Parasitology Research (2019) 118:2843–2855
https://doi.org/10.1007/s00436-019-06421-8

GENETICS, EVOLUTION, AND PHYLOGENY - ORIGINAL PAPER

Functional characterization of the translation initiation factor eIF4E of *Echinococcus granulosus*

Filipe Santos Pereira-Dutra [1,2] • **Martin Cancela** [1,2] • **Bruna Valandro Meneghetti** [1,2] • **Henrique Bunselmeyer Ferreira** [1,2,3] • **Karina Mariante Monteiro** [1,2,3] • **Arnaldo Zaha** [1,2,3]

Received: 28 January 2019 / Accepted: 2 August 2019 / Published online: 10 August 2019

Abstract

The eukaryotic initiation factor 4E (eIF4E) specifically recognizes the 5′ mRNA cap, a rate-limiting step in the translation initiation process. Although the 7-methylguanosine cap (MMGcap) is the most common 5′ cap structure in eukaryotes, the trans-splicing process that occurs in several organism groups, including nematodes and flatworms, leads to the addition of a trimethylguanosine cap (TMGcap) to some RNA transcripts. In some helminths, eIF4E can have a dual capacity to bind both MMGcap and TMGcap. In the present work, we evaluated the distribution of eIF4E protein sequences in platyhelminths and we showed that only one gene coding for eIF4E is present in most parasitic flatworms. Based on this result, we cloned the *Echinococcus granulosus* cDNA sequence encoding eIF4E in *Escherichia coli*, expressed the recombinant eIF4E as a fusion protein to GST, and tested its ability to capture mRNAs through the 5′ cap using pull-down assay and qPCR. Our results indicate that the recombinant eIF4E was able to bind preferentially 5′-capped mRNAs compared with rRNAs from total RNA preparations of *E. granulosus*. By qPCR, we observed an enrichment in MMG-capped mRNA compared with TMG-capped mRNAs among Eg-eIF4E-GST pull-down RNAs. Eg-eIF4E structural model using the *Schistosoma mansoni* eIF4E as template showed to be well preserved with only a few differences between chemically similar amino acid residues at the binding sites. These data showed that *E. granulosus* eIF4E can be used as a potential tool to study full-length 5′-capped mRNA, besides being a potential drug target against parasitic flatworms.

Keywords Capped RNAs · mRNA purification · *Echinococcus granulosus* · eIF4E

Introduction

Hydatidosis or cystic echinococcosis caused by parasitic flatworms of the genus *Echinococcus* affects humans and animals all over the world (WHO 2013). The metacestode or hydatid cyst (HC) is the larval stage of the parasite and is located mainly in liver and lungs of the intermediate host, including cattle and sheep. Once within its host, HC can grow and produce the pre-adult form, the protoscolex (PSC), the infective stage of definitive canine host. Molecular aspects associated with parasite gene expression and regulation during development and establishment within its hosts are relevant in order to develop rational strategies for parasitic control.

Translation initiation in eukaryotes involves several proteins associated in a large protein-RNA complex that directs the ribosome to the initiation codon. The key and rate-limiting step of this process involves the participation of the eukaryotic

---

Filipe Santos Pereira-Dutra and Martin Cancela contributed equally to this work.

Section Editor: Christoph G. Grevelding

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00436-019-06421-8) contains supplementary material, which is available to authorized users.

✉ Arnaldo Zaha
zaha@cbiot.ufrgs.br

[1] Laboratório de Biologia Molecular de Cestodeos, Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, UFRGS, Avenida Bento Gonçalves, 9500, Caixa Postal 15053, Porto Alegre, RS CEP 91501-970, Brazil

[2] Programa de Pós-Graduação em Biologia Celular e Molecular, Centro de Biotecnologia, UFRGS, Porto Alegre, Brazil

[3] Departamento de Biologia Molecular e Biotecnologia, Instituto de Biociências, UFRGS, Porto Alegre, Brazil

translation initiation factor 4E (eIF4E), a cap-binding protein of about 25 kDa (Gingras et al. 1999; Prévôt et al. 2003; Goodfellow and Roberts 2008). For most eukaryotes, especially for vertebrates, the canonical cap-dependent translation begins with the specific recognition of the mRNA 5′-terminal monomethylguanosine cap (MMGcap) by eIF4E (von der Haar et al. 2004; Rutkowska-Wlodarczyk et al. 2008). In spite of the fact that the MMGcap is the most common 5′ cap structure in eukaryotes (Rutkowska-Wlodarczyk et al. 2008), a hypermethylated cap, the trimethylguanosine cap (TMGcap) (Davis 1996; Blaxter and Liu 1996), can be added to mRNAs by a trans-splicing process, present for example in several parasitic and non-parasitic nematodes and in the phylum *Platyhelminthes* (Davis 1996; Blaxter and Liu 1996; Brehm et al. 2000; Hastings 2005; Bitar et al. 2013). In these organisms, generally both cis- and trans-splicing processes occur simultaneously and the transcripts from both origins coexist (Davis 1996; Blaxter and Liu 1996; Hastings 2005). Trans-splicing differs from cis-splicing on the origin of the transcripts involved in the process. While cis-splicing is concerned with exons from a single transcript, trans-splicing can be subdivided into two groups, one involving the junction of exons from different pre-mRNA transcripts, and another that involves a specialized spliced leader RNA which donates a short exon to 5′ UTR of another RNA (Lasda and Blumenthal 2011; Bitar et al. 2013).

The vertebrate eIF4E has very low affinity for TMGcap in comparison with the MMGcap (Niedzwiecka et al. 2004). In contrast, some nematode eIF4E isoforms, and the eIF4E from the flatworm *Schistosoma mansoni* can recognize both the TMGcap and MMGcap (Liu et al. 2011). In the case of *S. mansoni* and *Schistosoma japonicum*, another peculiarity is the presence of only a single eIF4E isoform, atypical for metazoan (Liu et al. 2009). This characteristic could be explored to develop novel anthelminthic drugs (Liu et al. 2009). The 5′-cap-binding proteins, including human eIF4E, and 7-methylguanosine antibodies are being used as tools to deplete rRNA from total RNA samples in order to improve yield in RNA-seq experiments (Blower et al. 2013) and to enrich samples in full-length transcripts (Bajak and Hagedorn 2008; Papic et al. 2012). The development of a complementary and sensitive mRNA purification methodology for parasitic flatworms may be a key tool for elucidating important mechanisms associated with flatworm development and its pathogenicity. Based on the single ability of helminth eIF4E to interact with both MMGcap and TMGcap, we evaluated in this work the potential of the eIF4E protein *E. granulosus* as a tool for enrichment of mRNA samples from total parasite RNA. Based on the above, in the present work, we analyzed the presence and distribution of eIF4E genes in *E. granulosus* and other species of the phylum *Platyhelminthes.* Furthermore, we cloned the *E. granulosus* eIF4E (Eg-eIF4E) coding sequence, expressed and purified the recombinant Eg-eIF4E as fusion protein to GST, and tested its potential binding properties using total RNA from *E. granulosus* protoescoleces.

Material and methods

Sequence analyses

eIF4E-like protein sequences were surveyed in the available sequence databases Wormbase ParaSite (http://parasite.wormbase.org/), Planmine (http://planmine.mpi-cbg.de/planmine/begin.do), and GenBank (http://www.ncbi.nlm.nih.gov/) for 25 flatworms (*Clonorchis sinensis*, *Dugesia japonica*, *Echinococcus canadensis*, *Echinococcus granulosus*, *Echinococcus multilocularis*, *Gyrodactylus salaris*, *Hydatigera taeniaeformis*, *Hymenolepis diminuta*, *Hymenolepis microstoma*, *Hymenolepis nana*, *Macrostomum lignano*, *Mesocestoides corti*, *Opisthorchis viverrini*, *Protopolystoma xenopodis*, *Schistocephalus solidus*, *Schistosoma curassoni*, *Schistosoma japonicum*, *Schistosoma mansoni*, *Schistossoma mattheei*, *Schistosoma margrebowiei*, *Schistosoma rodhaini*, *Schmidtea mediterranea*, *Taenia solium*, *Taenia asiatica*, and *Trichobilharzia regenti*). To identify eIF4E-encoding genes, we performed in silico analyses in tblastN using the previously identified eIF4E protein sequences from *S. mansoni* (G4VCF5) and *E. granulosus* (U6IZR7) as queries. Sequences from the representative species within the flatworm classes Cestoda, Trematoda, Monogenea, and Turbellaria (*E. granulosus*, *S. mansoni*, *P. xenopodis*, and *D. japonica*, respectively) were used as queries in subsequent blast searches to improve representation. This iterative process was repeated until no novel eIF4E family members were found (Jones et al. 2015). We selected only the search results that consistently returned pairwise alignments of > 120 aligned amino acids. As an external group, sequences of *Saccharomyces cerevisiae* (P07260), *Ascaris suum* (Q6PKX2), *Mus musculus* (P63073), *Caenorhabditis elegans* eIF4E-1 (O45551), eIF4E-2 (E3MFQ1), and eIF4E-5 (P56570) were retrieved from Universal Protein resource, UniProt (http://www.uniprot.org).

