Correspondence: Marcos Sterkel, Laboratorio de Neurobiología de Insectos (LNI), Centro Regional de Estudios Genómicos, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CENEXA, CONICET, La Plata, Buenos Aires, Argentina. Email: msterkel@conicet.gov.ar

*These authors contributed equally to this work.

ORIGINAL ARTICLE

eIF3 subunit M regulates blood meal digestion in *Rhodnius prolixus* affecting ecdysis, reproduction and survival

Pilar Ameijeiras[1,*], Natalia Capriotti[1,*], Sheila Ons[1], Pedro L. Oliveira[2,3] and Marcos Sterkel[1]

[1]*Laboratorio de Neurobiología de Insectos (LNI), Centro Regional de Estudios Genómicos, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CENEXA, CONICET, La Plata, Buenos Aires, Argentina;*

[2]*Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil;*

[3]*Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (INCT-EM), Rio de Janeiro, Brazil*

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Abstract In triatomines, blood-feeding triggers many physiological processes including post-embryonic development and reproduction. Different feeding habits, such as hematophagy, can shape gene functions to meet the challenges of each type of diet. The gut of blood-sucking insects faces particular challenges after feeding due to the quantity and the quality of the food ingested. A comparison of transcriptomic and proteomic data indicates that post-transcriptional regulation of gene expression is crucial in the triatomine gut. It was proposed that eukaryotic translation initiation factor 3 subunit m (eIF3m) and eIF3e define two different eIF3 complexes with a distinct affinity for the different mRNAs, thus selecting the set of mRNAs to be translated and constituting a post-transcriptional mode of regulation of gene expression. Because the eIF3m is mainly expressed in the gut, we evaluated its relevance in *Rhodnius prolixus* physiology through RNA interference-mediated gene silencing. The knockdown of eIF3m reduced the digestion rate, affecting the processes triggered by a blood meal. Its silencing inhibited moulting and caused premature death in nymphs while impaired ovary development, oviposition and increased resistance to starvation in adult females. The survival of males after feeding (resistance to starvation) was not affected by eIF3m knockdown. The information regarding the eIF3m function in insects is scarce and the phenotypes observed in *R. prolixus* upon eIF3m silencing are different and more severe than those previously described in *Drosophila melanogaster*, indicating a pleiotropic role of this gene in triatomines.

Key words egg hatching; hematophagous vectors; lifespan; oviposition; post-embryonic development; starvation resistance

Introduction

Triatomines, vectors of the parasite that causes Chagas disease, feed on hosts that are thousands of times larger than themselves, so the timing of feeding represents a high risk to their survival. Although hematophagy arose on many independent occasions during the evolution of arthropods (Lehane, 2005), a common adaptive strategy has been to decrease the frequency of feeding, compensating for it with the ingestion of an enormous volume of blood that is equivalent to several times their body weight during each visit to the host (Lehane, 2005). The adaptations that allow blood-sucking arthropods to tolerate their diet constitute a unique operating model regarding cell signalling, immunity and metabolism (Sterkel *et al.*, 2017). A blood meal impacts on triatomine physiology and triggers several processes, such as diuresis, digestion, moulting in nymphs and reproduction in adults, involving a massive synthesis of new proteins synchronized with digestion (Guarneri & Lorenzo, 2021).

According to the so-called “ribosome filter” hypothesis, eukaryotic translation initiation factors (eIF) recruit specific sets of mRNAs on the ribosome without causing a drastic change in the existing mRNA pool (Mauro & Edelman, 2007). The eukaryotic translation initiation factor 3 (eIF3) is required to form a stable 40S preinitiation complex and stabilizes the binding of the eIF2-GTP-Met-RNAt complex to the 40S ribosomal subunit (Chaudhuri *et al.*, 1999). eIF3 contains 13 subunits, named alphabetically as eIF3a-eIF3m (Zhou *et al.*, 2008). This complex has constituent subunits conserved in all eukaryotes (eIF3a, eIF3b and eIF3c, eIF3g and eIF3i) and variable subunits (Masutani *et al.*, 2007). The eIF3 complex associates with the mRNA 5' untranslated portion of mRNAs related to cell growth, cell cycling, differentiation and apoptosis (Lee *et al.*, 2015). It was proposed that eIF3m and eIF3e define two different eIF3 complexes with a distinct affinity for the different mRNAs, thus selecting the set of mRNAs to be translated and constituting a post-transcriptional mode of regulation of gene expression (Zhou *et al.*, 2005). Reduction in the expression of eIF3 subunits led to

developmental disorders in zebrafish, plants and mice (h and m subunits. Kim *et al.*, 2004; Choudhuri *et al.*, 2013; Zeng *et al.*, 2013), reduced malignant properties of the cells (a, -m, -e and -h subunits. Grzmil *et al.*, 2010; Robichaud *et al.*, 2019) and increased longevity in *Caenorhabditis elegans* (k and l subunits. Cattie *et al.*, 2016).

