Egypt. Acad. J. Biolog. Sci., 4(1):31-42 (2012) C. Physiology & Molecular Biology
Email: egyptianacademic@yahoo.com ISSN: 2090-0767
Received: 25/2/2012 www.eajbs.eg.net

Transgenic *Drosophila melanogaster* Carrying RNAi Against *gce* Gene Proved the Involvement of *gce* in Juvenile Hormone Action

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ABSTRACT

Juvenile hormone (JH) is important for multiple aspects of insect development and physiology. Although roles of JH have received considerable studies, JH signaling pathway at the molecular level is still not well understood. *Methoprene-tolerant* (*Met*) in *Drosophila melanogaster* fulfills many of the requirements as a hormone receptor gene. A paralogous gene, *germ-cell expressed* (*gce*), possesses homology to *Met* and is a candidate as a *Met* partner in mediating JH action. To probe roles of this gene in JH action, we carried out in vivo *gce* underexpression studies using RNAi technique. Precocious expression of *broad* (*br*) gene was found to occur when *gce* was knocked down in the young larvae. We also demonstrated that RNA interference-driven knockdown of *gce* expression in transgenic flies results in appearance of resistance to pyriproxyfen (IGRs). These results show that *gce* is a vital gene and appears to promote JH action in larval stages.

Keywords: *Drosophila melanogaster*, Transgenic**,** Juvenile Hormone**,** RNAi and *gce* gene

INTRODUCTION

Drosophila melanogaster (Order: Diptera; Family: Drosophilidae) provides an ideal system to examine the genes and molecular mechanisms of hormones that regulate the growth and differentiation of tissue. RNA interference (RNAi) is a mechanism that knocks down gene expression in variety of organisms (Nishikura 2001). In 2001, the *Drosophila* researchers started to inject dsRNA into adult *Drosophila* abdomen, trying to trigger RNAi of their favorite gene (Dzitoyeva *et al.* 2001). When a long dsRNA is introduced into the organism, its double-stranded structure is recognized by a ribonuclease III enzyme named Dicer, which subsequently cleaves it into smaller fragments named small interfering RNAs (siRNA). In turn, siRNA directs the cleavage of homologous messenger RNA after siRNA is incorporated into the RNA-induced silencing complex (RISC) (Nishikura 2001; Hannon 2002). The cleavage of messenger RNA then results in a substantial decrease in expression of that gene in the organism. The RNAi technique involves an inverted repeat of a sequence specific to the target gene, and clones the inverted repeat structure into a vector that contains a conditional promoter (Allikian *et al.* 2002). Since its discovery a decade ago, RNAi has become an important and powerful research tool to investigate gene function in an array of organisms, both in vivo and in vitro. Recently, researchers developed a large scale RNAi injection method for *Drosophila* embryos to identify and characterize the molecular functions of the 14,000 *Drosophila* genes (Cornell *et al.* 2008). More recent works based on RNAi experiments have shown

that the transcription factor Broad and Kruppel homolog 1 are also involved in the antimetamorphic action of JH (Minakuchi *et al.*, 2009). RNAi can be triggered by the expression of a long double stranded hairpin RNA from a transgene containing a gene fragment cloned as an inverted repeat. The expression of such transgenes under the control of a generic promoter containing the Gal4-responsive upstream activator sequence (UAS) element can target RNAi to any cell type at any stage of the insect for which a suitable Gal4 driver line is available (Bellés 2010).

To understand how Gce mediates JH action, we generated transgenic flies carrying the RNAi system to knockdown *gce* expression. The *gce* underexpression produced precocious *broad* expression in the immature stage and is functioning prepupal development by inhibiting *br* expression. Furthermore, we show that RNA interference-driven knockdown of *gce* expression in transgenic flies results in appearance of resistance to pyriproxyfen (IGRs). This study lays a foundation for further elucidation of the molecular mechanism of JH action in *Drosophila*.

MATERIALS AND METHODS

Insect used

The red-eyed *Oregon*-R strain was used as susceptible wild type control, Pin/Cyo, and UAS-mCD$_8$-GFP/Fm7c were from Bloomington *Drosophila* Stock Center at Indiana University (USA). Met^{27} was a gift from Dr. Thomas Wilson (*Ohio State University, USA).* Gal4- PG12 was a gift from Dr. Henri Bourbon (Centre de Biologie du De´veloppement, France). All *Drosophila* strains were grown on standard cornmeal/molasses/yeast food at 25°C.