For phylogenetic analysis, we selected only those sequences with 98% for maximum similarity and 30% for minimum similarity. Sequence alignment was performed using MAFFT7 (Katoh and Standley 2013) and MEGA 7.0 (Tamura et al. 2013). To reconstruct the phylogenetic tree, we performed the maximum likelihood method using PhyML v3.0 (Guindon et al. 2010) and bootstrap (1000 replicates) methods for branch support, and the tree was visualized and edited using Evolview (http://www.evolgenius.info).

Parasite material

E. granulosus hydatid cysts were obtained from the lungs and livers of naturally infected bovines donated by the abattoir for use in this work. Protoscoleces (PSC) were collected by aspiration of cyst content and then washed three times in sterile phosphate-buffered saline pH 7.4 (PBS). PSC viability was assessed by trypan blue exclusion and only batches with > 90% of viability were used for further analysis. Parasites were genotyped using Cox-1 gene as previously described (Santos et al. 2013).

Total RNA extraction and cDNA synthesis

Total RNA was extracted from 50 μL of *E. granulosus* PSC (approximately 5000 individuals) using the Trizol reagent (ThermoFisher, USA) following manufacturer's protocol. Extracted RNA was treated with DNase I (Thermo Fisher Scientific, USA) for 30 min at 37 °C to remove DNA contamination. The concentration of total RNA was determined using Qubit Quantitation Fluorometer and Quant-iT reagents (Thermo Fisher Scientific, USA). The first strand of cDNA was synthesized from 200 ng of total RNA using RevertAid reverse transcriptase (Thermo Fisher Scientific, USA) and Random Hexamer primes (Thermo Fisher Scientific, EUA, USA) (0.5 μg/μL) as the anchor primer. The reaction mixture was incubated at 45 °C for 1 h followed by 70 °C for 10 min to terminate the reaction. The final cDNA product was diluted 100-fold with nuclease-free water prior to use in qPCR analysis.

Molecular cloning, recombinant protein expression, and purification

Full-length cDNA sequence encoding *E. granulosus* eIF4E was amplified by PCR using gene-specific primers (forward 5′-ATGGCTGTAGTGGAGAGGG-3′ and reverse 5′-AGCACAGGGACAGAGGAAT-3′) and cloned into pGEX-TEV vector by in vivo recombination according to Lorenzatto et al. (2012), with some modifications. The recombinant protein fused to glutathione S-transferase (*Eg*-eIF4E-GST) was expressed using *Escherichia coli* strain BL21-CodonPlus-RIL (Stratagene, USA). The expression of the recombinant protein was induced with isopropyl β-d-1-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM (1 L of culture) at 25 °C for 3 h. After induction, the cells were harvested and lysed, and GST fusion protein was recovered from the soluble fraction by affinity chromatography in Glutathione Sepharose 4B (GE Healthcare, UK). The purified protein was analyzed by electrophoresis in 12% SDS-PAGE and protein concentration was measured using a Qubit quantitation fluorometer and Quant-iT reagents (Thermo Fisher Scientific, USA), and the yield was 12.8 mg of protein per liter of culture. The identity of the purified recombinant protein was confirmed by liquid chromatography–tandem mass spectrometry (LC-MS/MS), using a Waters nanoACQUITY UPLC system coupled to a Q-Tof Premier API mass spectrometer (MicroMass/Waters, EUA).

5′-capped mRNA capture using Eg-eIF4E-GST and cDNA synthesis

The fusion protein *Eg*-eIF4E-GST was used to purify mRNAs from total *E. granulosus* RNA according to Bajak and Hagedorn (2008), with some modifications. Purified *Eg*-eIF4E-GST (2 mg) was mixed with Glutathione Sepharose beads 4B (GE Healthcare) (1-mL packed volume) in PBS for 1 h at 4 °C and then washed three times with 3 mL of PBS buffer pH 7.4. Glutathione Sepharose-coupled *Eg*-eIF4E-GST beads (100 μL) were mixed with 10 μg of heat-denatured total RNA (70 °C for 10 min) in 500 μL of binding buffer (10 mM $KHPO_4$ pH 8.0, 100 mM KCl, 2 mM EDTA, 5% glycerol, 6 mM DTT, 1.3% polyvinyl alcohol (Sigma-Aldrich, USA), 0.005% Triton X-100, 20 units RNasin (Promega, EUA)) and incubated on ice for 1 h. The resin was washed five times with 1 mL of binding buffer. cDNA was synthesized directly from mRNA bound to *Eg*-eIF4E-GST beads using RevertAid reverse transcriptase (Thermo Fisher Scientific, USA) and Randon Hexamer primes (Life technology) (0.5 μg/μL) as the anchor primer. The reaction mixture was incubated at 45 °C for 1 h followed by 70 °C for 10 min to terminate the reaction. To recover cDNA bound to the resin, we added water to a final volume of 500 μL and boiled the solution for 5 min at 95 °C. Sample was then centrifuged 1 min at 10000×*g* to separate resin from cDNA sample. This cDNA was store at − 20 °C until use.

Real-time qPCR

For quantitative expression analysis, we selected five genes previously described as housekeeping for *Echinococcus* (TPx, TRx, EF-a, GADPH, and CYP) (Espínola et al. 2014). The 18S ribosomal gene was chosen to evaluate rRNA levels in the different samples. Moreover, we selected two genes previously found in a splice leader PSC cDNA library (SL1 and SL2) (derived from trans-splicing process) and two genes exclusive of GR library (GR1 and GR2) (derived from depletion of trans-spliced transcripts) (Parkinson et al. 2012). Gene sequences obtained from different databases (LophDB, Wormbase parasite, and GenBank) were used as templates to design gene-specific primers using Primer3 version 0.4.0 software. For primer design, the following characteristics were considered: an amplification product between 80 and 200 bp and annealing temperature of 60 ± 1 °C (Table 1).

For PCR reaction, 10 μL of diluted template cDNA was mixed with 0.1× SYBR Green I (Invitrogen), 0.1 mM of each primer, 1× PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 3 mM $MgCl_2$, 25 mM of each dNTP, 0.25 U of Platinum Taq DNA polymerase (Life Technologies, USA) in a final volume of 20 µL. All qPCR reactions were performed in technical duplicates. qPCR reactions were performed using a 7500 Fast Real-Time PCR system (Applied Biosystems) with the following settings: an initial activation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 60 °C for 10 s, and extension at 72 °C for 35 s. The specific amplification of each gene was analyzed by means of denaturation curves in SDS software. Denaturation was performed by increasing the temperature from 50 to 94 °C, higher by 0.1 °C/s. To determine the PCR efficiency for each set of primers, LinRegPCR software was used (Ruijter et al. 2009) (Table 1).

In order to quantify depletion of rRNA in samples after *Eg*-eIF4E-GST incubation, we analyzed the expression stability of the candidate reference genes in total RNA and *Eg*-eIF4E-treated RNA using geNorm algorithm (Vandesompele et al. 2002). Most stable reference genes were then used to perform a relative quantification analysis by the $\Delta\Delta$CT method (Livak and Schmittgen 2001).

Molecular modeling and docking of *Eg*-eIF4E

Tridimensional molecular model of *Eg*-eIF4E was built by modeling using the SWISS-MODEL (https://swissmodel.expasy.org/). The template used for modeling was the *S. mansoni* eIF4E (Sman-eIF4E) with 1.8-Å resolution (PDB code: 3HXI). The sequence alignment between *Eg*-eIF4E and Sman-eIF4E was done using PRALINE (http://www.ibi.vu.nl/programs/pralinewww/). The *Eg*-eIF4E model was evaluated with PROSA-web (https://prosa.services.came.sbg.ac.at/prosa.php), RAMPAGE (http://mordred.bioc.cam.ac.uk/ ~rapper/rampage2.php), TM-score (http://zhanglab.ccmb.med. umich.edu/TM-score/), QMEAN (https://swissmodel.expasy.org/qmean/), and SuperPose (http://wishart.biology.ualberta.ca/cgi-bin/SuperPose). The visualization and manipulation of the molecular images were performed with PyMOL version 1.3 (www.pymol.org).

Molecular docking was performed using the *Eg*-eIF4E model with MMGcap and TMGcap ligands, whose structures were extracted from *Ascaris suum* eIF4E bound to each ligand (PDB code: 3M93 and PDB code: 3M94, respectively). Also, a control docking with another eIF4E proteins was done for *Caenorhabditis elegans* (PDB code: 5ABY), *S. mansoni* (PDB code: 3HXI), and *Homo sapiens* (PDB code: 2V8W) to

