

## Characterization of TcSTI-1, a homologue of stress-induced protein-1, in *Trypanosoma cruzi*

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*The life cycle of the protozoan Trypanosoma cruzi exposes it to several environmental stresses in its invertebrate and vertebrate hosts. Stress conditions are involved in parasite differentiation, but little is known about the stress response proteins involved. We report here the first characterization of stress-induced protein-1 (STI-1) in T. cruzi (TcSTI-1). This co-chaperone is produced in response to stress and mediates the formation of a complex between the stress proteins HSP70 and HSP90 in other organisms. Despite the similarity of TcSTI-1 to STI-1 proteins in other organisms, its expression profile in response to various stress conditions, such as heat shock, acidic pH or nutrient starvation, is quite different. Neither polysomal mRNA nor protein levels changed in exponentially growing epimastigotes cultured under any of the stress conditions studied. Increased levels of TcSTI-1 were observed in epimastigotes subjected to nutritional stress in the late growth phase. Co-immunoprecipitation assays revealed an association between TcSTI-1 and TcHSP70 in T. cruzi epimastigotes. Immunolocalization demonstrated that TcSTI-1 was distributed throughout the cytoplasm and there was some colocalization of TcSTI-1 and TcHSP70 around the nucleus. Thus, TcSTI-1 associates with TcHSP70 and TcSTI-1 expression is induced when the parasites are subjected to stress conditions during specific growth phase.*

Key words: STI-1 - Hop - HSP70 - stress response - *Trypanosoma cruzi*

The stress-induced protein-1 (STI-1), also known as Hop (HSP70/HSP90-organising protein), is a co-chaperone that interacts with the heat-shock proteins HSP70 and HSP90 (Smith et al. 1993). This multi-chaperone complex is required for steroid receptor (SR) assembly (Chen et al. 1996, Dittmar et al. 1996, Chang et al. 1997) in which STI-1 acts as a mediator, potentially increasing the rate of SR maturation (Morishima et al. 2000). Furthermore, this chaperone complex is able to reconstitute hepadnavirus reverse transcriptase activity in vitro (Hu et al. 2002), suggesting that a purified assembly system could be used to mediate various biological processes requiring HSP90 function (Pratt & Toft 2003).

STI-1 acts as an adaptor protein, mediating the interaction between HSP70 and HSP90 through its tetratricopeptide repeat (TPR) domains. The N-terminal TPR domain (TPR1) specifically recognizes the C-terminus of HSP70, whereas the central TPR domain (TPR2A) binds the C-terminus of HSP90 (Chen & Smith 1998, Scheufler et al. 2000). The C-terminal domain of STI-1 seems to be involved in the interactions with both HSPs (Flom et al. 2007, Onuoha et al. 2008). STI-1 may also modulate HSP activities (Johnson et al. 1998, Richter et al. 2003, Odunuga et al. 2004).

The *STI-1* gene was first isolated and characterized in *Saccharomyces cerevisiae* during a genetic screen for proteins involved in the heat-shock response. The gene was named "stress-inducible" because, like other well-characterized HSP genes, it was induced by heat shock and by the arginine analogue canavanine. However, the nucleotide sequence of *STI-1* displayed no similarity to the sequences of other HSP-encoding genes (Nicolet & Craig 1989). Like their counterpart in yeast, the mouse and *Caenorhabditis elegans* *STI-1* homologues are induced by heat shock (Lässle et al. 1997, Song et al. 2009). Interestingly, a soybean homologue has been shown to be induced by either heat or cold stress (Zhang et al. 2003). In addition, the upregulation of *STI-1* observed in human SV-40-transformed fibroblasts or mouse macrophages exposed to bacterial lipopolysaccharide demonstrates that stress conditions unrelated to temperature may also lead to the induction of *STI-1* expression (Honoré et al. 1992, Heine et al. 1999). The murine STI-1 was identified as a cytoplasmic protein (Lässle et al. 1997). However, a small fraction of STI-1 was found bound to a cellular prion protein at the cell membrane, where it promotes neuroprotection against cell death (Zanata et al. 2002). Moreover, STI-1 accumulates in the nucleus of murine fibroblasts after treatment with leptomycin B, a nuclear export inhibitor (Longshaw et al. 2004). A human homologue has also been found in the Golgi apparatus and in small vesicles within fibroblasts (Honoré et al. 1992).

