

TcZFP8, a novel member of the *Trypanosoma cruzi* CCHC zinc finger protein family with nuclear localization

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ABSTRACT. In a 17-kb genomic fragment of *Trypanosoma cruzi* chromosome XX, we identified three tandemly linked genes coding for CX₂CX₄HX₄C zinc finger proteins. We also showed that similar genes are present in *T. brucei* and *Leishmania major*, sharing three monophyletic groups among these trypanosomatids. In *T. cruzi*, TcZFP8 corresponds to a novel gene coding for a protein containing eight zinc finger motifs. Molecular cloning of this gene and heterologous expression as a fusion with a His-tag were performed in *Escherichia coli*. The purified recombinant protein was used to produce antibody in rabbits. Using Western blot analysis, we observed the presence of this protein in all three forms of the parasite: amastigote, trypomastigote and epimastigote. An analysis of cytoplasmic and nuclear cell extracts showed that this protein is present in nuclear extracts, and indirect immunofluorescence microscopy confirmed the nuclear localization of TcZFP8. Ho-

mologues of *TcZFP8* in *T. brucei* are apparently absent, while one candidate in *L. major* was identified.

Key words: CCHC-type zinc finger, Nuclear protein, TcZFP8, *Trypanosoma cruzi*, Trypanosomatid

INTRODUCTION

Trans-acting factors such as DNA- or RNA-binding proteins have conserved structural domains that mediate and stabilize their binding to nucleic acid molecules. The zinc finger domain is a very ubiquitous structural element, whose hallmark is the coordination of a zinc atom by several amino acid residues (cysteines and histidines, and occasionally aspartate and glutamate). These structural elements are associated with protein-nucleic acid recognition as well as protein-protein interactions (Leon and Roth, 2000), where they are found in proteins involved in differentiation and growth signals, in proto-oncogenes, in general transcription factors, and in the development of eukaryotic organisms (Pabo and Sauer, 1992). One subtype of this zinc finger domain is the CX₂CX₄HX₄C structure that occurs twice in the NCp7 protein of the nucleocapsid of HIV-1. This domain contributes to multiple steps of the viral life cycle, including the proper encapsidation of the HIV RNA through interactions with single-strand nucleic acids and viral proteins (Ramboarina et al., 1999). Another protein that contains seven CX₂CX₄HX₄C zinc finger domains, eukaryotic CNBP, a single-strand DNA-binding protein, plays a fundamental biological role across different species as a transcription factor. For instance, mouse CNBP is located in the nucleus of cells and stimulates cell proliferation and increases *c-myc* promoter activity during embryogenesis (Shimizu et al., 2003).

Several zinc finger proteins (ZFP) sharing the motif CX₂CX₄HX₄C have been identified in trypanosomatids and have been shown to be involved in different cellular functions. In *Leishmania major*, the protein HEXBP, containing nine zinc finger CX₂CX₄HX₄C motifs, binds to the hexanucleotide repeat sequence found in the intervening region of the GP63 gene cluster, the most abundant surface glycoprotein of this protozoan, and it is likely to be involved in DNA replication, structure and repair (Webb and McMaster, 1993). In *Crithidia fasciculata*, a protein containing five CX₂CX₄HX₄C zinc finger domains designated UMSBP binds to the conserved universal sequence of kinetoplast DNA minicircles (Tzfati et al., 1992, 1995). Probable homologues of this protein are PDZ5 in *Trypanosoma cruzi* (Coelho et al., 2003) and TbZFP protein in *T. brucei* (Radwanska et al., 2000). In *T. brucei*, the RRM1 protein containing three consensus RNA recognition motifs (RRM), two tandem copies of CX₂CX₄HX₄C zinc finger domain and an arginine-serine-rich region was localized in the trypanosome nucleus, and it was suggested that it plays a role in *trans*-splicing (Manger and Boothroyd, 1998). Recently, in *T. cruzi*, a poly-ZFP with seven CX₂CX₄HX₄C motifs designated as PZFP1 was identified and shown to bind specifically to the single-strand DNA or RNA oligonucleotides possessing binding sequences of other CX₂CX₄HX₄C proteins, such as the *C. fasciculata*-conserved universal minicircle sequence and *L. major* hexanucleotide of GP63 (Espinosa et al., 2003).

