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Molecular identification and lipid mobilization role of adipokinetic hormone receptor in *Spodoptera litura* (F.)

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Research Paper

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Abstract

Energy homeostasis is essential for organisms to maintain fluctuation in energy accumulation, mobilization. Lipids as the main energy reserve in insects, their metabolism is under the control of many physiological program. This study aimed to determine whether the adipokinetic hormone receptor (AKHR) was involved in the lipid mobilization in the *Spodoptera litura*. A full-length cDNA encoding AKHR was isolated from *S. litura*. The *Sl*AKHR protein has a conserved seven-transmembrane domain which is the character of a putative G protein receptor. Expression profile investigation revealed that *SlAKHR* mRNA was highly expressed in immatural stage and abundant in fat body in newly emerged female adults. Knockdown of *SlAKHR* expression was achieved through RNAi by injecting double-stranded RNA (dsRNA) into the 6th instar larvae. The content of triacylgycerol (TAG) in the fat body increased significantly after the *SlAKHR* gene was knockdown. And decrease of TAG releasing to hemolymph with increase of free fatty acid (FFA) in hemolymph were observed when the *SlAKHR* gene was knowned-down. In addition, lipid droplets increased in fat body was also found. These results suggested that SlAKHR is critical for insects to regulate lipids metabolism.

Introduction

The insect fat body is a dynamic tissue involved in energy storage and metabolism, and regulation. Insect adipocytes store a great amount of lipid reserves as cytoplasmic lipid droplets (Arrese and Soulages, 2010). The intermediary metabolism of the fat body is regulated by many hormonal signals. Lipids metabolism is tightly coupled with a large array of physiological processes such as feeding preference and frequency change (Konuma *et al*., 2012; Fukumura *et al*., 2018), digestion regulation (Bil *et al*., 2014), egg-laying (Lindemans *et al*., 2009) etc. Adipokinetic hormone (AKH), a peptide hormone, is the first factor that stimulates lipid mobilization (Stone *et al*., 1976; Gäde *et al*., 1997). Reports had proven that the major function of the AKH is the regulation of triacylglycerol lipase to break down the stored glycogen and triacylglycerol (TAG) in the fat body. When the AKH is released into the hemolymph and binded by a G protein-coupled receptor, adipokinetic hormone receptor (AKHR), various cellular signaling pathways come into play (Cae Rs *et al*., 2012; Alves-Bezerra *et al*., 2016). The detailed signaling cascades for many insect species is not well known yet.

Lipid storage and mobilization are important for energy homeostasis in insect development, reproduction etc. And the lipid droplets for the main energy store are primarily TAG (Canavoso *et al*., 2001; Zhou *et al*., 2018*b*). Signaling molecule can affect gene transcription (Xu *et al*., 2012). When energy needed, AKH signaling pathways are activated and the ester bonds in TAG are hydrolyzed to form diacylglycerols which are released into the hemolymph (Ryan and van der Horst, 2000). Then the alanine/acetyl-CoA system is open. And the free fatty acids that are released from triacylglycerols undergo β-oxidation, and the ATPs are generated for vital movement (Auerswald *et al*., 2005). But which enzyme(s) are being activated by the AKHs is still not known.

In the present study, we identified the adipokinetic hormone receptor (*SlAKHR*) in the the tobacco cutworm (*Spodoptera litura*) which was not reported in genome database using molecular biological approaches. The function of the *SlAKHR* gene in the mobilization of TAG was confirmed by dsRNA injection knockdown. The obtained data will confirm whether *SlAKHR* gene is involved in lipid mobilization related to the tobacco cutworm.

Table 1. Primers used in this study

Primer name	Primer sequence (5′–3′)
For gene cloning	
SlAKHR-F1	GCATTTRGCWGTNGCYGAYYT
SlAKHR-R1	CCGTAVACRATGGGRTTBGCRC
GSP1	GATTACGCCAAGCTTACGTGGAATATGAAGCTCTGCGGC
NGSP1	GATTACGCCAAGCTTATAGCCCGAAGGTCCTGGTGAAC
GSP2	GATTACGCCAAGCTTTGTACGTCATCCCTCTGCTGTCCAC
NGSP2	GATTACGCCAAGCTTTAATGATAAGATGCGACGGAGCGGC
For qTR-PCR	
SlAKHR-qF1	TGTACGTCATCCCTCTGCTGTC
SlAKHR-qR1	TGTCATCTTGAGTGTCCGAGCC
Sl RPL10-qF1	GATGACATGGAATGGATG
Sl RPL10-qR1	GACTTGGGTAAGAAGAAG
For *SlAKHR* dsRNA synthesis	
SlAKHR ds-F1	CCTCTCCAGCTTCGTGCTTATC
SlAKHR ds-R1	ACATGAGAACAACCACATCGCC
T7 *SlAKHR* ds-F	GGATCCTAATACGACTCACTATAGGGCCTCTCCAGCTTCGTGCTTATC
T7 *SlAKHR* ds-R	GGATCCTAATACGACTCACTATAGGGACATGAGAACAACCACATCGCC
EGFP dsRNA-F1	GCTGACCCTGAAGTTCATCTG
EGFP dsRNA-R1	GAACTCCAGCAGGACCATGT
T7 EEGFP ds-F	GGATCCTAATACGACTCACTATAGGGGCTGACCCTGAAGTTCATCTG
T7 EEGFP ds-R	GGATCCTAATACGACTCACTATAGGGGAACTCCAGCAGGACCATGT

