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Review Article

PUF Proteins as Critical RNA-Binding Proteins in TriTryp Parasites: A Review Article

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Received 10 May 2024
Accepted 20 Aug 2024

Keywords:
RNA-binding proteins; PUF proteins; *Trypanosome brucei*; *Trypanosome cruzi*; *Leishmania*

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Abstract

In eukaryotes, translation is a fundamental step in the long pathway of protein synthesis within the cell. In this process, several proteins and factors have involved directly or indirectly, individually or in association with other elements to contact mRNA. For perfect translation, many essential modifications should be done, such as cis-splicing to remove introns and two main events for capping and poly A polymerization in 5' and 3' end of mRNA, respectively. Gene expression is then regulated at both translation and stability of the target mRNA molecule levels. Pumilio/FBFs (PUFs) are the main group of RNA-binding proteins which bind to the 3'-UTR of target RNA and thereby regulate the fate, stability and subcellular localization of mRNAs and adjust the translated protein level. PUF proteins have been found both in nucleus where that bind to precursor mRNA, for processing and maturation of rRNA, and in cytoplasm where that bind to mRNA, stall the ribosomes, suppress the translation and localization of the mRNA. They can regulate the expression of mRNAs through activation or suppression of translation. Therefore, these proteins have recently garnered much attention as new generation of therapeutic targets against diseases such as cancer and neurological disorders. In comparison to other eukaryotes, trypanosomatids have a high number of PUF proteins, which function not only as gene expression regulatory factors but also in several biological processes such as differentiation and life-cycle progression of the cells. Here, we review the molecular and biological roles of known PUF proteins in TriTryp parasites (*Trypanosome brucei*, *T. cruzi* and *Leishmania*) beside some other parasites.

Introduction

During growth cycle of eukaryotic cells, there are three important phases, transcription, post-transcription and translation. There is a principal difference between prokaryotes and eukaryotes at the early stage of mRNA translation (1). In prokaryotes, translation begins before the end of transcription to prevent mRNA degradation. Hence, no modification to preserve mRNA molecule is necessary. In contrast, in eukaryotes, transcription and translation are completely separated and proceed in two different locations, nucleus and cytoplasm, respectively. Therefore, eukaryotic precursor mRNAs need some modifications in different regions in order to maintain their stability, to protect them from degradation and to enable the delivery of intact molecules to cytoplasm for use in translation (2). Three main modifications include: 1) 5' capping through addition of one guanine nucleotide; 2) RNA splicing to generate intron free transcripts; and 3) polyadenylation through adding a long poly A-tail at the 3'-end. Also, the adaptation of cells with the environment is essential and directly depends on the regulation of gene expression (2). Therefore, any deficiencies in regulation of gene expression, e.g. at post-transcriptional level, has a drastic effect on protein synthesis and eventually the proper functionality of the protein and the cell.

In eukaryotes, 5' and 3'-untranslated regions (UTRs) play very important roles in post-transcriptional gene regulation (3). Translation initiation (in 5'-UTR) and termination (in stop codon) are controlled by some Cis- and trans-acting elements. Several elements like RNA-binding proteins (RBPs) are involved individually or together or even in association with other factors. Among the known RBPs are zinc finger proteins, proteins with K homology domains, DEAD/DEAH boxes, and PUFs (also named Pumilio (PUM)) which are able to associate directly or indirectly with RNA molecules through some specific binding motifs or domains (4).

