University of South Bohemia in České Budějovice, Faculty of Science,

Department of Molecular Biology

The expression pattern of CG18446 gene in *Drosophila melanogaster*

Bachelor Thesis

Dajana Tanasić

Biological Chemistry

Supervisor: RNDr. Alena Krejci, Ph.D.

Laboratory of Developmental Biology and Genomics

Tanasić, D., Investigating the expression pattern of CG18446 gene in *Drosophila melanogaster*. BSc. Thesis, in English – 47 p., Faculty of Science, The University of South Bohemia, České Budjějovice, Czech Republic.

Annotation

The aim of this thesis was to investigate expression pattern of CG18446 gene in fruit fly *Drosophila melanogaster* and correlate it to the Notch signaling pathway.

Affirmation

I hereby declare that, in accordance with Article 47b of Act No. 111/1998 in the valid wording, I agree with the publication of my bachelor, in full form in the Faculty of Science archive, in electronic form in publicly accessible part of the STAG database operated by the University of South Bohemia in České Budějovice accessible through its web pages.

Further, I agree to the electronic publication of the comments of my supervisor and thesis opponents and the record of the proceedings and results of the thesis defense in accordance with aforementioned Act No. 111/1998. I also agree to the comparison of the text of my thesis with the Theses.cz thesis database operated by the National Registry of University Theses and a plagiarism detection system.

In České Budějovice, _____

Acknowledgment

I am grateful to RNDr**.** Alena Krejci, Ph.D. for giving me an opportunity to work in the Laboratory of Developmental Biology and Genomics. I would also like to thank her for helpful guidance through all the laboratory work, detailed explanations, invested time and effort and friendly approach.

I would like to acknowledge Mgr. Zorana Vujin and Mgr. Aleksandar Mihajlović for honest consultation and support, inspiration and friendship.

Many thanks to all the other laboratory members for help and motivational working atmopshere. I would also like to thank laboratory of Mgr. Tomas Dolezal, Ph.D. for collaboration.

I want to thank Prof. RNDr. Libor Grubhoffer for great help throughout my bachelor study, especially during my stay in Budweis in Czech Republic.

I would like to thank my sister Aleksandra, mother Jelena and father Miroslav for their endless support in my studies, understanding, patience and love.

A great support I got from Lili and her family helped me to feel loved and safe despite being away from home and I would like to say special thanks to them.

Many thanks to my cousin Danka and friends Kristina, Bojana and Elisabeth for warm words and strong friendship. I would also like to thank to Edita and Johanna for encouragment and for providing the unique humor and amusement.

Table of contents

1. INTRODUCTION

1.1. NOTCH SIGNALING PATHWAY

1.1.1 Notch signaling pathway

As cells in the embryo need to be directed to their positions and roles during animal development there is a need for cell to cell communication. This is maintained by several signaling pathways responsible for the exchange of information between neighbor cells to adapt each others' behaviors. These pathways are highly conserved in evolution and they are used repeatedly during the development. One of the most broadly used pathway during development is the Notch signaling pathway which operates in many different cell types and developmental stages. Notch signaling pathway accounts for the simplest and the most direct signaling pathway known that conveys information from the cell-surface to the nucleus. Notch receptor is a transmembrane protein which depends on proteolysis in order to signal. It is subjected to four proteolytic cleavages; nevertheless, only the last two occur after the receptor binding one of its ligands (Delta or Serrate in Drosophila). At the very beginning, the protease *furin* operates in the Golgi apparatus in order to cleave the newly emerging Notch protein. This breakage converts the molecule into a heterodimer which is then transported to the cell surface to form a mature receptor. Binding of Delta initiates the second cleavage of Notch at the membrane by ADAM-family metalloproteases [1]. This releases so-called NEXT, Notch Extracellular Truncation, which is activated membrane-bound form. Soon after that the third and final cleavage follows by a protease complex called *γ-secretase* and the cytoplasmic tail of the Notch receptor is released (Notch intracellular domain, ^{Nicd}). N^{icd} is further moved into the nucleus where it initiates the transcription of a set of Notch-response genes. Crucial part here plays a transcription factor called CSL (CBF in mammals, SuH in Drosophila, Lag1 in C. elegans). N^{icd} binds to CSL as well as to nuclear co-activator Mastermind (MAM) and other proteins to activate transcription of Notch target genes. (Fig. 1) In the absence of signaling, CSL binds to the DNA in the enhancers of target genes but it works as a repressor to shut down gene expression (recruiting co-repressor complexes instead of N^{icd} containing co-activator complexes) (Fig. 2) [1].

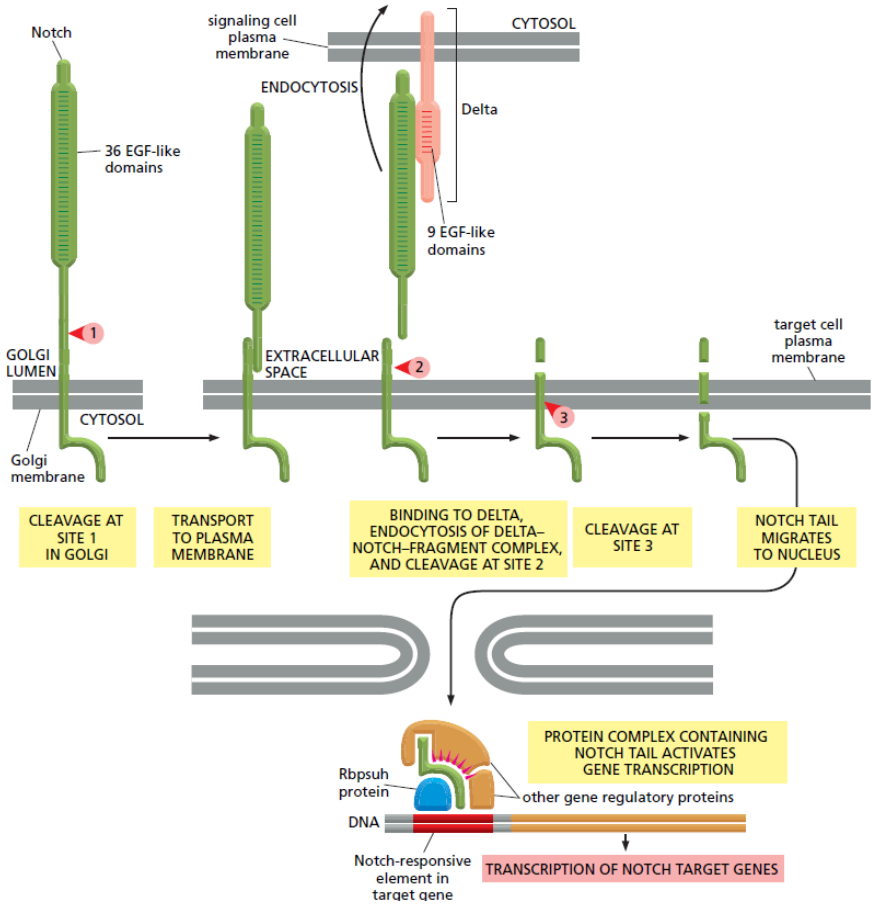

Figure 1: The processing and activation of the Notch by proteolytic cleavages [2].

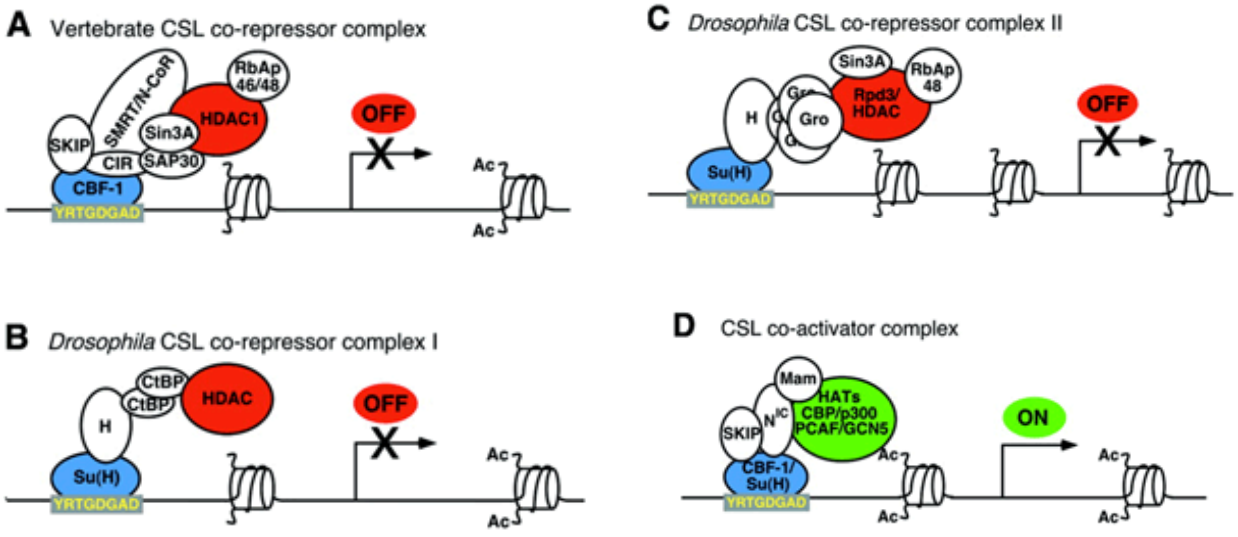

Figure 2: CSL proteins associate with co-repressor and co-activator complexes [3].

1.1.2. Regulation of Notch signaling pathway

Because a cleavage of a protein is an irreversible process, a characteristic feature of a Notch signaling is a fact that each receptor molecule can only signal once. Signaling is relatively simple and direct and therefore the possibilities for cross-talk between different signaling pathways are limited. Nevertheless, there are multiple levels of regulation of this signaling pathway [4].

1) Gene dosage sensitivity

Notch is very sensitive to gene dosage. If females carry three copies or only one copy of Notch gene (rather the normal two copies), mutant phenotype will be shown. This indicates that animal is "counting" the number of Notch copies and it is reflected in the intensity of Notch signaling. One of the possible reasons for this extreme sensitivity could be the fact that Notch does not rely on enzymatic amplification step, but on stoichiometric relation between the elements of its pathway [5].

2) Regulation in *cis* and in *trans*

It has been shown that Delta trans-activates Notch in neighboring cells and cis-inhibits Notch in the same cell [2]. Notch's response to cis-Delta is very sharp and switch-like, opposed to trans-Delta response that is rather graded. Competition between cis and trans interactions can be crucial for deciding which of two neighboring cells will become signal-sending versus signal-receiving [5].

3) Post-translational modifications

Post-translational modifications of either receptor or ligand such as ubiqitylation, glycosation and phosphorylation also play a major regulatory role during signaling. For example, both the receptor and ligands need to be endocytosed and monoubiquitinylated in order to signal. Glycosylation of Notch receptor may favor its interaction with Delta versus Serrate ligands. And phoshorypation of N^{icd} leads to its degradation [4].

4) Target gene selection

There is a whole range of regulations at the level of Notch target genes selection. There are several thousands of predicted CSL binding site in the genome but only a few hundreds of them are occupied in a given cell type. Moreover, the set of the occupied target enhancers differ significantly amongst different tissues. Whether a gene is regulated by Notch in a given tissues depends on the presence of CSL binding sites in its enhancers as well as on the activity of other signaling pathways and presence of other transcription factors regulating the same gene. For example, Su(H) needs to cooperate with the transcription factor Twist in order to trigger the expression of Notch target genes in muscle progenitor cells [6].

