Characterization of messenger RNA from epimastigotes and metacyclic trypomastigotes of *Trypanosoma cruzi*

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The cell-free translation products of polyribosomal and post-polyribosomal mRNAs from the non-infective epimastigotes and the infective metacyclic trypomastigotes of the parasitic protozoan *Trypanosoma cruzi* were compared by two-dimensional polyacrylamide gel electrophoresis. The result show that although many polypeptides are conserved, quantitative and qualitative differences are observed between both differentiation stages. The results also indicate the existence of post-polyribosomal mRNAs in equilibrium with polyribosomal counterparts. The immunoprecipitation of the in vitro synthesized polypeptides with chagasic human serum and the serum raised against an 85-kDa glycoprotein (P2-WGA), potentially involved in the process of *T. cruzi* penetration into mammalian cells, shows that while the chagasic serum recognizes the same 72-kDa, 68-kDa and 46-kDa polypeptides in both differentiation stages, the anti-P2-WGA serum immunoprecipitates a single 48-kDa polypeptide from in vitro translation products of metacyclic trypomastigotes.

T. cruzi *mRNA* *Cell-free translation* *Surface antigen* *Chagas' Disease*

1. INTRODUCTION

Trypanosoma cruzi, the etiological agent of American trypanosomiasis, also known as Chagas' disease, possesses a life-cycle involving mammalian and insect hosts, comprising various morphological and functional types. The principal features of this cycle are: infected hematophagous reduviidae insects (triatomines) bite the mammal releasing metacyclic trypomastigotes in the excreta. Metacyclics invade the mammal through the wound or mucosa and penetrate or are phagocytized by the mammalian host cells. Inside the host cell, metacyclic trypomastigotes are transformed to the non-flagellated amastigotes prior to replication, as the infective form *T. cruzi* does not replicate. The amastigotes are transformed again into trypomastigotes which are released from the infected cell. Once in the blood stream, trypomastigotes either invade new cells or may be ingested by the reduviidae insect. In the latter case, they are transformed into the replicative epimastigotes in the insect midgut, completing their life-cycle and being finally released as metacyclic trypomastigotes in the excreta of the insect [1,2].

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Abbreviations: P2-WGA, *T. cruzi* trypomastigote glycoprotein fraction which binds to wheat germ agglutinin-Sepharose and contains a major 85-kDa component; TAUS, triatomine artificial urine supplemented with 10% newborn calf serum; LIT, liver infusion tryptose medium

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As yet, very little is known about the molecular mechanisms involved in control of gene expression within *T. cruzi*. We have recently observed that stage-specific gene expression precedes the morphological changes which occur in the differentiation of epimastigotes to metacyclic trypomastigotes [3]. This differentiation must involve the expression of stage-specific genes and among them those coding for surface antigens.

Here, we pursue our investigation of the differentiation of *T. cruzi* epimastigotes to metacyclic trypomastigotes by comparing the in vitro translation products of polyribosomal and post-polyribosomal poly(A)$^+$ mRNAs from both differentiation stages. Furthermore, in vitro translation polypeptides are characterized by their immunoprecipitation with human chagasic serum and with the serum raised against the 85-kDa WGA-binding glycoprotein possibly involved in *T. cruzi* adhesion and penetration into mammalian cells [4–6].

2. MATERIALS AND METHODS

2.1. *Cells*

T. cruzi Dm 28c cells [7] were grown in LIT medium [8] for 6 days without agitation. For trypomastigote induction epimastigotes from LIT-medium were incubated in TAUS for 6 days [3] and metacyclic trypomastigotes were finally purified on DEAE-cellulose [9].

2.2. *Isolation of polyribosomes*

Cycloheximide (final concentration 10 μg/ml) was added to 5×10^{10} cells in LIT or TAUS media 30 min prior to harvesting. Cells were centrifuged at 8000 × *g* for 15 min at 10°C (Beckman rotor JA-14) and washed 3 times with 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 10 mM Hepes, pH 7.4, 5 μg/ml cycloheximide and 10 U/ml heparin. Cells were lysed in 5 vols of 10 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris–HCl, pH 7.6, 5 mM 2-mercaptoethanol, 5 μg/ml cycloheximide and 10 U/ml heparin for 3 min at 4°C with agitation. Sucrose and Nonidet P-40 were added to final concentrations of 0.25 M and 1%, respectively. Lysed cells were centrifuged at 2500 × *g* at 4°C followed by centrifugation at 10000 × *g* at 4°C for 30 min (Beckman JS 13 rotor). Polyribosomes were obtained by centrifugation of the post-mitochondrial supernatant containing 0.5% sodium deoxycholate through a 1 M sucrose cushion at 365000 × *g* for 90 min at 4°C (Beckman SW 50.1 rotor).

