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A head-to-tail tandem organization of hsp70 genes in *Trypanosoma cruzi*

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ABSTRACT.

We describe the isolation and characterization of the T. cruzi hsp 70 DNA coding region which was found to be formed by multigene copies organized in a tandem array in a head-to-tail manner. The restriction pattern of one of the repetition units whithin the largest clone obtained from the genomic library, clone Tc70.6, shows that the hsp70 coding region should be formed by at least seven identical copies of 2.5 kb. We have found, however, the presence of restriction polimorphisms (Pvu II) within these repeats. Subsequent analysis of the time course of nuclear DNA digestion has revealed that the copy number per haploid genome could be as greater as 10. The analysis of the DNA and amino acid sequence of a fragment (70%) of one of the repetition units has shown the existance of a high homology with all the hsp70 genes of other organisms. The protein sequence homology of the fragment analyzed is as high as 88% when compared with that of the T. brucei hsp70. On the other hand, there are significant restriction site variations between both. The T. cruzi hsp70 contains at the C-terminal end a tetrapeptide repeat of the structure $(GMPG)_9$.

INTRODUCTION.

The hemaflagellate Trypanosoma cruzi is the etiological agent of Chagas' disease, a chronic and progressive disease that is known to afflict at least 12 millions people in South and Central America (1, 2). T. cruzi has a complex life cycle which involves infection of vertebrate hosts and transmission by insect vectors. T. cruzi has three major morphological stages (3). The epimastigote form proliferates in the insect vector and eventually differentiates to produce metacyclic trypomastigotes. After passing into the blood stream and entering the cells of the vertebrate host, the metacyclic trypomastigotes transform into amastigotes. The amastigotes, the dividing forms inside the cells of the vertebrate host, finally differentiate to blood stream trypomastigotes which may initiate the next cycle of infection.

Thus, these parasitic protozoa are subjected to a heat shock period when they pass from the 25°C ambient temperature of the insect vector to the 37°C temperature of their mammalian host. Since the synthesis of heat shock

proteins is dramatically induced at 37°C in different mammalian parasitic protozoa (4-6), it has been suggested that these proteins may play an important role in their life cycle during differentiation and morphogenesis. In fact, it has been shown that in Leishmania major a temperature shift from 25°C to 37°C is sufficient to induce in vitro transformation from promastigote to amastigote forms (7). Similar data have been reported for L. mexicana (8).

Because of that and due to the evolutionary conservation of the hsp70 genes, we have attempted to determine the structural relationship between the T. cruzi hsp70 genes and other hsp70 genes of parasitic and non parasitic origen. In contrast with the organization of this gene in eucaryotic organisms (9), we have found that in T. cruzi the hsp70 genes are organized as a tandem array of DNA copies, with similar restriction patterns, in a head-to-tail fashion. Similar organizations have been shown also to exist in other genes from T. brucei (10-12).

MATERIALS AND METHODS.

Isolation of DNA and Southern analysis.

The strain of Trypanosoma cruzi used in this study was isolated in the Institute of Malariology in Maracay (Venezuela) and kindly provided by Dr. A. Osuna (Dept. of Parasitology. Granada). The parasites were cultured in LIT medium at 28°C (13).

10^{10} epimastigotes were harvested by centrifugation, washed several times in 0.9 % saline solution and suspended in 10 ml of 0.15 M NaCl, 0.1 M EDTA, 100 µg/ml proteinase K, and 0.5 % SDS. After incubation for 30 min at 50ºC, the DNA was extracted twice with phenol at 60°C, one time with phenol-chloroform-isoamyl alcohol (25:24:1), and a second time with chloroform-isoamyl alcohol (24:1). Finally, the DNA was washed two times with diethyl ether and collected by ethanol precipitation.