1.2. DROSOPHILA MELANOGASTER AS A MODEL

1.2.1. Why *Drosophila melanogaster*?

The fruit fly, *Drosophila melanogaster*, is the most commonly used model organism in genetics and developmental biology. It has the longest history of all model organisms, serving the purpose of science for more than one century. There are couple of reasons for the statement mentioned above. One of them is rather of a practical nature; Drosophila melanogaster is a small organism, which is fed on the simple diet. This implies that numerous fruit flies can be held in laboratory at relatively low expense. Moreover, methods for manipulation of flies in laboratories are well-established and numerous data about it can be easily accessed and applied. The life cycle of Drosophila is only about two weeks, implying that crosses can be set and examined through several generations within rather short period of time. Quite a remarkable point is also the fact that significant number of genes are conserved to human. The fly genome is 165 million base pairs in length, spread over four chromosomes, and contains approximately 14 000 genes. Even though humans have nearly twice as many genes as flies, they still have the same number of gene families (human has about 25 000 genes). This implies that relationship between human and fly genome is unusually close and that discoveries in the field of *Drosophila melanogaster* can directly help in developing drugs for human population. Significant advantage of using Drosophila to study Notch pathway is the fact that there is only one receptor and two ligands in the fly as oppose to four receptors and five ligands in human. Therefore genetic manipulations of the pathway are easier in the fly and certain question can be answered by significantly shorter time than in mammals [7].

1.2.2. Life cycle of Drosophila

The life cycle of *Drosophila melanogaster* lasts 14 days (at 25 °C). There are four stages for complete development: egg, larva, pupa and adult. Twenty-four hours after fertilization and laying eggs, a larva hatches. Drosophila has three larval instars, during which cuticle, mouth hooks, and spiracles are casted off. Metamorphosis occurs during the third stage of life cycle, the pupa. Pupa turns dark before adult fly arises. Folded wings as well as pigments of eyes can be noticed on the pupa twenty-four hours before adult emerges. At the very beginning, fruit fly is very light in the color and has long abdomen and unexpended wings. After few hours, it turns darker. Females are capable of laying eggs forty-eight hours after arising from puparium. Nevertheless, only in the first 8 hours after leaving puparium female of fruit fly is considered to be a virgin because it has not mated yet (8 hours at 25 °C, 18 hours at 18 °C).

1.2.3 The Drosophila genome

Drosophila melanogaster genome was sequenced and determined in a partnership between Celera and the Berkeley Drosophila Genome Project. It has been described on 24th March 2000 in Science. Nevertheless, the Berkeley Drosophila Genome Project carried out additional sequencing and re-assembly. The 5th release was published in March 29, 2006. [8]. There are in total 4 chromosomes. Chromosome 1 is a sex chromosome, whereas chromosomes 2 to 4 are autosomes. Sex determination is of XY type, where XX are females and XY are males. However, chromosome Y does not play a role in the sex determination. Crucial factor for this is the ratio of X chromosome to the number of copies of each autosome. Chromosomes 2 and 3 are large and metacentric, while chromosome number 4 is rather small and presents only 2% of the size of the autosomes in total (Fig. 3).

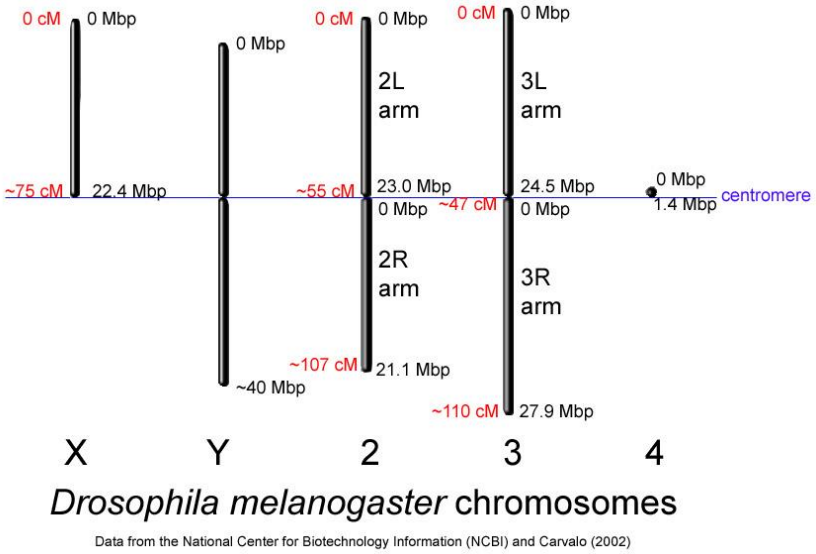

Figure 3: *Drosophila melanogaster* chromosome map [8].

1.2.4. Balancer chromosomes

The most important genetic tool in fly genetics is the balancer chromosome. Balancer chromosomes are chromosomes that carry multiple overlapping inversions and are resistant to crossover (that can occur only in females in Drosophila). This special chromosome type is useful for many genetic manipulations where it allows a precise tracking of the segregation of chromosomes and mutant alleles. The word "balancer" arose from the usage of these chromosomes in stock keeping where they balance the number of autosomes. Stock is usually made by crossing males and females carrying desired mutation on an autosome and a balancer chromosome with a nonmutated allele of the same gene. Balancer carries dominant mutations that allow its phenotypic distinguishing in progeny and also recessive lethal mutations ensuring that the progeny that with two the same balancer chromosome will not survive [9].

1.3. LYMPH GLAND – STRUCTURE AND DEVELOPMENT

1.3.1. Hematopoiesis of *Drosophila melanogaster*

Two distinct phases of hematopoiteic development in *Drosophila melanogaster* can be distinguished. Early embryonic phase generates mature hemocytes that are present in the circulation from the larval stages (plasmatocytes and crystal cells). Nevertheless, hemocytes produced in the second phase, by the lymph gland, do not enter the circulation until the metamorphosis starts [10-11].

Mammalians have two different immune responses – innate and adaptive. In *Drosophila melanogaster* innate immune system is present and a primitive form of adaptive exists as well [12]. The innate system in *Drosophila* consists of two main elements – humoral and cellular system. The fat body is crucial for inducing humoral response. It gives a rise to the antimicrobial peptides, that are then secreted into hemolymph [13]. In the case of the cellular response, it includes phagocytosis, formation of nodules and pathogens encapsulation. It consists of three basic types of circulating hemocytes (Fig. 4):

a) Plasmatocytes are the most abundant type of immune surveillance cells, constituting 90 – 95% of mature hemocytes population. These cells are highly motile and are responsible for removal of dead cells and pathogens by phagocytosis and encapsulation. Differentiation of plasmatocytes in the lymph gland is tightly related to the transcription factors Glial cells missing (Gcm) and Glial cells missin-2 (Gcm2) [11, 14].

b) Crystal cells are cells representing around 5% of mature hemocytes. They are non-phagocytic cells, but are involved in wound-healing and process of melanization. Their name comes from the big crystals of zymogen proPhenolOxydase (proPO1 and proPO2), a component of the melanization enzymatic complex in their cytoplasm. Transcription factor *Lozenge* forms a complex with one of the five GATA factors, Serpent (Srp), and this complex then initiates the crystal cells production in the lymph gland [11, 14].

c) Lamellocytes cells are the biggest and the least abundant hemocytes. They are functioning in encapsulation of foreign bodies too large for phagocytosis. They are usually produced in the case of infections and they are rarely observed under the normal condition (in a healthy organism). These cells can be identified for example by the expression of the *misshapen* gene [11].

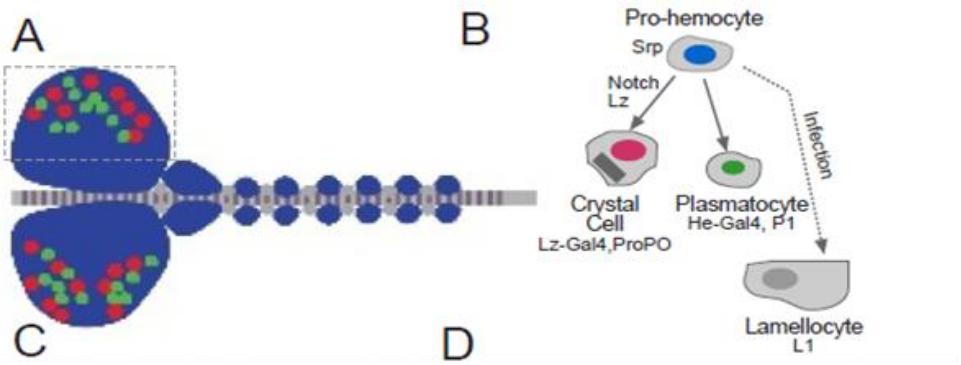

Figure 4: Types of hemocytes, their location in the lymph gland and pathways affecting their proliferation [15].

1.3.2. Encapsulation

The reaction of encapsulation can be observed in the case of a parasitic wasp laying eggs in the hemocoel of *Drosophila*. This induces a strong response reflected in the release of plasmatocytes from the lymph gland. Additionally, it also triggers the proliferation of lamellocytes. When the parasitic egg is recognized, plasmatocytes are changing from non-adhesive to adhesive in order to attach to the foreign body and create a capsule. There are three phases of capsule formation. In the first one, plasmatocytes are binding and spreading around the chorion of the egg (the outer membrane or shell of the egg). Afterwards, cells separate the egg from hemocoel by creating septate junctions. In the last phase, lamellocytes are attached and melanization occurs as a result of the crystal cells degranulation (Fig. 5) [13].

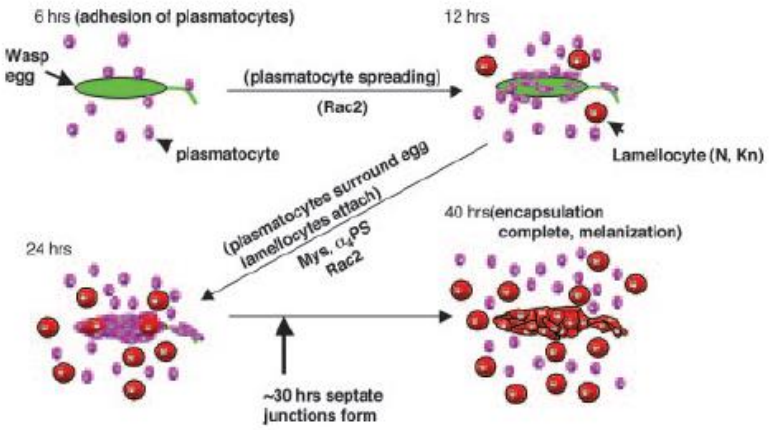

Figure 5: Diagram of the encapsulation of a parasitoid wasp egg [13].

1.3.3. Lymph gland

The lymph gland arises from the cardiogenic mesoderm of the embryo and it grows by cells' differentiation during the larval instars. In the late embryonic phase, lymph gland comprises only one single pair of lobes. These lobes are rather poor in cell number, containing approximately 20 cells each. In the second larval instar, lymph gland has slightly different structure. Primary lobes are significantly increased in size, being consisted of c.a. 200 cells per lobe. Moreover, two to three new pairs of posterior lobes are formed. Late third larval instar shows additional enlargement of a primary lobes for approximately ten folds more. However, the arrangement of the posterior lobes and cells stays the same. Two structurally different regions can be observed in the primary lobes of the third larval instar lymph gland. Cells of the periphery of the lobes are arranged in such way that they make very few cell – cell contacts. This is displayed in the granular appearance of the region. Periphery zone of the primary lobes is called *cortical zone*. On the contrast, cells in the medial region are compactly arranged with almost no intercellular space in between. As a result, this region appears as a smooth zone and is called a *medullary zone*. It has been proven that the presence of all three types of hemocytes is restricted to the cortical zone. Medullary zone is a zone of non-differentiated prohemocytes that will later change into mature hemocytes in the cortical zone. According to various markers for gene expression, cortical zone has been formed already in the second larval instar. In addition to these two zones, that can be distinguished structurally and morphologically, there exists third zone called *posterior signaling center* (PSC) (Fig. 6).

PSC is shown to be crucial for maintaining the balance between pro-hemocytes and differentiating hemocytes. The significance of preserving the differentiating potential of prohemocytes can be appreciated in the case of parasitic wasp infections when these cells can differentiate into lamellocytes [11].

On the other hand, expression of mature hemocytes has not been observed in the secondary lobes. Secondary lobes have a smooth structure similar to the medullary zone of the primary lobe. Lymph gland developes via the silencing and activation of prohemocytes. In the second instar larval stage proliferation of prohemocytes stagnates as they settle in the medullary zone. During the conversion of the prohemocytes into mature hemocytes in the cortical zone, reproduction is once again activated. The fact that the prohemocytes in the secondary lobes are distributed equally even in the third instar lymph gland implies that prohemocytes here do not even go through the silencing of cell proliferation while populating medullary zone. This all illustrates that the cells of the lymph gland are experiencing clear and specific proliferative phases throughout hematopoietic development [11].