Materials and methods

Insect rearing

The *Spodoptera litura* population was gotten from School of Life Sciences, Sun Yat-sen University. And they were reared on an artificial diet (Chen *et al.*, 2000) at 25 ± 1°C in a 14L:10D photoperiod and 70–80% relative humidity in intelligent light incubator from 2013. The newly emerged adults were transferred to a one-end open cylindrical plastic cage ($\Phi = 12$ cm, $H = 25$ cm) covered with filter paper in inner around and a cotton cloth at the open end, honey added as a diet supplement.

Gene cloning

Total RNA was isolated from about 100 mg fat body from 6th larvae using the Trizol reagent according to the manufacturer's specifications (Invitrogen, USA). First-strand complementary DNA was synthesized from 2 μg total RNA using a first strand synthesis kit (PrimeScript™ II 1st Strand cDNA Synthesis Kit, TaKaRa, Japan). The intermediate fragment of *AKHR* was amplified by a pair of degenerate primers which were designed based on the conserved amino acid sequences of the reported insect *AKHR*s in NCBI database (table 1). The PCR was carried out with the following conditions: initial preheating for 5 min at 94 °C, 35 cycles at 94 °C for 30 s, 48 °C for 30 s and 72 °C for 1 min, and with a final extension at 72 °C for 10 min using the primer pair *SlAKHR*-F1and *SlAKHR*-R1.

For the full-length cDNA cloning, A Rapid Amplification of cDNA Ends (RACE) Kit (Clontech, Japan) was used. Specific primers for the 5′-and 3′-Rapid RACE were designed based on partial *SlAKHR* cDNA sequence amplified by the degenerate primers *SlAKHR*-F1 and *SlAKHR*-R1. The specific primers GSP1 and NGSP1 were used for 5′-RACE, while GSP2 and NGSP2 used for 3′-RACE (table 1).

Expression profiles of SlAKHR

The *AKHR* transcript levels were detected by quantitative real-time PCR (qRT-PCR) using an iCycler iQ (BIO-RAD, Hercules, CA) and SYBR Premix Ex Taq (Takara, Japan). Briefly, each qRT-PCR mixture contained 10 μl total volume: 5 μl TB Green Premix Ex Taq II (Tli RNaseH Plus) (Takara), 0.4 μl forward and 0.4 μl reverse primers (10 μM), 3.2 μl MilliQ water and 1 μl cDNA. The target gene primer pair *SlAKHR*-qF1 and *SlAKHR*-qR1 was used with a housekeeping gene (Ribosomal protein L10, RPL10, GenBank Acc. No. KC866373) primers *SlRPL10*-qF1 and *SlRPL10*-qR1. The qRT-PCR was performed in a CFX Connect Real-Time System (BIO-RAD) under the following conditions: one cycle for 30 s at 95°C, followed by 40 cycles of 10 s at 95°C, 30 s at 60°C and 30 s at 72°C. The data were normalized to the reference gene (RPL10) expression. The relative expression levels were calculated by $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

To investigate the expression pattern of different developmental phases and tissues, we got the 1st to 6th larvae, pupae, newly female adults. And the head, ovaries, midgut, fat body of newly female adults were carefully separated and collected. The specimen or tissues were rinsed in PBS buffer (0.01 M, pH = 7.4)

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several times, and each tissue was pooled from 8–10 individuals. Total RNA was isolated from all samples and obtained the first-strand cDNA as above.

Gene characterizationand phylogenetic analysis

The signal peptide of the AKHR was predicted with the SignalP server (http://www.cbs.dtu.dk/services/SignalP). And the transmembrane domain predicting was executed under the the TMHMM server (http://www.cbs.dtu.dk/services/TMHMM). The alignment of all sequences were generated using the MultiAlin website server (http://multalin.toulouse.inra.fr/multalin/multalin.html). The neighbor-joining (NJ) tree was produced in MEGA 6.0 software (Tamura *et al.*, 2013) with Maximum-likelihood analysis using the Jones-Taylor-Thornton model (1000 bootstrap replicates). AKHR amino acid sequences used for phylogenetic analysis derived from other reported sequences.