3 µg genomic DNA per sample were digested with restriction endonucleases in the appropiated reaction buffer (3-5 units/µg for 5 hrs) and resolved in 0.8 % agarose gels. Before transfer to nitrocellulose filters (14), the gels were treated with 0.25 M HCl for 10 min. Prehybridization was carried out at 42°C in 50% formamide, 0.1% SDS, 0.1% sodium pyrophosphate pH 6.9, 5 x Denhardt's solution, 5 x SSC and 100 µg/ml sheared, denatured herring sperm DNA. Hybridization was carried out in the same way in the presence of 10% dextran sulfate. As probe the 1.1 Kb PstI fragment of clone 2.32.1, containing the Drosophila melanogaster hsp70 gen (15), was used. The ^{32}P-labeled nick translated probes had a specific activity of 10^{8} cpm/µg. Routinely we have

used the probe at 10 ng/ml. After hybridization, the filters were washed four times for five min in 2 x SSC - 0.1 % SDS at room temperature and three times for 20 min in 0.1 x SSC - 0.1 % SDS. Each one of the experiments was performed with different stringency conditions (ranging temperature from 40°C to 65°C).

Construction and screening of the genomic library.

The DNA was partially digested with the restriction endonuclease Sau3A and the fragments ranging from 15 to 20 Kb in length were isolated from the agarose gels by electroelution (16). The EMBL-3 replacement vector DNA was digested with internal flanking restriction enzymes BamHI and EcoRI, and precipitated with 0.6 volumes isopropanol and 0.15 volumes 3 M Na acetate. Ligation was carried out at 3:1 molar ratio (vector:insert) at a concentration of 0.1 µg/µl and 1 unit (Weiss) of T4-DNA ligase. The mixture was incubated over night at 12°C. After ligation the DNA was packaged *in vitro* and plated on *E. coli* Q359. 5 10^5 recombinant clones were obtained. Assuming that the complexity of the *T. cruzi* genome is 10^8 base pairs, we calculated that the genomic library may contain at least 70 genomes. The genomic clones were then screened for inserts corresponding to the hsp70 gene by *in situ* plaque hybridization (17). The positively hybridizing clones were purified and then used for subsequent characterizations. The positive clones were named Tc70.1 to Tc70.14.

The number of repeats in each clone was determined by partial digestion of the inserts with SmaI and by microdensitometric measurements of the intensity of the ethidium bromide bands generated by SmaI digestion as revealed in photografic negatives. The exposure time was such that the blackening of the bands in the negatives was in the linear range of response of the film (18).

Subcloning and DNA sequence analysis.

The 2.5 Kb BglII fragment from clone Tc70.6 was isolated and subcloned into the BamHI site of both pUC8 plasmid and the replicative form of M13mp18 phage. The recombinant molecules were called p.Bg.2 and p.Bg.5, when cloned in pUC8, and M.Bg.5 and M.Bg.8 when cloned into M13 (the DNA insert was cloned in both orientations). *Escherichia coli* strains JM83 and JM109 were transformed with the recombinant plasmids or phages, respectively. Additional subcloning was carried out by internal deletion of the BglII repeat. For that purpose 0.1 µg of the recombinant plasmid p.Bg.2 was digested either with PstI, SmaI or XmnI, and ligated in a 50 µl of the ligation buffer at room temperature for two hrs. Bacterial strain JM83 were again transformed as before with the recombinant molecules. The resulting delection recombinant plasmids are shown in figure 2.

The nucleotide sequence was carried out by the dideoxy chain termination method (19). The DNA fragments subcloned into M13mp18 were sequenced as detailed by Sanger et al., (20). The DNA in double-stranded forms into pUC8 were sequenced by the chain termination method as described by Korneluk et al. (21) with the modifications introduced by Haltiner (22). For the DNA sequence shown in figure 5 both DNA strands were analyzed.

RESULTS.

Genomic organization of T. cruzi hsp70 genes.

Figure 1 (A and B) shows the distribution of genomic restriction fragments of the T. cruzi hsp70 coding DNA. When the T. cruzi DNA was digested with EcoRI, BamHI and XhoI and the Drosophila melanogaster Pst I fragment of clone 2.32.1 (15; Fig 1A) was used as probe only one band greater than 25 Kb was generated. In contrast, the pattern observed after cleavage with BglII and SmaI consisted of only a single band of 2.5 kb in length. When the DNA was digested with AluI and HhaI only one band of about 0.75 kb and 0.35 kb, respectively, was observed. The strong signal detected in the hybridization blots suggests that the degree of homology between the hsp70 genes of Drosophila and T. cruzi should be very high. When the plasmid p.Bg.2 containing the BglII DNA insert was used as probe (Fig. 1B), a similar band greater than 25 Kb was observed after digestion of the DNA with EcoRI, BamHI and XhoI. Also the 2.5 Kb band was revealed after digestion with Sma I and BglII but the pattern obtained after AluI and HhaI digestion consisted of two bands of about 0.75 and 0.9 Kb, and 0.35 and 0.5 Kb, respectively. Obviously, a fraction of the AluI or HhaI digestion products of the T. cruzi hsp70 gene is not contained in the 1.1 Kb PstI fragment of the plasmid 2.32.1. Since a single hybridization band was observed after digestion of nuclear DNA with the enzymes BglII, SmaI, EcoRI, BamHI and XhoI either when the 1.1 Kb PstI fragment of clone 2.32.1 or the 2.5 Kb BglII was used as probe it is likely that the hsp 70 coding DNA is located at a single locus in the T. cruzi genome. We cannot exclude, however, the presence of pseudogenes or other hsp70 related sequences in the T. cruzi genome, since at lower stringency (40°C) additional weakly hybridizing bands were also observed.

