Characterization of an interspersed repetitive DNA element in the genome of *Trypanosoma cruzi*

J. ARAYA[1,2], M. I. CANO[1], H. B. M. GOMES[1], E. M. NOVAK[1], J. M. REQUENA[3], C. ALONSO[3], M. J. LEVIN[4], P. GUEVARA[5], J. L. RAMIREZ[5] *and* J. FRANCO DA SILVEIRA[1]*

[1] *Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, UNIFESP, Rua Botucatu, 862-CEP 04023-062, SP, Brazil*
[2] *Unidad de Parasitologia, Universidad de Antofagasta, Av. Coloso s/n, Casilla 170, Antofagasta, Chile*
[3] *Centro de Biologia Molecular Severo Ochoa, CSIC-UAM, Canto Blanco 28049, Madrid, Spain*
[4] *Ingebi, Obligado 2490, 1428 Buenos Aires, Argentina*
[5] *Centro De Biologia Celular, UCV, Apdo 47525, Caracas, Venezuela*

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SUMMARY

We report the molecular characterization of a middle repetitive DNA sequence, named C6, isolated from the *Trypanosoma cruzi* genome. C6 appears to be a composite repeated element since 3 subregions may be defined within it on the basis of sequence similarities with other *T. cruzi* genomic sequences. Sequences homologous to C6 are interspersed in the genome and can be mapped out on most chromosomal bands of different *T. cruzi*. strains. The copy number of the C6 element is about 1000 per haploid genome. Given the species specificity and different genomic distribution of C6 homologous sequences among the *T. cruzi* strains the C6 element could be a useful probe for diagnosis and typing of parasites. C6 is a polymorphic marker with potential as a tool for physical mapping of the *T. cruzi* genome.

Key words: *Trypanosoma cruzi* repetitive DNA, sequence analysis, genomic distribution, polymorphic marker, diagnostic tool.

INTRODUCTION

Re-association kinetic studies on the genomic DNA of the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas' disease, have shown that repetitive DNA sequences account for a substantial portion of the nuclear genome of this parasite (Castro, Craig & Castaneda, 1981; Lanar, Levi & Manning, 1981). Tandemly repeated sequences (mini- and microsatellites) and interspersed elements (retroposon- and retrotransposon-like sequences) have been described in *T. cruzi* (Sloof *et al*. 1983; Frasch *et al*. 1983; Gonzalez *et al*. 1984; Cotrim, Cummings & Franco da Silveira, 1989; Wincker, Roizes & Goldenberg, 1990; Aksoy, 1991; Mendonça-Lima & Traub-Cseko, 1991; Requena *et al*. 1992, 1994; Requena, Soto & Alonso, 1993; Novak *et al*. 1993; Vazquez, Schijman & Levin, 1994; Martin *et al*. 1995). Based on the structure and organization of such sequences, several authors have suggested that these elements may be involved in genetic rearrangements and control of gene expression in trypanosomes (Hasan, Turner & Cordingley, 1984; Murphy *et al*. 1987; Aksoy, 1991; Novak *et al*. 1993; Requena *et al*. 1994; Vazquez *et al*. 1994).

From a practical point of view, repetitive DNA elements can be employed as versatile tools for diagnosis and typing of pathogens. The repetitive sequences are known to undergo rapid evolutionary change at a rate appropriate for taxonomic studies. The majority of the nuclear repeated sequences isolated from *T. cruzi* are species specific and have been shown to be useful tools for diagnostic and taxonomic purposes (Gonzalez *et al*. 1984; Aksoy, 1991; Requena *et al*. 1992; Gonzalez *et al*. 1994; Ramirez *et al*. 1994). Also, due to the high degree of interspersion and polymorphism of these elements they may constitute important tools for large-scale genome mapping projects (Levin *et al*. 1994; Cano *et al*. 1995; Zingales *et al*. 1997).