There is a superfamily of RBPs with a unique structure and function which is categorized into three crucial groups named PUF, Nop9 and PUM3. These proteins bind 3'-UTR sequences upstream of poly A-tail both in the nucleus and cytoplasm through a link between some puf domains and single RNA bases (5). Nop9 and PUM3 are single-copy and responsible for contact with precursor or immature RNA in nucleus, such as 18sRNA and rRNA, respectively. Nop9 contains 11 Puf domains that bind to specific sequences on RNA, but PUM3 binds nonspecific sequences on dsDNA or dsRNA. PUF proteins are the main members of PUF superfamily that usually bind mRNA. In contrast to Nop9 and PUM3, PUFs are multi-copy and cytoplasmic proteins that bind mature mRNAs (5). PUF proteins have been found in a wide range of eukaryotes from lower eukaryotes (like fungi and parasites) to high eukaryotes (4, 6, 7). At first, PUF protein was identified in *Drosophila melanogaster* embryo, where it disrupts translation of mRNA transcripts (8). Also, *Caenorhabditis elegans* has some proteins such as Fem-3-binding factors (FBF) that are structurally and functionally comparable with PUFs. Hence, RNA-binding PUF proteins have taken this name from Pumilio (Pum) and FBF of *D. melanogaster* and *C. elegans*, respectively (5).

Here, we review the function of these proteins in protozoan parasites and discuss their role in parasites with a focus on trypanosomatidae. These parasites cause diseases which are hardly curable due to lack of efficient therapy and are uncontrollable due to lack of effective preventive vaccine. Hence, knowing more about the biology of these parasites will help researches in developing more effective vaccines or therapeutics.

Puf-binding and RNA-binding domains

All proteins of PUF family have a highly conserved C-terminal domain to recognize and bind mRNA molecules as known Pumilio-homology domain (PUM-HD, ~36 aa) (9). Crystal structure observations have shown that the binding domain of these proteins contains eight tandem amino acid repeats as named Puf domain. These proteins also have a domain in the core of binding region which is located mostly in C-terminal (7) and sometimes in the center of the protein (10), and in rare cases in N-terminus (11). Point mutations in the core region of Puf-binding motif decreases binding affinity of the protein. This region has a unique structure that helps to bend and specifically bind RNA molecules through eight repeats of tripartite recognition motif. Each repeat interacts with one nucleotide of RNA in cytoplasm within PRE (PUF Response Element). This arrangement is conserved and makes three-helical structures on mRNA between two regions including the 3'-UTR and stop codon/Poly A-tail. The structure helps bend the protein and makes a crescent-shaped form to recognize and bind the sequences with variable size in the 3'-UTR on mRNA. The Puf domain is delimited by two conserved regions in N- and C-terminus.

These proteins bind through multiple repeat domains by interaction with different factors such as Nanos and brain Tumor in *Drosophila* (12), DAZ-like proteins in human cells (13), and Nos3 in *C. elegans* (14). In PUF proteins, each RNA-binding domain contains a 5'-UGU-3' triplet in the core. The UGU triplet in Nanos response Elements (NRE) is essential for interaction with RNA (8). It is reported that there are three repression domains in N-terminal of the PUF protein in *Drosophila* (15).

TriTryp parasites and high copy number of PUF genes

Tritryp is a name derived from three protozoa parasites belong to Kinetoplastida including, *T. brucei*, *T. cruzi* and *Leishmania* that cause emerging infection diseases in human after transmission to a mammalian host. These parasites are transmitted to human or animals through insect vectors. Despite many efforts and research, these diseases have no effective therapy approaches or vaccine (16-18). The study of these parasites is important because the world is facing a challenge in the field of treatment and vaccine against leishmaniasis (19).

PUF proteins are different with respect to their number, location, size, and structure in TriTryp parasites (11, 20). Although these proteins are conserved during evolution (21), the number of *puf* genes in different organisms is highly variable from 2 to 26 (Table 1). Each PUF protein presumably has its special RNA targets and some of the targets may be regulated by a complex of different PUFs (22).

The number of PUF genes is very different in parasites. For example at least two PUF genes in *Plasmodium* (Table 1) and at most 11 genes in trypanosomatidae have been identified (Table 2).

The number of PUF proteins in trypanosomatids is much more than other eukaryotes. Reason behind this event may be the importance of post-transcriptional regulation that needs more RNA-binding proteins. For instance, in *T. brucei*, 11 PUF proteins have been found which have different domain arrangements. Only PUF2, 3, 4 and 6 proteins keep the PUF eight repeats. Two proteins, PUF8 and PUF11, are conserved in Kinetoplastida. In these parasites, only three PUF proteins, i.e. PUF7, 8 and 10, are in the nucleolus (41).

