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Target of rapamycin (TOR) is necessary for the blood digestion and reproduction of *Aedes albopictus*

Chen Zhang[1] · Jiayan Shen[1] · Ruoyun Lan[1] · Jiaying Xu[1] · Tingting Ma[1] · Bin Tang[1] · Shigui Wang[1]

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Abstract

Aedes albopictus transmits the virus through repeated blood feeding behavior and can also vertically transmit the virus to its offspring. In this study, we analyzed the expression of the *TOR* gene in *Ae. albopictus* and found that it was highest in female mosquitoes 24 h after feeding, particularly in the head and thorax. Then, we used to feed method to silence the *TOR* gene of female mosquitoes and make them suck blood. Silencing *TOR* resulted in downregulation of amino acid transporter genes *SLC7A5* and *SLC3A2*, as well as regulatory intermediates *Rheb* and *Frizzled-2* in other signaling pathways. In terms of midgut blood digestion, silencing *TOR* led to reduced glucose metabolism while affecting trypsin (TRY) and chymotrypsin (CHY) enzymes involved in midgut blood digestion, thereby delaying the process. Regarding reproduction, silencing *TOR* resulted in decreased expression levels of *Vitellogenin 2* (*Vg2*) and *Vg3* gene, leading to reduced Vg content in the ovary. Additionally, downstream protein synthesis-related genes *4E-binding protein* (*4E-BP*) and *S6 kinase* (*S6K*) within the TOR pathway were affected. Although there were no significant changes observed in egg number or diameter, ovarian development cycle delay occurred along with signs of desiccation and shrinkage in some eggs. Moreover, both egg weight and hatching rate showed a significant decrease. The experimental results suggest that *TOR* regulates the blood digestion and reproduction process of *A. albopictus* by influencing post-vampire protease activity and protein synthesis, such as Vg. This provides a theoretical foundation for developing cost-effective and large-scale prevention and control measures for *A. albopictus*.

Keywords *A. albopictus* · Blood digestion · Reproduction · *TOR* · Vitellogenin

Introduction

Aedes albopictus, also known as the Asian tiger mosquito, is widely distributed in over 70 countries worldwide (Chiodini 2018). The species is globally recognized as the most invasive mosquito (Benedict et al. 2007), and dengue fever transmitted by it has been designated by the World Health Organization as the rapidly spreading and extensively prevalent arboviral disease, posing an increasingly significant threat to public health. When female *A. albopictus* feed on virus-infected patients or vertebrates, the virus enters and rapidly replicates within their bodies. Subsequently, when these mosquitoes bite other individuals, they excrete the virus, thereby establishing a "human–mosquito–human" transmission cycle. Mosquitoes infected with the virus can serve as lifelong carriers and transmit it to their offspring through reproduction (Rosen et al. 1983). Due to the intricate nature of pathogens, the development of vaccines against dengue fever has emerged as an arduous undertaking, thereby rendering vector control a pivotal aspect in the prevention and management of mosquito-borne diseases (Alphey et al. 2013). Currently, the control of adult *A. albopictus* primarily relies on chemical interventions, such as pyrethroid insecticides. However, the excessive and indiscriminate use of these insecticides has led to the emergence of resistance in *A. albopictus* populations (Hou et al. 2020; Piedra et al. 2023; Wang et al. 2022). Additionally, the environmental persistence of these insecticides poses a threat to non-target organisms, particularly beneficial insects (Xue et al. 2017). Therefore, it is imperative to explore alternative control strategies. In future, efforts to manage *A. albopictus*

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Chen Zhang and Jiayan Shen have contributed equally to this work.

✉ Shigui Wang
sgwang@hznu.edu.cn

[1] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, People's Republic of China

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will progressively shift toward environmentally friendly approaches encompassing green biological control methods like RNAi-based biological pesticides and transgenic technology.

In 1929, Roubaud first classified mosquitoes into autogeny and anautogeny based on whether female mosquitoes require vertebrate blood for ovarian development and reproductive behavior. Mandatory anautogeny mosquitoes, such as *A. albopictus*, are incapable of reproduction without a blood meal (Roubaud 1929). The necessity of blood intake exposes anautogeny mosquitoes to a diverse range of host organisms, thereby facilitating pathogen transmission between hosts. Following blood ingestion, neurosecretory cells in the brain are stimulated by blood to release insulin-like peptide (ILP) and ovarian ecdysteroidogenic hormone (OEH) (Brown et al. 1998). These hormones subsequently bind to their respective receptors, insulin receptor (IR) and OEH receptor (OEHR); this triggers the activation of insulin/insulin growth factor signaling (IIS) signal transduction pathway, ultimately inducing ecdysone production by follicle cells (Riehle and Brown 2002; Dhara et al. 2013; Vogel et al. 2015). Ecdysone enters the fat body and hydroxylates to 20 Hydroxyecdysone (20E). Subsequently, 20E forms a complex with the ecdysone receptor (ECR) and ultraspiracle (USP). This complex then binds to the ecdysone response element located in the promoter region of *Yolk protein precursors* (*YPPs*) gene, ultimately leading to the activation of *YPPs* gene transcription (Martín et al. 2001; Tran et al. 2001). Simultaneously, protease digests the blood in the midgut into amino acids, which then enter the hemolymph. The cationic amino acid transporter of SLC7 family within the fat body monitors and regulates the target of rapamycin (TOR) signaling pathway by assessing the level of amino acids in the hemolymph (Carpenter et al. 2012). Downstream of this pathway, TOR directly phosphorylates S6K protein kinase, thereby activating translation of GATA transcription factor (GATAa) within the fat body. Consequently, GATAa replaces transcription repressor GATA repressor (GATAr) in *Vitellogenin* (*Vg*) gene's promoter region and enhances *Vg* transcription (Jong-Hwa et al. 2006; Attardo et al. 2003). The activation of TOR can also induce hyperphosphorylation of the downstream translation repressor 4E-binding protein (4E-BP), thereby ensuring the efficient synthesis of YPPs (Roy and Raikhel 2012). YPPs production serves as a pivotal event in vitellogenesis, the secretion of vitellin into the hemolymph and its subsequent absorbed by developing oocytes through receptor-mediated endocytosis, facilitating embryonic development (Roy et al. 2018).

The TOR is a highly conserved serine threonine protein kinase that plays a crucial role in various cellular functions, including growth, development, aging, immunity, and autophagy (Diaz-Troya et al. 2008; Laplante and Sabatini 2012). The TOR signaling pathway can be activated by diverse extracellular and intracellular signals such as amino acids and insulin (Martin and Hall 2005). Conversely, TOR activity is inhibited under various stress conditions like cellular energy depletion and hypoxia stress. The role of the TOR pathway in insect tissue development, metabolism, aging, feeding behavior, autophagy, and protein synthesis has been intensive studied in *Drosophila melanogaster* (Partridge et al. 2011). Furthermore, *TOR* has been sequenced and characterized in *Bactrocera dorsalis*, *Bombyx mori*, *Nilaparvata lugens*, *Haemaphysalis longicornis* among others (Suganya et al. 2010; Zhou et al. 2010; Umemiya-Shirafuji et al. 2012; Zhuo et al. 2017). The involvement of the TOR pathway in regulating insect vitellin synthesis has been extensively demonstrated. Treatment with rapamycin or interference with the *TOR* gene in various insects, such as the brown *N. lugens*, *Periplaneta americana*, and *Bemisia tabaci*, resulted in arrested ovarian development and reduced Vg content (Liu et al. 2017; Puri and Jindal 2021; Zhu et al. 2020). Studies on *Aedes aegypti* have shown that the activity of TOR is essential to complete its reproductive cycle (Ling and Raikhel 2023). It not only influences YPPs production through the aforementioned pathways but also regulates early trypsin (TRY) at the translational level and impacts blood digestion (Brandon et al. 2008). Given its crucial regulatory role in mosquito blood digestion, egg development, and nutritional signal transduction, the TOR pathway has emerged as an ideal target for preventing and controlling *A. albopictus* and other vectors.

The vector *A. albopictus* was selected as the research subject in this study, and the expression of the *TOR* gene was examined in different developmental stages and tissues of *A. albopictus*. Subsequently, RNA interference was employed to silence the *TOR* gene specifically in female *A. albopictus*, aiming to investigate its impact on blood digestion, ovarian development, and egg formation. The results will contribute to a comprehensive understanding of the molecular mechanisms underlying *TOR* function in *A. albopictus* post-blood feeding and provide a theoretical foundation for large-scale control strategies targeting this species.

Materials and methods

Mosquitoes

Aedes albopictus were provided by Sun Yat-sen University (Guangdong Province, China) and were fed and stably passaged for more than three generations in an artificial climate chamber at 27 ± 1 °C with 70% relative humidity and light/dark (*L*:*D*) = 14:10. The first- and second-instar larvae were fed with the mixed powder of cat food and yeast powder in the ratio of 2:1. Cat chow powder was used to feed the third- and fourth-instar larvae, and post-eclosion adults were

Table 1 Primers for PCR

Primer name	Forward primer (5′-3′)	Reverse primer (5′-3′)
TOR	CCAAGCGTATGGTAGGGAC	TTGGTGCTCAACGGAATG

Table 2 Primers for synthesis of dsRNA

Primer name	Forward primer (5′-3′)	Reverse primer (5′-3′)
L4440-*TOR*	GCTGCAGGAATTCGATATCAAGCTTCCAAGCGTATGGTAGGGAC	CTCACTATAGGGCGAATTGGGTACCTTGGTGCTCAACGGAATG
L4440-*GFP*	GCTGCAGGAATTCGATATCAAGCTTAAGGGCGAGGAGCTGTTCACCG	CTCACTATAGGGCGAATTGGGTACCCAGCAGGACCATGTGATCGCGC

The underlined is the ligation sequence

fed 10% sugar water. Mice were used for mosquito blood feeding.

Cloning the *TOR* gene of *A. albopictus*

Total RNA was extracted from the fourth-instar larvae of *A. albopictus* using the TRIzol method. The quality and concentration of RNA were detected by agarose gel electrophoresis and microspectrophotometry (Thermo, USA). cDNA was synthesized according to the instructions of the Prime ScriptTM RT Reagent Kit (Haofeng, Hangzhou, China). The sequence of the *TOR* gene (LOC109429896) of *A. albopictus* was found using the National Center of Biotechnology Information (NCBI) database, and specific primers (Table 1) were designed using Primer premier 5.0 software to amplify intermediate sequence fragments with the cDNA template obtained in the PCR system. The primers had a start site of 766 bp and a termination site of 1229 bp, and the length of the PCR product was 463 bp. The reaction mixture included cDNA (1 μL), forwards and reverse primers (1 μL), dNTP (2.5 mM, 2 μL), 10 × buffer (Mg^{2+}, 2.5 μL), 5 U/μL Ex taq (0.2 μL), and ddH_2O (17.3 μL). PCR was performed using the following conditions: 5 min at 95 °C; 30 s at 95 °C, 30 s at 58 °C, and 1 min at 72 °C, for a total of 30 cycles, and then 10 min at 72 °C. The PCR products were separated and identified by electrophoresis on a 1% agarose gel, and the DNA in target bands was recovered by a DiaSpin PCR Product Purification Kit (Sangon Biotech, Shanghai, China), ligated into the pMDTM 18-T vector (Haofeng, Hangzhou, China) and transformed into Escherichia coli DH5α. After ampicillin screening, positive clones were selected sequenced (Shangya, Hangzhou, China).