We report here the identification of a tandemly linked family of CCHC ZFP genes in trypanosomatids. All predicted proteins share the zinc finger motif CX₂CX₄HX₄C that is present

in the NCp7 protein of the nucleocapsid of HIV-1. This type of structure seems to bind to single-strand nucleic acids (Ramboarina et al., 2004). We also report the characterization of TcZFP8 as a new member of this protein family with nuclear localization.

MATERIAL AND METHODS

Isolation and sequence analysis of a *Trypanosoma cruzi* 17-kb genomic region

A *T. cruzi* (Dm28c clone) genomic λ EMBL3 library (Fragoso and Goldenberg, 1992) was previously screened with the cDNA of the ALPHA6 proteasomal subunit (*TcPR29A* gene) (Bartholomeu et al., 2001), and one of the positive clones was further characterized and found to contain a 17,303-kb fragment of chromosome XX (Figure 1A). DNA was isolated from this selected positive clone, and restriction fragments were sub-cloned into the pBluescript SK+ (Stratagene) or pUC18 (Biolabs). All sub-clones were submitted to automatic sequencing, and computer-aided sequence analysis was performed using the University of Wisconsin GCG software package. Nucleotide sequence data reported in this paper are available in GenBank database under accession Nos. AF104214 and AY728266. The sequence alignment was carried out using version 1.82 of CLUSTALW (Thompson et al., 1994). A set of 1,000 sequences was generated with the SeqBoot program; the calculation of the distance matrix and the tree construction was carried out with the aid of ProtDist and Fitch programs, respectively. Finally, the consensus tree was obtained with the Consensus tree program. SeqBoot, ProtDist, Fitch and Consensus tree programs are part of the Phylip package, version 3.8 (Felsenstein, 2002).

Heterologous expression of TcZFP8, its purification and antibody production

The sub-clone pU5.4A was used to isolate the 1.8-kb fragment that contains the *TcZFP8* gene by digestion with *Bam*HI and *Sal*I, which was then ligated to the linearized plasmid pET28a+ (Novagen) (*Bam*HI and *Sal*I digestion) to obtain the proper frame with His tag coding sequence. The correct cloning was checked by automatic sequencing. The new plasmid called pETZ2.2 was used to transform *E. coli* strain BL21DE3 to express *TcZFP8* gene.

The BL21DE3 bacteria harboring plasmid pETZ2.2 were grown in 2YT medium (1.6% tryptone, 1.0% yeast extract, 0.5% sodium chloride, pH 7.0) supplemented with 30 μ g/mL kanamycin, to an absorbance of 0.6 at 600 nm. The TcZFP8 production was then induced with 1.0 mM isopropyl-beta-D-thiogalactopyranoside for 2 h at 37°C under aeration. Cells were harvested by centrifugation at 6000 g for 15 min. The pellet from each 250 mL of culture was resuspended in 6 mL PBS with protease inhibitors (5 mM phenyl methyl sulfonyl fluoride, 2 μ g/mL pepstatin, 0.5 μ g/mL leupeptin). The cells were disrupted by sonication (5 cycles/30 s) in ice, and Triton X-100 was then added to a final concentration of 1%. The suspension was centrifuged for 20 min at 15,000 g. The supernatant was incubated for 15 min at 4°C with 2 mL of solution I (20 mM Tris-HCl, pH 8.0, 4% SDS, 4% dithiothreitol) followed by the addition of 2.5 mL of solution II (20 mM Tris-HCl, pH 8.0, 0.5 M iodoacetamide) and incubation for 15 min at 28°C in a dark place; this is a modified method of alkylation described by Yan et al. (1999). After the treatment, the lysate was incubated for 1 h at 4°C with 1 mL Ni-NTA (Pharmacia) previously treated with equilibrium buffer (0.1 M sodium phosphate, 50 mM sodium chloride, pH 7.3). The protein was eluted with elution buffer (0.1 M sodium phosphate, 50 mM sodium

chloride, 7.5 M urea, 0.3 M imidazole, pH 6.3), and the protein concentration was determined using the method described by Ramagli and Rodriguez (1985).

