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# The FIP-1 like polyadenylation factor in trypanosomes and the structural basis for its interaction with CPSF30

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ABSTRACT

In trypanosomes transcription is polycistronic and individual mRNAs are generated by a *trans*-splicing/polyadenylation coupled reaction. We identified a divergent trypanosome FIP1-like, a factor required for mRNA 3′ end formation from yeasts to human. Here we showed that it is a nuclear protein with a speckled distribution essential for trypanosome viability. A strong interaction was found between TcFIP1-like and TcCPSF30, a component of the polyadenylation complex. We determined the specific amino acids in each protein involved in the interaction. Significant differences were found between the trypanosome interaction surface and its human counterpart. Although CPSF30/FIP1 interaction is known in other organisms, this is the first report mapping the interaction surface at the amino acid level.


## Introduction

Nuclear pre-mRNA 3′ end formation involves a two-step reaction in eukaryotes: an endonucleolytic cleavage at the poly(A) site, followed by the polyadenylation of the newly generated 3′ end. This reaction requires *cis*-acting signal sequences on the pre-mRNA and *trans*-acting protein components [1].

mRNA maturation in trypanosomes, a group of unicellular eukaryotic parasites causative agents of Chagas disease and Sleeping sickness in humans, clearly deviates from the same process in most eukaryotes primarily because protein-coding genes are transcribed into polycistronic RNAs separated by relatively short intergenic regions (IRs).

Precursor RNAs are processed into individual mRNAs by two coupled reactions, the addition of a 39-nucleotide capped RNA (spliced leader, SL), through a *trans*-splicing event [2,3], followed by cleavage/polyadenylation. Both reactions are governed by a common polypyrimidine rich sequence present in the IRs [4]. As a consequence, there is no recognizable polyadenylation signal, such as the AAUAAA sequence of mammalian pre-mRNAs [6]. Another outcome of this arrangement is that regulation of gene expression is not controlled by promoter activity but by mRNA processing and stability [5]. Little is known about the protein complex involved in the coupled process but it emerges as an interesting anti-parasitic drug target since the process diverges from its mammalian host [7–9].

In mammals, the *trans*-acting protein complexes required for the cleavage step are, the cleavage/polyadenylation specificity factor (CPSF), the cleavage-stimulatory factor (CstF), the cleavage factors Im and IIm (CF Im and CF IIm) and the RNA polymerase II (pol II). The poly(A) polymerase (PAP) and the poly(A)-binding protein II (PABP II) are involved in the polyadenylation reaction [10].

Previous *in silico* searches in the trypanosome genomes identified the members of CPSF complex and a few members of the CF but those of the CstF seemed to be absent [8].

In this report, we identified a new member of the trypanosome polyadenylation complex: a divergent FIP1-like factor.

The FIP1 protein (Factor Interacting with PAP) was first identified in yeast and it was shown to have a crucial regulatory function in the polyadenylation reaction by controlling the activity of poly(A) tail synthesis through multiple interactions with the CPSF30 ortholog (Yth1) and PAP within the polyadenylation complex [11,12]. The same interactions were reported for the identified human FIP1 [13]. The sequence of FIP1 orthologues from different eukaryotes is not conserved except for a short domain of 57 amino acids that is a signature of this protein [13].

Here, we showed that the identified FIP1-like interacts with the trypanosome CPSF30 subunit and is essential for cell viability. In addition, we analyzed in detail the contact-binding surface of the

*Abbreviations:* Y2H, yeast two-hybrid; ONPG, ortho-nitrophenyl-β-galactoside; SOEing PCR, splice overlap extension PCR; CPSF, cleavage and polyadenylation specificity factor; FIP, factor interacting with PAP.

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trypanosomatid CPSF30/FIP1 in comparison with its human counterpart showing significant differences that could lead to the postulation of this interacting pair as a putative drug target. Our results also described the first contact surface of a CPSF30/FIP1 at the amino acid level.

## Materials and methods

*Data mining.* Human polyadenylation factors were used as query sequences to screen the *Trypanosoma cruzi* and *Trypanosoma brucei* genome databases using TBLASTN (http://www.genedb.org/genedb/tcruzi/blast.jsp). Motifs and domains were searched in SMART (http://smart.embl-heidelberg.de/) databases.

*Trypanosoma brucei growth, transfection and RNAi experiments. T. brucei* procyclic cells (strain 1313) were grown at 27 °C in MEMpros media supplemented with 10% fetal bovine serum, and the appropriate antibiotics. Transfection and RNA knock-down analysis of *T. brucei* FIP1-like was done as previously described [14]. Total RNA samples from the induced (or uninduced) RNA knocked down cells was isolated by using the peqGold Trifast reagent (peqLab, Germany) according to the manufacturer's instructions and analyzed by Northern blot.

*Cloning of human and T. cruzi coding sequences into the gateway system.* Both TcCPSF30 and TcFIP1-like sequences were PCR amplified from 100 ng of total genomic DNA of *T. cruzi* CL-B clone using specific primers. *T. brucei* FIP1-like fragment for the RNAi experiment was PCR amplified from 100 ng of total genomic DNA of *T. brucei* 1313 strain using proper primers. The human orthologues were PCR amplify from human lymphocytes cDNA and cloned using specific primers (Supplementary file S1). All sequences were cloned into the gateway system as described [15].