RNA interference

According to the manufacturer recommendations of T7 RiboMAX™ Express RNAi System(Promega), two pairs of primers (SlAKHR ds-F1,SlAKHR ds-R1, T7 SlAKHR ds-F and T7 SlAKHR ds-R) (table 1) were designed to synthesize the 544-bp (734–1278 bp) region of the *SlAKHR* gene that included a T7 promoter region in both the sense and antisense strands, The *SlAKHR* cDNAs from fat body was used as a template, The amplification reactions protocol comprised 36 cycles of 95 °C for 40 s, 55 °C for 45 s and 72 °C for 60 s, with a final extension step of 72 °C for 10 min. The sequence was verified by sequencing (Huada gene company, Shenzhen, China). The *EGFP* gene (DQ768212.1) was used as a control dsRNA. The PCR primers EGFP dsRNA-F1 and EGFP dsRNA-R1 were used to amplify the *EGFP* fragment (547 bp) (126–672 bp) (table 1), and the dsRNA was synthesized by T7 RiboMAX™ Express RNAi System as above. The final dsRNA production corresponding to SlAKHR and EGFP genes were eluted into sterilized DEPC ddH2O, The quality and concentration of dsRNAs were determined by Nano-Drop Spectrophotometer (Implen, München, Germany) and their integrity were confirmed by a 1% agarose gel electrophoresis. Then they were stored at −80 °C and used up within 1 week.

For RNAi bioassays, newly 6th larvae were anesthetized on ice for 1 min before injection. dsRNAs were prepared the final concentrate 1 μg μl^{-1}. 5 μl dsRNA per larva was injected into the body cavity between the 3rd and 4th abdominal segment using a 10 μl micro-syringe syringe (Hamilton) and the injection point was sealed immediately with wax (Dong *et al.*, 2013). The injected larvae were then returned to the artificial diet under the conditions described above. The efficiency of *SlAKHR* gene knowdown was calculated using qRT-PCR at 12, 24, 36 and 48 h as described above. Larvae injected with EGFP dsRNA were used as controls.

Determination of triacylglycerol (TAG)

For TAG contents quantification, the TAG assay kit (Solarbio Biotech, Beijing, China) was used. Briefly, About 100 mg fat body was separated. After 1 ml n-heptane & isopropanol (1 : 1) was added, the mixture was homogenized in on ice for 5 min. Then the sample was centrifuged at 8000***g*** for 10 min at 4°C. 40 μl supernatant was transferred to a new 200 μl eppendorf tube. After added 125 μl reagent I and 25 μl reagent II, vortex shock 30 s. Then 15 μl supernatant was transferred to another new 200 μl eppendorf tube, 50 μl reagent III, 15 μl reagent IV, 50 μl reagent V and 50 μl reagent VI was added inturn. After incubated 15 min at 65 °C, 250μl compound was transferred to a 96-well plate, and the OD value was measured at 420 nm on a HIDEX Sense 425-301 (Turku, Finland) on room temperature. The ultrapure water was used for blank controls.

Measurement of free fatty acid (FFA)

The contents of FFA measurement was guided by Li and Yuan (2013). Simplely, 300 μl hemolymph sample from the 6th instar larvae were collected with 15 ml centrifuge tubes respectively, Then 1 ml 1/30 M PBS buffer (pH = 8.0) and 6 ml chloroform were added. After shook for 30 s, 2 ml copper reagent (triethanolamine solution: acetum: 6.45% copper nitrate solution = 9:1:10) was added. Another shaking for 15 min, stayed 10 min at room temperature. After centrifuged for 5 min at 3000***g***, 4 ml subnatant was tansfered to a new 15 ml centrifuge tubes. Then 0.5 ml developer (100 mg sodium diethyl dithiocarbamate trihydrate dissolved in 100 ml n-butyl alcohol) was added. 5 min later, the OD value were measured at 440 nm on a spectrophotometer UV-5500 (PC) (Metash instrument, Shanghai, China). Ultrapure water was used for blank controls.

Lipid droplet staining and microscopy

The dissected fat bodies were fixed with 4% paraformaldehyde on a glass slide for 2 h at room temperature and then washed with chilled PBS (0.01 M, pH = 7.4) three times (5 min × 3). Then these fat bodies were submerged in Nile red solution (5 μl Nile red (1 mg ml^{-1}) in 495 μl PBS) for overnight at 4°C (Wang *et al.*, 2017). After washing for 5 min with PBS for three times, the fat bodies were re-stained in DAPI (2 ng μl^{-1}) for 10 min, next 5 min washing with PBS for three times (Zhao and Huang, 2019). The fat body were mounted on glass slides in 80% glycerol and visualized under a fluorescence microscope (Nikon, Japan) at 510–560 nm excitation wavelength combined with a 580 nm emission filter.

Statistical analysis

Results were presented as means ± SD (standard deviation) based on three independent biological replications. Differences between two groups were analyzed by Student's *t*-test (*$P < 0.05$; **$P < 0.01$). One-way ANOVA followed by a Fisher's protected LSD multiple comparison was used for the comparison among more than two different conditions. Statistical analyses were performed using SPSS 20.0 (Chicago, USA).