Preparation of target DNA fragment

Genomic DNA was isolated according to the standard method. One pair of specific *gce* primers carrying restriction enzymes cut sites were used in the PCR reaction to amplify the target DNA fragment from the isolated genomic DNA. The primers were gce-DNA-F 5'-ATTGAATTCGC ATTGAA CAGCATTTGGG-3' (EcoR1) and gce-DNA-R 5'-ATTTCTAGAGTGAAGG GACTGAGATTGCG-3' (Xba1)

Preparation of target cDNA fragment

Total RNAs were isolated using the RNeasy Mini Kit (Qiagen, USA). One pair of specific primers (gce primer) carrying restriction enzymes cut sites were used in the RT-PCR reaction to amplify the target complementary DNA (cDNA) fragment from the isolated total RNA. The primers were gce-cDNA-F 5'-ATTGCGGCCGCGCATTGAACAGCA TTTGGG-3' (Not1) and gce-cDNA-R 5' ATTTCTAGAGATTCTGGATGAGCC GCA-3' (Xba1)

The *gce* RNAi construct

Expression of *gce* was subjected to RNAi-mediated knockdown. A gce RNAi-generating transgene was engineered from a fusion of genomic DNA and reverse complement cDNA and then subcloned into the injector plasmid vector, pCaSpeR-hs plasmid. Plasmid was sent to Best Gene Incorporation (USA) to be injected into Drosophila embryos (white eye strain, w^{1118}).

Green florescent protein (GFP) as a marker for the *Broad* (*br*) expression

Broad (*br*) gene is an important gene represents initiation of the metamorphosis, and its endogenous expression can be visualized in the larvae using green florescent protein (GFP) marker in specific fly (Gal4-PG12>UAS-mCD$_8$-GFP). That fly was crossed with gce-RNAi fly.

Heat shock procedure

Because the RNAi fragment for the *gce* gene was subcloned into pCaSpeR-hs plasmid, this insertion is under control of heat shock promoter (hs-promotor). By heat shock the first instar larvae of the transgenic fly for *gce* for one hour at 38^{o}C, the RNAi fragment are transcribed

into targeting mRNA as hair-pin structure. This structure functions as double stranded RNA (dsRNA) which interferes with endogenous mRNA of the targeting gene *gce*. This process, finally, reduce the mRNA level of this gene.

Immunohistochemistry and microscopy

Larval fat bodies were dissected from the 2nd instar larvae. Immunohistochemistry was performed as described previously (Wang *et al.*, 2002). Endogenous Br proteins were labeled with a Br-core antibody (25E9.D7, from the DSHB at the University of Iowa). Florescence signals were captured with a Zeiss LSM510 confocal microscope (Carl Zeiss).

JHA treatment

The JHA pyriproxyfen (Sigma) was dissolved in 95% ethanol to give a 300 ppm stock solution. JHA-containing fly food was prepared by adding JHA stock solution to the standard food at 50-55°C to a final concentration of 0.03-3 ppm. For the JHA resistance assay, 100 newly hatched Oregon-R, Met^{27}, or gce-RNAi larvae were reared in vials of food containing different concentrations of JHA. Survival rates were calculated based on the numbers of flies developing to adulthood.

RESULTS

Crossing screening for the successful integrated *gce*-RNAi into the genomic DNA of the injected flies:

The constructed plasmid for *gce*-RNAi was injected in the newly laid *Drosophila* eggs, w^{1118}, which marked by whit eye flies. Accordingly the constructed plasmid was designed to carry the *mini-white* gene as a red eye marker. The eye marker was used as an indicator for the successful integration by seeing the change in the eye color. As shown in Fig. (1) the survival injected males which carry the *gce*-RNAi integrated constructs were crossed to Pin/CyO virgin females. After about 10 days, males with red eyes which carry the Pin or CyO marker were selected from the progeny and crossed to Pin/CyO virgin females. After two generations, the P [hs-gce RNAi] on the second chromosome were selected independently and crossed to keep the stocks of the transgenic flies as shown in Fig. (2).

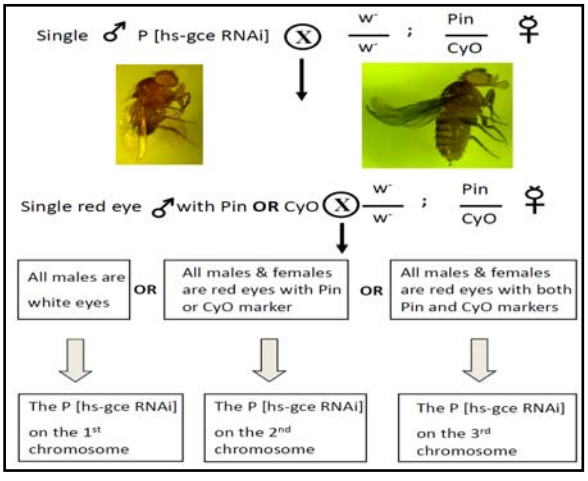

Fig.1: A Crossing screening to select the integrated P [hs-gce RNAi] construct in the second chromosome of the transgenic flies. B) Crossing steps to build the homozygous stock of the transgenic flies carrying the integrated P [hs-gce RNAi] construct in the second chromosome.