*Yeast two-hybrid assays and mutational analyses of CPSF30 and FIP1-like.* All interaction experiments were performed exactly as previously described in [15] measuring the activation of two independent reporter genes (*His3* and *LacZ*). All quantitative ONPG assays were done as indicated in the Proquest™ kit manual (Invitrogen) and they are the result of two independent experiments performed in duplicate. Variants were generated by SOEing PCR as described previously [16] using specific primers. Alanine scan mutations were also generated by SOEing PCR (Supplementary file S1). All amplifications were performed using the proofreading AccuTaq enzyme (Sigma–Aldrich) and sequenced.

*Production of polyclonal antibodies against TcFIP1-like and indirect immunoflorescence assay (IFA) on T. cruzi cells. T. cruzi* epimastigotes were grown as described previously [17]. Recombinant FIP1-like His tagged protein was produced in *Escherichia* coli and purified using Ni-NTA column following the manufacturer's instruction (QIAGEN). Antibodies were produced in mice as previously described [18]. The IFA was done as previously described [9] with the following modifications: nuclear staining was done using propidium iodide (PI) and confocal images obtained in an Olympus FV300 microscope.

## Results and discussion

### *Trypanosome FIP1-like diverged from the rest of its eukaryotic orthologues*

By data mining within the trypanosome genome database, we found a previously unidentified subunit of the CPSF complex, a FIP1-like factor (Fig. 1A and Supplemental Fig. S1).

The FIP1 protein in *Saccharomyces cerevisiae* and human, shares a similar domain organization that consists of an acidic N-terminal region and a highly conserved central region followed by a Proline-rich domain (Fig. 1A). However, trypanosome FIP1-like shows a different architecture and it only conserves the fip1 domain region showing a 48% of identity with the human domain (Fig. 1B and Supplemental Fig. S1). In contrast with other FIP1 proteins, trypanosome FIP1-like is shorter (280 amino acids in *T. brucei* and 287 in *T. cruzi*) and it is the only FIP1 ortholog containing a CCCH zinc finger motif followed by a Q-rich stretch in the C-terminal region (Fig. 1A).

### *Trypanosome CPSF30 presents a similar domain organization compared with other organisms*

The CPSF30 present in all eukaryotes is a poly-zinc finger protein with a highly conserved architecture from yeast to human. It contains five CCCH-type zinc finger motifs in *S. cerevisiae*, and it accommodates additional zinc finger motifs of the CCHC-type in human (one motif) and in *Drosophila melanogaster* (two motifs).

Trypanosome CPSF30, a 271 (*T. cruzi*) or 277 (*T. brucei*) amino acids protein, presents a high degree of similarity and the same domain organization compared with their orthologues (Supplemental Fig. S1). It has five CCCH-type zinc fingers and it also includes two CCHC-type zinc fingers in its C-terminal. The *T. brucei* protein was previously characterized as a *bona-fide* CPSF30 based on functional analyses [8].

### *FIP1-like is a nuclear protein essential for the parasite viability*

Immunoflorescence assays using a specific polyclonal serum showed FIP1-like localized exclusively in the nucleus of the trypanosome cells with a speckled distribution pattern (Fig. 2A), compartmentalization that is consistent with its predicted function.

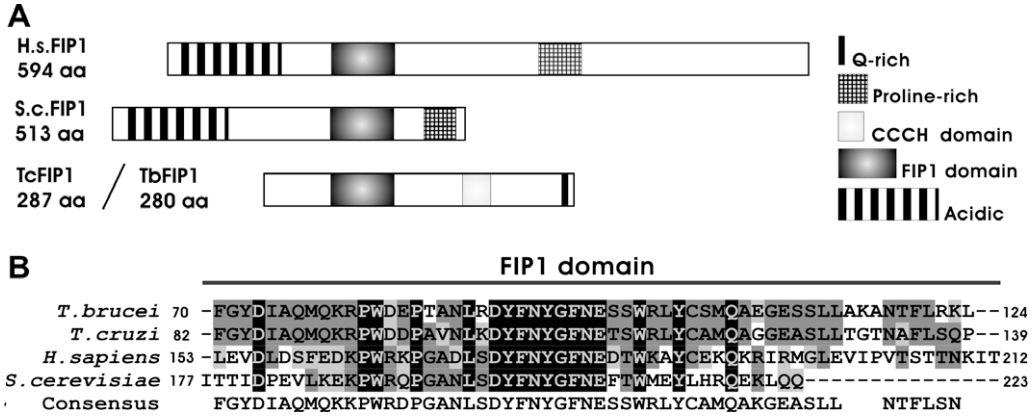


**Fig. 1.** Domain organization of trypanosome FIP1-like protein. (A) Schematic representation of FIP1 from human (H.s.FIP1.) and yeast (S.c.FIP1) compared with trypanosomes (Tc/TbFIP1). (B) Sequence alignment of the human and yeast fip1 domains compared with that of trypanosomes. Black box indicates 100% identical residues; white box corresponds to non-similar residues, light gray box indicates conservative changes; dark gray box shows blocks of similarity.