The EMBO Journal vol.8 no.12 pp.3911-3916, 1989

The 85-kd surface antigen gene of *Trypanosoma cruzi* is telomeric and a member of a multigene family

David S.Peterson[1], David L.Fouts and Jerry E.Manning

Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717,USA

[1]Present address: National Institute of Allergy and Infectious Disease, Building 5, Bethesda, MD 20892, USA

Communicated by H.Eisen

In previous studies we identified a 500 bp DNA fragment from the genome of *Trypanosoma cruzi* which encoded epitopes present in an 85 kd trypomastigote-specific surface antigen. A unique feature of this DNA insert was the presence of a 27 bp tandem repeat unit within the putative coding region. The findings presented here show that the gene which encodes this particular surface protein is a member of a multigene family, and that the 27 bp repeat unit defines a subset of this family. Only four separate members of the family contain sequences homologous to the 27 bp repeat unit. Of these, three have been cloned and shown by direct nucleotide sequence analysis not to contain the original 500 bp fragment. By restriction enzyme analysis, the 500 bp fragment is inferred to be present in a 5.4 kb *Eco*RI genomic DNA fragment that is refractory to isolation by standard cloning procedures. Preferential sensitivity of this fragment to digestion with *Bal*31 nuclease indicates that it is likely to be telomeric, thus explaining the inability to obtain it in several different recombinant DNA libraries. In order to determine which of the four members were transcribed, 26 cDNA recombinants having sequence homology with the 27 bp repeat were examined. Restriction enzyme maps and nucleotide sequence analysis of these cDNAs indicate that transcription occurs almost exclusively from the telomeric member of the family.

Key words: multigene family/surface antigen/telomere/*Trypanosoma cruzi*

Introduction

Trypanosoma cruzi is a parasitic protozoan which is the causative agent of Chagas' disease, a serious health hazard in most Central and South American countries (Brener, 1973). The life cycle of this parasite is complex and has multiple developmental stages in both the insect vector and the vertebrate host. In the insect vector the parasite replicates in the midgut as a non-infective epimastigote but transforms in the hindgut to an infective form, the metacyclic trypomastigote.

Morphologically different forms of the parasite are also found in the vertebrate host: the infective trypomastigote which is incapable of cellular division circulates in the bloodstream and following penetration of the host cell transforms into the replicative amastigote form. Since the circulating trypomastigote is exposed to the effector mechanisms of the host immune response, considerable attention has been focused on the identification of the surface proteins of this stage as prospective immunogens against infection (Snary and Hudson, 1979; Araujo and Remington, 1981; Nogueira *et al.*, 1981; Zingales *et al.*, 1982; Lanar and Manning, 1984; Beard *et al.*, 1985; Peterson *et al.*, 1986; Wrightsman *et al.*, 1986; Andrews *et al.*, 1988). In part, the potential of these proteins as vaccine candidates rests on the belief that the surface proteins of *T.cruzi* do not undergo antigenic variation of the type observed by the variant surface glycoproteins (VSGs) of the African trypanosomes. Standard serological approaches support this viewpoint (Snary, 1980); however, the question of antigenic variation has not been approached using probes that are specific for individual surface antigens.

We previously reported the cloning of a 500 bp genomic DNA fragment, Tcg-1, which encoded epitopes present in an 85 kd surface antigen of *T.cruzi* (Peterson *et al.*, 1986). Both the surface antigen and its mRNA were shown to be specific to the trypomastigote stage of the parasite. Nucleotide sequence analysis revealed the presence of a

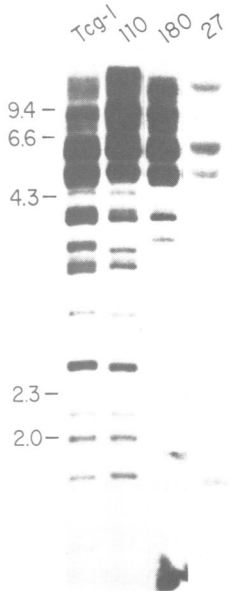

Fig. 1. Southern blot of *Eco*RI restricted trypomastigote nuclear DNA. For each lane 5 μg of trypomastigote nuclear DNA was digested with *Eco*RI, electrophoresed on an agarose gel and blotted onto nitrocellulose. Individual lanes were hybridized in 6 × SSC, 1 × Denhardt's solution at 68°C for 24 h with either the ^{32}P-labeled 500 bp insert of Tcg-1, the 110 bp *Eco*RI–*Sau*3A fragment from the 5′ end of the insert, the 180 bp *Hha*I–*Eco*RI fragment from the 3′ end of the insert, or the 27 nt synthetic oligomer respectively. Filters were washed in 0.1 × SSC, 0.1% SDS for 4 × 30 min at 52°C followed by two 30 min washes in 0.1 × SSC at 52°C (Fouts *et al.*, 1981).

