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CLONING OF TRYPANOSOMA CRUZI STAGE SPECIFIC GENES.

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INTRODUCTION

Trypanosoma cruzi displays a complex life-cycle comprising at least three well-distinct morphological and functional types (Brener, 1973; De Souza, 1984). From these, only trypomastigote forms are considered to be infective to the vertebrate host. However, the morphological and functional differences between the various developmental forms, at least in the case of epimastigotes and metacyclic trypomastigotes, can not be attributed to major differences in their mRNA population. Indeed, the comparison of the *in vitro* translation products from both differentiation stages has shown a high conservation of the expressed mRNA sequences (Goldenberg et al, 1985).

T. cruzi is a very suitable biological system for the study of lower eukaryotes cell differentiation (Bonaldo et al, 1987). Although the mechanisms involved in *T. cruzi* differential gene expression regulation remain to be determined, stage specific genes are a very good target for such studies. Furthermore, gene expression products from trypomastigotes are important in view of their relevance for the parasite life-cycle as well as for their potential use in the diagnosis and therapeutics of Chagas' Disease.

In the present report we describe our attempts to clone trypomastigote genes in the search for those which are stage specific or potentially relevant for the diagnosis of Chagas' Disease.

RESULTS

The first approach to clone T.cruzi trypomastigote stage specific genes consisted in the selection of these sequences by chromatography on hydroxylapatite (Alt et al,1978; Hedrick et al, 1984). Metacyclic trypomastigote cDNA was hybridized with a 30 fold excess of epimastigote mRNA and chromatographed on hydroxylapatite at 65ºC. The fraction eluted at 0.1M-0.12M phosphate was digested with RNase and the single stranded cDNA was used for second strand cDNA synthesis and cloning in λgt11 (Young & Davies, 1983). Rot analysis showed 98% homology between epimastigote and trypomastigote mRNAs, thus corroborating our previous results (Goldenberg et al, 1985). This method resulted in the obtention of 200 recombinant clones containing inserts with an average size of 600 base pairs. From these, 80% were specific to the trypomastigote stage (Fig. 1) and 40% displayed fusion polypeptides.However, none of the fusion polypeptides was recognized by both a trypomastigote antiserum (Andrews et al, 1985) and Human chagasic sera, suggesting that antigenic determinants of T.cruzi antigens are preferentially located in other sites than the carboxy terminal portion of the proteins.

In order to identify the recombinant clones, we choose 20 of them for mice immunization. The antisera were then used in Western blots of total trypomastigote proteins and for the immunoprecipitation of 125-iodine and 35S-methionine labelled T.cruzi proteins. The results showed that although the mice antisera recognized the fusion polypeptides, they did not recognize any T.cruzi protein. Indeed, the β-galactosidase portion of the fusion polypeptides proved to be the antigenic determinant.

In view of these facts, an alternative method was used for isolating trypomastigote genes, which consisted in the immunological screening of a T.cruzi genomic expression library in λgt11. This methodology has proven to be useful with T.cruzi (Peterson et al, 1986; Ibañez et al, 1987) since the genes from the parasite