After complete larval development, which occurs in the third larval instar, lymph gland disperses a mixture of both differentiated and non-differentiated hemocyets. The function of non-differentiated one is still not elucidated [14].

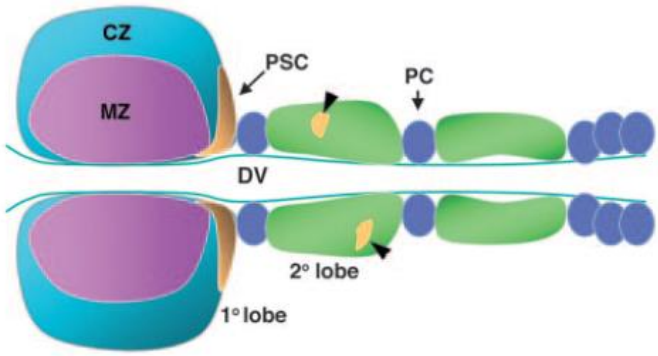

Figure 6: Schematic diagram of the third instar lymph gland; primary lobe is consisted of cortical zone (CZ), medullary zone (MZ) and posterior signaling center (PSC) - it flanks the dorsal vessel (DV) and each lobe is separated by pericardial cells (PC) [14].

1.3.4. Notch Signaling Controls Lineage Specification

It has been shown that Notch signaling pathway plays an essential role in the crystal cell fate determination [11, 16]. When second instar larvae bearing the temperature sensitive N^{ts1} allele were shifted to prohibitive temperature (now having less Notch receptor) they had much less crystal cells than the one at the permissive temperature [11]. This implies that Notch is important for the differentiation of the crystal cells in the larval stage. Opposite experiment showed that proliferation of crystal cells is enhanced if Notch is overexpressed [11]. In the case of crystal cells differentiation, Serrate ligand (Ser) is crucial for triggering Notch pathway. This was proven by counting the crystal cells in mutants that had either Ser or DI loss-of-function [11]. Number of crystal cells was reduced in the case of Ser loss-of-function, whereas there was almost no effect where there was no DI function.

The formation of the third type of hemocytes – lamellocytes is also affected by the Notch activity. If Notch signaling is not present normal proliferation of lamellocytes after infection is arrested. Nevertheless, overexpression of Notch does not affect its number [11].

1.4. OOGENESIS AND SPERMATOGENESIS – STRUCTURE AND DEVELOPMENT

1.4.1. Oogenesis

The *Drosophila* adult ovary consists of varying number of ovarioles (16 - 20). Each of these ovarioles represents one separate line of egg production structures called the egg chambers. They contain 16 germline cells (1 oocyte and 15 nurse cells) surrounded by somatic follicle cells. Somatic and germline stem cells are situated in the germanium, placed at the anterior part of the ovariole. There are four different regions of germanium – 1, 2a, 2b and 3 (Fig. 7A). Egg chamber is maturing as it moves posteriorly in the ovariole. The larger and more posterior region of the ovariole after germarium is called the vitellarium. Usually six to seven increasingly mature egg chambers are present in the vitellarium of a single ovariole and they are separated by the interfollicular stalk cells. There are 14 stages of oogenesis based on morphological criteria. First stage is the formation of the egg chamber and the last, 14th stage, is a mature egg in an egg chamber [17][18].

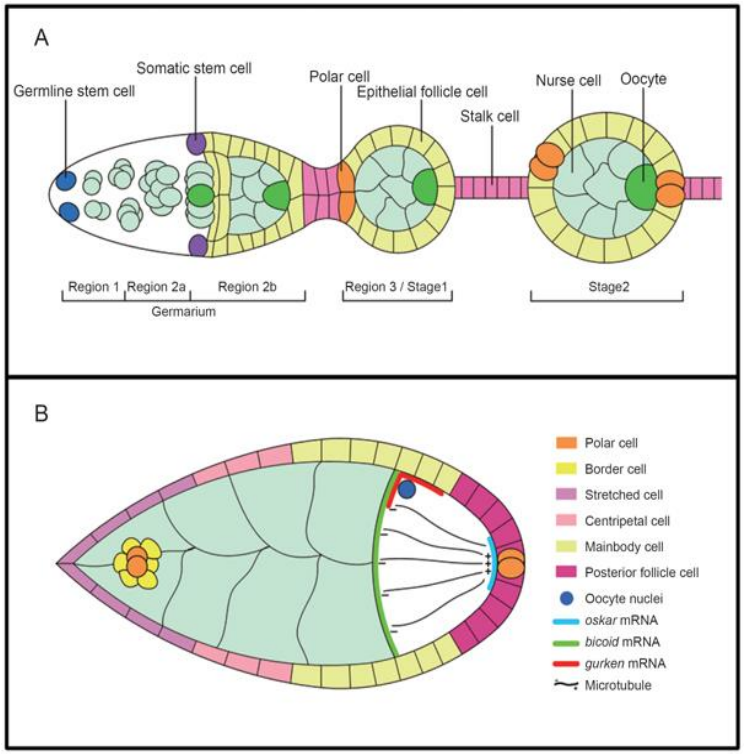

Figure 7A: Early stage of Drosophila oogenesis, germarium with its regions, stage 1 and stage 2 together with types of cells present are indicated. Anterior region is on the left [19].

Figure 7B: Drosophila egg chamber in the stage 9. Anterior region is on the left [19].

Germline stem cells (GSCs) are situated in their niches that allow their proliferation and self-renewal. These are germ line stem cells and somatic follicle stem cells. Each stem cells divides asymmetrically in order to produce another stem cell and its daughter. The daughter of the germline stem cell starts dividing further and differentiating into 16 cells connected by so called ring canals. Out of the 16 germline cell only oocyte will proceed to the meiosis while the other will become the nurse cells [17].

Precise coordination of both the germ line cells and somatic follicle cells is required for the proper development of the *Drosophila* egg. Follicle stem cells are situated in the 2b region of the germarium and they generate two different lineages – the epithelial cell precursors and the polar/stalk precursors. Epithelial cell precursors give a rise to a monolayer epithelium that envelopes each cyst and proliferates until the 6^{th} stage. After coating the cysts, polar/stalk cells terminate epithelial cells' division. They differentiate into the one pair of polar cells at each pole of the egg chamber and into the stalk cells that connect neighboring chambers. Every cyst determines posterior location of the oocyte and the polarization of the anteroposterior axis of the following cyst. This is followed by the differentiation of the follicular epithelium into five outcomes – border, stretched, centripetal, posterior and main-body cells (Fig. 7B). After 9^{th} stage, differentiated follicle cells show fate-specific markers and experience migrations and changes in morphology. Each of these subpopulations utilizes a certain role in the mature egg production [19].

Correct polarization of the oocyte is crucial, as it contains information about the axis formation in the future embryo. The establishment of the final oocyte polarity starts already in the stage 3 of the oogenesis. It is formed by the RNAs and associated proteins directed by microtubule polarity (mainly at stage 9), where microtubule plus end accumulate at the posterior and minus at the anterior region with certain extensions on the lateral base [17].

However, in order to completely understand oogenesis in *Drosophila*, morphology of the larval ovaries should be discussed as well. At the anterior part of each egg chamber (ovariole), two to three germline stem cells (GSCs) cooperate with somatic stem cells in order to influence their formation, maintenance and differentiation [20]. Development of these somatic cells across the larval gonad at the third larval instar allows separation into ovarioles already during early pupal development. The GSCs originate from so called primordial stem cells (PSCs), that are formed in the early embryo [20]. During larval development, primordial germ cells (PGCs) proliferate but remain undifferentiated and the entire gonad grows [21]. The number of PGCs primarily increases eightfold, from approximately 12 PGCs per embryonic gonad to 100 PGCs in the middle of third larval instar [20]. Larval gonad transformation into the adult ovary, so called ovarian morphogenesis, occurs at the larval - pupal transition state (Fig. 8) [21].

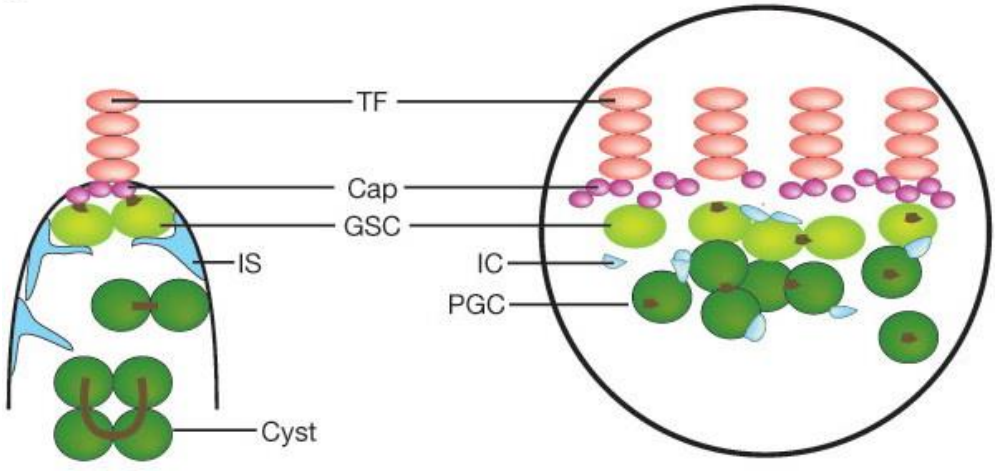

Figure 8: The adult germarium showed on the left and the late larval ovary showed on the right. Terminal filament (TF), cap (Cap), inner sheath (IS), and intermingled cells (IC). GSC stands for germline stem cells and PGC for primordial stem cells [20].

1.4.2. Notch signaling during Drosophila oogenesis

It has been shown that Notch signaling pathway plays an important role in *Drosophila* oogenesis [17]. It is necessary for the formation and conservation of the germline stem cell niche. Notch is activated by the Delta and Serrate protein on the surface of the GSCs and this triggers niche to induce and maintain the stem cell fate. Increased or decreased activation of Notch leads to higher GSC cell number and enlarged niche or lower cell number and reduced niche size, respectively. Notch signaling showed to be required to activate cap cell formation and supports the idea that cap cells are crucial component for controlling GSCs self-renewal. This activation is accomplished only during the late third-instar larval and early pupal stage [22]. Notch is important for the formation of the anterior – posterior polarity as well. From the germ line cyst (oocyst) of the newly formed egg chamber Delta is signaling to activate Notch in the neighboring polar/stalk precursors, causing them to change into polar cells. Delta signal from the germ line and Notch response in the follicle cells is also required for the shift of the follicle cells from mitotic cell cycle to endocycle. In endocycle, DNA is duplicated without cell division. It is after this switch that the follicle cells are differentiated into mature ones [17].

1.4.3. Spermatogenesis

Adult testis of *Drosophila* consists of two types of stem cells – germ line stem cells (GSCs) and cyst stem cells (CySCs). CySCs are clustered around the group of cells serving as a niche – hub cells. GSCs and CySCs are situated at the apical end of the testis. There are usually 6 to 9 GSCs present in testis and this number is tightly connected to the number of hub cells. Several signals involved in the maintaining the stem cells and their self-renewal are derived from the hub cells. Thus, hub cells are crucial for the survival of the stem cells.

GSCs experience asymmetrical division, generating two cells; one cell stays connected to the hub keeping stem cells identity while the other cell, gonialblast, moves out of the niche and differentiates. During differentiation germ cells are going through processes of dramatic changes characterized as spermatogenesis. Spermatogenesis is a ten-day process where gonialblasts divide four times in order to become an assemblage of 16 spermatogonia. Older spermatogonia and spermatocytes are being replaced with the new spermatogonial cysts as the spermatogenesis proceeds (Fig. 9) [23-24].

