Parasitology International 65 (2016) 625–631

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Recombinant *Trichomonas vaginalis* eIF-5A protein expressed from a eukaryotic system binds specifically to mammalian and putative trichomonal eIF-5A response elements (EREs)

Bertha Isabel Carvajal-Gamez [a], Laura Vázquez Carrillo [a], Julio César Torres-Romero [b], Minerva Camacho-Nuez [a], María Dolores Ponce-Regalado [c], César López Camarillo [a], María Elizbeth Alvarez-Sánchez [a,*]

[a] Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México (UACM), San Lorenzo # 290, Col. Del Valle, CP 03100 México City, México
[b] Laboratorio de Bioquímica y Genética Molecular, Facultad de Química de la Universidad Autónoma de Yucatán, Calle 43 No. 613 x C. 90 Col. Inalámbrica, CP 97069 Mérida, Yucatán, México
[c] Departamento de Clínicas, Centro Universitario de los Altos, Universidad de Guadalajara, Tepatitlán de Morelos, Jalisco, México

A R T I C L E I N F O

Article history:
Received 12 August 2016
Accepted 9 September 2016
Available online 9 September 2016

Keywords:
Trichomonas vaginalis
Eukaryotic translation initiation factor 5A
TveIF-5A
Polyamines
eIF-5A response elements EREs

A B S T R A C T

Trichomonas vaginalis eIF-5A-like protein (TveIF-5A) belongs to the highly conserved eIF-5A family of proteins that contains a unique polyamine-derived amino acid, hypusine. Recently, we determined that the polyamine putrescine is required for *tveif-5a* mRNA stability, and it is necessary for stability and maturation of the TveIF-5A protein. Eukaryotic eIF-5A is known to be involved in mRNA turnover and is capable of sequence-specific RNA binding to eIF-5A response elements (EREs). These ERE sequences are present in diverse mammalian mRNAs, including human cyclooxygenase-2 (*cox-2*). Here, we cloned the complete coding sequence of TveIF-5A and overexpressed it in a eukaryotic system. The recombinant protein (rTveIF-5A) was purified in soluble form using size-exclusion chromatography. Because of the polyamine-dependent regulation of TvCP39 (a protease of *T. vaginalis*) at the protein and RNA messenger (mRNA) levels, we looked for an ERE-like structure in the 3′ region of *tvcp39* mRNA. In RNA gel-shift assays, rTveIF-5A bound to transcripts at the EREs of *cox-2* or *tvcp39* mRNAs. This work shows the eIF-5A/ERE-like interaction in *T. vaginalis*.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Regulation of gene expression is of major importance for all living organisms. However, the regulatory mechanisms involved are considerably more complex and tightly controlled in eukaryotes. In eukaryotes, several of these mechanisms require polyamines or their diamine precursor, putrescine [1]. At the global level, polyamines may regulate translation *via* hypusination, a posttranslational modification unique to the eukaryotic translation initiation factor eIF-5A [2].

Recently, many studies have shown that eIF-5A is involved in other functions including RNA transport and mRNA stability. The viral protein Rev, required for human immunodeficiency virus-1 (HIV-1) gene expression, was one of the first reports of RNA that is processed *via* an eIF-5A-dependent mechanism [3]. It has been shown that active functional eIF-5A protein binds specifically to certain mRNAs, including the *cox-2* mRNA, *via* conserved motifs UAACCA and AAAUGU within the 3′-untranslated region (UTR), which are denominated eIF-5A response elements (EREs) [4,5,6]. These results suggest that the hypusinated form of eIF-5A participates in polyamine-dependent posttranscriptional regulation of gene expression.

In addition to higher eukaryotes, eIF-5A homologues have been found in invertebrates such as *Caenorhabditis elegans*, *Drosophila melanogaster* and leaf-feeding (*Spodoptera*) insects [7,8], in yeast [9], and even in archaea [10]. We have previously demonstrated that *Trichomonas vaginalis* has two *tveif-5a* genes and that putrescine is required for *tveif-5a* mRNA stability as well as for the expression, stability and maturation of the TveIF-5A protein [11,12]. Additionally, putrescine affected the expression, protein concentration, proteolytic activity, and cellular localization of TvCP39, a cysteine proteinase involved in *T. vaginalis* cytotoxicity [13].

Here, we report the expression of recombinant TveIF-5A (rTveIF-5A) in an active form. We also present evidence that rTveIF-5A binds specifically to a mammalian ERE and to a putative ERE within the 3'UTR of the *tvcp39* gene, suggesting a role of TveIF-5A in mRNA expression and stability.

2. Materials and methods

2.1. Parasites and HeLa cell culture

T. vaginalis isolate CNCD 147 was used in this study [14]. Parasites were grown to three weeks by daily passage in Diamond's trypticase-

* Corresponding author.
E-mail addresses: elizbeth2@gmail.com, maria.alvarez@uacm.edu.mx (M.E. Alvarez-Sánchez).

http://dx.doi.org/10.1016/j.parint.2016.09.004
1383-5769/© 2016 Elsevier Ireland Ltd. All rights reserved.

Downloaded for Anonymous User (n/a) at University of Guadalajara from ClinicalKey.com by Elsevier on June 20, 2022. For personal use only. No other uses without permission. Copyright ©2022. Elsevier Inc. All rights reserved.

yeast extract-maltose (TYM) medium [15] supplemented with 10% heat-inactivated horse serum (TYM-serum) for 24 h at 37 °C. HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco®, USA) supplemented with 10% heat-inactivated horse serum at 37 °C for 48 h at 5% CO_2 until confluent cell monolayers were formed [14].

2.2. Analysis of the 3'UTR tvcp39 sequence

The genomic clone Race 3N-C1-M13F [16] with a 94 bp (GenBank Accession No. XM_001316379) insert was sequenced. The sequence was analyzed by BLAST and EXPASY search, and predicted RNA secondary structures were analyzed using the Mfold program (http://bioinfo.rpi.edu/applications/mfold/).