The polyclonal anti-TcZFP8 immune serum was produced by immunization of rabbits with three intraperitoneal injections of 100 µg of recombinant purified protein TcZFP8 at 15-day intervals. Freund's complete adjuvant was used for the first injection and incomplete adjuvant in the subsequent injection. After two weeks from the last injection, blood was collected and the serum was stored at -20°C.

Cultivation of *Trypanosoma cruzi*

Epimastigote forms of *T. cruzi*, Dm28c and Y strains, were maintained in logarithmic growth phase at 28°C in liver infusion tryptose medium supplemented with 10% fetal bovine serum as described elsewhere (Camargo, 1964). Trypomastigote and amastigote cells were obtained by infecting monolayers of HeLa cells, and purified by centrifugation as previously described (Teixeira et al., 1994).

Western blot

To obtain the total cell lysates, *T. cruzi* epimastigote cell culture at late log phase and amastigote and trypomastigote cells were centrifuged at 6000 g for 15 min, washed twice in PBS and resuspended in SDS-PAGE sample buffer. Nuclear and cytoplasmic fractions of *T. cruzi* epimastigote cells were prepared as described by Batista et al. (1994). The cytoplasmic extract and nuclear enriched fraction were analyzed using 15% SDS-PAGE. Western blot analysis was performed following a standard protocol using polyclonal anti-TcZFP8 antiserum as the first antibody.

Indirect immunofluorescence assay

Exponentially growing *T. cruzi* epimastigote cells were centrifuged at 6000 g for 3 min, washed twice in PBS and resuspended in PBS supplemented with 1% BSA. Next, the cells were fixed in 100% methanol for 15 min at room temperature and placed on microscopic slides. After air-drying, parasites were permeabilized in 0.2% Triton X-100 in PBS for 15 min followed by saturation with 5% nonfat milk in PBS for 4 h at 4°C and probing for 4 h at room temperature with 1:50 polyclonal anti-TcZFP8 antiserum in 1% nonfat milk in PBS. Slides were then washed twice with PBS for 15 min and probed 4 h at room temperature with 1:50 FITC-conjugated goat anti-rabbit secondary antibody in 1% nonfat milk in PBS, followed by washing twice in PBS. After that, the slides were stained with 4'-6-diamidino-2-phenylindole (DAPI) for 30 min and washed twice in PBS. After air-drying, the parasites were observed with an Axiovert Carl Zeiss microscope with magnification of 1000X.

RESULTS

In previous study (Bartholomeu et al., 2001), we isolated and characterized the proteasomal ALPHA6 subunit gene, designated as *TcPR29A*, and showed that this gene is located on chromosome XX of *T. cruzi*. A genomic fragment of 17,303 kb was isolated and the gene

TcPR29A was localized at the end of this region (Figure 1A). Further sub-cloning and sequencing analysis of this fragment revealed the presence of five other open-reading frames (ORF) with the same transcription direction (Figure 1A). The first one, a 788-bp coding region (*TcBETA5*), was found at the other end of this fragment, and an analysis of databases showed it to be an incomplete gene coding for a proteasomal BETA5 subunit by homology with the BETA5 cDNA of *T. brucei* (GeneBank accession No. AJ132959) and other eukaryotic proteasomal BETA5 proteins. Upstream of the *TcPR29A* gene, a 5103-kb ORF (*TcCLAH*) was found coding for a clathrin heavy chain protein with 1701 amino acids in length. Between the *TcBETA5* and *TcCLAH* genes in a region with 8.6 kb of length, we identified three other ORFs encoding proteins containing five, seven and eight CX₂CX₄HX₄C zinc finger domains, respectively.