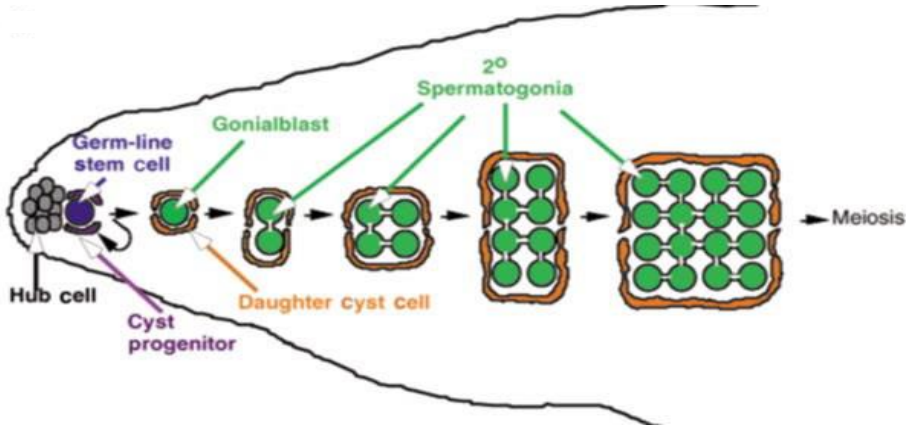

Figure 9: Testis apex showing germ-line stem cell together with double number of cyst progenitors and attached to hub cells [24].

1.4.4. Notch signaling during Drosophila spermatogenesis

Notch has a crucial role in specifying the hub cell fate. It has been shown that population precursor, somatic gonadal precursor (SGP), cannot accept hub cell fate without the Notch [25]. Even though Notch is not required for the differentiation of SGP into other types of somatic cells, it is necessary for the hub cell specification. Proper specifying of the hub cells occurs only in the case of early activation of Notch, in late 11^{th} or 12^{th} stage of gonadagenesis, as hub cell specification is performed during this period. Ligands involved in hub fate determination are both Delta and Serrate; nevertheless, it was shown that Delta has slightly more pronounced role here. Everything mentioned above is suggesting that endoderm expressing Delta activates Notch to specify hub cell amongst somatic gonadal precursor (SGP) in male testis [25].

1.4.5. Notch in oogenesis vs. Notch in spermatogenesis

Notch is responsible for niche cell specifications in three well-established stem cell-niche systems in Drosophila; two of them are niche for the female gonad and niche for the developing male gonad. However, it is important to emphases that there are differences between specifying above mentioned two systems. In the *Drosophila* oogenesis, only Delta ligand is involved in the niche cell specification, whereas in the spermatogenesis both Delta and Serrate can be used as activators. Second difference is the fact that in ovary the germ-line stem cells (GSCs) are expressing Delta whereas in the case of male gonade, cells from the distinct germ layer in the posterior midgut deliver ligand to the somatic gonadal precursors (SGPs) [25].

1.5. FAT TISSUE – STRUCTURE AND DEVELOPMENT

1.5.1. Fat body of *Drosophila melanogaster*

Constant energy supply independent of the food access is one of the crucial properties of animals. Metabolism of the fat tissue is highly affected by environmental conditions. Under the food rich conditions, lipids are deposited, whereas in the case of starvation as well as the insect pupation lipids are used up. Survival during these critical periods without food is possible only if the animal is capable of mobilizing stored energy that was accumulated during the time of excessive energy supply. The fat depository in *Drosophila* is in intracellular lipid droplets of a specialized organs called the fat body (corresponds to the adipose tissue in mammals). It is mostly composed of the triacylglycerols (TAG), as energy-rich diet components are converted into glycogen as a storage of carbohydrates (less frequent) and TAG as storage of fat (much more frequent). Fat body content of a fly can be used as a sensitive diagnostic phenotype that indicates disorders in the lipometabolism homeostasis. Moreover, the mobilization, storage and chemical composition of lipids in flies are very similar to the mammalian ones, that makes Drosophila a particularly valuable model system [26].

According to Grönke *et al* [26], there are 223 genes upregulated on the transcriptional level in starving *Drosophila*. They used (6-7 days old starved males) to perform genome-wide transcriptome analysis comparing gene expression of fed and food-deprived adult flies. *Brummer* (*bmm*) is one of the genes that controls fat storage in vivo. Bmm transcript encodes a 507 amino-acid-long protein similar to a starvation responsive gene found in mice and human, TTS-2/ATGL. This gene strongly affects energy storage tissues together with the food-absorbing parts of the digestive tract (i.e. gastric caeca and the larval midgut) [26].

The transcription factor *Twist* is necessary for the fat body development [27]. Namely, in the early *Drosophila* development selected mesodermal cells are repetitively segmented and assigned to specific tissue fates. Afterwards, each segment is subdivided into four domains – two across anterior-posterior axis and two across the dorsal-ventral axis. At the beginning, *Twist* is required for the specification of the mesoderm. *Twist* expression in a form of repeated pattern is constructed along the anterior-posterior axis of the embryo, dividing each segment into low and high *Twist* domain. Cells placed in the low *Twist* domain are the ones that will proliferate into visceral muscle, heart, mesodermal glia and, finally, fat body. High *Twists* levels generate somatic muscles and heart [27].

1.5.2. Role of Notch

There is no direct evidence of Notch playing a role in the fat tissue development or homeostasis. However, Notch can possibly regulate the expression of Twist in these tissues as it does in other contexts. It is necessary for the appropriate establishment of the low and high Twist domains at stage 10 of embryogenesis [27]. If Notch is absent, high Twist domain is dominant causing severe defects in establishing visceral mesoderm. Opposite was observed in the case of overexpression of Notch activity – very few cells were expressing high Twist. This implies that Notch represses the Twist expression at the stage 10.

1.6. CG18446

In previous we compared the expression profile of the *Drosophila* S2N, DmD8 and Kc cells and we found several hundred genes that were upregulated after the activation of Notch receptor [28]. We also performed chromatin immunoprecipitation experiments in these cells to locate the binding sites for Su(H). The CG18446 gene was amongst the genes that showed a robust Notch response and had a Su(H) peak in their vicinity suggesting that it is a primary target of the Notch pathway (Fig. 10). It is predicted Zn-finger protein that localizes into the nucleus (Fig. 11) but there is no information about its function. The mutants of this gene (exD flies) are viable with no obvious phenotype. In order to look more carefully for the specific phenotype of these flies we needed to know where it is expressed. Production of an antibody against this protein turned to be unsuccessful so we decided to take advantage of the FlyFos method that can be used to create an EGFP fusion of a protein of interest in the context of large BAC genomic construct. Transgenic flies with this construct were created. As the large BAC construct most probably contains all the regulatory regions of the CG18446 gene the GFP expression should reflect the endogenous expression pattern of this gene.

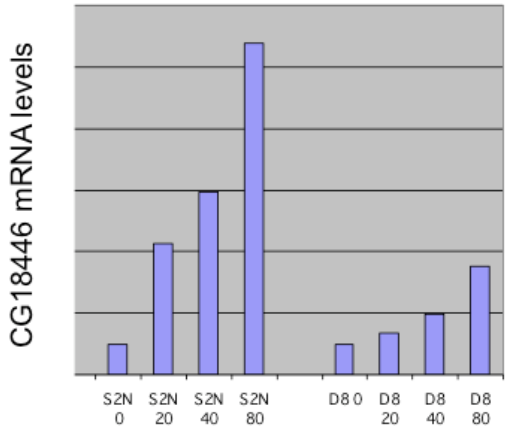

Figure 10: Temporal response of CG18446 following activation of Notch receptor in S2N and DmD8 cells (The y axis shows time in minutes).

Figure 11: Predicted structure of the CG18446 gene.

2. AIMS OF THE THESIS

1. Characterize the expression pattern of CG18446 using the FlyFos CG18446-GFP transgenic flies. We will use immunostaining with anti GFP antibody to look for the expression of CG18446-GFP in L3 larvae and in adult ovaries.

2. See how CG18446-GFP expression correlates with the activity of Notch pRR-NRE reporter.

3. Depending on the results from experiment (1) try to look for phenotypes in tissues expressing CG18446 on a CG18446 mutant background.

3.METHODS AND MATERIALS

3.1. Fly techniques

All flies were raised on agar food enriched with a cornmeal, glucose and yeast (agar 9 g, glucose 150 g, cornmeal 160 g, yeast 30 g, nipagin soln 50 mL, water 1900 mL) with additional dry yeast on the top of the food. They were kept on ambient light/dark cycles. The following Drosophila strains were used:

- CG18446 – GFP (II chromosome) (FlyFos construct containing about 30kbp of genomic DNA surrounding the CG18446 gene tagged with GFP on its C terminus, inserted on the III. chromosome. These flies also carry a dsRED selection marker that is visible in the adult eyes under a fluorescent microscope)
- If / cyo; TM3 / TM6 (double balancer stock)
- UAS-mCherry (II chromosome) (mCherry red reporter with upstream regulatory sequences in the promoter)
- w; Tft / cyo (balancer stock on the II. chromosome)
- yw (used as a control)
- pRR-NRE (Notch responsive elements driving the expression of a red fluorescent protein, a reporter for Notch activity)
- exD (II chromosome) (mutant for CG18446 gene)
- exD; CG18446-GFP (III chromosome) (CG18446 rescue flies)

3.2. Immunohistochemistry

Following protocol was used for all immunostainings performed:

1. Larvae/adult ovaries were dissected in PBS (room temperature) and collected into an Eppendorf tube on ice within 10 – 15 minutes;
2. Afterwards, larvae/adult ovaries were fixed for 20 – 30 minutes in 4% formaldehyde/PBS (room temperature);
3. Larvae/adult ovaries were first washed in PBX 2 x 10 minutes (on ice) and then in PBT for 10 minutes (on ice);
4. Final wash before adding antibody – in PBT for 1 hour (on ice);
5. Primary antibody was added and left overnight in cold room with slightly moving (1:500 in PBT, Rabbit serum anti - GFP, Life technology A6455);
6. Larvae/adult ovaries were washed in PBT 3 x 10 minutes;
7. Secondary antibody was added (1:1000 in PBT, Goat serum anti – Rabbit, Alexa Fluor 488), and larvae/adult ovaries were left for 2 hours with slightly moving and protected from light.
8. Final washing was done in PBT 3 x 10 minutes on room temperature with slightly moving and protected from light;
9. 70 % glycerol/PBS was added and left for 20 minutes (till overnight);
10. Finally, tissues were dissected in 70 % glycerol/PBS and mounted with Vectashield (alternatively CitiFluor).

Larvae were in the L3 stage (third instar larvae). For staining of adult ovaries, 3-day-old virgins were used. Two days after collecting, virgins were transferred to the fresh food and experiment was conducted third day after their hatching.

3.3. Starvation assay

Yellow – white flies, flies mutant for CG18446 gene (exD) and 18446 rescue stock (exD; CG18446-GFP) were propagated. Four vials of freshly flipped flies were taken and flipped five times every 2 days until a healthy population was established. Afterwards, we set 10 vials of each phenotype containing 6 virgin females and 3 males not older than 4 days. These vials are flipped every 2 days and kept moist. These synchronization steps were done to make sure all the flies of all the phenotypes were raised under similar conditions and their fat deposits were influenced by the same factors (for example if vials were overcrowded in some of the vials flies could have had problems to make good fat deposits and our results would be biased). Flies were then collected from the 10 vials for 2 days. 10 virgin females or 10 virgin males of the same age (hatched within 24 hours) were placed into separate vials. They were aged for 4 days on normal food and then transferred to a starvation food (0.8% agarose/PBS) and kept at 25°C. Survivals were counted every 6 hours.

3.4. Eggs counting experiment

Yellow – white flies, flies mutant for CG18446 gene (exD) and 18446 rescue stock (exD; CG18446-GFP) were propagated. 30 females and 15 males of each phenotype were taken and put into the very small cages. They were put on the apple juicy plates and a yeast paste. We set the cross and let them mate for 2 days, changing the plates every morning. Then, a plate with very little yeast on was put in the morning, left for four hours and replaced with a new plate. Eggs were counted. After replacing another plate, counting was done again. A plate was put overnight as well. This was done in the time period of 24 hours, with overall 4 plates per phenotype. Additionally, in order to see how many larvae actually hatched from the total amount of eggs, eggs and larvae were counted after 24 h for each plate of each phenotype.

4. RESULTS

4.1. CG18446 expression in the lymph gland

Expression pattern of CG18446 was observed in the lymph glands of FlyFos CG18446 transgenic flies (Fig. 12). Expression was present in the cortical zone of the primary lobe. No expression was noticed in medullary zone or secondary lobes (Fig. 13).