2.3. RNA isolation and RT-PCR assays

Total RNA from 1×10^7 parasites was extracted using TRIzol® Reagent (InvitrogenUSA) as recommended by the manufacturer. Using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR), total RNA (1 μg) was reverse-transcribed with the Superscript II reverse transcriptase kit (Invitrogen, USA) and an oligo (dT^{18}) primer, and 1 μg cDNA was used to amplified a 504 bp fragment corresponding to the *tveif-5a* complete ORF using the sense primer eIF5A1-S (5′-CCGGATCCATGTCTTCAGCTGAAGAAGA-3′) and the antisense primer eIF5A1-AS (5′-CCAAGCTTTTAGTTTGTGGACTTCTTGC-3′) [11].

2.4. Cytoplasmic extracts from HeLa cells and trichomonad parasites

HeLa cells were washed with cold phosphate buffered saline (PBS) and centrifuged (2500 ×*g* for 5 min at 4 °C). The cell pellet was suspended in lysis buffer A (10 mM HEPES–NaOH, pH 7.9, 15 mM MgCl2, 10 mM KCl), homogenized in a Dounce homogenizer (45 strokes), and centrifuged at 10,000 ×*g* for 30 min at 4 °C. Supernatants were diluted to a final protein concentration of 10 mg/ml and kept at −70 °C until use. Trichomonad cytoplasmic extracts were prepared from 1×10^8 trophozoites grown in the presence or absence of polyamines [17]. Parasites were lysed by vortexing in binding buffer (10 mM HEPES, pH 7.6, 3 mM $MgCl_2$, 40 mM KCl, 5% glycerol and 0.3% NP-40) containing 7.5 mM TLCK and 1.6 mM leupeptin, homogenized in a Dounce homogenizer (45 strokes), and centrifuged at 13,000 ×*g* for 5 min at 4 °C. Then, the supernatant was recovered, the protein concentration was determined by Bradford assays, and aliquots were kept at 70 °C.

2.5. Cloning of tveif-5a

The *tveif-5a* sequence was digested from the *pGEX6P1* (GE Healthcare, USA) vector using *Bam*HI and *Xho*I (New England Biolabs, USA), and the sequence was cloned into the pYES2 vector (Invitrogen, USA) followed by transformation into *E. coli* DH5α cells. Positive clones were analyzed by restriction digest as previously described. Insertion of the open reading frame in the correct orientation was confirmed by sequencing.

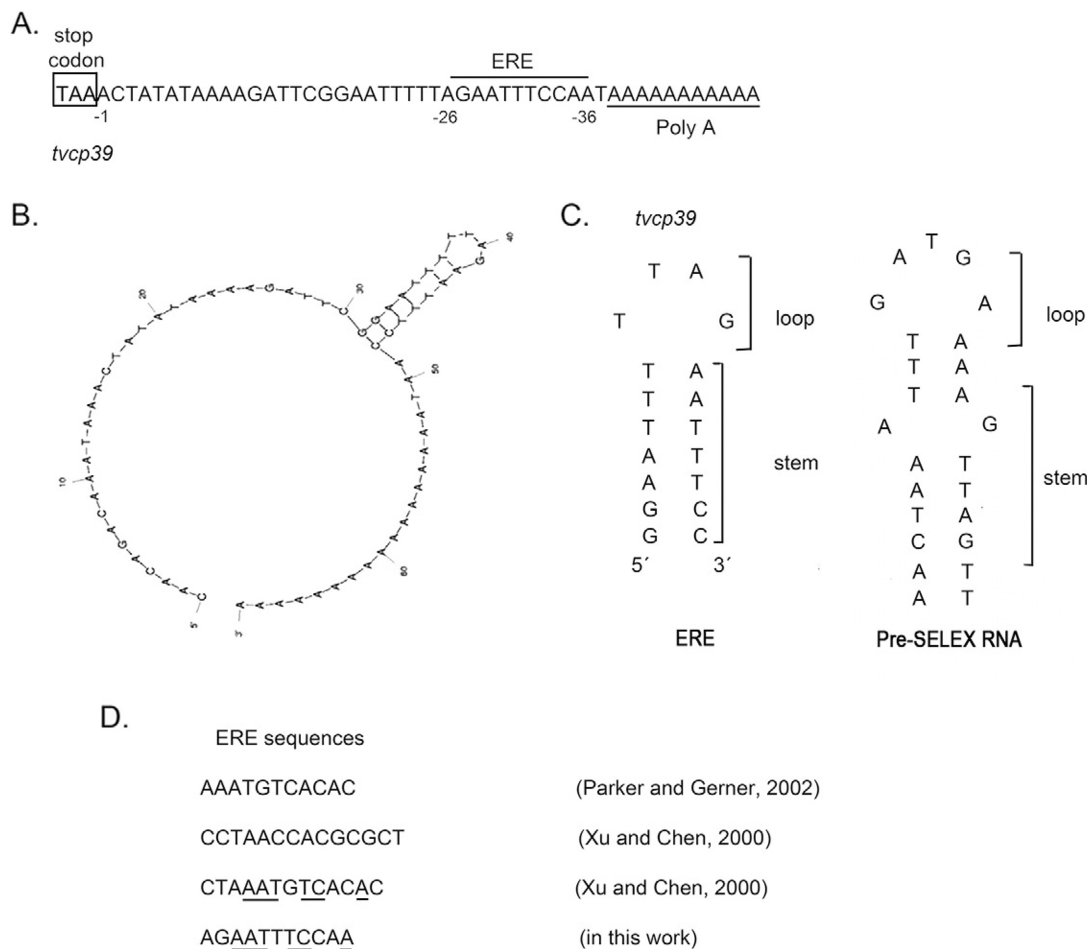

Fig. 1. Analysis of *tvcp39* mRNA 3'UTR. A. *tvcp39* 3'UTR sequence. The box shows the translation stop codon, the black line shows the ERE-like sequence of *tvcp39* and underlined nucleotides represents the polyadenylation site. B. Modeling of the mRNA 3'UTR with the "RNA fold." C. Comparison of stem-loop of ERE-like *tvcp39* with stem-loop of IRE *tvcp4*. D. ERE sequence comparisons.