27 bp tandem repeat unit within the coding region of the gene. Here we report that further characterization of this gene reveals it to be a member of a multigene family and that the 27 bp repeat unit defines a specific subset of this family. This gene family also shares several structural features in common with the VSG gene family of the African trypanosomes, suggesting the possibility that *T.cruzi* may have retained or acquired the mechanisms necessary for antigenic variation.

Results

Identification of the 85 kd gene as a member of a repetitive sequence family

In an initial attempt to clone the 85 kd gene, the size of the *Eco*RI restriction fragment(s) which contained the Tcg-1 sequence was determined by hybridization of a Southern blot of *Eco*RI restricted trypomastigote genomic DNA with ^{32}P-labeled Tcg-1 insert DNA. As shown in Figure 1 (lane 1), numerous *Eco*RI fragments hybridized with the Tcg-1 probe, indicating that sequences within the 500 bp insert are found in multiple copies within the genome. To define better which sequences within Tcg-1 were multicopy, three separate probes were constructed from the Tcg-1 insert. Probe one is a 110 bp *Eco*RI−*Sau*3A fragment containing the 5′ end of Tcg-1. Probe 2 is a 27 base synthetic oligonucleotide representing one unit of the tandem repeat. Probe 3 is a 180 bp *Hha*I−*Eco*RI fragment containing the 3′ end of Tcg-1. Hybridization of these probes labeled with ^{32}P to a Southern blot of genomic DNA restricted with *Eco*RI is shown in Figure 1(lanes 2−4). Both probes 1 and 3 hybridized to ~10−25 different size restriction fragments; however, probe 1 appears to hybridize to several fragments not identified by probe 3. Since these results indicate that the number of DNA sequences which are homologous to the 110 and 180 bp probes are not necessarily identical within the genome, the copy number of the sequences homologous to these two probes was more accurately determined by standard quantitative dot-blot hybridization experiments (data not shown). Consistent with the results from the Southern blot hybridization, the 110 bp fragment was determined to have a higher copy number (60 per haploid genome) than the 180 bp fragment (14 per haploid genome). In contrast, the 27 bp probe showed only three strong hybridization signals with fragments of lengths 13.5, 5.4 and 4.8 kb and one weaker signal with a fragment of length 1.7 kb, indicating that this repeat unit defines a limited subpopulation of the repetitive sequence family.

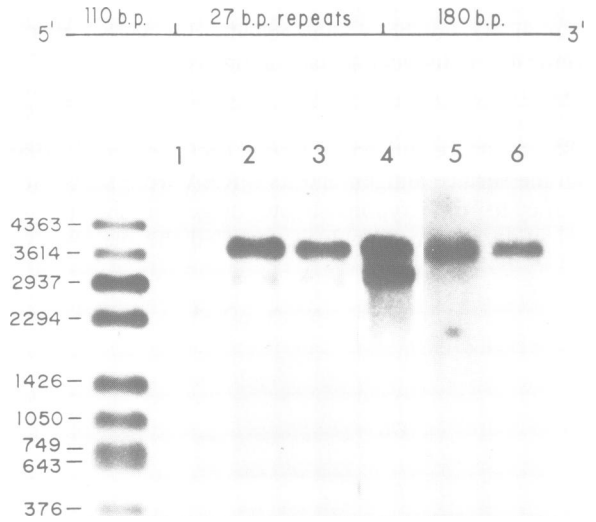

Fig. 2. Lanes 1−3 show epimastigote poly(A)$^+$ RNA, trypomastigote poly(A)$^+$ RNA, and trypomastigote total RNA respectively, hybridized with the ^{32}P-labeled 500 bp insert of Tcg-1. **Lanes 4−6** show trypomastigote poly(A)$^+$ RNA hybridized with the ^{32}P-labeled 110 bp *Eco*RI−*Sau*3A fragment from the 5′ end of the 500 bp insert of Tcg-1, the 27 nt synthetic oligomer and the 180 bp *Hha*I−*Eco*RI fragment from the 3′ end of the insert respectively. Amounts of poly(A)$^+$ RNA electrophoresed were 1.0−1.5 μg/lane. Total cellular RNA electrophoresed was 25 μg/lane.

Cloning of genomic DNA frgments containing the 27 bp repeat

The results of the copy number measurements clearly indicate that the 3.8 kb mRNA detected by hybridization with the 500 bp insert of Tcg-1 (Peterson *et al.*, 1986; Figure 2) may be transcribed by one or more members of the repetitive sequence family. Since those members which contain the 27 bp repeat define a subpopulation of the family, one approach for defining the transcriptional activity of the family is to determine whether the repetitive element is present in mRNA. As shown in Figure 2 (lane 5), the 27 bp repeat hybridized with a 3.8 kb poly(A)$^+$ RNA from trypomastigotes. No hybridization was observed with total cellular or poly(A)$^+$ RNA from epimastigotes (data not shown). These results are in keeping with those observed with the 500 bp insert in Tcg-1 and clearly shows that at least one of the four sites in the genome which contain the 27 bp repeat is transcriptionally active. In order to determine which site(s) were active, we attempted to clone and characterize a member of each.