According to several reports, locations of PUF proteins in different cells are variable (4, 5). Regarding the role of PUF proteins in translation, most of them have been found in cytoplasm (to bind cis-elements on mRNA target, suppress the translation and mRNA localization) and sometimes in nucleus (to bind the pre-mRNA and also to develop the processing and maturation of rRNA) (4, 5). Exceptionally, some PUF proteins such as

PUF7 in *T. brucei* (41) has been observed in both cytoplasm and nucleolus (32).

Digenetic microorganisms, including single-cell intracellular (*Plasmodium*, *Toxoplasma, Trypanosoma, Leishmania*) and extracellular (*Neospora caninu, Giardia*) parasites, have usually complex growth cycles and grow in at least two environments different in temperature, pH, and composition.

Table 1: The characteristics and function of PUF proteins in some organisms

Organism/genus	*Species*	*PUF family members*	*Description*	*Reference*
Drosophila	*D. melanogaster*	1	Posterior axis of embryo, mitotic arrest of primordial germ cells, migration of primordial germ cells and maintenance of germline stem cells.	(23)
Caenorhabditis	*C. elegans*	10	PUFs have different physiological roles including proliferation, differentiation and regulate of lifespan.	(23), (24)
Arabidopsis	*A. thaliana*	26	They are variable in number, position of Puf repeats (3-10) and identify of TMs.	(25)
Yeast	*S. cerevisiae*	6 proteins (5 cytoplasmic and one nuclear)	Regulate aging, mating-type switching and mitochondrial function.	(26)
			PUF1 and PUF2 regulate cellular response to environmental stress.	(27)
			Cytoplasmic PUF2 is involved in membrane-associated proteins and stress granules formation during glucose deprivation.	(28, 29)
			PUF3 is involved in mRNA localization to mitochondria.	(30)
			PUF4 has regulatory role via increasing nuclear ribosomal mRNA degradation.	(29)
			PUF5 is involved in mRNA localization to near peroxisome.	(31)
			PUF6 is expressed in both cytoplasm and nucleus.	(32)
Mammalian	Mice	2	Pum1 and pum2 promote differentiation and self-renewal of ESCs, respectively.	(33)
Neospora	*N. caninum*		PUF1 is identified as a key virulence and infectivity factor and may be used to develop live attenuated vaccines.	(34)
Toxoplasma	*T. gondii*	2	PUF1 is expressed in whole cell cycle and regulate the proliferation or/and differentiation.	(10)
Plasmodium	*P. berghei* *P. falciparum*	3	PUF1 and PUF2 are expressed in sporozoites and have regulatory roles through both 3' and 5'-UTRs. PUF1 has a vital role in whole cell cycle and PUF2 is essential for differentiation, complete sexual cycle and parasite infectivity. PUF3 is a nucleolar protein which participates in ribosomal biogenesis.	(34, 35)
Giardia	*G. lamblia* *G. intestinalis*	5-6	They are cytoplasmic localization and homologous with *S. cerevisae* PUF proteins.	(3, 37)

Table 2: Summarized information about known PUF proteins and their biological functions in TriTryp kinetoplastids

Organism/genus	*Species*	*PUF family members*	*Description*	*Ref*
Trypanosome	*T. brucei*	11	PUF1 is essential for cell viability, growth of blood-stream forms.	(36, 37)
			PUF2 is essential for growth of bloodstream forms.	(38)
			PUF3 is involved in parasite growth and differentiation.	(39)
			PUF4 has no effect on cell growth.	(37)
			PUF5 is cytoplasmic and its overexpression is lethal, but knockdown of *PUF5* has no effect on parasite differentiation.	(40)
			PUF7 is a nuclear protein and necessary for rRNA processing and growth.	(41)
			PUF8 is a nuclear protein and homologue with *S. cerevisiae* PUF6.	(41)
			PUF9 in multifunctional and controls transcripts through mRNA degradation in S-phase, cell division cycle.	(42)
			PUF10 is in nucleolus, involved in rRNA processing, maturation of 5.8S rRNA and expression of some specific genes.	(43)
	T. cruzi	>10	PUF6 is a cytoplasmic protein and expressed in whole cell cycle with no stage specific regulation. This protein is up-regulated in metacyclic form of the parasite and is involved in suppression of mRNAs expressed in the infective form.	(44)
Leishmania	*L. infantum*	11	Recombinant PUF proteins (especially PUF1 and PUF2) used for serodiagnosis or as vaccine candidate.	(11)
			PUF6 has a role in interaction with SIDER2 and mRNA destabilization.	(45)
			PUF 1, 4, 6, 7, 8 and 10 are expressed in the pro-mastigote.	(46)
	L. major	11	PUF1 is involved in protein synthesis.	(47)