Results

Gene character and phylogenetic analysis

The full-length *SlAKHR* cDNA was obtained after sequencing and splicing. It indicated that the *SlAKHR* gene was 2539 bp, including an ORF of 1197 bp that encoded a 398 amino acids protein (accession No. MZ424871). No signal peptide cleavage was found under website server. The multi-sequence alignment showed that AKHR protein shared a high homology with other insects AKHR. Seven transmembrane domains (TM) were

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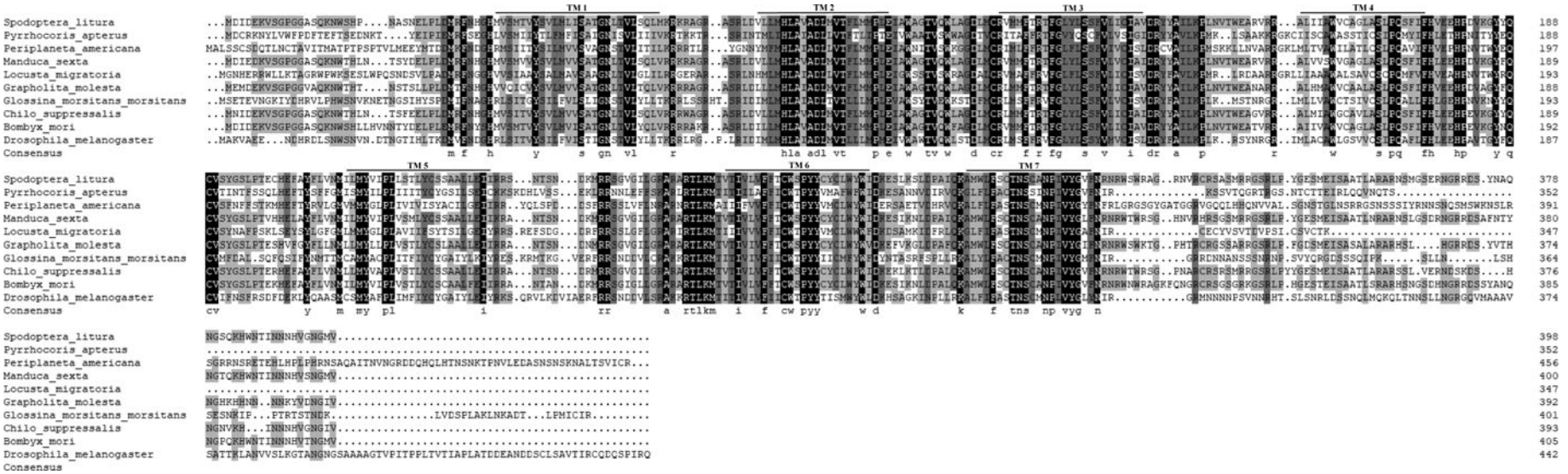

Figure 1. Alignment of the *Sl*AKHR protein with other species. The transmembrane domains (determined by TMHMM 2.0) are indicated with numbers from I to VII. *Bombyx mori* (GenBank Acc. No. NP_001037049.1), *Chilo suppressalis* (GenBank Acc. No. ALM88332.1), *Glossina morsitans* (GenBank Acc. No. AEH25943.1), *Grapholita molesta* (GenBank Acc. No. QPZ46758.1), *Locusta migratoria* (GenBank Acc. No. ANW09575.1), *Manduca sexta* (GenBank Acc. No. ACE00761.1), *Periplaneta americana* (GenBank Acc. No. ABB20590.1), *Pyrrhocoris apterus* (GenBank Acc. No. ARV86499.1), *Drosophila melanogaster* (GenBank Acc. No. NP_477387.1).

predicted in the protein, which indicates that this protein is a member of the GPCR superfamily (fig. 1).

For the phylogenetic analysis, it was chosen to compare the known and predicted insect AKHRs. Our tree showed that *SlAKHR* belongs to the same clade as the other AKHRs and was most related to the *Manduca sexta* AKHR (fig. 2).

Expression profiles of SlAKHR

To get more insights into the expression character of the *SlAKHR* gene in the *S. litura*, the transcription levels of *SlAKHR* gene in different developmental stages and a set of tissues in newly female adults were calculated by qRT-PCR. The result showed that *SlAKHR* gene was relatively low expression level during the early larval stage, and highly expressed when growing up (fig. 3a). In females, the mRNA was abundant in fat body and moderately expressed in midgut (fig. 3b).

Effects of SlAKHR *knockdown on lipid metabolism*

To confirm the role of *SlAKHR* in lipid metabolism, dsRNA-injection knockdown of *SlAKHR* was performed. qRT-PCR analysis were carried out after 12, 24, 36, 48 h and it showed a high silencing efficiency after 36 h (fig. 4a). To detect the function of *SlAKHR* on lipid metabolism, TAG contents in fat body and hemolymph were investigated, It showed that lipids content in fat body was increased but it was decreased in hemolymph after the *SlAKHR* gene knockdown (fig. 4b, c).