Downloaded for Anonymous User (n/a) at University of Guadalajara from ClinicalKey.com by Elsevier on June 20, 2022. For personal use only. No other uses without permission. Copyright ©2022. Elsevier Inc. All rights reserved.

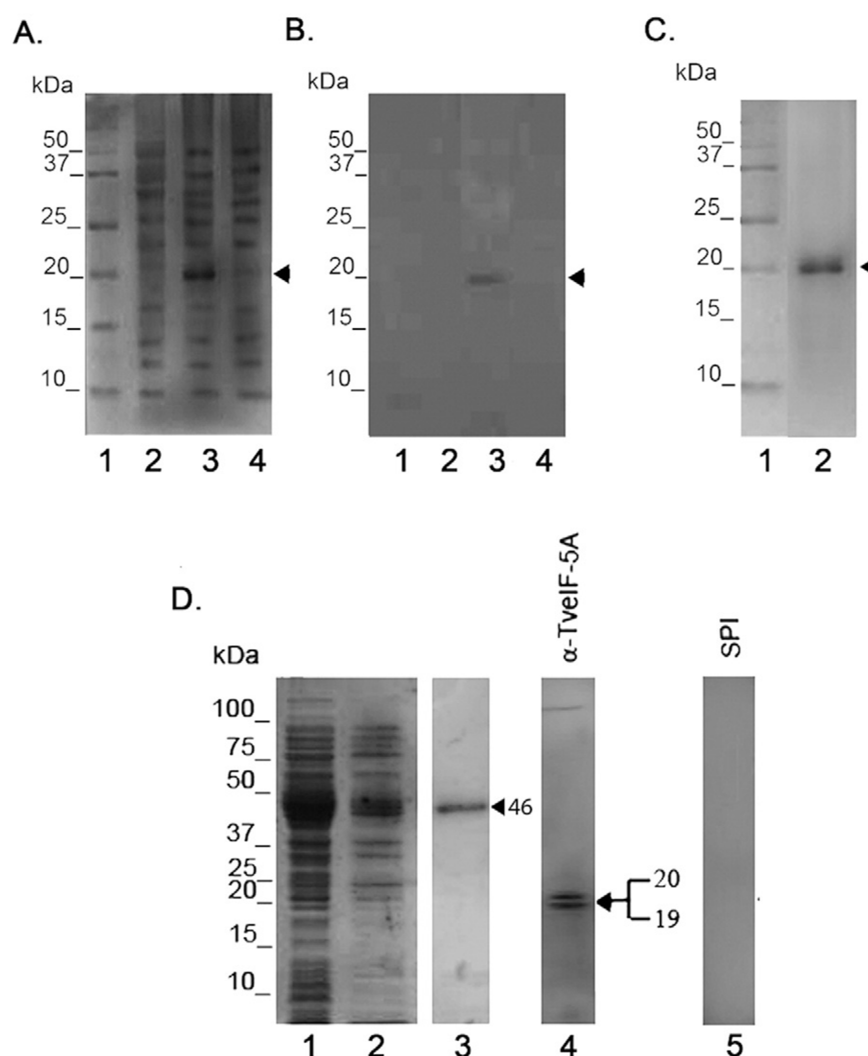

Fig. 2. Expression, and purification of mature TveIF-5A. A. SDS-PAGE analysis of mature expression TveIF-5A (20 kDa) in yeast strain *INVSC1*. Lane 1, molecular marker (kDa); lane 2, yeast strain *INVSC1* cell extracts; lane 3, cell extracts after induction with a band of 20 kDa, indicated by the arrow; lane 4, cell extracts before induction. B. Western blot analysis with anti-TveIF-5A (1:60,000) using the samples described in A. Lane 3, the 20 kDa band corresponding to mature TveIF-5Ar in cell extracts after galactose induction, indicated by the arrow. C. Chromatography purification of mature TveIF-5Ar from the soluble fraction (lane 2), indicated by the arrow; lane 1, molecular markers. D. SDS-PAGE analysis of the expression of the 46 kDa precursor rTveIF-5A in *E. coli*. Lane 1, soluble fraction of cell extracts after IPTG induction and insoluble fraction (lane 2). The prominent band of 46 kDa is indicated with the arrow; lane 3, GST-tagged precursor rTveIF-5A after induction; lane 4, western blot analysis using anti-rTveIF-5A antibody for the recognition of purified precursor and mature TveIF-5A; lane 5, preimmune normal mouse serum (SPI) was used as a negative control.

2.6. Expression and purification of precursor and mature recombinant TveIF-5A (rTveIF5A)

The precursor recombinant protein was obtained using the same protocol has been described [17]. Briefly, the recombinant TveIF-5A precursor (rTveIF-5A) protein in the pGEX6-*P*1 vector was expressed using the BL21 *E. coli* strain by adding 1 mM IPTG. Protein purification was performed according to the manufacturer's instructions using a 1 ml GST precast column (GE Healthcare, USA) under native conditions and a Biological HR Workstation (BioRad, USA) chromatographer. The rTveIF-5A precursor protein was eluted in elution buffer (50 mM Tris-HCl,10 mM reduced glutathione, pH 8.0).

The mature recombinant TveIF-5 A was obtained using pYES System: pYES2-*tveif-5a* construct was transformed into the yeast strain INVSC1 for expression. Recombinant TveIF-5A expression was induced for 4 h at 33 °C with 1 M galactose, and the protein was purified from supernatant by ionic chromatography according to the manufacturer's instructions, using a Biological HR Workstation (Bio-Rad, USA). The soluble fraction was filtered (0.22 μm Millipore filter) and applied to a Mono S Bio-Scale Mini Macro-Prep High S precast column of 5 ml (Bio-Rad, USA) equilibrated in buffer A (0.05 M Tris-acetate, pH 6.6, 0.1 mM EDTA, 1 mM dithiothreitol), followed by washing with 50 ml of this buffer (500 μl/min). The proteins was eluted with a linear gradient of 0–50 M KCl in the same buffer (500 μl/min), and 500 μl fractions were collected and analyzed by 12% sodium dodecyl sulphate-PAGE (SDS-PAGE), western blot and mass spectrometer (MS/MS) using 3200 Q TRAP hybrid tandem mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) with a nanoelectrospray ion source (NanoSpray II) to identify the hypusine residue in mTveIF5A fraction, the results were similar as we have reported [17].