In the course of studying the presence of repetitive sequences in the genome of *T. cruzi* we found a new family of species-specific sequences whose members are spread throughout the genome. In this report we show the characterization of a new repetitive DNA element establishing its relationship with other DNA repetitive families. It is present in several *T. cruzi* strains but absent in other trypanosomatids. Using 2 conserved oligonucleotide primers derived from the repetitive sequence, together with polymerase chain

* Corresponding author: Departamento de Microbiologia, Imunologia e Parasitologia, Escola Paulista de Medicina, UNIFESP, Rua Botucatu, 862-CEP 04023-062, SP, Brazil. Tel: +55 11 5764532. Fax: +55 11 5711095. E-mail: franco.dmip@epm.br

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A)

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        10         20         30         40         50         60         70         80
         :          :          :          :          :          :          :          :
GAATTCCAAC CGCTGATGCG CATTTGTTAC GAAAGGCAGG AATTATCGAG GACGCTTCGT CCAGATACGG GCGGATGGAT
TAATACCTTT CTCAGTTGTG GAGGAGAAAA CCACCGGTTT ACGACGACGA CGATGGATCG CGTGGCCACG CGACAAGAAC
AGAGACGCCC TTACGAGGCG AATGTTCCTC TTTTACATAT TCCATTATTA CCGCCTGTGA TGGCGAGGCG CTTCTCTTGA
TTAAGCATCC CTTTTTTAAG TCTCTTTACG CGGAGACTCG GATCTCTTTC GATGCCACGT GGAGTACGGC TGCGCTGGTG
GAGTCGACAC GGCTCCCAAT GGATACAAGG CCAGCCAGAA ATTCTCCAGT TATTACTTCC GACAATTGCG GGGGTGACAA
CGGTGGTTCG CCCCTCTGGG CCGCATCACC AATGGTCGAT CGACGTATGA TCATATATAC GCATAACACG GGTCAAAAAG
CGATGTGATA TCGTGGGAGG CCCAAGTGCT TTGCAATGCG GACAGTTGTC ACGCATCCAT GGGGGAAGAA CGCGAATCGG
GCGCTACACA GTACACCTTC TTGGGGTGCA GTTTGATCAC ACACACCGGG CGGTATCCCT GAGTGACAAG TTTGTCCGCT
CTGTACGCGC CATGCCGGCG TTAATTCTTT GACCATCGCG GAAATGGAGA TTATGCGTCA CGCTTTTTGT ACGCGGCTGC
CATTTTGGGC ACGCGTTTAT GTGACTACTA CTTTTTTATT AAGGCAGTGC GACGACGATT GTCCGCACTT AACCGGGGAT
TGTGCAGGAG ACATCCCCGG CGAACCTACG CCTACAGCGG TTGGTTTGGG CGAGAGATTG CGACACACCA TCGACGATAA
TCGTAAGCGA AACAGTCAAG CCCACGGAGA AGGCATCGGC TGCCATCATC ACACGGCACG CATCGCTCCA TGATGGGAGC
CGTTTTTATT CCAGACTTCC GGCGACGTTA AAATTGCCGG AGAAAAATGG GAGAGGAAGC CTTTTCTTAT CATGCAGGCC
GAGGCACGTG CGGTACGCTT AGCCTTATCG GCCTTTTCCG CCATTTTGCC ATCCACCATG GGCGTTTGGG TGGACAATAC
TTCGCTGCAA GGAGCGGCGA ATAAAGGCAG CTCAAAATCA CACGCGTTGA CGTGGGAGCT GCAACGGATA TACGAGTTTT
TGGACTCTCG CGGAATACAG GCAACATTTG CCTACGTGCG GTCTGCAGAA AACCCCGCAG ACGATATCAC GCGGTCGTGT
TTTTACACTT CAGACTTGGC GAAGGGGTGG AACTTCCGAA GGGGAGCGGC GGGGTCTTGT GGTTGTAGGA CCCCAAAGTC
TGCCACTTCG TAAGTAATAA TATTTTCAAA TCCTAACTGA GGACAAGGAC CATGCTAATG GTCCACGGAA TTC
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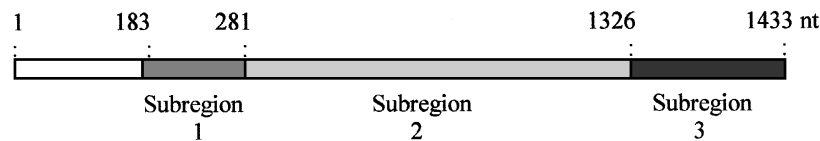
B)