To explore the further changes of lipid decomposition products, the content of FFA in hemolymph was also analyzed. The graph revealed that the content of FFA was increased in *SlAKHR* RNAi-treated group compared to control treated group (fig. 4d).

Effects of SlAKHR *knockdown on TAG accumulation*

Five μg *SlAKHR* dsRNA was injected to each 6th star larva. Twenty-four hour later, fat body was isolated and Nile-red & DAPI staining was performed to visualize the lipid droplets. The results showed that after *SlAKHR* knockdown, lipid droplets in the fat body was accumulated compared with the control (fig. 5).

Discussion

In the present study, we cloned and sequenced the full cDNA sequence encoding *AKHR* from the *S. litura (SlAKHR)* successfully. But it was not found in the reperted genome database. Several evidences confirm that the *SlAKHR* gene isolated here is indeed an AKHR of *S. litura*. Determination through TMHMM showed that there were seven transmembrane domains (fig. 1). AKHR is a glycoprotein hormone receptor containing seven transmembrane helices characteristic for the family of GPCRs (Wang *et al.*, 2017). Secondly, AKHRs and the vertebrate gonadotropin-releasing hormone receptors are structurally and evolutionary related (Hauser *et al.*, 1998; Lindemans *et al.*, 2011). Furthermore, *SlAKHR* was highly homologous to other receptors that had been reported (fig. 2). And the results of multiple amino acids sequence alignment showed that there were multiple conserved sites.

Our results indicate that *SlAKHR* was relatively low in earlier juvenile individuals and accumulated with their growing (fig. 3a).

