutant alleles. Her analysis established that there is an over proliferation and a massive differentiation of lamellocytes in the 2 gof alleles (dco L29Q and dcoL39QS101R), whereas no defect is observed in dco- null mutant. The lamellocyte is the third type of drosophila hemocyte, which only forms in response to an immune challenge such as wasp parasitism or stress conditions. The constitutive activation of the Toll pathway gives an over proliferation of circulating hemocytes and the abnormal formation of lamellocytes. Based on the similar phenotypes, it was interesting to determine if there was any connection between Dco and the Toll pathway. Unfortunately, no cross talk between Dco and the Toll pathway could be established.

In a second step, she looked in detail at the morphology of the LG by using several markers that either labeled the progenitors , present in a region called the medullary zone (MZ), the differentiated hemocytes that constituted the cortical zone (CZ) and the hematopoietic niche called the PSC. For the 4 alleles studied no change in the PSC was observed. To follow the MZ she looked at the expression of Domeless (dome), which is the receptor of the JAK/STAT pathway, by using the domeless-gal4>GFP driver. In two gof dco alleles (L29Q and dcoL39QS101R) she observed an ectopic expression of *dome* in many larval tissues

including the epidermis, the ring gland, the cardiac tube and the brain. Very interestingly, such ectopic expression of *dome* is also observed in response to an immune challenge such as wasp parasitism. These observations raise the interesting question of the potential link between the constitutive activation of Dco and the response to wasp infestation.

In the last step, Lucie Jonatova established the expression pattern of Dco in larvae by using either a FlyFos vector expressing a Dco-Gfp fusion protein or antibodies directed against Dco. This analysis indicated that Dco is expressed in most larval cells and may have either a nuclear or cytoplasmic localization, depending on the tissue. This analysis has been completed by performing Q-RTPCR on different larval tissues, allowing one to define the relative level of Dco expression in different larval tissues.

This master thesis manuscript is very well written and very easy to read. The introduction is well done, complete and facilitates understanding the scientific questions addressed in this study. In addition, the chapter on "Material and Methods "is complete, precise and is very helpful to follow the experiments performed. The results obtained during this study are very interesting and open new fields of investigation. Two key questions that remain are i) to define in which larval cells Dco constitutive expression is required to induce hemocyte over proliferation and lamellocyte differentiation and ii) to decipher the molecular process involved.

This study is very complete, very well performed and analyzed. During this work, Lucie Jonatova mastered a range of molecular and cellular biology techniques. I am convinced that Lucie Jonatova has the potential to pursue her studies and successfully perform a PhD .

Based on these conclusions, Miss Lucie Jonatova has completely fulfilled all the criteria for the obtention of a master diploma.

P.I: Michèle CROZATIER

Review of the Master Thesis of Bc. Lucie Jonátová "The Role of Dco in Drosophila Haematopoiesis"

The Master thesis by Bc. Lucie Jonátová is dedicated to the topical biological problematique of the structural and functional correspondence of homologous genes and proteins in *H. sapiens* and in the model organism (*Drosophila melanogaster*). Clarifying these relationships is an important approach for finding novel interactions and missing links in the evolutionary conserved regulatory pathways, elucidation of which is required for better understanding and finding of the new therapeutical approaches of various human inherited diseases, including cancer, hurt disorders, neurological abnormalities and many other pathological syndromes.

This particular work describes the new phenotypes caused by mutations in the conserved sites of the gene *discs overgrown* (*dco*), a *Drosophila* homologue of a human *Casein kinase Iδ/ε* (*CKIδ/ε*), which is known to be associated with breast cancer. The very mutations seen in cancer patients were reproduced in the region exactly conserved between two homologues, human and *Drosophila* ones, and have been demonstrated to induce a new tissue-excessive phenotype. This phenotype is different from the imaginal disc overgrowth phenotype by the dco^{3} allele, the only presently known tissue-surplus phenotype caused by *dco*. Unlikely to that phenotype, the mutations dco^{L39Q} and $dco^{L39Q\,S101R}$ characterized by the Applicant cause excessive multiplication of hemolymph elements, the haemocytes, together with the lack of differentiation of their vast majority, determined morphologically and by the expression of hemolectin, a differentiation marker. These new phenotypes also include overgrowth of the lymph gland, the likely source of the excessive haemocytes. It was shown that imaginal discs are mostly not affected by those new mutations. These phenotypes, finally lethal for the larvae, were characterized through the course of individual development of the mutants. Along with dramatic increase in the population of haemocytes, the substantial growth of their derivative cells, lamellocytes, were reported, together with higher incidence of the melanotic pseudotumors, especially in the mutants for the $dco^{L39Q\,S101R}$ allele with two mutations introduced.

