J. Eukaryot. Microbiol., 47(3), 2000 pp. 271–279

Trypanosoma cruzi Infection Affects Actin mRNA Regulation in Heart Muscle Cells

MIRIAN CLAUDIA S. PEREIRA,[a] ROBERT H. SINGER[b] and MARIA DE NAZARETH S. L. DE MEIRELLES[a]

[a]*Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Av. Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brazil, and*

[b]*Department of Anatomy and Structural Biology and Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461, USA*

ABSTRACT. We have previously described alterations in the cytoskeletal organization of heart muscle cells (HMC) infected with *Trypanosoma cruzi* in vitro. Our aim was to investigate whether these changes also affect the regulation of the actin mRNAs during HMC differentiation. Northern blot analysis revealed that α-cardiac actin mRNA levels increased during cell differentiation while β-actin mRNA levels declined. Nonmuscle cells displayed β-actin mRNA signal localized at the cell periphery, while α-cardiac actin mRNA had a perinuclear distribution in myocytes. *Trypanosoma cruzi*-infected cells showed 50% reduction in α-cardiac actin mRNA expression after 72 h of infection. In contrast, β-actin mRNA levels increased approximately 79% after 48 h of infection. In addition, in situ β-actin mRNA was delocalized from the periphery into the perinuclear region. These observations support the hypothesis that *Trypanosoma cruzi* affects actin mRNA regulation and localization through its effect on the cytoskeleton of heart muscle cells.

Key Words. Actin mRNA, cardiomyocytes, in situ hybridization, *Trypanosoma cruzi*.

CHAGAS' disease or American trypanosomiasis results from infection with the protozoan hemoflagellate *Trypanosoma cruzi*. This parasitosis is an important cause of cardiomyopathy in Latin America, where approximately 16–18 million individuals are infected (Araújo-jorge, Barbosa, and Meirelles 1992; Dias 1997; Tanowitz et al. 1992). Although most people have mild symptoms during the acute phase of Chagas' disease, a small number of patients, mainly children, develop severe myocarditis. The chronic phase, however, may result in severe myocardial dysfunction.

Many investigators have been interested in clarifying biological and molecular events involved in *T. cruzi*-host cell interaction. There is evidence that trypomastigote forms of *T. cruzi* invade muscle cells by an endocytic mechanism (Meirelles et al. 1986; Meirelles et al. 1987; Barbosa et al. 1993), which requires host-cell cytoskeleton participation (Barbosa and Meirelles 1995). In addition, there have been reports of an active invasion of these parasites in other cell lines (Kipnis, Calich, and Dias da Silva 1979; Schenckman, Andrews, and Nussenzweig 1991; Schenkman and Mortara 1992).

Trypanosoma cruzi alters the organization of the target cell cytoskeleton. We have previously demonstrated that *T. cruzi* infection induces cytoskeletal disruption of heart muscle cells and that actin polygonal configuration occurs as a result of myofibrillar breakdown (Pereira et al. 1993). Stress fibers and vimentin filament disruption were also reported in *T. cruzi*-infected fibroblasts (Low, Paulin, and Keith 1992). Depolymerization of actin filaments underlying the cell membrane was observed in NRK cells at early stages of invasion (Tadiex et al. 1992). Since actin filaments play an important role in the cytoarchitecture and many cellular processes in all eukaryotic cells (Bretscher 1991; Carlier 1991; Kabsch and Vandekerckhove 1992), and in particular serve to anchor and localize actin mRNA (Bassel and Singer 1997; Sundel and Singer 1991), it became of interest to investigate actin gene regulation during myocardiogenesis and its expression after *T. cruzi* infection.

It has been well established that actin genes are expressed in a temporal- and tissue-specific manner during cell differentiation (Delvin and Emerson 1979; Hayward and Schwartz 1986; Lawrence and Singer 1986; Lawrence, Taneja, and Singer 1989). Proliferative myoblasts expressed high levels of β-actin mRNA, while sarcomeric α-actin mRNAs are induced at relatively high levels after multinucleated myotubes form. Early studies have demonstrated that many mRNAs are localized in specific cellular regions, which may be a mechanism to compartmentalize protein assembly sites within the cytoplasm (Bassel et al. 1998; Singer 1992; St. Johnston 1995; Wilhelm and Vale 1993). There are some examples of this specific localization: a selective distribution of actin mRNA in the apical region of intestinal epithelium (Cheng and Bjerknes 1989); a distinct localization of mRNAs coding for actin, vimentin, and tubulin in chicken embryo fibroblast (Lawrence and Singer 1986); the localization of microtubule-associated proteins (MAP-2) in dendrites (Garner, Tucker, and Matus 1988) and the restriction of Gap-43 and α-tubulin mRNAs to the cell body in neurons (Bruckenstein et al. 1990).

In this work, we have investigated the sequential actin gene expression during myogenesis of heart muscle cells and its regulation after *T. cruzi* interaction in vitro. Our data demonstrate a distinct pattern of isoactin mRNAs expression during cardiac cell development and indicate that changes occur in β-actin and α-cardiac actin mRNA regulation, which are induced by *T. cruzi* infection.

MATERIALS AND METHODS

Cell culture. Heart muscle cells (HMC) were isolated from 18-day-old mouse embryos as previously described (Meirelles et al. 1986). Cells were plated (1×10^6 cells/cm^2) into 100-mm culture dishes containing glass coverslips and incubated in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% horse serum, 5% fetal bovine serum, 2.5 mM $CaCl_2$, 1 mM L-glutamine, and 2% chicken embryo extract. The cell cultures were maintained at 37 °C in an atmosphere of 5% CO_2. After 24 h of incubation, most cells were beating spontaneously and synchronously. For in situ hybridization, cells grown on gelatin-coated coverslips for 2–4 d were washed with Hanks' balanced salt solution (HBSS) and fixed for 15 min with 4% paraformaldehyde in phosphate-buffered saline (PBS) containing 5 mM $MgCl_2$. After fixation, the cells were stored in 70% ethanol at 4 °C. For Western and Northern blot analysis, the cells were plated at a density of 3×10^6 cells/100-mm culture dishes and processed as described below.

Parasites. Bloodstream trypomastigote forms of *Trypanosoma cruzi*, Y strain, were obtained from Swiss Webster mice at the peak of the parasitemia as described (Meirelles, Souto-Padrón, and De Souza 1984). Muscle cell cultures were infected at an infection ratio of 10 parasites per host cell. After 24 h, free trypanosomes in the medium were removed by washing the cultures with Ringer's solution and fresh medium was placed in the culture plates. The time course of infection was interrupted after 24, 48,72, and 96 h.