Three separate genomic libraries were constructed using trypomastigote DNA partially digested with *Mbo*I, and one library was constructed using a limit digest with *Eco*RI. Approximately 400 000 independent recombinant plaques

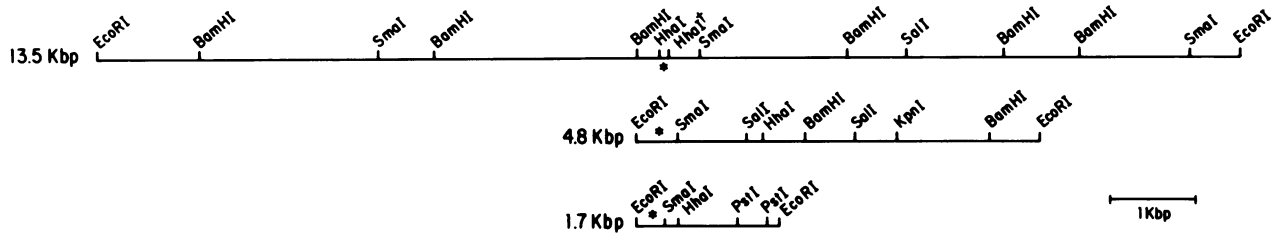

Fig. 3. Restriction map of the three *Eco*RI genomic DNA fragments that share homology with the 27 bp repeat unit. The asterisk marks the approximate position of homology with the 27 bp repeat. The dagger denotes that not all *Hha*I sites within the 13.5 kb *Eco*RI fragment have been mapped.