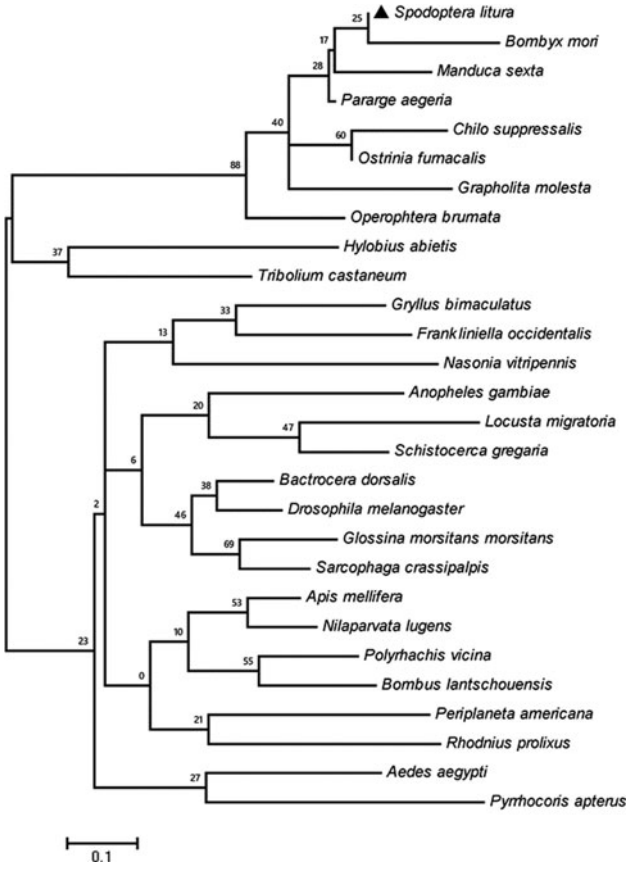

Figure 2. Phylogenetic tree of SlAKHR and other insect AKHRs. The tree was constructed by MEGA 6.0 using the Neighbor-joining (NJ) method with 1000 bootstrap replicates. SlAKHR was marked in triangle. The scale bar represents 0.1 amino acid substitutions per site. AKHR amino acid sequences used for phylogenetic analysis derived from *Aedes aegypti* (GenBank Acc. No. CAY77164.1), *Anopheles gambiae* (GenBank Acc. No. ABD60146.1), *Apis mellifera* (GenBank Acc. No. NP_001035354.1), *Bactrocera dorsalis* (GenBank Acc. No. AQX83416.1), *Bombyx mori* (GenBank Acc. No. NP_001037049.1), *Chilo suppressalis* (GenBank Acc. No. ALM88332.1), *Frankliniella occidentalis* (GenBank Acc. No. KAE8753142.1), *Glossina morsitans* (GenBank Acc. No. AEH25943.1), *Grapholita molesta* (GenBank Acc. No. QPZ46758.1), *Gryllus bimaculatus* (GenBank Acc. No. ADZ17179.1), *Hylobius abietis* (GenBank Acc. No. AVI00624.1), *Locusta migratoria* (GenBank Acc. No. ANW09575.1), *Manduca sexta* (GenBank Acc. No. ACE00761.1), *Nilaparvata lugens* (GenBank Acc. No. AZP54622.1), *Operophtera brumata* (GenBank Acc. No. KOB73379.1), *Periplaneta americana* (GenBank Acc. No. ABB20590.1), *Polyrhachis vicina* (GenBank Acc. No. ADK55068.1), *Pyrrhocoris apterus* (GenBank Acc. No. ARV86499.1), *Sarcophaga crassipalpis* (GenBank Acc. No. AOC38019.1), *Schistocerca gregaria* (GenBank Acc. No. AVG47955.1), *Tribolium castaneum* (GenBank Acc. No. NP_001076809.1), *Ostrinia furnacalis* (GenBank Acc. No. AXF67446.1), *Bombus lantschouensis* (GenBank Acc. No. QGN75353.1), *Nasonia vitripennis* (GenBank Acc. No. XP_031784266.1), *Rhodnius prolixus* (GenBank Acc. No. AKO62856.1), *Drosophila melanogaster* (GenBank Acc. No. NP_477387.1), *Pararge aegeria* (GenBank Acc. No. JAA79325.1).

The chronic accumulation and acute lipid mobilizing is mainly regulated by AKH signaling pathway (Grönke *et al.*, 2007; Lu *et al.*, 2018). Insect fat body is an important organ of biosynthetic and metabolic location (Law and Wells, 1989). And its cells control the synthesis and utilization of energy stores (Arrese and Soulages, 2010). The highest expression of *SlAKHR* in the fat body of females was observed in this study as other insects, *D. melanogaster* (Staubli *et al.*, 2002), *M. sexta* (Ziegler *et al.*, 2011), *G. morsitans* (Caers *et al.*, 2016), *R. prolixus* (Alves-Bezerra *et al.*, 2016) and *B.dorsalis* (Hou *et al.*, 2017). These findings most probably support the main lipids mobilization organ is fat body (Alves-Bezerra *et al.*,

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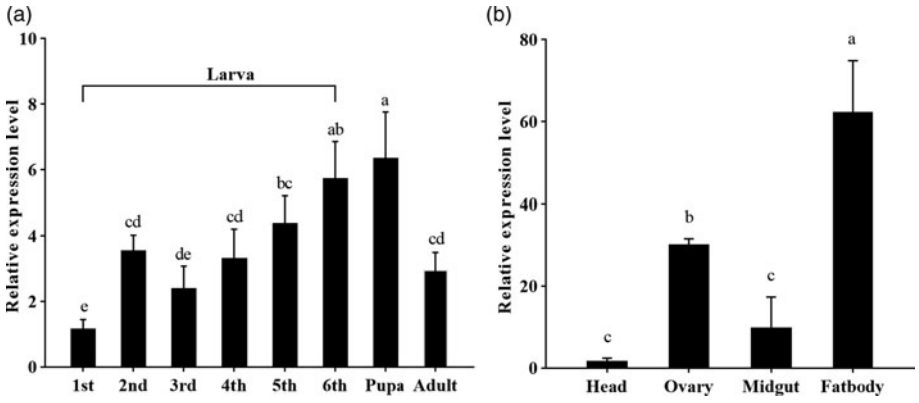

Figure 3. Expression profile of *SlAKHR* in different developmental stages and tissues. (a) qRT-PCR analysis of *SlAKHR* expression levels from first to sixth instar larva and pupal stage and female adults. (b) qPCR analysis of *SlAKHR* expression levels in different tissues of female adults. Results are represented as means ± SD of three independent samples, and samples are normalized to *RPL10* expression levels. Different lowercase letters represent significant difference of *SlAKHR* levels among developmental stages and various tissues determined by One-way ANOVA.