Double stranded RNA (dsRNA) preparation and RNA interference

The L4440 carrier was digested with *KpnI* and *HindIII* enzymes, followed by separation and recovery of the linearized carrier using 2% agarose gel electrophoresis. The sequenced *TOR* fragment served as a template, with the ligation sequence added to the 5′ end of the PCR primer (Table 2). Subsequently, the PCR product was ligated to the linear vector using DNA ligase, and the resulting ligation product was transformed into HT115 (DE3). Following ampicillin and tetracycline screening, positive bacteria clone was selected and sent for sequencing at Shangya Biological Company (Hangzhou, China). The positive monoclonal colonies with successful sequencing were inoculated into 30 mL of 2 × YT liquid medium and cultured at 37 °C, 250 rpm until reaching an optical density (OD) of approximately 0.5. Subsequently, the expression of ds*TOR* in HT115 was induced by Isopropyl-beta-D-thiogalactopyranoside (IPTG) at a concentration of 0.4 mM, incubated at 37 °C and 250 rpm for 4 h. The bacterial pellet was harvested by centrifugation and resuspended in sterile 10% sugar water prior to feeding. In the same way, a L4440-*GFP* recombinant vector was constructed and induced to express ds*GFP*, which served as the control group for feeding *A. albopictus*.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Twenty third-instar larvae, ten fourth-instar larvae and ten pupae of *A. albopictus* were collected from the normal rearing, female mosquitoes that had first emerged (within 2 h of emergence), female mosquitoes that had emerged 24 h, 48 h and 4 d after eclosion, and 10 female mosquitoes that had absorbed blood 24 h and 48 h after eclosion. At the same time, 50 female mosquitoes were dissected and collected 24 h after blood sucking, and the remaining parts of the female mosquito head, thorax, ovary, midgut and abdomen were collected. RNA was extracted and cDNA was synthesized for real-time fluorescent quantitative PCR reaction (Bio-Rad, California, USA). The qPCR reaction mixture (10 μL) contained 5 μL of TB Green® Premix Ex Taq ™, 3.2 μL of ddH_2O, 0.4 μL each of upstream and downstream primers and 1 μL cDNA. The qPCR procedure was as follows: 10 min at 94 °C; 30 s at 94 °C, 30 s at 59 °C, 45 s at 72 °C for a total of 30 cycles, and then 10 min at 72 °C. A default dissolution curve analysis was performed. The *actin* gene was used as an internal control, and the $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression of *TOR* gene in the tissues of *A. albopictus* at different developmental stages and after blood sucking. Ten female *A. albopictus* fed with dsRNA for 3 d were collected. The expression of *TOR* gene and the expression of *SLC3A2*, *SLC7A5*, *Frizzled-2*, *Rheb*

genes in the entire body of female mosquitoes after *TOR* silencing were detected by the same method. Ten female mosquitoes were collected after 24 h and 48 h of blood sucking after *TOR* silencing, and *CHY1*, *CHY2*, *TRY*, *Vg1*, *Vg2*, *Vg3*, *VgR*, *4E-BP* and *S6K* genes in the entire body of female mosquitoes expression was detected by the same method. Primers for all genes are shown in Table 3.

Detection of sucrose content and trehalase activity in A. albopictus

Ten female *A. albopictus* with *TOR* silencing were collected in EP tubes after 24 and 48 h of blood feeding. The mosquitoes were homogenized in 200 µL of PBS buffer, followed by immersion in an ice-water mixture and ultrasonic crushing for 30 min using Biomuptor's (Diagenode, Belgium) high-power (320 W) ultrasound with a cycling pattern of 30 s on and 30 s off to obtain the ultrasonic crushing product. Then the crushed sample was supplemented with 800 µL PBS, centrifuged at 4 °C, ×1000 *g* for 20 min, and 350 µL of supernatant was used to determine the content of glycogen, trehalose, and protein, another 350 µL of the supernatant underwent ultracentrifugation at 4 °C, ×20,800 *g* for 60 min. After ultracentrifugation, 300 µL of the supernatant was used for to determine glucose concentration, protein content, and soluble trehalase activity. The remaining precipitate was suspended in 300 µL PBS and utilized for the assessment of glucose content, membrane-bound trehalase activity, and protein content. The protein contents of samples were determined using the BCA Protein Assay Kit. The trehalose content was determined using the anthrone method, following the protocol described by Zhang et al. (2017). Total glycogen and glucose content were measured using a combination of glucose oxidase/peroxidase reagent and amylase. The specific principle of detecting glucose content is that amylase degraded polysaccharides to glucose, which was then catalyzed by oxidase to produce gluconic acid and H_2O_2. Peroxidase further catalyzed H_2O_2 to generate red compounds, with color intensity proportional to the glucose content. The reference Guan et al. (2024) employed trehalose (sigma) for detecting trehalase activity.

Detection of protease activity in midgut and Vg content in ovary

The midgut and ovary of 20 female *A. albopictus* mosquitoes with *TOR* silencing was dissected and collected into EP tubes after 24 and 48 h of blood feeding. The midgut was diluted with saline at a ratio of mass (mg) to dilution solution (µL) of 1:19, and then Ultrasonication was performed using the same method as above. The activities of total protease, High-alkaline trypsin, Low-alkaline trypsin and chymotrypsin in the midgut were detected according to Zhao et al. (2018). The principle entails employing azo casein, Na-benzoy-DL-arginine-pnitroanilide HC1 (BAPNA), nalpha-tosyl-L-arginine methyl ester hydrochloride (TAME), and N-benzoyl-L-tyrosine ethyl ester (BTEE) as the respective color substrates for the four enzymes. The resulting color intensity is directly proportional to the enzyme activity, which is detected by colorimetric analysis. Sterile steel balls and 200 µL of PBS were added to the ovary, which were crushed using a 50 Hz crusher for 180 s. Subsequently, ultrasonic fragmentation was performed once again following the aforementioned method. The content of vitellogenin in the ovaries was detected by the insect vitellogenin (VTG) ELISA kit (Jingkang, Shanghai, China), and the specific experimental steps were in accordance with the instructions. The specific principle is that Vg in the sample is captured by pre-coated antibodies, and the absorbance is measured at 450 nm through the colorimetric reaction of the substrate tetramethylbenzidine (TMB). The color intensity is positively correlated with the vitellogenin in the sample.

Ovarian morphology and egg-related indicators were observed

Following *TOR* silencing, female mosquitoes were housed in a mosquito cage, and a random selection of 3–5 individuals was dissected every 24 h to examine the midgut and ovary, which were subsequently documented using a microscope camera system (Axio Observer A1 + Stemi2000, ZEISS, Germany). Ten female mosquitoes with blood sucking after *TOR* silencing were randomly selected and kept in empty mosquito cages. The daily egg production was recorded. Gather all the eggs, 30 eggs were randomly selected and placed in a petri dish, and the number of hatched larvae was determined statistically to calculate the hatching rate. The observation was continued until no new larvae emerged. Finally, after the remaining eggs had dried, one hundred eggs were randomly selected and weighed on a balance paper. The length (from the front to the rear end of the egg) and the short diameter (the diameter at the widest point of the egg) of the eggs were measured under a microscope, and the morphology of the eggs was observed using a microscope imaging system.

Data analysis

IBM SPSS Statistics 23.0 was used for the statistical analysis. The gene expression data from various developmental stages and tissues were analyzed using one-way analysis of variance and Duncan's test. (Different lowercase letters above the bar indicate that the difference is statistically significant.) Remaining data were analyzed using the independent sample *t* test ($P < 0.05$ indicated a significant difference, annotated with "*"; $P < 0.01$ indicated a significant

difference, annotated with "* *"). SigmaPlot 10.0 was used for the plot. Data normality and homogeneity were evaluated based on three replicates for each treatment concentration and tissue sample quantity.

Result

The developmental period and tissue expression of the *TOR* gene in *A. albopictus*

With the development of *A. albopictus* larvae, the expression of the *TOR* gene gradually increased and reached its peak during the pupal stage. Following emergence, there was a subsequent decrease in *TOR* expression in female *A. albopictus*, which then gradually increased again. The highest level of *TOR* expression was observed 24 h after blood feeding, followed by a gradual decline (Fig. 1A). The *A. albopictus* were dissected 24 h post-blood feeding, although not significantly different among various tissues, *TOR* expression was predominantly detected in the head and thorax of *A. albopictus* (Fig. 1B).

Efficiency of RNAi silencing mediated by *Escherichia coli* feeding method

In sugar-fed/dsRNA mosquitos, there was a significant decrease in the expression of the *TOR* gene ($t=3.25$, df = 4, $p=0.047$). This suggests that the expression of the *TOR* gene in *A. albopictus* can be effectively inhibited by feeding them with *E. coli* induced to express dsRNA (Fig. 2A). Following blood feeding, *A. albopictus* continued to be provided with sugar water containing dsRNA, resulting in a sustained reduction in *TOR* expression; however, the interference efficiency was observed to diminish (24 h: $t=3.615$, df = 4, $p=0.015$; 48 h: $t=2.814$, df = 4, $p=0.048$) (Fig. 2B).

Expression of TOR pathway-related genes after *TOR* silencing

After silencing the *TOR* gene in *A. albopictus*, the expression levels of upstream genes *SLC3A2* and *SLC3A5* were significantly reduced at 24 h and 48 h post-blood feeding (*SLC3A2* 24 h: $t=8.036$, df = 4, $p=0.01$; 48 h: $t=7.838$, df = 4, $p=0.001$; *SLC3A5* 24 h: $t=5.984$, df = 4, $p=0.009$; 48 h: $t=5.948$, df = 4, $p=0.01$) (Fig. 3A, B). The expression level of *Frizzled-2* decreased at 24 h after blood feeding but increased at 48 h (24 h: $t=3.346$, df = 4, $p=0.029$; 48 h: $t=-5.721$, df = 4, $p=0.011$) (Fig. 3C), while the expression level of *Rheb* increased at both time points (24 h and 48 h) following blood feeding (24 h: $t=-3.736$, df = 4, $p=0.02$; 48 h: $t=-6.34$, df = 4, $p=0.003$) (Fig. 3D).