Corresponding Author: M. Pereira—Telephone number: (5521) 598-4330. FAX number: (5521) 260-4434; Email: mirian@gene.dbbm.fiocruz.br

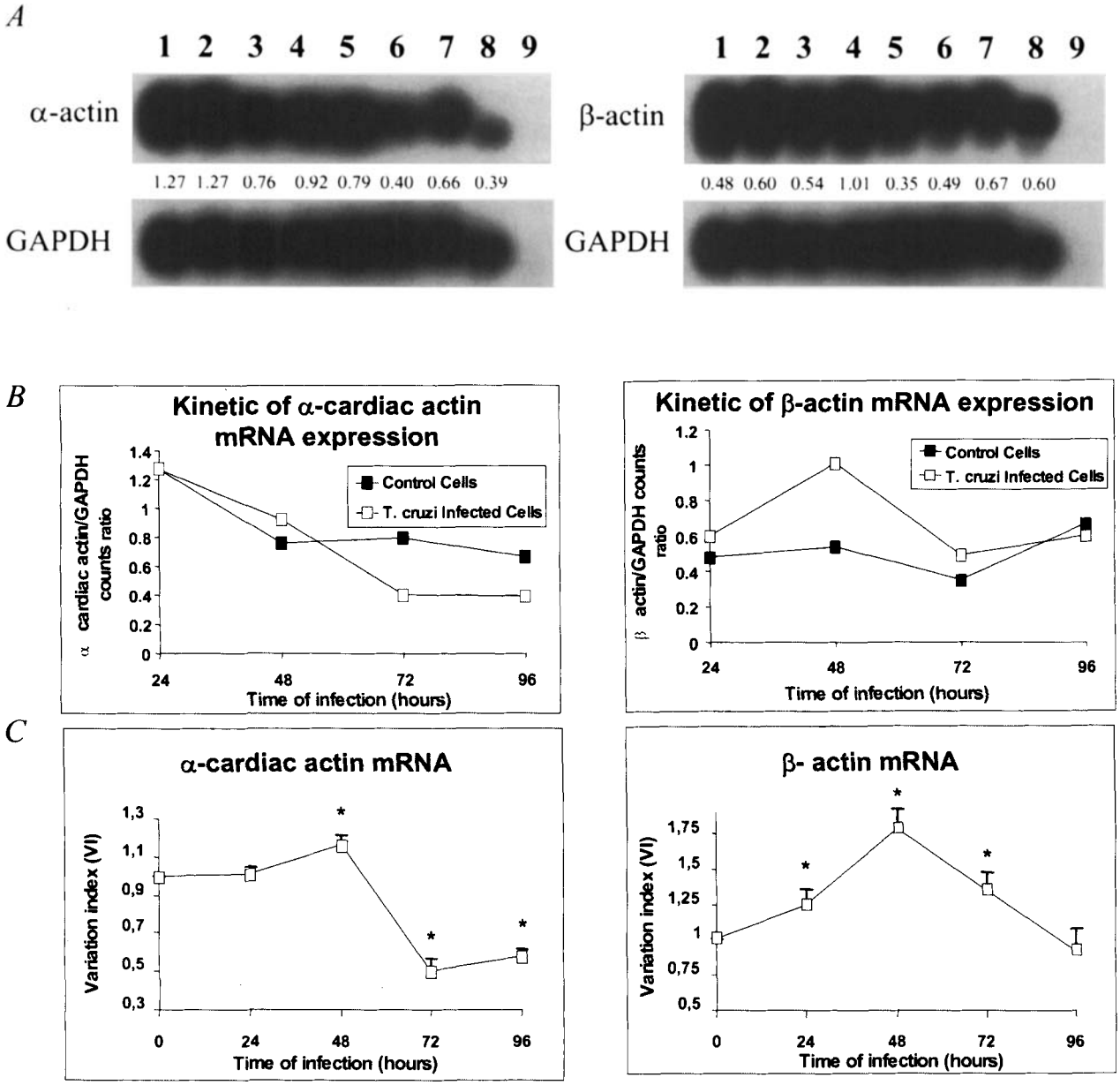

Fig. 1. Representative Northern blot of β and α-cardiac actin mRNAs in uninfected and *Trypanosoma cruzi*-infected heart muscle cells (A). Heart muscle cells (HMC) were infected 24 h after plating. Lane 1, control cells 2 d in culture; lane 2, HMC 24 h after *T. cruzi* infection; lane 3, control cells 3 d after plating; lane 4, infected cells, 48 h; lane 5, control cells, 4 d; lane 6, infected cells, 72 h; lane 7, control cells, 5 d; lane 8, infected cell, 96 h; lane 9, epimastigote forms of *T. cruzi*. (B) Kinetic expression of actin mRNAs in uninfected and *T. cruzi*-infected heart muscle cells. Quantitation of β and α-cardiac actin mRNAs was normalized with GAPDH mRNA. The variation index of 3 different experiments revealed changes in expression of actin mRNAs infected cells with reduction of α-cardiac actin and increase of β-actin mRNAs levels. * Student's *t*-test: Statistically significance $p < 0.05$.

Indirect immunofluorescence. Normal and infected cultures grown on coverslips were fixed for 5 min at room temperature (22 °C) in 4% paraformaldehyde in PBS, followed by three rinses in PBS for 15 min each. Cells were then incubated for 1 h at 37 °C with anti-β-actin antibody (Sigma Chemical Co., St. Louis, MO) in a 1/100 dilution. After rinsing, the cells were incubated in a 1/200 dilution of tetramethylrhodamine isothiocyanate (TRITC)-coupled anti-mouse IgG (Jackson Laboratories, Inc., West Grove, PA) for the same lapse of time. Phosphate-buffered saline (PBS) containing 0.5% Triton X-100 was used for all antibody dilutions and for washing. Some cells were stained for 30 min at 37 °C with fluorescein isothiocyanate (FITC)-labeled phalloidin (Molecular Probes, Inc., Eugene, OR) at a concentration of 4 μg/ml in PBS. To stain nuclei, 4',6-diamidino-2-phenilyndole (DAPI) was used. Coverslips were mounted in Vectashield® mounting medium. The samples were observed in a Nikon microscope equipped with epifluorescence and pictures were taken with a 63× immersion objective.

Drug treatment. Muscle cells were treated with nocodazole and Cytochalasin D to depolymerize the microtubules and actin filaments, respectively. Cells were incubated with nocodazole (10 μg/ml) or Cytochalasin D (1 μg/ml) for 1 h at 37 °C. The

