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IDENTIFICATION OF THE 85 KDA SURFACE ANTIGEN GENE OF TRYPANOSOMA CRUZI AS A MEMBER OF A MULTIGENE FAMILY

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Introduction: The parasitic protozoan Trypanosoma cruzi is the causative agent of Chagas disease, a major health problem throughout Central and South America. The parasite infects human and other vertebrate hosts by direct penetration of host cells by trypomastigotes transmitted by the insect vector. The surface antigens of the trypomastigotes have been implicated in the process of cell penetration. In particular antibody neutralization experiments show that an 85 kDa surface glycoprotein is necessary for efficient interiorization of bloodstream trypomastigotes in mammalian cells. We have recently described the molecular cloning of a genomic DNA fragment that encodes antigenic determinants present in an 85 kDa surface antigen and have confirmed previous studies that show this antigen is specific to the trypomastigote stage of the parasite. Nucleotide sequence analysis of the cloned DNA infers the presence of a nonapeptide unit that is tandemly repeated five times. Here we show that the 85 kDa gene is a member of a complex multigene family of which only three members contain the repeated sequence. Only one member appears to be expressed and it is located on a telomere. Also, the nonapeptide unit is recognized by serum antibodies of mice infected with T. cruzi.

Results and Discussion:

Genomic Organization of the 85 kDa Surface Antigen Gene:

In order to determine the size of genomic DNA restriction fragments which flank the DNA sequences present in the cloned 85 kDa sequence, designated Tcg1, a southern blot of EcoRI restricted trypomastigote genomic DNA was hybridized with Tcg1 insert DNA. Numerous EcoRI fragments hybridized (ie. 20-25), indicating that sequences within the 500 bp clone are found in multiple copies within the genome. To better define which sequences within Tcg1 are multicopy, three separate probes were constructed from the Tcg1 insert. Probe one is a 110 bp EcoRI/Sau3A fragment containing the 5' end of Tcg1. Probe 2 is a 27 base synthetic oligonucleotide representing one unit of the tandem repeat. Probe 3 is a 180 bp HhaI/EcoRI fragment containing the 3' end of Tcg1. Hybridization of these probes to a Southern blot of genomic DNA restricted with EcoRI showed that probes 1 and 3 hybridized to 10-20 different size restriction fragments while the 27 nucleotide probe detected only three restriction fragments.

Cloning of Genomic DNA Fragments Containing the 27 Nucleotide Repeat.

The above results indicate that mRNA detected by hybridization with the 500 bp insert of Tcg1 may be transcribed

by one or more members of this multigene family. To confirm that the repeat sequence is present in mRNA, the 27 nucleotide probe was shown to hybridize with a 3.8 kb RNA in both total cellular and poly A+ RNA from trypomastigotes. No hybridization was observed with total cellular or poly A+ RNA from epimastigotes. These results are identical to those observed with the 500 bp insert in Tcg1 and clearly show that at least one of the three sites in the genome which contain the 27 bp repeat is transcriptionally active. In order to determine which of these sites are transcriptionally active we attempted to clone and characterize a representative of each.

Three separate genomic libraries were constructed in the cloning vector EMBL4. Approximately 400,000 independent recombinant lambda plaques were screened with the 27 nucleotide probe and 59 positive plaques were identified. A 4.8 kbp EcoRI fragment was observed to hybridize with the 27 nucleotide probe in 48 of the DNAs, and a 1.7 kbp EcoRI fragment hybridized in the remaining 11 DNAs. Surprisingly, no phage DNAs were observed to contain a 5.4 kbp fragment homologous to the 27 nucleotide probe.

For the 4.8 kbp and 1.7 kbp fragments the sequences homologous to the 27 bp repeat were localized to EcoRI/SmaI fragments of length 490 bp and 325 bp, respectively. The nucleotide sequence of these fragments clearly showed that Tcg1 is not present in either fragment. Tcg1 must, therefore, reside elsewhere within the genomic DNA, possibly within the 5.4 kbp

fragment.