The novel mutations were tested for a genetic interaction with the members of signaling pathways, known to mediate immune response (the Toll-Cactus-Relish pathway, orthologous to mammalian NFκB pathway) and the JAK-STAT pathway. It was shown that combination of the novel *dco* mutations with Dorsal and Dif mutations results in further amplification of haemocytes, which, however, was found to be a multiplication of two independent effects from *dco* and *Dorsal* mutants from the Toll pathway. Interaction with the JAK-STAT pathway was examined with a different approach, namely visualizing of the expression of its apical component *domeless* and external non-autonomous stimulator *collier* by the use of the Gal4-drivers fused to the appropriate promoters and a UAS-GFP reporter. Their expression was studied in the lymph gland, ring gland and imaginal discs. Those patterns of expression and morphology traits of the lymph gland were found comparable in the novel *dco* mutants, and wild type flies under a wasp parasitic attack, with activated JAK/STAT pathway. It was shown a correspondence of their expression strength and pattern with the morphology of lymph glands and their parts.

Also the expression of *dco* was studied in the *dco* mutants and normal flies, quantitatively, by RT-PCR, and its pattern in imaginal discs and lymph glands upon visually, with the help of a FF:dco (a dco::GFP fusion protein) and confocal microscopy.

The study beautifully combines the diversity of modern methods of molecular and cellular biology, and visualizing with the power of classic genetic approaches. Using the targeted recombination methods, the *dco* locus was duplicated to the X-chromosome from the chromosome III, in which the original *dco* locus was still preserved, in hemizygous condition over the deficiency *Df(3R)A177der22* covering it. So, the novel dco^{L39Q} and $dco^{L39Q\,S101R}$ mutations were tested in a heteroallelic combination (or , in other words, background) with dco^{+}, dco^{3} disc overgrowth allele and dco^{le88} null alleles of *dco*, and the number of copies of the mutant allele of interest was always kept the same with the "background" allele.

As a reviewer, I have following critical comments for the thesis.

1. The literature review could contain more information about human CKI, its function in the context of cell proliferation and its homology to the *Drosophila dco*.

2. In the Materials and Methods section (and hereinafter in the text) the author should follow the standard genetic nomenclature rules and use the terms correctly, avoiding lab jargon. For example, it is incorrect to name the flies $Dp(3;1)dco^{L39Q}/+$; $dco^{le88}/Df(3R)A177der22$, *dco* homozygotes for dco^{L39Q}, in spite of that dco^{L39Q} is the only functional copy of the gene in these flies, because the term homozygosity is only applicable to alleles in homologous chromosomes. Typically, genotype formulas in a standard notation are shorter and more certain than verbal descriptions of the respective genotypes.

3. In the Results, where haemocytes counts are described, attention should be paid not only to absolute numbers of the hemolymph elements but also to their titer, as the author compares phenotypically normal larvae to the *dco* mutant larvae.

However, the differences in the haemocyte population in the *dco* mutants compared to the phenotypically wild type control were drastic. This does not compromise the conclusion of the haemocyte population substantial increase and specially the conclusion about relative preponderance of the non-differentiated haemocytes fraction in the mutants.

4. The pictures should be always supplied with a complete self-explaining legend, and all the parts of the organs in the photos showing the expression pattern of the genes must be signed.

5. The Conclusions should be given in short, clear and robust statements, preferably numbered ones.

However, these critical comments are not principal and can be easily fixed by minor corrections. The work in general is bright and elegant, and the variety of the applied methods, ranged from the formal genetics to cell counts, gene expression visualizing, RT-PCR and confocal microscopy, is very impressive. To my opinion, this master thesis work after correction the uncertainties and conducting the additional experiments defined by the author in Discussion, has a good potential to grow in the future into a PhD thesis. The Master Thesis work by Bc. Lucie Jonátová meets all the requirements for Master theses, and the applicant deserves the Master degree.

Dr. Roman A. Sidorov, PhD. 18.01.2013