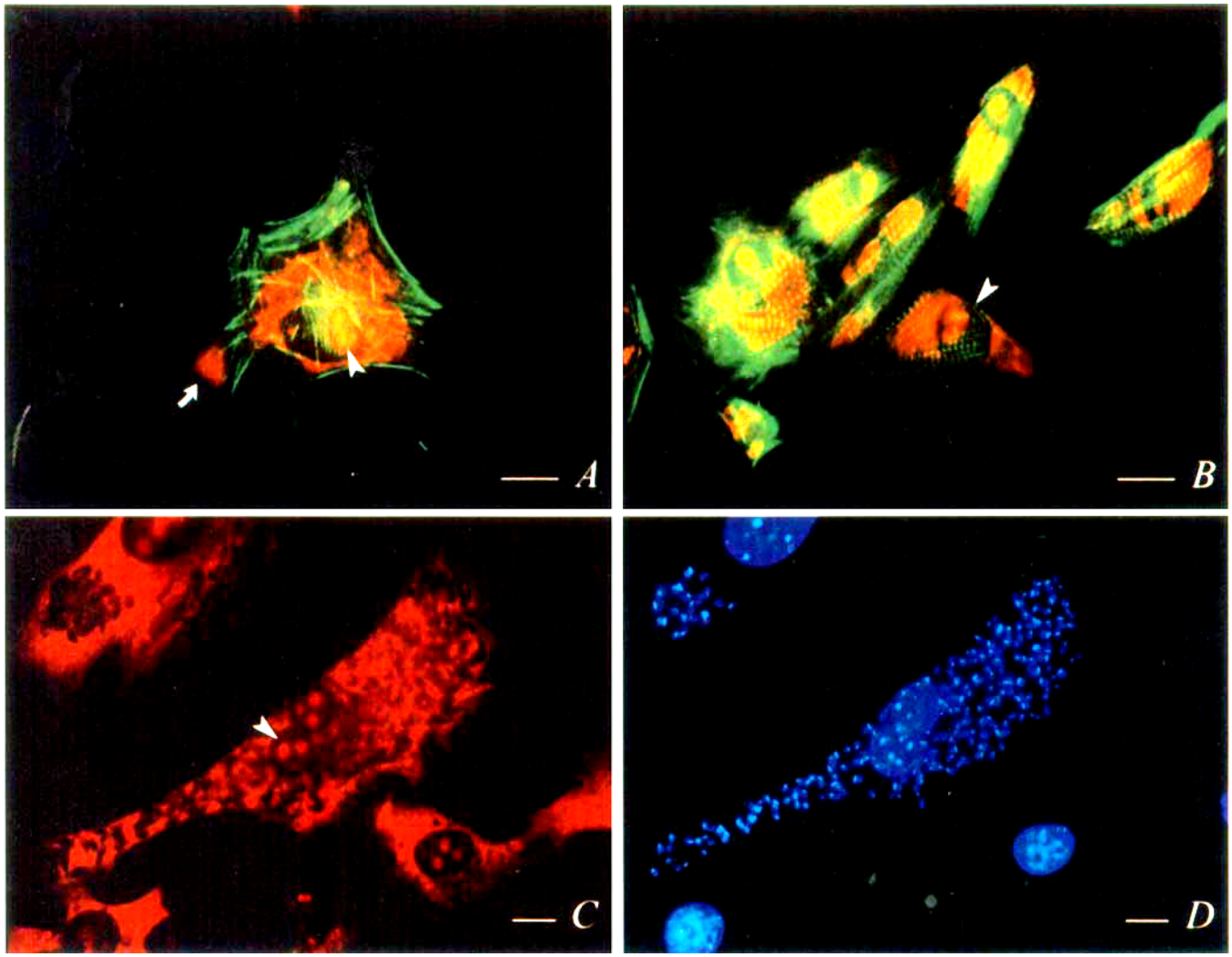

Fig. 2. Distribution of 18S rRNA in uninfected and *Trypanosoma cruzi*-infected cells. Double labeling of actin filament and rRNA: FITC-Phalloidin staining revealed actin stress fibers in non-muscle cells (A) and well-developed myofibrils in myocytes (B). In situ hybridization using CY3-labeled rRNA oligonucleotide probes demonstrated an intense signal in the nucleoli (arrowhead) and cell cytoplasm. Ribosomal RNA also located at the cell lamellae of non-muscle cells (A; arrow) while only a perinuclear signal was observed in myocytes (B). A similar distribution was observed in highly infected cells (C). DAPI stained host cell's nucleus and parasite's nucleus and kinetoplast (D). Bar = 20 μm.

cells were then rinsed in HBSS, and fixed in paraformaldehyde as described above.

Probes. Oligonucleotide probes (50–55 bases) were prepared as described (Taneja and Singer 1990). The probes were made to the 3′ untranslated region (UTR) of rat β and γ actin mRNA sequence and mouse α-cardiac actin mRNA sequence. Five probes were made to β-actin (Accession No: nucleotide (nt) 3135–3185, 3315–3365, 3437–3487, 3490–3540, 3544–3594) and γ-actin (Accession No: nt 1130–1178, 1259–1309, 1386–1437, 1447–1500, 1512–1567) mRNAs and two probes to α-cardiac actin mRNA (Accession No: nt 1129–1170, 1184–1226). Rat β- and γ-actin probes were used because their 3′ UTR sequences were available and have high homology to mouse sequences. Oligonucleotide probes were labeled by incorporating either digoxigenin-UTP or a specific fluorochrome. β-actin probes were labeled with Cy-3 (Biological Detection Lab, Pittsburgh, PA) and α-cardiac actin probes were labeled with FITC. Coding region sequences were not used in order to avoid cross-reaction between the actin isoforms.

In situ hybridization. Fixed cells on coverslips stored in ethanol (70%) were hydrated in PBS containing 5 mM $MgCl_2$ and then permeabilized with PBS containing 0.5% Triton X-100 for 10 min. Prior to hybridization, coverslips were incubated in 40% formamide/4× SSC (0.06 M saline sodium citrate and 0.6 M NaCl) for 10 min at room temperature using oligonucleotide probes β- and γ-actin. Hybridizations were performed for 3 h at 37 °C in a humidified chamber with the mixture of specific oligonucleotide probes in 40% formamide/4× SSC containing 1% bovine serum albumin (BSA), 10% dextran sulfate, 10 mM Vanadyl complex and 10 μg each of *Escherichia coli* tRNA, and sonicated Salmon sperm DNA. Cells were then washed in 40% formamide in 4× SSC at 37 °C for 30 min, followed by washes in 4× SSC, 2× SSC, 1× SSC, and PBS at room temperature, with gentle agitation, for 10 min each. α-cardiac actin oligonucleotide probes hybridization was performed in 50% formamide/4× SSC. For alkaline phosphatase detection, cells were incubated in anti-digoxigenin alkaline phosphatase-conjugated antibody diluted 1:200 in PBS containing 1% BSA at 37 °C for 30 min. Cells were washed 2× with PBS and equilibrated in buffer 3 (100 mM Tris HCl, pH 9.5,

100 mM NaCl, 50 mM $MgCl_2$) for 5 min at room temperature. The coverslips were then incubated in buffer 3 containing 3 mg of nitroblue tetrazolium (NBT) and 2 mg of bromochloroindolyl phosphate (BCIP). Color development was monitored by light microscopy and stopped by addition of water when the signal was evident. The coverslips were then mounted in gel mount (Biomeda, Foster City, CA). For fluorescent in situ hybridization (Fish), Cy3 or FITC-labeled oligonucleotide probes were used. After hybridization and wash steps described below, the coverslips were mounted in Vectashield® mounting medium. The samples were observed in a Nikon microscope equipped with epifluorescence and pictures were taken with 63× immersion objective, N.A. 1.4.

RNA isolation and Northern blot. Total RNA was isolated from confluent heart muscle cells (HMC) plated on 100-mm culture dishes by using TRI REAGENT-RNA/DNA/Protein isolation reagent (Molecular Researcher Center, Inc., Cincinnati, OH). Cells were lysed with 1 ml of TRI reagent added directly into the plate. The solution was transferred to a microfuge tube and 0.2 ml of chloroform was added to the lysate. After vortexing, the solution was kept at room temperature for 10 min and centrifuged at 12,000 *g* for 20 min at 4 °C. The RNA precipitation was obtained by adding 0.5 ml of isopropanol to the aqueous phase at room temperature for 10 min. After centrifugation at 12,000 *g* for 20 min at 4 °C, the pellet was resuspended in 75% ethanol. RNA was dissolved in formamide and separated electrophoretically on a 1% agarose/formaldehyde gel. The RNA was transferred to a nylon membrane (Zeta probe membrane, Bio Rad Laboratories, Richmond, CA) and irreversibly bound by UV crosslinking. β, γ and α-cardiac actin oligonucleotide probes were ^{32}P-labeled with T4 polynucleotide kinase. Messenger RNAs levels were compared to an internal standard, using oligonucleotide probes for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Membrane was exposed for 2 h in the Betascope equipment and also to the X-ray film (Kodak, Rochester, NY) for 48 h at −70 °C.