The results above suggest that the 5.4 kbp fragment may contain the sequences found in Tcg1. If this is the case restricting genomic DNA with enzymes that cut adjacent to the repeat region should yield fragments whose sizes can be predicted from the restriction maps of Tcg1, the 4.8 and the 1.7 kbp EcoRI fragments. Restriction analysis of the three fragments revealed that each contains a HhaI site 3' of the repeat region. Digestion of the subcloned Tcg1, 4.8 and 1.7 kbp fragments with EcoRI and HhaI yielded fragments of 320, 1500, and 500 bp respectively, that hybridized with the 27 nucleotide probe. When genomic DNA was digested with EcoRI and HhaI three fragments of length 1500, 500 and 320 bp were observed to hybridize with the 27 nucleotide probe. As the 1500 and 500 bp fragments can be identified as having originated from the 4.8 and 1.7 kbp fragments respectively, the 320 bp fragment found in Tcg1 is putatively present in the 5.4 kbp fragment.

Confirmation that the 5.4 kbp fragment contains Tcg1 could be obtained by cloning, restriction mapping and sequencing the 5.4 kbp fragment. Therefore an effort was made to directly clone the 5.4 kb fragment as an EcoRI insert into the EcoRI site of lambda gt10. Total trypomastigote genomic DNA was digested with EcoRI, size fractionated by agarose gel electrophoresis and fragments of size 4.5-6.0 kbp were excised and cloned into lambda gt10. Approximately 200,000 independent clones were screened

with the 27 nucleotide probe and 69 plaques were identified and rescreened positive. In each of the 69 phage DNA hybridization with the 27 nucleotide probe was observed only to a single 1.5 kb EcoRI/HhaI fragment identical in size to the EcoRI/HhaI fragment found in the cloned 4.8 kbp fragment, and no phage were observed to contain a 5.4 kbp insert.

Nuclease Bal 31 Sensitivity

The inability of the 5.4 kbp fragment to be cloned as an EcoRI insert led us to consider the possibility that one end of the fragment was not an EcoRI termini but is found in the telomeric region of the chromosome. To test this possibility, aliquots of high molecular weight trypomastigote genomic DNA were digested for increasing times with nuclease Bal 31. The digested DNAs were then restricted with EcoRI, electrophoresed through agarose, blotted to nitrocellulose, and probed with the 27 nucleotide repeat. A progressive decrease in the size of the 5.4 kbp fragment was observed with increasing digestion with Bal 31. Neither the 4.8 kbp nor the 1.7 kbp EcoRI fragment changed significantly in size over the time course of the experiment. This result strongly implies that in the trypomastigote the 5.4 kbp fragment is telomeric.

DNase I Sensitivity

Sensitivity to DNase I is a characteristic of actively

transcribed genes. As a first step in determining which of the EcoRI fragments that hybridize with the 27 nucleotide probe are expressed, the relative DNase 1 sensitivity of the 3 genomic EcoRI fragments was assayed. Aliquots of trypomastigote nuclei were reacted with increasing concentrations of DNase 1, and DNA was isolated from each sample. After digestion with EcoRI nuclease and agarose gel electrophoresis, the DNA was Southern blotted and probed with the 27 base oligonucleotide. At progressively higher concentrations of DNase I, the 5.4 kbp fragment was found to be preferentially digested. The DNA present in each hybridizing band was quantitated by densitometric measurement and the relative amounts of the three DNA bands were plotted as a function of the different DNase I concentrations. The results showed that while the amount of 4.8 kbp and 1.7 kbp EcoRI fragments changes little with respect to each other, the amount of 5.4 kbp fragment decreases markedly relative to each of these fragments. The hypersensitivity of the 5.4 kbp fragment to digestion by DNase 1 argues that it, and not the 4.8 or 1.7 kbp fragments, contains sequences which are being actively transcribed.