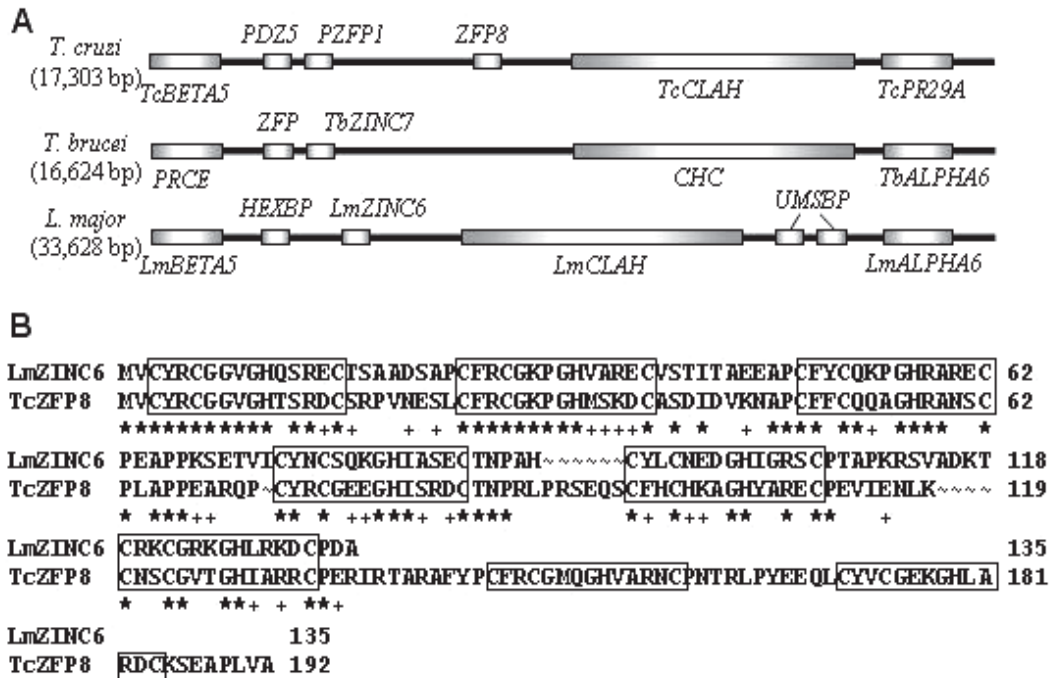


Figure 1. Comparison of *Trypanosoma cruzi*, *T. brucei* and *Leishmania major* homologous genomic regions and characterization of a gene family encoding CX₂CX₄HX₄C zinc finger proteins. **A.** Scheme showing the *T. cruzi* chromosome XX 17-kb region, where rectangles represent genes in the following order: *TcBETA5* - proteasomal BETA5 subunit gene; *PDZ5*, *PZFP1* and *ZFP8* - genes coding for proteins containing five, seven and eight zinc finger domains, respectively; *TcCLAH* - clathrin heavy chain gene, and *TcPR29A* - proteasomal ALPHA6 subunit gene. The same region between the proteasomal BETA5 and ALPHA6 subunit genes is shown in *T. brucei* (chr 10, TIGR database) and *L. major* (contig 36.1, Sanger database). **B.** Alignment of the ZFP8 and LmZINC6 proteins. Asterisks indicate conserved amino acids and plus signs indicate similar amino acids. Zinc finger domains are boxed.

Blast search of the predicted protein with five zinc finger domains showed that this 14.32-kDa protein corresponds to the PDZ5 of *T. cruzi* (Coelho et al., 2003), homologous to UMSEBP of *C. fasciculata* (Tzfati et al., 1992) and CCHC ZFP of *T. brucei* (Radwanska et al., 2000). PDZ5 was described from a cDNA clone of CL Brener strain and the corresponding gene was located on chromosome XX using pulse field electrophoresis and Southern blot (Coelho

et al., 2003). The corresponding gene was identified in *T. cruzi* Dm28c strain with its localization in this 17-kb fragment of chromosome XX downstream of the *TcBETA5* gene. In *T. brucei* the same position of the *ZFP* gene in relation to the proteasomal *BETA5* gene was observed in chromosome X (Figure 1A). Radwanska et al. (2000) performed a cDNA library screening and identified a 2.3-kb fragment encoding a proteasomal *BETA5* subunit and a putative *ZFP*, which they showed to be an mRNA precursor or a processing by-product. Thus, *PDZ5* and *ZFP* are strong candidates to be the *T. cruzi* and *T. brucei* homologues of the *C. fasciculata* *UMSBP*, a single-stranded DNA-binding protein that binds specifically to the 12 conserved nucleotides termed universal minicircle sequence (UMS) located at the heavy strand of the replication origin of the kinetoplast DNA minicircles (Tzfati et al., 1992). In *L. major* we found two identical copies in tandem of the homologous *C. fasciculata* *UMSBP* genes, downstream of the *LmCLAH* gene (Figure 1A), which we designated as *LmUMSBP* genes. The predicted protein from these genes shows 80% identity and 92% similarity with the *C. fasciculata* *UMSBP* (Genebank accession Nos. AAC32814 and A54598).