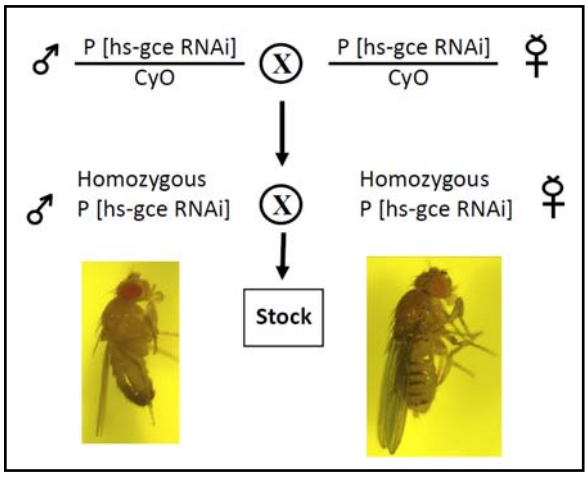

Fig. 2: Crossing screening to select the integrated P [hs-gce RNAi] construct in the second chromosome of the transgenic flies.

Knockdown validation of *gce* in the gce-RNAi flies:

In a qualitative RT-PCR test the expression level of *gce* was compared in the wild type and heat shocked insects at the same conditions. The results were presented in Fig. (3).It can be noticed from Fig. (3) that the expression level of *gce* was dramatically decreased in the heat shocked insects compared with the expression level in the wild type insects. This indicated that gce-RNAi was active and targeted the *gce* gene which leads to produce nonfunctional mutant *gce* gene. The control gene, *Rp49*, was expressed normally in both heat shocked insects and in the wild type insects as well. The positive expression of the control gene was validating that the mRNA level of the target gene was the only affected one. As shown in Fig. (3 B).

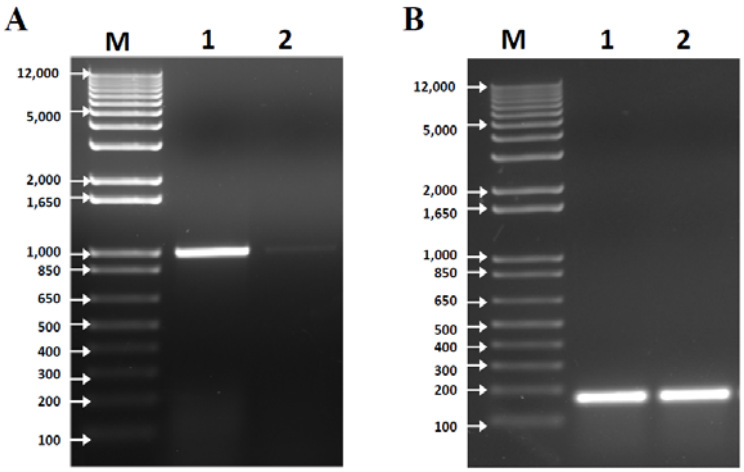

Fig. 3: DNA agarose gel electrophoresis of the RT-PCR products to validate the RNAi activity in reducing the expression level of *gce*

A: M: DNA marker in bp
1: *gce* expression in the wild type insects
2: *gce* expression in the heat shocked insects

B: M: DNA marker in bp
1: *Rp49* expression in the wild type insects
2: *Rp49* expression in the heat shocked insects

Resistance bioassay to juvenile hormone agonist, pyriproxyfen (IGRs) in three different fly types:

Met gene is well known as a candidate receptor for juvenile hormone signaling path way and mediates its action. When this mediator (*Met*) is knocked down and become mutant, the flies which carry this mutation become resistant to JHA such as methoprene and pyriproxyfen in the larval stages. Based on that, gce-RNAi larvae were contentiously heat shocked in order to activate gce-RNAi and subsequently knock down the expression of *gce*. This type of larvae in addition to the susceptible wild type Oregon R larvae and Met^{27} larvae as a resistant strain were treated by 0, 0.03, 0.1, 0.3, or 1 ppm pyriproxyfen independently. The mean percentage numbers of the survival adult flies from each concentration were counted and presented in Table (1). Results represented in Table (1) and Fig. (4) revealed that the mean percentage number of adult survival from the four different fly genotype had a positive relation with the different concentrations of pyriproxyfen.It can be noticed that the survival percentages of adult flies were decreased significantly ($p < 0.05$) with the increase in the concentration of pyriproxyfen. From table (1) & Figure (4) it is clear that at conc. 0.1ppm, the highest tolerance which leads to a high adult survival rate was recorded as 87.3±9.7% for the Met mutant flies (Met^{27}), and recorded as

52.3±7.3% in the gce-RNAi flies.In comparing with Oregon R (34.2±3.3%), the previous two survival rate percentages showed high significant difference at the same concentration. At concentration 0.3 ppm, the wild type flies showed significant low survival rate 4±1.3% compared with the survival rate 29.2±6.3, and 83±8.7 % for gce-RNAi, and Met^{27} flies, respectively.