C)

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C6     183   TGTTCCTCTTTTACATATTCCATTATTACCGC---CTGTGATG-GCGAGGCGCTTCTCTTGATTAAGCA
E22    489   TGTTTTTCTTTACTTAATGTCTGGTTGCTGCACACTGTAATGAGCGAAGTGCTTCCCG----------
             ****   *  ***** ** *    *   ** * **    **** **   **** * ***** *

C6     249   TCCCTTTTTTAAGTCTCTTTACGCGGAGACTCGG   281
E22    548   -CTCTTGTTTGCTTTTGTTTTCGCCCACACGCGG   580
              * *** ***    * * *** * *   * ** ***

C6    1326   GCGGCGGGGTCTTGTGGTTGTAGGACCCCAAAGTCTGCCACTTCGTAAGTAATAATATTTTCAAATCCTA
H2A    994   GCGGTGGGGTTTTGTGGCTGGAGGACCCCAAAGTCTGCCACTTCGTAAGTAATAATATT------TCGGA
SIRE   218   GCGGCGGGGTCTTGTGGTTGGAGGACCCCAAAGTCTGCCACTTCGTAAGTAATAATATTTC-AAATCCCA
HEX   2383   GCGGCGGGGTCTTGTGGCTGGAGGATCTGAAATTCTGCCACTTCTTATGTAATGAACATTTCGAATTCGA
             **** ***** ****** ** **** *   *** *********** ** ***** *    **** ***** *

C6    1395   ACTGAGGAC-AAGGACCATGCTAATGGTCCACGGAATTC   1433
H2A   1057   TCTGAGTACAAAAGACCACGTGAGTAGTCAACAGAATAC   1096
SIRE   286   ACTGAGGACAAAGGACCATGCTAATGGTCCACAGAATTC    326
HEX   2432   A--------------------------------------   2433
              ***** ** ** ***** *  * * *** ** **** *

C6    1406   TTGTCCTCAGTTAGGATTTGAAAATATTATTACTTACGAAGTGGCAGACTTTGGGGTCCTACAACCACAA
24Sp  1547   TTGAACCTCGGTTAGGATCTGAAATATTTATCGCTTACGAAGTGGCAGACTTTGGGGTCCTCCAACCACA
             ***  *   * *  *  *  *******  *    *    *    *      ** ***  *  * * *   *

C6    1337    GACCCCGCCGCTCCCCTTCG-GAAGTTCCACCCCTTCGCCAAGT-CTGAAGTGTAAAAACACGACCGCGT
24Sp  1616    GACCCCGCCGCTCCCCTTGCAAAGTTCCAGCCCCTTCGCCAAGTCCTGAAGCGTAAACACGCGATTACG-
              ******************    *  * *  ************** ************ ** ***   **

C6    1268    GATAT--CGTCTGCGGGGTTTTCTGCAG   1243
24Sp  1685    GATATGCCGCCTGCGGGGTTTTCTGCAG   1715
              *****  ** *******************
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Fig. 1. Sequence analysis of C6 repeated element. (A) Nucleotide sequence of the C6 repeated element. The nt sequence of the C6 element has been deposited in GeneBank database under accession No. U16295. Subregions 1 and 3 which share homology with previously reported *Trypanosoma cruzi* sequences are underlined. (B) Schematic representation of C6. Subregion 1, sequence with similarity to the repeated element E22; subregion 2, without homology to previous reported *T. cruzi* sequences; subregion 3, sequence with similarity to the flanking region of the 24Sα rDNA pseudogene, the intergenic spacer of the H2A histone gene, the 3′ untranslated region of the hexose

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reaction technique, a specific band can be readily detected in all *T. cruzi* DNA samples, suggesting that this sequence could be used for diagnostic purposes.

MATERIALS AND METHODS

Parasites, nucleic acid isolation, genomic library construction and screening

T. cruzi was cultured and nucleic acid was isolated as previously described (Novak *et al*. 1993). A partial genomic library was constructed in plasmid Bluescript. The genomic nuclear DNA was digested with *Eco*RI and the resulting fragments separated in 0·8% agarose gel. After ethidium bromide staining, a highly stained band of 1·5 kb was cut from the gel and the purified DNA was inserted into the *Eco*RI site of the vector. After transformation of competent *Escherichia coli* cells, recombinant plasmids were selected by *in situ* hybridization using labelled *T. cruzi* nuclear DNA.

