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Minichromosomal repetitive DNA in *Trypanosoma cruzi*: Its use in a high-sensitivity parasite detection assay

(trypanosomes/hybridization/satellite DNA)

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ABSTRACT **We have isolated genomic clones containing members of a tandemly repeated DNA family from *Trypanosoma cruzi*. This family, which contains a 195-base pair (bp) repeating unit, is the most abundant repetitive DNA in this organism. DNA sequencing analysis of three adjacent tandem repeats as well as two independent nonadjacent repeats showed relatively little sequence heterogeneity. Surprisingly, the three tandem elements contained a 585-bp open reading frame. However, blot hybridization of RNA from epimastigotes as well as blood-form trypomastigotes failed to show evidence for transcription of these sequences. Fractionation of whole *T. cruzi* DNA in sucrose gradients or in agarose gels followed by hybridization with appropriate radioactive probes showed that the size distribution of DNA bearing the 195-bp repetitive element is distinct from that of kinetoplast DNA as well as from that of DNA bearing tubulin genes. Hybridization of the 195-bp element probe with DNA from six different *T. cruzi* strains was positive; hybridization with DNA of other protozoa was negative with the single exception of *Leptomonas collosoma*, which displayed a weak cross-hybridization signal. Clones bearing this repetitive element are shown to be useful as probes for identification and counting of *T. cruzi* cells by dot-blot hybridization. The sensitivity of this assay permits detection of the DNA of 30 parasites in blood samples.**

Tandemly repeated DNA sequences known as "satellite" DNAs are a common constituent in the genomes of higher eukaryotes. These elements typically contain a simple consensus sequence that is repeated thousands of times, albeit imperfectly. Such sequences are often located in the more condensed heterochromatic regions of chromosomes and, with few exceptions (1–3), appear to be transcriptionally inactive. It has been proposed that satellite DNA can influence the specificity of pairing between homologous chromosomes (see refs. 4 and 5 for reviews), but this or any other specific functional role remains to be proven.

Renaturation studies on the genomic (non-kinetoplast) DNA of trypanosomes have revealed the existence of a large proportion of highly repetitive sequences. In *Trypanosoma brucei*, this fraction constitutes 12% of the genome (6), while in *T. cruzi* the figure is about 9% (7, 8). The work of Borst *et al.* (6) and Frasch *et al.* (9, 10) demonstrated the existence of repetitive sequences distinct from kinetoplast DNA in *T. brucei*, *T. equiperdum*, and *T. cruzi*. The major satellite-like sequence in *T. brucei* and *T. equiperdum* contained a repeating unit of about 177–180 base pairs (bp), while in *T. cruzi* the repeat was 194 bp. These trypanosome repetitive DNAs are interesting biological objects because of the recent demonstration by Sloof *et al.* (11) that the *T. brucei* 177- and 70-bp repetitive elements reside in minichromosomal structures. In this paper we characterize clones representative of a 195-bp repetitive DNA family that is the major component of the genome of *T. cruzi* and report some interesting features of their sequence. We have used these clones as probes to investigate the size distribution of chromosomal elements bearing this repetitive DNA family. In addition, we show that this repetitive DNA sequence is species specific and may be used in a very sensitive assay for the presence of *T. cruzi* DNA in blood samples.

METHODS

Trypanosomes. Epimastigotes of the Y strain were harvested from 2-week-old cultures in LIT medium as described (12, 13). Other strains used were CL, Peru (14), Brasil, and Tulahuen. The last two strains were kindly provided by J. Dvorak from the National Institutes of Health.