2.7. Western blot analysis of mature TveIF-5A

Total protein extract of induced and uninduced cells was obtained after 4 h of culture or total trichomonad proteins from 2×10^7 parasites [18]. Solubilized proteins were boiled in sample buffer [19] before analysis with 12% SDS-PAGE. After electrophoresis, proteins were transferred onto nitrocellulose membranes, blocked with 5% skim milk in PBS-0.1% Tween 20 (PBST) for 18 h at 4 °C, and the membranes were

Downloaded for Anonymous User (n/a) at University of Guadalajara from ClinicalKey.com by Elsevier on June 20, 2022. For personal use only. No other uses without permission. Copyright ©2022. Elsevier Inc. All rights reserved.

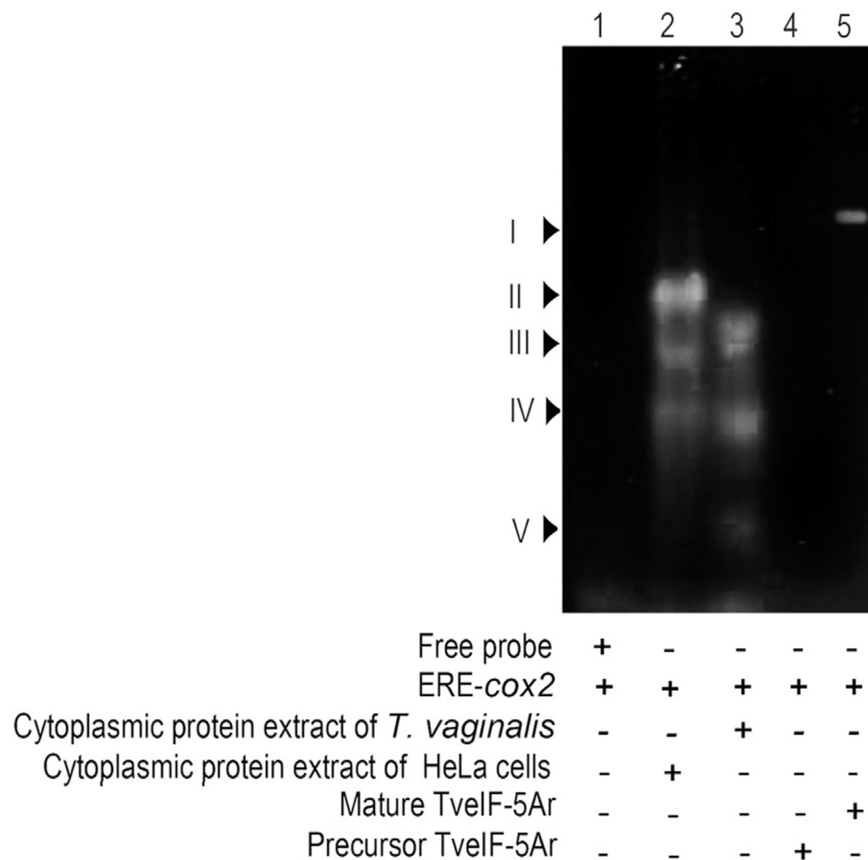

Fig. 3. Hypusine is required for TveIF-5A-ERE *cox-2* and ERE *tvcp39* binding. Lane 1, free probe ERE *cox-2*; lane 2, ERE-*cox-2* incubated with cytoplasmic proteins of HeLa cells; lane 3, ERE-*cox-2* incubated with cytoplasmic protein of *T. vaginalis*; lane 4, ERE-*cox-2* incubated with precursor rTveIF-5A; lane 5, ERE-*cox-2* incubated with mature rTveIF-5A. Complexes observed between native eIF-5A from cytoplasmic protein of *T. vaginalis* and HeLa cells with ERE-*cox-2* are indicated with arrows (II to V). The complex observed between mature rTveIF-5A with ERE-*cox-2* is indicated with the arrow (I).

probed with an anti-peptide antibody (anti-TveIF-5A) (1:60,000 dilution) for 18 h at 4 °C. Then, membranes were washed five times with PBST, incubated with peroxidase-conjugated secondary antibody (1:3000 dilution) (Amersham, USA) for 2 h at 25 °C, and visualized by enhanced chemiluminescence using the ECL Plus Western Blotting Detection System (GE Healthcare, USA) according to the manufacturer's instructions.

2.8. In vitro transcription of ERE sequences

The ERE-like *tvcp39* sequence was *in vitro* transcribed in the presence of biotin-14-uridine triphosphate (UTP) (Invitrogen, USA) to produce labeled ERE-like structures containing the consensus sequence AGAATTTCCAA. Briefly, amplicons were obtained by PCR using sense 5′TAATACGACTCACTATAGGGGTTTAGAATTTCCAATAA-3′ and antisense 5′ATTATGCTGAGTGATATCCCCAAATCTTAAAGGTTATT-3′ primers. The PCR sense primer contained a bacteriophage T7 promoter sequence and an additional GG sequence for enhancing transcription. PCR products were used as templates for RNA synthesis using an *in vitro* transcription MEGA short script kit (Ambion, USA). DNA templates and unincorporated nucleotides were removed by DNase RQ1 (Promega, USA) treatment in the presence of RNase inhibitors (Promega, USA).

2.9. Electrophoretic mobility-shift assay

Three hundred nanograms of mRNA ERE-like structures were incubated with precursor or mature TveIF-5A protein for 25 min on ice in binding buffer. The transfer RNA (tRNA) (sequence) and heparin were added as nonspecific competitors. The mixture was separated on a 1.25% agarose gel. Complex formation between TveIF-5A and ERE-like structures was visualized using SYBR® green (Invitrogen, USA) staining [17].