The intracellular parasites have more than one morphological forms in their cell cycle (at least two forms in *Leishmania* and *Plasmodium*, and four forms in *T. brucei* and *T. cruzi*), and their differentiation occurs through inactivation and activation of genes specific for each stage. For rapid differentiation from intracellular to intercellular form and vice versa**,** these pathogens use a complex gene regulatory mechanism at post-transcription stage. Moreover, gene arrangement and mechanism of gene expression is highly different in trypanosomatidae from other eukaryotes. Two major differences are: 1) lack of promoters as conventional regulators of gene expression for most protein-coding genes; indeed, initiation of transcription by RNA polymerase II is not regulated; and 2) transcription of mRNAs as long polycistronic transcripts which are later on processed by trans-splicing and poly A addition (48).

To regulate the amount of mRNA transcripts, the cytoplasmic mRNA level should be modulated by degradation. Therefore, mRNA lifespan is short and depends on exonucleolytical processes which are controlled by cis-elements in 3'-UTR of mRNA and also trans-acting elements (49). Hence, in trypanosomatids these proteins are able to suppress or activate gene expression (20). For this reason, the high number of PUF proteins indicates their important roles and function in gene regulation. Furthermore, sequence homology of PUF proteins in trypanosomatids are much more prevalent; for example, TriTryp parasites including *T. brucei*, *T. cruzi* and *Leishmania* spe-

cies contain at least 10 PUF proteins with a high level of homology to each other (50).

So far, most of our knowledge on parasites has been obtained from *Plasmodium*, *Trypanosome* and *Toxoplasma*. Given the structural similarities and differences, as well as differences in the number of PUF proteins, much more studies are needed to determine their roles. Table 2 summarizes the informations such as gene and protein length and chromosome number that carries the gene in different species and strains of TriTryp parasite family.

General function of PUF proteins

Several reports have shown that PUF proteins have a life stage-specific expression and are involved in different cellular processes from molecular level to biological functions. Generally, regulation of gene expression is associated with both translation control and increasing or decreasing of the mRNA stability. These proteins bind to the 3'-UTR of RNA molecule and regulate translation at early stages. The outcome of protein binding to mRNA is the protection of molecule from enzymatic degradation, which leads to the increase in mRNA half-life and stability (51). Unlike the function of other RBPs, binding of PUF proteins to RNA lead to repression of mRNA translation either poly A-tail and also increasing of mRNA degradation and subcellular localization of transcripts' targets. Indeed, the main role of PUF proteins binding to specific cis-elements on their mRNA target and decreasing of translation, and also ribosome stalling (4, 51).

Despite the preservation of PUF proteins, there is no conservation in mRNA targets which are controlled by these groups of proteins (52). It is proposed that the PUF proteins function through binding to specific ribonucleotide sequences in the 3'-UTR of different mRNA molecules, thus, they can control gene expression at post-transcriptional level. Any structural modification in these proteins or RNA sequences can block the activity of protein or decreases translation and protein synthesis.

Some other studies have suggested other biological roles for these proteins such as effect on infectivity, precise translation, mRNA stability and localization. So, probably, the engineering of these proteins can be considered as anti-infective agents (9, 53). Some of these roles are more interesting, particularly in parasites that usually have a complex life cycle and gene regulation that is modulated by UTR regions. Based on the available reports, here we aim to review the role of these proteins in parasites. However, more studies are needed to clarify their specific roles.