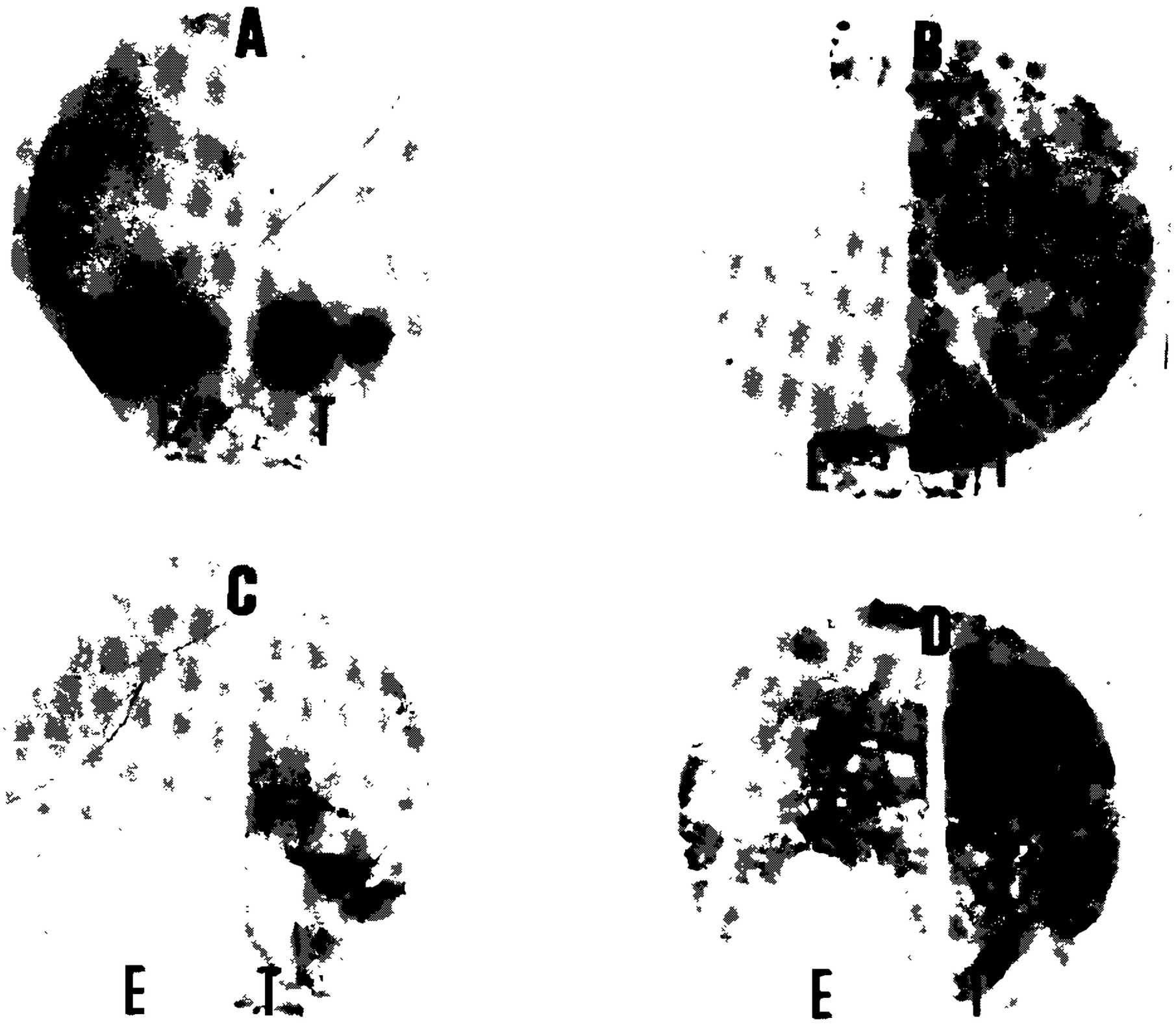

Figure 1 - Stage specificity of hydroxylapatite-selected cDNA clones. The clones were transfered to nitrocellulose, the filters were cut and hybridized with 32P-labelled epimastigote (E) and trypomastigote (T) cDNAs. A- Control with spotted parasites; B and C - Trypomastigote stage specific clones; D- Clone common to epimastigotes and trypomastigotes.

are apparently intronless. Furthermore, such procedure should avoid the bias commonly observed on cDNA cloning primed by oligo--dT, rendering possible the detection of clones whose fusion polypeptides corresponded to the central or amino terminal regions of the antigens.

T.cruzi nuclear DNA was sheared to an average size of 1.2Kb and cloned in λgt11 after DNA repair with DNA polymerase I and EcoRI-linkers' addition. The screening of 100.000 clones with a trypomastigote antiserum (Andrews et al, 1985) extensively adsorbed with bacteria, resulted in the obtention of 12 clones.