TOR silencing reduces protease activity in the midgut of *A. albopictus* after blood sucking

After silencing the *TOR* gene, the expression of the *CHY1* gene in female *A. albopictus* was suppressed at 24 h post-blood feeding and subsequently increased at 48 h (24 h: $t=4.157$, df = 4, $p=0.014$; 48 h: $t=-5.345$, df = 4, $p=0.006$) (Fig. 4A). The *CHY2* gene exhibited an initial increase at 24 h after blood feeding, followed by a return

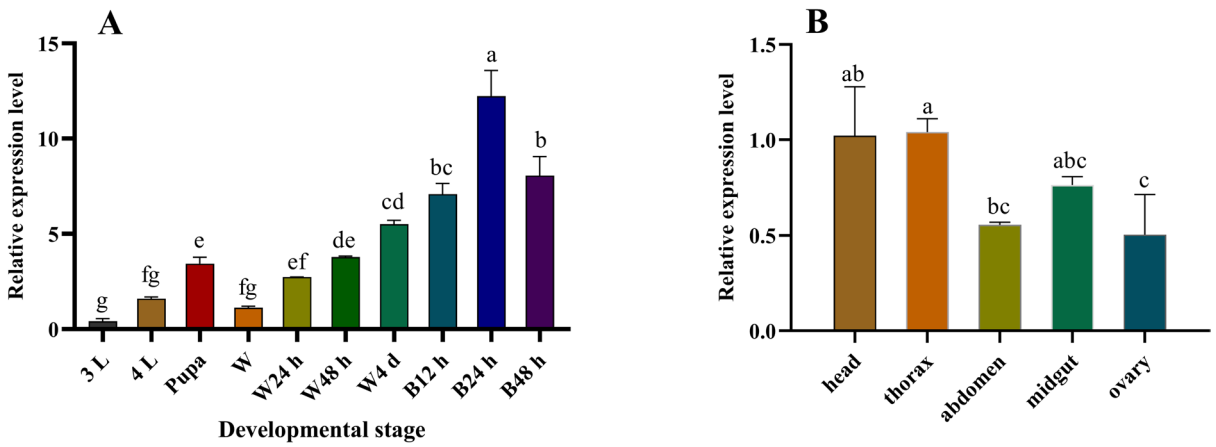

Fig. 1 Expression of *TOR* gene in different developmental stages of *A. albopictus* and in different tissues of female mosquitoes 24 h after blood feeding. **A** Expression of *TOR* gene in different developmental stages of *A. albopictus*. (3 L: third-instar larvae; 4 L: fourth-instar larvae; W: initial eclosion; W24 h: 24 h after eclosion; W48 h: 48 h after eclosion; W4 d: 4dafter eclosion; B12 h: 12 h after blood feeding; B24 h: 24 h after blood feeding; B48 h: 48 h after blood feeding; **B** The expression of *TOR* gene in different tissues of female *A. albopictus* at 24 h after blood feeding. Values represent mean ± SE. Relative expression levels were calculated in, respectively, comparison with that of the newly emerged adult and the head, which was ascribed an arbitrary value of 1. Different lowercase letters above the bar indicate that the difference is statistically significant (Duncan's test, $p<0.05$)

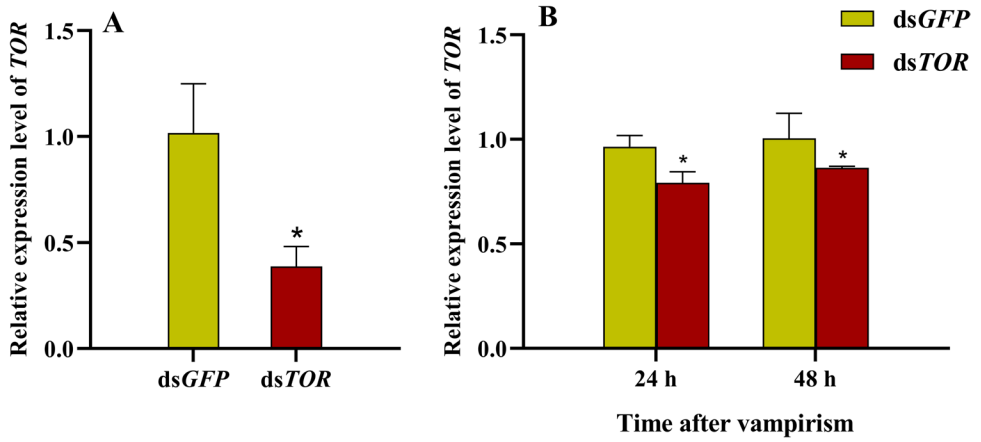

Fig. 2 Expression of *TOR* gene in RNAi-treated mosquitoes. **A** Expression of the *TOR* gene was examined after sugar-feeding/dsRNA during 3 days. **B** The expression of the *TOR* gene in *A. albopictus* mosquitoes was analyzed at different time points following blood feeding for 3 days, subsequent to their consumption of a sugar solution containing dsRNA. Values represent mean ± SE. Relative expression levels were calculated in comparison with that of the ds*GFP* group, which was ascribed an arbitrary value of 1. The asterisks * on the bars indicate significant differences in the expression levels between ds*GFP* and ds*TOR*, (Student's t test, * $p < 0.05$)

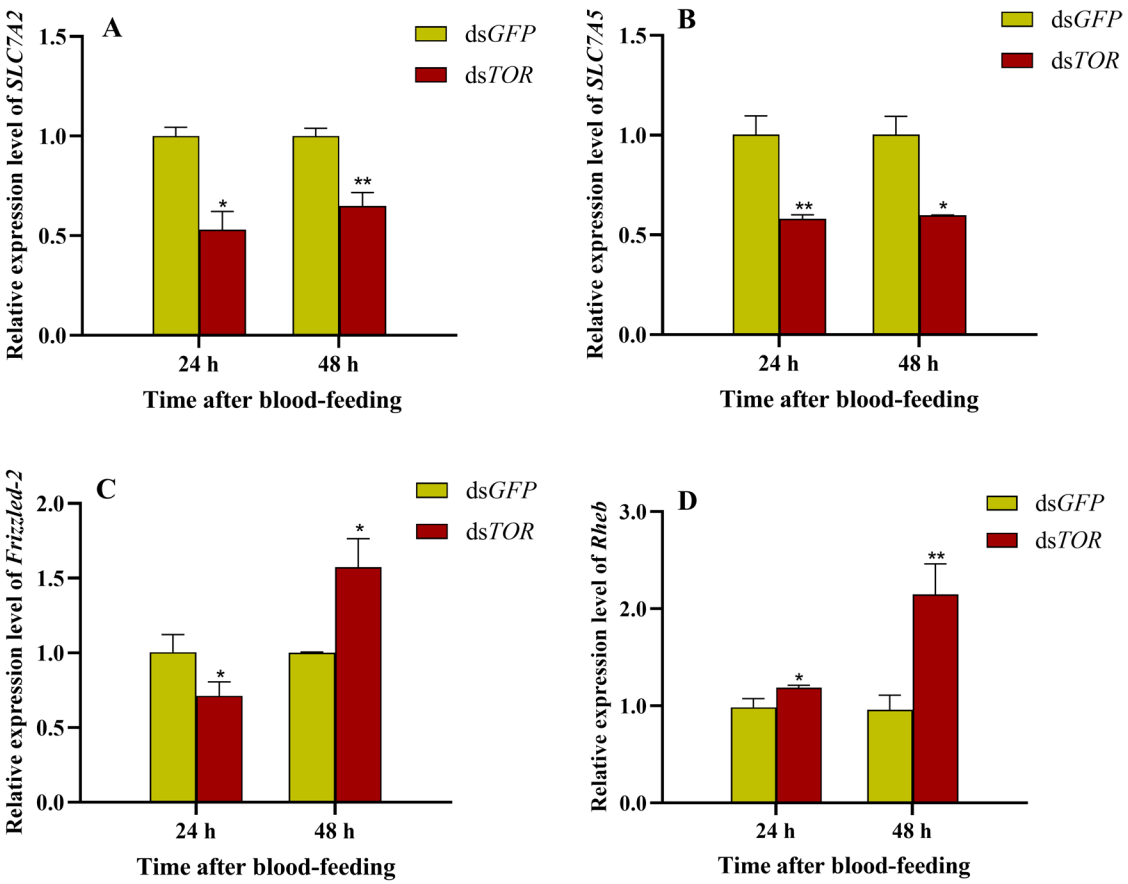

Fig. 3 Expression of *TOR* pathway-related genes in *A. albopictus* after blood feeding after *TOR* gene silencing. Values represent mean ± SE. Relative expression levels were calculated in comparison with that of the ds*GFP* group, which was ascribed an arbitrary value of 1. The asterisks * on the bars indicate significant differences in the expression levels between ds*GFP* and ds*TOR*, (Student's t test, * $p < 0.05$; ** $p < 0.01$)

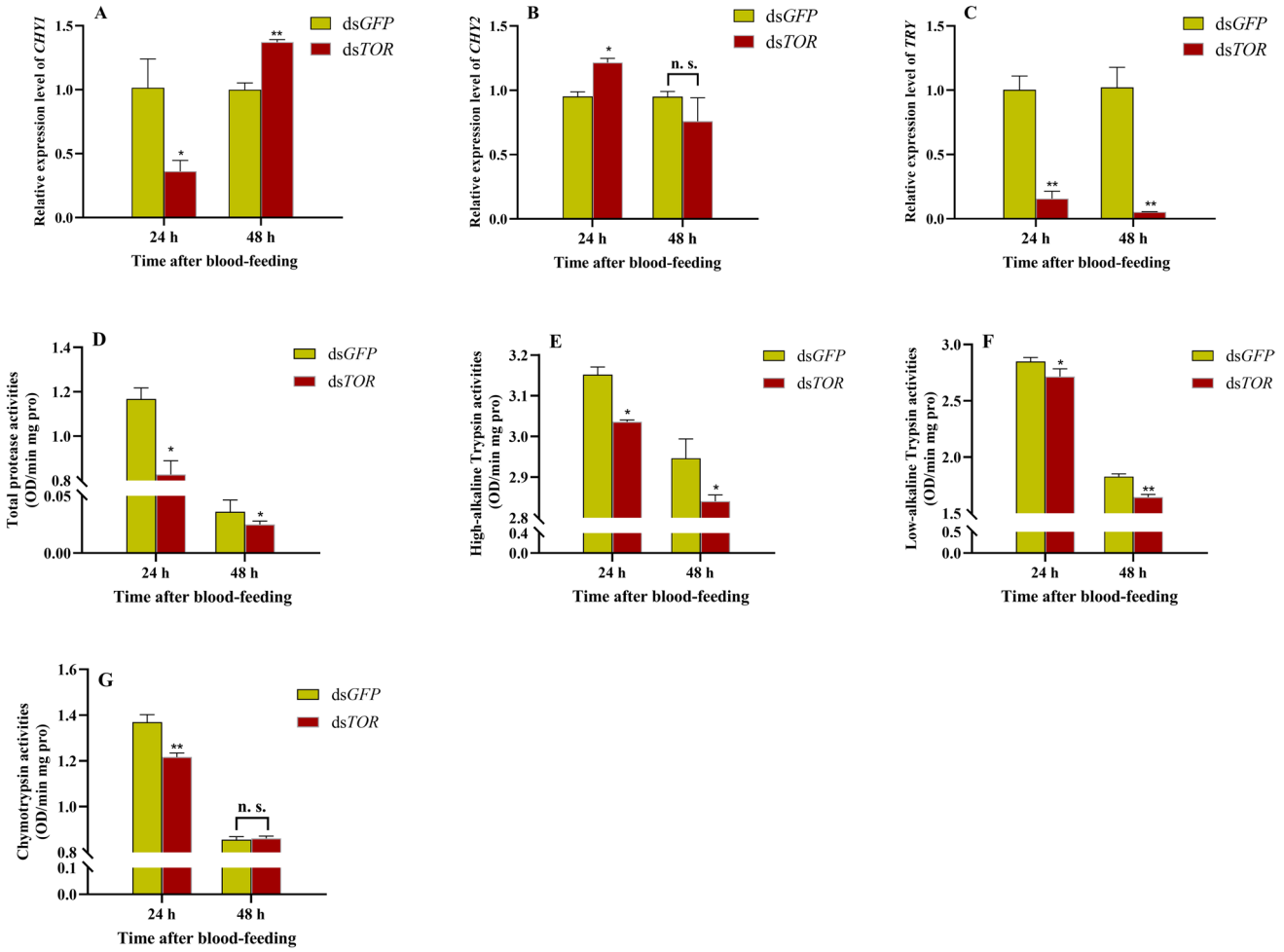

Fig. 4 Expression and activity of digestive enzymes in the midgut of *A. albopictus* following blood feeding subsequent to *TOR* gene silencing. Most of the protease genes and enzyme activities were significantly decreased. Values represent mean ± SE. Relative expression levels were calculated in comparison with that of the ds*GFP* group, which was ascribed an arbitrary value of 1. The asterisks * on the bars indicate significant differences in the expression levels between ds*GFP* and ds*TOR*, (Student's t test, n.s. not significant; * $p < 0.05$; ** $p < 0.01$)