DNA sequencing

Nucleotide sequences were determined by the dideoxy-nucleotide chain termination method (Sanger, Nicklen & Coulson, 1977), using Sequenase (United States Biochemical). Progressive deletions were obtained with exonuclease III. Parts of the sequence were confirmed by using overlapping subclones generated by digestion of insert with appropriate restriction enzymes. The sequences were analysed and compared by PCGENE SOFTWARE and the University of Wiscosin Genetics and Computer Group programs (Devereux, Haerberli & Smithies, 1984).

Southern and dot blot analyses

T. cruzi genomic DNA samples were digested with the enzymes listed above, fractionated in a 0·8% agarose gel, transferred onto nylon filters and hybridized with the insert of clone C6 labelled with [α-^{32}P]dATP by the random primed synthesis method. The hybridizations were carried out in 50% formamide/5 × SSC (1 × SSC = 15 mM NaCl/1·5 mM Na_3 citrate, pH 7·5)/0·1% SDS/5 × Denhart's solution/50 μg ml^{-1} yeast tRNA/100 μg ml^{-1} sonicated herring sperm DNA/10 μg ml^{-1} poly (A) for 16 h at 42 °C. The final wash was done in stringent conditions in 0·1 × SSC/0·1% SDS at 65 °C for 1 h.

Known amounts of DNA were denaturated by heat (3 min in boiling water), applied onto nylon filters and incubated for 2 min in 0·5 M NaOH. After neutralization, the filters were hybridized with the labelled insert of clone 6. The hybridizations were carried out as described above. The washes (twice) were done in 0·1 × SSC/0·1% SDS at 50 °C for 30 min (Novak *et al*. 1993).

Pulsed-field gel electrophoresis (PFGE)

The preparation of the agarose blocks, parasite pellets and PFGE separation were carried out as described (Cano *et al*. 1995). PFGE was done in the Pharmacia Gene Navigator apparatus with a hexagonal electrode array. The separations were carried out in 1·2% agarose gel in 0·5 × TBE (45 mM Tris/45 mM boric acid/1 mM EDTA, pH 8·3) as running buffer at 13 °C using 5 phases of homogenous pulses with interpolation for 132 h at 80 V. The separated chromosomal bands were hybridized with the insert of clone C6 labelled with [α-^{32}P]dATP by the random primed synthesis method. The hybridizations were carried out as described before. After hybridization the filters were washed twice (30 min) in 0·1 × SSC/0·1% SDS at 56 °C.

PCR amplification

Genomic samples (10 ng) were used as template for PCR using 2·5 units of *Thermus aquaticus* (*Taq*) DNA polymerase in buffer composed of 10 mM Tris, pH 8·3/50 mM KCl/5·0 mM $MgCl_2$/0·25 mM of each of the 4 nucleotide triphosphates/0·4 mM of each primer. Samples (25 μl) were denaturated for 5 min at 95 °C and amplified in a programmable thermal cycle. The profile of amplification was: 1 min at 95 °C, 1 min at 60 °C and 1 min at 72 °C (repeated 35 times). Afterwards, the samples were incubated for 2 min at 60 °C and 2 min at 72 °C. Samples were analysed on 3·0% NuSieve agarose gels. The oligonucleotides used were 5′GATGCGCATTTGTTACGA 3′ (sense, nt 15 to nt 32) and 5′CTGGCTGGCCTTGTATCC 5′ (reverse, nt 358 to nt 340).

RESULTS AND DISCUSSION

Isolation and nucleotide sequence analysis of C6 element

When the DNA from *T. cruzi* G strain was digested with *Eco*RI endonuclease, after gel electrophoresis

transporter gene and SIRE repeated element. (C) Comparison of the C6 sequences with previously *T. cruzi* reported sequences. E22 repeated element (Genebank accession number X95485); 24Sp, 24Sa rDNA pseudogene (M28886); H2A, intergenic spacer of histone H2A gene (X67287); SIRE repeated element (X75033); Hex, 3′ untranslated region of hexose transporter gene (U05588). Asterisks indicate identical bases. The underlined nucleotide represents a difference with respect to the C6 sequence. The homology with the 24Sα rDNA pseudogene is with the complementary strand of the sequence comprised between nt 1547 and 1715 (Arruda *et al*. 1990).