Nucleotide Sequence of cDNA Having Homology to the 27 bp Repeat

The results of the DNase 1 sensitivity experiment provide indirect evidence that only the 5.4 kbp fragment is transcribed. More direct evidence could be obtained by determining the nucleotide sequence of cDNA inserts selected by hybridization with the 27 bp repeat unit. Since the nucleotide sequences

adjacent to the repeat in the three genomic clones clearly differ, comparing the cDNA sequence with that of the genomic clones should identify which genomic sequence is being transcribed.

Two separate cDNA libraries were constructed from trypomastigote poly A+ RNA, the first was synthesized with oligo dT as the primer, the second used small oligonucleotides of random sequence . After the addition of EcoRI linkers, the libraries were cloned into the EcoRI site of lambda gt10. Approximately 100,000 recombinant plaques from each library were screened with the 27 nucleotide probe and 11 plaques, 8 from the random primed library and 3 from the oligo dT primed library, were isolated and plaque purified. DNA was isolated from each phage and restricted with EcoRI. The cDNA inserts were sized on an acrylamide gel, subcloned into Bluescript and sequenced. All of the cDNA inserts isolated from the random primed library, as well as the largest clone from the dT primed library, matched the sequence of Tcg1 through the ninth base of the sixth repeat unit, with two notable exceptions. Each cDNA lacked two adjacent bases in the sixth repeat, a adenine and a thymidine at positions 269 and 270 respectively, found in Tcg1. One other consistent difference between the sequence of Tcg1 and that of the cDNA's, as well as that of the 490 bp EcoRI/SmaI, is the presence of a cytosine at position seven in Tcg1. This cytosine is lacking in all other fragments sequenced and is believed to be an artifact of the EcoRI linkers used in the construction of the genomic

library from which Tcg1 was isolated.

None of the nucleotide sequences of the cDNA inserts match the nucleotide sequence of the 1.7 kbp EcoRI fragment. The nucleotide sequence of Tcg1 and the 490 bp EcoRI/SmaI fragment are identical through the third repeat unit after which no homology is observed. Thus, only cDNA inserts whose sequence extends 3' downstream of the third repeat unit can be used to distinguish between Tcg1 or the 4.8 kbp fragment as the putative transcriptional template. The nucleotide sequence of ten cDNAs extends beyond the third repeat and in each case matches the sequences found in Tcg1 but not that of the 4.8 kbp EcoRI fragment. Although this result does not rule out a low level of transcription of either the 1.7 kbp or the 4.8 kbp EcoRI fragment, it does suggest that the most abundant transcript originates from a site other than of the 1.7 kbp or 4.8 kbp fragments. Based upon the results of the restriction enzyme mapping, and the Bal 31 and DNase I digestion experiments, we suggest that the transcription site for these cDNAs is the 5.4 kbp telomeric fragment.

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TDR-4 CLONING OF GENES FOR ANTIGENICALLY RELEVANT PROTEINS OF TRYPANOSOMA CRUZI

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INTRODUCTION

Trypanosoma cruzi has a complex antigenic make up. Several (glyco)proteins have been identified on their surface membrane (1-2) some of which are specific of a given stage of the parasite life-cycle (3-5). In addition, T. cruzi isolates differ in several biological and biochemical properties (6) including antigenic composition (7-8). Analysis of parasite antigens is essential not only to work out practical problems like diagnoses (9-10) and eventually, vaccination (11-12), but also to better understand the immunopathology of Chagas' disease. Since each of the several T. cruzi antigens is a minor component of the parasite, recombinant DNA technology may contribute to identify and obtain in sufficient amounts, relevant parasite proteins. In order to be able to answer some of these questions, we isolated and characterized those parasite genes whose products are detected by sera from Chagasic patients (13).

In this paper we summarize previous observations and describe new results on the structural organization of the genes and the antigenic relevance of the fusion proteins.

RESULTS

Isolation of T. cruzi antigen genes and identification of the corresponding parasite proteins.