The eIF3m subunit is conserved from fission yeast to higher eukaryotes, but it is absent in budding yeast (Zeng *et al.*, 2013), and it is the only unidentified eIF3 subunit in trypanosomatid genomes (Rezende *et al.*, 2014). In humans, eIF3m regulates the expression of tumorigenesis-related genes in colon cancer (Goh *et al.*, 2011) and promotes the malignant phenotype of lung adenocarcinoma by the up-regulation of the oncogene *CAPRIN1* (Liu *et al.*, 2021). EIF3m silencing inhibits herpes virus protein translation but has little effect on cellular RNA or protein expression and is not cytotoxic (Cheshenko *et al.*, 2010). In mice, eIF3m is critical for eIF3 structure and is required for embryonic development, homeostasis and organ size control (Zeng *et al.*, 2013). The knockdown of eIF3m has a limited impact on mRNA-specific translation but affects ribosome biogenesis and transcription in mice liver (Smekalova *et al.*, 2020). The information regarding the eIF3m function in insects is scarce. In *Drosophila melanogaster*, it is a pro-apoptotic factor (Chew *et al.*, 2009). It positively regulates *Dronc* caspase levels through a post-transcriptional mechanism. Its silencing prevents death induced by multiple caspase-dependent apoptotic stimuli (Chew *et al.*, 2009). However, eIF3m has functions in cells that are not destined to die, such as cell remodelling by the apoptosome (D'Brot *et al.*, 2013). EIF3m regulates the caspase-dependent remodelling process necessary for sperm development (D'Brot *et al.*, 2013) and regulates *Dronc* caspase activity at the cortex in salivary glands of *D. melanogaster* (Kang *et al.*, 2017).

After sequencing the *Rhodnius prolixus* genome, the first triatomine genome available (Mesquita *et al.*, 2015), several transcriptomic studies correlated mRNA level profiles of different tissues with physiology, immunity, reproduction and development (Ribeiro *et al.*,

2014; Latorre-Estivalis *et al.*, 2017, 2020; Leyria *et al.*, 2020a, 2020b; Coelho *et al.*, 2021). However, the results obtained with a proteomic approach in the gut showed an expression profile distinct from the mRNA, suggesting that post-transcriptional regulation of gene expression is crucial in this tissue (Ouali *et al.*, 2021). The gut of hematophagous arthropods is a central tissue responsible for blood digestion and nutrient absorption. It undergoes several physiological changes after feeding, such as huge potentially toxic amounts of amino acids and heme, and a drastic increase in the microbiota (Sterkel *et al.*, 2017). It is also the tissue where *T. cruzi* develops. It is composed of three regions called anterior midgut, posterior midgut and rectum. Transcriptomic data indicated that the anterior midgut presented the highest eIF3m mRNA level (Ribeiro *et al.*, 2014). It is also expressed in the posterior midgut, rectum and testes, but its expression was not detected in the fat body, Malpighian tubules or ovaries (Ribeiro *et al.*, 2014). The eIF3m mRNA was also detected in the central nervous system (Ons *et al.*, 2016) and antennae (Latorre-Estivalis *et al.*, 2017). These observations and the fact that the role of the non-conserved eIF3 subunits is essentially untested in insects, led us to evaluate the impact of RNAi-mediated gene silencing of eIF3m expression on *R. prolixus* physiology. Its knockdown reduced the digestion rate affecting development, reproduction and survival. Interestingly, the phenotypes observed upon reduction of the expression of eIF3m in *R. prolixus* are different and more severe than those reported in *D. melanogaster*, in which the effects of its silencing were mild and tissue-specific (Chew *et al.*, 2009; D'Brot *et al.*, 2013; Kang *et al.*, 2017). The results point to a particular and crucial role of eIF3m in triatomine physiology.

Materials and methods

Rearing of insects

Experiments were performed with animals obtained from two colonies in the Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro (UFRJ), Brazil, and another in the Centro Regional de Estudios Genómicos, Universidad Nacional de La Plata (CREG), Argentina. In both cases, insects were maintained at 28°C and 50%–60% relative humidity under a photoperiod of 12 h of light/12 h of dark. In UFRJ, the insects were fed on rabbit blood at 3-week intervals. In CREG, the insects were fed on chickens at 3–4 weeks intervals. All the work with animals was conducted according to the guidelines of the Institutional Animal Care and Use Committee (Committee for Evaluation of Animal Use for Research from the Federal University of Rio de Janeiro), which are based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3). The protocols received registry number 149-19 from the Animal Ethics Committee (Comissão de Ética no Uso de Animais, CEUA). Technicians at the animal facility at the Institute of Medical Biochemistry (UFRJ) performed all aspects related to rabbit husbandry under strict guidelines to ensure careful and consistent handling of the animals. Biosecurity considerations are in agreement with CONICET resolution 1619/2008, which is in accordance with the WHO Biosecurity Handbook (ISBN 92 4 354 6503).