2.10. Production of anti-TveIF-5A

A four-week-old male New Zealand rabbit was immunized subcutaneously with 150 μg of purified rTveIF-5A using Freund's adjuvant (Sigma, USA) according to the manufacturer's instructions. Immune serum was assayed by western blot against total protein extract from *T. vaginalis* obtained by trichloroacetic acid (TCA)-precipitation [14,18].

3. Results

3.1. Analysis of the ERE sequence in the 3'UTR of tvcp39

Recent reports showed that the cathepsin L-like TVCP39 is encoded by the *tvcp39* gene with an ORF of 915 bp in *T. vaginalis*. Analysis of the genomic clone Race3N-C1-M13F showed that *tvcp39* contains a TAA stop codon and non-coding regions downstream with the putative polyadenylation regulatory sequences (Fig. 1A). The non-coding regions downstream had the AATTTCCAA putative ERE sequence. Analysis of the 57 nt downstream of the TAA codon by RNAfold revealed a sequence with a possible stem-loop RNA secondary structure with a ΔG −2.39 kcal/mol, and the 9 nt hairpin contained the AAATTCTAA sequence (Fig. 1B). This structure was similar to the hairpin loop of mRNA from HeLa cells, mRNA *cox-2* and the iron-responsive element (IRE) of *tvcp4* that binds IRP proteins in a post-transcriptional regulatory mechanism

Downloaded for Anonymous User (n/a) at University of Guadalajara from ClinicalKey.com by Elsevier on June 20, 2022. For personal use only. No other uses without permission. Copyright ©2022. Elsevier Inc. All rights reserved.

mediated by iron in *T. vaginalis* (Fig. 1C). The ERE-like *tvcp39* sequence was compared with previously reported sequences (Fig. 1D) [4,5,6].

3.2. Expression of mature TveIF-5A in a eukaryotic system

To characterize the function of the TveIF-5A, we cloned the mature protein of *tveif-5a* using a yeast system (mTveIF-5A) (Fig. 2A, lane 3), expressed the recombinant protein, which was in the soluble fraction, and purified the protein by chromatography (Fig. 2B, lane 3). TveIF-5A was confirmed by western blot assays using an anti-TveIF-5A peptide antibody (Fig. 2C, lane 2), and a band the expected size of 20 kDa was observed.

3.3. Recombinant mature TveIF-5A interacts with tvcp39 ERE hairpin structures

The interaction of TveIF-5A and the RNA hairpin structure of ERE of the *cox-2* gene and the TvCP39 gene *tvcp39* was evaluated with *in vitro* transcription [16] and electrophoretic mobility shift assays (EMSA). We used the mature and the precursor TveIF-5A prepared from yeast and *E. coli*, respectively, as described in the methods. The results showed the RNA-protein complex formed between the ERE-*cox 2* and mature TveIF-5A (Fig. 3 lane 4). In contrast, no complex formation was observed with precursor TveIF-5A (Fig. 3 lane 5).

3.4. The ERE-like tvcp39 sequence interacts with TveIF-5A

To determine whether the mature TveIF-5A bound to the RNA hairpin of *tvcp39* ERE-like sequence due to the ability of this protein to interact with the ERE structure of *cox-2*, we analyzed the RNA *tvcp39* ERE-like structure by EMSA. We observed a slight decrease in the mobility of mature TveIF-5A and ERE-*tvcp39* due to the RNA-protein complex formation between the two (Fig. 4, lane 5). In contrast, no complex formation was observed with precursor TveIF-5A (Fig. 4 lane 4). The electrophoretic profile showed three RNA-protein complexes (II, III, IV) formed between the ERE-*tvcp39* and HeLa cell extracts (Fig. 4 lane 2) containing the eIF-5A protein (used as a positive control). The profile using *T. vaginalis* with native TveIF-5A showed five RNA-protein complexes (Fig. 4, lane 3). The interaction of ERE-*tvcp39* and TveIF-5A was diminished with the antibody anti-rTveIF-5A (Fig. 4 lane 6), which inhibited the complex formation. The interaction of the *tvcp39* ERE-like sequence with the precursor TveIF-5A and the anti-rTveIF-5A antibody did not show any complex formation (Fig. 4, lane 7). With the ERE-*tvcp39* and an unrelated glutathione-S-transferase (GST) protein, no complex formation was observed (Fig. 4, lane 8). The unrelated antibody anti-tubulin and the unrelated GST protein interactions with the cytoplasmic protein extract of *T. vaginalis* were used as negative controls (Fig. 4, lane 9 and 10, respectively). These results showed that only mature TveIF-5A binds the ERE-like sequence of *tvcp39*, suggesting that the mature TveIF-5A may participate in mRNA stability by specifically binding to ERE-like sequences.