Protein extraction and Western blot. Normal and *T. cruzi*-infected confluent cultures were extracted with lysis buffer (50 mM Tris-HCl pH 8, NaCl 150 mM, sodium azide 0.02%, PMSF 100 μg /ml, aprotinin 1 μg/ml, and 1% Triton X-100) and protein assay was performed according to Lowry. Total protein (7 μg/slot) was electrophoresed through 10% SDS-polyacrylamide gel using standard procedures. The proteins were electrophoretically transferred to a PVDF membrane. Then, after incubating with blocking buffer (1% I-block, 0.1% Tween 20 in PBS), the specific protein was detected using anti β-actin or anti α-sarcomeric actin monoclonal antibodies (Sigma Chemical Co., St. Louis, MO) diluted 1/2500. Afterwards, the membrane was incubated with rabbit anti-mouse IgG conjugated with alkaline phosphatase and was developed with the enzyme substrate (NBT/BCIP).

RESULTS

Actin gene expression during myogenesis in culture. Isoactin mRNAs expression was analyzed in vitro during myogenesis of mouse heart muscle cells (HMC). The time course of actin mRNAs was determined by RNA Northern blots and GAPDH mRNA served as an internal hybridization standard. Northern blot analysis revealed a distinct expression of β, γ and α-cardiac actin mRNAs during cell differentiation. β-actin mRNA displayed low expression from days 2–4, followed by a slight increase after this time, due to contaminating fibroblasts in the cultures (Fig. 1 A and B). Similar results were obtained with γ-actin mRNA (data not shown). In contrast, α-cardiac actin mRNA content was greater than that of the γ and β actin mRNAs by day 2 in culture. Thereafter, the expression decreased to relatively constant levels up to 5 d in culture (Fig. 1 B). The high levels of α-cardiac actin mRNA were coincident with the visualization of an elevated number of contracting cells by phase contrast microscopy under the standard growth conditions used. In addition, indirect immunofluorescence using monoclonal anti β-actin antibody labeled a few myoblasts and fibroblasts but not myocytes while FITC-phalloidin stained stress fibers of nonmuscle cells and well-developed myofibrils in myocytes (data not shown). The probe specificity was evaluated using L6 and 3T3 cell lines. These cell lines expressed β and γ actin mRNAs, but did not express α-cardiac actin mRNA as expected (data not shown).

Actin mRNA regulation in Trypanosoma cruzi infected cells. To address the question of whether expression of β cytoplasmic actin mRNAs was affected during the interaction of *T. cruzi* and heart muscle cells, total RNA was isolated from control cultures and bloodstream trypomastigote-infected cultures 24, 48, 72, and 96 h after infection. Cellular actin mRNA levels were obtained by measuring the ratio between actin and GAPDH mRNAs (Fig. 1 A). The percentage of infection reached approximately 32% after 24 h of interaction and eventually more than 65%. Although *T. cruzi* expresses actin from its own genome, no significant sequence homology was shared between the probes and the *T. cruzi* actin mRNA sequence. In addition, no signal was detected when total RNA from epimastigotes was used to detect any possible cross-reaction under our hybridization conditions (Fig. 1 A). Northern blot analysis revealed an increase of 16% in steady state levels of muscle-specific actin mRNA up to 48 h after infection (Fig. 1 B,C). However, after 72 h of infection *T. cruzi*-infected cells displayed a reduction of 50% in the α-cardiac actin mRNA and it remained low thereafter. In contrast, 24 h after interaction β-actin mRNA levels increased approximately 24% showing a peak of enhancement of 79% 48 h of infection later (Fig. 1 B,C).

To determine whether the actin mRNA levels in infected cells represented changes in actin isoforms or alteration in the GAPDH mRNA expression, ribosomal RNA (rRNA) was used as an alternate internal standard. We first analyzed the distribution of 18S rRNA of uninfected- and *T. cruzi*-infected cells by in situ hybridization to determine that there was no alteration with this RNA during infection; we then performed the analysis of the GAPDH mRNA by Northern blot using 18S rRNA as internal standard. The intracellular distribution of 18S rRNA was revealed using CY3-labeled mouse 18S rRNA oligonucleotide probes, which presented an intense signal both in the nucleoli and in the cytoplasm of the cell (Fig. 2). Double labeling of actin filaments and 18S rRNA revealed that myoblasts and fibroblasts showed rRNA localization at the leading lamellae (Fig. 2 A), like the β-actin mRNA, while myocytes displayed no peripheral cytoplasmic localization (Fig. 2 B). In highly infected cells, rRNA signal remained unaffected (Fig. 2 E, F). No hybridization was observed with the rRNA of the parasite (Fig. 2 E, 3). Northern blot analysis revealed no significant alterations of GAPDH levels at any time of infection (Fig. 3).

Cellular localization of actin isoform mRNAs. Intracellular localization of isoactin mRNAs was determined by in situ hybridization using oligonucleotide probes specific to the 3′ UTR of each actin isoform sequence. Actin mRNAs were located in different intracellular regions. The β-actin mRNA was detected at the perinuclear region and the periphery of fibroblasts and undifferentiated cells (Fig. 4 A), while myocytes presented very low or no detectable signal. The use of fluorochrome-conjugated probes allowed the visualization of the transcription sites of β-actin mRNA in the nucleoplasm of nonmuscle cells. The transcription sites were frequently observed as two or more intense,