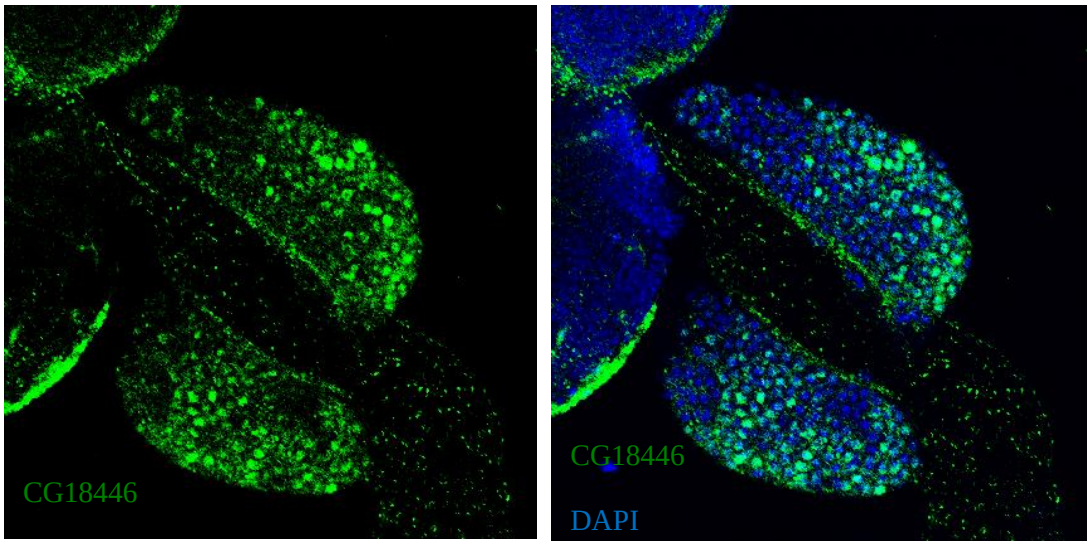

Figure 12: The expression of CG18446 in the primary lobes of the lymph gland.

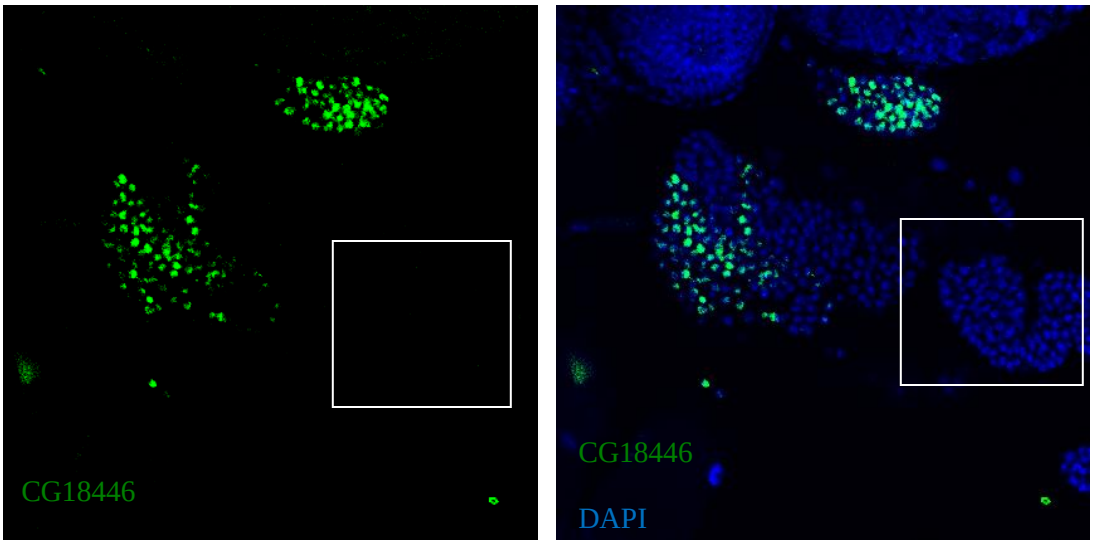

Figure 13: The expression of the CG18446 in the primary lobes of the lymph gland. Secondary lobes (framed) showed no expression.

4.2. Stronger expression of CG18446 in lymph gland of immune-challenged larvae

Stronger expression pattern of CG18446 was noticed after injecting wasp eggs to adult flies (in collaboration with Tomas Dolezal lab, Fig. 14). Lymph glands dissected from the third larval instar larvae showed an overlapping expression pattern with the lymph glands dissected from healthy flies. However, the signal in infected flies was obviously stronger.

We also found a 18446 signal in the encapsulating wasp eggs in the third instar larvae after infections (Fig. 15).

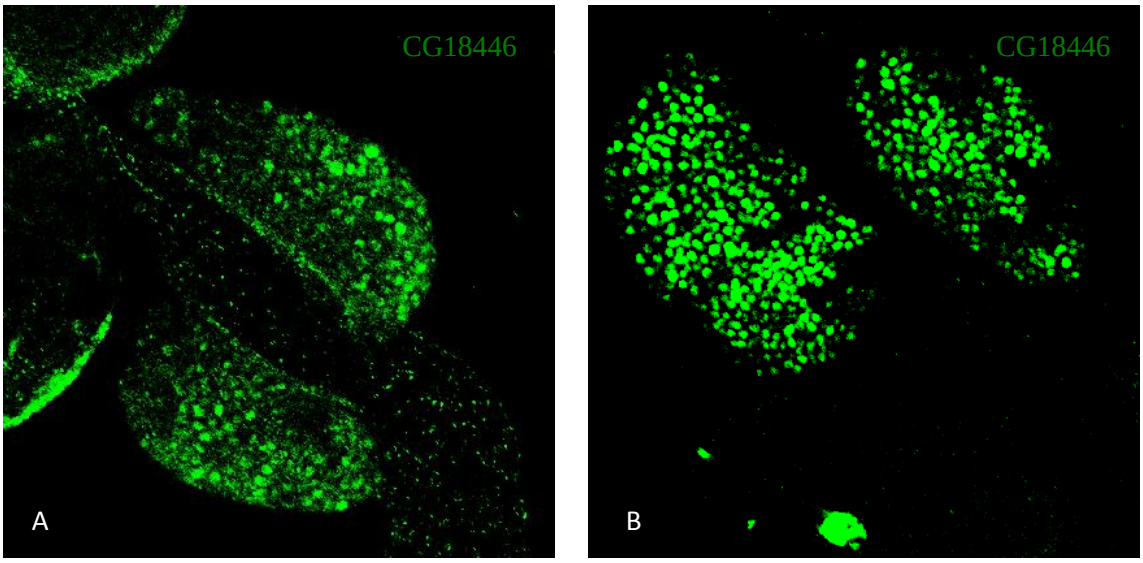

Figure 14: The expression of CG18446 in primary lobes. (A) The expression noticed in the non-infected fly. (B) The expression noticed in the infected lymph gland. The settings to take picture A had to be adjusted to much more sensitive conditions to pick up the GFP signal.

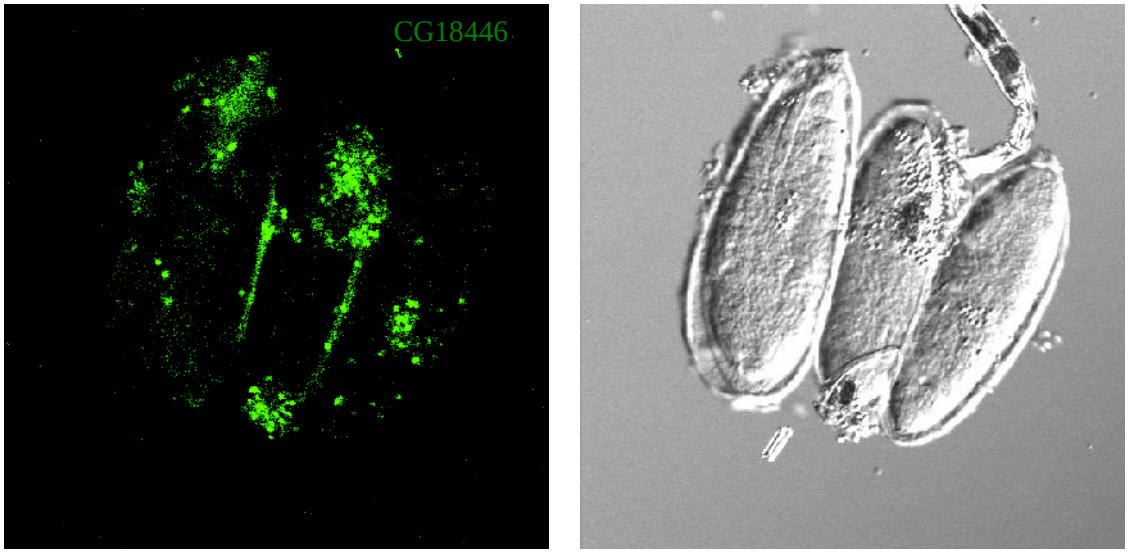

Figure 15: Infected eggs dissected from the infected third larval instar.

4.3. The expression pattern of CG18446 in adult ovaries

The expression of CG18446 was observed in the adult ovaries, on the outside surface of most of the developing egg chambers in the ovariol (Fig. 16). We presume these are the somatic follicular cells that are known to have an active Notch pathway. The expression was missing in germline cells.

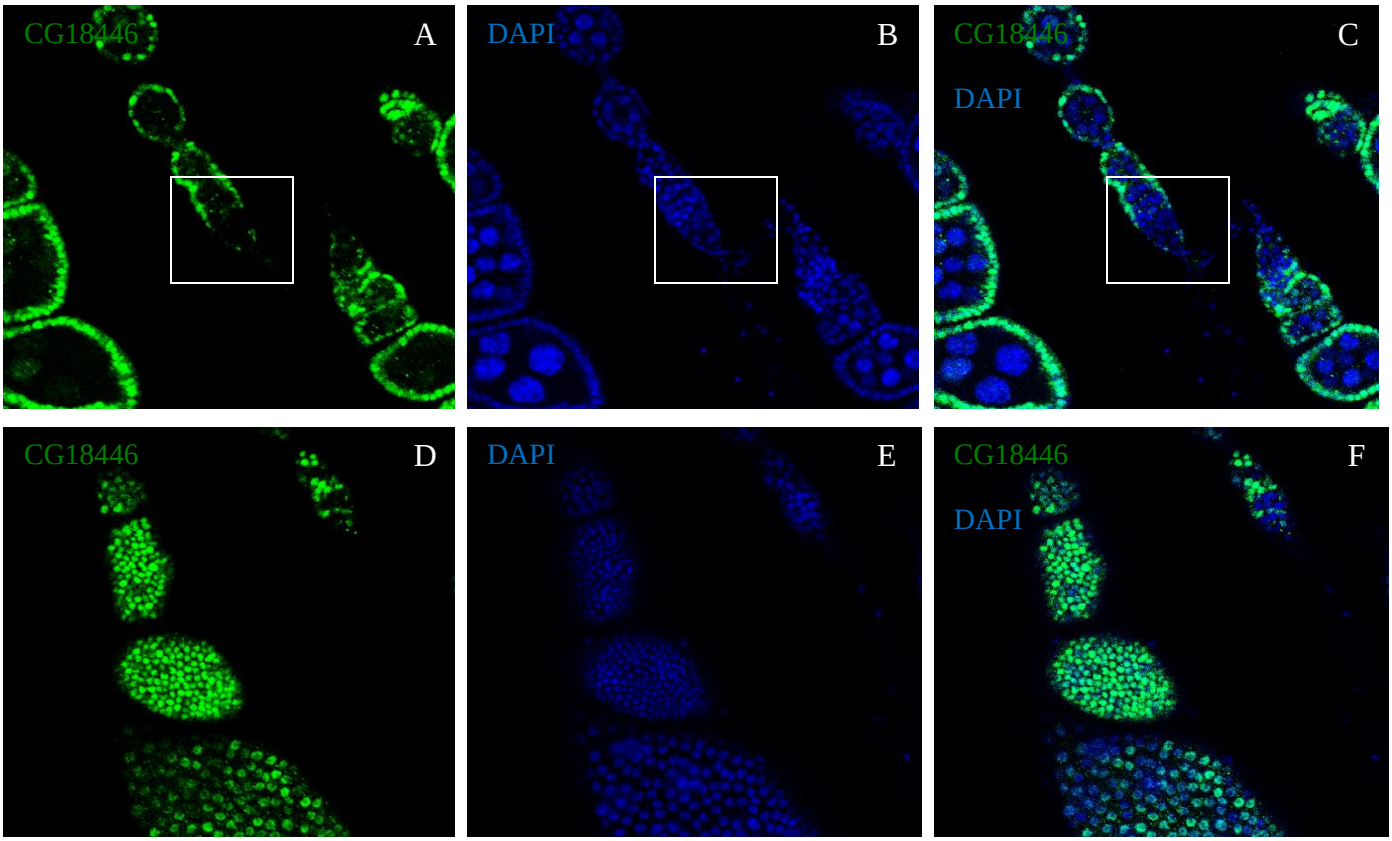

Figure 16: (A-C) The expression of CG18446 in the germarium, no signal observed in the tip of the germarium (framed) but in the developing follicular cells. Signal in the nuclei of nurse cells is a background that was also present in the yellow - white flies stained with GFP. (D-F) The expression of CG18446 observed in the interior of the egg chamber.