Blast search of the predicted protein with seven zinc finger domains (193 amino acids, 20.42 kDa) showed that this protein corresponds to *PZFP1* of *T. cruzi* (Espinosa et al., 2003). It was shown that *PZFP1* is located mainly in the cytoplasm and binds to single-stranded DNA or RNA oligonucleotides carrying recognition sequences of other $CX_2CX_4HX_4C$ proteins, such as *C. fasciculata* *UMS* and *L. major* *GP63* hexamer sequences (Espinosa et al., 2003). In *T. brucei*, we found a *PZFP1* homologous gene designated as *TbZINC7* in the same position in relation to the *ZFP* gene (Figure 1A). Alignment analysis between the predicted proteins *PZFP1* and *TcZINC7* showed 67% identity and 70% similarity. These two proteins are also similar to the *T. equiperdum* nucleic acid-binding protein (Genebank accession No. AAB47542), showing 49% identity and 53% similarity with *PZFP1* and 74% identity and 75% similarity with *TbZINC7*. These proteins still have unknown functions.

Alignment of *PDZ5* and *PZFP1* showed an identity of 95% in 375 nucleotides and 94% in 125 amino acids among the five domains of *PDZ5* and the last five domains of *PZFP1*. This suggests that these two genes could be derived from a gene duplication. The same was observed in *T. brucei* when *ZFP* and *TbZINC7* were compared.

The predicted protein with eight zinc finger domains revealed a novel gene designated as *TcZFP8* that contains an ORF encoding a 192-amino acid protein. An analysis of databases showed the presence of this gene in another strain of *T. cruzi*, CL Brener, where three genes with minor differences were observed. Two gene sequences were obtained from TIGR database, accession Nos. 7413 and 5774, and one from GeneBank accession No. AF204398. A few changes in the predicted proteins showed us three patterns, suggesting three alleles of this gene in CL Brener (data not shown). Alignment of the *TcZFP8* protein with other known CCHC *ZFP* of trypanosomatids (*TcPDZ5*, *TcPZFP1*, *TbZFP*, *TbZINC7*, and *LmUMSBP*) revealed that the similarity among them is limited to the cysteines and histidines at the $CX_2CX_4HX_4C$ zinc finger domains. Quantitative alignment data showed that the identity is around 27 to 34% and the similarity is 40 to 49%, confirming that *TcZFP8* is a novel gene/protein in *T. cruzi*.

Interestingly, no similar *TcZFP8* genes were found either in the same region (Figure 1A) or in the whole genome of *T. brucei* (TIGR and Sanger database). In *Leishmania major*, the presence of the *HEXBP* gene (Webb and McMaster, 1993) downstream of the proteasomal *BETA5* gene was observed (Figure 1A). A candidate for the *ZFP8* homologous gene was identified in this region designated as *LmZINC6* (Figure 1A), but the predicted protein contains

only six zinc finger domains. Figure 1B shows the alignment of TcZFP8 with LmZINC6. Note that these proteins have a higher degree of identity in their three first domains.

In order to evaluate the evolutionary relationship between these ZFP, we performed a phylogenetic analysis, as shown in Figure 2, where three distinct monophyletic groups may be observed. TcZFP8 is more closely related to LmZINC6 than to the other proteins, which suggests that both proteins could have the same cellular functions in *L. major* and *T. cruzi*.