to control levels (24 h: $t = -3.667$, df = 4, $p = 0.021$; 48 h: $t = 1.906$, df = 4, $p = 0.153$) (Fig. 4B). The silencing of *TOR* led to a significant decrease in *TRY* gene expression (24 h: $t = 11.878$, df = 4, $p = 0.001$; 48 h: $t = 15.146$, df = 4, $p = 0.004$) (Fig. 4C). Silencing *TOR* resulted in notable reductions in total protease activity, as well as high-alkaline TRY and low-alkaline TRY activities within the female mosquito intestines (total protease activity 24 h: $t = 6.64$, df = 4, $p = 0.03$; 48 h: $t = 3.095$, df = 4, $p = 0.036$; high-alkaline TRY 24 h: $t = 4.445$, df = 4, $p = 0.011$; 48 h: $t = 3.637$, df = 4, $p = 0.022$; low-alkaline TRY 24 h: $t = 2.955$, df = 4, $p = 0.042$; 48 h: $t = 6.456$, df = 4, $p = 0.003$) (Fig. 4D–F), while CHY activity significantly decreased at 24 h post-blood feeding but remained consistent with the control group at 48 h (24 h: $t = 5.379$, df = 4, $p = 0.006$; 48 h: $t = 1.287$, df = 4, $p = 0.268$) (Fig. 4G).

Sugar content and trehalose activity of female mosquitoes after *TOR* silencing and blood sucking

The silencing of *TOR* had minimal impact on the levels of total glycogen and trehalose in female *A. albopictus*. There were no significant changes observed in total glycogen at 24 h and 48 h post-blood feeding, compared to the control group (24 h: $t = -2.296$, df = 4, $p = 0.105$; 48 h: $t = 0.197$, df = 4, $p = 0.857$) (Fig. 5A). Trehalose content remained unchanged at 24 h after blood feeding but exhibited a slight increase after 48 h (24 h: $t = 0.109$, df = 4, $p = 0.92$; 48 h:

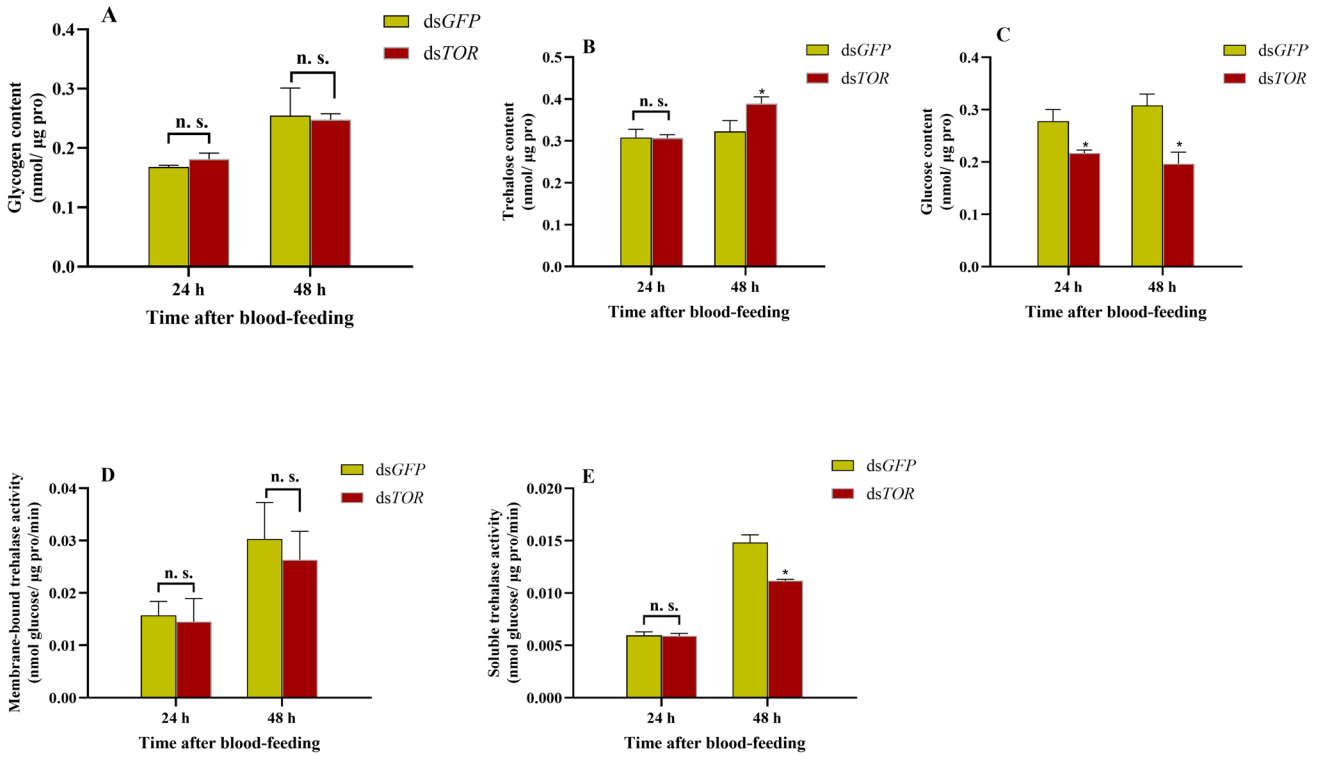

Fig. 5 Sugar content and trehalase activity of *A. albopictus* after blood feeding after *TOR* gene silencing. Values represent mean ± SE. The asterisks * on the bars indicate significant differences in the expression levels between ds*GFP* and ds*TOR*, (Student's t test, n.s. not significant; * $p < 0.05$)

$t = -3.746$, df = 4, $p = 0.02$) (Fig. 5B). However, glucose content in female *A. albopictus* significantly decreased at both time points following blood feeding when compared to the control group (24 h: $t = 4.6$, df = 4, $p = 0.01$; 48 h: $t = 5.1$, df = 4, $p = 0.036$) (Fig. 5C), suggesting that *TOR* plays a role in energy metabolism by affecting glucose levels in vivo. Both membrane-bound trehalase and soluble trehalase showed no significant change in enzyme activity at 24 h post-blood feeding but exhibited a significant decrease at 48 h post-feeding (membrane-bound trehalase 24 h: $t = 0.41$, df = 4, $p = 0.703$; 48 h: $t = 0.778$, df = 4, $p = 0.48$; soluble trehalase 24 h: $t = 0.191$, df = 4, $p = 0.861$; 48 h: $t = 5.226$, df = 4, $p = 0.014$) (Fig. 5D, E).

Expression of reproductive related genes in female mosquitoes after *TOR* silencing and blood sucking

The silencing of *TOR* has a significant impact on the expression of reproduction-related genes in female *A. albopictus*. Following *TOR* silencing, there was a notable decrease in the expression levels of *Vg2* and *Vg3* genes (*Vg2* 24 h: $t = 5.66$, df = 4, $p = 0.011$; 48 h: $t = 4.413$, df = 4, $p = 0.048$; *Vg3* 24 h: $t = 6.823$, df = 4, $p = 0.002$; 48 h: $t = 6.434$, df = 4, $p = 0.003$) (Fig. 6B, C). Additionally, *Vg1* expression decreased at 24 h after blood feeding but showed a significant increase at 48 h (24 h: $t = 3.216$, df = 4, $p = 0.049$; 48 h: $t = -12.418$, df = 4, $p = 0.006$) (Fig. 6A). *VgR* expression significantly decreased at 24 h after blood feeding but eventually reached similar levels as the control group (24 h: $t = 4.936$, df = 4, $p = 0.008$; 48 h: $t = -0.896$, df = 4, $p = 0.421$) (Fig. 6D). Among the downstream reproduction-related genes regulated by the TOR pathway, there was a substantial reduction in the expression level of *4E-BP* at both 24 h and 48 h after blood feeding (24 h: $t = 3.975$, df = 4, $p = 0.016$; 48 h: $t = 3.993$, df = 4, $p = 0.016$) (Fig. 6E), while *S6K* gene expression initially declined and then exhibited a significant increase (24 h: $t = 7.195$, df = 4, $p = 0.002$; 48 h: $t = -5.96$, df = 4, $p = 0.004$) (Fig. 6F).