A protozoan orthologue of *STI-1* has been characterized in *Leishmania major* and shown to be upregulated when the parasites are exposed to heat stress conditions (Webb et al. 1997). This parasite, like other trypanoso-

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matids, is exposed to various environmental conditions during its life cycle in which it alternates between invertebrate and vertebrate hosts. Experiments in vitro have shown that environmental changes, such as acidification or heat shock, trigger a change in the developmental program of the parasites, promoting their differentiation from the promastigote to the amastigote form (Zilberstein & Shapira 1994). The differentiation of *Trypanosoma brucei* (the causal agent of sleeping sickness) from the vertebrate bloodstream trypomastigote form to the invertebrate procyclic form is also induced by exposure to mild acidic stress in vitro (Rolin et al. 1998). Similarly, the incubation of *Trypanosoma cruzi* trypomastigote forms in culture medium at pH 5.0 for 2 h is sufficient to trigger their transformation into amastigote forms (Tomlinson et al. 1995). The differentiation of *T. cruzi* epimastigotes into metacyclic trypomastigotes is induced by nutritional stress in the insect midgut and in chemically defined conditions in vitro (Contreras et al. 1985, Kollien & Schaub 2000).

As stress conditions trigger the transformation of trypanosomatids from one form to another throughout their life cycle, the characterization of stress response proteins should contribute to our understanding of these differentiation processes. Therefore, this work describes the first characterization of the conserved eukaryotic STI-1 orthologue in *T. cruzi* and investigates the interaction of STI-1 in *T. cruzi* (TcSTI-1) with TcHSP70. We also report the effects of various stress conditions on TcSTI-1 expression in exponentially growing epimastigotes.

## MATERIALS AND METHODS

**Parasite culture and stress conditions** - Epimastigotes of *T. cruzi* clone Dm28c (Contreras et al. 1988) were maintained in the exponential growth phase by subculturing approximately  $2 \times 10^7$  cells/mL every three days at 28°C in liver infusion tryptose (LIT) medium supplemented with 10% foetal bovine serum (Camargo 1964). Epimastigotes in late growth phase were cultured for five days under the same conditions. Parasites were subjected to different stress conditions by incubation for 2 h either in triatomine artificial urine medium (nutritional stress), LIT medium adjusted to pH 5.0 (pH stress) or LIT medium at 37°C (heat stress).

**Cloning and analysis of the sequence of the TcSTI-1 gene** - The coding region of the *T. cruzi* Dm28c STI-1 gene was amplified by polymerase chain reaction (PCR) using primers based on the CL Brener sequence (GeneDB systematic: Tc1047053506321.290) (TcSTI-1F 5'GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGACGCAACAGAACTTAAGAACAG3', TcSTI-1R 5'GGGGACCACTTTGTACAAGAAAGCTGGGTCCTTTCCAAAACGAATGATTCCAGC3'), with recombination sites appended to the 5' end for insertion into the pDONR221 vector (Gateway™ Platform). PCR was carried out on 100 ng of total DNA from *T. cruzi* epimastigotes. PCR reactions contained 10 pmol of each primer, 200 µM of each dNTP, 2.5 units of *Pfu* DNA polymerase and *Pfu* DNA polymerase buffer (Stratagene). They were cycled as follows: 94°C for 5 min, 10 cycles of 94°C for 30 s, 62°C for 30 s and 72°C for 2 min, followed by

25 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 2 min, with a final extension step of 72°C for 4 min. The amplified fragment was inserted into pDONR221 (entry vector) and transferred to pDEST17 (expression vector) according to the manufacturer's protocol (Invitrogen).

DNA sequences were analyzed using BLAST (Altschul et al. 1990) and ClustalW2 (Larkin et al. 2007) algorithms and the Lasergene sequence analysis package (DNASTAR Inc).

**Production and purification of recombinant TcSTI-1** - The expression construct (PCR-amplified DNA fragment inserted into pDEST17) was introduced into *Escherichia coli* strain BL21(DE3)pLysS (Invitrogen). Production of the His<sub>6</sub>-tagged recombinant protein was induced by adding 0.1 mM IPTG (isopropyl-β-D-thiogalactoside) and incubating for 3 h at 37°C. The recombinant protein was purified under denaturing conditions by immobilized metal affinity chromatography using a Ni-NTA agarose column (Qiagen) according to the manufacturer's protocols. Purified protein was subjected to preparative SDS-PAGE and electroelution. The concentration and purity of TcSTI-1 were assessed with a Quant-IT Kit (Invitrogen) and by SDS-PAGE, respectively.

**Polyclonal antiserum production and western blot analysis** - Swiss mice were immunized intraperitoneally with 50 µg of purified recombinant TcSTI-1 and Freund's incomplete adjuvant (Sigma) for the first inoculation and with 50 µg of the recombinant protein and Alu-Gel-S suspension (SERVA) for four booster injections, administered at two-week intervals. Antiserum was obtained seven days after the last booster injection. The antiserum reacted with recombinant TcSTI-1 and specifically recognized a protein with an approximate molecular mass of 65 kDa in *T. cruzi* whole-parasite extracts. No cross-reaction was observed with preimmune serum at dilutions of up to 1:200. The same inoculation protocol was used to obtain a polyclonal antiserum against TcGAPDH, whose molecular mass is 39 kDa. A rabbit polyclonal antiserum against TcHSP70 was kindly provided by Dr Silvana Murta, from the René Rachou Research Institute-Fiocruz.