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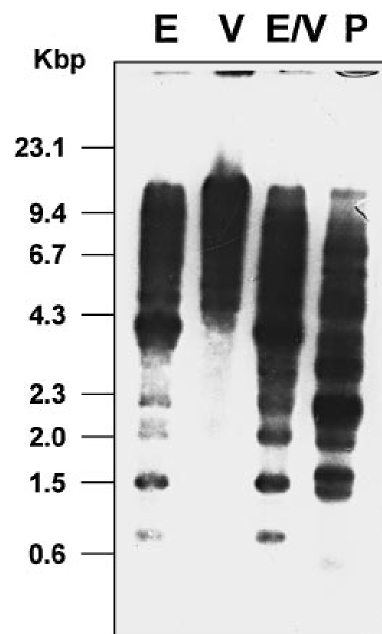

Fig. 2. Genomic distribution of C6 element. Southern blot of restriction endonuclease digestions of *Trypanosoma cruzi* (CL strain) genomic DNA probed with the insert of clone C6. Restriction enzymes used were: E (*Eco*RI), V (*Eco*RV) and P (*Pst*I). Size markers (kbp) are shown on the left.

and ethidium bromide staining, a 1·5 kb band was clearly visible suggesting the presence in the band of a highly repetitive sequence (data not shown). The band was sliced from the agarose gel and, after purification of the DNA, it was inserted into the BlueScript plasmid. After transformation of competent *E. coli* cells, recombinant plasmids were selected by *in situ* hybridization using labelled *T. cruzi* nuclear DNA. From the positive clones we selected one harbouring a 1·5 kb insert (clone C6).

Figure 1 shows the nucleotide sequence of C6, its physical map and a comparison of the element with other *T. cruzi* repetitive sequences reported in the literature. We have identified 2 subregions (1 and 3) of C6 with sequence homology to previously reported *T. cruzi* sequences. Both sequences flank a central region (subregion 2) of 962 bp which did not share homology with any *T. cruzi* sequences. Subregion 1, from nt 183 to nt 281, has 65% sequence identity with a repeated DNA sequence called E22 (Requena, J. M. and Alonso, C., unpublished data).

Subregion 3, from nt 1243 to nt 1433, presents a high sequence identity with repetitive sequences associated to several *T. cruzi* genes: 24Sα rDNA, histone H2A, the ribosomal P2β protein and the hexose transporter protein. Most of this sequence homology is due to the presence in subregion 3 of a truncated form of a mobile element named SIRE element (Vasquez *et al*. 1994). Subregion 3 has 83% sequence identity with a repetitive sequence found in the intergenic spacer of the H2A histone gene (Puerta *et al*. 1994) and the intergenic region (SIRE element) of the ribosomal P2β protein gene (Vasquez *et al*. 1994) and 88% sequence identity (reverse and complementary) with the 3′ flanking region of the 24Sα rDNA gene (Arruda *et al*. 1992). In subregion 3, there is also a 70 nt sequence (nt 1326–1396) that displays 78% identity with the 3′ untranslated region of the mRNA for the *T. cruzi* hexose transporter protein (Tetaud *et al*. 1994). The C6 element appears to be a composite repeated element since 3 subregions may be defined within it on the basis of sequence similarities with other *T. cruzi* sequences.

Genomic organization of the C6 element

We estimated the copy number of C6 using the element as probe against dot blots of known amounts of *T. cruzi* genomic DNA. Assuming a haploid genomic content of $1{\cdot}8 \times 10^8$ bp (Castro *et al*. 1981; Lanar *et al*. 1981), we calculated that there are about 1000 copies of C6 per *T. cruzi* haploid genome (data not shown). The insert of clone C6 was hybridized with *T. cruzi* genomic DNA digested with several restriction enzymes (Fig. 2). A complex hybridization pattern was obtained suggesting a high level of dispersion and restriction polymorphism of the C6 sequences throughout the *T. cruzi* genome.