All the experiments were carried out first in the UFRJ laboratory (Brazil) and later replicated in the UNLP laboratory (Argentina), except for haemoglobin and protein content in ovaries quantification experiments which were only performed in Brazil. We obtained identical phenotypes upon eIF3m silencing in both laboratories, despite minor experimental differences in the protocols used.

RNA isolation and complementary DNA (cDNA) synthesis

R. prolixus tissues were dissected in ice-cold PBS (NaCl 0.15 mol/L, Na^+-phosphate 10 mmol/L, pH 7.4). The total RNA from different tissues was extracted using TRIzol reagent (Ambion, USA), according to the manufacturer's instructions. Following treatment with DNAse I (Invitrogen, USA), first-strand cDNA synthesis was performed using 1 μg of total RNA with MMLV Reverse Transcription Kit (Applied Biosystems, Brazil) and poly-T primer, according to the manufacturer instructions.

Synthesis of double-stranded RNA (dsRNA).

Two pairs of specific primers for the eIF3m transcript (VectorBase ID: RPRC009587) containing the T7 RNA polymerase binding sequence at 5' end, required for dsRNA synthesis, were designed. We ruled out off-target effects by injecting two dsRNAs covering different regions of the eIF3m mRNA in independent experiments. This was done to confirm that the phenotypes observed were due to eIF3m knockdown and not to a possible non-specific silencing of other genes due to sequence similarity. dsRNA-1 was designed in exons 1 and 2 (primers: Fw 5′-TAATACGACTCACTATAGGGAGATCTGGACGTTCCGGCAGT-3′ and Rv 5′-TAATACGACTCACTATAGGGAGATGGGCATCTTCCCTGGC-3′. dsRNA-2 was design in exon 3 (primers: Fw 5′-TAATACGACTCACTATAGGGAGAGCCCTAGCCGATCCTAACACT-3′and Rv 5′-TAATACGACTCACTATAGGGAGATTTCATTAGCACCTTCAGCC-3')

A fragment from the β-lactamase gene, absent in the *R. prolixus* genome, was PCR-amplified from the pBluescript plasmid (primers: Fw 5′-TAATACGACTCACTATAGGGGAACTGGATCTCAACAG-3′ and RV: 5′-TAATACGACTCACTATAGGGGGATCTTCACCTAGATC-3′) and used as a control to

assess the putative non-specific effects of dsRNA injections. All the PCR products were sequenced (Macrogen, Korea) to confirm their identity. One μL of the PCR product was used for a second PCR reaction using T7-full promoter primer (5′-ATAGAATTCTCTCTAGAAGCTTAATACGACTCACTATAGGG-3′). The product of the second PCR was used as the template for dsRNA synthesis. The dsRNAs were *in vitro* transcribed using T7-RNA polymerase (Invitrogen, USA), according to the manufacturer's instructions. dsRNAs were precipitated with isopropanol and resuspended in ultrapure water (Milli-Q). The dsRNA fragments were visualized by agarose (1% w/v) electrophoresis gel to verify their size, integrity and purity. The dsRNAs were quantified from images of the gels using the ImageJ software (National Institute of Health, USA). A 100 pb molecular weight marker was used as standard for quantification (Productos Bio-logicos, Argentina). The dsRNAs were stored at −20°C until use.

RNAi to determine loss-of-function phenotypes

Fourth instar nymphs, adult males and females *R. prolixus* were injected into the thorax with 2 μL of each dsRNA (1 μg/μL) dissolved in ultrapure water using a 10 μL Hamilton microsyringe. Control insects were injected with 2 μg of β-lac dsRNA. Only mated males and females that had been previously fed once during the adult stage were used to perform the experiments. Adult insects were injected with the dsRNA 21 days after the first feeding during the adult stage, while fourth instar nymphs were injected 15–21 days after moulting. On that day, the intestine of some starved insects was collected in TRIzol reagent (Invitrogen, USA) to check the efficacy of gene knockdown by qPCR.