Cloning and Sequencing of DNA. High molecular weight DNA from *T. cruzi* cultures or from blood samples was prepared by using sodium dodecyl sulfate/proteinase K and phenol/chloroform (15, 16). Restriction endonucleases were obtained from Boehringer Mannheim and used under the conditions suggested by the supplier. Electrophoresis of DNA in horizontal agarose gels was carried out in a buffer containing 40 mM Tris acetate at pH 8.0, 20 mM sodium acetate, and 2 mM EDTA in the presence of ethidium bromide at 0.4 μg/ml. *Hin*dIII-digested λ phage DNA and *Hae* III-digested φX174 phage DNA were used as molecular weight markers. DNA fragments in the size range of 200–5000 bp were isolated on a preparative scale by band interception with DEAE-membrane strips (NA-45, Schleicher & Schuell). Techniques for recovery of DNA from DEAE-membranes have been published (17). For DNA fragments larger than 5 kilobases (kb), preparative isolation was carried out by partition into 1-butanol containing cetyltrimethylammonium bromide (18) after electrophoresis in low-melting agarose gels (same buffer as above, but 5 mM sodium acetate). DNA transfers for blot hybridization were performed as described by Southern (19). Probes were labeled by using a nick-translation kit (Bethesda Research Laboratories) and [α-^{32}P]dCTP (New England Nuclear). The origin of the different clones used as probes is as follows: pTCNRE repetitive elements, see *Results*; pTCβ tubulin, *T. cruzi* cDNA clone containing a 400-bp insert of the β-tubulin gene (unpublished); and pTCKm, *T. cruzi* DNA clone containing 1200-bp insert of kinetoplast minicircle DNA (unpublished). Nucleic acid hybridizations were carried out in 50% (vol/vol) formamide/750 mM NaCl/75 mM sodium citrate/0.1% sodium dodecyl sulfate/100 μg of carrier *Escherichia coli* DNA per ml/10% dextran sulfate, at 42°C (unless otherwise specified). The nitrocellulose filters were washed by standard procedures, with a final wash in 15 mM NaCl/1.5 mM sodium citrate/0.1% sodium dodecyl sulfate at 37°C. Autora-

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Abbreviations: bp, base pair(s); kb, kilobases.

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diography was carried out at −80°C, using two intensifying screens.

Specific DNA fragments were cloned either at the *Sst* I or *Bam*HI sites of pUC13 and pUC12 (20). Recombinant plasmids were isolated on a preparative scale by using the alkaline sodium dodecyl sulfate method of Birnboim and Doly (21) as modified by Ish-Horowicz and Burke (22), with a final isopycnic banding step in CsCl/ethidium bromide. Appropriate DNA fragments were labeled with polynucleotide kinase and sequenced according to Maxam and Gilbert (23).

Size Fractionation of Chromosomal DNA. Sucrose density gradients were prepared essentially as described by Sloof *et al.* (11) except that sodium dodecyl sulfate was present throughout the gradient at a concentration of 0.05%. Live trypanosomes were layered directly over a 0.5-ml layer that contained 2.0% sodium dodecyl sulfate and proteinase K at 250 μg/ml. After 3 hr at room temperature, the samples were spun for 16 hr at 10,000 rpm (20°C) in a Beckman 50.1 rotor. The gradients were fractionated into 12 0.4-ml fractions, and the DNA was bound to nitrocellulose by using standard dot-blot techniques (24). Gel electrophoresis of trypanosome DNA after *in situ* lysis in the gel slot was carried out essentially as described (11), except that the agarose gel buffer system was the Tris acetate system as given in the previous section. The DNA was blotted to nitrocellulose (19) after brief treatment with acid and alkali (25) to reduce the average size of DNA fragments. Hybridization of the blots was performed as described in the previous section.

RESULTS

A 195-bp Element Is a Major Component of the *T. cruzi* Genome. *T. cruzi* DNA was cut with a variety of restriction enzymes, of which *Sst* I gave a particularly striking pattern (see Fig. 1*A*). A sharp band of polynucleotide approximately 200 bp long accounted for about 9% of the ethidium bromide-stained material in the *Sst* I lane. The DNA from this band was recovered from the gel and inserted into the *Sst* I site of pUC13. Two clones were selected for further analysis and named pTCNRE1 and pTCNRE2. An equimolar mixture of DNA obtained from these two clones was labeled by nick-translation and hybridized to a Southern blot of *T. cruzi* DNA. Fig. 1*B* shows the hybridization of the pTCNRE probe to DNA digested with *Sst* I, *Pst* I, and *Hin*dIII. The radioactive signal consists of a ladder of bands starting at approximately 200 bp for *Sst* I and *Pst* I and 600 bp for *Hin*dIII. The ladder contains multiples of 200 bp for all three enzymes, but only the *Sst* I digest generates a predominant band of monomer; the other samples were digested exhaustively, yet generated little monomer. A simple interpretation of this data is to envision a tandem array of nearly identical elements, many of which lack *Pst* I or *Hin*dIII sites as a result of sequence heterogeneity. Precisely this kind of tandem sequence arrangement is characteristic of the satellite DNAs of higher eukaryotes.