Analysis of tandemly repeated genomic clones.

Using as probe the hsp70 PstI insert of clone 2.32.1, 14 positively hybridizing clones were isolated from the genomic library. The clones were called Tc70.1 to Tc70.14. In all of them repetition units of 2.5 Kb were detected, after digestion with BglII or SmaI. Figure 2 shows the organization

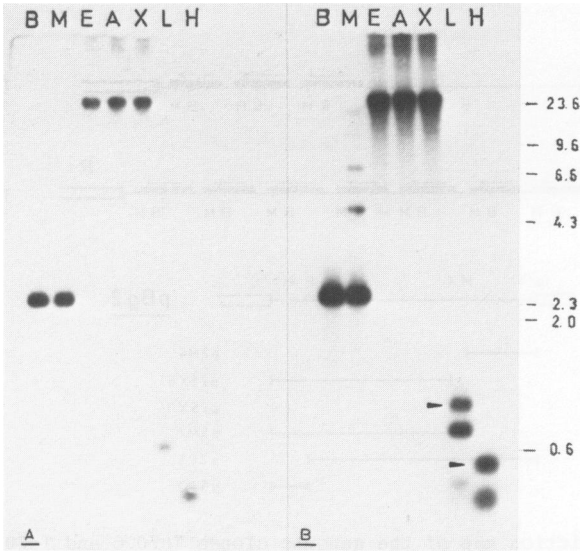

Fig. 1. Analysis of T. cruzi hsp70 gene by Southern blotting. Total DNA was cleaved with the restriction enzimes: B, BglII; M, SmaI; E, EcoRI; A, BamHI; X, XhoI; L, AluI; H, HhaI. The electrophoresis in A and B was carried out in parallel. In A the DNA was hybridized with the ^{32}P-labeled PstI fragment of clon 2.32.1 (the D. melanogaster hsp70 gene insert; 15); in B the ^{32}P-labeled plasmid p.Bg.2 was used (see Fig. 2). The arrows note the additional bands appearing when the DNA was digested with AluI or HhaI and the plasmid p.Bg.2 was used as probe. The size markers correspond to HindIII digestion of lambda phage in Kb.

of the repeating units in clones Tc70.6 and Tc70.11. Tc70.6 has six BglII-BglII or SmaI-SmaI complete repetition units while Tc70.11 has only two. We think that the repeating units in clon Tc70.11 are the first ones located at the 5′ end of the tandem array of the T. cruzi hsp70 genes since most of the DNA in the insert does not have homology with the probe. At present we cannot determinethe exact localization of the repetition units in clone Tc70.6 within the tandem array.

Figure 2 also shows the restriction map of one of the six BglII repeats within clone Tc70.6. Only one restriction site was found for SmaI, XmnI and PstI while PvuII cut in two sites. In order to determine if all the repeats in the Tc70 clones had the same restriction pattern, the restriction fragments generated by digestion with the above mentioned enzymes were probed with the 2.5 Kb insert from plasmid p.Bg.2. Assuming that all the repetition units present in the clones had the same restriction pattern as the one shown in