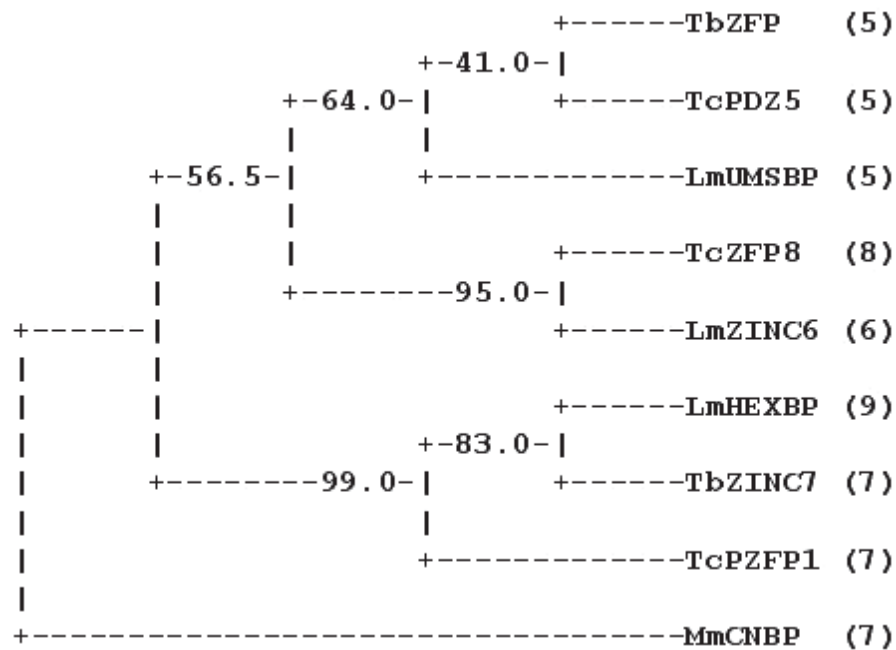


Figure 2. Phylogenetic tree depicting the evolutionary relationships between the zinc-finger proteins. Numbers in parentheses represent the number of zinc finger motifs in the proteins. MmCNBP - accession No. P53996; the others in legend of Figure 1 and Material and Methods.

Although there are other homologous genes of CL Brener strain in the databases, no descriptions about the TcZFP8 gene/protein were found in the literature. Therefore, we pursued the characterization of this protein in *T. cruzi*.

To further characterize TcZFP8 in *T. cruzi* cells, the heterologous expression of the gene *TcZFP8* in *E. coli* was examined. Figure 3A shows the expression of TcZFP8 as a fusion protein with six histidines (His-tag), as expected with 21 kDa (lane I and arrow 1), which is not present at lane N (uninduced sample). The recombinant TcZFP8 fusion protein was purified from bacterial lysates using Ni²⁺ affinity columns (Figure 3A, lane P). Note that the purified protein shows a higher molecular mass (26 kDa) than we expected (20 kDa); the alkylation process, to which the protein was submitted during the purification, explains that. This purified recombinant protein was used to produce a polyclonal antibody by immunization in rabbits. Western blot analysis using this antibody confirmed that the antiserum recognized the recombinant protein in *E. coli* total extracts (data not shown).