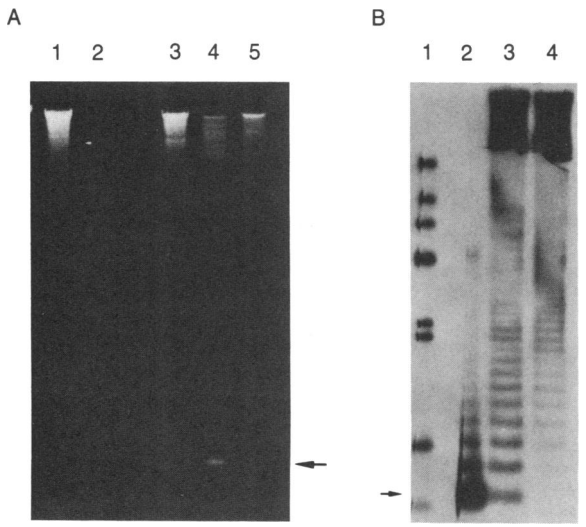

FIG. 1. Restriction patterns of *T. cruzi* DNA and hybridization of a Southern blot with the *Sst* I repetitive element clone. (*A*) *T. cruzi* DNA was cut with different restriction enzymes and fractionated in a 1.2% agarose gel. Lane 1, *Hin*dIII; lane 2, *Pst* I; lane 3, *Sma* I; lane 4, *Sst* I; and lane 5, *Xho* I. The arrow points to a band at approximately 200 bp. (*B*) *T. cruzi* DNA was cut with three different restriction enzymes, fractionated in an agarose gel, and blotted on nitrocellulose. The blot was hybridized with nick-translated pTCNRE DNA. Lane 1, *Hin*dIII-digested λ DNA markers; lane 2, *Sst* I; lane 3, *Pst* I; and lane 4, *Hin*dIII.

To study the sequence of these repetitive elements we constructed additional genomic clones containing tandem elements. A partial digest of *T. cruzi* DNA was generated with *Sst* I and clones were selected by hybridization with the pTCNRE1 probe. Two of these, pTCNRE3 and pTCNRE4, were found to contain inserts of approximately 600 bp (3 repeats) and 2400 bp (12 repeats) and were selected for further study. Fig. 2 shows the nucleotide sequence of the first element (A) of pTCNRE3, as well as the sequence differences found in the other two contiguous elements (B, C) and the two independent clones pTCNRE1 and pTCNRE2. In all five sequences the repeat length is 195 bp, bounded by *Sst* I sites. Note that *Pst* I sites are found in only two of the clones. Base differences are detected at 12 positions; for 7 of these positions divergence was observed in more than one element. The clustering of changes that occur in more than one clone suggests that the distribution of divergent bases is nonrandom. The sequence of pTCNRE3A is the consensus sequence for the five elements examined; its most striking property is the presence of an open reading frame in phase 2 (starting with the codon AGC). In fact, all five sequences in Fig. 1*A* have an open reading frame in phase 2. Fig. 3 shows that an open reading frame extends over the entire sequence of pTCNRE3, giving this unit a coding potential of 195 amino acids. Normally one would expect to find a terminator codon every 35-50 nucleotides for a random sequence of this base composition. Yet, since the consensus sequence contains an

```
pTCNRE3A  GAGCTCTTGCCCACACGGGTGCTGCACTCGGCTGATCGTTTTCGAGCGGC
pTCNRE3B                  A       C                     T  C
pTCNRE3C                  C     ^^^^^G                  C  T
pTCNRE1                   C     ^^^^^G                  C  T
pTCNRE2                   C       C                     C  C

pTCNRE3A  CGCTGCATCACACGTTGTGGTCCAAATTTTTGTTTCCGATTGTGAATGGT
pTCNRE3B  T  A                  C                  G
pTCNRE3C  C  G                  T                  A
pTCNRE1   C  G                  T                  A
pTCNRE2   T  G                  C                  G

pTCNRE3A  GGGAGTCAGAGACACTCTCTGTCAATATCTGTTTGCGTGTTCACACACTG
pTCNRE3B            A  CA
pTCNRE3C            G  GT
pTCNRE1             G  GT
pTCNRE2             A  CT

pTCNRE3A  GACACCAAACAACCCTGAACTATCCGCTGCTTGGAGGAATTTCGC
pTCNRE3B         G
pTCNRE3C         C
pTCNRE1          C
pTCNRE2          C
```