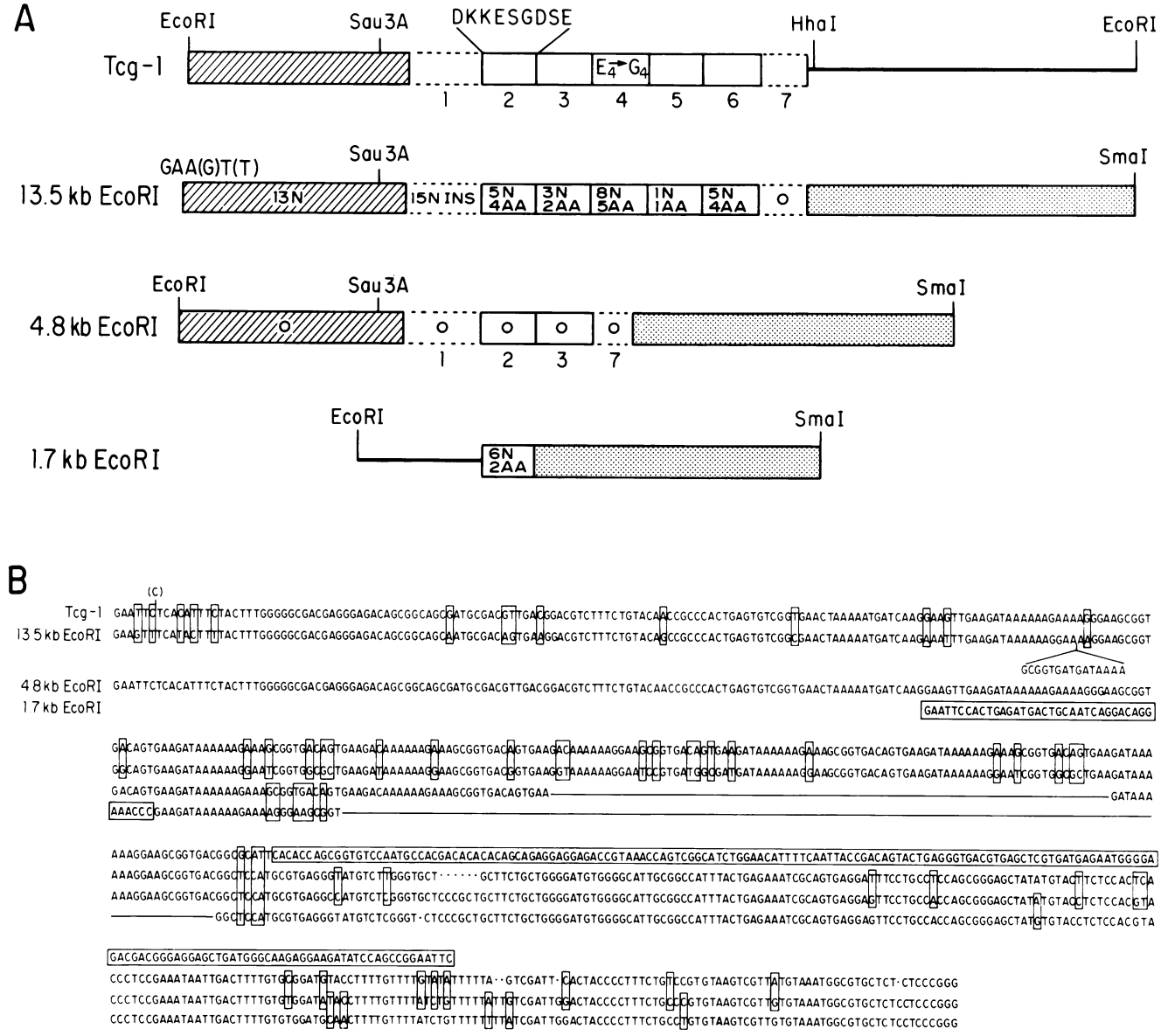

Fig. 4. A schematic representation (**A**) and a direct comparison (**B**) of the nucleotide sequence of Tcg-1 and those regions of the three cloned genomic DNA fragments which show homology with the 27 bp repeat unit. In (**A**) the hatched box denotes sequence homology 5′ upstream of the repetitive sequence region while the shaded box denotes homology 3′ downstream of the repetitive region. For Tcg-1 the numbers below the dashed and solid boxes denote the seven tandem repeat units. The dashed boxes represent degenerate repeats while the conserved repeats are shown as solid boxes. The predicted amino acid sequence of the repeat unit is shown above repeat 2. Numbers present in the boxed areas of the three genomic DNA fragments denote the number of nucleotide substitutions (N) or predicted amino acid substitutions (AA) between Tcg-1 and the respective genomic fragment. In (**B**) nucleotide differences between the four sequences are denoted by open boxes. The (C) residue shown above the *Eco*RI site in Tcg-1 is not present in the cDNAs (Figure 7), and is presumed to be part of the *Eco*RI synthetic linker. The $E_4 \rightarrow G_4$ shown in the fourth repeat unit of Tcg-1 denotes a transition from glutamic acid to glycine in the fourth repeat unit.

from the *Mbo*I libraries were screened with the 27 nucleotide probe and 59 plaques that rescreened positive were identified. DNA from each phage was isolated, digested with *Eco*RI, blotted to nitrocellulose and hybridized with the 27 nucleotide probe. A 4.8 kb fragment was observed to hybridize in 48 of the DNAs, and a 1.7 kb fragment hybridized in the 11 remaining DNAs. A similar screening of 100 000 plaques from the *Eco*RI library resulted in the identification of three phages, each of which contained a single *Eco*RI fragment of 13.5 kb. Surprisingly, no phage DNAs from either the *Mbo*I or *Eco*RI libraries were observed to contain a 5.4 kb *Eco*RI fragment which had homology with the 27 bp probe.

To determine whether the sequence of Tcg-1 was a subset of the sequences present in either the 13.5, 4.8 or 1.7 kb *Eco*RI fragments, a restriction map of these fragments was generated (Figure 3), and the nucleotide sequence of regions containing homology with the 27 bp repeat unit was determined (Figure 4). Examination of these sequences clearly shows that Tcg-1 is not present in any one of these three *Eco*RI fragments. Tcg-1 must, therefore, reside elsewhere within the genomic DNA, presumably in the 5.4 kb fragment.

Further examination of the data in Figure 4 shows that the repeat sequence in Tcg-1 and the 4.8 kb *Eco*RI fragment would be expected to be present in different size restriction fragments following digestion with *Hha*I. Specifically, digestion of the 500 bp insert in Tcg-1 and the 4.8 kb *Eco*RI fragment should yield fragments of 320 bp and 1500 bp

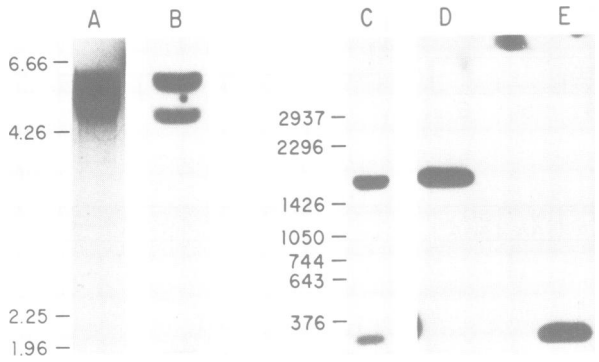

Fig. 5. Southern blot analysis of genomic DNA restricted with *Eco*RI and *Hha*I. Total genomic DNA (60 μg) was digested with *Eco*RI, electrophoresed on an agarose gel and fragments of size 4.5–6.5 kb were excised and isolated by electroelution. **Lane A** shows an ethidium bromide stained gel of the 4.5–6.5 kb fragments isolated by this procedure. **Lane B** shows hybridization of the fragments in lane A with the ^{32}P-labeled 27 nt oligomer. The size-fractionated *Eco*RI fragments were further restricted with *Hha*I, electrophoresed on an agarose gel, Southern blotted and hybridized with the 27 nt oligomer (**lane C**). Hybridization of the 27 nt oligomer to the 4.8 kb *Eco*RI fragment (see Figure 3) and to Tcg-1 after restriction with *Eco*RI–*Hha*I is shown in **lanes D** and **E** respectively.