Table 1 Gene-specific primers used in qPCR experiments

Gene symbol	Gene accession	Gene name	Primers (F: forward, R: reverse)	Ta (°C)	Amplicon size	Efficiency (%)
SL1	EgrG_000147050	Conserved Protein Expressed	F 5′-GGAGAAGGAGGAGGAGGAGA-3′ R 5′-GGTTGTGAAGGGAGATGGAA-3′	60	184 bp	90
SL2	EgrG_000513400	Intersectin 2	F 5′-GCATCATCTACCGCCTGAGT-3′ R 5′-CGACGAAAGGGCACATAAAT-3′	60	151 bp	92
GR1	EgrG_000183800	60S ribosomal protein L44:L36a	F 5′-CTCGGTCGTGTGTGCTTG-3′ R 5′-CCGTAGCCTGACTGCTTTCT-3′	60	179 bp	92
GR2	EgrG_000372400	Tegument protein	F 5′-GCAGGTGGTCATTCTCACAG-3′ R 5′-GGCACACAGATAGGCAGGTAG-3′	60	192 bp	92
18S	U27015.1	18S nuclear ribosomal gene	F 5′-CCTTTGGATGCCCTTGATT-3′ R 5′-CCCGCCTGTCTCTTTTGAT-3′	60	179 bp	93
TPx	EgrG_000791700	Thioredoxin peroxidase	F 5′-CGTACTGATCGAGGATCAAGG-3′ R 5′-ATGGTGATTTGACGCAGAACT-3′	60	81 bp	100
Trx	EgrG_000360300	Thioredoxin	F 5′-TCCTCGTTTGTGATTTCTTCG-3′ R 5′-ACTCGATACTTCTCCGCAACA-3′	60	148 bp	90
EF-a	EgrG_000982200	Elongation factor	F: 5′-TTGAGAAAGAGGCGGCTGAGATG-3′ R: 5′-TAATAAAGTCACGATGACCGGGCG-3′	60	174 bp	99
GAPDH	EgrG_000254600	Glyceraldehyde-3-phosphate dehydrogenase	F: 5′-ACTCCGTCAATGTTGTCGCTGTCA-3′ R: 5′-TAACCAACTTGCCGCCATCAACCT-3′	60	128 bp	93
CYP	EgrG_000920600	Cyclophilin	F: 5′-CGACATCTCCATTGGCGGTAAGC-3′ R: 5′-TTGTATCCGAAACCCTTCTCACCG-3′	60	120 bp	97

MMGcap and TMGcap ligands. The protonation state analysis was performed by PROPKA program. Docking studies were performed by Dockthor (https://dockthor.lncc.br/v2/) and SwissDock (http://www.swissdock.ch/). The best score of binding mode docking was shown for each MMGcap and TMGcap ligands. We estimated the binding affinity from predictive ΔG° values using the following equation: $\Delta G^\circ = RT\ \ln Kd$ (Yugandhar and Gromiha 2014; Guedes et al. 2018). Where ΔG° is the predicting Gibbs free energy, Kd is the dissociation constant, *R* is the universal

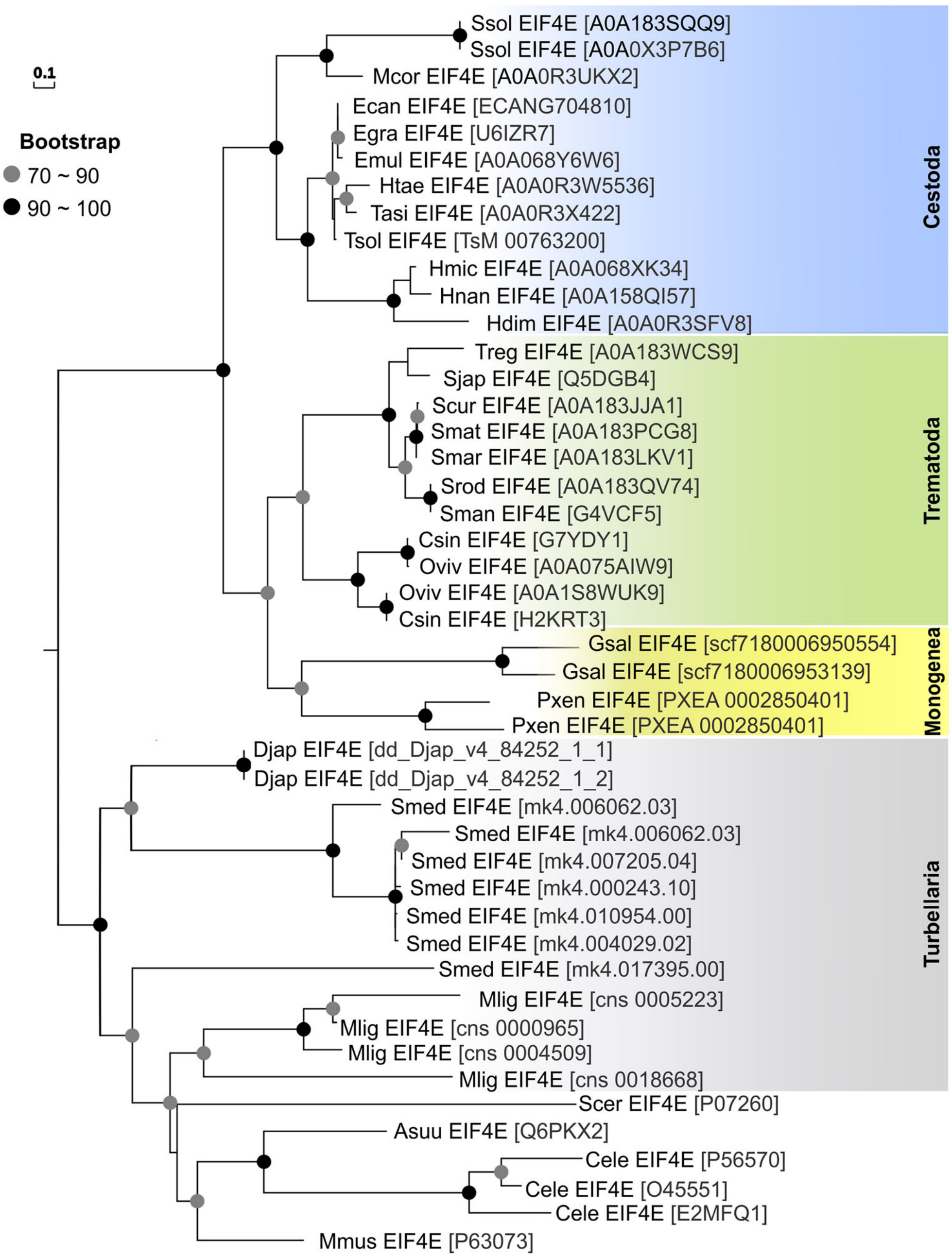


Fig. 1 Phylogenetic analysis of flatworms eIF4E. Phylogenetic tree was generated using the maximum likelihood method and visualized using Evolview. eIF4E protein sequences obtained from different sequence databases are described by their accession numbers (WormBase parasite, Uniprot). Species included are flatworms *Clonorchis sinensis* (*Csin*), *Dugesia japonica* (*Djap*), *Echinococcus canadensis* (*Ecan*), *Echinococcus granulosus* (*Egra*), *Echinococcus multilocularis* (*Emul*), *Gyrodactylus salaries* (*Gsal*), *Hydatigera taeniaeformis* (*Htae*), *Hymenolepis diminuta* (*Hdim*), *Hymenolepis microstoma* (*Hmic*), *Hymenolepis nana* (*Hnan*), *Macrostomum lignano* (*Mlig*), *Mesocestoides corti* (*Mcor*), *Opisthorchis viverrini* (*Oviv*), *Protopolystoma xenopodis* (*Pxen*), *Schistocephalus solidus* (*Ssol*), *Schistosoma curassoni* (*Scur*), *Schistosoma japonicum* (*Sjap*), *Schistosoma mansoni* (*Sman*), *Schistosoma mattheei* (*Smat*), *Schistosoma rodhaini* (*Srod*), *Schistosoma margrebowiei* (*Smar*), *Schmidtea mediterranea* (*Smed*), *Taenia solium* (*Tsol*), *Taenia asiatica* (*Tasi*), and *Trichobilharzia regenti* (*Treg*). As external group, the peptide sequences are from *Saccharomyces cerevisiae* (*Scer*), *Mus musculus* (*Mmus*), *Caenorhabditis elegans* (*Cele*), and *Ascaris suum* (*Asuu*)

gas constant ($1.987\ 10^{-3}$ kcal mol^{-1} K^{-1}), and T is the absolute temperature (assumed to be room temperature, 298.15 K).

Results

Sequence identification and phylogenetic analysis of eIF4E in flatworms

By mining *E. granulosus* genome in the public databases, we found a single eIF4E-like coding sequence. This sequence had a CDS of 585 bp and encoded a 184 amino acid protein. This sequence was used as a query in subsequent blast searches to improve identification of other *E. granulosus* eIF4E-like proteins. However, no novel *E. granulosus* eIF4E family members were found. We also did not find any splicing variant of eIF4E, which indicates that *E. granulosus* would have only one isoform of this protein.

In order to analyze if this feature is exclusive of *E. granulosus*, we performed blast searches for eIF4E sequences in 25 flatworm genomes deposited in public databases, and we found differences in the number of eIF4E sequences between parasitic and free-living flatworms. Most parasitic flatworms had a single eIF4E sequence, with the exception of one cestode (*S. solidus*), two trematodes (*C. sinensis* and *O. viverini*), and two monogeneans (*P. xenopodis* and *G. salaris*) with two sequences encoding eIF4E-like proteins. In free-living flatworms, we identified four sequences for *M. lignano*, 7 for *S. mediterranea*, and two sequences for *D. japonica.*

The phylogenetic analysis of *E. granulosus* eIF4E amino acid sequence and the related sequences identified in platyhelminths is presented in Fig. 1. Sequences from *S. cerevisiae*, *A. suum*, *M. musculus*, and *C. elegans* were included as an external group. The parasitic flatworm eIF4E sequences were assigned to one clade. Parasitic eIF4E orthologues were subdivided into two subclades where the Cestoda sequences (55.2–99% sequence similarity) grouped separately from Trematoda and Monogenea sequences (55.6–98.5%). However, in our analysis, Turbellaria class did not cluster together with the parasitic flatworms, being grouped together with sequences from external group organisms *C. elegans*, *M. musculus*, and *S. cerevisae* sequences.

