Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
International Journal for Parasitology 36 (2006) 87–96
INTERNATIONAL Journal for PARASITOLOGY
www.elsevier.com/locate/ijpara

Control mechanisms of tubulin gene expression in *Trypanosoma cruzi*

Rosiane A. da Silva [a], Daniella C. Bartholomeu [b], Santuza M.R. Teixeira [a,*]

[a] *Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-010, Brazil*
[b] *The Institute for Genomic Research, Rockville, MD, USA*

Received 31 May 2005; received in revised form 29 August 2005; accepted 13 September 2005

Abstract

α- and β-Tubulin mRNAs are three to six-fold more abundant in the epimastigote forms than in trypomastigote and amastigote forms of *Trypanosoma cruzi*. It has been previously shown that the increased abundance of α- and β-tubulin mRNAs found in epimastigotes is due to an increase in their half-lives. By analysing soluble and cytoskeletal protein fractions of the parasite, we found an inverse correlation between tubulin mRNA and the protein levels of free α- and β-tubulin subunits, which are more abundant in trypomastigotes and amastigotes than in epimastigotes. Here we investigated a possible autoregulatory mechanism responsible for the differential accumulation of tubulin mRNAs in *T. cruzi* by treating epimastigotes with vinblastine and taxol, drugs that disrupt microtubule dynamics by different mechanisms: vinblastine causes significant depolymerisation of tubulin whereas taxol stabilises microtubules. Vinblastine treatment caused significant morphological alterations in epimastigotes whereas taxol does not alter the parasite morphology. Vinblastine, but not taxol, had a specific effect on the levels of α- and β-tubulin mRNAs, causing a five to nine-fold reduction in the steady-state levels of both mRNA populations, whereas the levels of other mRNAs such as *gapdh* remained unchanged. The reduction in RNA levels caused by vinblastine treatment is mediated by changes in tubulin mRNA half-lives. In an attempt to identify regulatory elements within tubulin mRNAs, plasmids containing luciferase reporter gene associated with 5′-untranslated (UTR), 3′-UTR and part of coding sequence of the tubulin genes were constructed and used for transient DNA transfections of epimastigotes. Determination of luciferase activity in transfected parasites cultured in the presence and absence of vinblastine indicated that sequences located within the α-tubulin 3′-UTR and coding region may be involved in modulating the stability of these transcripts in response to changes in the dynamics of *T. cruzi* microtubules.

Keywords: Trypanosoma cruzi; Tubulin; Gene regulation; Untranslated region

1. Introduction

Trypanosoma cruzi, the causative agent of Chagas disease, remains a significant public health concern throughout South and Central America (http://www.who.int/tdr/diseases/chagas/). The life cycle of this unicellular eukaryote involves three stage-specific forms in the mammalian host and the triatomine vector, each one adapted for proliferation and/or cell invasion. Epimastigotes multiply in the insect vector and migrate to the hindgut where they differentiate into nondividing, infective trypomastigotes. Trypomastigotes, excreted in the feces, are inoculated through skin punctures or mucous membranes of the mammalian host. After reaching the bloodstream, trypomastigotes invade a variety of cells and convert into amastigote forms, which replicate in the cytosol by binary division. After multiple rounds of replication, amastigotes transform into trypomastigotes that are released by rupture of the host cell plasma membrane. Trypomastigotes and amastigotes present in the blood of the infected mammal complete the cycle when they are taken up in a blood meal by a reduviid bug (reviewed by Tyler and Engman, 2001).

Morphological changes during the *T. cruzi* life cycle most likely involve alterations in the expression of genes encoding components of the cytoskeleton of the parasite. A peculiar feature of trypanosomatids is the subpellicular corset of microtubules that encloses the entire cell body and it is very closely associated with the overlying cell membrane (Seebeck et al., 1990). In addition to the subpellicular corset, three other types of microtubule structures have been described in the trypanosoma cell: the flagellar axoneme, the basal body and the mitotic spindle (Kohl and Gull, 1998). As found in all eukaryotes, the basic building block of the microtubule is the heterodimeric

* Corresponding author. Tel.: +55 31 3499 2665; fax: +55 31 3499 2614.
E-mail address: santuzat@icb.ufmg.br (S.M.R. Teixeira).

0020-7519/$30.00
doi:10.1016/j.ijpara.2005.09.005

protein αβ-tubulin that assembles in a head-to-tail arrangement to form a linear protofilament, which are assembled into cylindrical microtubules (Nogales et al., 1999).

Genes encoding *T. cruzi* α- and β-tubulin are arranged in a cluster with an alternating α/β array with a basic repeat unit length of 4.3 Kb (Maingon et al., 1988). One class of mRNA encoding α-tubulin and two classes of β-tubulin mRNAs have been identified in various *T. cruzi* strains (Gonzales-Pino et al., 1997; Bartholomeu et al., 2002). Earlier studies indicated that, in contrast to *T. brucei*, the expression of *T. cruzi* tubulin genes are regulated during the parasite life cycle. A significant reduction in the levels of both α- and β-tubulin mRNAs is observed during the growth of epimastigotes from the logarithmic to the stationary phase and during metacyclogenesis (Rondinelli et al., 1986; Gonzalez-Pino et al., 1999). Lower levels of the α-tubulin and the two β-tubulin transcripts are found in amastigotes and trypomastigotes compared to epimastigotes (González-Pino et al., 1997; Bartholomeu et al., 2002).