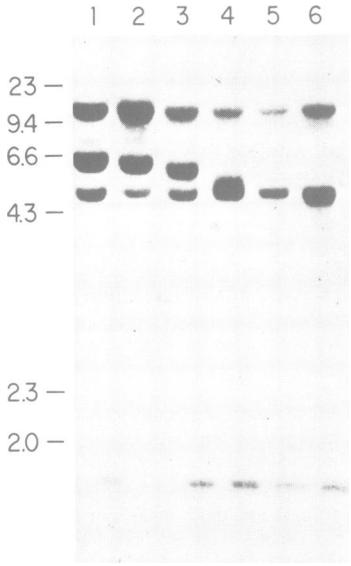

Fig. 6. Nuclease *Bal*31 sensitivity of genomic sequences with homology to the 27 bp repeat unit. Aliquots of trypomastigote genomic DNA were digested for increasing times with *Bal*31 nuclease, restricted with *Eco*RI, electrophoresed on an agarose gel and probed with the ^{32}P-labeled 27 nt oligomer.

respectively that hybridize to the 27 nucleotide (nt) repeat unit. Therefore, to confirm the supposition that the 5.4 kb *Eco*RI fragment is the origin of the Tcg-1 insert, total genomic DNA was digested with *Eco*RI, electrophoresed on agarose, and DNA fragments of 4.5–6.5 kb were isolated (Figure 5). The size-selected DNA was then digested with *Hha*I, fractionated by agarose gel electrophoresis, Southern blotted, and hybridized with the 27 bp probe. Hybridization signals were observed only with fragments of 1500 and 320 bp. Since the 1500 originated from the 4.8 kb *Eco*RI fragment, the 320 bp fragment must, therefore, reside within the 5.4 kb fragment.

Since further confirmation that the 5.4 kb fragment contains the Tcg-1 insert could be obtained most convincingly by cloning, restriction mapping and partially sequencing the 5.4 kb fragment, a further attempt to clone this fragment was made by constructing a library in λgt10 using the size-selected fragments described above. Of 200 000 plaques screened with the 27 nt probe, 69 positives were identified. Of these phage, none were observed to contain a 5.4 kb *Eco*RI insert.

Nuclease Bal31 sensitivity

The inability of the 5.4 kb fragment to be cloned as an *Eco*RI insert suggested the possibility that one end of the fragment was not an *Eco*RI terminus. In a related organism, *Trypanosoma brucei*, the expressed copies of the genes encoding the VSGs are found in the telomeric region of the chromosome and have been shown to be preferentially sensitive to digestion with exonuclease *Bal*31 (De Langer and Borst, 1982; Parsons *et al.*, 1983; Pays *et al.*, 1983). If the 5.4 kb fragment is present as a chromosome end (telomere), it also would be expected to be both sensitive to nuclease *Bal*31 and lack one of the *Eco*RI cohesive ends required for cloning as an *Eco*RI insert.

To test this possibility aliquots of high mol. wt trypomastigote DNA were digested for increasing times with nuclease *Bal*31 (Figure 6). The digested DNAs were then restricted with *Eco*RI, electrophoresed through agarose, blotted to nitrocellulose, and probed with the 27 nt repeat. Figure 6 clearly shows a progressive decrease in the size of the 5.4 kb *Eco*RI fragment with increasing digestion with *Bal*31 while neither the 13.5, 4.8 or 1.7 kb *Eco*RI fragments changed significantly in size over the time course of the experiment. This result strongly implies that in the trypomastigote the 5.4 kb fragment is telomeric. An identical series of digestions was performed on genomic DNA from epimastigotes, and, as with the trypomastigote DNA, the 5.4 kb fragment was progressively shortened by increased digestion with *Bal*31 with no apparent reduction in size of the other three fragments (data not shown). Therefore, it appears that the sequences present in the 5.4 kb fragment are telomeric in the genomes of both epimastigotes and trypomastigotes.