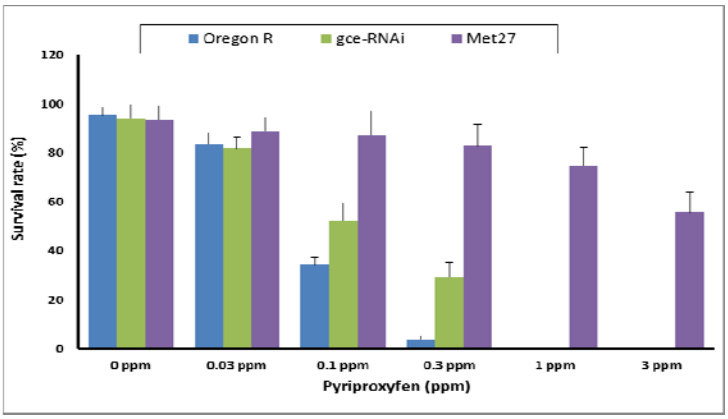

Fig. 4: Comparison between the mean percentage number of survival Oregon R, gce-RNAi, and Met^{27} adult flies after treatment by different concentrations of pyriproxyfen (IGRs).

Table 1: Mean percentage number of survival Oregon R, gce-RNAi, and Met^{27} adult flies after treatment by different concentrations of pyriproxyfen (IGRs).

	Mean % number of survival adult flies ± SE		
	Oregon R	**gce-RNAi**	**Met^{27}**
0 ppm	95.3±3.4 a	94.2±5.5 a	93.6±5.7 a
0.03 ppm	83.6±4.5 b	81.7±4.7 b	88.9±5.5 b
0.1 ppm	34.2±3.3 c *	52.3±7.3 c *	87.3±9.7 b *
0.3 ppm	4±1.3 d *	29.2±6.3 d *	83±8.7 b *
1 ppm	0	0	74.6±7.6 c
3 ppm	0	0	55.7±8.2 d

Mean values in vertical columns having different small letters are statistically significant ($p < 0.05$)
Mean values in horizontal rows followed by asterisk (*) are statistically significant ($p < 0.05$)

The use of *Gal4-PG12* to recapitulate the *br* expression pattern in gce-RNAi flies:

The *broad* (*br*) gene is a molecular marker for pupal commitment and specifies the larval-pupal metamorphosis in a variety of holometabolous insect species. It is the most important one of the early response genes in the Ecdyson signaling path way where it is highly expressed in the late third instar larvae. The *broad* (*br*) gene has been identified as a key regulator in mediating the cross-talk between Ec and JH signaling pathways. JH through its receptors inhibit the expression of *br* during the larval stages so *br* is a key gene for monitoring the JH signaling bath way through its receptor. The expression patterns of the *Gal4-PG12* enhancer-trap line inserted near the *br* gene was used to monitor *br* expression in live organisms. Where the expression pattern of the *Gal4-PG12* was found to be closely resembled the temporal and spatial expression pattern of the endogenous *br* gene in tissues other than the salivary gland. In this study *Gal4-PG12* was used to drive *UASmCD8GFP* on the X chromosome as a reporter of *br* expression. The gce-RNAi insertion on the 2nd chromosome was made homozygous in combination with *Gal4-PG12>UASmCD8GFP* by genetic crosses as illustrated in figures (5) to produce the flies stock:

Gal4-PG12>UASmCD8GFP/Fm7c/Y; gce-RNAi.