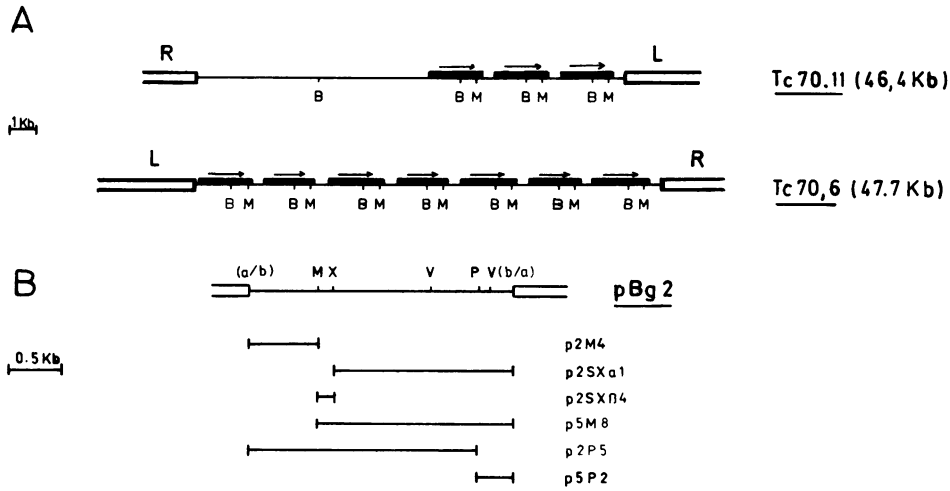

Fig. 2. A) Restriction map of the genomic clones Tc70.6 and Tc70.11. The black boxes represent the hsp70 coding repetition unit and the arrows indicate the the direction of transcription (see also fig 5). B) Subcloning of one of the 2.5 Kb-BglII repetition units from Tc70.6 was carried out into the BamHI site of pUC8. The resulting recombinant molecule was called p.Bg.2. In order to subclone specific fragments of the BglII repeat, plasmid p.Bg.2 was digested either with SmaI, XmnI or PstI. After ligation plasmids p.2.M.4, p.2SXα1, p.2SXβ4, p.5.M.8, p.2.P.5 and p.5.P.2 were obtained. M, SmaI; B, BglII; X, XmnI; P, PstI; V, PvuII; a/b, (insert-vector ligation site BamHI-BglII) ; R and L, right and left arms of EMBL-3 phage, respectively.

figure 2, we expected to obtain a single band of 2.5 kb after digestion with BglII, SmaI, XmnI and PstI and two fragments of 1.9 Kb and 0.6 Kb after digestion with PvuII. The result obtained was, in fact, the expected except for PvuII. After digestion of clones Tc70.4, Tc70.5 and Tc70.6 with this enzyme an extra band of 0.8 Kb was observed, in addition to the 1.9 and 0.6 Kb bands expected. The PvuII restriction pattern of Tc70.11 was even more surprising, since the 1.9 Kb band was not observed and two additional bands of 1.0 and 4.5 kb appeared. It was interesting to detect that all these bands are present in PvuII digested genomic DNA when probed with the BglII repeat (Fig 3). From these results, we conclude that the BglII repeating units are polymorphic for PvuII. Since the length of each one of the repetition units is very similar to the length of the coding region of the hsp70 gene from *D. melanogaster* and the repetition units are, moreover, very similar in the pattern of restriction sites we postulated that the 2.5 kb repetition units are members of a hsp70 multigene family, in which the copies are organized in

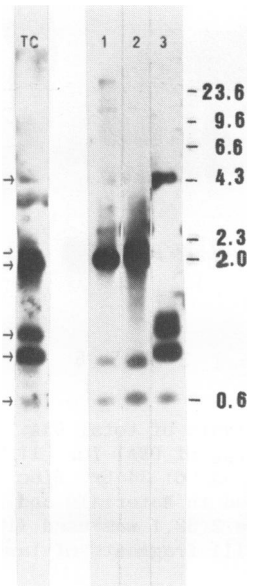

Fig. 3. Polymorphism for the restriction enzyme PvuII. T. cruzi total DNA (line TC), Tc70.5 DNA (line 1), Tc70.6 DNA (line 2) and Tc70.11 DNA (line 3) were digested with PvuII. A different pattern of hybridizing bands exists between the clones; in clone Tc70.11 two extrabands of aprox. 1Kb and 4.5kb are detected which are not present in clones Tc70.5 and 6. All these bands are present in line TC. The size markers, indicated in Kb, are the HindIII fragments of lambda phage.

a head-to-tail fashion separated by short non coding sequences. This hypothesis was further supported when the sequence analysis of one of the repetition unit was carried out (see Fig. 5).