Analysis of cDNAs having homologies with the 27 nucleotide repeat

To determine which of the four *Eco*RI fragments functions as the template for transcription of the 3.8 kb mRNA, ~200 000 phage from three separate λgt10 recombinant trypomastigote cDNA libraries were hybridized with the 27 nucleotide probe, and 26 positive phage were identified. DNA was isolated from each phage, digested with *Eco*RI/*Hha*I, Southern blotted, and probed with the 27 nt repeat (data not shown). Twenty-five of the DNAs showed a single band of hybridization at 320 bp, consistent with the transcription template being the 5.4 kb *Eco*RI fragment. However, one DNA showed hybridization to a fragment of 1.3 kb, suggesting that it may have been transcribed from the 4.8 kb fragment. To confirm these assignments, the nucleotide sequence of a 432 bp *Eco*RI–*Sac*I fragment from four of the phage containing the 320 bp *Eco*RI–*Hha*I fragment was determined. A comparison of these sequences with those of Tcg-1 and the three genomic *Eco*RI fragments shown in Figure 4 clearly indicates that the sequences of the four cDNAs match the sequence of Tcg-1 but not the sequence of any of the other genomic fragments (Figure 7).

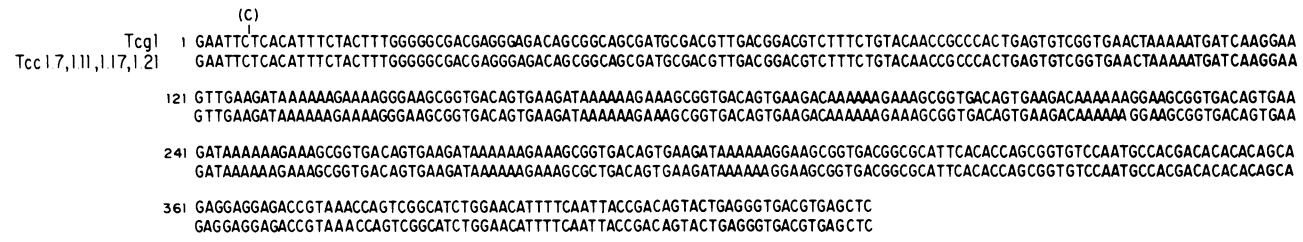

Fig. 7. The nucleotide sequence of identical *Eco*RI−*Sac*I fragments isolated from four independent cDNAs which showed positive hybridization with the 27 bp repeat unit is compared to the nucleotide sequence of the *Eco*RI−*Sac*I fragment containing the 27 bp repeat in Tcg-1.

In contrast, a partial nucleotide sequence of the 1.3 kb insert showed that it contained a repeat array identical to that observed in the 4.8 kb *Eco*RI fragment (not shown). In the comparison of the four cDNA sequences with that of Tcg-1, one exception is noted. The cytosine at position 7 in Tcg-1 is absent in the four cDNAs and is believed to be a result of the *Eco*RI linkers used in the construction of the genomic library from which Tcg-1 was isolated.

Transcription of other members of the repetitive sequence family

Results of the analysis of the 26 cDNAs indicate that stable, poly(A)$^+$ RNA transcripts arise almost exclusively from only one member of the repetitive family that contains the 27 bp repeat unit. However, since this gene is clearly a member of a higher order family of repetitive sequences (Figure 1), many members of which lack the 27 bp repeat, it was of interest to examine the possibility that one or more of these other members of the family may be transcribed. Northern blots of trypomastigote RNA were probed with the 500 bp insert of Tcg-1, the 110 bp *Eco*RI−*Sau*3A fragment from the 5′ end of Tcg-1, the 27 nt repeat, and the 180 bp *Hha*I−*Eco*RI fragment from the 3′ end of Tcg-1 (Figure 2). The 27 bp repeat and the 180 bp *Hha*I−*Eco*RI fragment hybridize only to a single band of 3.8 kb. Interestingly, the 110 bp *Eco*RI−*Sau*3A fragment detects two bands of 3.8 and 3.4 kb respectively. The 3.4 kb band is not detected by hybridization with either the 27 nt probe or the 180 bp *Hha*I−*Eco*RI fragment, but is present as a faint band after hybridization with the Tcg-1 probe.

Discussion

Organization of the 85 kd gene family

The results described above show that the gene encoding the 85 kd surface antigen of *T.cruzi* is a member of a complex multigene family. The 27 bp repeat unit and its flanking sequences each define subpopulations of the family. The sequences present in the 110 bp fragment 5′ upstream of the tandem repeats in Tcg-1 have a repetition frequency ~4 times that of the 180 bp sequences which are 3′ of the tandem repeats. The disparity in copy number is further evidenced by the hybridization of these two restriction fragments to a genomic Southern blot (Figure 1). The 110 bp fragment hybridized to at least 20 distinguishable bands, while the 180 bp fragment hybridized to < 10 bands. Most surprising is the observation that few of the genomic fragments which showed homology with the 110 bp probe also hybridized with the 180 bp sequence. This suggests that these two sequences are generally not adjacent in the genome, and the close association seen in Tcg-1 is rare and may be unique to this single genomic location. In support of this possibility is the observation that screening of ~200 000 plaques from a partial *Mbo*I genomic library constructed in EMBL4 with both the 110 and 180 bp fragments identified no plaques that hybridized to both probes.