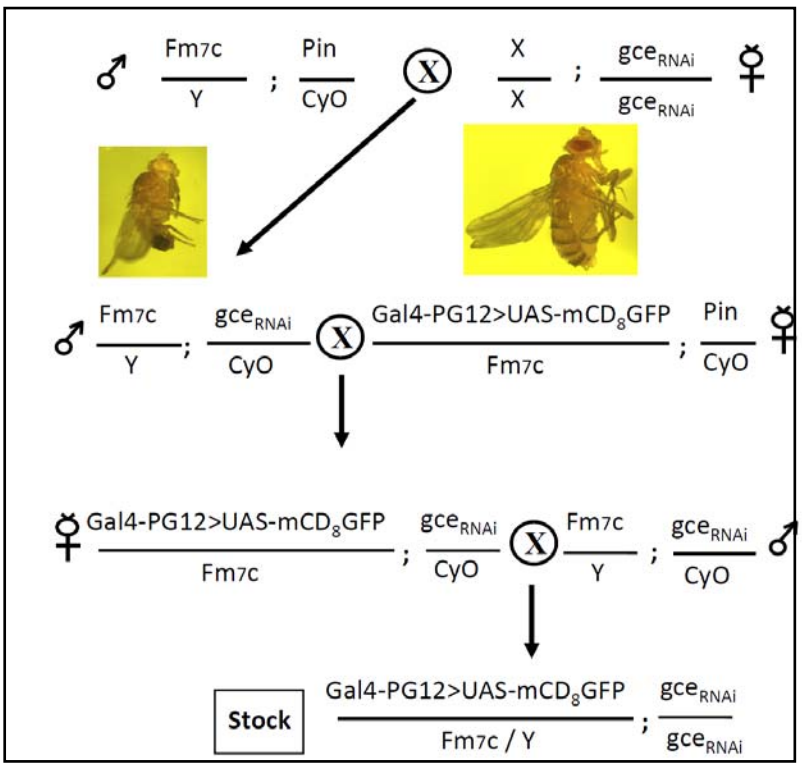

Fig. 5: Schematic diagram of genetic crosses for combining *gce*-RNAi on the second chromosome with *Gal4-PG12> UAS-mCD$_8$GFP* on X chromosome which used to monitor *br* expression.

Monitoring the precocious *br* expression in the gce-RNAi 2nd instar whole larvae:

Because JH represses *br* expression during early larval stages, it was reasoned that mutations that reduce the JH titer or disrupt JH action should cause precocious *br* expression in the young larvae (2nd instar). Accordingly *br* expression was monitored in gce-RNAi 2nd instar larvae by using special Gal4-UAS binary system with a specific Gal4 driver, *Gal4-PG12*. It is expressed resemble to the expression of the endogenous *br* expression, and drives *UAS-mCD$_8$GFP* which produces florescent green color. The gce-RNAi flies were combined with *Gal4-PG12> UAS-mCD$_8$GFP* on X chromosome by genetic crosses independently. The GFP expression was examined at the 2nd instar and images were captured under the fluorescent microscope and presented in figure (6).Images in figure (6) displayed that the expression of *Gal4-PG12>UAS-mCD8GFP* was restricted to salivary glands in the wild type 2nd instar larvae but ubiquitous expression of *Gal4-PG12>UAS-mCD8GFP* was detected at the same stage in the *gce* mutant larvae. These results suggest that *gce*is required to repress *br* expression during the early larval stages, possibly by regulating either the JH titer or JH signaling.In order to distinguish whether *gce*is involved in the JH titer or JH signaling, Gal4-PG12>UAS-mCD8GFP/Fm7c/Y ; gce-RNAi larvae were fed on food containing 0.1 ppm juvenile hormone analogue (pyriproxyfen) and heat shocked. The GFP expression was examined at the 2nd instar and images were captured under the fluorescent microscope and presented in figure (7). Images in figure (7) demonstrated that the expression of *Gal4-PG12>UAS-mCD8GFP* was restricted to salivary glands in the wild type 2nd instar treated larvae with pyriproxyfen while the *gce* mutant treated larvae with pyriproxyfen showed ubiquitous expression of *Gal4-PG12>UAS-mCD8GFP* at the same stage. The precocious *br* expression was not prevented by pyriproxyfen treatment. These results propose that *gce* is required to repress *br* expression during the early larval stages, possibly by regulating the JH signaling.

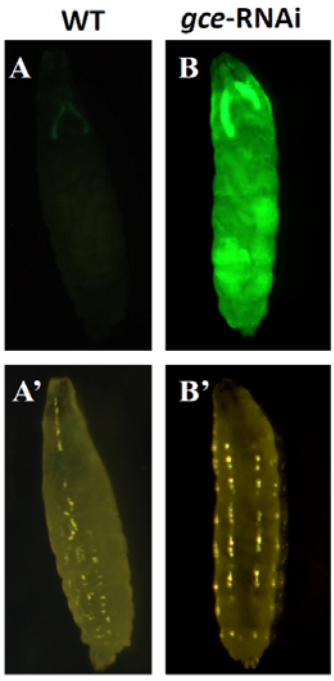

Fig. 6: GFP images show the expression of *Gal4-PG12>UAS-mCD8GFP* in the gce-RNAi 2nd instar larvae after the heat shock process. GFP was detected and captured under the fluorescent microscope. (60 X magnification). **A**: Wild type larva. **B**: Gal4-PG12>UAS-CD8GFP/Fm7c/Y; gce-RNAi larva. **A**',**B**': White light images of the same larvae are shown in[**A,B**].