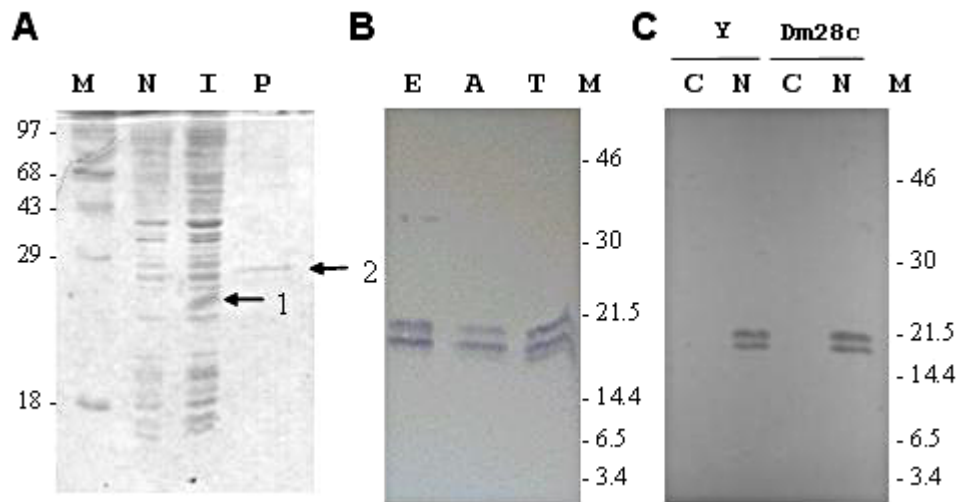


Figure 3. TcZFP8 expression and nuclear localization. **A.** Heterologous expression in *Escherichia coli* of the TcZFP8 with His tag fusion and purification of the recombinant protein analyzed in SDS-PAGE. The N and I lanes represent *E. coli* total cell lysates of non-induced and 2 h isopropyl-beta-D-thiogalactopyranoside-induced, respectively (arrow 1, induced TcZFP8). The P lane shows the purified recombinant TcZFP8 (arrow 2) purified by Ni²⁺ affinity chromatography, after alkylation treatment. M, molecular size markers in kDa. **B.** Western blot of *Trypanosoma cruzi* lysates from three forms of the parasite, epimastigote (lane E), amastigote (lane A), and trypomastigote (lane T), with the anti-TcZFP8 antiserum. M - molecular size markers in kDa. **C.** Western blot of cytoplasmic (lane C) and nuclear (lane N) lysates from *T. cruzi* epimastigotes Y and Dm28c strains, with the anti-TcZFP8 antiserum. M, molecular size markers in kDa.

In order to analyze the TcZFP8 protein in *T. cruzi*, total cell extracts from amastigote, trypomastigote and epimastigote forms of the parasite were used and Western blot analysis revealed the presence of this protein in all three forms (Figure 3B). As observed, a double band of proteins with slightly lower molecular weight was revealed by the anti-TcZFP8 antiserum, which probably represents processed or modified forms of the protein. The same was observed by Hendriks et al. (2001) working with a CCCH ZFP of *T. brucei*.

The analysis by Western blot using cytoplasmic and nuclear cell extracts of *T. cruzi* epimastigote cells from two different strains (Dm28c and Y) and the anti-TcZFP8 antiserum showed that the TcZFP8 protein is present in the nuclear extracts (Figure 3C). In order to confirm this result and discard a possible contamination of the nuclear extract with kinetoplast proteins, immunofluorescence microscopy analysis was performed on *T. cruzi* epimastigote cells using the anti-TcZFP8 antiserum. A strong nuclear signal was obtained, which co-localized with DAPI labeling (Figure 4A and B). An enlarged image of one parasite (Figure 4C and D) illustrates this general pattern: a DAPI labeling of the kinetoplast and nuclear DNA and the co-localization of the antiserum labeling in the nucleus, confirming that TcZFP8 is a nuclear ZFP.

In order to determine the binding sequence of TcZFP8, the SELEX (systematic evolution of ligands by exponential enrichment) experiment (Ellington and Szostak, 1990) was performed using purified recombinant GST-TcZFP8 and single-strand DNA oligonucleotides with 30 degenerated bases. After six cycles of binding and PCR, the final amplified products were cloned and automatically sequenced. The resulting sequences did not show any consensus sequences (data not shown), suggesting that the binding site is not restricted to one specific sequence.

