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Protein synthesis in purified trypo- and epimastigote forms of *Trypanosoma cruzi*

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Summary

In order to develop the experimental background for studies on the differentiation of the medically important lower eukaryote, *Trypanosoma cruzi,* the polypeptides synthesized by purified epimastigotes and trypomastigotes were examined. The in vivo synthesized proteins were compared with polypeptides synthesized in reticulocyte lysate systems programmed with total RNA of the two forms. Qualitative and quantitative differences between the protein populations of the two forms were detected. The most prominent differences concern three proteins of 73,000, 64,000 and 55,000 daltons. The possible use of these proteins as model systems for studies on differential gene expression is discussed.

Key words: *Trypanosoma cruzi;* Chagas' disease; differentiation; protein synthesis; regulation; gene expression.

Introduction

Trypanosoma cruzi is the causative agent of Chagas' disease which affects millions of South Americans (Hudson, 1981). Its life-cycle displays a well-characterized polymorphism: trypomastigote blood-stream forms are taken up from a vertebrate host by haematophagous insects, reduviid bugs, and differentiate in the insect's midgut to epimastigote forms that multiply by mitosis. Upon reaching the hindgut and rectum of the insect, some of these epimastigotes transform into metacyclic trypomastigotes which are excreted with the faeces simultaneously when the bug ingests another blood-meal (Brener, 1973). These trypomastigotes then infect the new vertebrate host, usually via the opening wound through which the blood-meal had been ingested.

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With this, *T. cruzi* represents an example of differentiation of a unicellular eukaryote. In contrast to the African trypanosomes, where the expression of the variable-surface-glycoprotein genes is well investigated (Borst and Cross, 1982; Englund et al., 1982), reports on the regulation of gene expression in *T. cruzi* are scarce.

It is now possible to obtain large quantities of trypomastigote and epimastigote culture forms (Chiari et al., 1980) which opens the way for studies aiming at the identification of genes that are differentially expressed in these two forms. In this paper we present in vivo and in vitro studies on the polypeptides synthesized by *T. cruzi* epi- and trypomastigotes.

Materials and Methods

Production of epi- and trypomastigotes

Epimastigotes. This form was obtained from a *T. cruzi* Y 10 strain (Prof. H. P. de Azevedo, Department of Cell Biology, University of Brasília, Brazil) maintained in log phase in LIT (Camargo, 1964) medium at 28° C.

Trypomastigotes. Trypomastigote forms were produced according to Chiari (1980). In short: cells in exponential phase in LIT medium were transferred to M-16 medium (0.4% NaCl, 0.04% KCl, 0.8% Na_2HPO_4, 0.2% glucose, 0.125% tryptose and 2.5% fetal bovine serum, pH: 6.7, filter-sterilized after 1 h at 68° C) and maintained for 8 days at 28° C. After this period between 30–40% trypomastigote forms were present which were isolated subsequently using ion-exchange chromatography on Whatman DE-52 cellulose (Al-Abbassy et al., 1972).

The purity of epi- and trypomastigotes was checked in the light microscope using methanol-fixed and Giemsa-stained slides.

RNA extraction

Either trypo- or epimastigotes were pelleted at $2{,}000 \times g$ for 10 min, and washed two times in 0.2 M NaCl. Total nucleic acids were then extracted according to Aviv and Leder (1972). During the initial phase of the ethanol precipitation the DNA was spooled on a glass rod; RNA was pelleted after an overnight precipitation at −20° C and resuspended in 20mM Tris-HCl, pH: 7.5, 0.1 mM EDTA. One unit of absorbance at 254 nm was considered to equal 40μg of RNA.