Purification of *E. granulosus* mRNAs by binding to eIF4E

The sequence encoding *E. granulosus* eIF4E was cloned and expressed in *E. coli* as GST fusion protein (Fig. 2). High yields of the pure recombinant protein (12.8 mg L^{-1} culture medium) were obtained, with the expected apparent molecular weight of 48 kDa (Fig. 2). Mass spectrometry analysis confirmed the identity of the eIF4E from *E. granulosus* (data not shown).

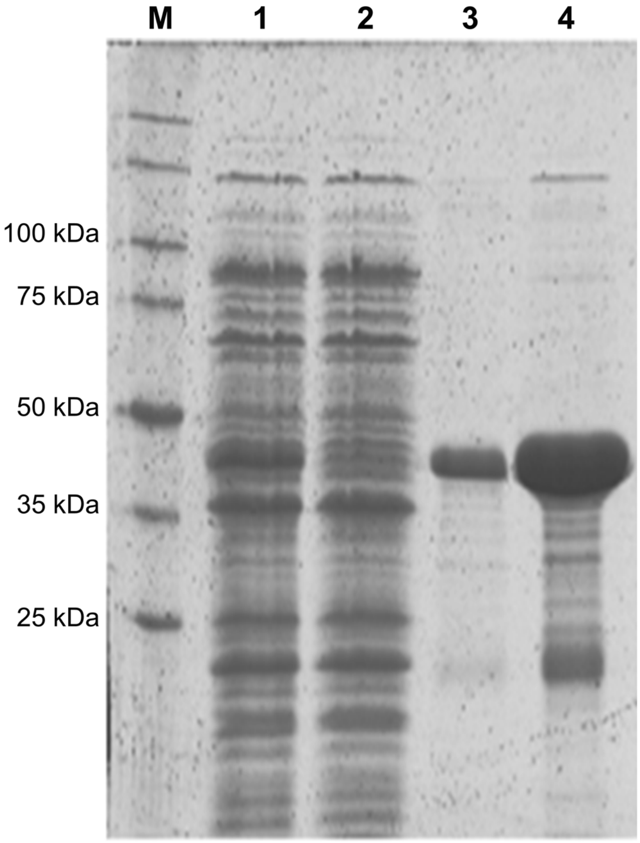


Fig. 2 Purification of recombinant protein *Eg*-eIF4E. GST fusion protein was purified as described in material and methods and analyzed in SDS-PAGE 12%. 1: Cell lysate soluble fraction; 2: column flow through; 3: sample of the resin after fusion protein binding; 4: eluate fraction. M: molecular weight marker

In order to assess the ability of recombinant *E. granulosus* eIF4E (*Eg*-eIF4E-GST) to recognize the 5′ cap in mRNAs and deplete ribosomal RNA from total RNA samples, we performed the pull-down experiment illustrated in Fig. 3A. Briefly, *Eg*-eIF4E-GST fusion protein was bound to Glutathione Sepharose beads and then incubated with total RNA extracted from *E. granulosus* protoescoleces. RNA-bound to eIF4E-sepharose was on-bead reverse transcribed to avoid losing of RNA during the elution process. By RT-qPCR, we first analyzed the level of 18S rRNA, a component of the small eukaryotic ribosomal subunit (40S), in cDNAs obtained from RNA before and after binding to eIF4E. We observed a marked CT difference for 18S rRNA, being 6–7 in RNA before (Fig. 3B) and 12–14 after incubation with *Eg*-eIF4E-GST (Fig. 3C).

To perform a quantitative analysis of rRNA (18S RNA), mRNAs (TPX, TRX, EF-A, CYP, GAPDH, GR1 and GR2), and trans-spliced mRNA (SL1 and SL2) levels in total RNA and *Eg*-eIF4E-bound RNA, we first selected the most suitable reference gene using the GeNorm algorithm. Regarding the average expression stability values (M), *GR1* and *GR2* were the most stable genes (Fig. 4A), and considering the value V,

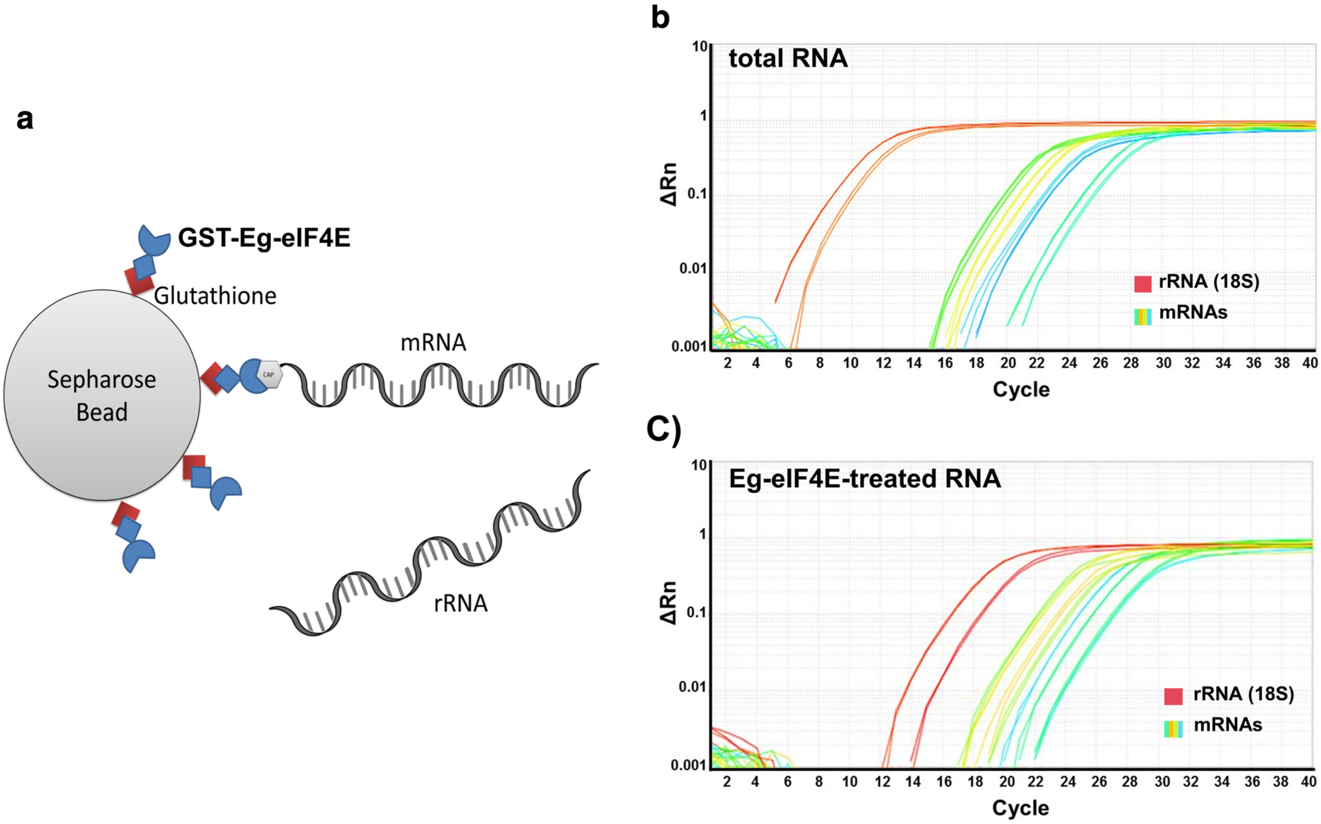


Fig. 3 qPCR analysis of total RNA and *Eg*-eIF4E-GST pull-down RNA to evaluate 18S RNA depletion. (A) Experimental design of *Eg*-eIF4E-GST pull-down and amplification plots of 18S rRNA and mRNAs before (B) and after (C) *Eg*-eIF4E-GST pull-down

V2/3 with a cut-off value < 0.15 (Fig. 4B), the optimal number of reference genes for normalization is 2. Using the ΔΔCT method for relative quantification and GR1 and GR2 as reference genes, we observed a strong depletion of 18S rRNA in eIF4E-treated samples confirming the efficiency of this method in removing rRNA from total RNA samples (Fig. 4C). We also observed a significant reduction of trans-spliced mRNA SL1 and SL2 in RNA-bound to *Eg*-eIF4E-GST (Fig. 4C). We estimated a 61-fold change (FC) depletion of 18S rRNA and 7 and 25-FC depletion for SL1 and SL2, respectively. Interestingly, we did not observe differences in TPX, EF-A, and TRX levels between total and *Eg*-eIF4E-bound RNA. These data indicate that there was no significant loss, neither enrichment of these mRNAs throughout the process relative to GR1 and GR2. However, for two transcripts (GAPDH and CYP), we observed a significant enrichment (near 1.5-fold change) between total and *Eg*-eIF4E-bound RNA. We also estimated mRNA enrichment in *Eg*-eIF4E-bound RNA sample calculating ΔCT between gene targets and 18S ribosomal RNA and comparing RNA sample before and after eiF4E treatment using the $2^{-\Delta\Delta CT}$ method. We observed an important enrichment in SL and capped mRNA in eiF4E-RNAs compared with 18S RNA amount obtained after eiF4E pull-down. 5′-capped mRNAs were more enriched (almost an increase of one order of magnitude) than SL mRNA transcripts (Table 2). In order to analyze eIF4E-specific binding to capped RNAs, we performed a pull-down experiment using GST as a negative control and compared with pull-down using eIF4E-GST. By using conventional PCR, we observed amplification of 18S RNA, TPX, and EF-a in cDNAs obtained after eIF4E-GST pull-down experiment and no amplification was detected in pull-down experiment using GST as negative control (data not shown), confirming eIF4E-capped RNA binding specificity.