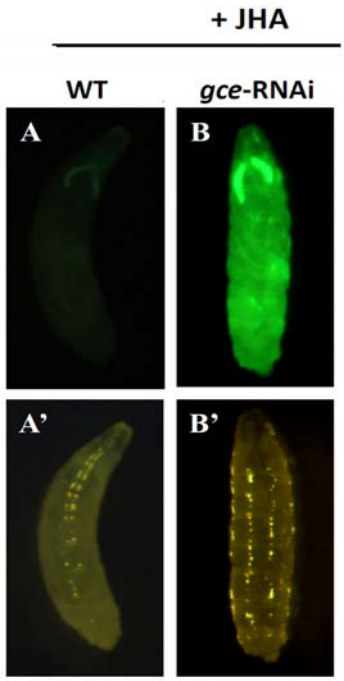

Fig. 7: GFP images show the expression of *Gal4-PG12>UAS-mCD8GFP* in the gce-RNAi 2nd instar of treated larvae with 0.1 ppm pyriproxyfen after the heat shock process. GFP was detected and captured under the fluorescent microscope (60 X magnification). **A:** Wild type larva. **B:**Gal4-PG12>UAS-mCD8GFP/Fm7c/Y;gce-RNAi larva. **A'-B':** White light images of the same larvae are shown in [A-C].

Detection of the precocious *br* expression in the fat bodies of the 2nd instar larvae of gce-RNAi:

The *br* gene represents the key gene which is prevented to be expressed in the immature stages where the JH acts through its receptors and its signaling to suppress the *br* expression. Accordingly the *br* expression was examined in the mutant *gce* 2nd instar larvae by Br-core antibody in the fat body (FB) cells by the immunohistochemical assay and compared to the *br* expression in the same stage of the wild type. The samples were inspected under the florescent microscope and the captured photos were presented in Fig. (8). Immunohistochemical results in Fig. (8) revealed that the precocious *br* expression was found to be highly expressed in the fat body cells of gce-RNAi mutant larvae at the 2nd instar marked by the red color and was not detected in the wild type larvae at the same stage. Therefore, suppression of *gce* by RNAi gene targeting in the early larval instars caused precocious *br* expression and premature metamorphosis. To figure out whether Gce protein is required for JH-mediated *br* suppression in the FB cells, wild type, gce-RNAi, larvae were reared on a diet containing 0.1 ppm pyriproxyfen. The *br* expression was examined in the 2nd instar larvae by Br-core antibody in the FB cells by the immunohistochemical assay. The samples were perceived under the florescent microscope and the captured photos were presented in Fig. (9).Photos of the immunohistochemical results in Fig. (9) confirmed that precocious *br* expression was highly expressed in the FB cells of *gce* treated mutant larvae. The results revealed that precocious *br* expression was not suppressed by exogenous JHA in the FB of these mutant larvae and was not detected in the FB cells of the wild type larvae at the

same conditions. Therefore, at the early larval stages, the presence of the Gce protein in the FB cells is required for JH-mediated *br* suppression in these cells.