Functions of PUF proteins in parasites other than TriTryp: Neospora

Disruption of a gene encoding cytoplasmic PUF1 protein in *Neospora caninum* (as a pathogenic parasite for cattle and dogs) through CRISPR/Cas9 system does not influence the survival, differentiation and cyst formation of this extracellular parasite (54). However, it decreases virulence and infectivity of the parasite, which are critical for the development of a live attenuated vaccine against this parasitic disease in cattle, dogs and birds (54).

Toxoplasma

In *T. gondii*, PUF proteins control some vital processes such as proliferation, differentiation and parasite development (10). PUF1 is expressed in cytoplasm in both stages, bradyzoites and tachyzoites, but the expression rate of PUF1 is very different during cell cycle and is much higher in bradyzoites compared to tachyzoites (10). Therefore, it causes tachyzoite-bradyzoite transformation and helps respond rapidly to environmental changes (10). So far two PUF proteins have been identified in *T. gondeii*. They have different RNA targets due to variation in length and sequence (55).

Plasmodium

Early studies have demonstrated that *Plasmodium* parasites have three different genes

encoding PUF proteins. PUF1 and PUF2 are transcribed in gametocytes and spoeozoites (infectious form of the malaria parasite) forms, respectively (35). The size of PUF1 and PUF2 in *P. berghei* is different (1183 versus 477 aa), but the two proteins have partial homology (~27%) in Puf domains (56, 57). Although PUF proteins mostly recognize motifs in 3'-UTR, however, PUF2, which represses translation of pfs25 and pfs28 mRNAs, recognizes motifs in 5'-UTR (39). It seems, PUF2 in *Plasmodium* is involved in differentiation and transformation of parasite (58). Therefore, PUF proteins as multifunctional translation regulators play their roles by attachment to 3' or 5'-UTRs (59).

In *P. falciparum*, only PUF2 and in *P. berghei*, both PUF1 and PUF2 proteins are expressed in sporozoites (57). PUF1 in *P. falciparum* is able to recognize NRE sequence both in vitro and in vivo (56). In *P. berghei*, PUF1 may have a vital role throughout the growth cycle (57). In *Plasmodium*, unlike PUF1 that is important in whole cell cycle, PUF2 has a critical role in differentiation and transformation of parasite between the two hosts, namely an insect and a mammal. Other reports have shown that deletion of Puf2 in *P. falciparum* or *P. berghei* promote the differentiation of gametocytes in these parasites (25). Indeed, knocking out Puf2 gene in *P. berghei* leads to inhibition of differentiation of the parasite and also its inability to initiate the infection (57). On the other side, overexpression of PUF2 in *P. falsiparum* leads to the repression of mRNA, and knockdown of this protein improves gametocytogenesis (58). It is very important to complete sexual cycle of parasite transmission from insect vector to human in order to maintain the infectivity. Therefore, before and during parasite differentiation process between the two hosts, regulation of mRNA translation is critical. This is enabled by several RNA-binding proteins including PUFs (60). Furthermore, in *Plasmodium*, disruption of PUF2 showed that this protein regulates the transition stage in sporozoites and has a critical role in gametocytes, while PUF1 gene had no effect on this cellular stage (10). It is also indicated that PUF2 in *Plasmodium* is able to inhibit expression of UIS2 (Upregulated in Infective Sporozoites 2), which is highly expressed in salivary gland sporozoites and is essential for the parasite's survival in liver stage in mammalian cell. So repression of UIS2 expression may be useful in anti-malaria therapy (35). Recently, another PUF protein (PUF3) was recognized in *P. falciparum* that is located in nucleus and participates in ribosomal biogenesis (34).