FIG. 2. Sequence of five repeats of the *T. cruzi* repetitive element. Elements A, B, and C are contiguous in pTCNRE3 but are listed separately for clarity. pTCNRE1 and pTCNRE2 are independent clones containing one single repeat insert. Note that pTCNRE3A is the "consensus" sequence of all five variants of the element. Single capitals denote base changes in each clone. The ^ symbol indicates the presence of a *Pst* I recognition sequence.

```
      1        10        20        30        40        50        60        70
      GAGCTCTTGCCCACACGGGTGCTGCACTCGGCTGATCGTTTTCGAGCGGCCGCTGCATCACACGTTGTGG
1
2       S  S  C  P  H  G  C  C  T  R  L  I  V  F  Q  R  P  L  H  H  T  L  W
3                                   XXX
          80        90        100       110       120       130       140
  TCCAAATTTTGTTTCCGATTGTGAATGGTGGGAGTCAGAGACACTCTCTGTCAATATCTGTTTGCGTGTTCACA
1
2  S  K  F  L  F  P  I  V  N  G  G  S  Q  R  H  S  L  S  I  S  V  C  V  F  T
3                     XXX
    150       160       170       180       190       200       210       220
  CACTGGACACCAAACAACCCTGAACTATCCGCTGCTTGGAGGAATTTCGGCGAGCTCTTGCCCACAAGGGTGCTGC
1                   XXX
2  H  W  T  P  N  N  P  E  L  S  A  A  W  R  N  F  A  S  S  C  P  Q  G  C  C
3
          230       240       250       260       270       280       290
  ACTCGGCTGATCGTTTTCGAGTGGCTGCTACATCACACGTTGTGGTCCAAATTTTTGTTTCCGATTGTGAATGGT
1
2  T  R  L  I  V  F  E  W  L  L  H  H  T  L  W  S  K  F  L  F  P  I  V  N  G
3        XXX                                                         XXX
    300       310       320       330       340       350       360       370
  GGGAGTCAGAGACACACTCTGTCAATATCTGTTTGCGTGTTCACACACTGGACACCAAAGAACCCTGAACTATCC
1                                                               XXX
2  G  S  Q  R  H  T  L  S  I  S  V  C  V  F  T  H  W  T  P  K  N  P  E  L  S
3
          380       390       400       410       420       430       440
  GCTGCTTGGAGGAATTTCGCGAGCTCTTGCCCACACGGGTGCTGCAGTCGGCTGATCGTTTTCGAGCGGTCGCTG
1
2  A  A  W  R  N  F  A  S  S  C  P  H  G  C  C  S  R  L  I  V  F  E  R  S  L
3                                                     XXX
    450       460       470       480       490       500       510       520
  CATCACACGTTGTGGTCTAAATTTTTGTTTCCGATTATGAATGGTGGGAGTCAGAGGCAGTCTCTGTCAATATCT
1              XXX
2  H  H  T  L  W  S  K  F  L  F  P  I  M  N  G  G  S  Q  R  Q  S  L  S  I  S
3
          530       540       550       560       570       580  585
  GTTTGCGTGTTCACACACTGGACACCAAACAACCCTGAACTATCCGCTGCTTGGAGGAATTTCGC
1                                  XXX
2  V  C  V  F  T  H  W  T  P  N  N  P  E  L  S  A  A  W  R  N  F
3
```

FIG. 3. Open reading frame in pTCNRE3. The numbers at the left denote the three possible reading frames. Standard capital letter symbols specify coded amino acids. XXX demarcates termination codons. The complementary sequence was found to contain terminators in all reading frames.

open reading frame, the potential for coding may extend over three or even more elements.