Structural and docking analyses of Eg-eIF4E protein

We performed a protein modeling of *Eg*-eIF4E using as template the Sman-eIF4E which has 50% identity (Fig. 5A). Our structural model of *Eg*-eIF4E has eight beta-strands and three alpha-helices, forming a glove-shaped structure (Fig. 5C and Tables S1) similar to Sman-eIF4E (Liu et al. 2009) (Fig. 5B). The major amino acid residues and features involved in the cap binding are conserved between *Eg*-eIF4E and Sman-eIF4E (Fig. 5D). The *Eg*-eIF4E residues involved in the cap binding are Glu-81, Trp34, Trp80, Gln132, and Arg134 that correspond to Glu90, Trp43, Trp89, Gln141, and Arg143 in the *S. mansoni* protein, respectively. However, the residues Lys148, Arg132, and Arg188 in Sman-eIF4E were changed to Arg139, Lys183, and Lys179 in *Eg*-eIF4E respectively,

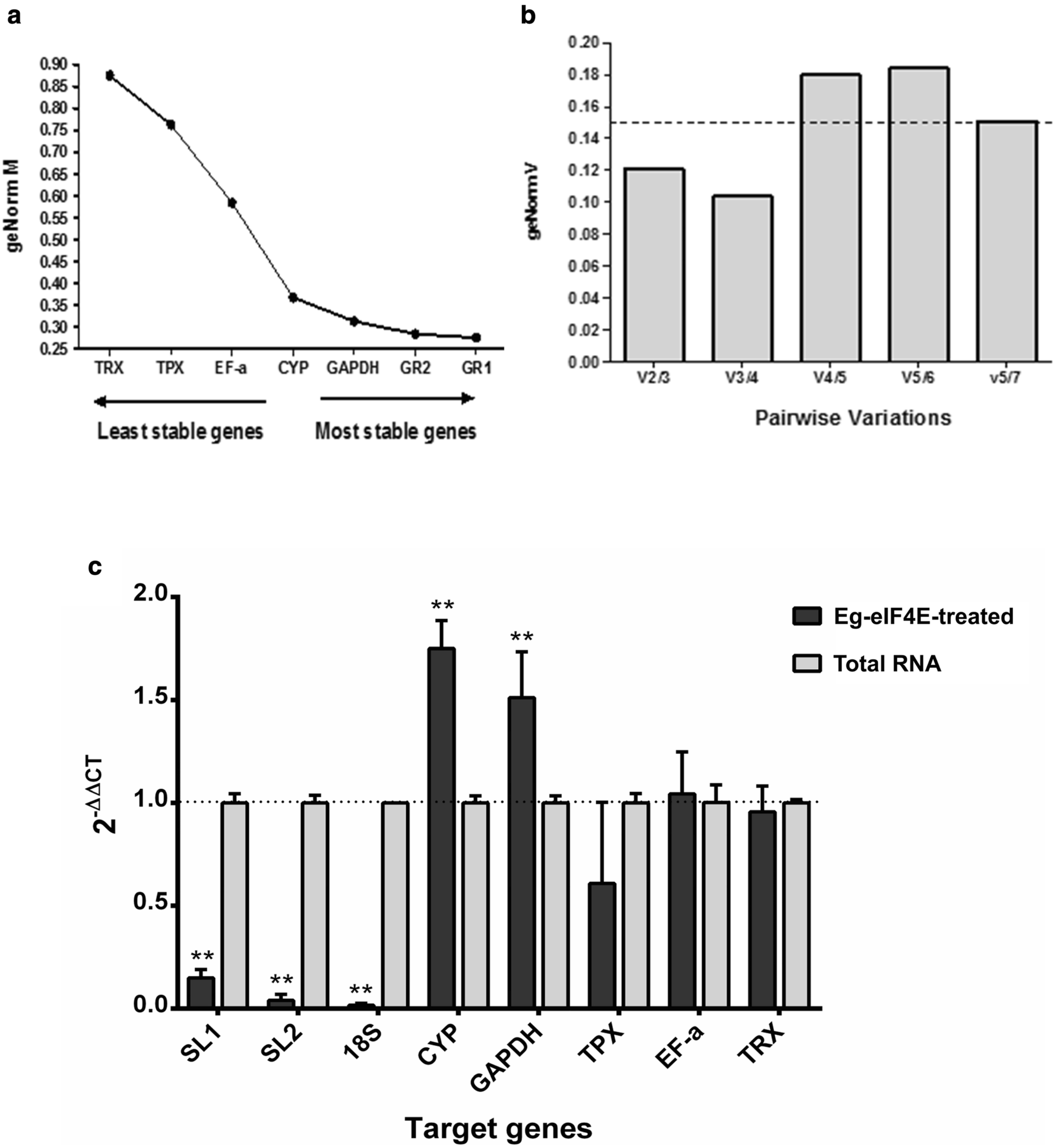


Fig. 4 Relative qPCR analysis of total RNA and *Eg*-eIF4E-GST pull-down RNA. Gene expression stability (A) and determination of the optimal number of reference genes (B) by geNorm. The dotted line represents the cut-off of 0.15 proposed by geNorm authors to determine the optimal number of reference genes to be used. (C) qPCR analysis of relative mRNA expression of 18S rRNA, trans-spliced and capped mRNA using GR1 and GR2 as reference genes. Differential gene expression between *Eg*-eIF4E-GST pull-down and total RNA is shown (**$p < 0.05$). Genes used in qPCR analyses are described in Table 1

although these changes in the amino acids residues were conservative (Grosdidier et al. 2011).

Molecular docking (composed by parameters related to intermolecular interactions, ligand entropy, desolvation, and lipophilic contacts) was performed using SwissDock (Grosdidier et al. 2011) and DockThor (de Magalhães et al. 2004, 2014) to predict the binding affinity of Eg-eIF4E for both MMGcap and TMGcap ligands (Tables S2 and

Table 2 mRNA enrichment relative to 18S ribosomal RNA after eiF4E sepharose 4B pull-down

Genes	$\Delta\Delta CT^{*a}$ (ΔCT eiF4E-ΔCT total RNA)	$2^{-\Delta\Delta CT}$ (mRNA enrichment after Eg-eiF4E pull-down)
SL1	−3.5	11
SL2	−2.1	4
GR1	−6.4	84
GR2	−6.4	84
TPx	−6.0	64
EF-a	−7.2	147
Trx	−7.0	128

^{*a} ΔCT was calculated as follow (CT gene-CT 18S RNA)

Table S3). Eg-eIF4E showed similar predicted binding affinities to MMGcap and TMGcap ligands according to score values (−7.638 for MMGcap and −7.377 for TMGcap) calculated by DockThor. Similar binding affinity result in MMGcap and TMGcap (score value of −7.820 and −7.244, respectively) was also observed for human eIF4E.