Further confirmation of the high degree of interspersion of C6 was obtained by analysis of the chromosomal distribution of sequences homologous to C6 in 4 *T. cruzi* isolates. The chromosomes separated by PFGE were transferred onto nitrocellulose filters and probed with C6 (Fig. 3). Most of the chromosomal bands from the 4 isolates were labelled by the C6 probe. Figure 3A shows the intensity of the ethidium bromide fluorescence was not evenly distributed along all chromosomal bands showing a good correlation with the hybridization pattern obtained by the C6 probe.

In addition, when we screened *T. cruzi* genomic libraries with C6, many phage plates reacted. Randomly selected positive clones had different restriction enzyme patterns, indicating that they represent different regions of *T. cruzi* genome (data not shown). Thus, in conjunction with other reported repetitive elements C6 may be a useful tool for large-scale physical mapping of the *T. cruzi* genome (Levin *et al*. 1994; Cano *et al*. 1995; Zingales *et al*. 1997). At present, we are applying C6 element as a probe for the analysis of *T. cruzi* yeast artificial chromosomes (YACs) (Levin *et al*. 1994; Zingales *et al*. 1997). Preliminary results showed that the *T. cruzi* YACs can be ordered as collections of overlapping genomic fragments called contigs using the hybridization patterns obtained with the C6 element.

Species-specificity of the C6 element

Sequences homologous to C6 were detected by dot blot hybridization in all *T. cruzi* strains tested: G, Y,

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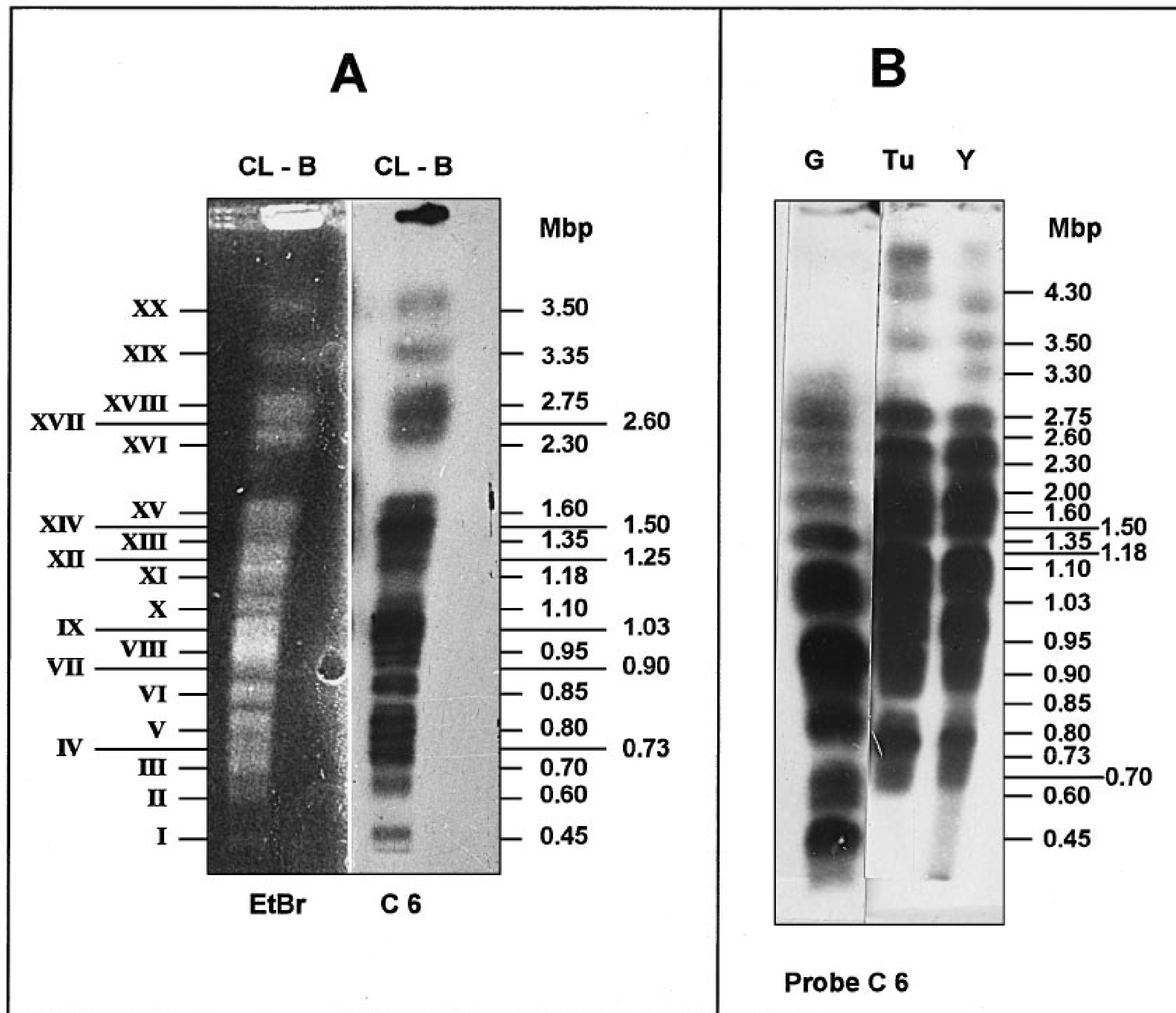