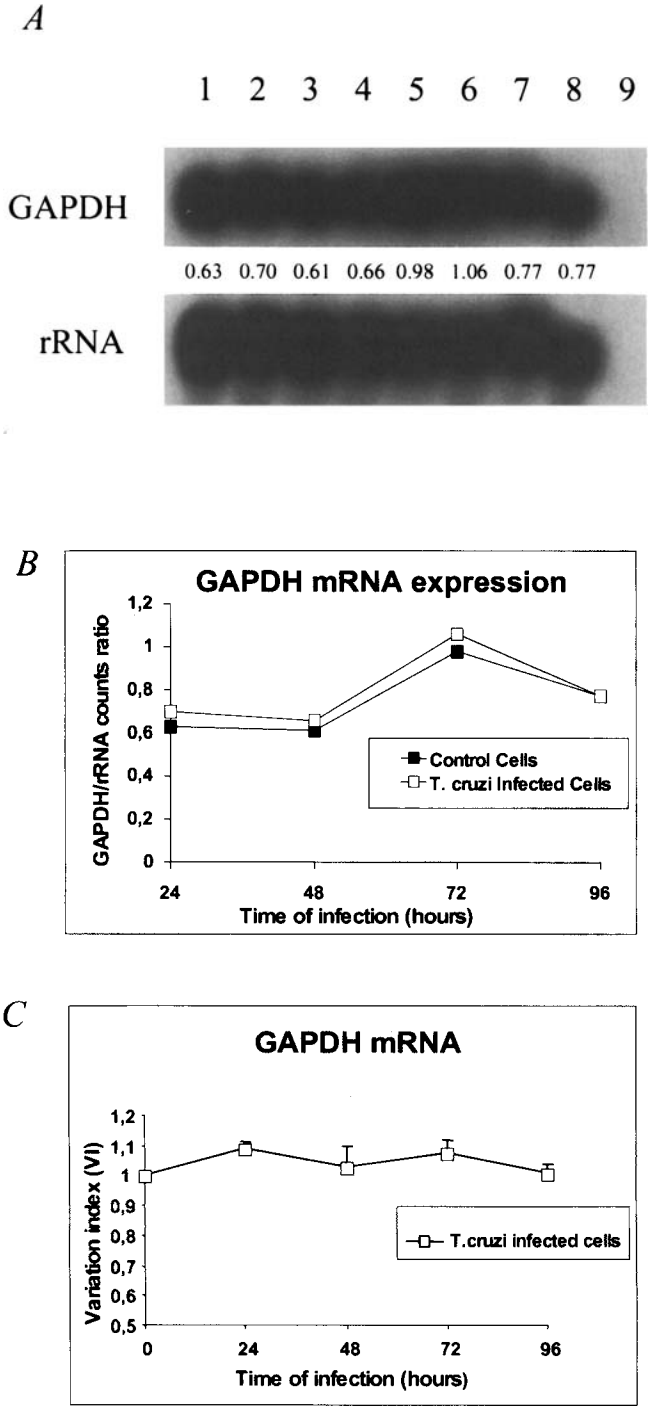

Fig. 3. Representative Northern blot of glyceraldehyde phosphate dehydrogenase (GAPDH) mRNAs in uninfected and *Trypanosoma cruzi*-infected heart muscle cell (HMC) (A). Lane 1, control cells 2 d in culture; lane 2, HMC 24 h after *T. cruzi* infection; lane 3, control cells 3 d after plating; lane 4, infected cells, 48 h; lane 5, control cells, 4 d; lane 6, infected cells, 72 h; lane 7, control cells, 5 d; lane 8, infected cell 96 h; lane 9, epimastigote forms of *T. cruzi*. (B) Kinetic expression of GAPDH mRNAs in uninfected and *T. cruzi*-infected heart muscle cells. Quantitation of GAPDH mRNAs was normalized with 18S rRNA. The variation index of 3 different experiments revealed no changes in GAPDH mRNA expression in infected cells.

intranuclear punctate signals. No specific localization was detected, although they were often visualized near the nuclear envelope (Fig. 4 A). The alkaline phosphatase detection demonstrated a similar pattern of cytoplasmic distribution, but no reaction was found in the nucleus (data not shown). Cells in the process of division also displayed high β-actin mRNA labeling in ruffling edges (data not shown). On the other hand, α-cardiac actin mRNA localization was restricted to the perinuclear region (Fig. 4 B), showing no peripheral signal in well-spread cells. Similar results were obtained with γ-actin mRNA (data not shown).

The effect of intracellular parasites on actin mRNA localization. In situ hybridization was performed to evaluate changes in the spatial distribution of β-actin mRNA during heart muscle cell-parasite interaction. After 24 h of infection with *T. cruzi,* the distribution of β-actin mRNA was similar to the control cells showing β-actin mRNA located at the cell periphery (Fig. 5 A). A strong signal throughout the cytoplasm with no peripheral localization was observed in 48-h infected cells (Fig. 5 C). This signal intensification may be indicative of the increase in the steady state level of the β-actin mRNA observed by Northern blot. However, 72 h after infection the β-actin mRNA delocalized, showing an intense signal surrounding the nucleus (Fig. 5 E). It was possible to visualize the β-actin mRNA transcription site even in highly infected cells, demonstrating that the intracellular parasites did not interfere with transcription in the cells. The visualization of the intracellular parasites was obtained with DAPI, which stained the host-cell nuclei and the parasite's nucleus and kinetoplast (Fig. 5 B, D, F).

In order to investigate whether the delocalization was associated with cytoskeletal disruption, we incubated control cells with drugs known to depolymerize actin filaments and microtubules (data not shown). Incubation with Cytochalasin D, which depolymerizes actin filaments, showed delocalization of β-actin mRNA from the periphery of the cell to the perinuclear region. Nocodazole treatment, which disrupts microtubules, resulted in no major effects on their distribution.

The fact that β and α-cardiac actin mRNA expression and β-actin mRNA localization were affected by *T. cruzi* infection suggested that the cellular G-actin pool could also be altered. SDS-PAGE gel electrophoresis of total protein extracted from control and infected cells and analyzed by Western blot using specific monoclonal antibodies showed no change in the β and α actin total intracellular pool observed even with high levels of infection. (data not shown).

DISCUSSION

The major focus of this paper was to elucidate actin mRNA regulation during the cytopathology induced in myocardial cells by *T. cruzi.* Since cardiac tissue is highly affected by *T. cruzi* infection, our laboratory has developed a mouse embryo primary system to characterize events occurring in the early stage of parasite invasion and during their interaction with host cells (Barbosa and Meirelles 1993; Contreras et al. 1988; Meirelles et al. 1986; Soeiro 1995). One of the most striking events is the disruption of the actin cytoskeleton, even in well-formed sarcomeres (Pereira et al. 1993). Because of this cytoskeletal disruption, we hypothesized that the regulation of actin mRNA, which depends on this cytoskeleton for its localization would likewise be affected.

We first evaluated actin mRNA expression during normal heart myogenesis in vitro. An increase in α-cardiac actin mRNA level was concomitant with the differentiation of myoblasts to cardiomyocytes in culture. After differentiation, the cells maintained a steady state level of α-cardiac actin mRNA