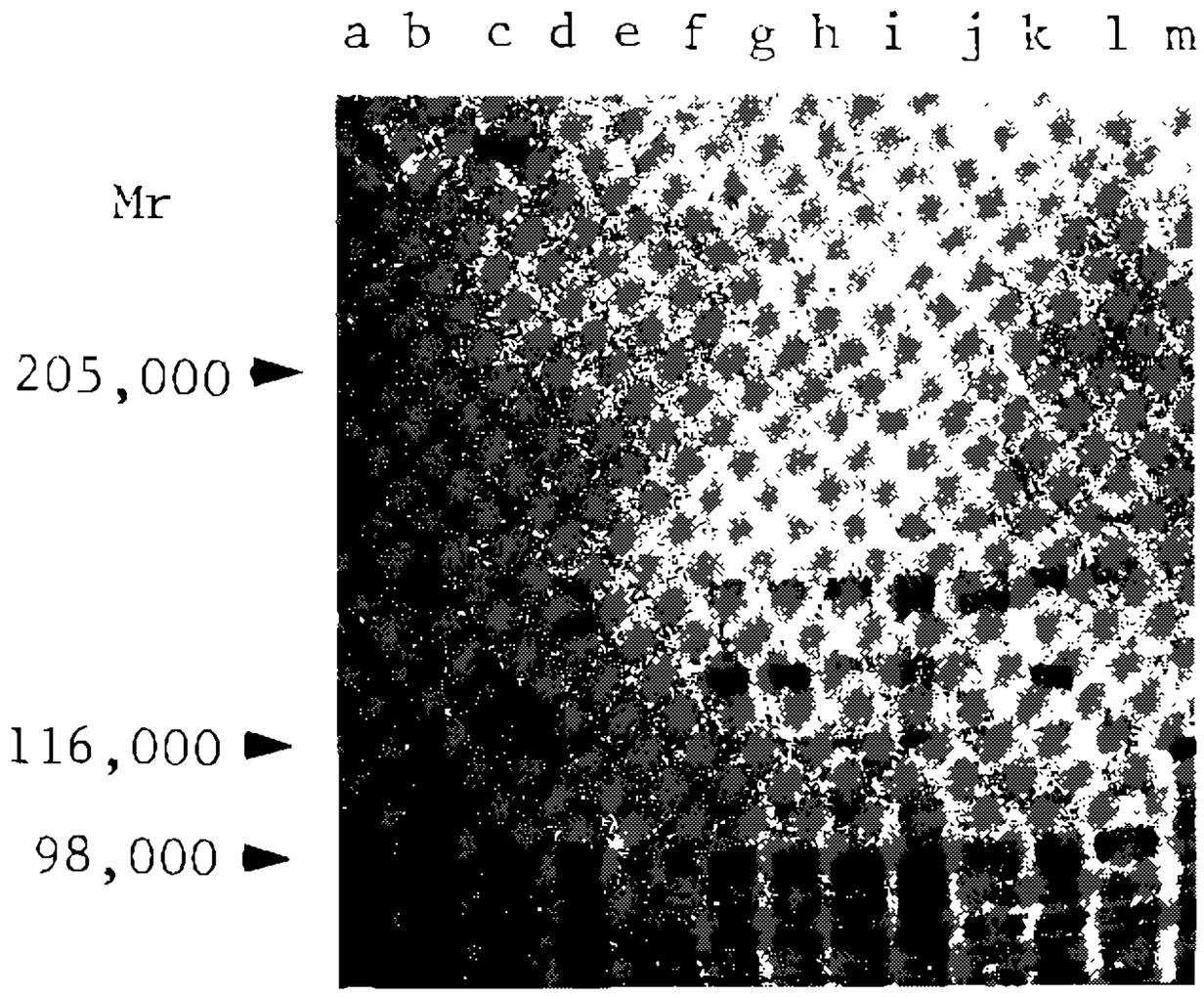

Figure 2- SDS-PAGE analysis of fusion polypeptides - The lanes refer to clones: a-6/2; b-7/2; c-8/2; d-8/5; e-15/3; f-21/5; g-25/5; h-28/7; i-31/2; j-31/3; k-33/1; l-40/6; m- native λgt11

These clones were characterized in terms of their respective fusion polypeptides (fig. 2) and reaction with different sera after Western blot of total proteins from IPTG-induced bacteria(Table I).

In order to determine which clones might be useful for diag nostic purposes, we made Western blots of the fusion polypeptides with different sera from Human patients bearing Chagas' Disease, visceral and tegumentar leishmaniasis, toxoplasmosis, malaria, paracoccidio mycosis and schistosomiasis and with a T.rangeli antiserum. The results showed that only clones 7/2 and 33/1 are specifically recognized by all chagasic sera.

We are presently characterizing these clones in terms of their nucleotide sequence, genome organization and coding products. Preliminary data indicate that clones 7/2 and 33/1, although localized in the same chromosome, correspond to different genes. In addition, clone 7/2 contains a 14-mer repetitive epitope (fig.3) and might code for a 60-65Kd surface antigen, as judged by the immunoprecipitation of iodine-labelled trypomastigotes with the serum from mice immunized with recombinant bacteria.

TABLE I

Characterization and reactivity of trypomastigote recombinant clones with different sera

Clone number	Mr Fusion Polypeptide	Trypomastigote Antiserum	Epimastigote Antiserum	Human Chagasic sera	Coomassie blue Staining
6/2	170Kd	+	-	+	++
7/2	150Kd	++	++	+++	++
8/2	290Kd	+	-	-	++
8/5	140Kd	++	-	++	++
15/3	-	+	-	+	-
21/5	130Kd	+	-	+	++
25/5	130Kd	+	-	+	++
28/7	175Kd	+++	-	-	++
31/2	148Kd	+	-	++	++
31/3	145Kd	++	++	++	+
33/1	130Kd	+	-	++	++
40/6	-	+	-	+	-