Giardia

Human intestinal parasites as *Giardia* have highly compact genome with short UTRs. In *G. lamblia* five genes have been identified as puf repeats. Four of them have five to eight repeats in the C-terminal half of the PUF protein and the other one has three repeat domains in N-terminus. Therefore, the latter should be considered as a pseudogene. Moreover, *in silico* prediction identified six PUF proteins in *G. intestinalis* that all have cytoplasmic localization (5). BLASTP results from *Giardia* PUF proteins and *Saccharomyces* genome database indicated homology with *S. cerevisae* PUF proteins.

Functions of PUF proteins in TriTryp parasites: Trypanosome brucei

T. brucei (African trypanosome) is an extracellular parasite that is transmitted by tsetse fly to human and inhabits the blood plasma and body fluids and cause sleeping sickness. This parasite has a large PUF family with 10 main members, one gene for each PUF and two genes for PUF9 (37, 40, 41). Single-cell RNA sequencing has shown that PUFs beside other proteins like RBPs, zinc-finger and U-rich RNA binding protein families are presented in the highest number in these parasites (61). PUF1 is essential for survival of *T. brucei*. Moreover, overexpression of this protein could increase parasite virulence (36). Like PUF1 in *Plasmodium* that is critical parasite

growth (62). PUF2 is a cytosolic protein and RNAi targeting showed that its expression is necessary for growth of parasite in vivo (38). PUF3 is conserved in all kinetoplastids (39). In *T. brucei*, PUF3 binds mRNAs with UGUA[U/C]AUU recognition motif. Depletion of PUF3 also slightly delays differentiation into the procyclic form. Furthermore, in this parasite, knockdown of PUF5 did not have any impact on normal growth of parasite in procyclic forms (40), but PUF5 overexpression is lethal (37). Furthermore, it has been shown by epitope tagging that PUF7 is found in the nucleolus and it is essential for effective processing of rRNA precursor (41). In addition, PUF10 as another nuclear PUF protein besides other factors particularly PUF7 is involved in processing and maturation of 5.8SrRNA (63). In contrast, PUF9 may be a multifunctional protein, because it is responsible for the control of transcription rate in specific time points during replication and cell cycle and also the copy number of organelles in *T. brucei* (42). Knock-down of the PUF9 using RNAi has shown that its presence is essential for mRNA stability in the S-phase and cell growth (42). Furthermore, interaction of PUF9 with consensus sequence in the 3'-UTR of mRNA transcripts specially EIF4E2 (Eukaryotic translation initiation factor 4E type 2) plays an important role in DNA replication in the S-phase (42). In the latest research it is reported that PUF9 may interact with EIF4E2-SLBP2 complex to stabilize the mRNAs in S or early G2 phase that is ready for translation (64).

Trypanosome cruzi

T. cruzi (American trypanosomiasis) is the causative agent of Chagas disease and is transmitted to the host through triatomine insect vectors. *In silico* analysis has identified ten PUF proteins in haploid genome of *T. cruzi* with orthologue in *T. brucei* (20). Like *T. brucei*, *T. cruzi* has two isoforms of PUF9. PUF1 in *T. cruzi* is the homologue of PUF1 in *T. brucei*. Some RNA binding proteins such as UBP1 control the stability of mRNA through interaction with ARE (RNA instability element) that is an AU-rich sequence in 3'-UTR region. This interaction causes instability of RNA molecule (65). PUF6 protein is expressed in the cytoplasm and is involved in all growth cycles of parasite and metacyclogenesis process (6). However, co-localization of PUF6 and DHH1 helicase in epimastigotes might lead to associated mRNA instability while in metacyclic stage, these proteins do not show such interaction (44). Similar to PUF4 in Yeast, three trypanosomatidae PUF proteins (TcPUF1, TcPUF6 and TbPUF6) interact with nuclear proteins and cause foci localization.

Leishmania

Intracellular *Leishmania* protozoan parasites are another group of TriTryp family which belongs to trypanosomatida that cause a complex leishmaniasis disease with different clinical manifestations from cutaneous to visceral leishmaniasis. Leishmanial infection takes place during sand-fly blood meal and transfer promastigote form of parasite into macrophages. In different species of *Leishmania*, PUF family has 11 members, including one more isoform of PUF9. It seems that protein 9 gene has been duplicated during evolution (11, 47) although, this phenomenon is not rare in *Leishmania* and often occurs in stress situation. So far only few studies have been done to identify the role/s of these proteins.