The copy number of the repetitive element DNA was estimated by dot-blot hybridization on nitrocellulose filters containing total *T. cruzi* epimastigote or blood form DNA and known amounts of pTCNRE1 DNA (a one-copy clone). The copy number in either case was approximately 120,000 repeats per genome, based on a DNA content of 0.33 pg per cell (7). This implies that one genome contains about 2.3×10^7 bp of repetitive element DNA. To investigate whether this repetitive DNA is transcribed *in vivo* we carried out several blot hybridization experiments using RNA from epimastigotes as well as blood form trypomastigotes. Hybridization of the blots with nick-translated probes failed to show evidence for transcribed RNA (data not shown).

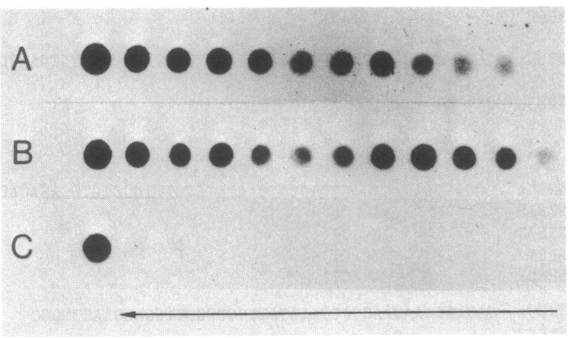

FIG. 4. Dot-blot hybridization of sucrose gradient-fractionated DNA with three cloned probes. Approximately 7×10^5 *T. cruzi* epimastigotes were lysed over a sucrose gradient in the presence of sodium dodecyl sulfate and proteinase K and centrifuged for 16 hr at 10,000 rpm in a Beckman 50.1 rotor. The gradient was then fractionated into 12 fractions, and identical aliquots of each fraction were denatured and blotted in nitrocellulose to yield three identical strips. Each strip was hybridized separately with ^{32}P-labeled DNA from the following clones: *A*, pTCNRE1; *B*, *T. cruzi* kinetoplast minicircle DNA; *C*, *T. cruzi* β-tubulin cDNA. The radioautographic exposures were identical for *A* and *B* but 5 times longer for *C*.

The 195-bp Repetitive Sequence Is Located in Minichromosomal Structures. To investigate the chromosomal localization of the *T. cruzi* 195-bp repetitive element family we performed a size fractionation of DNA from parasites lysed over sucrose gradients in the presence of sodium dodecyl sulfate and proteinase K. Fig. 4 shows a dot-blot experiment in which fractions from a gradient were alternatively hybridized with probes from a 195-bp repetitive element clone, a *T. cruzi* kinetoplast minicircle DNA clone, or a *T. cruzi* β-tubulin clone. Virtually all the material hybridizing with the β-tubulin probe was found at the bottom, while the 195-bp repetitive DNA signal was spread over the lower two-thirds of the gradient. The kinetoplast DNA probe hybridized with heavy as well as light material. The light material probably represents kinetoplast minicircle DNA, while the heavier material may consist of kinetoplast DNA networks.