Quantitative polymerase chain reaction (qPCR)

Total RNA was extracted from the gut (anterior midgut, posterior midgut and rectum) of females, males and fourth instar nymphs seven days after dsRNA injection. The cDNAs were synthesized as previously described. Specific primers targeting the eIF3m gene (Fw 5′-GGAAGAAGTAGAGGCTTTCGTG-3′ and Rv 5′-TGTTCCCATTGGGCTACTCC-3′) were designed to amplify a different region from that amplified by the RNAi primer pairs to prevent the amplification of the injected dsRNA, which may be retrotranscribed during synthesis of the cDNA together with insect RNA. They were also designed in different exons (exons 3 and 4) to prevent genomic DNA amplification. Primers were tested for dimerization, efficiency (85.7%) and amplification of a single product. The *glucose-6-phosphate dehydrogenase (G6DPH)* gene was used as reference gene (Fw 5′-AGCCTGGAGAAGCGGTTTACGTTA-3′ and Rv 5′-GTGAGCCACAGAATACGTCGAGT-3′. Omondi *et al.*, 2015). qPCR was performed using qPCR SYBR Green Master Mix (Productos Bio-lógicos, Argentina) under the following conditions: 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 15 s. Finally, the melting temperature of the PCR product generated was calculated (1 cycle of 95°C for 30 s, 65°C for 30 s and 95°C for 30 s) in an Agilent AriaMx Real-time PCR System (Applied Biosystems, USA). For each sample, ΔCT values were calculated (CT_{eIF3m}–CT_{G6DPH}). The $2e^{-\Delta CT}$ values obtained for dseIF3m and dsβ-lac-injected insects were used to evaluate gene-silencing efficacy (Livak & Schmittgen, 2001).

Haemoglobin quantification.

The anterior midguts were collected immediately after the blood meal (between 0.5–2 h), 2, 4, 7 and 14 days post-blood meal (PBM) and homogenized in 250 μL of PBS (10 mmol/L

Na–phosphate, 0.15 mol/L NaCl, pH 7.4) in a 1.5 mL plastic tube. The volume was adjusted to 1 mL with PBS. Haemoglobin content was assayed with a colorimetric kit (K023 kit, Bioclin, Brazil). Two μL of each sample were mixed with 198 μL of reagent for measurements. The standard curve was performed with the provided haemoglobin standard (15 mg/mL). Then, 5, 4, 3, 2, and 1 μL of the standard were mixed with 195, 196, 197, 198 and 199 μL of reagent, respectively. The absorbance of the supernatants was read at 540 nm with a plate spectrophotometer (Spectramax M3, Molecular Devices, USA).

Protein content measurement in ovaries.

The ovaries from the same females dissected for haemoglobin quantification were collected in 300 μL of PBS and macerated in a 1.5 mL plastic tube. The volume was adjusted to 1 mL with PBS. A total of 50 μL were used for protein quantification by the Bradford method, according to Bradford (1976). Bovine serum albumin (BSA) was used as a standard for protein quantification.

Oviposition and eclosion

Fully engorged females were individually separated into vials and kept at 28°C and 50%–60% relative humidity under a photoperiod of 12 h of light/12 h of dark. The number of eggs laid by each female was counted daily. The eclosion ratios were calculated by dividing the number of hatched first instar nymphs by the number of eggs laid by each female.

Survival and ecdysis measurements

Insect survival and/or ecdysis occurrence was scored daily post-blood meal (PBM).

Statistical analysis

At least two independent replicates were performed for each experiment. Each replicate included N = 7–20 insects per experimental group. The data from different replicates were combined into a single graph for the design of the figures. The statistics were performed on both, each independent experimental replicate and the final data containing the information from the different replicates combined. The *P*-values indicated in the text are from the data of the independent experiments combined. Statistical analysis and graph designs were performed using Prism 8.0 software (GraphPad Software). Survival was evaluated by Kaplan-Meier curves and log-rank (Mantel-Cox) test. Statistical differences between the experimental and control groups in oviposition, egg hatching and qPCR were evaluated by unpaired *t*-test. Time course differences between groups in haemoglobin and protein content, and in ecdysis were assessed by two-way ANOVA.

Results

A high reduction of eIF3m mRNA levels was achieved in nymphs (89.94%. Unpaired *t*-test. $P = 0.033$) and adult insects injected with dsRNA (Fig. S1A–C. Female: 96.83%. Unpaired *t*-test. $P = 0.1032$. Male: 93.68%, Unpaired *t*-test. $P = 0.0355$). Even though similar volumes of blood were ingested during feeding (Fig. 1A. Time 0 PBM. two-way ANOVA. $P = 0.8463$), the amount of haemoglobin in the anterior midgut was higher in eIF3m-silenced insects by day 7 PBM (two-way ANOVA. $P < 0.0001$.), indicating that eIF3m knockdown reduced the blood meal digestion rate. By day 14 PBM, control females had already digested all the blood meal, while eIF3m-silenced insects still possessed ~30% of the ingested haemoglobin (Fig. 1A). Coincident with the reduction in the rate of blood meal digestion, the blockage of ovary development was observed. The protein content of the ovaries was