Screening of a T. cruzi genomic DNA library made in gt11 lambda vector was performed with serum from one chronic Chagasic patient (13). Out of 300,000 recombinant phages, 53 positive clones were obtained. Thirty-two of them were analyzed. Nine different groups were defined according to the sequence homologies of the recombinant clone inserts and to the reactivity of the cloned gene products with selected antibodies (14). That is, clones included in one group have cross-hybridizing inserts and their products react with the antibodies selected from a serum with one of them and, consequently, have similar or identical epitopes. Clones included in each group are shown in Table I. Some groups have a single clone while in others, up to 8 homologous clones were detected. These results may be related, in part, to the particular structure of some of the clone inserts and to the size of the corresponding gene (see below).

To identify the parasite proteins that have antigenic determinants homologous to each of the cloned gene products, we used the antibody selection method (14) followed by reaction of these antibodies with Western blots of protein extracts from axenic culture-epimastigotes and cell-culture trypomastigotes of the RA T. cruzi strain (15). These results are summarized in Table I. Most proteins detected were of high relative molecular weight, the smallest ones being those of clones 2, 13, 36 and 54 (85-90 kDa). Interestingly, several groups have identified 85-90 kDa (glyco)proteins on the surface of T. cruzi related either to parasite internalization (16) or to the binding of fibronectin (17), and also

Table I

Clone N°	Homologous clones	Parasite protein size (kDa)	E or T
1	3-6-33-42-43-44-53	>205	E-T
2	16-20-38	85	T
7	9-12-41	205/200/190/175/165	T
10	45-47	150/140/125	E-T
13	-	85	T
26	15-17	>205	T
30	4-46	205/195/160	E
36	21-27-40-50	85	E-T
54	-	90	T

T. cruzi proteins detected with each recombinant clone. The number of clones per group and size of the parasite protein identified with each recombinant clone are indicated. The column E or T indicates whether proteins have been identified in epimastigotes (E), trypomastigotes (T) or both stages (E-T).

a heat-shock protein (18). Since our four clones whose products are homologous to parasite proteins of 85-90 kDa do not share sequence homologies (unpublished results) with those of the two genes sequenced (18-19) we conclude that there are at least six antigenic proteins within this size range in T. cruzi. The putative mRNA detected with each cloned DNA was described previously (13).

Preliminary immunofluorescence studies (unpublished results) with living parasites and selected antibodies showed that the parasite proteins homologous to the products of clones 1, 2, 10, 13 and 54 are located on the surface. It remains to be studied whether the proteins homologous to the products of the other clones, which are also antigenically active during an infection (see below) are proteins secreted by the parasite.

Genomic organization and structure of the isolated genes

Nuclear DNA of one T. cruzi strain (CA1-65, ref 15) was digested with Bam HI, EcoRI (Fig. 1), Hind III, PstI, HpaII and HaeIII (not shown) and hybridized with ^{32}P-labelled clone inserts. Most clones detected bands compatible with a single gene but for two of them, clones 7 and 13. In the case of clone 7, several bands are detected in PstI, HpaII and HaeIII digest, and interestingly, the overall pattern is the same with the three enzymes but for the differences in the sizes of the bands (Fig. 1B). Clone 13, on the other hand, detected a great number of bands (see also PstI, HpaII and HaeIII digests, Fig. 1C) even under high stringent hybridization conditions. Since a single RNA species was observed with this clone (13), it is possible that the insert of clone 13 has a repetitive sequence which is scattered throughout the T. cruzi genome.