Total protein extracts were prepared by washing parasites ( $2 \times 10^8$  cells) in phosphate-buffered-saline (PBS) and resuspending them in 100 µL of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. Protein concentration was determined with a 2D-Quant Kit (Amersham). Lysate proteins were separated by SDS-PAGE through a 10% polyacrylamide gel (Laemmli 1970), transferred onto nitrocellulose membranes (Amersham) (Towbin et al. 1979) and probed with polyclonal antisera at dilutions of 1:800 (anti-TcSTI-1), 1:5000 (anti-TcHSP70) or 1:1000 (anti-TcGAPDH). The resulting antigen-antibody complexes were detected using horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies (1:7500) and the enhanced chemiluminescence western blotting system (Amersham). Relative quantities of TcSTI-1 were normalized to TcGAPDH levels to control for variations in protein loading. Statistical analysis was performed by one-way ANOVA.

**Quantitative PCR (qPCR)** - Parasites in exponential growth phase and subjected to different stress conditions (heat, pH and nutritional stresses) were used to obtain poly-some-associated RNAs, as described by Goldenberg et al. (1985). Polysomal RNAs from three independent experiments were purified with the RNeasy kit (QIAGEN) and 0.5 µg of RNA was converted into cDNA using oligo(dT)<sub>15</sub> and ImProm-II reverse transcriptase (Promega). Using a modified version of the method described by Nardelli et al. (2007), real-time PCR assays were performed on an ABI PRISM 7500 sequence detection system (Applied Biosystems), with specific primers for *STI-1* (TcSTI-1qPCR: 5'GAAGGACGCATGCGGACTCTG3', TcSTI-1qPCR: 5'GCGCGTTTTGCCACTTCATCTC3'), H2B histone (TcH2B: 'CGGTGGTGCGCGTCAACAA-GAAGC3', TcH2B: 5'CCAGGTCCGCGGCGAGCAGCAGAG3') or ribosomal protein L9 (TcL9F:5'CCTTCACTGCCTTCGTTGGTTTG3', TcL9R:5'ATGCGAGAGTGCCTGTTGATGGT3'). The reaction mixture contained 10 ng of cDNA, 250 nM primer and the recommended concentration of SYBR Green master mix (Applied Biosystems). Amplifications were carried out in triplicate, with an initial denaturation step at 95°C for 10 min followed by 45 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 1 min. Thermal dissociation and 5% PAGE confirmed that PCR generated specific amplicons. A standard curve method, based on cycle threshold values, was used to assess expression of the *TcSTI-1* gene. Triplicate 1:5 dilutions of known concentrations of cDNA were used to generate standard curves extending from 50 ng-80 pg of cDNA for each gene. Relative quantities of *TcSTI-1* mRNA were normalized with respect to transcript levels of TcH2B and TcL9 control genes in various stress conditions and plotted, with unstressed epimastigotes as the reference population. Statistical analysis was performed using one-way ANOVA and Statgraphics Plus 5.0 software.

**Immunofluorescence assay** - Parasites were washed and resuspended at a density of 10<sup>7</sup> cells/mL in PBS. The cells were incubated for 20 min at room temperature (RT) to allow them to adhere to poly-L-lysine-coated slides. The samples were then fixed by incubation with 4% paraformaldehyde for 10 min, washed in PBS and treated with 50 mM NH<sub>4</sub>Cl for another 10 min. Fixed cells were permeabilized by incubation with 0.1% Triton X-100 in PBS for 2 min and blocked by incubation overnight with 1.5% bovine serum albumin (BSA) in PBS. The cells were incubated for 1 h with polyclonal antisera at a dilution of 1:60 (anti-TcSTI-1) and 1:250 (anti-TcHSP70). The samples were then washed and incubated for 1 h with Alexa 488-conjugated goat anti-mouse antibody and Alexa 546-conjugated anti-rabbit antibody (Sigma) diluted at 1:500, as well as with DAPI (1 µg/µL). Stained slides were observed with an epifluorescence microscope (Nikon Eclipse E600) using a 100X objective.