drastically reduced on days 7 and 14 PBM (two-way ANOVA. $P < 0.01$) compared with controls, while no differences were observed on days 2 PBM (before oviposition began) or 21 PBM (after oviposition finished) (Fig. 1B). As a result, the oviposition was drastically reduced (Fig. 2A–B. Two-way ANOVA. $p < 0.0001$). Besides, the few eggs that were laid presented malformations (Fig. S2), and no nymph hatched from them (Fig. 2C–D. Two-way ANOVA. $P < 0.0001$). Therefore, reproduction was completely blocked. As a consequence, eIF3m-silenced females were more resistant to starvation and survived longer than controls after a blood meal (Fig 2E–F). Control female survival was 46 ± 2.6 (mean ± SEM) days PBM, while eIF3m-silenced females lived for 70.97 ± 2.7 days (log-rank test. $p < 0.0001$). In contrast, a similar survival period was observed in males upon eIF3m silencing. Both the control (69.82 ± 4.26 days PBM) and eIF3m-silenced males (68.38 ± 4.91 days PBM) survived for a similar period than eIF3m-silenced females (Fig 2E–F. Log-rank test. $P = 0.8037$). Different from control insects, the eIF3m-silenced nymphs did not moult to the next instar (two-way ANOVA. $p < 0.0001$) and died 3–4 weeks after the blood meal.

Discussion

The different feeding habits can shape the function of genes; the loss of function could have diverse, sometimes opposite, effects in animals with different diets. For example, the inhibition of the tyrosine catabolism pathway extends the lifespan of *D. melanogaster* (Parkhitko *et al.*, 2020) and *C. elegans* (Lee *et al.*, 2003; Ferguson *et al.*, 2013) but causes the death of hematophagous arthropods after a blood meal due to tyrosine accumulation and precipitation (Sterkel *et al.*, 2016, 2021; Sterkel & Oliveira, 2017; Ramirez *et al.*, 2021). Here we demonstrated that the silencing of the eIF3m, different from the phenotypes described in *D. melanogaster* that were only mild and tissue-specific (D'Brot *et al.*, 2013; Kang *et al.*,

2017), drastically affected *R. prolixus* physiology, causing a delay in blood meal digestion that affected moulting, reproduction and survival.

The knockdown of eIF3m prevented ecdysis. The nymphs did not moult even though they survived longer than the expected moulting period. This phenotype is different from the characteristic phenotype of interrupted ecdysis observed upon the knockdown of the neuropeptides *crustacean cardioactive peptide* (Lee *et al.*, 2013), *orcokinin A* (Wulff *et al.*, 2017) and *ecdysis triggering hormone* (Sterkel *et al.*, 2022). The nymphs that presented reduced levels of these neuropeptides underwent moulting but died shortly after due to failure in the ecdysis process, while eIF3m-silenced insects did not undergo moulting and died 3–4 weeks after feeding, probably because of starvation since they were unable to digest the blood meal correctly.

The phenotypes observed in females upon eIF3m silencing are similar to those reported in non-hematophagous organisms upon dietary restriction (DR). DR extends lifespan and reduces the reproductive fitness of females in many animal species (Moatt *et al.*, 2020; Green *et al.*, 2022), except for *Musca domestica* (Cooper *et al.*, 2004). Lifespan extension by DR has been proposed to be due to a shift in resources from growth and reproduction towards somatic maintenance allowing the animals to survive nutrient-poor environments until they find favourable conditions to reproduce (Moore & Attisano, 2011; Attisano *et al.*, 2012). Low levels of methionine and its derivate metabolite S-adenosyl-methionine (SAM) were reported to be involved in the phenotypes observed upon DR (Obata *et al.*, 2018; Green *et al.*, 2022). The depletion of dietary methionine (or inhibition of SAM synthesis) reduces the division rate of intestinal stem cells. Homeostatic epithelial turnover is suppressed when SAM is lost (Obata *et al.*, 2018). EIF3m knockdown may emulate DR by reducing the digestion rate and limiting nutrient availability.