The copy number of the BglII repeats in each clone was determined by microdensitometric measurements. From the ethidium bromide intensity of the 2.5 Kb DNA length band revealed in agarose gels relative to the intensity of the EMBL-3 arms, we have calculated that in clone Tc70.6 there are six complete BglII-BglII repetition units, and that Tc70.4, Tc70.5, and Tc70.11 have 4, 5 and 2 repeats, respectively. These calculations were confirmed by partial digestion experiments of the clones. Since the short genomic DNA sequences ligated to both arms of the EMBL-3 vector, generated by restriction with BglII or SmaI, in clone Tc70.6 also hybridize with the probe DNA (p.Bg.2), we think that there might be at least seven repetition units in the T. cruzi genome. In an attempt to estimate the total copy number of the BglII

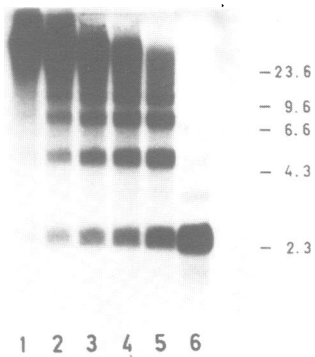

Fig. 4. Partial digestion analysis of total DNA: 5 µg of total DNA were digested with BglII (0.2 unit/µg of DNA) for [1] 5 min, [2] 20 min, [3] 40 min, [4] 60 min, [5] 90 min, and [6] 24 hr. Electrophoresis and Southern blot hybridization were as described in Materials and Methods. As probe the 32P-labeled PstI fragment of clone 2.32.1 was used (15). The size markers, indicated in Kb, are the HindIII fragments of lambda phage.

repeats present in the T. cruzi genome, nuclear DNA was submitted to partial digestion with BglII. Figure 4 shows the typical ladder pattern of bands in which the sizes are multiple of 2.5 Kb. Since the longest ladder containing the BglII repeats include DNA fragments larger than 25kb in length we think that it is likely that at least 10 copies of these repeats should be present in the T. cruzi genome.

Partial nucleotide sequence of T. cruzi hsp70 gen.

In order to locate the hsp70 gene within the 2.5 Kb BglII repetition unit we carried out the nucleotide sequence analysis of both ends of the repetition unit in plasmids p.Bg.2, p.2.SXβ4, p.2.SXα1, p.5.M8, p.5.P.2, p.2.M.4 and p.2.P.5 and in phages M.Bg.5 and M.Bg.8 (Fig. 2). Since the D. melanogaster hsp70 coding DNA sequence is 2.0 Kb long (23), we assumed that either the T. cruzi hsp70 gene is included in the repetition unit or each repeat contains two fragments of contiguous genes. The nucleotide sequence obtained is shown in figure 5. By comparation with the complete nucleotide sequence of the hsp70 genes of several organisms the 5′ end of the fragment analyzed has to be located upstream of the PstI site. Thus, we conclude that transcription, within the unit, goes in the PstI-BglII-SmaI direction. The 3′ terminal end of the coding sequence for the T. cruzi hsp70 gene is located 129 nucleotides downstream of the SmaI restriction site. It is clear, therefore, that the 2.5 kb BglII repetition unit does not associate with a single gene copy but

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AAG GCG ACG AAC GGC GAC ACG CAC CTG GGC GGC GAG GAC TTT GAC AAC CGT CTC GTT GCG
                                                    100
CAC TTC ACG GAC GAG TTC AAG CGC AAG AAC AAG GGC AAG GAC CTG AGC ACA AAC TTA AGG

GCC CTC CGC CGC CTC CGC ACC GCC TGC GAG CGC GCG AAG CGC ACG CTG TCG TCC GCG GCA
                        200
CAG GCG ACG ATT GAG ATC GAC GCG CTG TTC GAC AAC GTG GAC TTC CAA GCA ACC ATC ACT
                                                          PstI              300
CGC GCC CGC TTC GAG GAG CTC TGC GGC GAG CTC TTC CGA GGG ACT CTG CAG CCG GTG GAG