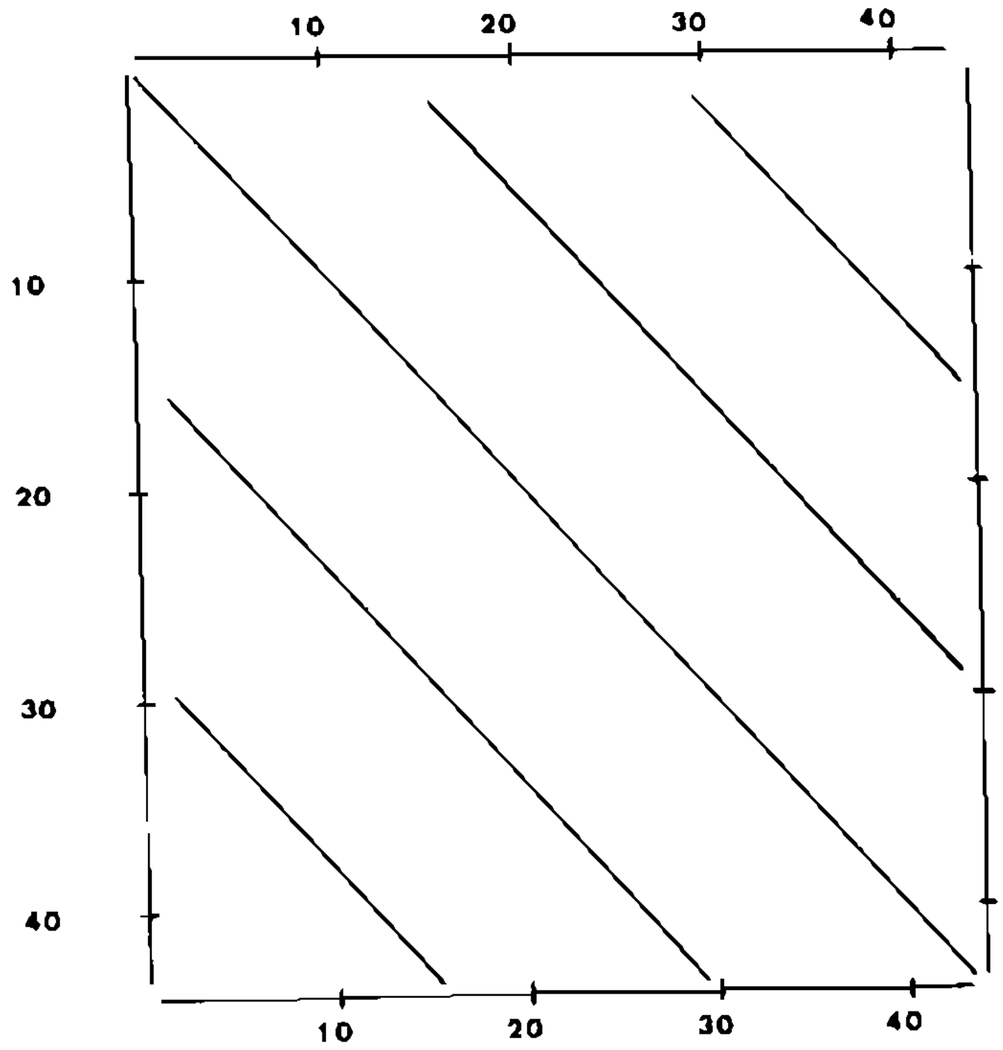

Figure 3 - Dot-matrix analysis of the aminoacid sequence from clone 7/2 fusion protein.

CONCLUSIONS AND PERSPECTIVES

Our results suggest that the most antigenic portion of *T.cruzi* proteins do not lie on the carboxy terminal region of the molecules. Indeed, neither a trypomastigote antiserum nor Human chagasic sera recognized the fusion polypeptides from a trypomastigote stage specific cDNA expression library.

However, the immunological screening of a genomic expression library with a trypomastigote antiserum, although more laborious, resulted in the obtention of recombinant clones whose fusion polypeptides are recognized by several Human chagasic sera. These clones are being characterized and preliminary results indicate that at least two of them (clones 7/2 and 33/1) might be useful for Chagas' Disease diagnosis. Also, a partial nucleotide sequencing showed that clone 7/2 contains a repetitive epitope and encodes a protein different from those cloned by others (Peterson et al, 1986; Ibañez et al, 1987).

Our present effort is centered in confirming the nucleotide

sequence of clone 7/2 and determining the sequence of the other clones. In addition, we are purifying the fusion polypeptides in order to obtain a purest reagent for diagnostic purposes and for the production of monospecific polyclonal and monoclonal antibodies.

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