Studies performed with a large number of developmentally regulated genes in trypanosomatids have shown that gene expression in these organisms is mainly controlled at the post-transcriptional level (for a review see Clayton, 2002). Accordingly, in spite of the differences in their mRNA steady state levels, nuclear run-on assays demonstrated that the transcription rates of α- and β-tubulin genes are similar in different stages of the *T. cruzi* life cycle. Further analyses also demonstrated that the changes in mRNA levels are due to mechanisms affecting their half-lives (Bartholomeu et al., 2002).

In mammalian cells it has been shown that when the levels of free tubulin subunits increase in the cytoplasm, polysomal tubulin mRNAs are destabilised by the binding of protein factors to the nascent *N*-terminal tubulin tetrapeptide. The binding of these yet to be identified factors results in the recruitment of RNAses that degrade polysomal tubulin mRNAs (Cleveland, 1988; Yen et al., 1988). For mammalian β-tubulin mRNA but not for α-tubulin, site directed mutagenesis and transient DNA transfection have shown that the elements required for autoregulation of mRNA half-life correspond to the 13 translated nucleotides encoding the amino-terminal tetrapeptide MREI (Bachurski et al., 1994).

To investigate whether a similar autoregulatory mechanism operates in *T. cruzi*, we analysed the effect of drugs that disrupt microtubule dynamics on the levels of tubulin mRNAs in epimastigotes. We also investigated the involvement of sequences within tubulin mRNAs using plasmids containing the luciferase reporter gene associated with 5′-untranslated (UTR), 3′-UTR and tubulin coding sequences in transient DNA transfection assays. Our results indicate that an autoregulatory mechanism involving elements present at the beginning of the coding region and in the 3′-UTR of α-tubulin mRNA controls the expression of *T. cruzi* tubulin genes by affecting their mRNA half-lives in response to changes in the concentration of unpolymerised tubulin.

2. Materials and methods

2.1. Parasite cultures

Epimastigotes of the CL Brener clone and Tulahuén strain of *T. cruzi* were maintained in logarithmic growth phase at 28 °C in liver infusion tryptose (LIT) medium supplemented with 10% fetal bovine serum as described by Camargo (1964). Amastigote and trypomastigote forms were obtained from infected Vero cells grown in RPMI medium supplemented with 10% fetal bovine serum, at 37 °C and 5% CO_2, and purified by centrifugation in discontinuous metrizamide gradients as described previously (Teixeira et al., 1994). Epimastigote cultures were treated with 50 or 100 μM vinblastine (Vinblastine sulfate salt—Sigma) or 10 μM taxol (Paclitaxel—Sigma) during various periods of time.

2.2. Protein extract preparations

Total parasite proteins were extracted from epimastigotes, amastigotes and trypomastigotes by directly suspending 10^8 cells in 100 μl of Laemmli's sample buffer. Volumes corresponding to 10 μg of total parasite proteins were loaded on a 12.5% SDS-PAGE gel. To analyse soluble protein fractions, 10^8 parasites were washed in PBS and suspended in 100 μl of lysis buffer (10 mM tris, pH 7.5, 0.4% Triton X-100, 1 mM PMSF, 1 μg/ml pepstatin A and 2.0 μg/ml leupeptin). The cells were passed through a 29-gauge needle, centrifuged at 1000×*g* for 10 min, 4 °C, after which the supernatant was collected. Protein concentration was determined using the DC Protein Assay kit (Bio-Rad). Ten micrograms of protein from each supernatant were separated on a 12.5% SDS-PAGE gel and stained with Coomassie Brilliant Blue. For Western blot analyses, total protein extracts or soluble fractions were transferred to polivinylidene difluoride (PVDF) membranes after electrophoresis (Bio Rad trans-blot transfer medium), stained with Ponceau S (Sigma) and blocked by incubation with 5.0% nonfat dry milk. α- and β-tubulin were detected on Western blots using commercially available monoclonal antibodies anti α-tubulin from sea urchin and anti rat β-tubulin (Sigma) diluted at 1:1000 in PBS 0.1% Tween-20 and 1.0% nonfat dry milk. The blots were revealed with an anti-mouse IgG peroxidase conjugate at a dilution of 1:3000 and ECL Plus western blotting detection reagents (Amersham Biosciences). Extraction of cytoskeleton-associated proteins was performed by a modification of the procedures of Robinson et al. (1991); Bangs et al. (1993). Briefly, 10^8 epimastigotes were washed in PBS, suspended in 200 μl of lysis buffer (0.1 M PIPES, pH 6.9, 1% NP-40, 2 mM EGTA, 1 mM magnesium sulfate, 0.1 mM EDTA and the protease inhibitors: pepstatin A (5 μg/ml), PMSF (5 μg/ml) and leupeptin (50 μg/ml)) and incubated at room temperature for 5 min. After centrifugation at 1000×*g* for 10 min at 4 °C the supernatant was removed and the pellet, containing cytoskeleton-associated tubulin, was suspended in the same buffer. An amount of proteins corresponding to 2×10^6 parasites in each lane was separated on a 12.5% SDS-PAGE and stained with Coomassie Brilliant Blue.