Only four members of this multigene family show homology with the 27 bp repeat unit in Tcg-1. Three of these members have been cloned, restriction mapped and partially sequenced. It is clear that these three members do not represent the origin of Tcg-1, nor do they serve as templates for any abundant class of poly(A)$^+$ RNA. The fourth member, a 5.4 kb *Eco*RI, has not been identified in either partial *Mbo*I recombinant libraries or *Eco*RI libraries. A feasible explanation for the difficulty encountered in the cloning of this fragment is its chromosomal location. Based upon the preferential sensitivity of this fragment to *Bal*31 nuclease, it is very likely that the 5.4 kb fragment is telomeric and represents a chromosome end.

The observation that the 500 bp insert in Tcg-1 is not present in the three members of the subfamily which have been cloned invites the suggestion that it is present in the 5.4 kb fragment. This possibility is strongly supported by the results shown in Figure 4. It is clear that in 4.5−6.5 kb *Eco*RI-digested genomic DNA, the only *Eco*RI−*Hha*I fragments that contain the repeat motif are those present in Tcg-1 and the 4.8 kb *Eco*RI fragment. Furthermore, since the vast majority of the cDNA sequences examined (25 of 26) are identical to Tcg-1, it is reasonable putatively to assign the 5.4 kb fragment as containing all or part of the gene that encodes the 85 kd antigen.

Transcription of the gene family

Of the four members of the gene family defined by homology with the 27 bp repeat, only one member, the 5.4 kb fragment, appears to encode an abundant RNA transcript. It is very likely, however, that other members of the gene family which lack the repeat are actively transcribed. The observation that the 110 bp *Eco*RI−*Sau*3A fragment detects two prominent RNA bands in a Northern blot of trypomastigote RNA (Figure 2), while the 27 nt probe detects only one band, suggests that at least one member of the family which lacks the repeat sequence is actively transcribed in the trypomastigote.

Comparison with the surface antigen genes of T.brucei

One striking result of this study is the similarities between the *T.cruzi* 85 kd surface antigen gene and the genes encoding the VSGs of the African trypanosomes. The trypanosome VSG genes comprise a large gene family of the order of 1000 different genes, most of which are located at either an internal chromosomal site or at a telomere-linked

site (see Donelson, 1988). The VSG genes are expressed in the infective trypomastigote forms of the parasite, but are inactive in the replicative stage found in the midgut of the insect vector. Genes located at an intrachromosomal site are never transcribed and expression of these VSG genes requires duplication followed by translocation to a telomeric location. Of the telomere-linked VSG genes, some require chromosomal rearrangement prior to expression, while others are activated by mechanisms which do not involve any detectable chromosomal changes.

The similarities between the VSG gene family and the 85 kd gene family are several fold. Firstly, as with the VSG gene, the 85 kd gene is actively transcribed only in the infective trypomastigote stage and no transcripts of this gene are observed in the replicative stage of the parasite found in the gut of the insect vector. Secondly, the genes that encode both surface antigens are members of a multigene family. Although the relative sizes of the two families appear quite different (i.e. ~1000 versus ~60), the size of the VSG gene family was estimated by hybridization under low-stringency conditions (Van der Ploeg, 1982). Although we have not carefully examined the potential size of the 85 kd gene family under similar hybridization conditions, preliminary studies show that decreases in the stringency of the hybridization conditions result in substantial increases in the number of restriction fragments that hybridize to the 500 bp insert in Tcg-1 in Southern blots of restricted genomic DNA (D.L.Fouts and J.E.Manning, unpublished results). Thus the size of the family is potentially greater than that presented here. Thirdly, the expressed gene for the 85 kd surface antigen is located at a telomere-linked site, as are all transcriptionally active VSG genes.

The striking structural similarities between these two genes families suggests the possibility that *T.cruzi* has retained or acquired the mechanistic properties required for antigenic variation. Studies to determine whether such variation actually occurs within this gene family in clonal populations of this organism are being undertaken.

Materials and methods

Parasites strains and cultures

Trypanosoma cruzi Peru, Y, and Cl strains were obtained from Stuart M.Krassner, University of California, Irvine. The cloned *T.cruzi* strains of Esmeraldo clone 3 and Silvio X10 clone 1 were obtained from James Dvorak, National Institutes of Health, Bethesda, MD. Growth and maintenance of epimastigotes and tissue-culture derived trypomastigotes of these five strains are as described elsewhere (Beard *et al.*, 1985).