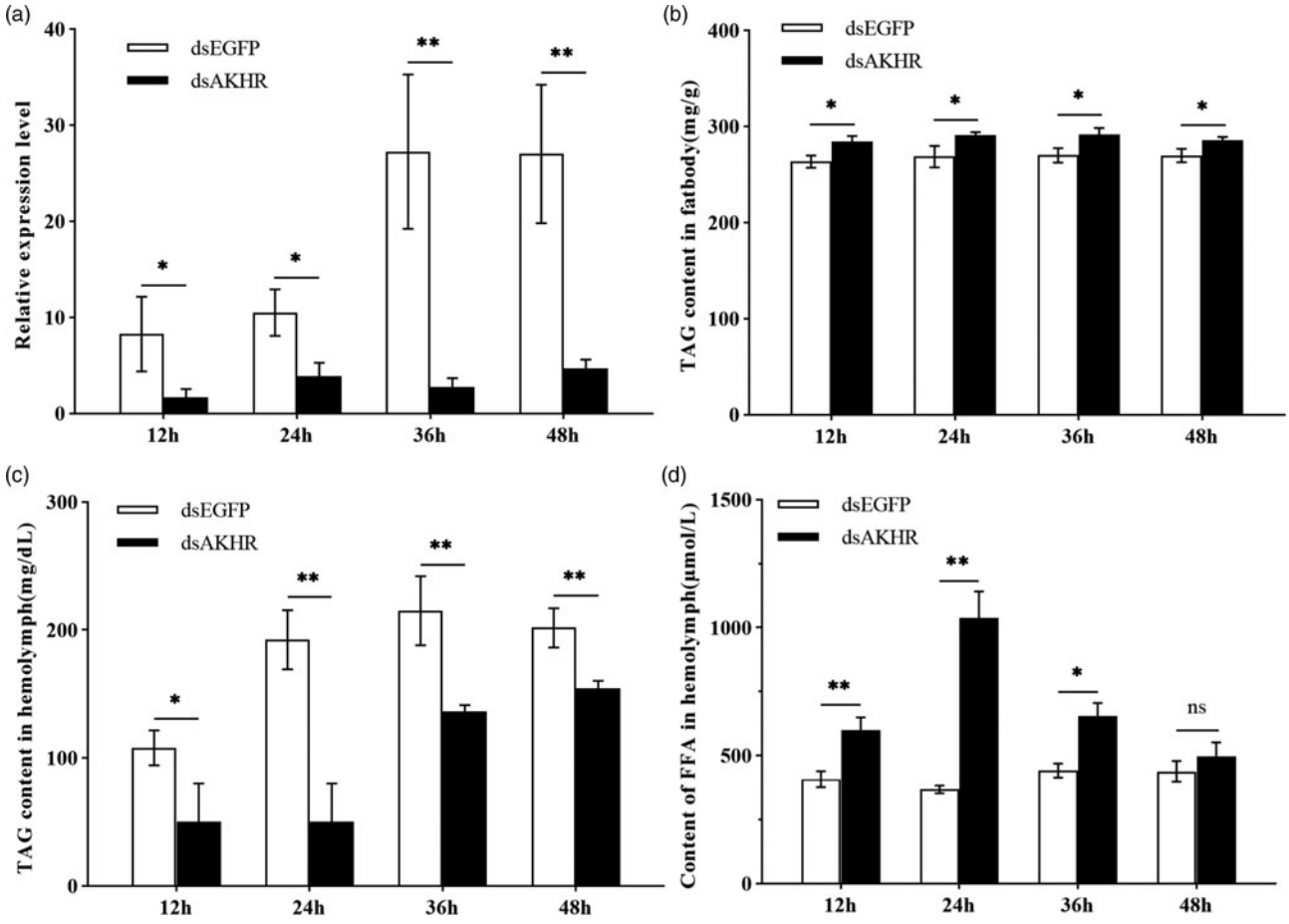

Figure 4. Efficiency of *SlAKHR* RNAi and effects of *SlAKHR* knockdown on TAG and FFA. (a) Efficiency of RNAi of at 12, 24, 36 and 48 h after *SlAKHR* dsRNA injection. TAG contents in fat body (b) and hemolymph (c) were determined at 12, 24, 36 and 48 h after dsRNA injection ($n = 25$). (d) FFA contents in Hemolymph at 12, 24, 36 and 48 h after *SlAKHR* dsRNA injection ($n = 25$). Results are represented as means ± SD of three independent replicates. Differences between two groups were analyzed by Student's *t*-test (*$P < 0.05$; **$P < 0.01$).

2016). Insect mid-gut is closely associated with food digestion and immerse in fat body cells. qRT-PCR indicated that *SlAKHR* was moderately expressed in the midgut (fig. 3b) next to the fat body. This result might hint this tissue also be involved in lipid metabolism functionally, as reported in *D. melanogaster* (Hauser *et al.*, 1998), *P. regina* (Stoffolano *et al.*, 2014), *P. americana* (Bodláková *et al.*, 2017), and *B. dorsalis* (Wang *et al.*, 2017).

Focusing on the intended function, *SlAKHR* mRNA was specifically knocked-down via dsRNA injection (fig. 4a). The intermediary metabolism of the fat body is regulated by many hormonal signals. The amount of TAG present in the cells act in insect energy homeostasis of lipid synthesis and breakdown. Both the amount and size of lipid droplets in fat body cells can be altered by lipogenesis and lipolysis (Grönke *et al.*, 2005). In this study, Knockdown of *SlAKHR* resulted in the accumulation of TAG in fat bodies and prevent it releasing to hemolymph (figs 4b, c and 5). This result indicated that *SlAKHR* controlled the metabolism of TAG in fat body. Similar results were obtained in *G.*