**Immunoprecipitation assay** - Anti-TcSTI-1 (8 µL) or anti-TcHSP70 (2 µL) antiserum was incubated with about 50 µL of protein G-Sepharose or protein A-Sepharose beads (Sigma), respectively, for 2 h at 4°C. Mouse anti-IgG and rabbit preimmune antisera were used as

controls (control beads). The beads were blocked with 1 mg/mL BSA in PBS. Antibodies were cross-linked to beads by treatment with 6.5 mg/mL dimethyl-pimide-late (Sigma) and 200 mM triethanolamine in PBS for 30 min at RT, followed by washing with 200 mM triethanolamine in PBS (the incubation and washing steps were then repeated twice). Cross-linking was stopped by adding 50 mM ethanolamine in PBS and unbound antibodies were washed off with 200 mM glycine (pH 2.3). Conjugated beads were washed with PBS and incubated overnight at 4°C in low-salt lysis buffer (100 mM KCl, 5 mM MgCl<sub>2</sub>, 10 mM HEPES pH 7.0, 0.5% NP-40, 1 mM PMSF, 1 µM E-64) containing a soluble protein fraction obtained from 10<sup>9</sup> exponentially growing epimastigotes. After three washes with 10 volumes of washing buffer (100 mM KCl, 5 mM MgCl<sub>2</sub>, 10 mM Hepes pH 7.0, 1% NP-40, 1 mM PMSF, 1 µM E-64), bound proteins were eluted with one volume of 200 mM glycine (pH 2.3). Immunoprecipitated proteins were separated by SDS-PAGE through a 10% polyacrylamide gel and analyzed by western blotting.

**Ethics** - Animal experiments were approved by the Oswaldo Cruz Foundation's Ethical Committee on Animal Experimentation (CEUA/FIOCRUZ, protocol P-0434/07).

## RESULTS

**The *STI-1* homolog of *T. cruzi*** - A single copy of the putative *STI-1* orthologue has already been identified in the *T. cruzi* genome. The open reading frame of 1671 nucleotides encodes a protein containing 556 amino acid residues with a predicted molecular weight of 63.1 kDa (El-Sayed et al. 2005).

The amino acid sequence of the protein encoded by *TcSTI-1* gene displays significant similarity to the sequences of other STI-1 proteins from *L. major* (64%), humans (40%), mice (39%), soybeans (37%) and yeast (37%). A comparison of the predicted STI-1 proteins is presented in Fig. 1, with the conserved amino acids indicated. Furthermore, most of the conserved residues are present in TPR motifs that mediate binding to HSP70 and HSP90 (TPR1, TPR2A and TPR2B), such as a 10-amino-acid conserved sequence (NHVLYSNRSA) in TPR1 and a six-amino-acid conserved sequence (YSN-RAA) in TPR2B.

**Effect of different stress conditions on *STI-1* expression in *T. cruzi*** - We investigated whether *STI-1* production in *T. cruzi* was induced by stress conditions, as reported for other STI-1 proteins. Levels of mRNA and protein were analyzed in epimastigotes subjected to nutritional, heat or pH stress for 2 h. Relative levels of polysomal *STI-1* mRNA from stressed parasites were quantified by qPCR, but no significant difference ( $p > 0.05$ ) was found between these levels and those in unstressed parasites (Fig. 2A). A western blot analysis of steady-state levels of TcSTI-1 protein revealed no differences between stressed and unstressed epimastigotes in exponential growth phase (Fig. 2B), confirming the results obtained by qPCR. Increasing the duration of stress up to 6 h did not significantly alter TcSTI-1 levels (Supple-