2.3. *Isolation of poly(A)$^+$ mRNAs*

Isolated polyribosomes or post-polyribosomal supernatants precipitated with ethanol, were made 10 A_{260} units/ml in 10 mM Tris–HCl, pH 7.4, 0.5% SDS and extracted by the hot phenol method [10]. Poly(A)$^+$ RNAs were isolated as in [11].

2.4. *mRNA in vitro translation and immunoprecipitation*

mRNA in vitro translation was performed in a nuclease-treated rabbit reticulocyte lysate [12]. Immunoprecipitation was performed as in [3].

2.5. *Polyacrylamide gel electrophoresis*

One-dimensional SDS-polyacrylamide gel electrophoresis was performed in linear 10–15% gradients [13]. Two-dimensional polyacrylamide gel electrophoresis was performed using uniform 13% SDS-polyacrylamide gels in the second dimension [14]. Gels were treated for fluorography [15] and exposed to Sakura RX-films, using Kodak X-O-Matic intensifying screens.

2.6. *Reagents*

These were analytical grade and nuclease free. [^{35}S]Methionine (1045 Ci/mmol) was from Amersham (England). Micrococcal nuclease was from P.L. Biochemicals and the other enzymes were from Sigma. Oligo(dT) cellulose was from Collaborative Research Inc.

3. RESULTS

3.1. *Analysis of in vitro translation products of polyribosomal mRNA*

The comparison, by two-dimensional polyacrylamide gel electrophoresis, of the in vitro translation products directed by polyribosomal mRNAs from epimastigotes (fig.1A) and metacyclic trypomastigotes (fig.1B) of *T. cruzi* Dm 28c shows a complex polypeptide pattern. Some of the polypeptides are conserved in both differentiation stages (e.g., polypeptides 1–6, fig.1). However, quantitative (e.g., polypeptides 7–12) and qualitative (e.g., polypeptides 13–17, fig.1A and 18–26, fig.1B) differences are detected