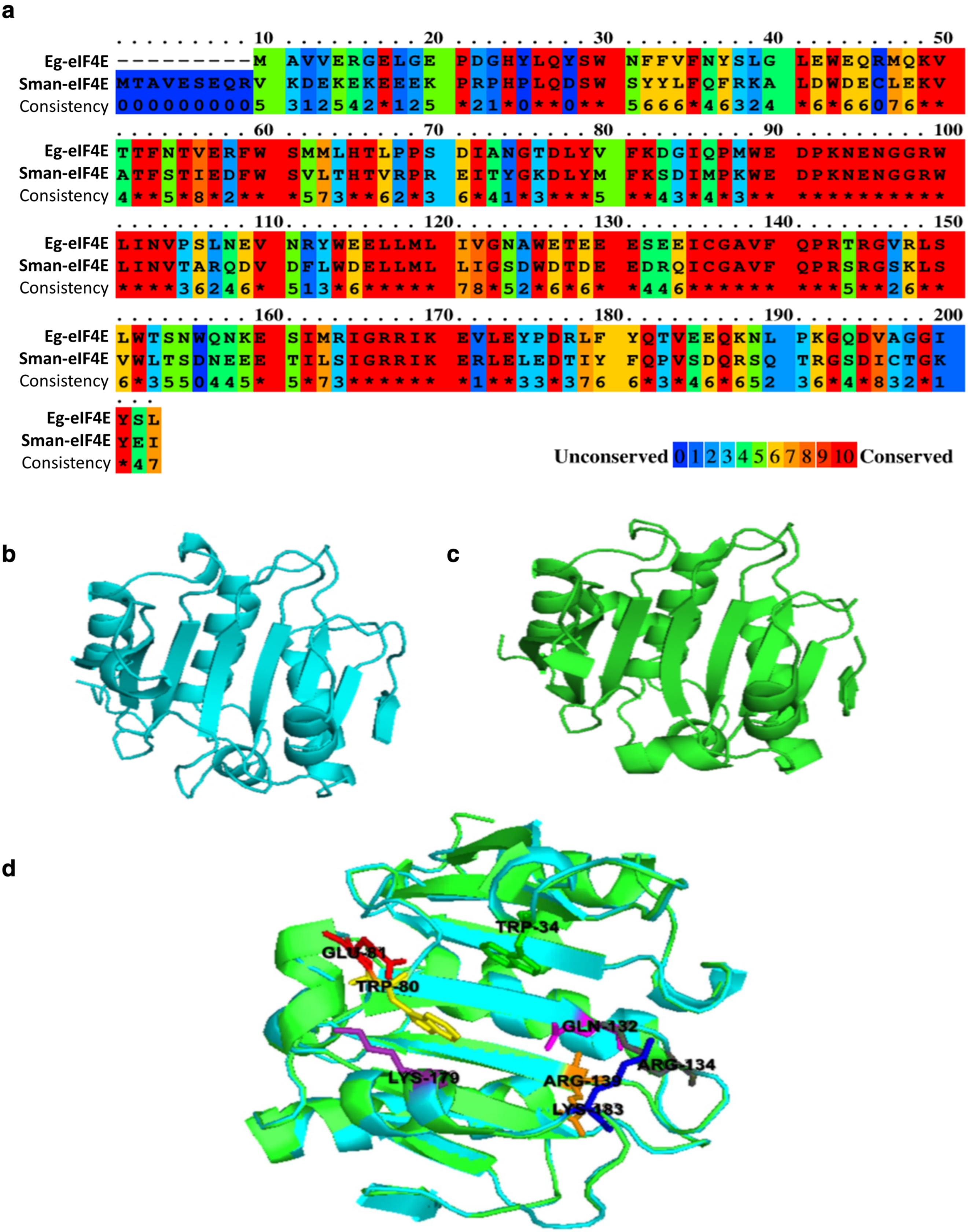
On the other hand, Eg-eIF4E molecular docking analysis using SwissDock showed a more positive total energy value (44.510) and a more negative FullFitness value (−1417.17) for MMGcap than TMGcap. Similar results of total energy and FullFitness values were also found in molecular docking using other helminths eIF4E proteins and human eIF4E. In contrast, the estimated ΔG values of Eg-eIF4E showed a more negative value for TMGcap (ΔG of −10.46 kcal/mol) than MMGcap (ΔG of −8.78 kcal/mol), indicating a higher affinity for TMGcap. Based on this difference of 1.68 kcal/mol of estimated ΔG values, we estimated a 17-fold greater affinity for TMGcap than MMGcap. The other eIF4E proteins presented a discrete difference between the estimated ΔG values. Based on ΔG values, we estimated that *S mansoni*, *C. elegans*, and human eIF4E have a slightly greater affinity for MMGcap than TMGcap (2.8-fold, 2.7-fold, and 1.44-fold, respectively).

Discussion

eIF4E is a cap-binding protein with important roles in controlling cellular proteome through translation regulation (Borden 2016). Moreover, recent studies demonstrated the participation of eIF4E factor in another process than translation, including mRNA stability, nuclear exportation, and polycistronic mRNA processing, demonstrating its importance not only for protein translation, but also for post-transcriptional regulation in eukaryotes (Volpon et al. 2017). The eIF4E family from most eukaryotes has been reported as a protein family with greater diversity and degree of gene duplication (Keiper et al. 2000; Joshi et al. 2005; Rhoads 2009; Freire et al. 2011). The number of eIF4E family members varies between the eukaryotic groups, generally the organisms contain two or more genes (i.e., mammalians have been reported to have three (Joshi et al. 2005; Rhoads 2009) and *C. elegans* five (Hernández and Vazquez-Pianzola 2005)). The phylogeny and specific amino acid changes mapped onto the protein structure suggest that metazoan eIF4E family members may be involved in different cellular roles related to post-transcriptional regulation of gene expression (Joshi et al. 2005). The duplication events observed in nematodes may have resulted in eIF4E having the capacity to interact with both mono- and tri-methylated cap structures (Joshi et al. 2005), as is the case of *C. elegans* eIF4E-1, eIF4E-2, and eIF4E-5. However, only eIF4E-3, which binds MMGcaps, is essential for *C. elegans* cell viability (Jankowska-Anyszka et al. 1998; Keiper et al. 2000).

Our phylogenetic analyses indicated that the process of diversification resulting from duplication of eIF4E probably arose in Turbellaria and Monogenea groups, but not in most of parasitic flatworms, due to the presence of only one eIF4E isoform in these organisms, as reported in *S. mansoni* and *S. japonicum* (Liu et al. 2009). All the predicted eIF4E proteins from parasites formed a single phylogenetic cluster, which was divergent from the other organisms analyzed. This result is in agreement with the data reported by Liu et al. (2009), which found that *Schistosoma* eIF4E was more highly divergent from human eIF4E than nematode eIF4Es.

The identification of only one eIF4E isoform for most of parasite flatworms suggests that these proteins would have the capacity of binding to both types of 5′ cap similar to *S. mansoni* eIF4E. Interestingly, a mutant form of the human eIF4E with MMGcap affinity was previously used to develop an efficient system for mRNA purification in mammalian model (Choi and Hagedorn 2003; Bajak and Hagedorn 2008). In order to functionally characterize the eIF4E protein from a cestode platyhelminth, we have cloned and purified a recombinant *E. granulosus* eIF4E factor and tested the ability of this protein to bind parasite mRNAs. Our results indicate that *Eg*-eIF4E bind 5′-capped mRNA preferentially over rRNA, allowing the enrichment of mRNA transcripts and depletion of rRNAs in total RNA samples obtained from *E. granulosus* PSC. Previous studies demonstrated the influence of RNA extraction method and the experimental strategies to deplete rRNAs, to purify mRNAs (Sultan et al. 2014), and to synthesize cDNA (Parkinson et al. 2012) in the results obtained in transcriptomic analysis. Based on our results, recombinant eIF4E from *E. granulosus* probably would not be a good strategy for mRNA purification for transcriptome analysis, because Eg-eIF4E has a stronger binding for MMGcap when compared with TMGcap. However, this purification procedure could be useful to study full-length 5′-capped RNA with low representation in RNA samples.



a

```
             . . . . . . . . . 10 . . . . . . . . . 20 . . . . . . . . . 30 . . . . . . . . . 40 . . . . . . . . . 50
Eg-eIF4E     ---------M AVVERGELGE PDGHYLQYSW NFFVFNYSLG LEWEQRMQKV
Sman-eIF4E   MTAVESEQRV KDEKEKEEEK PRPHPLQDSW SYYLFQFRKA LDWDECLEKV
Consistency  0000000005 312542*125 *21*0**0** 5666*46324 *6*66076**

             . . . . . . . . . 60 . . . . . . . . . 70 . . . . . . . . . 80 . . . . . . . . . 90 . . . . . . . . . 100
Eg-eIF4E     TTFNTVERFW SMMLHTLPPS DIANGTDLYV FKDGIQPMWE DPKNENGGRW
Sman-eIF4E   ATFSTIEDFW SVLTHTVRPR EITYGKDLYM FKSDIMPKWE DPKNENGGRW
Consistency  4**5*8*2** *573**62*3 6*41*3***5 **43*4*3** **********

             . . . . . . . . . 110. . . . . . . . . 120. . . . . . . . . 130. . . . . . . . . 140. . . . . . . . . 150
Eg-eIF4E     LINVPSLNEV NRYWEELLML IVGNAWETEE ESEEICGAVF QPRTRGVRLS
Sman-eIF4E   LINVTARQDV DFLWDELLML LIGSDWDTDE EDRQICGAVF QPRSRGSKLS
Consistency  ****36246* 513*6***** 78*52*6*6* *446****** ***5**26**

             . . . . . . . . . 160. . . . . . . . . 170. . . . . . . . . 180. . . . . . . . . 190. . . . . . . . . 200
Eg-eIF4E     LWTSNWQNKE SIMRIGRRIK EVLEYPDRLF YQTVEEQKNL PKGQDVAGGI
Sman-eIF4E   VWLTSDNEEE TILSIGRRIK ERLELEDTIY FQPVSDQRSQ TRGSDICTGK
Consistency  6*3550445* 5*73****** *1**33*376 6*3*46*652 36*4*832*1

             . . .
Eg-eIF4E     YSL
Sman-eIF4E   YEI
Consistency  *47
```

Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved

Fig. 5 Structure and molecular modeling of eIF4E from *E. granulosus* (*Eg*-eIF4E) and *S. mansoni* (*Sman*-eIF4E). (A) Alignment of the amino acid sequences from *Eg*-eIF4E and *Sman*-eIF4E. Alignment is colored based on conservation of amino acids. (B) *Sman*-eIF4E structure (PDB code: 3HXI), (C) *Eg*-eIF4E molecular modeling, (D) superposition of both *Eg*-eIF4E and *Sman*-eIF4E three-dimensional structures (blue: *Sman*-eIF4E and green: *Eg*-eIF4E) and amino acids in the active site (Glu-81, Trp34, Trp80, Gln132, and Arg134) from *Eg*-eIF4E are highlighted

Although our results indicate a weaker binding of Eg-eIF4E to TMGcap, it may not have a direct impact in the translation levels of the trans-spliced transcripts. In vivo, the binding of eIF4G to mRNA can enhance exponentially the binding of eIF4E to the 5′ cap (Haghighat and Sonenberg 1997). In addition, the lowest affinity of nematodes eIF4E to TMGcap could be partially compensated by interaction between the splice leader RNA sequence spliced, a 5′-RNA UTR sequence of trans-spliced transcript, with eIF4E-eIF4G complexes (Wallace et al. 2010; Liu et al. 2011). This interaction of splice leader with eIF4E-eIF4G complexes facilitates recruitment of trans-spliced transcripts and its translation (Wallace et al. 2010; Liu et al. 2011). However, the role of eIF4G in flatworms still needs to be investigated.