To further clarify the distinction between the size distributions of 195-bp repetitive element DNA and kinetoplast DNA we fractionated total *T. cruzi* DNA by agarose gel electrophoresis and then did Southern blotting. Hybridization with the 195-bp repetitive element probe (Fig. 5*A*) showed a band in the region of 24–50 kb (the gel system does not fractionate very precisely in this size range). There is also a weak hybridization signal with material at the origin, which probably corresponds to the rapidly sedimenting material in

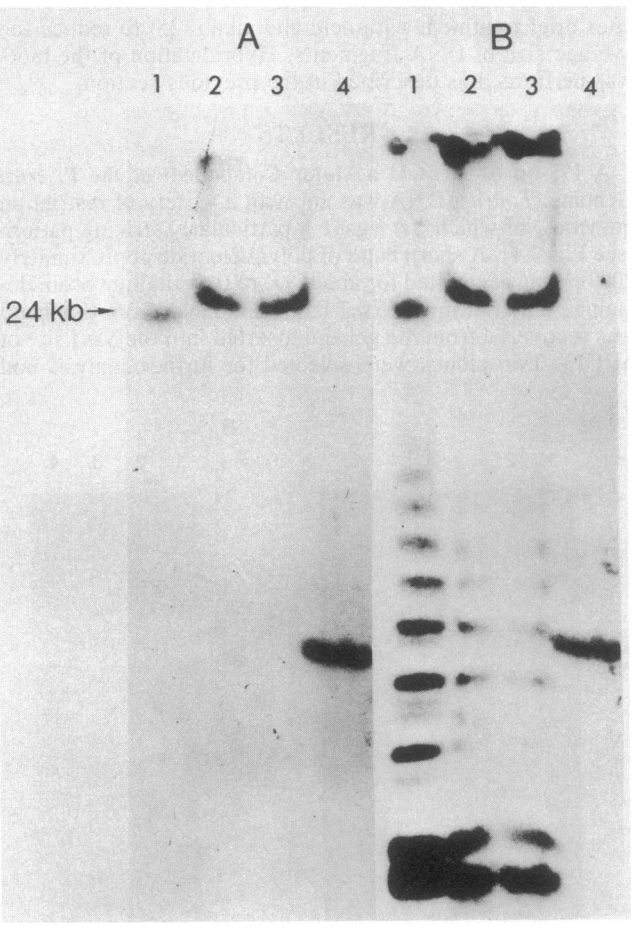

FIG. 5. Southern hybridization of *T. cruzi* total DNA fractionated in an agarose gel. Approximately 1.5×10^5 *T. cruzi* cells were lysed *in situ* in several slots of an agarose gel, and after electrophoresis the DNA was blotted on to nitrocellulose. A single blot was hybridized with nick-translated pTCNRE1 DNA, exposed for radioautography (*A*), and then rehybridized with labeled DNA from a kinetoplast minicircle clone (*B*). Lanes 1, blood form trypomastigotes; lanes 2, tissue culture epimastigotes; lanes 3, same as 2; lanes 4, DNA from the repetitive element clone pTCNRE3. The arrow marks the position of a phage λ DNA marker fragment (23.5 kb, detected by staining).

Fig. 4*A*. While this large material probably represents about 50% of the repetitive DNA, it is underrepresented in the Southern blot because of lack of penetration or poor transfer to nitrocellulose. In any event, we can conclude that a large proportion of the repetitive DNA resides in DNA molecules of minichromosome size (24–50 kb). It is worth noting that the signal from blood form DNA (lane A1) is weaker. Other experiments (data not shown) demonstrate that the net contents of 195-bp repetitive element DNA are identical in epimastigotes and blood forms. Whether or not the two forms differ in the relative amount of minichromosomal/large repetitive element DNA deserves further study.

The second part of Fig. 5 shows the rehybridization of the same blot with the kinetoplast DNA probe. This experiment (Fig. 5*B*) produced a ladder of rapidly migrating bands, the smallest of which correspond to kinetoplast minicircle (≈1300 bp). The ladder probably consists of oligomers of concatenated minicircles.