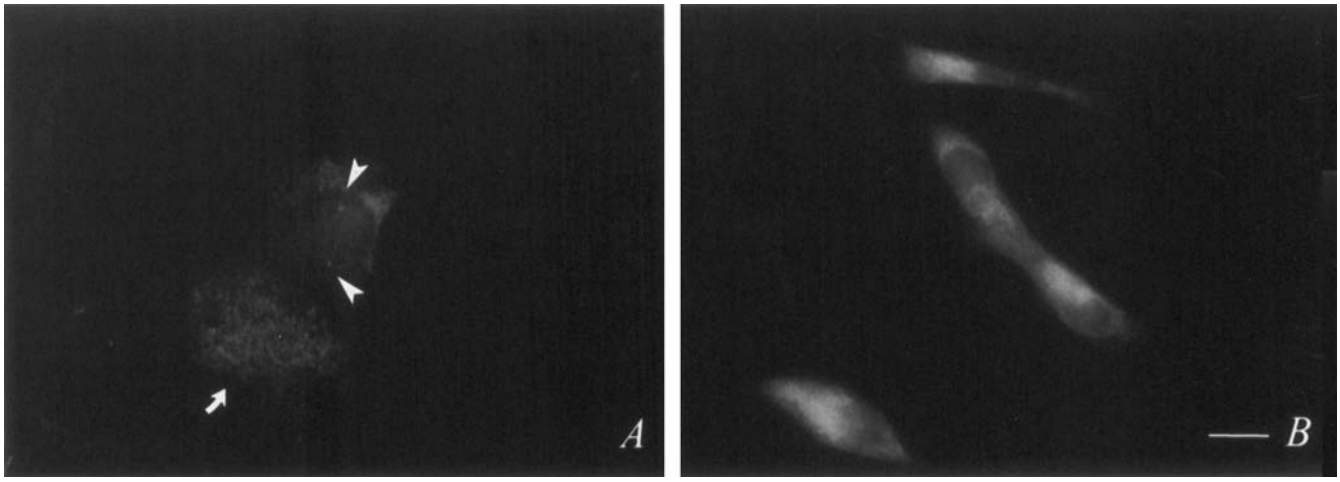

Fig. 4. Intracellular localization of β-cytoplasmic and α-cardiac actin mRNAs during differentiation of heart muscle cells. (A) In situ hybridization using CY3-labeled β-actin probes revealed myoblasts with β-actin mRNA signal located at the periphery of lamellipod. Arrowheads indicate β-actin mRNA transcription sites. (B) Perinuclear distribution of α-cardiac actin in myocytes. Bar = 20 μm.

along with high levels of well developed myofibrils, γ and β-actin mRNA displayed low levels of expression in these cells.

In other experimental studies, the actin genes were also found to display a temporal and sequential expression during cardiogenesis (Mchugh, Crawford, and Lessard 1991; Ruzicka and Schwartz 1988; Sawtell and Lessard 1989). In addition, skeletal muscle differentiation also presented a distinct regulation in the expression of the cytoplasmic and striated-muscle isoactins (Lawrence, Taneja, and Singer 1989; Hayward, Zhu, and Schwartz 1988). The co-expression of α-actin genes in amphibian embryos (Mohun et al. 1984) and mouse myoblast cultures (Minty et al. 1982) has been demonstrated.

The steady state levels of α-cardiac and β-actin mRNA were affected in *T. cruzi*-infected heart muscle cells. The infection caused a 50% decrease in α-cardiac actin mRNA but a 79% increase in the β-actin mRNA. These effects were independent of *T. cruzi* RNA and did not result from changes in GAPDH mRNA. Furthermore, the in situ hybridization with 18S rRNA revealed that rRNA was also co-localized, like β-actin mRNA, in motile cells, which supports the concept of mRNA localization as a mechanism for targeting protein synthesis at their sites of action, and its synthesis was not altered in infected cells, despite the high number of intracellular parasites. This provides an additional indication of the functional state of the cytoplasmic polysomes. Thus it is possible that the parasites induced transcription shutdown and/or degradation of the muscle-specific actin mRNA.

Rowin and co-workers (1983) demonstrated that *T. cruzi* infection inhibits biochemical differentiation of L_6E_9 cells but the parasite infection is not able to interfere with the expression of actin genes after myotube formation. In their report the authors did not observe any alteration in the β-actin mRNA expression. The results presented here do not suggest an inhibition of cell differentiation, since the expression of α-cardiac actin gene was already activated at early stages of infection. The downregulation of α-cardiac actin and upregulation of the β-actin gene may indicate reactivation the "nonmuscle cells program" under pathological conditions. The capacity of differentiated cardiac cells to reactivate fetal genes, while other genes, active in adult cardiomyocytes, become downregulated was also observed in overload cardiac hypertrophy (Boheler and Schwartz 1992; Eppenberger, Hertig, and Eppenberger-Eberhardt 1994).

We have demonstrated that α-cardiac, γ and β-cytoplasmic actin mRNAs are located in different compartments within the cytoplasm during the differentiation of heart muscle cells. This corroborates the concept that most mRNAs are targeted to the functional protein compartment (Kislauskis and Singer 1992; Singer 1993). Hill and Gunning (1993) also observed β and γ-actin mRNAs localized in different intracellular region of C2 myoblasts. We have first demonstrated the intracellular localization of α-cardiac actin mRNA, whose protein integrates myofibrils in cardiomyocytes. Other investigators also reported perinuclear localization of α-cardiac actin mRNA in small myotubes (skeletal muscle) and localization of β-actin mRNA in non-muscle cells to regions of cell motility (Kislauskis et al. 1993; Hill, Schedlich and Gunning 1994; Shestakova et al. 1999).

Our previous report revealed that *T. cruzi* infection induced myofibrillar breakdown and destruction of several cytoskeletal filaments in heart muscle cells (Pereira et al. 1993). This suggested that actin mRNA localization could be affected. Our analysis provided evidence of β-actin mRNA translocation from the cell periphery to the perinuclear region in highly infected cells. However, the intense in situ hybridization signal observed at the perinuclear region could not be only related solely to the mRNA translocation, but also may be correlated with an increase in its expression. Disruption of microfilaments in uninfected cells treated with Cytochalasin D translocated the β-actin mRNA to the perinuclear region. Its signal intensity was lower than in *T. cruzi*-infected cells (data not shown), indicating that in addition to the mRNA translocation, the β-actin mRNA of infected cells was also being excessively transcribed while its movement to the cell periphery was inhibited by disassembly of actin filaments. Delocalization of β-actin mRNA was also observed in fibroblasts treated with Cytochalasin D, demonstrating that intact microfilaments are required to maintain this mRNA at its functional region (Sundel and Singer 1991).

In conclusion, these data provide evidence that *T. cruzi* affects actin mRNA regulation. The mechanism involved in this process has not yet been elucidated and will be the focus of further investigations. However, this is the first case of a pathological mechanism that disrupts the normal cytoplasmic localization of mRNA.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Krishan Taneja for constructing the oligonucleotide probes and Dr. Maurílio José Soares for