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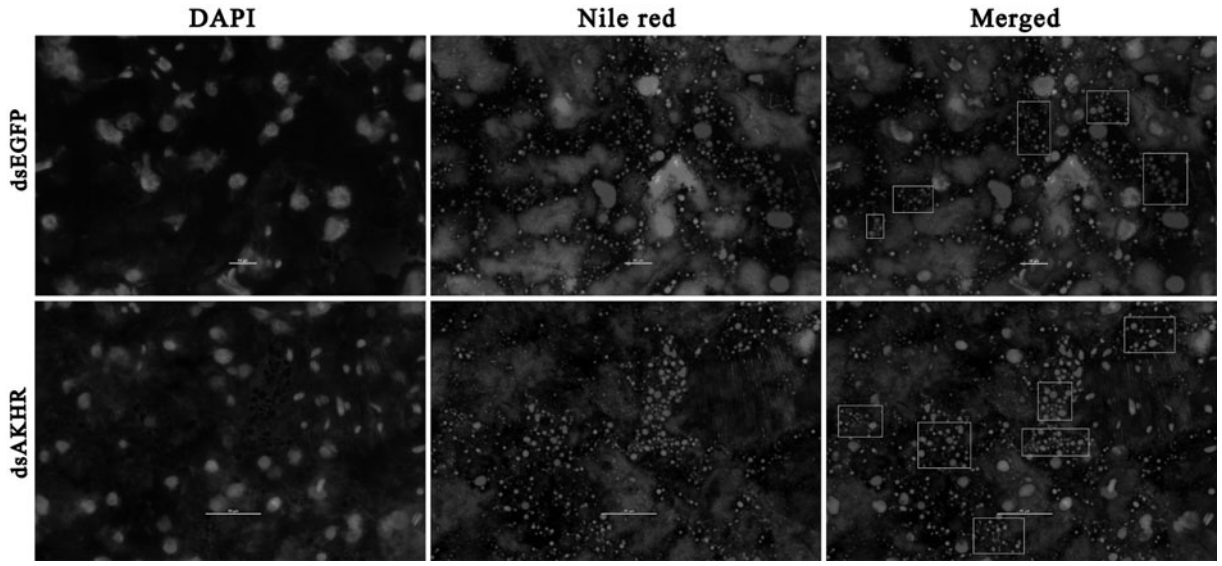

Figure 5. Effects of *SlAKHR* knockdown on lipid accumulation. Nile-red & DAPI staining of the fat body after the *SlAKHR* dsRNA injection 24 h. Scale bars = 50 μm.

bimaculatus (Konuma *et al.*, 2016), *R. prolixus* (Jedličková *et al.*, 2015), *B. dorsalis* (Hou *et al.*, 2017) etc. Deprivation of *Drosophila* AKHR also accumulated high levels of TAG in fat bodies (Grönke *et al.*, 2007). But the step of TAG breakdown into FFA was not stop (fig. 4d) (Katewa *et al.*, 2012; Fukumura *et al.*, 2018).

Gene transcription and translation sometimes be triggered by signaling molecules, including nutrition, hormones, insulin-like peptides and other chemicals (Xu *et al.*, 2012). Lipids metabolism routes had already well documented. The triacylglycerol were broken-down to form free fatty acid and glycerin, then bound by acyl-CoA and undergone β-oxidation for ATP generation (Auerswald *et al.*, 2005). In this physiological process, the adipokinetic hormone (AKH) was linked with context of a batch of gene expression, the lipase brummer (Zhou *et al.*, 2018*a*), the Glycerol-3-phosphate acyltransferase (Alves-Bezerra *et al.*, 2016), receptors for JHIII and 20E, Hepatocyte nuclear factor 4 (HNF4) (Wang *et al.*, 2017), uncoupling protein 4 (UCP4) (Slocinska *et al.*, 2013), insulin pathway genes (Xu *et al.*, 2012) and so on. Utilization of lipids for will direct support energy of flight (Beenakkers *et al.*, 1985; Canavoso *et al.*, 2003). Releasing of trehalose and proline is coupled to TAG degeneration. This physiology will supply enengy for insect locomotion (McDougall and Steele, 1988; Giulivi *et al.*, 2008). Lipids metablism also contribute to embryogenesis in insects (Ciudad *et al.*, 2007) and body defence (Mullen and Goldsworthy, 2003; Mendes *et al.*, 2008; Bodláková *et al.*, 2021). But the role of AKH on the regulation or feedback of these gene expression is poorly understood. In our study, we also found the female lay less egg when the SlAKHR was knocked-down (not present in this paper). If the entire physiology process of lipid metabolism is clear, it might be a potential target for application in pest control. So the function of the AKH signaling should be worth further study.

Conclusions

Overall, the molecular characteristics of AKHR in *S. litura* as well as its structure properties have been identified and described. *SlAKHR* gene knockdown could affect the lipids metabolic including lipids contents increasing and TAG accumulation in fat body, and lipid content decreased in hemolymph. Our results provide further insights into the role of AKHR on lipid metabolism in *S. litura* larvae and potential target for pest control.

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