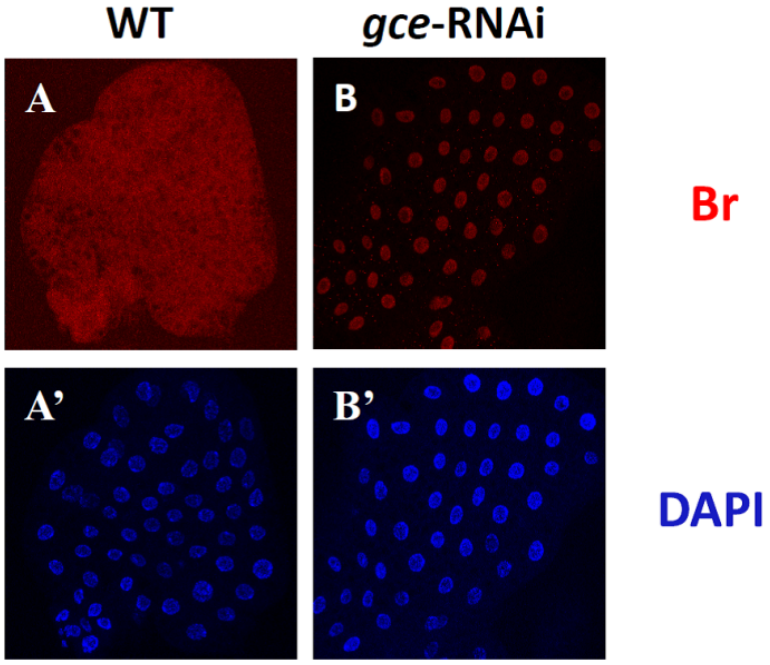

Fig. 8: Photos of immunohistochemical results showing that the fat bodies of the 2nd instar larvae were stained with a Br-core antibody (red) [A,B]. The nuclei of the same tissues were stained with DAPI (blue) [A',B']. **A:** Fat body cells of wild type larvae showing no *br* expression. **B:** Fat body cells of *gce*-RNAi larvae showing precocious *br* expression.

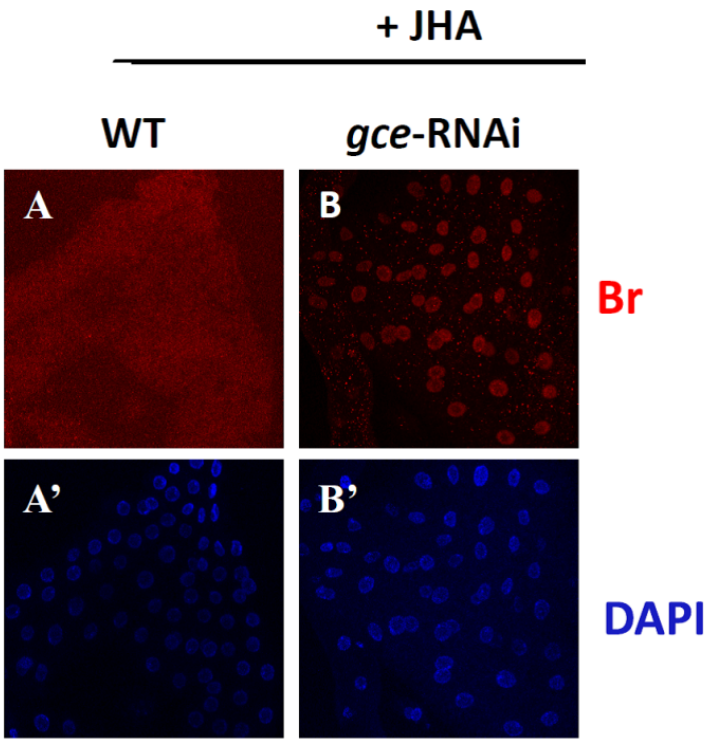

Fig. 9: Photos of immunohistochemical results showing that the fat bodies of treated 2nd instar larvae with pyriproxyfen (JHA) were stained with a Br-core antibody (red) [A-C]. The nuclei of the same tissues were stained with DAPI (blue) [A', B']. **A:** Fat body cells of wild type larvae showing no *br* expression. **B:** Fat body cells of *gce*-RNAi larvae showing precocious *br* expression.