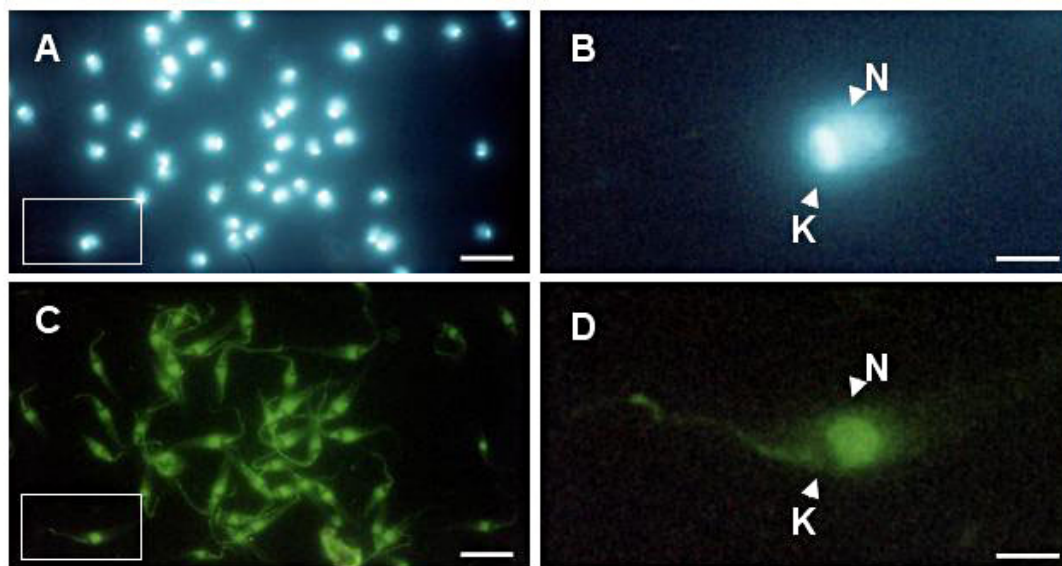


Figure 4. Nuclear localization of TcZFP8 in *Trypanosoma cruzi* epimastigotes. **A.** Cells stained with DAPI. Scale bar = 10 μ m. **B.** Cells stained with DAPI. Magnified area of the rectangle in Figure 4A. Scale bar = 2 μ m. **C.** FITC-indirect immunofluorescence analysis with anti-ZFP8 antiserum. Scale bar = 10 μ m. **D.** FITC-indirect immunofluorescence analysis with anti-ZFP8 antiserum. Magnified area of the rectangle in Figure 4C. Scale bar = 2 μ m. K, kinetoplast, N, nucleus.

DISCUSSION

In the present study, we identified three ZFP genes in a cluster of chromosome XX of *T. cruzi*. The predicted proteins, named TcPDZ5, TcPZFP1 and TcZFP8, share the zinc finger motif CCHC found in a diverse range of DNA/RNA-binding proteins involved in various aspects of the control of cell homeostasis and differentiation. Specifically, TcPDZ5, TcPZFP1 and TcZFP8 have the $CX_2CX_4HX_4C$ zinc finger structure that is present in the NCp7 protein of the nucleocapsid of HIV-1. This kind of structure is shown to bind to single-strand nucleic acids.

TcPDZ5 protein is homologous to the *C. fasciculata* UMSBP (Tzfati et al., 1992) and *T. brucei* CCHC (Radwanska et al., 2000). UMSBP, a sequence-specific single-strand DNA-binding protein, binds specifically to the 12 conserved nucleotides termed UMS located at the heavy strand of the origin of replication of kinetoplast DNA minicircles (Tzfati et al., 1992). TcPDZ5 protein was also shown to bind to the UMS sequence dodecamer, which strongly suggests that this protein is the *T. cruzi* UMS-binding protein (Coelho et al., 2003).

The TcPZFP1 protein is located mainly in the cytoplasm and binds to single-strand DNA or RNA oligonucleotides possessing recognition sequences of other $CX_2CX_4HX_4C$ proteins, such as *C. fasciculata* UMS and *L. major* GP63 hexamer sequences (Espinosa et al., 2003).

The third gene identified here is the novel *T. cruzi* ZFP8 gene, coding for a ZFP with eight domains $CX_2CX_4HX_4C$ showing a high degree of identity and similarity with the *L. major* ZINC6. We found three gene sequences corresponding to the alleles in the CL Brener strain, but the description about their characterization was missing. We opted to study and characterize

this protein in *T. cruzi* cells. In this way, we obtained the heterologous expression of TcZFP8 and the production of a polyclonal antibody against this recombinant protein. Using this antiserum, we could detect TcZFP8 in equal amounts in all three forms of the parasite, amastigote, trypomastigote and epimastigote, suggesting that TcZFP8 is not developmentally regulated and is probably involved in a process occurring in all three forms. Since trypomastigotes are non-replicative cells and since this protein is present in the same amount as in the replicative cells, epimastigote and amastigote, it is hardly probable that this protein is involved in replication control as suggested for TcPDZ5.

Analysis of nuclear and cytoplasmic extracts by Western blot showed that TcZFP8 is a nuclear-specific protein, which was confirmed by immunofluorescence microscopy. Different from PZFP1 which was localized mainly in the cytoplasm, TcZFP8 was present in the nucleus of the cell, and since its zinc finger domains have a high degree of identity and similarity with the same domains in single-strand DNA/RNA-binding proteins, we hypothesize here that this protein may be a nuclear protein involved in gene expression.

As shown in this study, the zinc finger family in trypanosomatids is very complex, and it will be necessary to study and to determine the exact functions of these genes/proteins. The identification and characterization of *trans*-acting factors that bind to DNA and RNA molecules will help us to understand the mechanisms of gene expression and regulation in trypanosomatids.

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