For flatworms and nematodes, it has been estimated that 25–70% of transcripts would come from trans-splicing process (Davis et al. 1995; Brehm et al. 2000; Allen et al. 2011). In *E. multilocularis*, the trans-splicing process may be responsible for 13% of all transcripts (Tsai et al. 2013). Typically, the eIF4E relative affinities to each cap have been measured using cap analogs and physicochemical methods (Liu et al. 2009, 2011; Ruszczyńska-Bartnik et al. 2011). In this approach, human and nematode eIF4Es have several hundred-fold lower affinity for TMGcap (Niedzwiecka et al. 2002; Liu et al. 2011), while flatworm *S. mansoni* eIF4E has a dual capacity to bind TMGcap and MMGcap (Liu et al. 2009). Despite the flatworm *S. mansoni* discriminates less against the TMGcap compared with other eukaryotic eIF4E proteins, the *S. mansoni* eIF4E has a lower affinity for TMGcap (between 5-fold and 20-fold) as was shown by fluorescence titration assay and isothermal titration calorimetry (ITC) (Liu et al. 2009). This difference could explain both the recovery of TMG-capped and MMG-capped mRNAs after *Eg*-eIF4E-GST pull-down and the difference in the enrichment of mRNAs from different origins. Interestingly, some authors demonstrated a distinct selectivity of eIF4E to 5′-capped mRNA through binding to additional RNA elements (Sonenberg and Gingras 1998; Liu et al. 2011). This data could explain some difference in 5′-capped mRNA recovery after *Eg*-eIF4E incubation observed for CYP and GAPDH mRNAs.

To better understand the greater Eg-eIF4E affinity to MMG-capped mRNA over TMG-capped mRNAs, we performed a molecular docking of eIF4E from *E. granulosus* and different organisms, and we compared the binding affinity for MMGcap and TMGcap. The molecular docking results using SwissDock indicated that eIF4E proteins from *E. granulosus* and *S. mansoni* have a binding mode to MMGcap alike to *C. elegans* IFE-3, which have a known greater affinity for MMGcap (Jankowska-Anyszka et al. 1998; Keiper et al. 2000). However, human eIF4E, which has a well-known greater affinity for MMGcap, presented similar scores of affinity prediction calculated by Dockthor to both 5′ caps in our in silico analysis, odds with previous research of human eIF4E (Niedzwiecka et al. 2002). In line with the latter result, the binding affinity of Eg-eIF4E estimated by ΔG values showed a greater affinity for TMGcap (about 17-fold), which is the opposite found in our experimental data. These results indicated that in silico affinity prediction of eIF4E to MMGcap and TMGcap is not an accurate strategy since part of our in silico results are divergent from each other, with our experimental results and with the current literature. The divergence between in silico data from experimental literature was also reported by Ruszczyńska-Bartnik et al. (2011), whose molecular dynamics simulation of eIF4E from different species showed that discrimination between MMGcap and TMGcap consists neither in the differences in the stacking energy nor in structural differences inside the cap-binding pocket. Also, this work showed that the anchoring of 5′ caps inside of eIF4E cap-binding pockets is not a “static” net of stabilizing contacts, but a dynamic process that must take into account the difference between the formation and dissociation of the eIF4E-cap complexes (Ruszczyńska-Bartnik et al. 2011).

In conclusion, the present work described for the first time eIF4E sequence analysis for free-living and parasite species. Our results indicate the presence of only one gene coding for eIF4E for most of parasite flatworms, while free-living flatworms have multiples eIF4E sequences. Recombinant *E. granulosus* eIF4E had a stronger affinity for 5′-capped RNAs compared with rRNA. In this sense, recombinant *Eg*-eIF4E promises to be a good tool to study full-length 5′-capped RNA with low representation in RNA samples. The importance of eIF4E at the initial step of protein translation together with the identification of a single copy in flatworm genomes suggest that eIF4E proteins could be potential targets for parasitic control. eIF4E inhibitors were previously developed (Soukarieh et al. 2016) and tested as anticancer agents (Lu et al. 2016) and could be used for drug repurposing against platyhelminth infections.

Author contributions Co-wrote and edited the manuscript, retrieved sequences, performed phylogenetic analysis, and performed the experiments: FSPD and MC; performed the structural and docking analyses of proteins: BVM; conceived and designed the experiments: FSPD, MC, KMM, and AZ. Contributed reagents/materials/analysis tools: HBF and AZ. Wrote the paper: FSPD, MC, BVM, and AZ. All authors read and approved the final manuscript.

Funding This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES) (Finance Code 001 and grant number 1278/2011) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant number 472316/2013–3). FSPD and BVM were recipients of a CAPES MSc. fellowship and MC was recipient of a CAPES postdoctoral fellowship.

References

Allen MA, Hillier LW, Waterston RH, Blumenthal T (2011) A global analysis of C. elegans trans-splicing. Genome Res 21:255–264. https://doi.org/10.1101/gr.113811.110

Bajak EZ, Hagedorn CH (2008) Efficient 5' cap-dependent RNA purification: use in identifying and studying subsets of RNA. In: Methods in Molecular Biology. pp 147–160

Bitar M, Boroni M, Macedo AM, Machado CR, Franco GR (2013) The spliced leader trans-splicing mechanism in different organisms: molecular details and possible biological roles. Front Genet 4:199. https://doi.org/10.3389/fgene.2013.00199

Blaxter M, Liu L (1996) Nematode spliced leaders–ubiquity, evolution and utility. Int J Parasitol 26:1025–1033. https://doi.org/10.1016/S0020-7519(96)00060-4

Blower MD, Jambhekar A, Schwarz DS, Toombs JA (2013) Combining different mRNA capture methods to analyze the transcriptome: analysis of the Xenopus laevis transcriptome. PLoS One 8:e77700. https://doi.org/10.1371/journal.pone.0077700

Borden KLB (2016) The eukaryotic translation initiation factor eIF4E wears a “cap” for many occasions. Translation 4:e1220899. https://doi.org/10.1080/21690731.2016.1220899

Brehm K, Jensen K, Frosch M (2000) mRNA trans-splicing in the human parasitic Cestode Echinococcus multilocularis. J Biol Chem 275: 38311–38318. https://doi.org/10.1074/jbc.M006091200

Choi YH, Hagedorn CH (2003) Purifying mRNAs with a high-affinity eIF4E mutant identifies the short 3′ poly(A) end phenotype. Proc Natl Acad Sci 100:7033–7038. https://doi.org/10.1073/pnas.1232347100

Davis RE (1996) Spliced leader RNA trans-splicing in metazoa. Parasitol Today 12:33–40. https://doi.org/10.1016/0169-4758(96)80643-0

Davis RE, Hardwick C, Tavernier P, Hodgson S, Singh H (1995) RNA trans-splicing in flatworms: analysis of trans-spliced mRNAs and genes in the human parasite, Schistosoma mansoni. J Biol Chem 270:21813–21819. https://doi.org/10.1074/jbc.270.37.21813

de Magalhães CS, Barbosa HJC, Dardenne LE (2004) Selection-insertion schemes in genetic algorithms for the flexible ligand docking problem. In: GECCO 2004: genetic and evolutionary computation, pp 368–379

de Magalhães CS, Almeida DM, Barbosa HJC, Dardenne LE (2014) A dynamic niching genetic algorithm strategy for docking highly flexible ligands. Inf Sci (Ny) 289:206–224. https://doi.org/10.1016/j.ins.2014.08.002

Espínola SM, Ferreira HB, Zaha A (2014) Validation of suitable reference genes for expression normalization in Echinococcus spp. larval stages. PLoS One 9:1–9. https://doi.org/10.1371/journal.pone.0102228

Freire ER, Dhalia R, Moura DMN, da Costa Lima TD, Lima RP, Reis CRS, Hughes K, Figueiredo RCBQ, Standart N, Carrington M, de Melo Neto OP (2011) The four trypanosomatid eIF4E homologues fall into two separate groups, with distinct features in primary sequence and biological properties. Mol Biochem Parasitol 176:25–36. https://doi.org/10.1016/j.molbiopara.2010.11.011

Gingras A-C, Raught B, Sonenberg N (1999) eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annu Rev Biochem 68:913–963. https://doi.org/10.1146/annurev.biochem.68.1.913

Goodfellow IG, Roberts LO (2008) Eukaryotic initiation factor 4E. Int J Biochem Cell Biol 40:2675–2680. https://doi.org/10.1016/j.biocel.2007.10.023

Grosdidier A, Zoete V, Michielin O (2011) SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic Acids Res 39:W270–W277. https://doi.org/10.1093/nar/gkr366

Guedes IA, Pereira FSS, Dardenne LE (2018) Empirical scoring functions for structure-based virtual screening: applications, critical aspects, and challenges. Front Pharmacol 9:1–18. https://doi.org/10.3389/fphar.2018.01089