Partial sequencing of the inserts of the nine clones showed that seven of them have an internal tandemly repeated structure. The size of the repeats

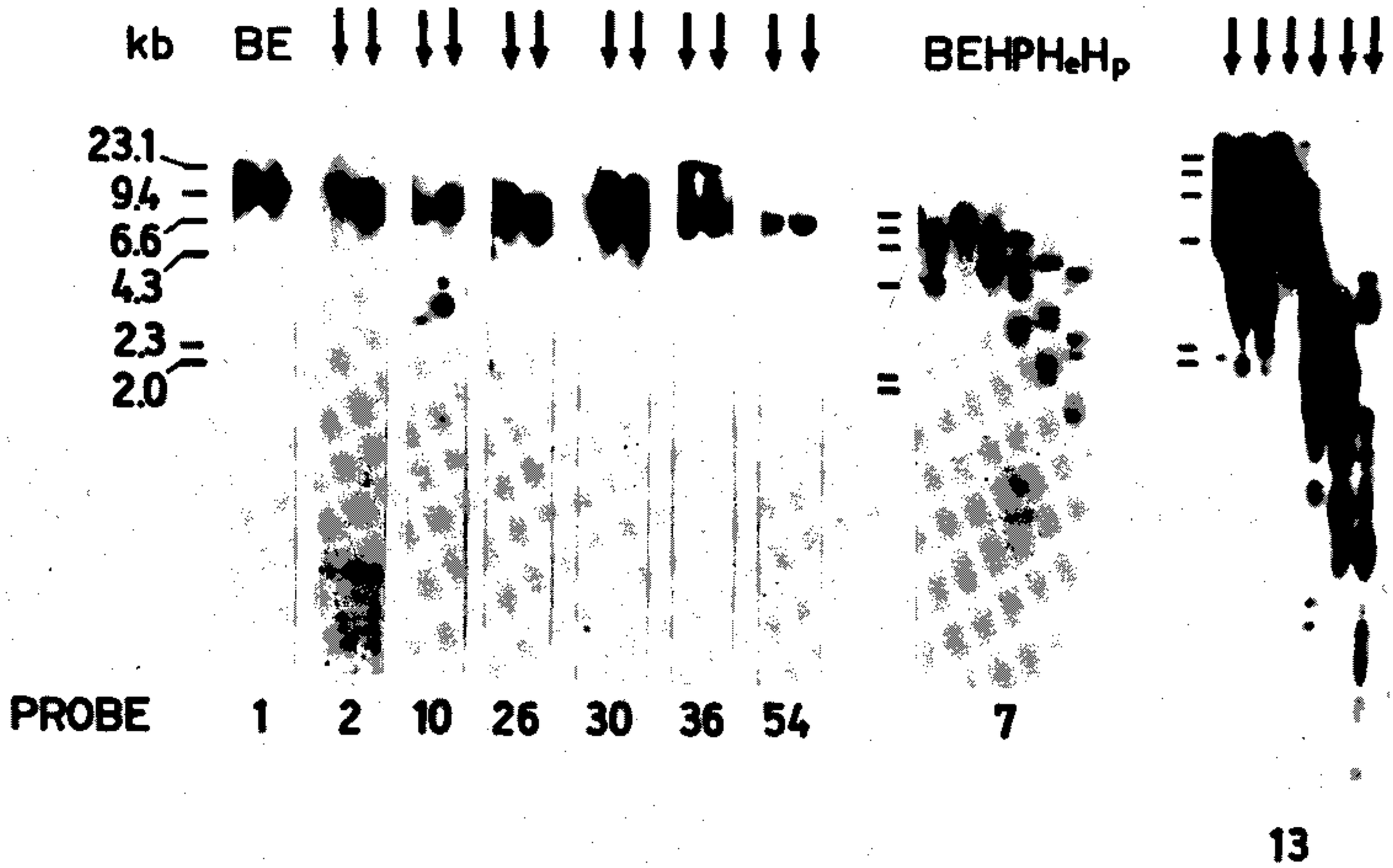

Figure 1.

Genomic organization of the isolated recombinant clones. Nuclear DNA from epimastigotes (CA1 strain) was digested with the indicated restriction endonuclease, run on 0.8% agarose gel, blotted onto nitrocellulose filters and hybridized with the indicated clone inserts. B BamI, E EcoRI, H HindIII, P PstI, He HaeIII and Hp HpaII.