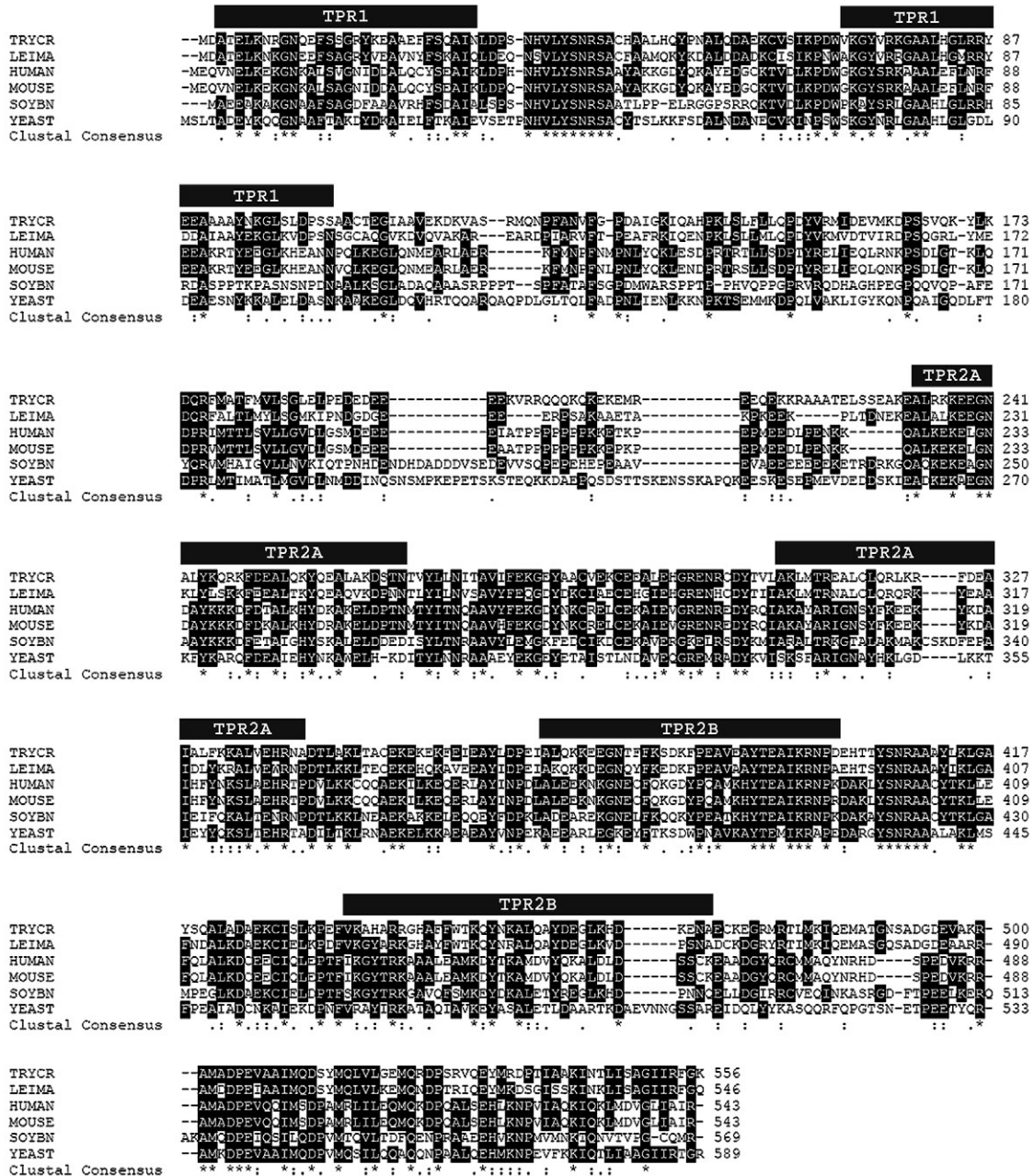


Fig. 1: comparative alignment of amino acid sequences from stress-induced protein-1 (STI-1) homologs. Translated sequences of *STI-1* from *Trypanosoma cruzi* (TRYCR), *Leishmania major* (LEIMA), human, mouse, soybean (SOYBN) and yeast were analyzed with ClustalW. Tetratricopeptide repeat motifs are indicated by boxes above the residues. Residues identical in all sequences are shaded in black.

mentary data). Similarly, no changes in protein steady-state levels of TcHSP70 were observed in either assay (Fig. 2B, Supplementary data). A significant increase in TcSTI-1 levels (Fig. 3) was observed in epimastigotes in late growth phase subjected to nutritional stress (Epi 5d stressed) compared with exponentially growing epimastigotes (Epi 3d) ( $p < 0.05$ ). These results indicate that stress-induced expression of TcSTI-1 in *T. cruzi* epimastigotes differs according to the growth phase during which the epimastigotes are subjected to stress.

*TcSTI-1 co-immunoprecipitates and co-localizes with TcHSP70* - STI-1 is a co-chaperone known to bind heat shock proteins, such as HSP70. Co-immunoprecipitation assays were performed to determine whether this interaction also occurred in *T. cruzi*. Western blot analysis of the fractions eluted from beads loaded with anti-TcSTI-1 antiserum showed that TcSTI-1 and TcHSP70 co-eluted from protein extracts (Fig. 4A). Similarly, these proteins were also identified in an eluted fraction when the beads were loaded with anti-TcHSP70 serum (Fig. 4B).

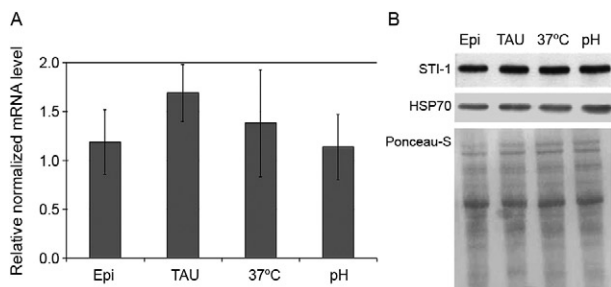


Fig. 2: stress-induced protein-1 (STI-1) in *Trypanosoma cruzi* polysomal mRNA and protein levels in various stress conditions. Data were obtained from unstressed *T. cruzi* epimastigotes in the exponential growth phase (Epi) and from epimastigotes subjected to nutritional [triatomine artificial urine (TAU)], heat (37°C) or pH stress (pH) for 2 h. A: relative levels of polysome-associated *STI-1* RNAs were quantified by quantitative polymerase chain reaction and normalized against mean values for the *L9* and *H2B* control genes. Each column shows the mean values  $\pm$  standard deviation for three independent experiments; B: western-blot analysis of *T. cruzi* whole-protein extracts (20  $\mu$ g) probed with anti-TcSTI-1 antibody (1:800) or anti-TcHSP70 (1:5000). A Ponceau-S stained membrane is shown below the western blot as a control for protein loading. This figure shows one representative result from three independent experiments.