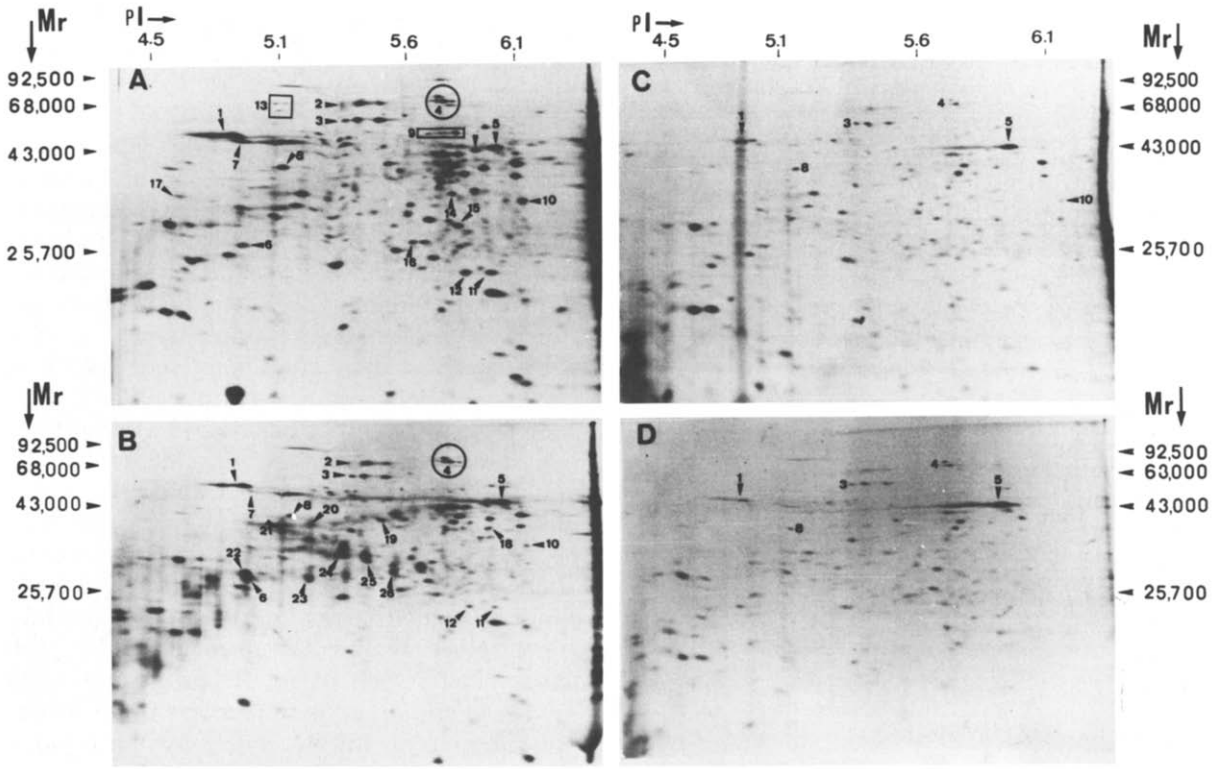

Fig.1. Two-dimensional polyacrylamide gel electrophoresis of the in vitro translation products of: (A) epimastigote polyribosomal mRNA; (B) metacyclic trypomastigote polyribosomal mRNA; (C) epimastigote post-polyribosomal mRNA; (D) metacyclic trypomastigote post-polyribosomal mRNA. The first dimension is an isoelectric focusing and the second dimension is a 13% SDS-polyacrylamide gel.

between both differentiation stages. Some of the polypeptides specific to metacyclic trypomastigotes are faintly represented in the epimastigote mRNA in vitro translation products profile (e.g., polypeptides 23–26); this is probably due to the small percentage of metacyclic trypomastigotes normally found in epimastigote cultures [8] or to the presence of morphological epimastigotes displaying metacyclic trypomastigote gene expression products [3]. Most of the epimastigote in vitro translation products are concentrated in the range pH 5.6–6.1 (fig.1A) while the metacyclic trypomastigote stage specific in vitro synthesized polypeptides are localized in the range pH 5.1–5.6.

3.2. *The existence of* T. cruzi *post-polyribosomal mRNAs*

To investigate whether *T. cruzi* polyribosomal mRNAs could be stored as non-polyribosomal mRNAs and whether mRNA sequences from a particular differentiation stage could be stored in the other form as non-translatable post-polyribosomal mRNA, we have analysed the in vitro translation products of post-polyribosomal poly(A)$^+$ mRNAs from epimastigotes (fig.1C) and from metacyclic trypomastigotes (fig.1D). The results show that although *T. cruzi* possesses mRNA stored in the cytoplasm, these mRNA sequences are in equilibrium with the homologous polyribosomal population. As we will discuss later, we do not believe these post-polyribosomal mRNAs arise from polyribosome run-off.

3.3. *Immunoprecipitation of in vitro translation products*

The in vitro translation products from polyribosomal and post-polyribosomal mRNAs isolated from epimastigotes and from metacyclic

trypomastigotes were immunoprecipitated with human chagasic patient serum and with the serum raised in rabbits against the P2-WGA fraction which contains an almost pure 85-kDa glycoprotein [5]. The human chagasic antiserum recognizes two major polypeptides of M_r 72000 and 68000 and minor polypeptides of M_r 82000, 55000, 46000 and 32000 within epimastigote polyribosomal and post-polyribosomal mRNA in vitro translation products (fig.2, lanes A and B, respectively). Three polypeptides of M_r 72000, 68000 and 46000 are immunoprecipitated from metacyclic trypomastigote polyribosomal and post-polyribosomal mRNA in vitro translation products by the chagasic antiserum (fig.2, lanes C and D, respectively). The comparison of these results with those obtained for epimastigotes shows that fewer proteins are immunoprecipitated from metacyclic trypomastigotes and that there is a relative enrichment of the 46-kDa polypeptide in the metacyclic trypomastigote immunoprecipitated polypeptides. Conversely, the immunoprecipitation of epimastigote and metacyclic trypomastigote mRNA in vitro translation products with the P2-WGA antiserum recognizes specifically a 48-kDa polypeptide within the in vitro synthesized proteins of polyribosomal mRNA from metacyclic trypomastigotes (fig.2, lane H). The fact that no polypeptides are specifically immunoprecipitated either from epimastigote polyribosomal and post-polyribosomal mRNA in vitro translation products (fig.2, lanes F and G, respectively) or from metacyclic trypomastigote post-polyribosomal mRNA in vitro translation products (fig.2, lane I), indicates that the mRNA coding for this polypeptide is stage specific and is not stored in the cytoplasm. The control immunoprecipitations with human and rabbit normal sera is shown in fig.2, lanes E and J, respectively.