In vivo protein synthesis

Epi- or trypomastigote forms obtained as described were pelleted at $2{,}000 \times g$ for 10 min and washed one time in PSG buffer (0.6% $Na_2HPO_4 \times 7\ H_2O$, 0.67% NaCl, 0.0035% KCl and 0.9% glucose, pH: 7.0). Incorporation was performed at 28° C in a volume of 1 ml PSG containing 5×10^7 cells, 32 μl of an amino-acid mixture (twice as concentrated as the one described by Hunt and Jackson, 1974, from which the respective radioactive amino acids were omitted) and either 10 μl of leucine (56 Ci/mmol, 0.4 mCi/ml) for incorporation kinetics, or 10 μl of ^{35}S-methionine (1280 Ci/mmol, 4 mCi/ml) for fluorographic analysis of the products (Bonner and Laskey, 1974). The incorporation kinetic was established by withdrawing 10 μl aliquots at 1 hour intervals which were subsequently pipetted into 1 cm^2 Whatman 3 MM filter papers previously soaked with 5% trichloracetic acid. After drying, the papers were washed 3 times in cold TCA 5%, once in ethanol/ether (1:1) and the radioactivity determined in a Beckman liquid scintillation counter.

In vitro protein synthesis

Reticulocyte lysate systems (Pelham and Jackson, 1976) were programmed with total RNA from either epi- or trypomastigotes. Typically the systems contained, in a total volume of 100 μl, 6 μg of total RNA and 10 μCi of ^{35}S-methionine. Incubation was for 30 min at 28° C. The labelled proteins were detected in 16% polyacrylamide gels according to Laemmli (1970) via fluorography (Bonner and Laskey, 1974).

Results

In Fig. 1 representative sections from slides of the epi- and trypomastigote forms used in this work are shown. As can be seen, a purity of 95% for epi- and of 98% trypomastigotes was achieved.