A High-Sensitivity Hybridization Diagnostic for *T. cruzi* DNA in Blood. We have utilized dot-blot hybridization assays to ascertain whether sequences closely related to the 195-bp element exist in different strains of *T. cruzi* or in other protozoan species. Hybridization at high stringency (50% formamide, 47°C) gave positive signals with all other *T. cruzi* strains tested (CL, Peru, Brasil, and Tulahuen; data not shown). On the other hand, high-stringency hybridization with DNA from 12 other hemoflagellate species was negative (Fig. 6 *Upper*). The only strong positive signal is the control *T. cruzi* (Y strain) DNA. There is a discernible signal with *Leptomonas collosoma* DNA, but it is about 1/20th as strong as the *T. cruzi* signal. Other protozoa that gave negative hybridization signals were *T. lewisi*, *T. equiperdum*, *Leishmania brasiliensis*, *Leishmania mexicana*, and *Leishmania donovani*.

A simple dot-blot assay using phenol-extracted DNA from blood permits an extraordinarily sensitive parasitemia count in blood samples of animals infected with *T. cruzi*. Fig. 6 *Lower* shows the detection of the parasite in DNA obtained from 0.3 μl of blood from an infected mouse (H4). Comparison with the purified *T. cruzi* DNA standards (H3, I3) shows that the signal corresponds to that expected from about 0.02 ng of *T. cruzi* DNA. Microscopic examination of the infected blood shows that sample H4 corresponds to approximately 60 trypomastigotes. Likewise, the weak signal in I3 (0.01 ng) corresponds to about 30 *T. cruzi* genome equivalents, which agrees with the genome size estimate of 0.33 pg per cell published by Lanar *et al.* (7). Other samples in this blot contained normal mouse blood DNA, human DNA, and *Leishmania donovani* DNA; all of them were negative for hybridization with the pTCNRE1 probe.

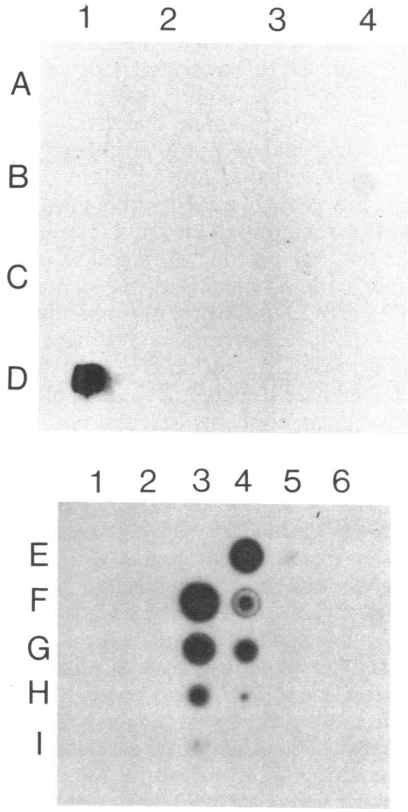

FIG. 6. Demonstration of species specificity and sensitivity of repetitive DNA probe in dot-blot hybridization experiments. (*Upper*) The first dot blot was prepared with 2.5 μg per dot of DNA from 12 different protozoa, plus a *T. cruzi* control. Hybridization with the nick-translated pTCNRE1 probe was carried out at 50°C. A1, *T. brucei brucei* 164 (ISTAT); A2, *T. brucei rhodesiense* ILRAD 2002; A3, *T. brucei gambiense* U2; A4, *T. equinum*; B1, *T. congolense*; B2, *T. evansi*; B3, *Crithidia fasciculata*; B4, *Leptomonas collosoma*; C1, *Herpetomonas muscarum*; C2, *Phytomonas davidi*; C3, *Endotrypanum* sp.; C4, *Leishmania tropica*; D1, *T. cruzi*. (*Lower*) The dot blot contained the following samples: column 1, rows E–I, human DNA, 10 μg, 1 μg, 200 ng, 20 ng, 2 ng. Column 2, rows E–I, *Leishmania donovani* DNA, 10 μg, 1 μg, 100 ng, 10 ng, 1 ng. Column 3, rows F–I, *T. cruzi* DNA, 10 ng, 1 ng, 0.1 ng, 0.01 ng. Column 4, rows E–H, phenol-extracted DNA from *T. cruzi*-infected mouse blood, extracted blood volumes 300 μl, 3 μl, 30 μl, 0.3 μl. The parasitemia level of these blood samples was approximately 2×10^5 trypomastigotes per ml. Column 5, rows E–H, DNA from normal mouse blood, otherwise same volumes as column 4. Column 6, same as column 5, but blood is from a different uninfected animal. Hybridization was performed at 47°C.