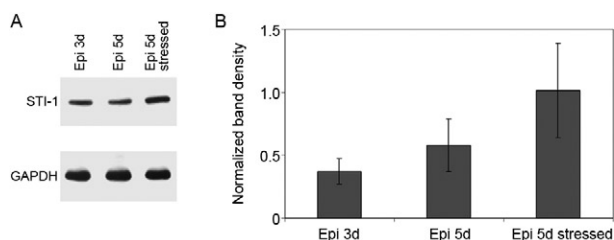


Fig. 3: stress-induced protein-1 (STI-1) in *Trypanosoma cruzi* (TcSTI-1) levels in epimastigotes in late growth phase. A: western-blot analysis of *T. cruzi* whole-protein extracts (20  $\mu$ g) obtained from unstressed *T. cruzi* epimastigotes in the exponential growth phase (Epi 3d), epimastigotes in the late growth phase (Epi 5d) and from epimastigotes in the late growth phase subjected to nutritional (Epi 5d stressed). Membranes were probed with anti-TcSTI-1 antibody (1:800) or anti-GAPDH (1:800) as a control for protein loading. This figure shows one representative result from three independent experiments; B: bands density were quantified by ImageJ program and normalized against mean values for the GAPDH control protein. Each column shows the mean values  $\pm$  standard deviation for three independent experiments.

Neither TcSTI-1 nor TcHSP70 was identified in fractions eluted from control beads. The co-immunoprecipitation of these two proteins suggests that STI-1 is associated with HSP70 in *T. cruzi* epimastigotes.

The cellular distribution of TcSTI-1 was determined by indirect immunofluorescence analysis, which showed this protein to be present in the cytoplasm of cultured (unstressed) epimastigote forms (Fig. 5B) and in epimastigotes subjected to nutritional stress (Fig. 6B). A similar pattern was observed for TcHSP70 in unstressed epimastigote forms (Fig. 5C), but a stronger signal was detected in the nucleus of stressed epimastigotes (Fig.

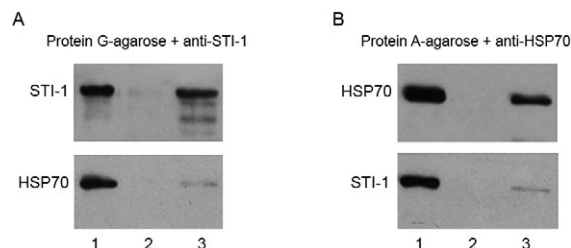


Fig. 4: co-immunoprecipitation of stress-induced protein-1 (STI-1) in *Trypanosoma cruzi* and TcHSP70. Agarose beads loaded with specific antisera were incubated with soluble protein extracts from exponentially growing *T. cruzi* epimastigotes. Eluted proteins were separated by SDS-PAGE in 10% polyacrylamide gels and analyzed by western blotting. Lane 1: input protein extract; 2: fraction eluted from control beads; 3: fraction eluted from beads with specific antiserum. This figure shows one representative result from two independent experiments.

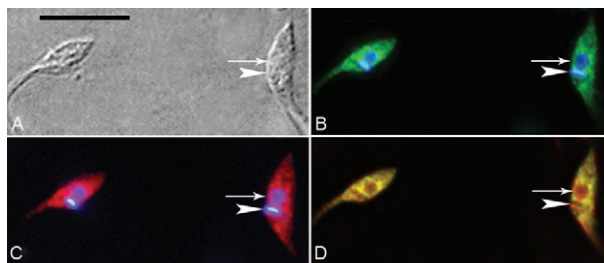


Fig. 5: immunolocalization of stress-induced protein-1 (STI-1) in *Trypanosoma cruzi* (TcSTI-1) protein in unstressed *T. cruzi* epimastigotes. Parasites were immunostained with a specific antiserum against TcSTI-1 and TcHSP70 and then incubated with AlexaFluor 488 (B, D) and with AlexaFluor 546 (C, D) secondary antibodies. A: cell bodies, as visualized by interferential contrast (DIC); B: merged image, showing nuclei (arrow) and kinetoplasts (arrowhead) stained in blue (DAPI) and green fluorescence for TcSTI-1; C: red fluorescence for TcHSP70. Nuclei and kinetoplasts are stained blue with DAPI; D: merged image showing green fluorescence for TcSTI-1 and red fluorescence for TcHSP70. The two proteins are colocalized throughout the cell body. Note the intense colocalization of TcSTI-1 and TcHSP70, visualized as yellow patches around the nuclei. Bar = 10  $\mu$ m.