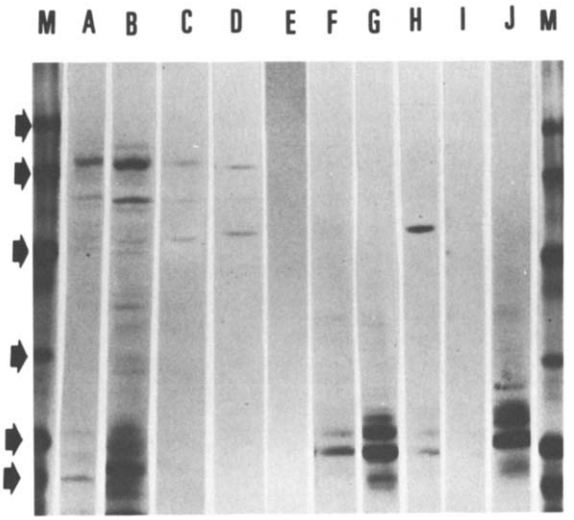

Fig.2. Polyacrylamide gel electrophoresis of in vitro translation products of: (A) epimastigote polyribosomal mRNA immunoprecipitated with chagasic sera; (B) epimastigote post-polyribosomal mRNA immunoprecipitated with chagasic sera; (C) metacyclic trypomastigote polyribosomal mRNA immunoprecipitated with chagasic sera; (D) metacyclic trypomastigote post-polyribosomal mRNA immunoprecipitated with chagasic sera; (E) epimastigote polyribosomal mRNA immunoprecipitated with human normal sera; (F) epimastigote polyribosomal mRNA immunoprecipitated with anti-P2 WGA sera; (G) epimastigote post-polyribosomal mRNA immunoprecipitated with anti-P2 WGA sera; (H) metacyclic trypomastigote polyribosomal mRNA immunoprecipitated with anti-P2 WGA sera; (I) metacyclic trypomastigote post-polyribosomal mRNA immunoprecipitated with anti-P2 WGA sera; (J) metacyclic trypomastigote polyribosomal mRNA immunoprecipitated with rabbit normal sera; (M) ^{14}C molecular mass markers of 92.5, 68, 43, 25.7, 18.4 and 12.3 kDa.

4. DISCUSSION

The results point to the complexity of the mRNA population of *T. cruzi* non-infective epimastigotes and infective metacyclic trypomastigotes, contrasting with results obtained by others showing an extremely simple profile for the in vitro synthesized polypeptides [17]. Several mRNAs are conserved among both differentiation stages, in agreement with the fact that epimastigotes and metacyclic trypomastigotes must share common structural proteins and enzymes. Notwithstanding, differences are observed among mRNAs of both differentiation stages. These stage-specific gene expression products could be involved in the biological properties characteristic of each *T. cruzi* differentiation form.

The presence of mRNAs in the post-polyribosomal supernatant is not surprising since this appears to be a common feature for different

eukaryotic cells [18,19]. We do not believe that the post-polyribosomal mRNAs have been artifactually generated both because run-off inhibitors (cycloheximide or emetine) have been used during cell manipulation and because one of the major polyribosomal mRNA in vitro translation products (polypeptide 1) is almost absent within the post-polyribosomal in vitro translation products. In the case of *T. cruzi*, these hitherto in vivo translation repressed mRNAs would be in equilibrium with counterparts being actively translated, contrasting with other cell systems (e.g., duck reticulocytes) where the analytical complexity of post-polyribosomal mRNAs is much greater than the polyribosomal population [20]. However, we cannot rule out the possibility that minor polypeptides have not been detected or, alternatively, that some *T. cruzi* mRNAs are not translatable in the reticulocyte lysate. Conversely, it has been shown that the intracellular transformation of *T. cruzi* trypomastigotes to amastigotes is performed without RNA synthesis [21], suggesting the existence of stored mRNAs that would translate the new proteins required in the transformation process. In addition, it has been suggested that *Leishmania mexicana* tubulin mRNA is sequestered to the post-polyribosomal compartment during differentiation [22].

The immunoprecipitation of the in vitro translation products with two different antisera shows that while human chagasic serum recognizes common polypeptides of M_r 72000, 68000 and 46000 in all 4 mRNA populations, the anti-P2 WGA serum recognizes a single polypeptide of M_r 48000 within the metacyclic trypomastigote polyribosomal mRNA population. As yet we cannot explain the difference in molecular mass of the in vivo 85-kDa protein [4–6] and the in vitro synthesized 48-kDa polypeptide (fig.2, lane H). It could be at least partially due to the post-translational glycosylation of the polypeptide chain inside *T. cruzi*. Indeed, several *T. cruzi* proteins are post-translationally modified [23], implying that care must be undertaken when comparing *T. cruzi* in vivo and in vitro translation products [17].

Finally, the fact that sera raised against surface antigens relevant to *T. cruzi* penetration in mammalian cells recognize polypeptidic epitopes raises the possibility of parasite antigen production by cDNA cloning in expression vectors.

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