It has been reported that PUF proteins show an antigenic reaction with sera of infected hamsters and human patients. Researches generated recombinant proteins of ten PUF genes from *L. infantum* and assessed the level of antibody responses in sera of infected animals. In contrast to hamsters that generated specific antibodies against all recombinant proteins, in human patients, just two PUF proteins (PUF1 and PUF2) showed strong activity. They suggested that these proteins may be used as serodiagnosis or vaccine can-

didates against *L. infantum*, although these antigens induced cross-reactivity with *T. cruzi* (11). In addition, 6 members of this protein family including PUF1, PUF4, PUF6, PUF7, PUF8 and PUF10 are expressed in promastigote or extracellular form of *L. infantum* (46) and others are expressed in amastigote. The same research group used a proteomics approach and co-immunoprecipitation with anti-PUF1 antibody to recognize PUF1 protein partners in *L. major*. They identified at least 90 proteins that directly or indirectly interact with PUF1. Their functions are mostly in protein synthesis, transport, post-translational modifications, ATP synthesis and RNA binding protein (46, 47). In addition, in *Leishmania*, one of the important sequences that is critical for post-transcriptional regulation is SIDER2 (Short Interspersed Degenerate Retroposons) in 3'-UTR of mRNA (45). Interestingly, PUF6 is a candidate protein to interact with mRNA through SIDER2 and decrease the mRNA half-life. Also, *Leishmania* PUF6 binds a retroposon-like sequence in the 3'-UTRs of mRNA and has a direct role in maintaining mRNA stability (45). In fact, removing these sequences from mRNA blocks mRNA degradation (66). However, biological functions and localization of PUF proteins in *Leishmania* parasites remains to be further elucidated.

Conclusion, perspective and future application of PUFs as mRNA regulators

In recent years, the involvement of PUF proteins in various diseases has attracted attention and opening up new ways for therapeutic mediation (67). Dysregulation of Puf-family RNA-binding proteins is linked to some diseases such as certain cancers, neurodegenerative disorders, and metabolic diseases (68). Targeting PUF proteins could provide innovative therapeutic strategies against these diseases by influencing the expression of key genes involved in disease progression (67). The expression of these proteins is directly related to the reduction of oncogenes (69). Therefore, understanding the intricate mechanisms through which PUF proteins contribute to pathophysiological conditions is essential for the development of targeted therapies. As these proteins are involved in the biogenesis of ribosomes, preventing their expression causes a significant decrease in their precursors, including 5.8srRNA.

In TriTryp parasites, since UTRs have significant roles in post-transcriptional regulation of gene expression, identifying the role of each RNA-binding protein will enable better characterization of the mechanisms that control RNA stability and protein level, and finally improvement of the vaccine or drug candidates. It seems that, there is a direct relation between the number of PUF proteins in a species and regulation potential of transcription. Considering that, these proteins are necessary for normal differentiation, so maybe they can be given more attention as a drug target in future studies. Hopefully, the genomics advancements will help more accurate analysis through whole and transcriptome sequencing and comparative genomics. Furthermore, these studies need complementary tests such as disruption of many genes stepwise or simultaneously and also restoration of the same disrupted genes in their original locus to restore native phenotypes. The most studies were performed using old methods such as RNAi, but, with novel gene manipulation tools such as CRISPR/Cas system, study of gene function is very easy and rapid. The role of many PUF proteins is poorly or not completely understood; therefore, more studies are needed to identify their molecular characterization, cellular localization, potential PUF-interacting proteins, and also to develop a diagnosis tool or vaccine candidate based on these proteins.

Acknowledgements

We thank Dr. Amir Mizbani for critical reading and valuable comments on the manu-

script. This work received no specific grant from any funding agency.

Conflict of Interest

The authors declare that there is no conflict of interests.

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