Ovarian development and egg indicators of female mosquitoes after *TOR* silencing and blood sucking

Analysis of daily and cumulative egg production revealed that, compared to the control group, there was no significant difference in the average total number of eggs laid per female mosquito in the experimental group ($t = -1.237$, df = 6, $p = 0.262$) (Fig. 7A). However, a significant increase in spawning duration was observed (Fig. 7B). This phenomenon was further substantiated by the development of ovaries in female mosquitoes. Daily

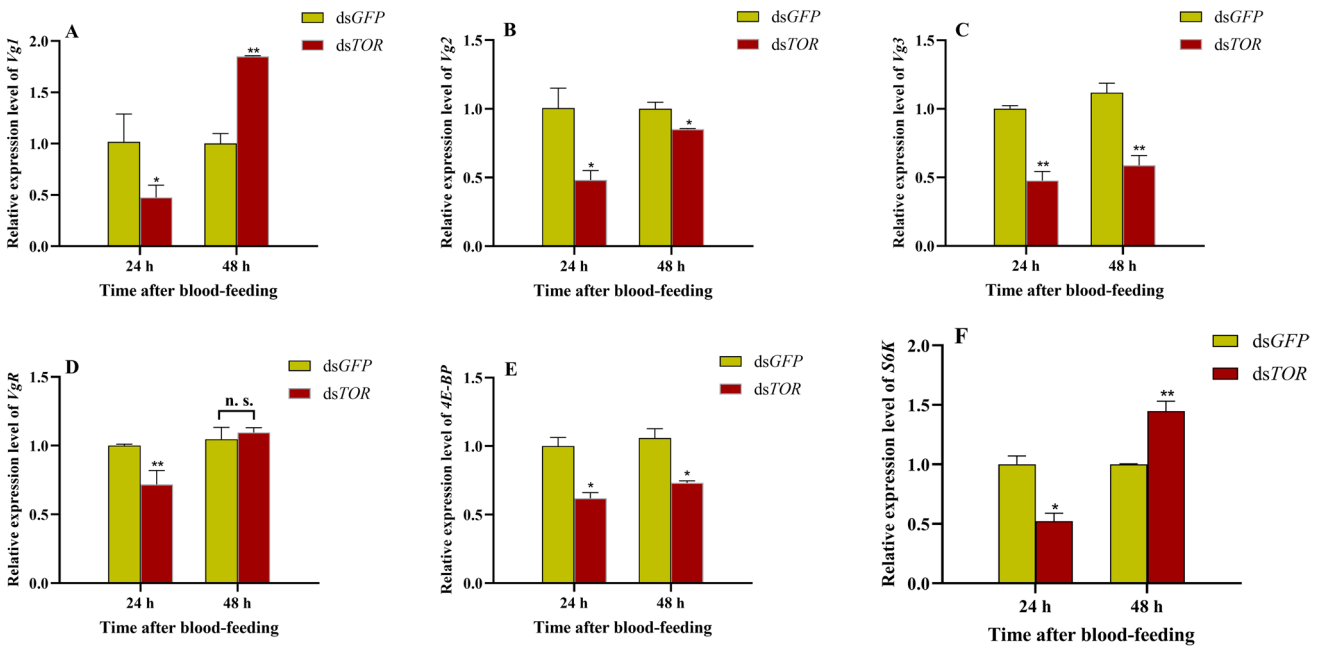

Fig. 6 Expression of reproductive genes in *A. albopictus* after blood feeding after *TOR* gene silencing. Values represent mean ± SE. Relative expression levels were calculated in comparison with that of the ds*GFP* group, which was ascribed an arbitrary value of 1. The asterisks * on the bars indicate significant differences in the expression levels between ds*GFP* and ds*TOR*, (Student's t test, n.s. not significant; * $p < 0.05$; ** $p < 0.01$)

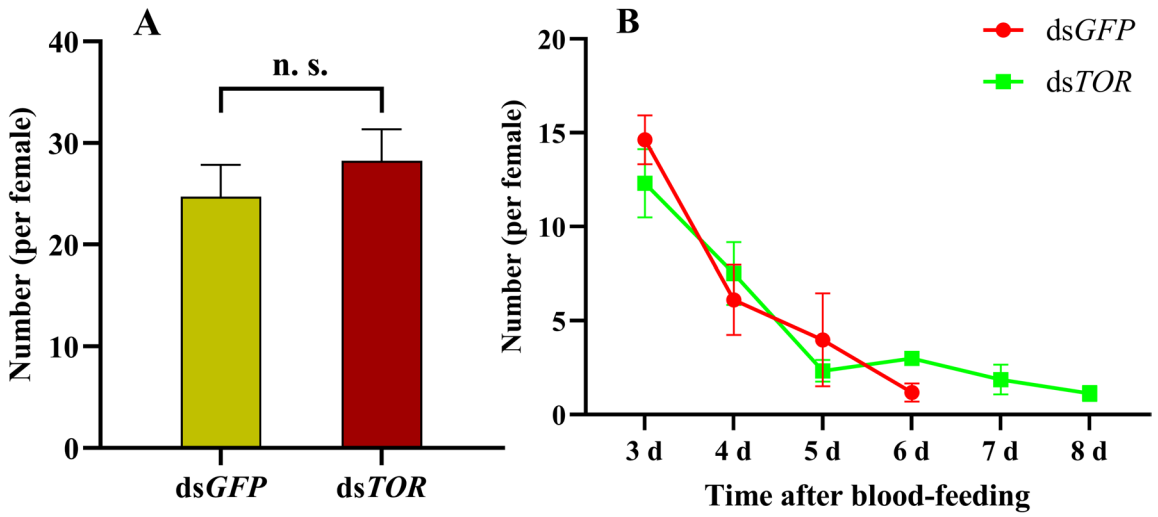

Fig. 7 Egg production of *A. albopictus* after *TOR* gene silencing. **A** Total egg production per female mosquito; **B** Daily fecundity of single female mosquito. Values represent mean ± SE. (Student's t test, n.s. not significant)

anatomical observations of ovarian phenotype revealed that while the control group completed oviposition on average 5 days after blood feeding, mosquitoes with *TOR* gene silenced had numerous eggs in their ovaries even at day 5 post-blood meal and only completed oviposition by day 8 (Fig. 8). We also measured the amount of Vg in the ovaries, compared to the control group, there was a significant decrease in Vg content in the ovaries of female *A. albopictus* after *TOR* gene silencing (24 h: $t = 20.97$, df = 4, $p < 0.001$; 48 h: $t = 4.665$, df = 4, $p = 0.019$) (Fig. 9A). Evaluation of egg quality demonstrated no significant difference in egg diameter between groups (length diameter: $t = 1.858$, df = 18, $p = 0.137$;


A
0.5 mm
B
0.5 mm
C
0.5 mm
D
0.5 mm
E
0.5 mm
F
0.5 mm
G
0.5 mm
H
0.5 mm
I
0.5 mm
J
0.5 mm
K
0.5 mm
L
0.5 mm
M
0.5 mm

◂**Fig. 8** Anatomical map of ovarian development of *A. albopictus* after *TOR* gene silencing. **A-E** ds*GFP*; **F-M**: ds*TOR*; **A, F** 24 h after blood feeding; **B, G** 48 h after blood feeding; **C, H** 3 d after blood feeding; **D, I** 4 d after blood feeding; **E, J** 5 d after blood feeding; **K** 6 d after blood feeding; **L** 7 d after blood feeding; **M** 8 d after blood feeding. The scale is 50 μm, and the white triangle points to the ovary, and the red arrow points to the hindgut (blood storage)

short diameter: $t = 1.609$, df = 18, $p = 0.183$) (Fig. 9B, C); however, some eggs laid by mosquitoes with *TOR* gene silenced were underdeveloped or even shriveled to just shells (Fig. 9G). Additionally, there was a reduction in dry weight of eggs ($t = 3.905$, df = 8, $p = 0.0045$) (Fig. 9D) and a significantly decreased hatching rate ($t = 4.027$, df = 8, $p = 0.004$) (Fig. 9E).

Discussion

As a central gene in the TOR pathway, *TOR* exhibits high conservation across eukaryotes and plays crucial roles in various physiological processes including cell proliferation, immune response, autophagy, and protein synthesis. Previous studies have demonstrated its differential expression throughout different developmental stages and tissues of diverse insect species due to its involvement in distinct physiological activities. In this study, we employed RT-qPCR to investigate the expression pattern of *TOR* during different developmental stages of *A. albopictus*. The results demonstrated that the expression level of *TOR* in pupae reached its peak prior to eclosion, potentially attributed to the substantial synthesis of ecdysone regulated by the TOR signaling pathway (Liu et al. 2017). Upon emergence as adult mosquitoes, the expression of *TOR* gene gradually increased, reaching its maximum at 4 days old. In blood-fed mosquitoes, *TOR* expression was highest after 24 h. Subsequently, we examined the tissue-specific distribution of *TOR* gene expression in female mosquitoes at 24 h after blood feeding. Notably, higher levels of *TOR* gene expression were observed in the head and thorax regions. Moreover, distinct patterns of *TOR* gene distribution were evident among different insect species. For instance, in *Bombyx mori*, the highest expression levels of *TOR* gene were detected in the head and nerve cord (Zhou et al. 2010).

RNA interference (RNAi), as a method for regulating biological gene expression, exhibits remarkable specificity, rapid efficacy, and minimal susceptibility to drug resistance. Consequently, it has emerged as a prominent research area in the field of pest control. The key to harnessing RNAi lies in the synthesis and delivery of double-stranded RNA (dsRNA). However, current laboratory techniques such as in vitro transcription synthesis and microinjection delivery are inadequate in terms of cost-effectiveness, technological feasibility, and efficiency for large-scale pest control applications. Therefore, it is widely believed that employing microorganisms like Escherichia coli to produce dsRNA and deliver it through feeding represents the primary avenue for utilizing RNAi as a biological pesticide in future. Currently, RNAi feeding experiments have been conducted in *Mythimna separata*, *Bactrocera dorsalis*, and other insect species (Li et al. 2011; Ganbaatar et al. 2017). The ability of transformed bacteria to colonize the gut and release dsRNA, which is subsequently internalized by intestinal epithelial cells through Sil proteins, has been demonstrated (Tian et al. 2009). The dsRNA of the *TOR* gene was successfully expressed in *E. coli* by constructing and inducing expression of a recombinant vector in this study. Subsequently, adult *A. albopictus* were fed with *E. coli* containing dsRNA and sugar water, resulting in significant inhibition of *TOR* gene expression, thereby demonstrate the efficacy of feeding RNAi in effectively suppressing the expression of the *TOR* gene in adult *A. albopictus*. However, it was observed that while *TOR* expression remained inhibited after blood feeding, the interference efficiency notably decreased; the reason for this phenomenon is not clear. After blood feeding, the intestinal environment of *A. albopictus* becomes more complex, potentially impeding bacterial colonization and dsRNA release in the midgut. Additionally, upstream gene regulation caused by *TOR* silencing before blood feeding may contribute to the decreased interference efficiency. Furthermore, a previous laboratory studies have primarily shown *CHS-2* expression in the midgut of *A. albopictus*, is crucial for midgut peritrophic membrane formation (Zhang et al. 2023). However, following blood feeding, *CHS-2* gene expression in *A. albopictus* is upregulation. Therefore, we hypothesize that blood feeding leads to a thicker midgut peritrophic membrane, hindering dsRNA penetration into midgut epithelial cells. Additionally, blood intake induces an elevation in nuclease levels within the female mosquito's midgut, further enhancing dsRNA degradation and subsequently reducing interference efficiency. Further studies are required to validate these specific mechanisms.