In order to correlate the CG18446 signal in the follicle cells to the activity of the Notch pathway, pRR-NRE reporter line was used to look for colocalization by an immunostaining. As expected, we found an overlap in the expression pattern of CG18446 and the activity of the Notch pathway in the follicular cells (Fig. 17).

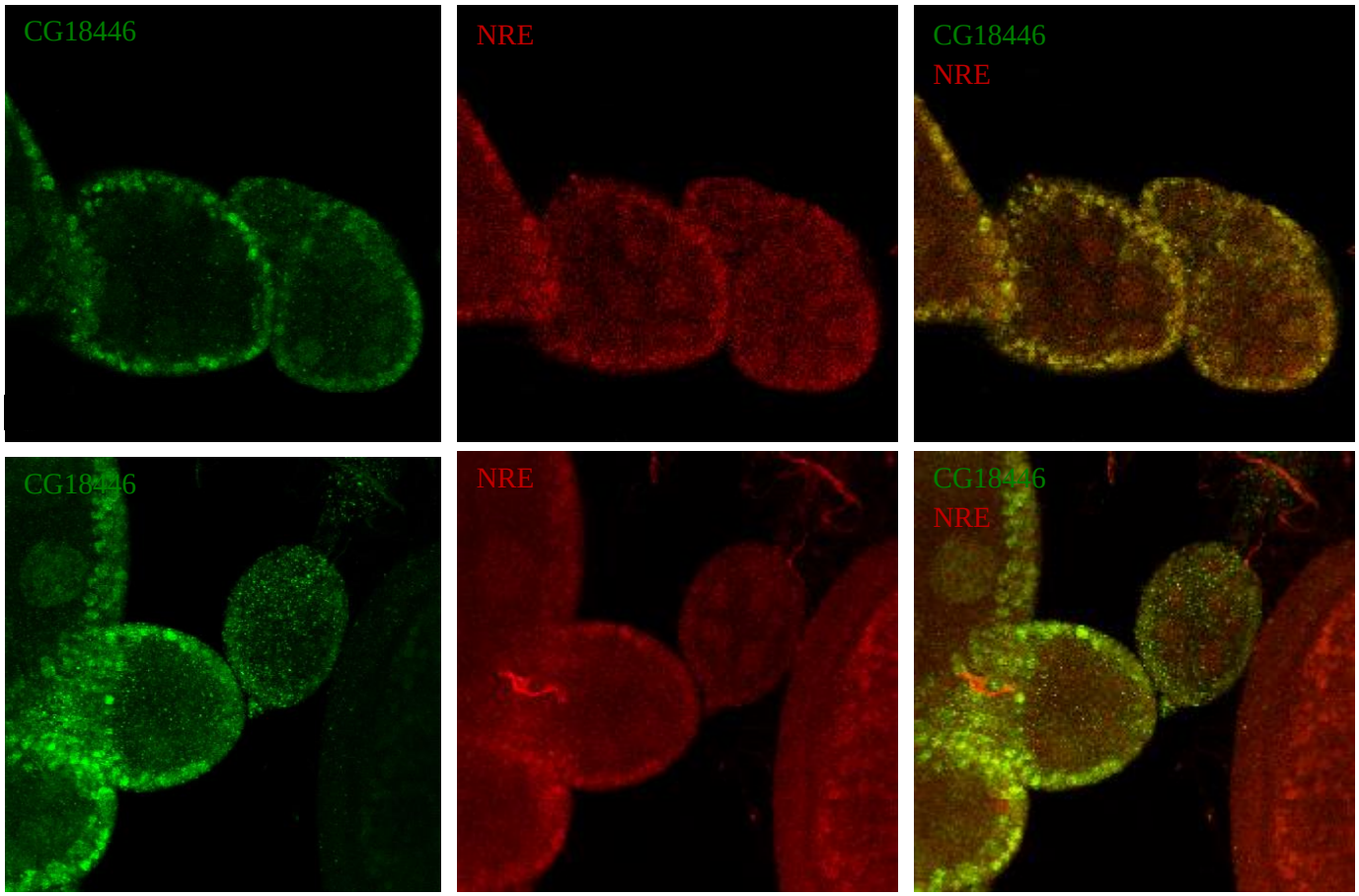

Figure 17: The expression of CG18446 in adult ovaries (green) correlates with the activity of the Notch reporter NRE (red).

As the expression of CG18446 is so widespread in the adult ovaries we wanted to test whether CG18446 has any impact on oogenesis. In a carefully controlled experiment (see the Method section) we counted the number of eggs laid by the wild type (yw), CG18446 mutant (exD) and CG18446 rescue flies /Flyfos CG18446 construct on a exD background) as well as the number if hatched larvae.

The results of our experiment showed that CG18446 mutants were not capable of laying more than 200 eggs in the 24 hours (Fig. 18). On the contrary, the rescue CG18446 laid up to 1800 eggs in the same time period. As expected, the CG18446 rescue flies behaved very similarly to the controls, yellow - white flies.

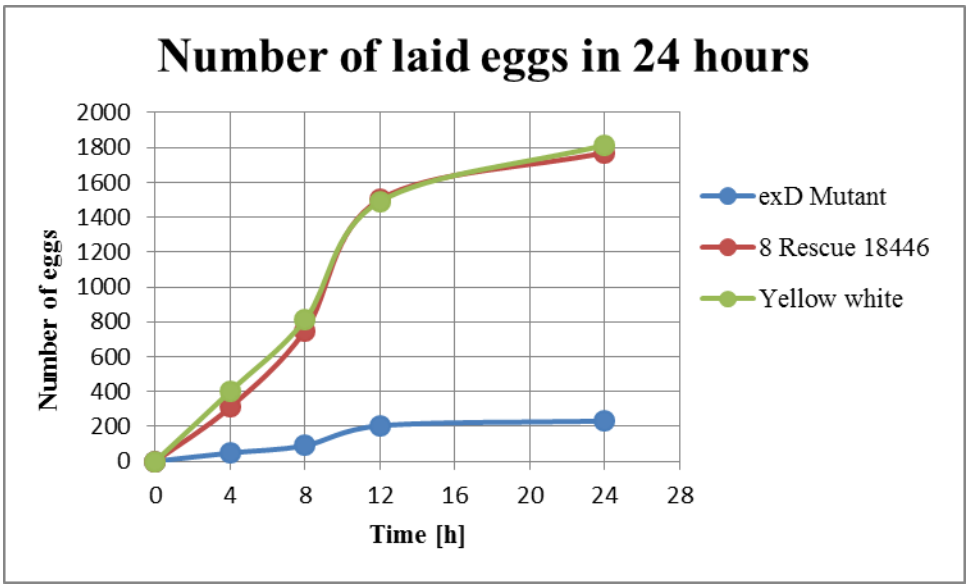

Figure 18: Number of laid eggs over 24 hours.

As previous experiment showed rather intriguing results, we decided test if it is only the production of eggs that is slowed down and the subsequent development of embryos is not affected or if there are less eggs production and these still have problems to develop further. We counted the larvae hatching from the total amount of the eggs laid over the 24 hour period in all phenotypes.

In the first 4 hours, larvae hatched in a similarly speed for the all three phenotypes, reaching 50% from the total amount of laid eggs (Fig. 19). The yellow - white and rescue flies continued hatching, reaching up to almost 95% of efficiency in 8 hours. However, only 60% of mutant flies hatched within the same time. This suggests that about 60% of the mutant CG18446 eggs developed normally but 40% of eggs were seriously affected during their development and the embryos did not reach the larvae stage. Our results clearly showed that CG18446 gene has impact on both the number of laid eggs and the percentage of the larvae hatched out of the eggs.

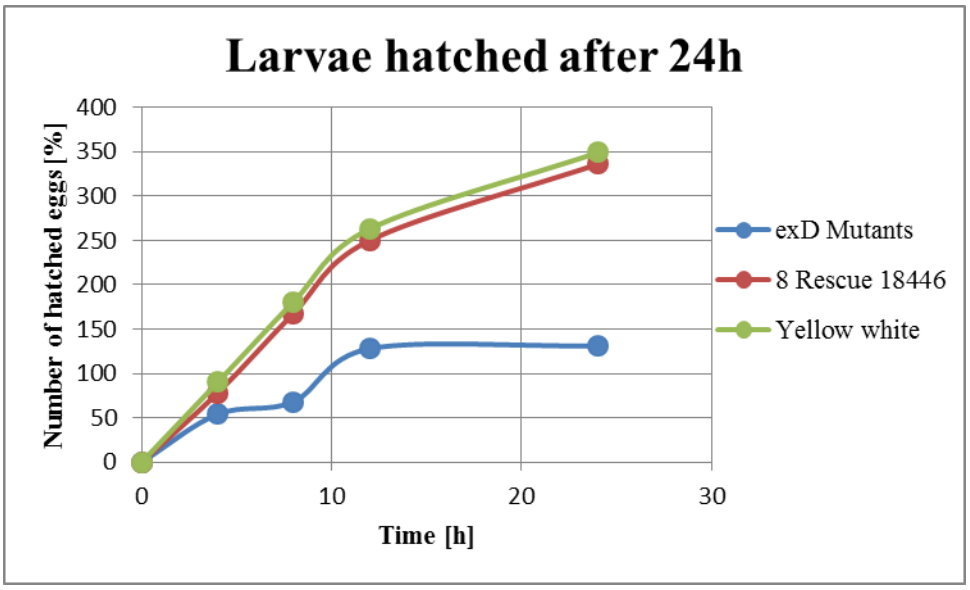

Figure 19: Larvae hatched over 24 hours (% of the total amount of the eggs laid).

4.4. The expression pattern of CG18446 in larval ovaries and testes

As it was detected that CG18446 plays role in adult ovaries and eggs formation, we decided to test whether larval ovaries will show an expression as well. Indeed, larval ovaries showed CG18446 expression in the zone between terminal filament and primordial germ cells (PGCs) (Fig. 20A). We also observed CG1844 expression in the larval testes (Fig. 20B).