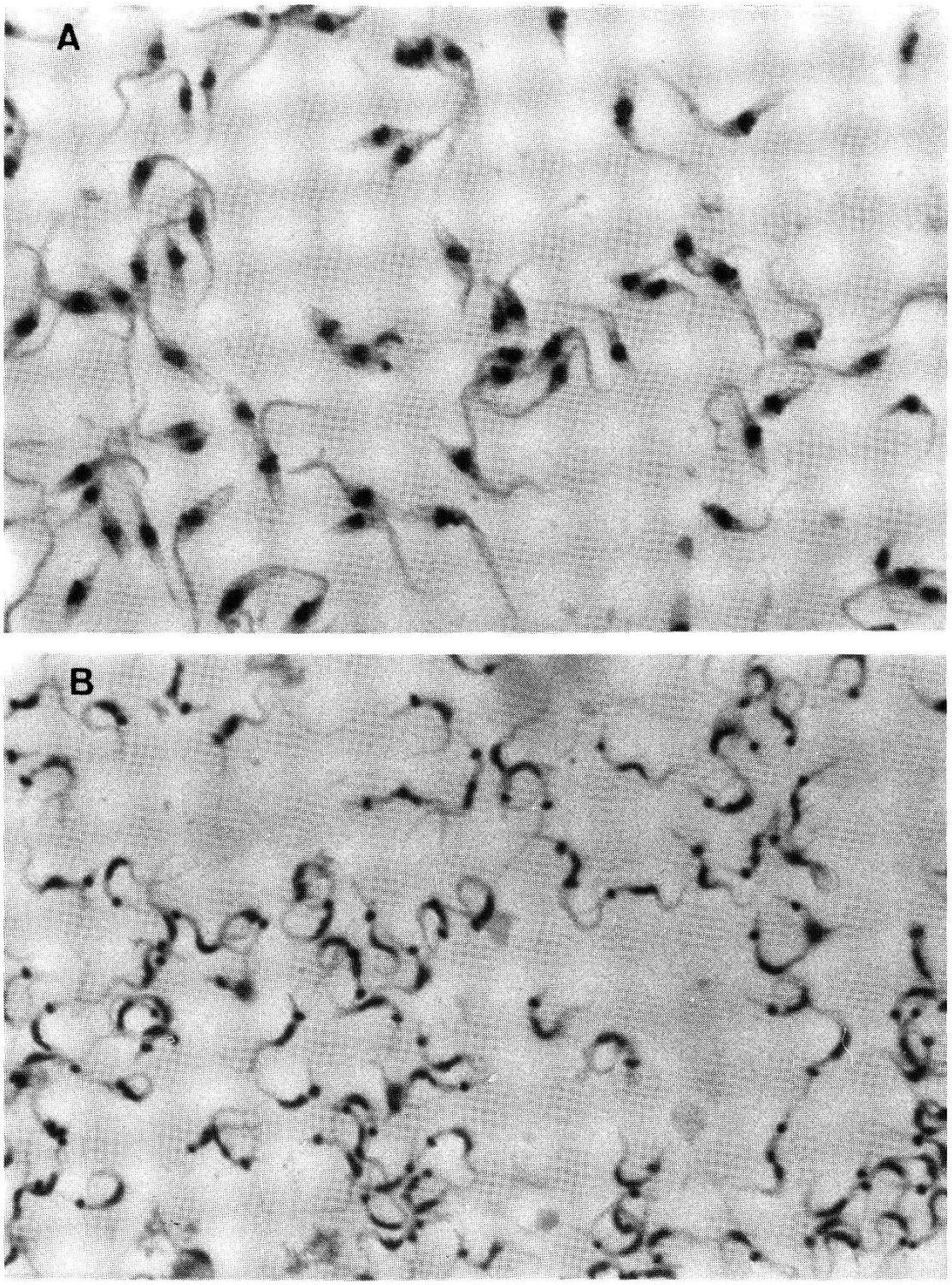

Fig. 1. Representative micrographs of epimastigotes (A) and trypomastigotes (B). Giemsa stained, $\times 1.000$.

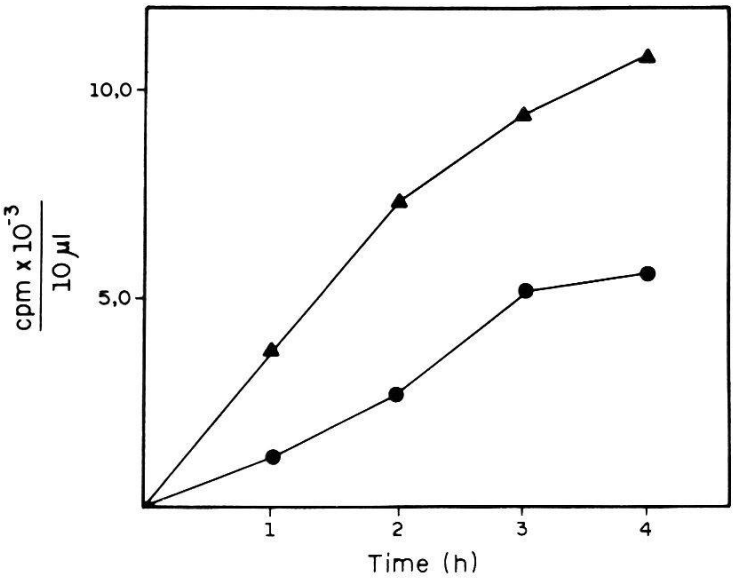

Fig. 2. Kinetics of ^{3}H-leucine incorporation into epimastigote (▲———▲) and trypomastigote (●———●) cells.

Both forms incorporate radioactive amino acids into proteins and in Fig. 2 the kinetics of this incorporation are shown. Both forms reach a plateau after about 4 hours and, interestingly, the epimastigote forms incorporate roughly two times more radioactivity per cell than the trypomastigotes. However, when the curves are equated with respect to the total proteins introduced into the incorporation assay, the incorporation patterns are practically superimposable.

Fig. 3 shows the electrophoretic analysis of the polypeptides present in vivo (3 A), of the proteins synthesized de novo in both forms (3 B) and of those synthesized in a rabbit reticulocyte lysate system programmed with total RNA extracted from epi- and trypomastigotes (3 C). The most prominent differences concern three polypeptides of 55,000, 64,000 and 73,000 daltons. In epimastigotes the 55,000 dalton protein is synthesized abundantly but is almost absent in trypomastigotes and, on the other hand, 64,000 and 73,000 dalton proteins are synthesized in large quantities in trypomastigotes but are nearly invisible in the epimastigote forms (arrows).