TOR (target of rapamycin) kinase is a nutrient and amino acid sensor that plays a central role in mediating the lifespan-extending effect of DR (Papadopoli *et al.*, 2019). Inhibiting the TOR pathway extends lifespan and suppresses protein translation, stimulates autophagy, and promotes metabolic health (Papadopoli *et al.*, 2019). Besides, a protein sensor for SAM, named SAMTOR, controls TOR complex 1 (TORC1) activation (Gu *et al.*, 2017). In *R. prolixus*, amino acids from blood digestion trigger the downregulation of superoxide via the TORC pathway in the midgut (Gandara *et al.*, 2016). In *D. melanogaster*, tyrosine is a nutritional cue in the fat body that is necessary and sufficient for promoting adaptive responses to a low-protein diet, which entails the reduction of protein synthesis and mTORC1 activity and increased food intake (Kosakamoto *et al.*, 2022). Therefore, a plausible hypothesis is that by reducing the digestion rate, eIF3m silencing might affect TOR complex signalling.

Sex differences in terms of lifespan and the costs of reproduction remain poorly understood in animals. In *D. melanogaster*, the magnitude of the response to DR and the food concentration that minimized adult mortality differed significantly between the sexes (Magwere *et al.*, 2004). Female flies subject to DR lived up to 60% longer than fully-fed females, whereas males lived only up to 30% longer (Magwere *et al.*, 2004). In *R. prolixus*, we did not observe differences in survival between eIF3m-silenced and control males. This fact also reinforces the hypothesis that the extended survival observed in eIF3m-silenced females was due to resource reallocation from reproduction to somatic maintenance, making them as resistant as males to starvation.

Digestion of a blood meal represents a challenge for intestine homeostasis due to the release of high and potentially toxic amounts of heme, iron, amino acids and a dramatic increase in the microbiota (Eichler & Schaub, 2002; Sterkel *et al.*, 2017). The damaged or senescent enterocytes must be continually replaced by new cells to maintain epithelial

integrity. Since in *D. melanogaster* eIF3m is a pro-apoptotic factor (Chew *et al.*, 2009), eIF3m knockdown may prevent enterocyte apoptosis and the cellular turnover of the intestine, affecting its functions (such as the digestion of the blood meal). Further experiments are required to test these hypotheses.

Many long-lived mutants with reduced reproduction were described (Shmookler Reis *et al.*, 2009). Reproduction tends to shorten lifespan in most organisms however, in some cases, the trade-off between reproduction and lifespan can be decoupled (Flatt, 2011). In *D. melanogaster*, mutations in the insulin receptor gene extend lifespan, but females are infertile with non-vitellogenic ovaries (Tatar *et al.*, 2001). In *R. prolixus*, insulin receptor deficiency improved longevity and reduced triacylglycerol storage in the fat body, whereas blood digestion remained unaffected for seven days after a blood meal. The females exhibited smaller ovaries and a marked reduction in oviposition (Silva-Oliveira *et al.*, 2021). The knockdown of *NADPH oxidases 5* and *Xanthine dehydrogenase* also prevents blood digestion and impairs egg production, but different from eIF3m knockdown, their silencing induces early mortality in *R. prolixus* (Gandara *et al.*, 2021). Similarly, the knockdown of *acetyl-CoA carboxylase* in females delays blood digestion and shortens lifespan. Their fat bodies show reduced *de novo* lipogenesis activity, do not accumulate triacylglycerol during the days after a blood meal, and have smaller lipid droplets. The females laid 60% fewer eggs and only 7% hatched (Moraes *et al.*, 2022). Besides, blood digestion relies on the massive synthesis of proteins, such as digestive enzymes and perimicrovillar membrane proteins. Since eIF3m is involved in translation, it is necessary to investigate how eIF3m silencing affects gene expression. For this, transcriptomic and quantitative proteomics experiments must be performed.

Similar to the phenotypes we observed in *R. prolixus* upon eIF3m knockdown, in *C. elegans* the knockdown of *eukaryotic translation initiation factor 4 gamma 3* (*eIF4G3*) and

S6 kinase, which resulted in the differential translation of genes, slows development, reduces fecundity and increases resistance to starvation and lifespan (Pan *et al.*, 2007). Altogether, these findings indicate a pleiotropic role of mRNA translation in modulating growth, reproduction, lifespan and the ageing process in different animals. Further studies are required to address the molecular mechanisms underlying the phenotypes observed upon eIF3m silencing in *R. prolixus*. Besides, the function of this gene must be studied in other hematophagous arthropods to evaluate if its physiological importance is conserved among blood feeders. A deeper understanding of the molecular mechanisms underlying the phenotypes observed upon eIF3m silencing may facilitate the identification of new targets for vector control.

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Disclosure

The authors declare no competing interests.

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Author contributions

P.A. performed the experiments and analysed the data. N.C. performed the experiments and contributed critical discussion of the experiments and the data. S.O. designed the experiments and wrote the paper. P. L. O. designed the experiments and wrote the manuscript. M. S. designed and performed the experiments, analysed the data and wrote the manuscript. All of the authors discussed the results and read and contributed to the final version of the manuscript.