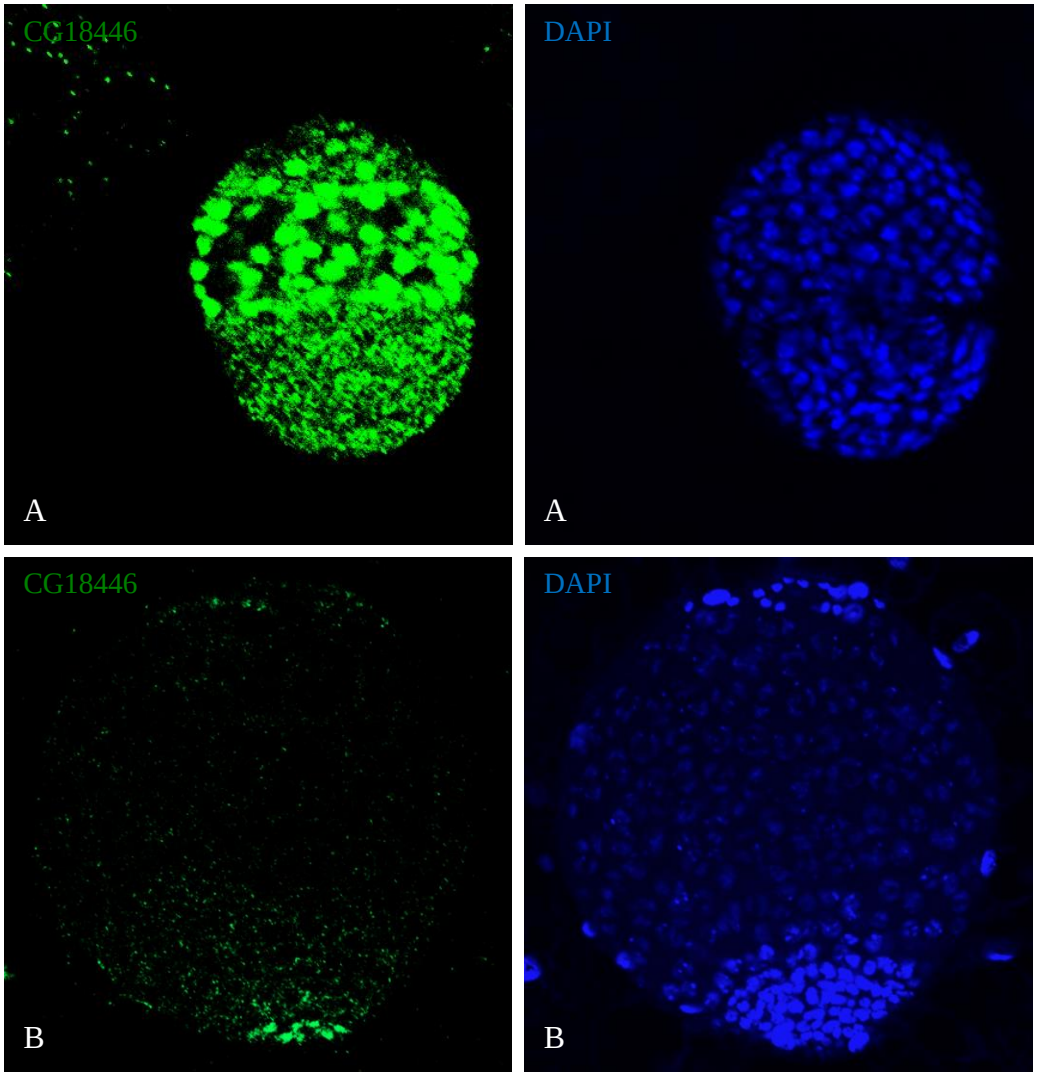

Figure 20: CG18446 expression observed in the (A) third larval instar ovaries and (B) third larval instar testes.

4.5. CG18446 signal in larval fat body tissue

The expression of CG18446 was also observed in the fat body tissue dissected from the third larval instar (Fig. 21).

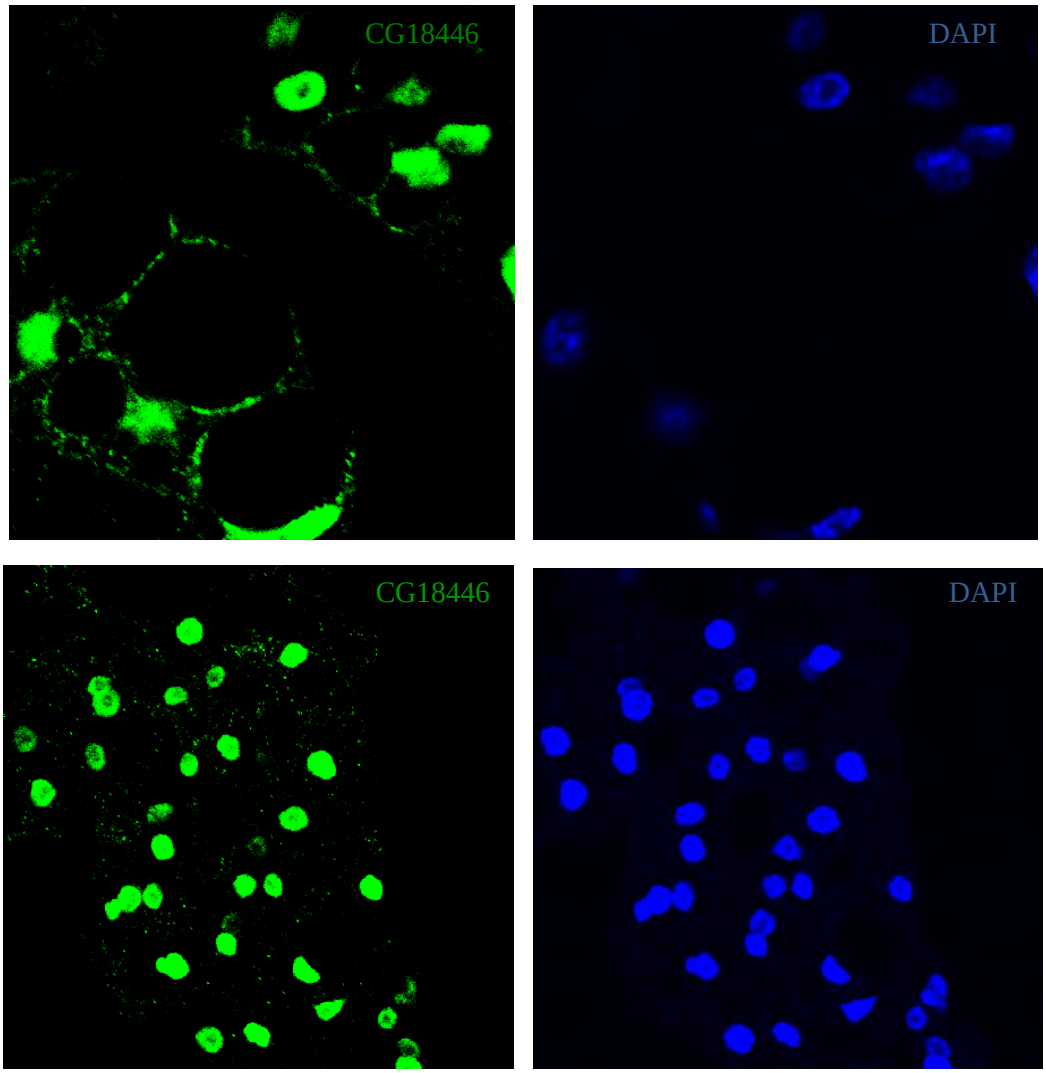

Figure 21: The expression of CG18446 in the fat tissue in third larval instar.

In order to connect the CG18446 expression to its function in the fat body tissue, starvation assay was conducted. Flies of three phenotypes were used – flies mutant for CG18446 gene (exD), CG18446 rescue stock (exD; CG18446-GFP) and control (yellow - white). In a carefully controlled experiment the exact number of both females and males flies were kept under starvation condition and the number of dying adults was counted (see the Method section). Our results showed that the CG18446 mutant female flies were much less resistant to starvation than both the control lines (Fig. 22). This suggests that they may have a problem with fat storage. Interestingly, the same effect was observed with males only in comparison to the yellow - white control line but not in comparison to the rescue CG18446 line; however, we did not have time to confirm these results in a repetition of the experiment (Fig. 23).

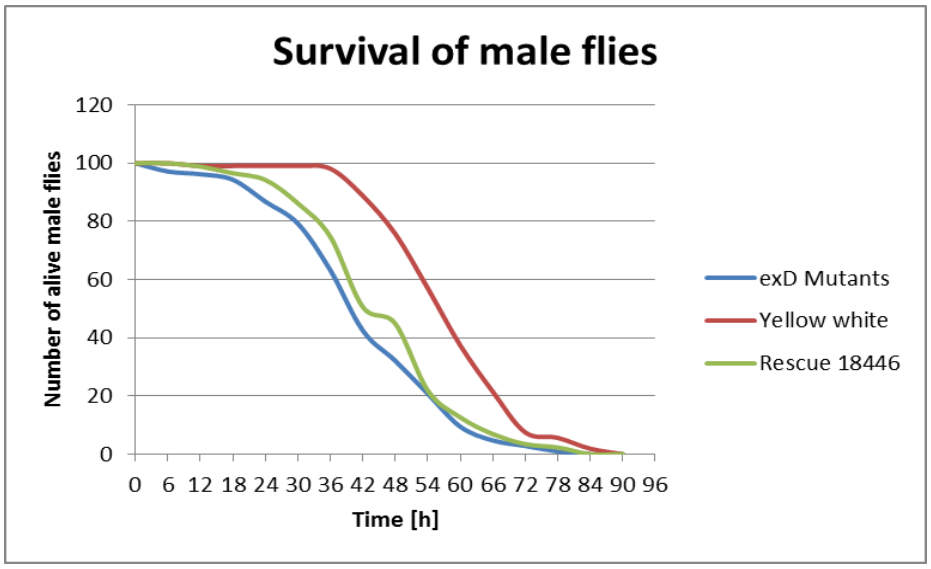

Figure 22: CG18446 mutant males are less resistant to starvation in comparison to the yellow - white strain but not in comparison to the 18446 rescue flies.

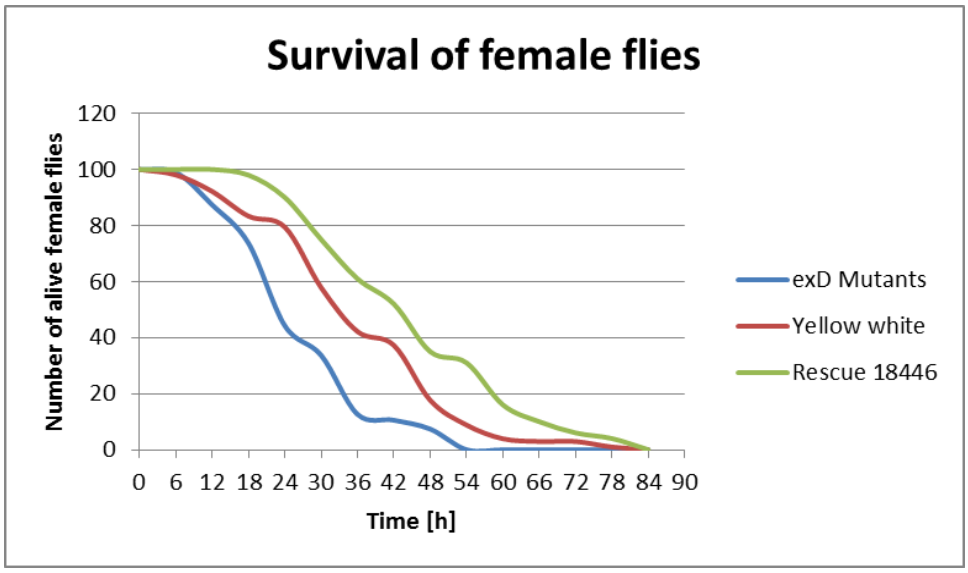

Figure 23: CG18446 mutant females are less resistant to starvation.

5. DISCUSSION

5.1. CG18446 expression in the lymph gland

The expression of CG18446 was present in the cortical zone of the primary lobe of the lymph gland, with no expression in medullary zone or secondary lobes (Fig. 12 and Fig. 13). This suggests that expression corresponds to one of the two mature hemocytes, either the plasmatocytes or crystal cells (lamellocytes are not present in healthy L3 larvae).

We noticed that the expression of CG18446 in the lymph gland was increased after the wasp infection (Fig. 14). Which cells do express CG18446 in these conditions? Most likely those are plasmatocytes, showing CG18446 expression in both the infected and non-infected larvae.

It has been shown that after wasp infestation the number of plasmatocytes increases rapidly in comparison to the amount of plasmatocytes in the healthy organism [29]. Same study came to the conclusion that very few crystal cells are present in the infected animals, what can be a consequence of the lamellocytes' proliferation at the expense of the crystal cells. If this is the case it would be interesting to connect the regulation of CG18446 by Notch with the differentiation of the plasmatocytes as no previous studies have pointed the role of the Notch pathway in this process.