Anautogenous mosquitoes such as *A. albopictus* require mammalian blood to complete their reproductive cycle. Protein constitutes the primary constituent of blood, with lipids and carbohydrates present in minimal quantities (Brackney et al. 2010). The conversion of blood protein into vitellin precursor for developing oocytes represents a crucial aspect of the reproductive cycle. Amino acids serve as fundamental units for protein synthesis. The blood intake of female *A. albopictus* exceeds her body weight every time, efficient protein degradation into amino acids is crucial in blood digestion. In female mosquitoes, blood digestion involves a diverse array of digestive enzymes, with the endoprotease primarily composed of TRY and CHY playing a pivotal role (Lu et al. 2006). Trypsin predominantly digests most of the ingested blood, releasing approximately two-thirds of the

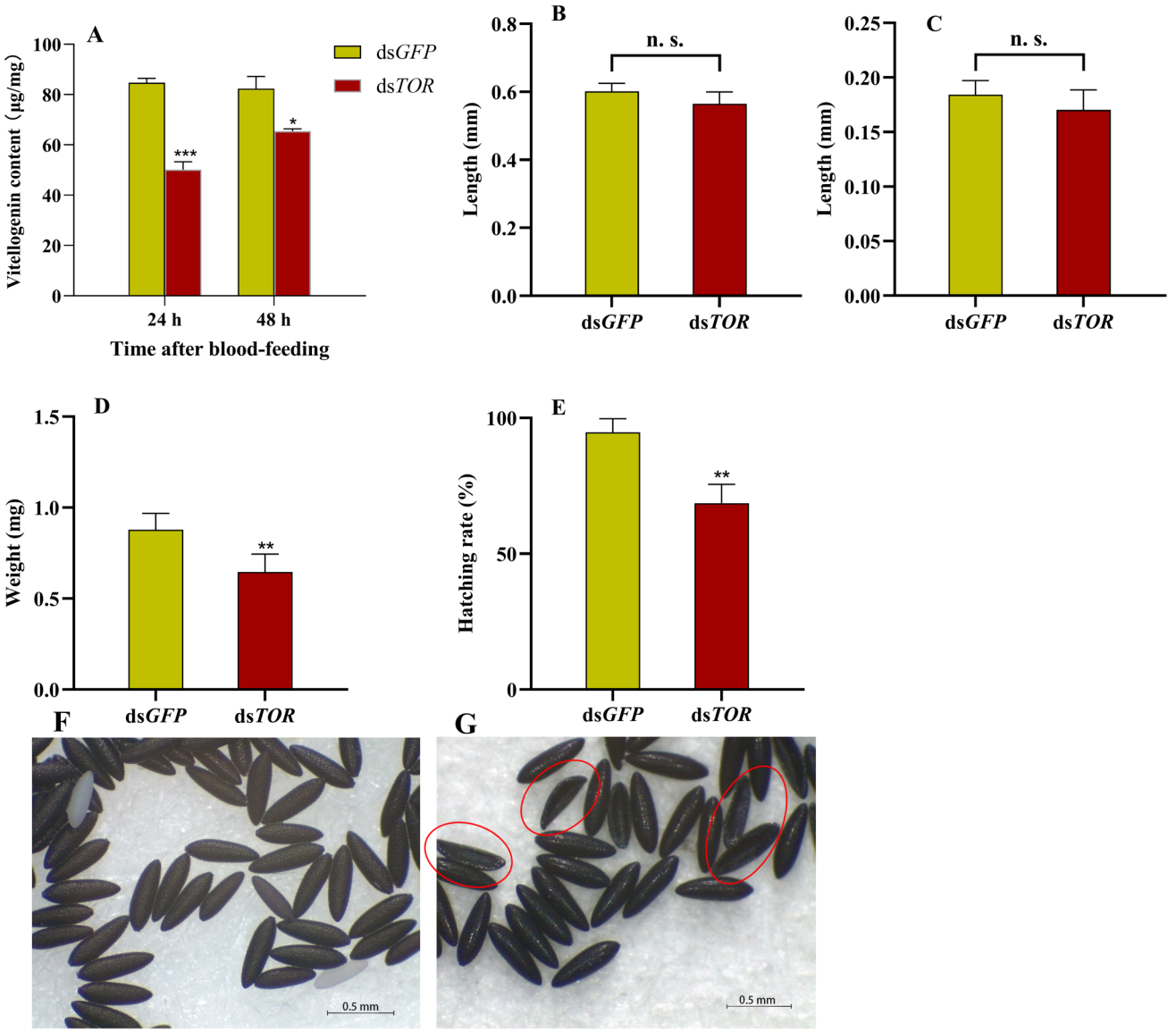

Fig. 9 Indicators related to *A. albopictus* eggs after *TOR* gene silencing. **A** Vg content in the ovary; **B** Long diameter of egg (anterior to posterior end of the egg); **C** Short diameter of egg (the diameter of the egg at its widest point); **D** Egg weight per 100 eggs; **E** Hatching rate of eggs; **F** ds*GFP*; **G** ds*TOR*. Values represent mean ± SE. The asterisks * on the bars indicate significant differences in the expression levels between ds*GFP* and ds*TOR*, (Student's t test, n.s. not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Inside the red circle are shriveled eggs

available amino acids that are subsequently transported to various tissues via the circulatory system and regulated by the TOR pathway (Gulia-Nuss et al. 2011). In the investigation of *Ae. aegypti*, either *TOR* gene silencing or rapamycin-induced inhibition of TOR protein would impact the translation process of early protease in the female mosquito midgut (Fernandes et al. 2020; Brandon et al. 2008). We observed the transcription and translation levels of TRY and CHY, revealing a significant inhibition in *TRY* gene expression upon *TOR* silencing, accompanied by a notable decrease in TRY activity. Thus, we propose that the *TOR* gene in *A. albopictus* regulates TRY activity at both transcriptional and translational levels. In the experimental group, *CHY1* expression significantly decreased 24 h after blood feeding but subsequently increased, while *CHY2* expression showed an increase. These findings suggest that *TOR* has a positive regulatory effect on *CHY1*, however, *CHY1* also possesses other upstream regulatory mechanisms independent of the TOR pathway. Moreover, the high expression of *CHY2* at 24 h post-blood feeding is speculated to compensate for reduced *CHY1* expression. Therefore, it is inferred that *CHY2* is not regulated by the TOR signaling pathway and

Table 3 Primers for qRT-PCR

Primer name	Forward primer (5′-3′)	Reverse primer (5′-3′)	NCBI
actin	GCTACGTCGCCCTGCACTT	AGGAACGACGGCTGGAAGA	DQ657949.1
TOR	CGAGTGGCTGATGGAGGAA	CCTTGATGGCAACGAATATGTG	LOC109429896
CHY1	GTAGGCATAGTGTCGTGGAGTA	GCATTCTGATGAAGTGGAAGGT	XM_029866654.1
CHY2	GAATCGTCGGAGGTGAAGAAG	AATGTGCGGCAGTAAGAACC	XM_019706941.2
TRY	GGCAATAGTGTTTCGCAGTT	GATCCAGCTCGTACTTTCAACT	XM_019696115.2
SLC3A2	ACCAAGGACGACCAACTGTT	ATTGCTGCGAGGCGACTT	XM_029855885.1
SLC7A5	ACCCATATCCACGACAACAC	CTTCAGGACGACCTTCTCAC	XM_029860051.1
Frizzled-2	ACAGTGCGACCAGATACGAC	CTACAAACGACCTTCTACAGTGAT	XM_029857134.1
Rheb	GTCCTCGCTCAGTATTCAGTTC	CAGTTTCACCTCGTAGTCGG	XM_029851959.1
Vg1	CTGAGCCGCAAGAACGATAC	CGAAGATGAGGACGAGGATGA	XM_019687400.2
Vg2	CAGCAGCAGCAGCATCATC	GCACATAACCGCAGATTCAGT	XM_029874019.1
Vg3	TCAGAACACCGACATCCG	CCAGCAGCATCGTAAAGG	XM_029866165.1
VgR	GTGCTGCTGGTCCTAATCCT	TGCGTTGTGGTCTGGTTGA	XM_019689430.3
S6K	AGAAGTGATCTGTCTAGCGTCTT	AATCTGCTCCAGTGCTGTTAT	XM_029876383.1
4E-BP	GAAAGGAAGCCCAGTAGGA	TTGTGGACCGTAACGAGAA	XM_029854536.1

its enzyme activity corresponds to gene expression. However, due to experimental constraints, this experiment merely detected the protease activity of female mosquitoes within 48 h after blood intake. Subsequent studies have indicated that silencing *TOR* prolongs the digestion period of blood. Hence, detailed research on the protease activity beyond 48 h after blood intake is necessary in future studies. After blood feeding, the female *A. albopictus* requires substantial energy support for efficient blood digestion and waste elimination, thereby its own energy metabolism is very important. In *Ae. aegypti*, upon blood ingestion, there is a notable reduction in trehalose levels within the female mosquito due to its essential role in sustaining energy metabolism (Hou et al. 2015). In this study, we observed alterations in glucose metabolism of *A. albopictus* following *TOR* silencing. The total glycogen content in female mosquitoes remained unaltered, possibly due to their continued suck sugar water after blood feeding. Conversely, 48 h post-blood meal, there was an increase in trehalose content accompanied by a decrease in trehalose enzyme activity and glucose levels. We propose two potential explanations for these findings: firstly, the absence of TOR signaling may hinder the mosquito's ability to respond to blood intake and consequently limit extensive degradation of trehalose for energy metabolism; secondly, *TOR* silencing could trigger a resistance mechanism in *A. albopictus* leading to elevated trehalose levels to improve its stress resistance.

The expression of the *Vg* gene is restricted to the fat body; therefore, the transport of amino acids across the adipose membrane is indispensable for successful vitellogenesis and reproduction. Amino acid transport is facilitated by various families of amino acid transporters, such as the SLC7 and SLC5 families. In *Ae. aegypti*, silencing members of the SLC7 family has been shown to disrupt signal transduction within the TOR pathway (Carpenter et al. 2012). The expression of *SLC7A5* and *SLC3A2* was significantly downregulated following *TOR* silencing post-blood feeding in this study. It is hypothesized that *TOR* silencing impacted the female mosquito's blood digestion process, leading to a decrease in amino acid content within the hemolymph and subsequently resulting in reduced transporter expression. Rheb, a GTPase that activates TOR protein kinase activity, serves as the key regulator of the TOR signaling pathway in eukaryotes in response to insulin signaling (Inoki et al. 2003). Silencing *Rheb* leads to significant downregulation of *Vg* transcription and translation in *Ae. aegypti* adiposomes (Roy and Raikhel 2010). In this study, silencing *TOR* results in a substantial increase in *Rheb* gene expression. It is hypothesized that the decreased expression of *TOR* triggered an upstream regulatory mechanism, ultimately restoring *TOR* expression levels through increased *Rheb* gene expression. Studies have demonstrated that the Wnt signaling pathway stimulates translation and cell growth by activating the mammalian TOR pathway (Inoki et al. 2006). It engages in interactions with other structural components on the cell surface via Frizzled protein, initiating a signal transduction cascade that ultimately leads to transcriptional regulation of gene expression (Staal et al. 2008). Deletion of *Frizzled-2* results in a significant reduction in S6K phosphorylation and *Vg* gene expression in *Ae. aegypti* (Weng and Shiao 2015). The expression of *Frizzled-2* in *TOR*-silenced *A. albopictus* exhibited a significant decrease after 24 h of blood feeding. The upregulation of *Frizzled-2* during later stages is speculated to be involved in the regulation of the Wnt signaling pathway.