6C). Some of the signal for both these proteins was colocalized in patches around the nucleus (Fig. 5D, 6D). These results are consistent with the immunoprecipitation data, providing further support for an association between STI-1 and HSP70.

## DISCUSSION

The Hop/STI-1 protein is a co-chaperone that mediates the formation of a complex between HSP70 and HSP90 (Smith et al. 1993). A recent study comparing 19 different eukaryotic organisms showed that this protein was the most widespread of the 10 co-chaperones studied (Johnson & Brown 2009). We have demonstrated the occurrence of a Hop/STI-1 homolog in the protozoan *T. cruzi* (TcSTI-1), thus confirming the conservation of this protein in diverse eukaryotes. Furthermore, the STI-

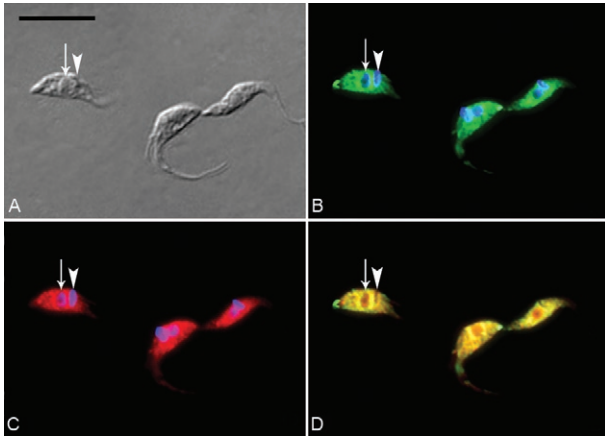


Fig. 6: immunolocalization of stress-induced protein-1 (STI-1) in *Trypanosoma cruzi* (TcSTI-1) protein in *T. cruzi* epimastigotes subjected to nutritional stress. Parasites were immunostained with a specific antiserum against TcSTI-1 and TcHSP70 and then incubated with AlexaFluor 488 (B and D) and with AlexaFluor 546 (C and D) secondary antibodies. A: cell bodies, as visualized by interferential contrast (DIC); B: merged image showing nuclei (arrow) and kinetoplasts (arrowhead) stained in blue (DAPI) and green fluorescence for TcSTI-1; C: red fluorescence for TcHSP70; nuclei and kinetoplasts are stained blue with DAPI; D: merged image showing green fluorescence for TcSTI-1 and red fluorescence for TcHSP70. Note the stronger red signal for TcHSP70 in the nuclei. Bar = 10  $\mu$ m.

1 protein of *T. cruzi* also presents the three TPR domains characteristic of Hop/STI-1 co-chaperones in its N-terminal, central and C-terminal portions, as deduced from its amino acid sequence. These TPR domains mediate the formation of a multi-chaperone complex between HSP70 and HSP90 (Chen & Smith 1998, Scheufler et al. 2000).

We demonstrated the association of TcSTI-1 and TcHSP70 in *T. cruzi* epimastigote forms using co-immunoprecipitation and immunolocalization assays, thus suggesting that TcSTI-1 acts as an adaptor protein. It has also been reported that the incubation of parasites for 2 h at 37°C induces the translocation of TcHSP70 from the cytoplasm to the nucleus (Martin et al. 1993, Olson et al. 1994). Our data indicate that TcHSP70 is also translocated to the nucleus when parasites are subjected to nutritional stress.

Heat shock and other stress conditions have been shown to induce *STI-1* expression in several organisms (Nicolet & Craig 1989, Honoré et al. 1992, Lässle et al. 1997, Heine et al. 1999, Zhang et al. 2003, Song et al. 2009). We evaluated the stress-induced expression of the *TcSTI-1* gene in exponentially growing *T. cruzi* epimastigotes subjected to nutritional, acidic or heat stress for 2 h. We found that the incubation of exponentially growing parasites under these stress conditions resulted in no significant change in the level of the TcSTI-1 protein. A similar result was obtained in a mouse model in which steady-state STI-1 protein levels were not affected by incubation for 150 min at 42°C, despite a large increase in mRNA levels (Lässle et al. 1997). Increased levels of TcSTI-1 were observed in epimastigotes in late growth phase subjected to nutritional stress (Epi 5d stressed)

compared with exponentially growing epimastigotes (Epi 3d), indicating that stress response and consequently TcSTI-1 induction in *T. cruzi* epimastigotes differs according to the growth phase during which the cells are subjected to stress.