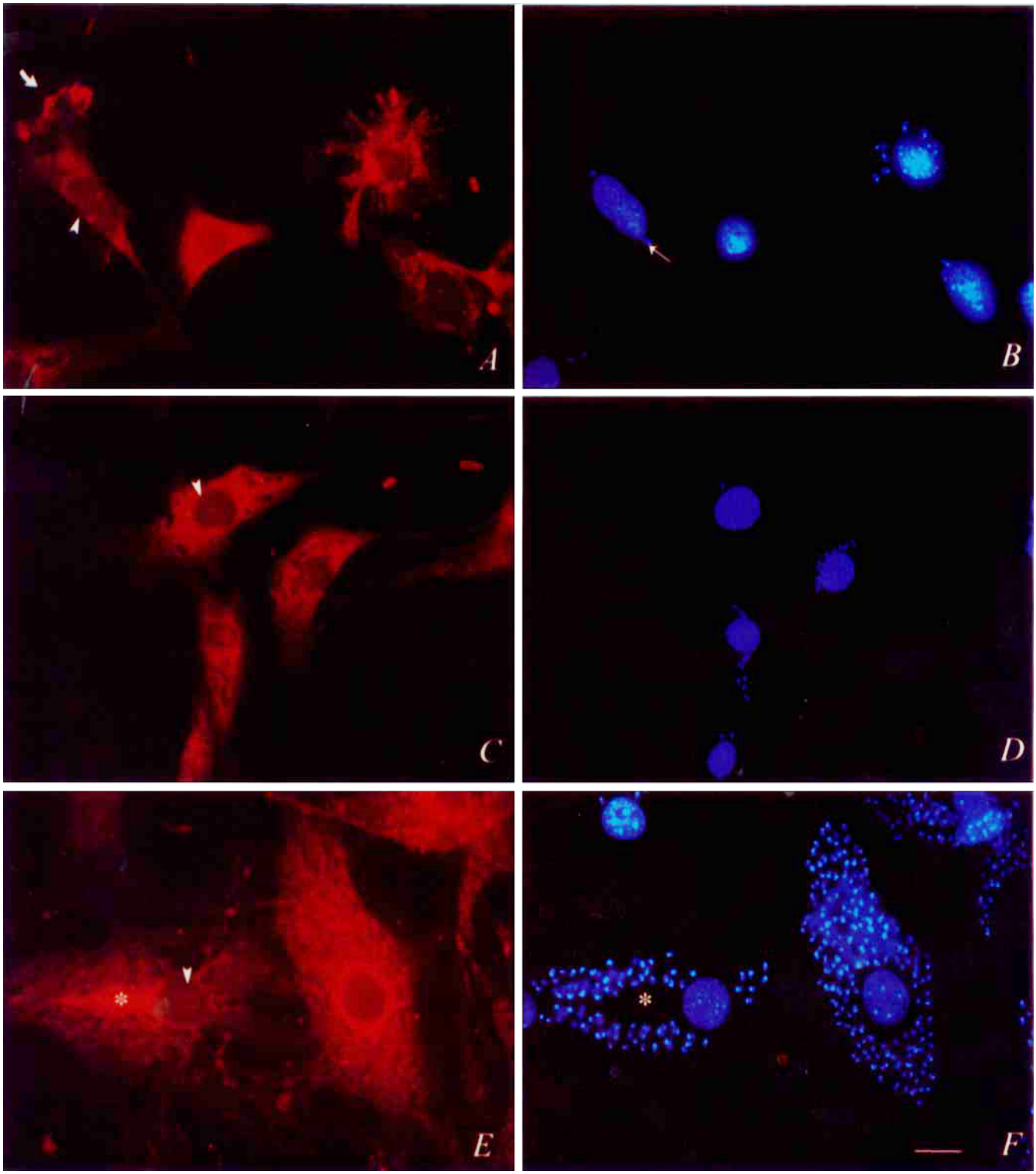

Fig. **5**. Effect of *Trypanosoma cruzi* infection on the β-actin mRNA localization. Cells were hybridized with CY3-labeled β-actin oligonucleotide probes. (A) Actin localization to the leading lamellae was unaffected after 24 h of infection (arrow). (C) After 48 h of interaction, an intense signal throughout the cell cytoplasm showed no localization. (E) Perinuclear distribution of β-actin mRNA in heavily infected cells (72 h post-infection). Note that transcription sites were still active even in highly infected cells (arrowhead). DAPI stained host cell's nucleus and parasite's nucleus and kinetoplast (B; D, and F; arrow). Bar = 20 μm.

his comments in the manuscript. This work was supported by grants from Fundação Oswaldo Cruz (FIOCRUZ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), FAPERJ PADCT/CNPq and PAPES/FIOCRUZ.

Part of this work was presented previously in abstract form at the XXIV Annual Meeting on Basic Research in Chagas' Disease, 1997, (Pereira, M. C. S., Singer, R. H. & Meirelles, M. N. L. Vol **92**:Suppl. I, 004).

LITERATURE CITED

Araújo-Jorge, T. C., Barbosa, H. S. & Meirelles, M. N. L. 1992. *Trypanosoma cruzi* recognition by macrophages and muscle cells: perspectives after 15-year study. *Mem. Inst. Oswaldo Cruz*; **87**:43–56.

Barbosa, H. S. & Meirelles, M. N. L. 1993. The role of RCA-biding sites in the adhesion of *Trypanosoma cruzi* to heart muscle cells, as revealed by electron spectroscopic imaging. *J. Submicrosc. Cytol. Pathol.*, **25**:47–51.

Barbosa, H. S. & Meirelles, M. N. L. 1995. Evidence of participation of cytoskeleton of heart muscle cells during invasion of *Trypanosoma cruzi*. *Cell Struct. Func.*, **20**:275–284.

Barbosa, H. S., Carvalho, E. L., Ferreira, C. F., Meirelles, M. N. L. 1993. *Trypanosoma cruzi*-heart muscle cell interaction: the presence of two or more parasites within a single endocytic vacuole. *J. Submicrosc. Cytol. Pathol.*, **25**:613–615.

Bassel, G. & Singer, R. H. 1997. MRNA and cytoskeletal filaments. *Curr. Opin. Cell Biol.*, **9**:109–115.

Bassel, G. J., Zhang, H., Byrd, A. L., Femino, A. M., Singer, R. H., Taneja, K. L., Lifshitz, L. M., Herman, I. M. & Kosik, K. S. 1998. Sorting of β-actin mRNA and protein to neurites and growth cones in culture. *J. Neurosci.*, **18**:251—265.

Boheler, K. R. & Schwartz, K. 1992. Gene expression in cardiac hyperthrophy. *Trends Cardiovasc. Med.*, **2**:176–182.

Bretscher, A. 1991. Microfilament structure and function in the cortical cytoskeleton. *Annu. Rev. Cell Biol.*, **7**:337–374.

Bruckenstein, D. A., Lein, P. J., Higgins, D., Jr. & Fremeau, R. T. 1990. Distinct spatial localization of specific mRNAs in cultured sympathetic neurons. *Neuron.*, **5**:809–819.

Carlier, M.-F. 1991. Actin: protein structure and filament dynamics. *J. Biol. Chem.*, **266**:1–4.

Cheng, H. & Bjerknes, M. 1989. Asymmetric distribution of actin mRNA and cytoskeletal pattern generation in polarized epithelial cells. *J. Mol. Biol.*, **210**:541–549.

Contreras, V. T., Araújo-Jorge, T. C., Bonaldo, M. C., Thomaz, N., Barbosa, H. S., Meirelles, M. N. L. & Goldenberg, S. 1988. Biological aspects of the DM28C clone of *Trypanosoma cruzi* after metacyclogenesis in chemically defined media. *Mem. Inst. Oswaldo Cruz*, **83**: 123–133.

Devlin, R. B. & Emerson, C. P., Jr. 1979. Coordinate accumulation of contractile protein mRNA during myoblast differentiation. *Dev. Biol.*, **69**:202–216.

Dias, J. C. P. 1997. Present situation and future of human Chagas' disease in Brazil. *Memórias Inst. Oswaldo Cruz*, **92**:13–15.

Eppenberger, H. M., Hertig, C. & Eppenberger-Eberhardt, M. 1994. Adult rat cardiomyocytes in culture: a model system to study the plasticity of the differentiated cardiac phenotype at the molecular and cellular level. *Trends Cardiovasc. Med.*, **4**:187–193.

Garner, C. C., Tucker, R. P. & Matus, A. 1988. Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. *Nature*, **336**:674–677.

Hayward, L. & Schwartz, R. J. 1986. Sequential expression of chicken actin gene during myogenesis. *J. Cell Biol.*, **102**:1485–1493.