These results of the in vivo incorporation of radiolabelled precursors into polypeptides is partially confirmed when reticulocyte lysates are programmed with the total RNAs isolated from the two forms. Again RNAs from epimastigotes cause the appearance of a prominent protein of 55,000 dalton but, most interestingly, the 64,000 and 73,000 dalton proteins are synthesized under the direction of the RNAs from both forms to a similar amount (Fig. 3 C, arrows).

Discussion

The aim of this work was to establish conditions for the use of the unicellular eukaryote *Trypanosoma cruzi* as a simple model system in which differential expression of defined genes during its life cycle can be observed.

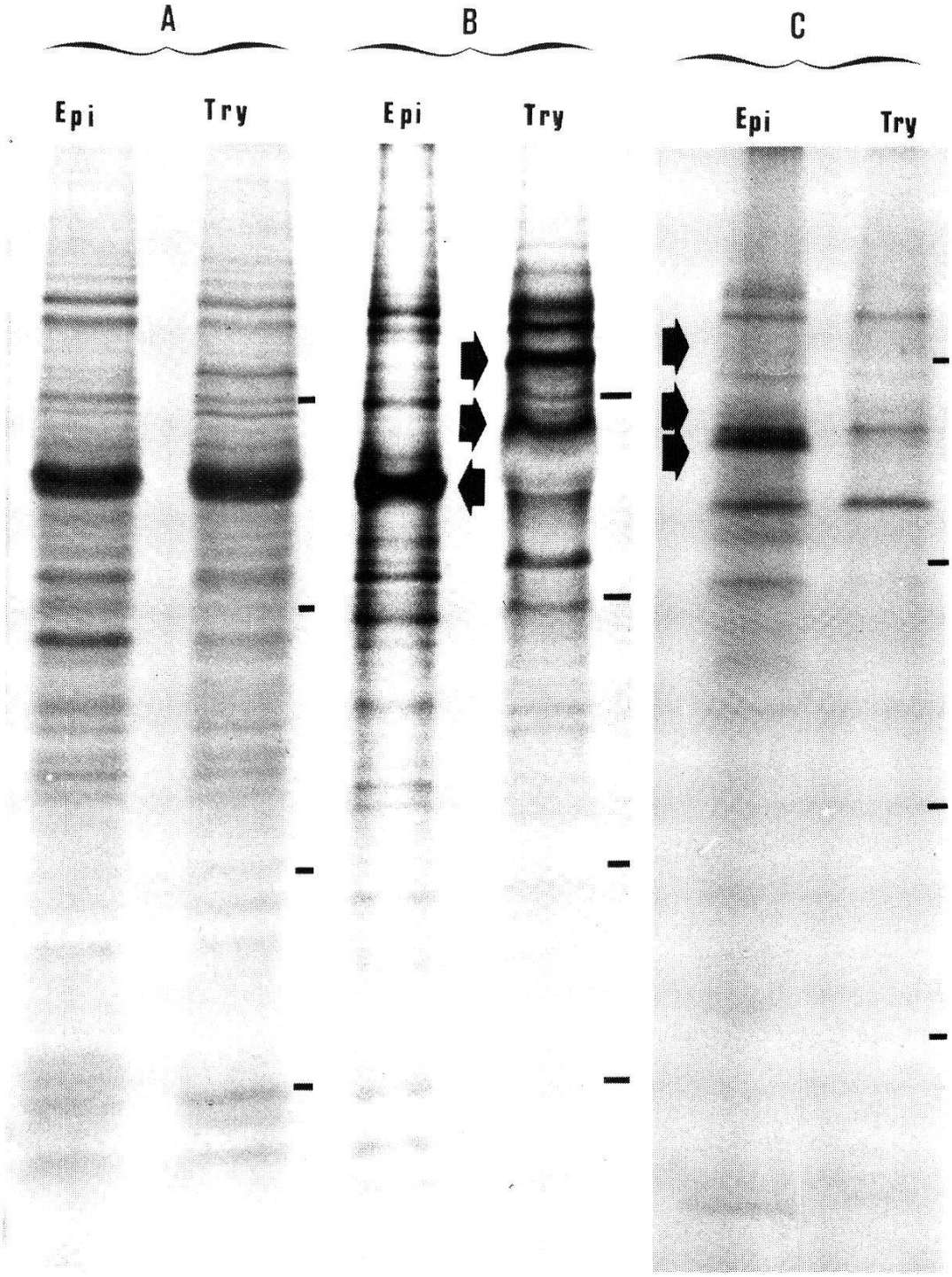

Fig. 3. Analysis on 16% SDS-polyacrylamide gels from epimastigotes and trypomastigotes. A: Coomassie Blue stained; about 100 μg of proteins charged per lane. B: Fluorography of the polypeptides synthesized in intact cells in the presence of ^{35}S-methionine. Incorporation: 90 min at 28° C, about 20.000 acid-precipitable counts were charged per lane. C: Fluorography of the proteins synthesized in a rabbit reticulocyte lysate system programmed with total RNA from epi- and trypomastigotes. Incorporation conditions as described in "Materials and Methods", about 4,000 acid-precipitable counts were charged per lane. The horizontal bars indicate the positions of the markers BSA (68,000), ovalbumine (45,000), trypsinogen (24,000) and β-lactoglobuline (18,400). Note: "A", "B" and "C" represent different gels.

Such studies, however, depend crucially on the purity of the cell populations used. In the present work we chose as a criterion morphological features and show that we are dealing with morphologically homogeneous populations of trypo- and epimastigotes.

Also, one needs to define the genes whose expression differ in the two forms. In the present approach purified cell populations are characterized for their proteins synthesized in vivo and for the informational content of their mRNA using a rabbit reticulocyte lysate system. The results show that clearcut qualitative and quantitative differences exist between the proteins synthesized by the two forms. This result is in contradiction to reports published earlier (Castro et al., 1980; Chiari, 1981) where the authors, using a similar one-dimensional gel system, claim to have found neither qualitative nor