Reproduction is the ultimate objective of all living organisms. The synthesis of YPPs in the fat body of *A. albopictus* plays a pivotal role in egg maturation, with *Vg* gene exhibiting the highest expression and serving as the most distinctive YPP. Previous investigations on *Drosophila*, *Apis mellifera*, *Blattella germanica*, and *Tribolium castaneum* have demonstrated a direct correlation between *Vg* and *TOR* (Sondergaard et al. 1995; Corona et al. 2007; Maestro et al. 2009; Parthasarathy and Palli 2011). *A. albopictus* possesses three *Vg* genes: *Vg1*, *Vg2*, and *Vg3* (Isoe and Hagedorn 2007). In this study, the expression levels of *Vg1*, *Vg2*, and *Vg3* were significantly reduced after 24 h of blood feeding by female mosquitoes as a result of *TOR* silencing. Additionally, the expression level of *VgR* was also decreased. However, the expression of *Vg1* increased at 48 h post-blood feeding, which contrasts with that of *Vg2* and *Vg3*. In Dittmer's study (2019), the expression patterns of *Vg1* and *Vg2* in *A. albopictus* fed with different sugars were also different. Concurrently, *Vg1* of *A. albopictus* is closely related to *VgC* of *Ae. aegypti*, which is also regarded as the putative ancestral vitellogenin gene of *Aedes* mosquitoes (Chen et al. 2010). Therefore, we speculate that *Vg1* also has other functions and regulatory mechanisms beyond the TOR pathway, but additional research is requisite to comprehend the related functions and regulatory mechanisms. The increase of *Vg1* expression also led to an increase of *VgR* expression levels consistent with the control group.

In addition to regulating *Vg* gene expression, *TOR* also exerts control over protein synthesis by phosphorylation-mediated inactivation of 4E-BP and phosphorylation-induced activation of S6K, thereby influencing yolk formation and egg development (Brunn et al. 1997; Burnett et al. 1998). In the study on *Ae. aegypti*, female mosquitoes exhibited immediate hyperphosphorylation of 4E-BP upon blood feeding, resulting in rapid inhibition of its translation inhibitory function. Furthermore, the expression level of *4E-BP* gene was relatively high during early vitellogenesis in female mosquitoes but decreased sharply to almost negligible levels at 3–12 h post-blood feeding. However, it started rising again at 24 h post-blood feeding and returned to the early vitellogenesis level within 48–72 h (Roy and Raikhel 2012). In this study, the expression of the *4E-BP* gene in female *A. albopictus* exhibited a significant decrease compared to the control group at 24 h and 48 h post-blood feeding. These findings demonstrate that *TOR* mediates the reduction in *4E-BP* gene expression within 12 h after blood feeding, and silencing of *TOR* attenuates the decline in *4E-BP* gene expression. S6K protein kinase is also rapidly phosphorylated after blood sucking. In *Ae. aegypti*, no significant alteration in *S6K* mRNA levels was observed post-blood meal, suggesting that the regulation of *S6K* expression is independent of pathways involved in blood digestion and reproduction. RNAi-mediated silencing of *S6K* effectively impedes mosquito egg development subsequent to blood feeding (Hansen et al. 2005). In this study, we observed a significant decrease in the expression of the *S6K* gene in female mosquitoes at 24 h after blood feeding, followed by an increase at 48 h. We speculate that *TOR* may regulate S6K solely at the translational level. Upon *TOR* silencing, phosphorylation of the S6K protein decreases, leading to reduced mRNA levels. Subsequently, *S6K* independently acts on an upstream regulator within the TOR pathway to enhance its expression; however, further investigation is required to elucidate the specific mechanism. Changes in gene expression at the molecular level will eventually be manifested at the macroscopic level. Currently, studies conducted on *Ae. aegypti*, *Ornithodoros moubata*, and *Bemisia tabaci* have demonstrated that *TOR* silencing can impact ovary development and egg production (Meryem et al. 2022; Hansen et al. 2004; Puri and Jindal 2021). Additionally, we quantified Vg content and observed its more intuitive appearance. Firstly, we quantified the Vg content in female mosquito ovaries and observed a significant decrease, which was consistent with *Vg* gene expression. Subsequently, we assessed the number of eggs laid and found that *TOR* silencing had minimal impact on total egg production but prolonged the spawning cycle, contradicting previous findings in *Ae. aegypti*. We hypothesize that this discrepancy may be attributed to the low efficiency of RNAi through feeding. Additionally, we conducted dissections and observed delayed ovarian development in *A. albopictus* upon *TOR* silencing, aligning with the oviposition patterns observed. Finally, we evaluated various parameters related to eggs and discovered that *TOR* silencing minimally affected egg diameter but significantly reduced both egg weight and hatching rate in the experimental group. Microscopic examination also revealed some shriveled eggs, and the mechanism through which their occurrence is associated with the silencing of *TOR* still requires further exploration.

In conclusion, the *TOR* gene of *A. albopictus* plays a pivotal role in female mosquito blood digestion, metabolism, and reproductive behavior following blood feeding. Silencing the *TOR* gene adversely affects *A. albopictus*' blood digestion efficiency by reducing protease activity and energy metabolism. Additionally, it disrupts Vg synthesis through downstream modulation of TOR pathway-related genes, leading to delayed ovarian development, prolonged spawning period, significantly diminished egg quality and hatching rate. These findings highlight the potential of *TOR* gene as a target for controlling *A. albopictus*. In future, our research will focus on three key areas: firstly, enhancing the efficiency of interference in feeding RNAi and improving the stability of dsRNA to withstand the intricate intestinal environment of female mosquitoes post-blood meal and prevent rapid degradation; secondly, delving deeper into the response and regulatory mechanisms of TOR signaling pathway, including its interplay with insulin signaling pathway and Wnt signaling pathway; and thirdly, investigating the heightened expression of *TOR* during *A. albopictus*

pupal stage to gain a comprehensive understanding of *TOR*'s role in *A. albopictus*.

Author contributions

CZ and SGW designed the study. CZ, JYS and RYL performed the research. CZ, JYS, TTM and BT analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Author contributions Chen Zhang and Jiayan Shen have contributed equally to this work.

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Data availability No datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate All animal procedures have been approved by the Experimental Animal Ethics Committee (AEWC) of Hangzhou Normal University (Approval No. HSD20220719-2).

References

Alphey L, McKemey A, Nimmo D, Neira Oviedo M, Lacroix R, Matzen K et al (2013) Genetic control of *Aedes* mosquitoes. Pathog Glob Health 107:170–179. https://doi.org/10.1179/20477732 13Y.0000000095

Attardo GM, Higgs S, Klingler KA, Vanlandingham DL, Raikhel AS (2003) RNA interference-mediated knockdown of a GATA factor reveals a link to anautogeny in the mosquito *Aedes aegypti*. Proc Natl Acad Sci U S A 100:13374–13379. https://doi.org/10.1073/pnas.2235649100

Benedict MQ, Levine RS, Hawley WA, Lounibos LP (2007) Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector Borne Zoonotic Dis 7:76–85. https://doi.org/10.1089/vbz.2006.0562

Brackney DE, Isoe J, Black WC V, Zamora J, Foy BD, Miesfeld RL et al (2010) Expression profiling and comparative analyses of seven midgut serine proteases from the yellow fever mosquito, *Aedes aegypti*. J Insect Physiol 56:736–744. https://doi.org/10.1016/j.jinsphys.2010.01.003

Brandon MC, Pennington JE, Isoe J, Zamora J, Schillinger A-S, Miesfeld RL (2008) TOR signaling is required for amino acid stimulation of early trypsin protein synthesis in the midgut of *Aedes aegypti* mosquitoes. Insect Biochem Mol Biol 38:916–922. https://doi.org/10.1016/j.ibmb

Brown MR, Graf R, Swiderek KM, Fendley D, Stracker TH, Champagne DE et al (1998) Identification of a steroidogenic neurohormone in female mosquitoes. J Biol Chem 273:3967–3971. https://doi.org/10.1074/jbc.273.7.3967

Brunn GJ, Hudson CC, Sekulic A, Williams JM, Hosoi H, Houghton PJ et al (1997) Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. Science 277:99–101. https://doi.org/10.1126/science.277.5322.99

Burnett PE, Barrow RK, Cohen NA, Snyder SH, Sabatini DM (1998) RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. Proc Natl Acad Sci U S A 95:1432–1437. https://doi.org/10.1073/pnas.95.4.1432

Carpenter VK, Drake LL, Aguirre SE, Price DP, Rodriguez SD, Hansen IA (2012) SLC7 amino acid transporters of the yellow fever mosquito *Aedes aegypti* and their role in fat body TOR signaling and reproduction. J Insect Physiol 58:513–522. https://doi.org/10.1016/j.jinsphys.2012.01.005

Chen S, Armistead JS, Provost-Javier KN, Sakamoto JM, Rasgon JL (2010) Duplication, concerted evolution and purifying selection drive the evolution of mosquito vitellogenin genes. BMC Evol Biol 10:142. https://doi.org/10.1186/1471-2148-10-142

Chiodini J (2018) Apps from the World Health Organization—The World Malaria Report and more. Travel Med Infect Dis 22:82–84. https://doi.org/10.1016/j.tmaid.2018.03.007

Corona M, Velarde RA, Remolina S, Moran-Lauter A, Wang Y, Hughes KA et al (2007) Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. Proc Natl Acad Sci U S A 104:7128–7133. https://doi.org/10.1073/pnas.0701909104

Dhara A, Eum JH, Robertson A, Gulia-Nuss M, Vogel KJ, Clark KD et al (2013) Ovary ecdysteroidogenic hormone functions independently of the insulin receptor in the yellow fever mosquito, *Aedes aegypti*. Insect Biochem Mol Biol 43:1100–1108. https://doi.org/10.1016/j.ibmb.2013.09.004

Diaz-Troya S, Perez-Perez ME, Florencio FJ, Crespo JL (2008) The role of TOR in autophagy regulation from yeast to plants and mammals. Autophagy 4:851–865. https://doi.org/10.4161/auto.6555

Dittmer J, Alafndi A, Gabrieli P (2019) Fat body-specific vitellogenin expression regulates host-seeking behaviour in the mosquito Aedes albopictus. PLoS Biol 17(5):e3000238. https://doi.org/10.1371/journal.pbio.3000238

Fernandes TLS, Pereira-Filho AA, Nepomuneno DB, Milagres TdF, Malta LGF, Pessoa GCDÁ et al (2020) Galactosamine reduces sandfly gut protease activity through TOR downregulation and increases *Lutzomyia* susceptibility to *Leishmania*. Insect Biochem Mol Biol 122:103393. https://doi.org/10.1016/j.ibmb.2020.103393

Ganbaatar O, Cao B, Zhang Y, Bao D, Bao W, Wuriyanghan H (2017) Knockdown of *Mythimna separata chitinase* genes via bacterial expression and oral delivery of RNAi effectors. BMC Biotechnol 17:9. https://doi.org/10.1186/s12896-017-0328-7

Guan L, Wang X, Wan S, Wang Y, Zhang X, Wang S et al (2024) The Role of *TcCYP6K1* and *TcCYP9F2* influences trehalose metabolism under high-CO_2 stress in *Tribolium castaneum* (Coleoptera). InSects 15(7):502. https://doi.org/10.3390/insects15070502

Gulia-Nuss M, Robertson AE, Brown MR, Strand MR (2011) Insulin-like peptides and the target of rapamycin pathway coordinately regulate blood digestion and egg maturation in the mosquito *Aedes aegypti*. PLoS ONE 6:e20401. https://doi.org/10.1371/journal.pone.0020401