Hayward, L. J., Zhu, Y. Y. & Schwartz, R. J. 1988. Cellular localization of muscle and nonmuscle actin mRNAs in chicken primary myogenic cultures: the induction of α-skeletal actin mRNA is regulated independently of α-cardiac actin gene expression. *J. Cell Biol.*, **106**:2077–2086.

Hill, M. A. & Gunning, P. 1993. Beta and gamma actin mRNAs are differentially located within myoblasts. *J. Cell Biol.*, **122**:825–832.

Hill, M. A., Schedlich, L. & Gunning, P. 1994. Serum-induced signal transduction determines the peripheral location of β-actin mRNA within the cell. *J. Cell Biol.*, **126**:1221–1230.

Kabsch, W., Vandekerckhove, J. 1992. Structure and function of actin. *Annu. Rev. Biophys. Biomol. Struct.*, **21**:49–76.

Kipnis, T. L., Calich, V. L. & Dias da Silva, W. 1979. Active entry of bloodstream forms of *Trypanosoma cruzi* into macrophages. *Parasitol.*, **78**:89–98.

Kislauskis, E. H. & Singer, R. H. 1992. Determinants of mRNA localization. *Curr. Opin. Cell Biol.*, **4**:975–978.

Kislauskis, E. H., Li, Z., Singer, R. H. & Taneja, K. L. 1993. Isoform-specific 3′-untranslated sequences sort α-cardiac and β-cytoplasmic actin messenger RNAs to different cytoplasmic compartments. *J. Cell Biol.*, **123**:165–172.

Lawrence, J. B. & Singer, R. H. 1986. Intracellular localization of messengers RNAs for cytoskeletal proteins. *Cell*, **45**:407–415.

Lawrence, J. B., Taneja, K. & Singer, R. H. 1989. Temporal resolution and sequential expression of muscle specific-genes by in situ hybridization. *Dev. Biol.*, **133**:235–246.

Low, H. P., Paulin, J. J. & Keith, C. H. 1992. *Trypanosoma cruzi* infection of BSC-1 fibroblast cells causes cytoskeletal disruption and changes in intracellular calcium levels. *J. Protozool.*, **39**:463–470.

Mchugh, K. M., Crawford, K. & Lessard, J. L. 1991. A comprehensive analysis of the developmental and tissue-specific expression on the isoactin multigene family in the rat. *Dev. Biol.*, **148**:442–458.

Meirelles, M. N. L., Souto-Padrón, T. & De Souza, W. 1984. Participation of cell surface anionic sites in the interaction between *Trypanosoma cruzi* and macrophages. *J. Submicrosc. Cytol.*, **16**:533–545.

Meirelles, M. N. L., Araújo-Jorge, T. C., De Souza, W., Moreira, A. C. & Barbosa, H. S. 1987. *Trypanosoma cruzi*: phagolysosomal fusion after invasion into non-professional phagocytic cells. *Cell Struct. Func.*, **12**:387–393.

Meirelles, M. N. L., Araújo-Jorge, T. C., Miranda, C. F., De Souza, W. & Barbosa, H. S. 1986. Interaction of *Trypanosoma cruzi* with heart muscle cells: ultrastructure and cytochemical analysis of endocytic vacuole formation and effect upon myogenesis in vitro. *Europ. J. Cell Biol.*, **41**:198–206.

Minty, A. J., Alonso, S., Caravatti, M. & Buckingham, M. E. 1982. A fetal skeletal muscle actin mRNA in mouse and its identity with cardiac actin mRNA. *Cell*, **30**:185–192.

Mohun, T. J., Brennan, S., Dathan, N., Fairman, S. & Gurdon, J. B. 1984. Cell type-specific activation of actin genes in the early amphibian embryo. *Nature (Lond.)*, **311**:716–721

Pereira, M. C. S., Costa, M., Chagas Filho, C. & Meirelles, M. N. L. 1993. Myofibrillar breakdown and cytoskeletal alterations in heart muscle cells during invasion by *Trypanosoma cruzi*: immunological and ultrastructural study. *J. Submicrosc. Cytol. Pathol.*, **25**:559–569.

Rowin, K. S., Tanowitz, H. B., Wittner, M., Nguyen, H. T. & Nadal-Ginard, B. 1983. Inhibition of muscle differentiation by *Trypanosoma cruzi*. *Proc. Natl. Acad. Sci., USA*, **80**:6390–6394.

Ruzicka, D. L. & Schwartz, R. J. 1988. Sequential activation of α-actin genes during avian cardiogenesis: vascular smooth muscle α-actin gene transcripts mark onset of cardiomyocyte differentiation. *J. Cell Biol.*, **107**:2575–2586.

Sawtell, N. M. & Lessard, J. L. 1989. Cellular distribution of smooth muscle actins during mammalian embryogenesis: expression of α-vascular but not the γ-enteric isoform in differentiated striated myocytes. *J. Cell Biol.*, **109**:2929–2937.

Schenkman, S. & Mortara, R. A. 1992. Hela cells extend and internalize pseudopodia during active invasion by *Trypanosoma cruzi* trypomastigotes. *J. Cell Sci.*, **101**:895—905.

Schenckman, S., Andrews, N. W. & Nussenzweig, V. 1991. Attachment of *Trypanosoma cruzi* to mammalian cells requires parasite energy, and invasion can be independent of the target cell cytoskeleton. *Infect. Immun.*. **59**:645–654.

Shestakova, E. A., Wyckoff, J., Jones, J., Singer, R. H. & Condeelis, J. 1999. Correlation of beta-actin messenger RNA localization with metastic potential in rat adenocarcinoma cell lines. *Cancer Res.*, **59**: 1202–1205.

Singer, R. H. 1992. The cytoskeleton and mRNA localization. *Curr. Opin. Cell Biol*, **4**:15–19.

Singer, R. H. 1993. RNA zipcodes for cytoplasmic addresses. *Curr. Biol.*, **3**:719–721.

Soeiro, M. N. C., Costa e, Silva Filho, F., Meirelles, M. N. L. 1995.

Alterations in the surface charge of heart muscle cells during interaction with *Trypanosoma cruzi*. *Cell Biophys.*, **26**:21–44.

St. Johnston, D. 1995. The intracellular localization of messenger RNAs. *Cell*, **81**:161–170.

Sundell, C. L. & Singer, R. H. 1991. Requirement of microfilaments in sorting actin messenger RNA. *Science*, **253**:1275–1277.

Tadieux, I., Webster, P., Ravesloot, J., Boron, W., Lunn, J. A., Heuser, J. E. & Andrews, N. W. 1992. Lysosome recruitment and fusion are early required for trypanosome invasion of mammalian cells. *Cell*, **71**:1117–1130.

Taneja, K. & Singer, R. H. 1990. Detection and localization of actin mRNA isoform in chicken muscle cells by in situ hybridization using biotinated oligonucleotide probes. *J. Cellular Biochem.*, **44**:241–252.

Tanowitz, H. B., Kirchhoff, L. V., Simon, D., Morris, S. A., Weiss, L. M. & Wittner, M. 1992. Chagas' disease. *Clinic. Microbiol. Reviews*, **5**:400–419.

Wilhelm, J. E. & Vale, R. D. 1993. RNA on the move: the mRNA localization pathway. *J. Cell Biol.*, **123**:269–274.

Received: 06-18-99, 11-10-99; accepted 01-28-00