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Supporting information

Fig. S1 Efficiency of elF3m gene silencing in *R. prolixus* assessed by quantitative real time PCR.

Fig. S2 Phenotype of the eggs laid by elF3m-silenced *R. prolixus* females.

Figure legends

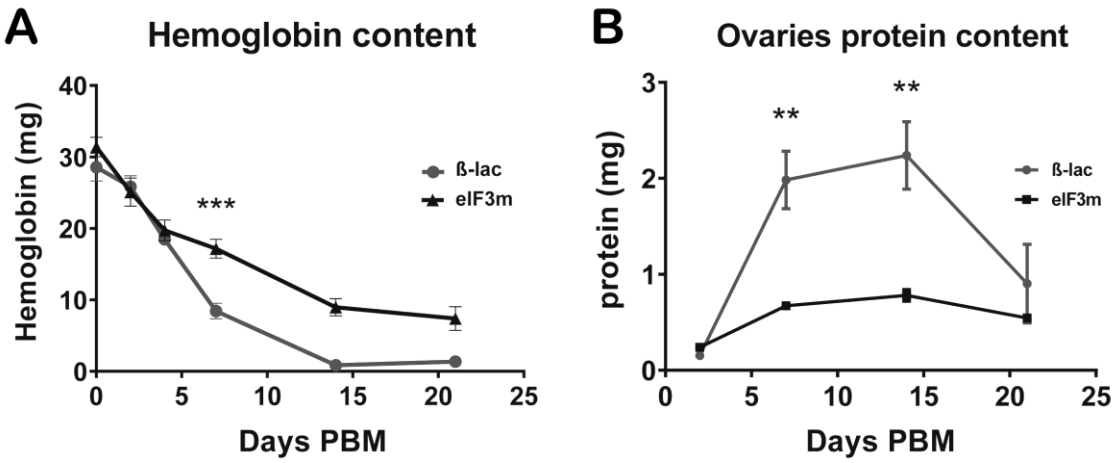

Fig. 1 Silencing of eIF3m reduces digestion rate and prevents ovaries development. (A) Amount of haemoglobin in the anterior midgut. (B) Quantity of protein in the ovaries. Two-way ANOVA was performed on the different time points to evaluate the difference between the control and dseIF3m injected group. $^{**}P < 0.01$, $^{***}P < 0.001$. Two independent experiments were carried out, each with $N = 3–5$ insects per experimental group at each time point. Data from both experiments were combined into a single graph. Data are plotted as mean ± SEM.