CGT GTG CTC CAG GAC GCC AAG ATG GAC AAG CGT GCC GTC CAC GAT GTG GTG CTC GTC GGC
                                    PvuII           400
GGC TCC ACC CGC ATT CCA AAG GTG ATG CAG CTG GTG TCG GAC TTT TTT CGT GGC AAG GAA

CTG AAG AAG AGC ATC CAA CCT GAT GAG GCT GTG GCG TAC GGT GCC GCC GTG CAG GCC TTC
                        500
ATC CTG ACG GGC GGC AAG AGC AAG CAG ACG GAG GGC CTG CTG CTG CTC GAC GTG ACG CCG
                                                                          600
CTG ACG CTT GGA ATC GAG ACG GCG GGT GGC GTC ATG ACG TCG CTG ATC AAG CGC AAC ACG
                              BglII
ACG ATT CCG ACC AAG AAA AGC CAG ATC TTC TCG ACG TAC GCG GAC AAC CAG CCG GGC GTG
                                                    700
CAC ATC CAG GTC TTT GAG GGA GAG CGT GCG ATG ACG AAG GAC TGC CAC CTG CTC GGC ACA

TTC GAG CTG TCC GGA ATC CCG CCG CCG CCG CGC GGT CTG CCC CAG ATT GAG GTG ACC TTT
                        800
GAC CTC GAC GCC AAC GGC ATC CTG AAC GTG TCC GCG GAG GAG AAG GGC ACC GGT AAG CGG
                                                                          900
AAC CAG ATT GTA CTC ACG AAC GAC AAG GGC CGC CTG AGC AGG GCA GAG ATT GAG AGG ATG
                                    1000
[ ~84 nt  ] TTT TCG ATG AAG AAC GCC GTG AAC GAC CCT AAC GTC GCT GGA AAG ATT GAG

GAG GCC GAC AAG AAG ACG ATT ACG AGT GCC GTG GAG GAG GCG CTC GAA TGG CTG AAC AAC
    1100
AAC CAG GAG GCC AGT AAG GAG GAG TAC GAG CAC CGC CAG AAG GAG CTG GAG AAC CTG TGC
                                                          1200
ACG CCC ATC ATG ACG AAT ATG TAC CAG GGC ATG GCC GGC GCG GGT ATG GCT GGC GGT ATG
                                                SmaI
CCT GGT GGA ATG TCT GGC GGT ATG CCT GGT GGA ATG CCC GGG GGC ATG CCT GGC GGT ATG
                                    1300
CCT GGC GGT ATG CCT GGC GGT ATG CCT GGT GGA ATG CCC GGT GGC ATG CCT GGC GGC GCG

AAC CCG TCG TCT TCG TCA GGA CCC GAA GTG GAG GAA GTT GAC TGA GAGCGCATCC
   XmnI 1400                                              *
CCTGAAGATG TTCTCATGGC GGCGTCTGTT CGCGAACGAA TACCGTTGGT TTTCTCCTTT GTAGAGCGTA
                                          1500
GTGTCTGCGA TCAAAACCCA GGCAGCCATC TACTATTTTT ATTATTGTTT TTTTTCCCTC TCCATTATTA
                                                                          1600
TTATTATTAT TATTGTTATT ATTATTGTTA TTATTATTAT TATTATTATT ATTGTTATTA TTATTGGTTA

CGGAGTTATT GGACTGCATG CGATGGCACT TGGCGTTGTA GGACACGGTA TAGTCTTGCA GAGTGTGCGC
                                    1700
CTCGGGGGCT TCCGATCTCC GTCCTCCTCG CTCC
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Fig. 5. Partial nucleotide sequence of the T. cruzi hsp70 gene. The nucleotides ordered in triplets represent the coding region of the gene. The nucleotides ordered in groups of ten are the intergenic region. The restriction recognition sites are also indicated. The gap of 84 nucleotides, indicated by [ ], shows the region of the gene for which the sequence has not been clearly determined. The opal termination codon is indicated by an *.

contains two fragments of two contiguous genes. The BglII repetition unit also contains the entire intergenic region, a fragment of which is shown in figure 5. Very strong similarities are observed between the intergenic region of T. cruzi and that of T. brucei mainly in the AT rich domain. When the nucleotide sequence was compared with the third 3' end of the hsp70 gene of several organisms, we find an 79% homology with the hsp70 gene of T. brucei and about 50% to the human and D. melanogaster hsp70 genes. We observed also that there is a bias in the choice of synonymous codons for particular amino acids and it ocurrs in many of the highly conserved proteins from trypanosomes (24).