Table II

Clone Nº	Repeat size (aa)	Nº of repeats sequenced
1	68	7
2	12	18
10	8	19
13	5	31
15 (26)	14	3
30	14	13
36	38	6

Structure of the cloned DNA sequences having internal repeated units. The number of aminoacids (aa) per repeat deduced from the nucleotide sequence and the number of repeats sequenced per clone are shown.

together with the number of units sequenced are shown in Table II. The repeats within each clone are highly conserved and ranged in size from 15 to 204 base pairs. No sequence homology between repeats of different clones was observed. They also differ from those present in the sporozoite and merozoite stages of plasmodium species (20-21) as well as from those T. brucei (22) and T. cruzi (18-19) genes reported. At least in three (1-30-36) of the six clones, the repeated region of the corresponding protein is antigenically active since their inserts are entirely made up of repeated units.

Antigenic relevance of the cloned gene products

In order to know the antigenic relevance of the cloned genes, each gene product was reacted with sera from Chagasic patients obtained in Argentina (23 sera), Brazil (49 sera) and Chile (12 sera) (see ref. 13 for the description of the method used).

Seventy of the 84 sera tested reacted with at least one of the nine cloned gene products (Table III). Most of the sera reacted with more than one clone product and, in this context, it is interesting to note that the products from clones 1, 2, 13 and 30 are enough to detect antibodies in the 70 sera tested but for one from Argentina and one from Brazil. Sera from Argentina and Chile detected in a higher proportion the products from clones 1, 2 and 30, while sera from Brazil detected mainly those from clones 1, 2, 13 and 36 (Table IV). Whether these variations are due to differences in T. cruzi strains and/or are of importance from the immunopathological point of view of Chagas' disease is still a matter of speculation.

Fourteen sera failed to react with any of the nine clone gene products (Table III). Some of these sera are now being used to screen the genomic DNA

Table III

Disease and control sera	Country of origin	Number of sera tested	Number of sera positive with one or more cloned gene product	Percentage
Chagas	Brazil	49	41	84
Chagas	Argentina	23	20	87
Chagas	Chile	12	9	75
Total Chagas		84	70	83
Leishmaniasis (Kala-Azar)	Brazil	21	0	0
Toxoplasmosis	Argentina	9	0	0
Controls	Brazil	14	0	0
Controls	Argentina	23	2	9

Reactivity of sera from Chagas, Leishmaniasis, Toxoplasmosis and controls with cloned gene products.

library in order to know whether they recognize a different set of parasite antigens.

Table IV

Country	No. of sera tested	Number of sera positive with each clone product								
		1	2	7	10	13	26	30	36	54
Argentina	20	16(80%)	15(75%)	6(30%)	5(25%)	9(45%)	9(45%)	18(90%)	7(35%)	3(15%)
Brazil	41	24(59%)	25(61%)	1(2%)	2(5%)	17(41%)	3(7%)	12(29%)	16(39%)	2(5%)
Chile	9	6(67%)	7(78%)	0	0	1(11%)	0	4(44%)	1(11%)	0
Total	70	46(66%)	47(67%)	7(10%)	7(10%)	27(39%)	12(17%)	34(49%)	24(34%)	5(7%)

Reactivity of sera from chronic Chagasic patients with individual products from recombinant clones. The number of sera (per country) reacting with each recombinant clone product are indicated as well as the total number of positive sera tested (see text).

Cloned genes products for the diagnosis of Chagas' disease

Current serological methods make use of whole parasites or parasite fractions (9). These heterogeneous populations of antigens give rise to false positives with control sera as well as with visceral Leishmaniasis (23). If individual proteins are to be used for diagnosis, they should not unspecifically react with control sera and with sera from persons affected with other parasitic diseases. In order to test this, we reacted the nine protein products with 23 control sera from Argentina, 14 control sera from Brazil, 9 sera from Argentina cases of Toxoplasmosis and 21 cases of visceral Leishmaniasis from Brazil (Table

III). All the above sera were negative with the nine proteins, except for two control sera from Argentina that reacted with some of the cloned gene products. Since these two sera also reacted with whole T. cruzi parasites, we consider likely that they are indeed derived from infected persons and were either not well diagnosed or mixed up with other control sera.