It is also possible that the majority of the cells expressing CG18446 in infected glands are differentiating lamellocytes. Lamellocytes are the biggest hemocytes which are normally not present in flies' hemolymph, but they differentiate in the case of infections of body by pathogens too huge to be phagocyted by plasmatocytes. The absence of Notch blocks normal differentiation of lamellocytes even though its overexpression does not affect lamellocytes production [16].

Last but not least, some of the CG18446 positive cells could also be crystal cells that encapsulate the pathogen ad help to kill it by their black pigment that contain – enzyme for humoral melanization [13]. Pigments can be observed on the chorion of the wasp egg and CG18446 expression was also found in the same place (Fig. 15).

However, one should keep in mind that plasmatocytes, lamellocytes and crystal cells are present in the lymph glands of the infected animals and on the wasp egg after infection. Most probably, CG18446 is expressed in plasmatocytes in normal conditions and in plasmatocytes and lamellocytes in immune challenged animals.

Immunostaining colocalization experiments with markers for all three types of hemocytes should be used in order to prove any of the above mentioned theories. For instance, Gal4 line for *Hemolectin* marker could be used to label plasmatocytes. Crystal cells could be stained using *Lozenge* antibody and, finally, *Misshapen* could serve as a marker for lamellocytes. *Serpent* would mark undifferentiated immune cells (prohemocytes). One way to test if CG18446 is regulated by Notch in these tissues could be to test its colocalization with the pRR-NRE reporter of Notch activity. Currently, we can only say that CG18446 is expressed in both infected and non-infected lymph gland. Which type of cells express it and in which way they are related to Notch, remains unknown.

5.2. The expression pattern of CG18446 in adult ovaries

The expression pattern of CG18446 was observed in the follicle cells of adult ovaries (Fig. 16). According to the results obtained, cells that seem to express CG18446 are follicle cells. Before we started the experiment, we expected and expression also at the anterior part of the germarium, as germline stem cell niche formation and maintenance demand Notch signaling [17]. However, the CG18446 expression only starts to be noticeable from the region 2b onwards, where follicle cells are starting to form. The signal in epithelial follicle cells is then present through all 14 stages. This correlates nicely with the findings that Notch signaling pathway is required for the differentiation of the follicle cells [17]. Moreover, it was suggested that initial Notch signal from germline stem cells is needed for follicle stem cells daughters to travel across the ovariole [30]. The differentiation of polar cells at both ends – anterior and posterior is necessary for proper follicle production and it depends on Notch signaling [30]. Indeed, expression of CG18446 can be clearly seen in polar cells in later stages. It was found that the formation of not only the polar cells but the stalk cells as well depends on the Notch pathway [17]. However, signal CG18446 in the stalk cells was not observed. Reason for this could be that development of these two types of the cells relies on different levels of activation.

Polar cells show high-level Notch activation originating from a germline Delta signal. This is not the case with the stalk cells. Stalk cells have low-level Notch activation and it comes from Delta signal in the polar cells [17].

In order to correlate the CG18446 signal in epithelial follicle and polar cells to Notch activity, pRR-NRE *Drosophila* reporter was used for immunostaining. The pattern of CG18446 and Notch activity reporter did overlap, supporting the connection between Notch, CG18446 and follicle stem cells (Fig. 17). Especially strong NRE expression was noticed in the polar cells, particularly in the one situated in the anterior part of the egg chamber. This could be correlated to the finding that Delta sends signal from the germline cyst in order to activate Notch in the neighboring anterior polar/stalk precursors, causing them to form polar cells [17]. This way the first anterior polar cells appear while the posterior ones migrate to the tip of the chamber. The Notch pathway is required for proper border cell migrations and it is triggered in these cells during their movements [17].

Remarkably low number of laid eggs by CG18446 mutant flies as well as the lower proportion of hatching larvae suggests an important role of CG18446 in *Drosophila* oogenesis (Fig. 18 and Fig. 19). As strong expression of CG18446 was noticed in follicle cells, reduced number of laid eggs can be related to the Notch duty to switch the follicle cells from mitotic cycle to endocycle [17]. It is after this switch when the follicle cells are actually differentiated into mature ones. Lack of Notch signal obviously can prevent this differentiation resulting in very few mature follicle cells. As a consequence, small number of eggs are properly formed and even fewer are able to provide protection until the complete embryo development and larvae hatching. Nevertheless, it still remains to be discovered whether this is true or not.

5.3. CG18446 signal in larval fat body tissue

The *Drosophila* fat body showed CG18446 expression (Fig. 21). To test the importance of CG18446 expression in this tissue starvation assay was conducted.

Flies mutant for the CG18446 gene were less prone to survive starvation periods (at least in the case of females). They either had less fat body tissue or they had certain disorders in mobilizing stored TAG. Another possible reason for this is that mutant flies had problems with storing the food at the first place. Additional experiments would need to be done to distinguish between these scenarios.

Could CG18446 be regulated by Notch signaling? There are almost no data connecting Notch to fat tissue. However, the transcription factor *Twist* could provide the link. Namely, it was shown that Notch signaling pathway regulates *Twist* expression and subdivision during embryogenesis [27]. Twist expression is conducted into stripes consisting of high and low levels. High Twist domains are differentiating into somatic muscles and heart, while low levels develop into visceral muscle, fat body and mesodermal glia. Exact and correct modulation into low and high expression domains can only be achieved by Notch signaling. In the absence of Notch for repression at stage 10, Twist is expressing exclusively uniformed high domains. Presence of only high Twist levels has serious consequences for proper development of the mesodermal tissues. Under normal condition, during the stage 9 – 10 mesodermal cells migrate dorsally and become specified as, amongst the others, fat body mesoderm CG18446 mutant flies were less prone to survive longer starvation periods [27]. This could be interpreted in a way that deletion of CG18446 led to improper development of Twist domains during embryogenesis that resulted in an irregular maturation of fat body tissue. Whether this is correct, remains to be tested.

Expression of CG18446 in the fat body tissue could also be related to the immune response. As a part if *Drosophila*'s reaction to an infection, there is a wide range of antimicrobial peptides produced in the fat body [30]. Serpent (srp) is the essential GATA factor expressed in this tissue during embryogenesis as well as in larvae where it is crucial for interfering the systematic immune response in the larval fat body [31]. Additional interesting detail is related to the *srp* – it is believed to be in charge of blood cells fate determination in the embryo [16]. Bearing in mind this information, it would be very intriguing to see the expression of CG18446 in the infected larval fat body tissue.

6. SUMMARY

The expression pattern of CG18446 was followed by immunostaining of Drosophila third instar larvae and adults. We found an expression in the cortical zone of the larval lymph gland, in larval ovaries, testes and fat tissue. We also observed CG18446 expression in the follical cells of adult ovaries. In agreement with these observations flies mutant for CG18446 laid less eggs, less larvae hatched from them and the surviving adults were less resistant to starvation. Our results point to an important role of CG18446 in egg development, fat tissue homeostasis and immune response.

7. REFERENCES

1. S.J. Bray, Notch signaling: a simple pathway becomes a complex, *Nature Reviews Molecular Cell Biology* **7** (2006) 678

2. Bruce Alberts, (2005), Molecular Biology of the Cell, Garland Science

3. E.C. Lai, Keeping a good pathway down: transcriptional repression of Notch target genes by CSL proteins, *EMBO Reports* **3** (2002) 840

4. F. Schweisguth, Regulation of Notch signaling activity, *Current Biology* **14** (2004) 129

5. K.G. Guruharsha, M.W. Kankel, S. Artavanis - Tsakonas, The Notch signaling system: Recent insights into the complexity of a conserved pathway, *Nature Reviews Genetics* **13** (2012) 654

6. F. Bernard, A. Krejci, B. Housden, B. Adryan, S.J. Bray, Specificity of Notch pathway activation: Twist controls the transcriptional output in adult muscle progenitors, *Development* **137** (2010) 2633

7. http://genome.wellcome.ac.uk/

8. http://www.ncbi.nlm.nih.gov/

9. Leland H. Hartwell, (2011), Genetics, McGraw-Hill Higher Education

10. M. Meister, M. Lagueux, *Drosophila* blood cells, *Cellular Microbiology* **5** (2003) 573

11. S.H. Jung, C.J. Evans, C. Uemura, U. Banerjee, The *Drosophila* lymph gland as a developmental model of hematopoiesis, *Development* **132** (2005) 2521

12. T.J. Little, D. Hultmark, A.F. Read, Invertebrate immunity and the limitis of mechanistic immunology, *Nature Immunology* **6** (2005) 651

13. M. J. Williams, The *Drosophila* Hemopoiesis and Cellular Immunity, *The Journal of Immunology* **178** (2007) 4711

14. J. Krzemien, M. Crozatier, A. Vincent, Ontogeny of the Drosophila larval hematopoietic organ, hemocyte homeostasis and the dedicated cellular response to parasitism, T*he International Journal of Developmental* Biology **54** (2010) 1117

15. A. Terriente-Felix, J. Li, S. Collins, A. Mulligan, I. Reekie, F. Bernard, A. Krejci, S.J. Bray, Notch cooperates with Lozenge/Runx to lock haemocytes into a differentiation programme, *Development* **140** (2013) 926

16. B. Duvic, J.A. Hoffmann, M. Meister, J. Royet, Notch Signaling controls lineage specification during *Drosophila* larval hematopoiesis, *Current Biology* **12** (2002) 1923

17. J. Xu, T. Gridley, Notch signaling during Oogenesis in *Drosophila melanogaster*, *Genetics Research International* **2012** (2012) 648207

18. R. Bastock, D.S. Johnston, *Drosophila* oogenesis, *Current Biology* **18** (2008) 1082

19. Q. Li, T. Xin, W. Chen, M. Zhu, N. Li, Lethal(2)giant larvae is required in the follicle cells for formation of the initial AP asymmetry and the oocyte polarity during Drosophila oogenesis, *Cell Research* **18** (2008) 372

20. L. Gilboa, R. Lehmann, Soma-germline interactions coordinate homeostasis and growth in the *Drosophila* gonad, *Nature* **443** (2007) 97

21. M. Asaoka, H. Lin, Germline stem cells in the Drosophila ovary descend from pole cells in the anterior region of the embryonic gonad, *Development* **131** (2004) 5079

22. X. Song, G.B. Call, D. Kirilly, T. Xie, Notch signaling controls germline stem cell niche formation in the *Drosophila* ovary, *Development* **134** (2007) 1071

23. E.L. Matunis, R.R. Stine, M. de Cuevas, Recent advances in Drosophila male germline stem cell biology, *Spermatogenesis* **2** (2011) 137

24. J. Tran, T.J. Brenner, S. DiNardo, Somatic control over the germline stem cell lineage during *Drosophila* spermatogenesis, *Nature* **407** (2000) 754

25. T.C. Okegbe , S. DiNardo, The endoderm specifies the mesodermal niche for the germline in Drodophila via Delta-Notch signaling, *Development* **138** (2011) 1259

26. S. Grönke, A. Mildner, S. Fellert, N. Tennagels, S. Petry, G. Müller, H. Jäckle, R.P. Kühnlein, Brummer lipase is an evolutionary conserved fat storage regulator in *Drosophila*, *Cell Metabolism* **1** (2005) 323

27. A. Tapanes-Castillo, M.K. Baylies, Notch signaling patterns *Drosophila* mesodermal segments by regulating the bHLH transcription factor *twist*, *Development* **131** (2004) 2359

28. A. Krejci, F. Bernard, B.E. Housden, S. Collins, S.J.Bray, Direct response to Notch activation: signaling crosstalk and incoherent logic, *Science Signaling* **2** (2009)

29. T. Nystul, A. Spradling, Regulation of epithelial stem cell replacment and follicle formation in the Drosophila Ovary, *Genetics* **184** (2010) 503

30. K. Senger, K. Harris, M. Levine, GATA factors participate in tissue specific immune responses in *Drosophila* larvae, *Proceedings of the National Academy of Sciences of the United States of America* **103** (2008) 15957

31. U.M. Petersen, L. Kadalayil, K.P. Rehorn, D.K. Hoshizaki, R. Reuter, Y. Engström, Serpent regulates *Drosophila* immunity genes in the larval fat body through an essential GATA motif, *The EMBO Journal* **18** (1999) 4014