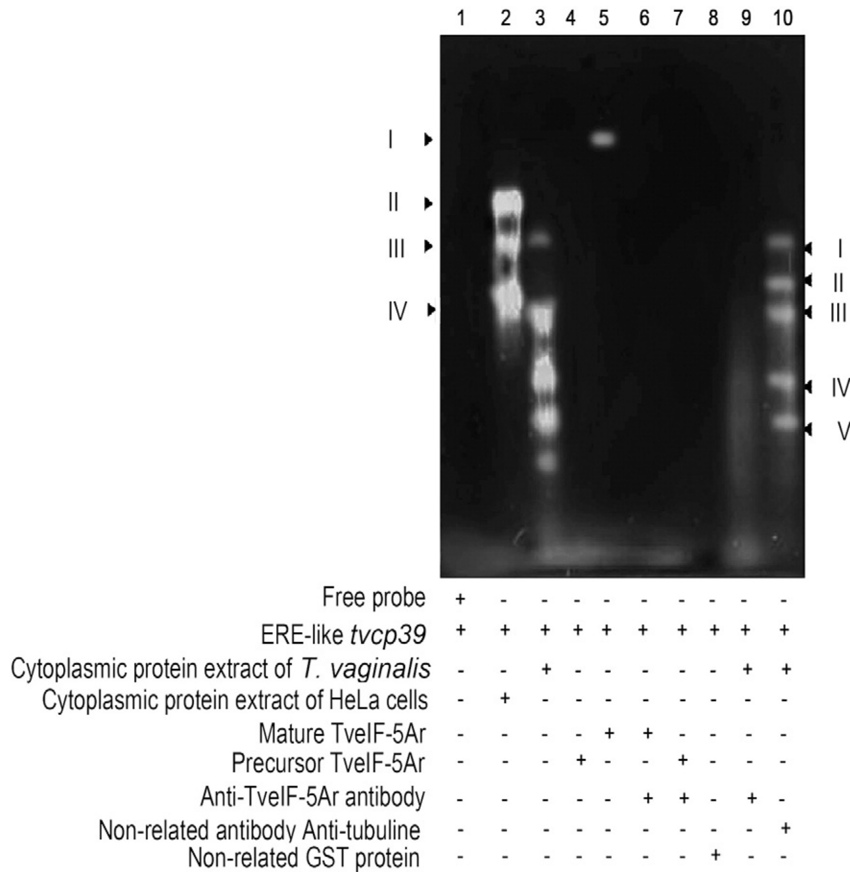

Fig. 4. Mature rTveIF-5A interacts with the ERE-like sequence of *tvcp39*. RNA-binding activity of TveIF-5A was assessed by electrophoretic mobility shift assays (EMSA). Lane 1, free probe ERE-*tvcp39*; lane 2, ERE-*tvcp39* incubated with cytoplasmic proteins of HeLa cells; lane 3, ERE-*tvcp39* incubated with cytoplasmic proteins of *T. vaginalis*; lane 4, ERE-*tvcp39* incubated with precursor rTveIF-5A; lane 5, ERE-*tvcp39* incubated with mature rTveIF-5A; lane 6, ERE-*tvcp39* incubated with mature rTveIF-5A and anti-TveIF-5A antibody.; lane 7, ERE-*tvcp39* incubated with precursor rTveIF-5A and anti-TveIF-5A antibody; lane 8, ERE-*tvcp39* incubated with unrelated GST protein; lane 9, ERE-*tvcp39* incubated with cytoplasmic proteins of *T. vaginalis* and unrelated antibody anti-tubulin. Complexes observed between native TveIF-5A from cytoplasmic proteins of *T. vaginalis* and HeLa cells with ERE-*tvcp39* are indicated with arrows (I to V). The complex observed between mature TveIF-5Ar with ERE-*tvcp39* is indicated with the arrow (I).

Downloaded for Anonymous User (n/a) at University of Guadalajara from ClinicalKey.com by Elsevier on June 20, 2022. For personal use only. No other uses without permission. Copyright ©2022. Elsevier Inc. All rights reserved.

As a negative interaction, control-loop probes (that do not contain the ERE sequence) were used, which showed no interaction with TveIF-5A (data not shown).

3.5. Specific interaction between the mature TveIF-5A and ERE-like sequence of tvcp39

To confirm the interaction of mature TveIF-5A with ERE-*tvcp39,* super shift assays were-performed using the recombinant antibody anti-rTveIF-5A at different concentrations (1 M, 2.5 M, 5 M or 10 M). The results showed that the antibodies super shifted the complex of mature TveIF-5A with the *tvcp39* ERE-like sequences (Fig. 5, lanes 2–5). Higher concentrations of anti-TveIF-5A decreased the amount of the complex TveIF-5A-ERE-*tvcp39* that was observed (Fig. 5, lane 4 and 5). These results suggest that the interaction of ERE-*tvcp39* and mature TveIF-5A is-specific.-As-a control experiment, we assessed the *tvcp39* ERE-like interaction with precursor-TveIF-5A and the anti-TveIF-5A at the same molar concentrations, and complex formation was not found (Fig. 6 lanes 6–10).

4. Discussion

Trichomonas vaginalis has several cysteine proteases CPs that are virulence factors, such as TvCP39 and TvCP65, which are involved in cytotoxicity toward host cells [14,16,18,20,21]. The expression of both of these proteins is dependent on a mechanism mediated by polyamines, possibly at the posttranscriptional level [22]. Recently, we reported two genes encoding TveIF-5A, and western blot assays using an anti-TveIF-5A antibody immunodetected two proteins of 19 and 20 kDa, which could correspond to the TveIF-5A precursor and mature forms, respectively [11,23]. eIF-5A undergoes a specific posttranslational modification called hypusination, which gives the protein biological activity [24]; this modification is irreversible and is carried out in seconds [7]. The first step in the two-step hypusination reaction involved the enzyme deoxyhypusine synthase (DHS), which is expressed in *T. vaginalis* [17,24] and may be responsible for the TveIF-5A precursor protein hypusination *in vivo*. The second step is carried out by the deoxyhypusine hydroxylase (DOHH) enzyme, which hydroxylates a 4-aminobutyl of lysine, although this step is not essential for hypusination in some organisms such as yeast. For example, *S. cerevisiae* with *dohh* gene mutations is viable and has a growth rate similar to wild type *S. cerevisiae*, suggesting that DHS is sufficient to carry out the hypusination [25,26] Mature eIF-5A has multiple functions [5,6,24] and binds to specific sequences called eIF-5A response elements (ERE) located in the 3'UTR of the mRNA [4,6]. The *cox-2* mRNA is regulated through eIF-5A polyamine-dependent binding to the ERE structure [6]. In this study we analyzed the 3'UTR *tvcp39* structure, which showed an atypical ERE sequence that forms a stem-loop structure (Fig. 1) and has two nucleotide changes compared to the consensus sequence reported in the 3'UTR of *cox-2* [4,6]. These results are consistent with the data that suggest that there may be up to three nucleotide changes in the ERE sequence. Through systematic evolution of ligands by exponential enrichment (SELEX), EREs sequences in ~800 mRNA and genes have been implicated in important cellular functions [5]. eIF-5A in eukaryotes