Isolation of cDNA clones

A cDNA library was constructed in phage λgt10 using trypomastigote poly(A)$^+$ RNA by methods described in Maniatis *et al.* (1982). The library was screened with a 27 nucleotide synthetic oligomer representing one unit of the tandem repeat array present in the 85 kd surface protein gene (Peterson *et al.*, 1986). The inserts present in those phage that showed hybridization were excised and a partial restriction map of each was constructed.

Isolation of genomic clones

DNA was isolated from culture form trypomastigotes, partially digested with endonuclease *Mbo*I and size fractionated on a 1% agarose gel. Fragments in the size range 9.0–20.0 kb were isolated and ligated into the *Bam*HI site of λEMBL4. Libraries were screened with the 27 nucleotide repeat unit. DNA inserts in phages showing positive hybridization were excised and characterized by both restriction enzyme mapping and direct nucleotide sequence analysis.

Nucleic acid isolation, radiolabeling, Southern and Northern blot transfer and restriction enzymes

Parasites were harvested and DNA, RNA and poly(A)$^+$ RNA were isolated as described previously (Maniatis *et al.*, 1982). Agarose gel electrophoresis of DNA and RNA, Southern transfer, Northern transfer, prehybridization, hybridization and filter washing were performed as described (Fouts *et al.*, 1981; Lanar *et al.*, 1981; Beard *et al.*, 1985). All restriction enzymes were purchased from International Biotechnologies, Inc. (New Haven, CT) and used as recommended.

Digestion with nuclease Bal31

Nuclease *Bal*31 was purchased from BRL (Bethesda Research Laboratories, Gaithersburg, MD) and used as recommended. Nuclear DNA (60 μg) was digested with 5 U *Bal*31 at 30°C in a 500 μl reaction volume. Aliquots (80 μl) were removed at 0 (immediately prior to addition of *Bal*31), 5, 10, 20, 30 and 40 min and immediately extracted with phenol. Following precipitation with ethanol, the DNA was digested with *Eco*RI and analyzed by Southern blot hybridization.

Acknowledgements

We thank Barbara Granger for assistance in maintenance and growth of parasite cultures, and Jim Massey for technical assistance. This work was supported by Public Health Service grant AI18873 from the National Institutes of Health.

References

Andrews,N.W., Robbins,E.S., Ley,V., Hong,K.S. and Nussenzweig,V. (1988) *J. Exp. Med.*, **167**, 300–314.
Araujo,F.G. and Remington,J.S. (1981) *J. Immunol.*, **127**, 855–859.
Beard,C.A., Wrightsman,R.A. and Manning,J.E. (1985) *Mol. Biochem. Parasitol.*, **16**, 199–212.
Brener,Z. (1973) *Annu. Rev. Microbiol.*, **27**, 347–383.
De Langer,T. and Borst,P. (1982) *Nature*, **299**, 451–453.
Donelson,J. (1988) In Englund,P.T. and Sher,A. (eds), *The Biology of Parasitism*. Alan R.Liss, New York, pp. 371–400.
Fouts,D.L., Manning,J.E., Fox,G.M. and Schmid,C.W. (1981) *Nucleic Acids Res.*, **9**, 7053–7064.
Lanar,D.E. and Manning,J.E. (1984) *Mol. Biochem. Parasitol.*, **11**, 119–131.
Lanar,D.E., Silven-Levy,L. and Manning,J.E. (1981) *Mol. Biochem. Parasitol.*, **3**, 327–341.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Nogueira,N., Chaplan,S., Tydings,J.D., Unkeless,J. and Cohn,Z. (1981) *J. Exp. Med.*, **153**, 629–639.
Parsons,M., Nelson,R., Newport,G., Milhausen,M., Stuart,K. and Agabian,N. (1983) *Mol. Biochem. Parasitol.*, **9**, 255–269.
Pays,E., Laurent,M., Delinte,K., Van Meirvenne,N. and Steinert,M. (1983) *Nucleic Acids Res.*, **11**, 8137–8147.
Peterson,D.S., Wrightsman,R.A. and Manning,J.E. (1986) *Nature*, **322**, 566–568.
Snary,D. (1980) *Exp. Parasitol.*, **49**, 68–76.
Snary,D. and Hudson,L. (1979) *FEBS Lett.*, **100**, 166–170.
Van der Ploeg,L., Valerio,D., De Langer,T., Bernards,A., Borst,P. and Grosveld,F. (1982) *Nucleic Acids Res.*, **10**, 5905–5923.
Wrightsman,R.A., Leon,W. and Manning,J.E. (1986) *Infect. Immun.*, **53**, 235–239.
Zingales,B., Andrews,N.W., Kuwajima,V.Y. and Colli,W. (1982) *Mol. Biochem. Parasitol.*, **6**, 111–124.

Received on June 6, 1989; revised on August 14, 1989