Hansen IA, Attardo GM, Park JH, Peng Q, Raikhel AS (2004) Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. Proc Natl Acad Sci U S A 101:10626–10631. https://doi.org/10.1073/pnas.0403460101

Hansen IA, Attardo GM, Roy SG, Raikhel AS (2005) Target of rapamycin-dependent activation of S6 kinase is a central step in the transduction of nutritional signals during egg development in a mosquito. J Biol Chem 280:20565–20572. https://doi.org/10.1074/jbc.M500712200

Hou Y, Wang XL, Saha TT, Roy S, Zhao B, Raikhel AS et al (2015) Temporal coordination of carbohydrate metabolism during mosquito reproduction. PLoS Genet 11:e1005309. https://doi.org/10.1371/journal.pgen.1005309

Hou J, Liu Q, Wang J, Wu Y, Li T, Gong Z (2020) Insecticide resistance of *Aedes albopictus* in Zhejiang Province, China. Biosci Trends 14:248–254. https://doi.org/10.5582/bst.2020.03194

Inoki K, Li Y, Xu T, Guan KL (2003) Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 17(15):1829–1834. https://doi.org/10.1101/gad.1110003

Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X et al (2006) TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell 126:955–968. https://doi.org/10.1016/j.cell.2006.06.055

Isoe J, Hagedorn HH (2007) Mosquito vitellogenin genes: comparative sequence analysis, gene duplication, and the role of rare synonymous codon usage in regulating expression. J Insect Sci 7:1–49. https://doi.org/10.1673/031.007.0101

Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149:274–293. https://doi.org/10.1016/j.cell.2012.03.017

Li X, Zhang M, Zhang H (2011) RNA interference of four genes in adult *Bactrocera dorsalis* by feeding their dsRNAs. PLoS ONE 6:e17788. https://doi.org/10.1371/journal.pone.0017788

Ling L, Raikhel AS (2023) Amino acid-dependent regulation of insulin-like peptide signaling is mediated by TOR and GATA factors in the disease vector mosquito *Aedes aegypti*. Proc Natl Acad Sci USA 120:e2303234120. https://doi.org/10.1073/pnas.2303234120

Liu, F, Li, K, Cai, W, Zhao, J, Zou, Y and Hua, H (2017) Knockdown of *TOR* causing ovarian diapause in a genetically stable brachypterous strain of *Nilaparvata lugens*. Arch Insect Biochem Physiol 95. https://doi.org/10.1002/arch.21400.

Lu SJ, Pennington JE, Stonehouse AR, Mobula MM, Wells MA (2006) Reevaluation of the role of early trypsin activity in the transcriptional activation of the late trypsin gene in the mosquito *Aedes aegypti*. Insect Biochem Mol Biol 36:336–343. https://doi.org/10.1016/j.ibmb.2006.01.011

Maestro JL, Juliana C, Xavier B (2009) Target of rapamycin (TOR) mediates the transduction of nutritional signals into juvenile hormone production. J Biol Chem 284:5506–5513. https://doi.org/10.1074/jbc.M807042200

Martín D, Wang S-F, Raikhel AS (2001) The vitellogenin gene of the mosquito *Aedes aegypti* is a direct target of ecdysteroid receptor. Mol Cell Endocrinol 173:75–86. https://doi.org/10.1016/s0303-7207(00)00413-5

Martin DE, Hall MN (2005) The expanding TOR signaling network. Curr Opin Cell Biol 17:158–166. https://doi.org/10.1016/j.ceb.2005.02.008

Meryem, B, Haruki, T, Keisuke, K, Shoko, N, H, OM and DeMar, T (2022) Production of the yolk protein precursor vitellogenin is mediated by target of rapamycin (*TOR*) in the soft tick *Ornithodoros moubata* (Acari: Argasidae). Insect SCI 29**:** 1299-1308. https://doi.org/10.1111/1744-7917.13025.

Park JH, Attardo GM, Hansen IA, Raikhel AS (2006) GATA factor translation is the final downstream step in the amino acid/target-of-rapamycin-mediated *vitellogenin* gene expression in the anautogenous mosquito *Aedes aegypti*. J Biol Chem 281:11167–11176. https://doi.org/10.1074/jbc.M601517200

Parthasarathy R, Palli SR (2011) Molecular analysis of nutritional and hormonal regulation of female reproduction in the red flour beetle, *Tribolium castaneum*. Insect Biochem Mol Biol 41:294–305. https://doi.org/10.1016/j.ibmb.2011.01.006

Partridge L, Alic N, Bjedov I, Piper MD (2011) Ageing in *Drosophila*: the role of the insulin/Igf and TOR signalling network. Exp Gerontol 46:376–381. https://doi.org/10.1016/j.exger.2010.09.003

Piedra LA, Rodriguez MM, Lopez I, Ruiz A, Martinez LC, Garcia I et al (2023) Insecticide resistance status of *Aedes albopictus* (*Diptera: Culicidae*) populations from Cuba. J Med Entomol 60:487–493. https://doi.org/10.1093/jme/tjac189

Puri H, Jindal V (2021) *Target of rapamycin* (*TOR*) gene is vital for whitefly survival and reproduction. J Biosci 46:60. https://doi.org/10.1007/s12038-021-00168-0

Riehle MA, Brown MR (2002) Insulin receptor expression during development and a reproductive cycle in the ovary of the mosquito *Aedes aegypti*. Cell Tissue Res 308:409–420. https://doi.org/10.1007/s00441-002-0561-8

Rosen L, Shroyer DA, Tesh RB, Freier JE, Lien JC (1983) Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti*. Am J Trop Med Hyg 32:1108–1119. https://doi.org/10.4269/ajtmh.1983.32.1108

Roubaud E (1929) Cycle autogene d'attente et generations hivrenales suractives inaparentes chez le moustique commun *Culex pipiens*. Acad Sci 180:735–738

Roy SG, Raikhel AS (2010) The small GTPase Rheb is a key component linking amino acid signaling and TOR in the nutritional pathway that controls mosquito egg development. Insect Biochem Mol Biol 41:62–69. https://doi.org/10.1016/j.ibmb.2010.10.001

Roy SG, Raikhel AS (2012) Nutritional and hormonal regulation of the TOR effector 4E-binding protein (4E-BP) in the mosquito *Aedes aegypti*. FASEB J 26:1334–1342. https://doi.org/10.1096/fj.11-189969

Roy S, Saha TT, Zou Z, Raikhel AS (2018) Regulatory pathways controlling female insect reproduction. Annu Rev Entomol 63:489–511. https://doi.org/10.1146/annurev-ento-020117-043258

Sondergaard L, Mauchline D, Egetoft P, White N, Wulff P, Bownes M (1995) Nutritional response in a *Drosophila* yolk protein gene promoter. Mol Gen Genet 248:25–32. https://doi.org/10.1007/BF02456610

Staal FJ, Luis TC, Tiemessen MM (2008) WNT signalling in the immune system: WNT is spreading its wings. Nat Rev Immunol 8:581–593. https://doi.org/10.1038/nri2360

Suganya R, Chen SL, Lu KH (2010) Target of rapamycin in the oriental fruit fly *Bactrocera dorsalis* (Hendel): its cloning and effect on yolk protein expression. Arch Insect Biochem Physiol 75:45–56. https://doi.org/10.1002/arch.20383

Tian H, Peng H, Yao Q, Chen H, Xie Q, Tang B, Zhang W (2009) Developmental control of a lepidopteran pest *Spodoptera exigua* by ingestion of bacteria expressing dsRNA of a non-midgut gene. PLoS ONE 4(7):e6225. https://doi.org/10.1371/journal.pone.0006225

Tran HT, Shaaban S, Askari HB, Walfish PG, Raikhel AS, Butt TR (2001) Requirement of co-factors for the ligand-mediated activity of the insect ecdysteroid receptor in yeast. J Mol Endocrinol 27:191–209. https://doi.org/10.1677/jme.0.0270191

Umemiya-Shirafuji R, Boldbaatar D, Liao M, Battur B, Rahman MM, Kuboki T et al (2012) Target of rapamycin (TOR) controls vitellogenesis via activation of the S6 kinase in the fat body of the tick, *Haemaphysalis longicornis*. Int J Parasitol 42:991–998. https://doi.org/10.1016/j.ijpara.2012.08.002

Vogel KJ, Brown MR, Strand MR (2015) Ovary ecdysteroidogenic hormone requires a receptor tyrosine kinase to activate egg formation in the mosquito *Aedes aegypti*. Proc Natl Acad Sci U S A 112:5057–5062. https://doi.org/10.1073/pnas.1501814112

Wang Y, An M, Stevens KM, Liu N (2022) Insecticide resistance in Alabama populations of the mosquito *Aedes albopictus*. J Med Entomol 59:1678–1686. https://doi.org/10.1093/jme/tjac085

Weng S, Shiao S (2015) Frizzled 2 is a key component in the regulation of TOR signaling-mediated egg production in the mosquito *Aedes aegypti*. Insect Biochem Mol Biol 61:17–24. https://doi.org/10.1016/j.ibmb.2015.03.010

Xue T, Zhang C, Xu Q, Li Z, Shao X (2017) Azobenzene-benzoylphenylureas as photoswitchable chitin synthesis inhibitors. Org Biomol Chem 15:3320–3323. https://doi.org/10.1039/c6ob02813f

Zhang L, Qiu LY, Yang HL, Wang HJ, Zhou M, Wang SG, Tang B (2017) Study on the effect of wing bud chitin metabolism and its developmental network genes in the brown planthopper, *Nilaparvata lugens*, by Knockdown of *TRE* Gene. Front Physiol 8:750. https://doi.org/10.3389/fphys.2017.00750

Zhang C, Ding Y, Zhou M, Tang Y, Chen R, Chen Y et al (2023) RNAi-mediated *CHS-2* silencing affects the synthesis of chitin and the formation of the peritrophic membrane in the midgut of *Aedes albopictus* larvae. Parasit Vectors 16:259. https://doi.org/10.1186/s13071-023-05865-3

Zhao A, Li Y, Leng C, Wang P, Li Y (2018) Inhibitory effect of protease inhibitors on larval midgut protease activities and the performance of *Plutella xylostella* (Lepidoptera: Plutellidae). Front Physiol 9:1963. https://doi.org/10.3389/fphys.2018.01963

Zhou S, Zhou Q, Liu Y, Wang S, Wen D, He Q et al (2010) Two *Tor* genes in the silkworm *Bombyx mori*. Insect Mol Biol 19:727–735. https://doi.org/10.1111/j.1365-2583.2010.01026.x

Zhu S, Liu F, Zeng H, Li N, Ren C, Su Y et al (2020) Insulin/IGF signaling and TORC1 promote vitellogenesis via inducing juvenile hormone biosynthesis in the American cockroach. Development 147:dev188805. https://doi.org/10.1242/dev.188805

Zhuo JC, Xue J, Lu JB, Huang HJ, Xu HJ, Zhang CX (2017) Effect of RNAi-mediated knockdown of *NlTOR* gene on fertility of male *Nilaparvata lugens*. J Insect Physiol 98:149–159. https://doi.org/10.1016/j.jinsphys.2017.01.002