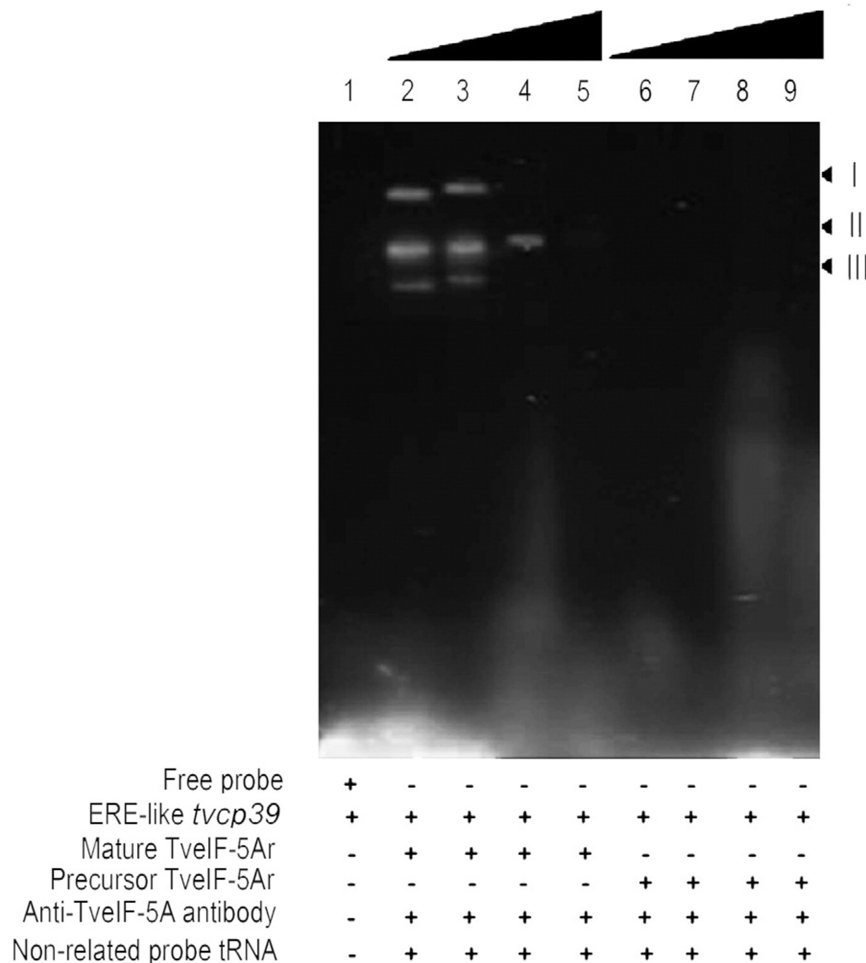

Fig. 5. RNA binding specificity of mature TveIF-5A and ERE-*tvcp39*. Super shift in agarose gel of mature rTveIF-5A interacting with ERE- *tvcp39*. Molar concentrations of anti-TveIF-5A, lanes: 2 and 6, anti-rTveIF-5A (1×); 3 and 7, anti-rTveIF-5A (2.5×); 4 and 8, anti-rTveIF-5A (5×); 5 and 9, anti-rTveIF-5A (10×).

Downloaded for Anonymous User (n/a) at University of Guadalajara from ClinicalKey.com by Elsevier on June 20, 2022. For personal use only. No other uses without permission. Copyright ©2022. Elsevier Inc. All rights reserved.

is essential for cell survival and proliferation; it may be involved in the translation of a specific subset of mRNAs involved in the cycle cell [24]. The depletion of eIF-5A in *Saccharomyces cerevisiae* has been shown to decrease protein synthesis by ~30%, resulting in the changes in several mRNAs that encode proteins involved in DNA replication and cell cycle progression from G1 to S phase [7]. Other studies have suggested that eIF-5A may serve as a carrier of proteins for the nuclear export of Rev or Rex, an HIV-I cofactor [3,27].

5. Conclusions

Our results suggest a possible posttranscriptional regulation mechanism in *T. vaginalis* through the stem-loop ERE-like structure in the 3'UTR of *tvcp39* mRNA which interacts with mature TveIF-5A.

Acknowledgments

This work was supported by UACM, and grant from CONACYT (83808) Mexico (to M.E.A.S.). We appreciate the excellent technical assistance of Laura Isabel Vazquez Carrillo M.Sc. and Brenda Herrera Villalobos of the UACM.