DISCUSSION

The major repetitive element of *T. cruzi* characterized in this paper bears some structural and functional analogies to the satellite DNAs of higher eukaryotes. Among these are the extensive tandem repetition, the occurrence of sequence microheterogeneity, and the apparent transcriptional inactivity. However, the 195-bp repetitive element family of *T. cruzi* is unlike satellite DNA because it contains long open reading frames. While this paper was in preparation, Sloof *et al.* (26) published a report on the characterization of satellite-like DNAs from *T. brucei* and *T. cruzi*. These DNAs were of nuclear origin and were shown to reside in nucleosomal structures with conventional repeat lengths. The *T. cruzi* repetitive element sequenced by these authors was 196 bp long and was claimed to lack open reading frames. We have examined the complementary sequence generated from the data of Sloof *et al.* and found that it corresponds almost exactly to our 195-bp consensus sequence, except for the introduction of an additional G residue between positions 86 and 87 in our map. Even with this insertion, the sequence of Sloof *et al.* does show an open reading frame in phase 2 (their sequence also contains three positions of uncertain base identification, but these bases do not correspond to potential terminators in the open reading frame).

Our observation that a large proportion of the 195-bp repetitive element family resides in minichromosomes suggests that the organization of this gene family is unlike that of most satellites of higher eukaryotes, which reside in chromosomes, intermingled with other sequences. A minichromosomal fraction containing a 177-bp satellite-like repetitive element has been described by Sloof *et al.* (11) for *T. brucei*. Thus, it appears that the organization of trypanosomal repetitive elements in minichromosomes may be a general feature of these parasites. An important question to be addressed by future studies is whether the *T. cruzi* minichromosomes consist exclusively of 195-bp repeats or contain other sequences adjacent to these. Conversely, it is critical to determine

whether "orphan" copies of the 195-bp repeat reside in the "large" chromosomes of *T. cruzi*. Since the 195-bp repetitive element gene family contains open reading frames, one could envision recombinational events that placed such reading frames inside large chromosomes and thereby created possibilities for new trypanosome structural gene products. Grimaldi and Singer (27) have shown that copies of the interspersed *Alu* family sequence exist within the α-satellite DNA of monkeys. It is tempting to envision that mobile elements may catalyze the transposition of trypanosome satellite-like sequences into coding domains of the genome. If, as has been proposed (28), certain trypanosomatidiae lack sexual stages, their repetitive elements could serve as alternative sequence pools for the generation of genetic diversity. While we have not been able to detect RNA complementary to *T. cruzi* repetitive element sequences, the possibility remains that transcripts may be produced in very small amounts, or perhaps transiently at specific developmental stages.

Our studies on the 195-bp repetitive element demonstrate that this sequence is shared by all *T. cruzi* strains tested so far and is absent from other protozoa, with the possible exception of *Leptomonas collosoma*. Since the 195-bp repetitive element family is the most abundant species-specific DNA in *T. cruzi* it is an ideal candidate for a *T. cruzi* diagnostic assay based on nucleic acid hybridization. Here we showed the detection of 30 *T. cruzi* genome equivalents in a dot-blot and in more recent experiments we have been able to push the sensitivity to about 10 parasite genomes in an overnight radioautographic exposure. We will show elsewhere the specific detection of *T. cruzi* in blood from Chagas patients.

Wirth and Pratt (29) have described hybridization assays for diagnosis and taxonomic typing of *Leishmania* based on the use of kinetoplast DNA probes. *T. cruzi* kinetoplast DNA is likewise a highly abundant sequence element, but it has been shown to display considerable strain-specific sequence variation (30). Satellite-like repetitive sequences such as the 195-bp repetitive element may be superior probes for diagnostic detection and typing of parasitic hemoflagellates because they display less strain variation within a species.

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