We observed that, at the amino acid level (Fig. 6), the fragment of the T. cruzi hsp70 analyzed has 65 % homology to D. melanogaster (23), 67 % to human (25), 73 % to P. falciparum (6) and 88 % to T. brucei hsp70's (12). However, the percentage of homology in different regions along the protein is variable. For example, between amino acids 228-242 and 263-281 the homology is 100 % or near to 100 % for all the hsp70's while the sequence of amino acids at the C-terminal end is more divergent. In the C-terminal end of the protein there is an unusual sequence of twelve short repeats (see Fig. 6) of the tetramer GMXG structure, where X can be P (9/12), A (2/12) or S (1/12). While similar types of repeats of this general structure have been also found at the C-terminal end of both T. brucei (12) and P. falciparum hsp70's (6, 26), they are absent in the human and Drosophila hsp70's. However the number of these short repeats is larger in T.cruzi than in T.brucei.

DISCUSSION.

As it has been reported for several genes of the T. brucei genome (10-12), we have also found that the T. cruzi hsp70 coding region contains several copies of the genes organized as a tandem array in head-to-tail fashion. They are futhermore located in a single site in the genome. Since this unusual organization is a common feature of many other structural genes in parasites it has been suggested that it could reflex an underlined peculiar trancriptional mechanism (27).

The already known evolutionary conservation of the hsp70's among eucaryotic organisms is really striking when the hsp70 of T. brucei and T. cruzi are compared, since at the amino acid level the T. cruzi hsp70 is 88% homologous to the T. brucei hsp70. A similar, but lower, level of homology is also maintained at the nucleotide level (79%). This level of nucleotide conservation is higher than the expected from the degeneracy of the code, suggesting that there should be a mechanism which restrict the appearance of