References

[1] A.C. Childs, D.J. Mehta, E.W. Gerner, Polyamine-dependent gene expression, Cell. Mol. Life Sci. 60 (2003) 1394–1406.
[2] M.H. Park, The post-translational synthesis of a polyamine-derived amino acid, hypusine, in the eukaryotic translation initiation factor 5 A (eIF5A), J. Biochem. 139 (2006) 161–169.
[3] V.W. Pollard, M.H. Malim, The HIV-1 Rev protein, Annu. Rev. Microbiol. 52 (1998) 491–532.
[4] A. Xu, K.Y. Chen, Hypusine is required for a sequence-specific interaction of eukaryotic initiation factor 5A with post systematic evolution of ligands by exponential enrichment RNA, J. Biol. Chem. 276 (2001) 2555–2561.
[5] A. Xu, D.L. Jao, K.Y. Chen, Identification of mRNA that binds to eukaryotic initiation factor 5A by affinity co-purification and differential display, Biochem. J. 384 (2002) 585–590.
[6] M.T. Parker, E.W. Gerner, Polyamine-mediated post-transcriptional regulation of *cox-2*, Biochimie 84 (2002) 815–819.
[7] M.M. van Oers, M. van Marwijk, M.S. Kwa, J.M. Vlak, A.A. Thomas, Cloning and analysis of cDNAs encoding the hypusine-containing protein eIF5A of two lepidopteran insect species, Insect Mol. Biol. 8 (1999) 531–538.
[8] M. Hanazawa, I. Kawasaki, H. Kunitomo, K. Gengyo-Ando, K.L. Bennett, S. Mitani, Y. Iino, The *Caenorhabditis elegans* eukaryotic initiation factor 5A homologue, IFF-1, is required for germ cell proliferation, gametogenesis and localization of the P-granule component PGL-1, Mech. Dev. 121 (2004) 213–224.
[9] H.A. Kang, J.W. Hershey, Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of *Saccharomyces cerevisiae*, J. Biol. Chem. 269 (1994) 3934–3940.
[10] K.K. Kim, L.W. Hung, H. Yokota, R. Kim, S.H. Kim, Crystal structures of eukaryotic translation initiation factor 5A from *Methanococcus jannaschii* at 1.8 Å resolution, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 10419–10424.
[11] B.I. Carvajal-Gamez, R. Arroyo, R. Lira, C. López-Camarillo, M.E. Alvarez-Sánchez, Identification of two novel *Trichomonas vaginalis eif-5a* genes, Infect. Genet. Evol. 10 (2010) 284–291.
[12] B.I. Carvajal-Gamez, R. Arroyo, M. Camacho-Nuez, R. Lira, M. Martínez-Benitez, M.E. Alvarez-Sánchez, Putrescine is required for the expression of eif-5a in *Trichomonas vaginalis*, Mol. Biochem. Parasitol. 180 (2011) 8–16.
[13] B.I. Carvajal-Gamez, L.I. Quintas-Granados, R. Arroyo, L.I. Vázquez-Carrillo, L.L. Ramón-Luing, E. Carrillo-Tapia, M.E. Alvarez-Sánchez, Putrescine-dependent re-localization of TvCP39, a cysteine proteinase involved in *Trichomonas vaginalis* cytotoxicity, PLoS One 9 (2014), e107293.
[14] M.E. Alvarez-Sánchez, L. Ávila-González, C. Becerril-García, L. Fattel-Facenda, J. Ortega-López, R. Arroyo, A novel cysteine proteinase (CP65) of *Trichomonas vaginalis* involved in cytotoxicity, Microb. Pathog. 28 (2000) 193–202.
[15] L.S. Diamond, The establishment of various trichomonads of animals and man in axenic cultures, J. Parasitol. 43 (1957) 488–490.
[16] L.L. Ramón-Luing, F.J. Rendón-Gandarilla, J. Puente-Rivera, L. Ávila-González, R. Arroyo, Identification and characterization of the immunogenic cytotoxic TvCP39 proteinase gene of *Trichomonas vaginalis*, Int. J. Biochem. Cell Biol. 43 (2011) 1500–1511.
[17] L.I. Quintas-Granados, B.I. Carvajal-Gamez, J.L. Villalpando, J. Ortega-Lopez, R. Arroyo, E. Azuara-Liceaga, M.E. Álvarez-Sánchez, Bifunctional activity of deoxyhypusine synthase/hydroxylase from *Trichomonas vaginalis*, Biochimie 24 (2015), http://dx.doi.org/10.1016/j.biochi.2015.09.027.
[18] M.E. Alvarez-Sanchez, B.I. Carvajal-Gamez, E. Solano-Gonzalez, M. Martınez-Benitez, A.F. García, J.F. Alderete, R. Arroyo, Polyamine depletion down-regulates expression of the *Trichomonas vaginalis* cytotoxic CP65, a 65-kDa cysteine proteinase involved in cellular damage, Int. J. Biochem. Cell Biol. 40 (2008) 2442–2451.
[19] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[20] R. Hernández-Gutierrez, L. Avila-González, J. Ortega-López, F. Cruz-Talonia, G. Gómez-Gutierrez, R. Arroyo, *Trichomonas vaginalis*: characterization of a 39-kDa cysteine proteinase found in patient vaginal secretions, Exp. Parasitol. 107 (2004) 125–135.
[21] M.E. Alvarez-Sánchez, E. Solano-González, C. Yañez-Gómez, R. Arroyo, Negative iron regulation of the CP65 cysteine proteinase cytotoxicity in *Richomonas vaginalis*, Microbes Infect. 9 (2007) 1597–1605.
[22] E.E. Figueroa-Angulo, F.J. Rendón-Gandarilla, J. Puente-Rivera, J.S. Calla-Choque, R.E. Cárdenas-Guerra, J. Ortega-López, L.I. Quintas-Granados, M.E. Alvarez-Sánchez, R. Arroyo, The effects of environmental factors on the virulence of *Trichomonas vaginalis*, Microbes Infect. 14 (2012) 1411–1427.
[23] B.I. Carvajal-Gamez, L.I. Quintas-Granados, R. Arroyo, G. Mendoza-Hernández, M.E. Alvarez-Sánchez, Translation initiation factor eIF-5A, the hypusine-containing protein, is phosphorylated on serine and tyrosine and O-glycosylated in *Trichomonas vaginalis*, Microb. Pathog. 52 (2012) 177–183.
[24] M.H. Park, The essential role of hypusine in eukaryotic translation initiation factor 4D (eIF-4D). Purification of eIF-4D and its precursors and comparison of their activities, J. Biol. Chem. 264 (1989) 18531–18535.
[25] G.M. Thompson, V.S. Cano, S.R. Valentini, Mapping eIF5A binding sites for Dys1 and Lia1: *in vivo* evidence for regulation of eIF5A hypusination, FEBS Lett. 555 (2003) 464–468.
[26] M.H. Park, K. Nishimura, C.F. Zanelli, S.R. Valentini, Functional significance of eIF5A and its hypusine modification in eukaryotes, Amino Acids 38 (2010) 491–500.
[27] M. Ruhl, M. Himmelspach, G.M. Bahr, F. Hammerschmid, H. Jaksche, B. Wolff, H. Aschauer, G.K. Farrington, H. Probst, D. Bevec, Eukaryotic initiation factor 5A is a cellular target of the human immunodeficiency virus type 1 Rev activation domain mediating trans-activation, J. Cell Biol. 123 (1993) 1309–1320.

Downloaded for Anonymous User (n/a) at University of Guadalajara from ClinicalKey.com by Elsevier on June 20, 2022. For personal use only. No other uses without permission. Copyright ©2022. Elsevier Inc. All rights reserved.