CONCLUSIONS AND PROSPECTS

Nine cloned genes for T. cruzi antigens have been identified. They code for parasite antigens ranging in size from 85 to larger than 205 kDa. Their antigenic relevance is shown by the fact that some of them reacted with most sera from Chagasic patients obtained in different geographical regions (Table III and IV). Since these fusion proteins do not react with control sera nor with sera from cases of Leishmaniasis and Toxoplasmosis, we consider some of them good candidates to be used for the serological diagnosis of Chagas' disease. However, it is still necessary to isolate other gene products in order to be able to detect sera from all Chagasic patients (see Table III).

The results in Table IV show that antibodies to some of the cloned gene products (No. 1, 2, 13, 30 and 36) are more frequently represented among sera from Chagasic patients. Interestingly, antibodies to some proteins (No. 26 and 30) are frequently observed in sera obtained in Argentina but not in those sera from Brazil. Further work is necessary to know whether the above differences can be related to the variations in the antigenic make-up of parasites from different geographical regions and/or to the clinical forms of the disease.

Partial sequencing of the nine cloned inserts showed that seven of them have an internal repeated structure, the size of the repeat being from 5 to 68 aminoacids in the corresponding protein. In the case of malaria, proteins with a

repeated structure are essential for parasite survival since they determine the binding domain that interacts with the host cell (21,24). Antibodies against the repeated sequence seem to be effective in experimental vaccination trials (20). Given the fact that in at least three of the seven T. cruzi cloned gene products the repeated unit is the region of the molecule antigenically active (see above), further work on the possibilities of these antigens as vaccines is desirable.

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REFERENCES

1. Andrews, N.W., Katzin, A.M. & Colli, W. (1984) Mapping of surface proteins and antigens of Trypanosoma cruzi by two-dimensional electrophoresis: a correlation with the cell invasion capacity. Eur. J. Biochem. 140, 599-604.
2. Araujo, F.G. & Remington, J.S. (1981) Characterization of stages and strains of Trypanosoma cruzi by analysis of cell membrane components. J. Immunol. 127, 855-859.
3. Nogueira, N., Unkeless, J. & Conn, Z. (1982) Specific glycoprotein antigens on the surface of insect and mammalian stages of Trypanosoma cruzi. Proc.

Natl. Acad. Sci. USA 79, 1259-1263.

4. Teixeira, M.M.G. & Yoshida, N. (1986) Stage-specific surface antigens of metacyclic trypomastigotes of Trypanosoma cruzi identified by monoclonal antibodies. Mol. Biochem. Parasitol. 18, 271-282.
5. Wrightsman, R., Leon, W. & Manning, J. (1986) Variation in antigenic determinants specific to the infective stage of Trypanosoma cruzi. Infect. Immun. 53, 235-239.
6. Brener, Z. (1985) General Review on Trypanosoma cruzi classification and taxonomy. Rev. Soc. Bras. Med. Trop. 18 (Supl.) 1-8.
7. Gonzalez, N.S., Sanchez, D.O., Frasch, A.C.C. and Algranati, I.D. (1984) Surface proteins in different isolates of Trypanosoma cruzi epimastigotes. Mol. Cell. Biochem. 63, 157-164.
8. Kirchhoff, L.V., Engel, J.C., Dvorak, J.A. & Sher, A. (1984) Strains and clones of Trypanosoma cruzi differ in their expression of a surface antigen identified by a monoclonal antibody. Mol. Biochem. Parasitol. 11, 81-89.
9. Krettli, A.U. (1982) Antibodies to Trypanosoma cruzi in experimental and human infections. Afr. J. Clin. Exp. Immunol. 3, 327-345.
10. Dragon, E.A., Brothers, V.M., Wrightsman, R.A. & Manning, J. (1985) A Mr 90.000 surface polypeptide of Trypanosoma cruzi as a candidate for a Chagas' disease diagnostic antigen. Mol. Biochem. Parasitol. 16, 213-229.
11. Brener, Z. (1986) Why vaccines do not work in Chagas Disease. Parasitol. Today 2, 196-197.
12. Ruiz, A.M., Esteva, M., Riarte, A., Subias, E. & Segura, E.L. (1986) Immunoprotection of mice against Trypanosoma cruzi with lyophilzed flagellar fraction of the parasite plus adjuvant. Immunol. Lett. 12, 1-4.
13. Ibanez, C., Affranchino, J.L. & Frasch, A.C.C. (1987) Antigenic determinants