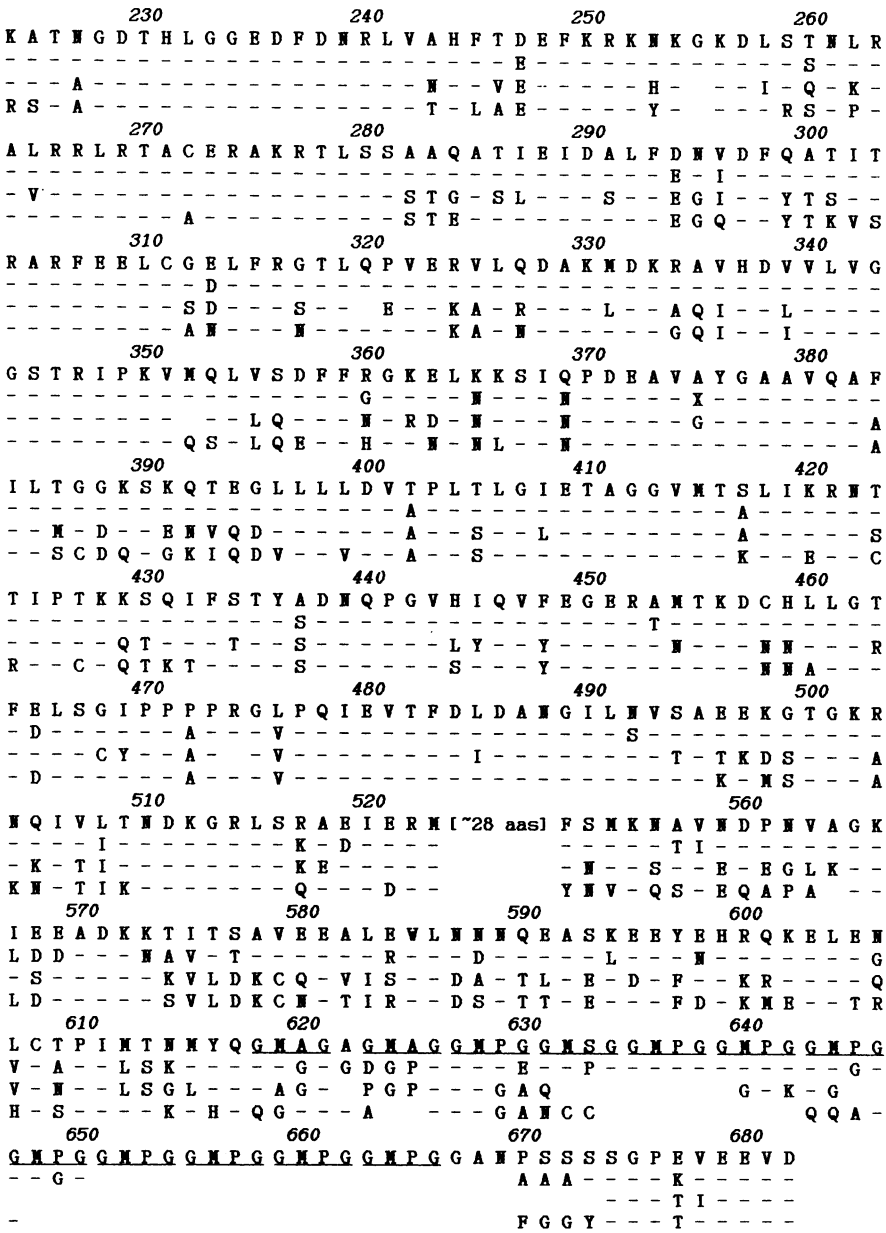

Fig. 6. Alignment of protein sequences and homologies among T. cruzi hsp70 (line 1), T. brucei hsp70 (line 2; 12), human hsp70 (line 3; 24) and D. melanogaster hsp70 (line 4, 22). The numbering is referred to the T. brucei hsp70. Homology is indicated by hyphens. Gaps were introduced to maximize homology. The terminal repeats are overlined.

variations in the nucleotide sequence. Hunt and Morimoto (25) has suggested that a specific function may exist in the nucleotide sequence itself. It may be also that the DNA structure specified by the nucleotide sequence at a particular site of the hsp70 gene may play an important informational role in the function of the protein or in the heat shock response..

In spite of the high level of homology at the amino acid and nucleotide level between the hsp70's of T. brucei and T. cruzi several restriction site variations were observed. While in T. brucei the repetition units are defined by HindIII (12), with an internal EcoRI site, the HindIII or EcoRI restriction sites are completely absent from the repetition units of the hsp70 genes of T. cruzi. In contrast, the BglII or SmaI restriction sites, which defined repetition units in the hsp70 gene of T. cruzi are absent from the repetition units of T. brucei. The similar tandem array organization of the hsp70 in both species and their strong homology at the nucleotide level seem to postulate the existance of a common progenitor, but the existence of identical variations in restriction sites in all the repetition units indicates that in the course of evolution and divergence novel mutations altered the existing restriction sites and created new ones. Since it is very unlikely that identical mutations, generating the specific restriction sites, appeared simultaneously in each one of the repetition units of the multigene family, we favor the idea that the progenitor species had only one copy of the gene and that in the course of speciation the genes had a different evolutionary life. The presence of identical restriction sites in all the repetition units is then best explained by amplification of the original copy. The possibility, however, that the tanden array existed in the progenitor species and that the restriction sites generated in one gene copy spreaded to the other copies by a homogeneization process cannot be excluded (28). In either case the existance of identical restriction sites in all the members of the multigene family should be explained by a homogenization process. It should be noticed that the the disapperance of one of the HindIII rectriction sites and the generation of the BglII and SmaI sites in T. cruzi do not imply change in the coded amino acids.

The repeats of the tretamer GMXG aminoacid structure (9 are GMPG) found in the C-terminal region of the T. cruzi hsp70 gene appear to be a common feature of the trypanosome and plasmodium heat shock proteins and of many other antigens of P. falciparum (29, 30). It is possible that these repeats play important immunological roles in the events occurring during the parasite-host interaction. In fact, the T. cruzi hsp83 (31), P. falciparum

hsp70 (6, 26), and Schistosoma mansoni hsp70 (32) have been recognized immunologically by antisera from human patients. Given the homology that exists among the heat shock proteins from different species it should be easy to speculate about the autoinmune consequences of the Chagas' disease (33). On the other hand, since the tetramer repeats are localized in the less conserved region of the hsp70, it is probable that the protein has the functional significance of the hsp70's of other organisms (34) and that the repeats play an additional role in relation to the immune system of the host.

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