DISCUSSION

The *gce* gene is another candidate gene for mediating the JH action. The selection of *gce* for this purpose was based on the similarity between this gene and other candidate gene known as *Met*. The *Met* gene was proposed previously by different authors (Wilson and Fabian, 1986; Miura *et al*., 2005; Konopova and Jindra, 2007). Our bioinformatics analysis showed that, Met and Gce were found to have highly similar amino acids sequences especially in the three conserved domains; bHLH, PAS-A, and PAS-B (data not shown). RNAi technique is used to knock down specific gene in the living organism. It was used successfully by different authors to knock down target genes in different insects (Uhlirova *et al*., 2003; Konopova and Jindra, 2007; Miller, *et al*., 2008; Parthasarathy, *et al*., 2008; Baumann *et al*., 2010; Huang *et al*., 2011). The functions of *gce* in the larval stage have been examined by RNAi experiments. RNAi-generating transgenes were engineered from a fusion of genomic DNA and reverse complement cDNA. Kalidas and Smith (2002) used the same technique and they found that it gives high knockdown of the gene of interest. The two RNAi construct was integrated into the genomic DNA of the living flies. The expression of these structures formed dsRNA as hairpin RNA expression. The hairpin structure was previously described by May and Plasterk (2005). We confirmed the knocks down in the gene by qualitative RT-PCR analysis. It was found that the *gce* transcript was dramatically reduced in the heat shocked 2nd instar larvae and was undetectable on the gel. These results verify the success of silencing *gce* gene by the RNAi technique. Flies which have mutation in the *Met* gene are resistant to JHA. This because *Met* gene is a candidate of JH receptor and mediate its function (Wilson and Fabian, 1986; Wilson and Ashok, 1998). Consequently, we hypothesized that if Gce is required as JH receptor and to mediate its function, the mutation in this gene would result in resistance to JHA as *Met* mutant flies. Our bioassay test revealed that flies with mutation in *gce* were resistant to JHA at high concentrations such as 0.1 and 0.3 ppm pyriproxyfen. The effect of knocking down *gce* on the appearance of precocious metamorphosis was addressed. This experiment was depending on the expression of *broad* (*br*) as a key gene connects the ecdysone signaling with the JH signaling. The *broad* (*br*) gene, an ecdysone-induced transcription factor, is the key regulator of the onset of metamorphosis, since amorphic *D. melanogaster* mutants of *br* can develop normally until the final larval instar but fail to begin metamorphosis (Kiss *et al*., 1988; DiBello, *et al*., 1991; Bayer, *et al*., 1996). It works as a key specifier of the pupal state. The expression of *br* is also regulated by JH (Zhou and Riddiford, 2002). In *Tribolium castaneum*, *br* is essential for promoting pupal development, and is inducible by exogenous JHA in the pupal stage (Konopova and Jindra, 2008; Parthasarathy*et al*., 2008; Suzuki *et al*., 2008). So, we decided to use *br* gene as an indicator for the initiation of the onset of metamorphosis. Accordingly we employed the *Gal4-UAS* system which developed by Brand and Perrimon (l993), which uses a *D. melanogaster* gene promoter ligated to yeast *Gal4,* resulting in Gal4 protein product to drive expression of *GFP* ligated to a UAS response element in transgenic flies. Transgenic flies (produced by Bourbon *et al*., 2002) carrying a specific *Gal4-PG12* driver inserted near the *br* gene were used in

this study. It resembled the temporal and spatial expression pattern of the endogenous *br* gene in tissues other than the salivary gland. The expression of *Gal4-PG12* in the salivary gland is a common feature for most Gal4 lines derived from the *P*{*GawB*} construct, which may carry a position-dependent, unidentified salivary gland enhancer (Brand *et al*., 1994). In this study, RNAi-mediated knockdown of *gce* causes a precocious larval–pupal transition which indicated by the precocious *br* expression. It is well documented by Riddiford *et. al*. (2003) that *br* is a molecular marker for pupal commitment and specifies the larval-pupal metamorphosis in a variety of holometabolous insect species. Also our study showed that, the precocious *br* expression was detected in the whole larvae by using the *GFP* as a reporter gene. It was also detected in the fat bodies and recognized by using specific Br-core anti bodies. The precocious *br* expression could not be prevented by treatment with pyriproxyfen (JHA) either in the whole larvae or in the fat bodies. This finding is in agreement with a reverse genetic study showed that precocious *br* expression was also detectable in *Met* mutant larvae (Huang *et al*., 2011). This study provides evidence that in *D. melanogaster gce* is essential mediators of the anti-metamorphic action of JH. Therefore it is necessary to maintain the larval state.

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ARABIC SUMMARY

حشرة *الدروسوفيلا ميلانوجستر* المهندسة وراثيا والتى تحمل تداخل للحمض الريبوزومي الرنا لجين ال جي سي اي تثبت مشاركة هذا الجين في اداء هرمون الشباب

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يعتبر هرمون الشباب هام في كثير من الجوانب لنمو وفسيولوجيا الحشرات.وعلى الرغم من ان أدوار هذا الهرمون كانت محل العديد من الدراسات، إلا ان آلية عمله علي المستوي الجزيئي لا تزال غير مفهومة جيدا. يوفر جين ال م ي ت (*Met*) في الدروسوفيلا كثير من المتطلبات كمستقبل للهرمون. يوجد جين مشابه وهو ال جي سي اي (*gce*) و الذي يمثل شريك لجين ال م ي ت (*Met*) فيالتوسط لعمل هرمون الشبابولمعرفة دور هذا الجين (*gce*) قمنا بعمل منع لتمثيل هذا الجين في الحشرات المعدلة وراثيا بتقنية التداخل في الحامض الريبوسومي (RNAi). ولقد وجد ان منع التمثيل لجين ال جي سي اي (*gce*) قد احدث تمثيل مبكر لجين البرود (*br*) في اليرقات صغيرة العمر. وقد اوضحنا ايضا ان منع التمثيل لجين ال جي سي اي (*gce*) قد احدث مقاومة في الحشرات المعدلة وراثيا لمبيد البيروبروكسيفين التابع لمجموعة منظمات نمو الحشرات. أوضحت هذه الدراسة ان جين ال جي سي اي (*gce*) له دور حيوى فعال في تحفيز هرمون الشباب في الطور اليرقي.