of Trypanosoma cruzi defined by cloning of parasite DNA. Mol. Biochem. Parasitol. (in press).

14. Saint, R.B., Beall, J.A., Grumont, R.J., Mitchell, G.F. & Garcia, E.G. (1986) Expression of Schistosoma japonicum antigens in Escherichia coli. Mol. Biochem. Parasitol. 18, 333-341.

15. Gonzalez Capa, S.M., Katzin, A.M., Anasco, N. & Lajmanovich, S. (1981) Comparative studies on infectivity and surface carbohydrates of several strains of Trypanosoma cruzi. Medicina (Buenos Aires) 41, 549-555.

16. Zingales, B., Katzin, A.M., Arruda, M.V. & Colli, W. (1985) Correlation of tunicamycin-sensitive surface glycoproteins from Trypanosoma cruzi with parasite interiorization into mammalian cells. Mol. Biochem. Parasitol. 16, 21-34.

17. Ali Ouaissi, M., Cornette, J. & Capron, A. (1986) Identification and isolation of Trypanosoma cruzi trypomastigote cell surface protein with properties expected of a fibronectin receptor. Mol. Biochem. Parasitol. 19, 201-211.

18. Dragon, E.A., Sias, S.R., Kato, E.A. & Gabe, J.D. (1987) The genome of Trypanosoma cruzi contains a constitutively expressed, tandemly arranged multicopy gene homologous to a major heat shock protein. Mol. Cell. Biol. 7, 1271-1275.

19. Peterson, D.S., Wrightsman, R.A. & Manning, J.E. (1986) Cloning of a major surface-antigen gene of Trypanosoma cruzi and identification of a nonapeptide repeat. Nature 322, 566-568.

20. Nussenzweig, V. & Nussenzweig, R.S. (1986) Development of a sporozoite malaria vaccine. Am. J. Trop. Med. Hyg. 35, 678-688.

21. Kochan, J., Perkins, M. & Ravetch, J.U. (1986) A tandemly repeated sequence

determines the binding domain for an erythrocyte receptor binding protein in P. falciparum. Cell 44, 689-696.

22. Roditi, I., Carrington, M. & Turner, M. (1987) Expression of a polypeptide containing a dipeptide repeat is confined to the insect stage of Trypanosoma brucei. Nature 325, 272-274.

23. Kirchhoff, L.V., Gam, A.A., Gusmao, R.D'A, Goldsmith, R.S., Rezende, J.M. & Rassi, A. (1987) Increased specificity of serodiagnosis of Chagas' disease by detection of antibody to the 72- and 90-kilodalton glycoprotein of Trypanosoma cruzi. J. Infect. Dis. 155, 561-564.

24. Hollingdale, M.R., Nardin, E.H., Tharavanij, S., Schwartz, A.L. and Nussenzweig, R.S. (1984) Inhibition of entry of Plasmodium falciparum and P. vivax sporozoites into cultured cells: an in vitro assay of protective antibodies. J. Immunol. 132, 909-913.

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