Fig. 3. Chromosomal mapping of C6-related sequences in clone Cl Brener (A) and strains G, Tulahuen (Tu) and Y (B). Chromosomal bands were separated by pulsed-field gel electrophoresis, stained with ethidium bromide (EtBr), transferred onto nylon filters and hybridized to the labelled insert of clone C6 (Probe: C6). The roman and arabic numerals indicate the numbers and sizes of the chromosomal bands, respectively.

DM30, CL, Tulahuen (Fig. 4A). No hybridization signal was found with other *Trypanosoma* species (*T. theileri*, *T. mega*, *T. equiperdum* and *T. rangeli*) or related trypanosomatids (Fig. 4A). Southern blots of endonuclease restricted *T. cruzi* DNA showed also the existence of both qualitative and quantitative differences in the hybridization patterns of C6 in different *T. cruzi* strains (data not shown).

We have also analysed whether specific primers derived from the C6 element could be used to detect the presence of *T. cruzi* DNA in a PCR amplification reaction (Fig. 4B). A 344 bp band can be readily amplified in all *T. cruzi* DNA samples using 2 conserved oligonucleotide primers derived from the repetitive sequence. No amplification band was observed in other trypanosomatids.

Since the C6 element does not show cross-reactivity with other trypanosomes (*T. theileri*, *T. mega*, *T. equiperdum* and *T. rangeli*) and related trypanosomatids, we suggest that C6 might be useful as a species-specific probe for *T. cruzi*. For instance, it could be used to detect the presence of *T. cruzi* in endemic areas where *T. cruzi* and *T. rangeli* can be found in the same triatominae vectors and reservoirs. Differentiation between *T. rangeli* and *T. cruzi* is of particular importance in the diagnosis of Chagas' disease since *T. cruzi* and *T. rangeli* have similar morphology and are antigenically cross-reactive (D'Alessandro, 1976) but *T. rangeli* does not cause any pathology to humans. We are carrying out a PCR-oligocapture assay in ELISA format for detection of *T. cruzi* in biological samples using primers derived from C6 sequences.

Sequences homologous to C6 were mapped among most of the chromosomal bands as an indication that these sequences are interspersed in the *T. cruzi* genome. These sequences are species specific and can be easily amplified by PCR or detected by hybridization. Given the species specificity and different genomic distribution of C6 homologous