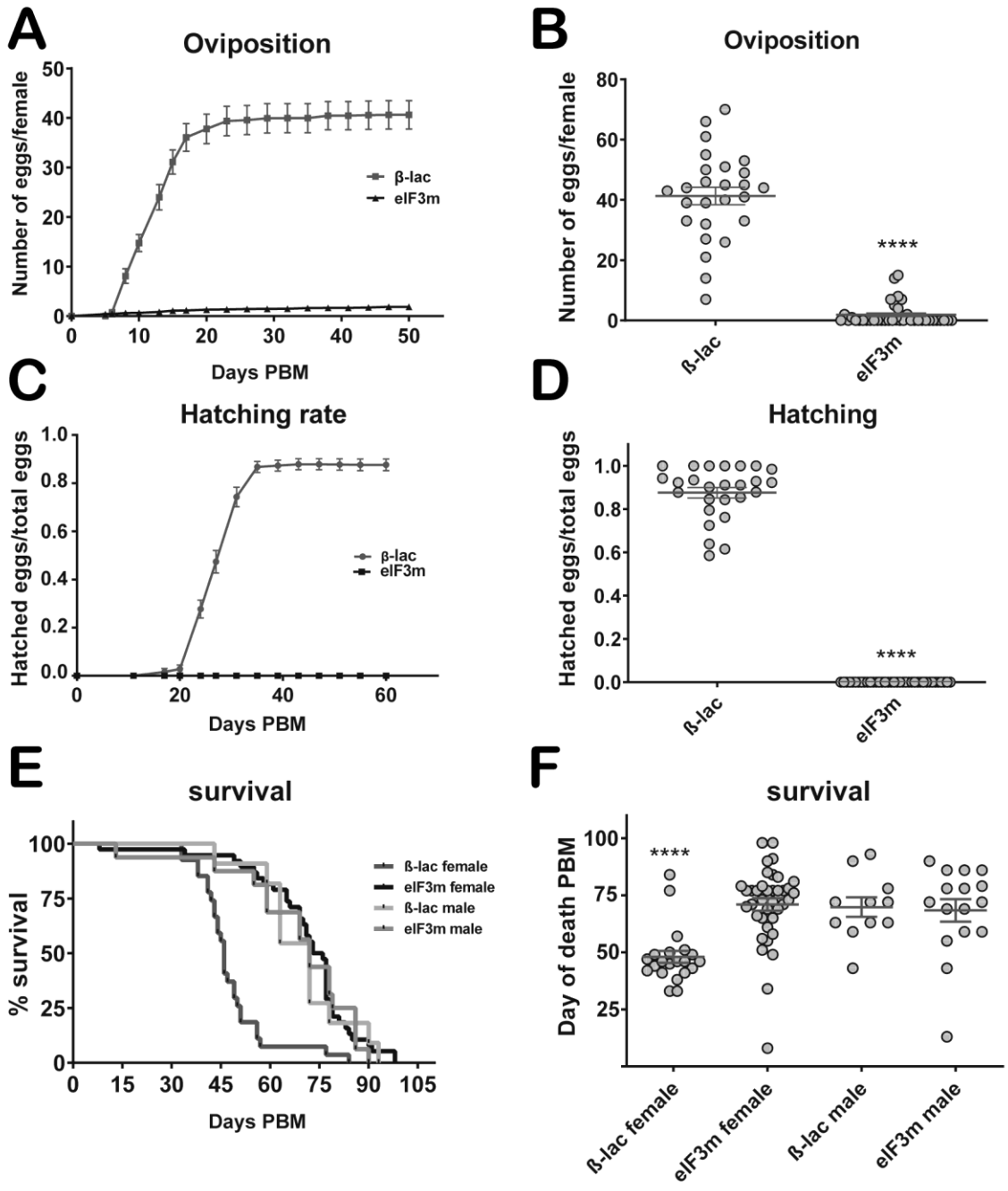

Fig. 2 eIF3m silencing impairs reproduction and increases resistance to starvation in females. (A–B) Oviposition. (C–D) The hatching of eggs. An unpaired *t*-test was performed. ****$P <$ 0.0001. Data are plotted as mean ± SEM. (E–F) Female and male survival. Data are plotted as Kaplan-Meier curves (E) and as the death day post-blood meal (F). Control females presented a reduced survival compared to the other groups (Log-rank (Mantel-Cox) test. $P <$

0.0001). Four independent experiments were carried out with $N = 5–12$ insects per experimental group. Data from independent experiments were combined into a single graph.

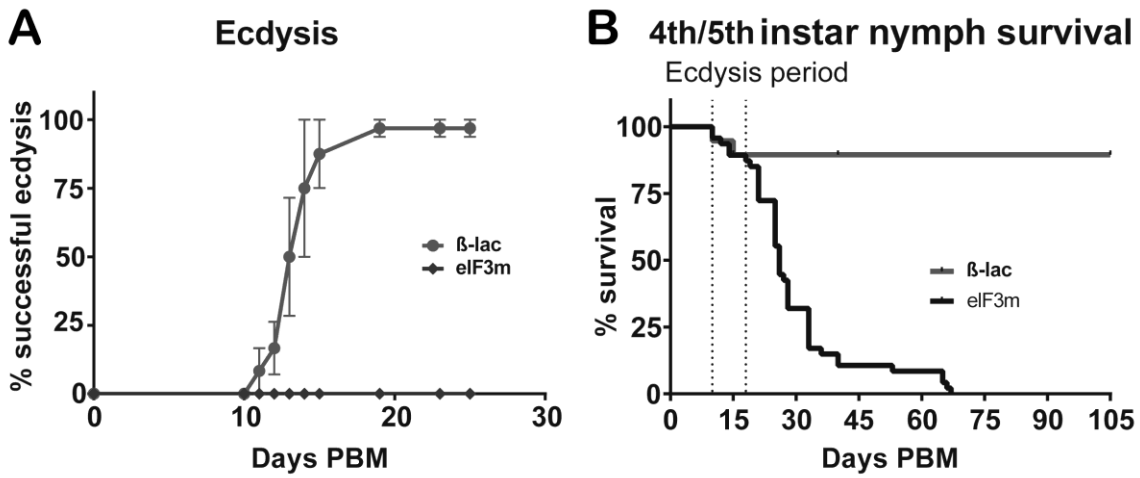

Fig. 3 eIF3m silencing prevented ecdysis and reduced nymphs' survival. (A) Ecdysis. Two ways ANOVA was performed ($P < 0.0001$). Data are plotted as mean ± SEM. (B) Survival. Data are plotted as Kaplan-Meier curves [Log-rank (Mantel-Cox) test. $P < 0.0001$]. Three independent experiments were carried out, each with $N = 8–15$ insects per experimental group. Data were combined into a single graph.