quantitative differences in the protein patterns of epi- and trypomastigotes. The reason of this discrepancy is not clear to us; it could be possible that, as they did not purify their trypomastigotes on DEAE-cellulose, enough cells in transient stage could have been present to mask the real differences. Furthermore, recently published results on the isoenzyme patterns during the life cycle of *T. cruzi* (Bogliolo, 1982) are consistent with our findings that at least some of the genes are expressed differentially in epi- and trypomastigotes. A broad population of de novo synthesized proteins is different in epi- and trypomastigotes, of which the most prominent alterations concern a 55,000, 64,000 and 73,000 dalton protein. It is highly possible, on the basis of the molecular weight, that the 55,000 dalton protein is tubulin. Its abundant sythesis in epimastigotes could be explained by the fact that these cells divide actively and need a high supply of tubulin to pass on to their daughter cells for the synthesis of microtubules. In the nondividing trypomastigotes de novo synthesis of microtubule-precursors can be expected to be much lower. With respect to this protein, the results obtained in vivo and in vitro match. One could suggest from these results that in trypomastigotes the gene for the 55,000 dalton protein is either silent or – if such mechanisms exist at all in trypanosomatids – the pre-mRNA for this protein is not processed down to the translational level. In contrast, the situation concerning the 64,000 and 73,000 dalton protein seems to be different. In this case the synthesis in epimastigotes and the in vitro translation of the total RNAs from both forms yielded roughly the same amount of these proteins but showed a significant stimulation in intact trypomastigote cells.

This might point to a translational control process governing the synthesis of these proteins and one could speculate that in intact trypomastigotes a stimulating factor, specific for this mRNAs, might be present.

Though we cannot offer absolute proof for the existence of different control levels in *T. cruzi*, our results demonstrate the existence of differentially expressed genes in this organism and also point out three specific proteins which are likely to serve as model systems for further studies on differentiation in this lower eukaryote.

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