Total mRNA levels have been analyzed in several organisms to evaluate the induction of *STI-1* gene expression by stress conditions (Lässle et al. 1997, Webb et al. 1997, Zhang et al. 2003, Song et al. 2009). We chose to analyze only the polysome-associated mRNA fraction because gene expression in *T. cruzi* is predominantly regulated at the posttranscriptional level (Teixeira 1998, Clayton 2002). The mechanisms thought to be involved in regulating *T. cruzi* gene expression determine mRNA half-life and accessibility to the translation machinery (Goldenberg et al. 1985, da Silva et al. 2006). The recent description of P-body-like structures in *T. cruzi* (Holetz et al. 2007), in which mRNA may be degraded or stored in the cytoplasm, provides evidence for the existence of posttranscriptional regulatory pathways in this organism. Although gene regulation is mainly regulated by posttranscriptional mechanisms, *TcSTI-1* polysomal mRNA levels were not significantly modified in stressed parasites compared with unstressed cells.

The stress response of HSP70 is not completely defined in *T. cruzi*. Some reports have shown that 42°C shock provokes mild to strong induction of HSP70 expression (de Carvalho et al. 1990, Requena et al. 1992, Olson et al. 1994). However, parasites from 48 h-old cultures do not display a classical response to heat treatment (de Carvalho et al. 1994). Our experiments, conducted using exponentially growing and stationary phase epimastigotes submitted to stress conditions, added new data to this subject. This variability of the stress responses in *T. cruzi*, together with the diversity of posttranscriptional gene regulation mechanisms at work in this organism, may account for the complexity in the stress response regarding to the canonical heat shock proteins expression profile. More experiments must be conducted to verify the differences in the expression of STI-1, HSP70 and other heat shock proteins subsequent to different stress challenges.

In addition to binding to HSP70 and HSP90, a small fraction of murine STI-1 binds to a cellular prion protein at the cell membrane (Zanata et al. 2002). It has been suggested that this interaction mediates neuronal survival and differentiation (Lopes et al. 2005, Arantes et al. 2009). Furthermore, STI-1 protein levels are modulated during mouse embryogenesis (Hajj et al. 2009). We are now trying to improve our understanding of the role played by TcSTI-1 during the *T. cruzi* life cycle by looking for other partners of STI-1 and analyzing the pattern of STI-1 gene expression during epimastigote-to-metacyclic trypomastigote differentiation (metacyclogenesis), induced by nutritional stress in chemically defined conditions in vitro.

#### ACKNOWLEDGEMENTS

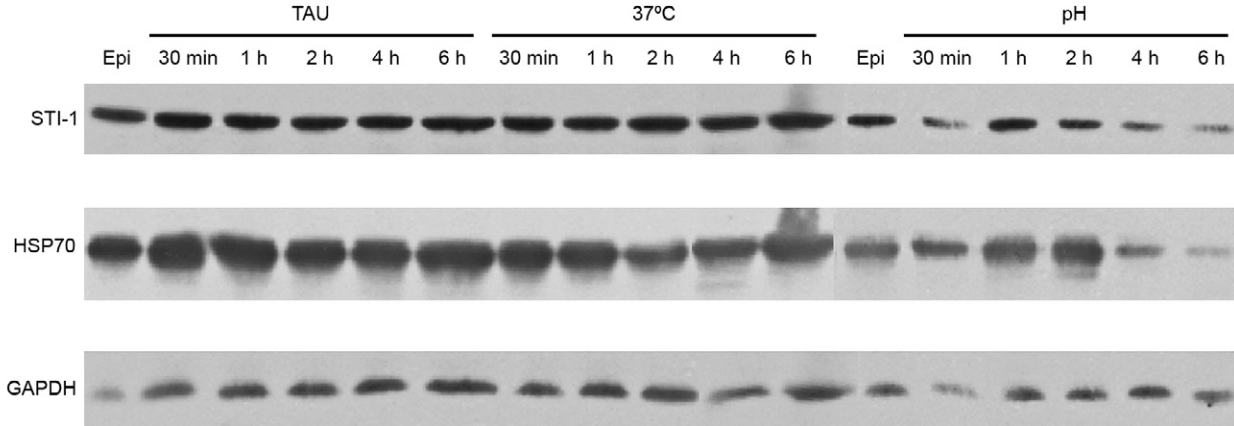
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Western blot analysis of *Trypanosoma cruzi* stressed up to 6 h. Whole-protein extracts (20  $\mu$ g), obtained from unstressed *T. cruzi* epimastigotes in the exponential growth phase (Epi) and from epimastigotes subjected to nutritional [triatomine artificial urine (TAU)], heat (37°C) or pH stress for 30 min, one, two, four and six hours. Membranes were probed with anti-TcSTI-1 antibody (1:800), anti-HSP70 (1:5000) or anti-GAPDH (1:800) as a control for protein loading. This figure shows one representative result from three independent experiments.