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies : assessing the performance of PhyML 3.0. Sist Biol 59:307–321. https://doi.org/10.1093/sysbio/syq010

Haghighat A, Sonenberg N (1997) eIF4G dramatically enhances the binding of eIF4E to the mRNA 5'-cap structure. J Biol Chem 272: 21677–21680. https://doi.org/10.1074/jbc.272.35.21677

Hastings KEM (2005) SL trans-splicing: easy come or easy go? Genet, Trends

Hernández G, Vazquez-Pianzola P (2005) Functional diversity of the eukaryotic translation initiation factors belonging to eIF4 families. Mech Dev 122:865–876. https://doi.org/10.1016/j.mod.2005.04.002

Jankowska-Anyszka M, Lamphear BJ, Aamodt EJ, Harrington T, Darzynkiewicz E, Stolarski R, Rhoads RE (1998) Multiple isoforms of eukaryotic protein synthesis initiation factor 4E in Caenorhabditis elegans can distinguish between mono- and trimethylated mRNA cap structures. J Biol Chem 273:10538–10542. https://doi.org/10.1074/jbc.273.17.10538

Jones GD, Williams EP, Place AR et al (2015) The alveolate translation initiation factor 4E family reveals a custom toolkit for translational control in core dinoflagellates:1–12. https://doi.org/10.1186/s12862-015-0301-9

Joshi B, Lee K, Maeder DL, Jagus R (2005) Phylogenetic analysis of eIF4E-family members. BMC Evol Biol 5:48. https://doi.org/10.1186/1471-2148-5-48

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Article Fast Track 30:772–780. https://doi.org/10.1093/molbev/mst010

Keiper BD, Lamphear BJ, Deshpande AM, Jankowska-Anyszka M, Aamodt EJ, Blumenthal T, Rhoads RE (2000) Functional characterization of five eIF4E isoforms in Caenorhabditis elegans. J Biol Chem 275:10590–10596. https://doi.org/10.1074/jbc.275.14.10590

Lasda EL, Blumenthal T (2011) Trans -splicing. Wiley Interdiscip Rev RNA 2:417–434. https://doi.org/10.1002/wrna.71

Liu W, Zhao R, McFarland C, Kieft J, Niedzwiecka A, Jankowska-Anyszka M, Stepinski J, Darzynkiewicz E, Jones DNM, Davis RE (2009) Structural insights into parasite eIF4E binding specificity for m7G and m2,2,7G mRNA caps. J Biol Chem 284:31336–31349. https://doi.org/10.1074/jbc.M109.049858

Liu W, Jankowska-Anyszka M, Piecyk K, Dickson L, Wallace A, Niedzwiecka A, Stepinski J, Stolarski R, Darzynkiewicz E, Kieft J, Zhao R, Jones DNM, Davis RE (2011) Structural basis for nematode eIF4E binding an m 2,2,7 G-cap and its implications for translation initiation. Nucleic Acids Res 39:8820–8832. https://doi.org/10.1093/nar/gkr650

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Lorenzatto KR, Monteiro KM, Paredes R, Paludo GP, da Fonsêca MM, Galanti N, Zaha A, Ferreira HB (2012) Fructose-bisphosphate aldolase and enolase from Echinococcus granulosus: genes, expression patterns and protein interactions of two potential moonlighting proteins. Gene 506:76–84. https://doi.org/10.1016/j.gene.2012.06.046

Lu C, Makala L, Wu D, Cai Y (2016) Targeting translation: eIF4E as an emerging anticancer drug target. Expert Rev Mol Med 18:e2. https://doi.org/10.1017/erm.2015.20

Niedzwiecka A, Marcotrigiano J, Stepinski J, Jankowska-Anyszka M, Wyslouch-Cieszynska A, Dadlez M, Gingras AC, Mak P, Darzynkiewicz E, Sonenberg N, Burley SK, Stolarski R (2002) Biophysical studies of eIF4E cap-binding protein : recognition of mRNA 5 0 cap structure and synthetic fragments of eIF4G and 4E-BP1 proteins. J Mol Biol 2836:615–635. https://doi.org/10.1016/S0022-2836(02)00328-5

Niedzwiecka A, Darzynkiewicz E, Stolarski R (2004) Thermodynamics of mRNA 5′ cap binding by eukaryotic translation initiation factor eIF4E †. Biochemistry 43:13305–13317. https://doi.org/10.1021/bi0491651

Papic N, Maxwell CI, Delker DA, Liu S, Heale BSE, Hagedorn CH (2012) RNA-sequencing analysis of 5’ capped RNAs identifies many new differentially expressed genes in acute hepatitis C virus infection. Viruses 4:581–612. https://doi.org/10.3390/v4040581

Parkinson J, Wasmuth JD, Salinas G, Bizarro CV, Sanford C, Berriman M, Ferreira HB, Zaha A, Blaxter ML, Maizels RM, Fernández C (2012) A transcriptomic analysis of Echinococcus granulosus larval stages: implications for parasite biology and host adaptation. PLoS Negl Trop Dis 6:e1897. https://doi.org/10.1371/journal.pntd.0001897

Prévôt D, Darlix J-L, Ohlmann T (2003) Conducting the initiation of protein synthesis: the role of eIF4G. Biol Cell 95:141–156. https://doi.org/10.1016/S0248-4900(03)00031-5

Rhoads RE (2009) eIF4E: new family members, new binding partners, new roles. J Biol Chem 284:16711–16715. https://doi.org/10.1074/jbc.R900002200

Ruijter JM, Ramakers C, Hoogaars WMH, Karlen Y, Bakker O, van den Hoff MJB, Moorman AFM (2009) Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res 37:e45–e45. https://doi.org/10.1093/nar/gkp045

Ruszczyńska-Bartnik K, MacIejczyk M, Stolarski R (2011) Dynamical insight into Caenorhabditis elegans eIF4E recognition specificity for mono-and trimethylated structures of mRNA 5′ cap. J Mol Model 17:727–737. https://doi.org/10.1007/s00894-010-0773-x

Rutkowska-Wlodarczyk I, Stepinski J, Dadlez M, Darzynkiewicz E, Stolarski R, Niedzwiecka A (2008) Structural changes of eIF4E upon binding to the mRNA 5′ monomethylguanosine and trimethylguanosine cap. Biochemistry 47:2710–2720. https://doi.org/10.1021/bi701168z

Santos G, Espínola S, Ferreira H, Margis R, Zaha A (2013) Rapid detection of Echinococcus species by a high-resolution melting (HRM) approach. Parasit Vectors 6:327. https://doi.org/10.1186/1756-3305-6-327

Sonenberg N, Gingras A-C (1998) The mRNA 5′ cap-binding protein eIF4E and control of cell growth. Curr Opin Cell Biol 10:268–275. https://doi.org/10.1016/S0955-0674(98)80150-6

Soukarieh F, Nowicki MW, Bastide A, Pöyry T, Jones C, Dudek K, Patwardhan G, Meullenet F, Oldham NJ, Walkinshaw MD, Willis AE, Fischer PM (2016) Design of nucleotide-mimetic and non-nucleotide inhibitors of the translation initiation factor eIF4E: synthesis, structural and functional characterisation. Eur J Med Chem 124:200–217. https://doi.org/10.1016/j.ejmech.2016.08.047

Sultan M, Amstislavskiy V, Risch T, Schuette M, Dökel S, Ralser M, Balzereit D, Lehrach H, Yaspo ML (2014) Influence of RNA extraction methods and library selection schemes on RNA-seq data. BMC Genomics 15:675. https://doi.org/10.1186/1471-2164-15-675

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197

Tsai IJ, Zarowiecki M, Holroyd N et al (2013) The genomes of four tapeworm species reveal adaptations to parasitism. Nature 496:57–63. https://doi.org/10.1038/nature12031

Vandesompele J, De Preter K, Ilip P et al (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3:34–31. https://doi.org/10.1186/gb-2002-3-7-research0034

Volpon L, Culjkovic-Kraljacic B, Sohn HS, Blanchet-Cohen A, Osborne MJ, Borden KLB (2017) A biochemical framework for eIF4E-dependent mRNA export and nuclear recycling of the export machinery. RNA 23:927–937. https://doi.org/10.1261/rna.060137.116

von der Haar T, Gross JD, Wagner G, McCarthy JEG (2004) The mRNA cap-binding protein eIF4E in post-transcriptional gene expression. Nat Struct Mol Biol 11:503–511. https://doi.org/10.1038/nsmb779

Wallace A, Filbin ME, Veo B, McFarland C, Stepinski J, Jankowska-Anyszka M, Darzynkiewicz E, Davis RE (2010) The nematode eukaryotic translation initiation factor 4E/G complex works with a trans-spliced leader stem-loop to enable efficient translation of trimethylguanosine-capped RNAs. Mol Cell Biol 30:1958–1970. https://doi.org/10.1128/MCB.01437-09

WHO WHO (2013) Sustaining the drive to overcome the global impact of neglected tropical diseases. Second WHO reported on neglected tropical diseases., 2nd edn. Paris, France

Yugandhar K, Gromiha MM (2014) Protein–protein binding affinity prediction from amino acid sequence. Bioinformatics 30:3583–3589. https://doi.org/10.1093/bioinformatics/btu580