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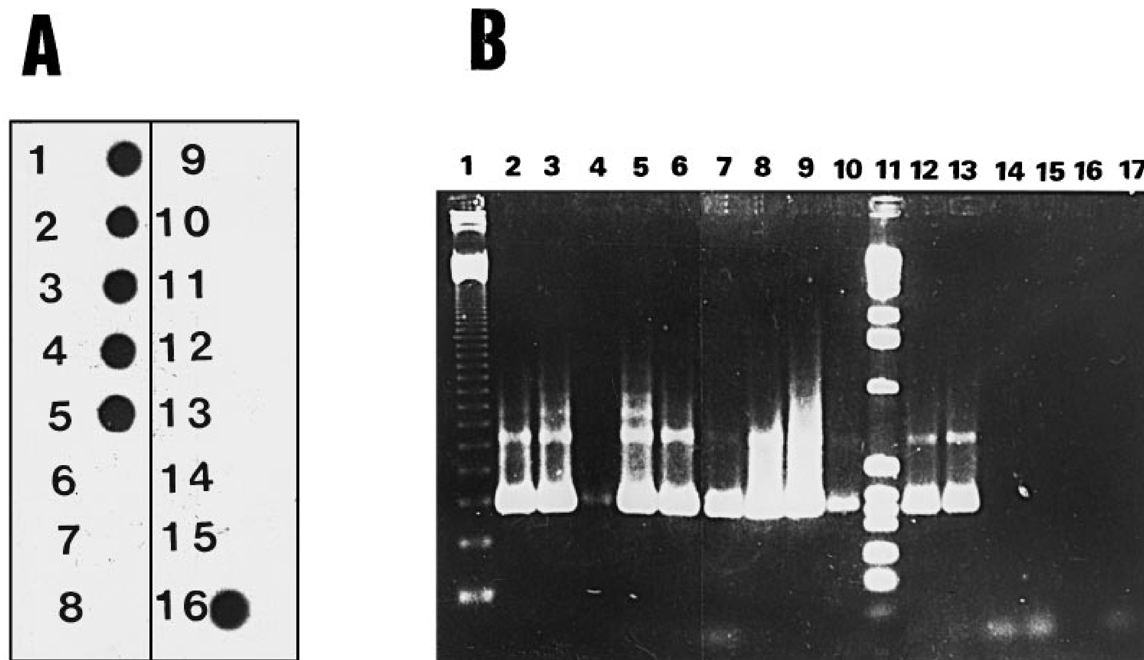

Fig. 4. (A) Demonstration of species-specificity of DNA sequences in dot blot experiments. Genomic DNA from *Trypanosoma cruzi* (1 μg) and other trypanosomatids (5 μg) was denaturated by heat, bound to nylon filters and hybridized to labelled C6. The trypanosomatids tested were: 1 to 5, *T. cruzi*, strains G, DM30, CL, Y and Tulahuen, respectively; 6, salmon sperm DNA (5 μg); 7, *Endotrypanum schaudini*; 8, *T. theileri*; 9, *T. equiperdum*; 10, *T. rangeli*; 11, *Leptomonas collosoma*; 12, *Leishmania donovani chagasi*; 13, *Leishmania mexicana amazonensis*: 14, *Crithidia luciliae termophila*; 15, *Phytomonas davidii*; 16, plasmid C6 (1 μg). (B) Specificity of PCR amplification of *T. cruzi* DNA using the C6-derived primers. The reaction was performed in the presence of 10 ng of genomic *T. cruzi* DNA or other trypanosomatids. Lanes 2–13 contain the DNA of different *T. cruzi* strains and a recent isolate obtained from opossum and identified by the current methods as *T. cruzi*. Lanes: (2) CL28; (3) DM30; (4) Quafitas; (5) DM28; (6) F; (7) EP; (8) Y; (9) G; (10) CL; (12) DM28; (13) an oppossum isolate. Lanes 14–16 contain: (14) *Leishmania guyanensis* (M4147); (15) *Leishmania amazonensis* (LBT0016); (16) *Crithidia fasciculata*. Lanes 1 and 11 contain molecular weight markers, the 123 bp ladder and 1 kb ladder, respectively. Lane 17 is a DNA-free sample (negative control).

sequences among the *T. cruzi* strains the C6 element could be a useful tool for diagnosis and typing of parasites.

Relationship of C6 element with other T. cruzi *repeated sequences*

Recently, Requena *et al*. (1992, 1993, 1994) have described the characterization of several *T. cruzi* repetitive sequences (E13, E12, E22) of about 1 kb in length which were isolated from a genomic library constructed with fragments obtained by digestion with *Eco*RI. The C6 element and these repetitive sequences share some common structural features. For instance, we have also found many short poly(dT) stretches distributed along these elements. For instance, a CT_n motif ($n = 3$–6) is repeated 11 times in C6, and 5 and 6 times in E12 and E13, respectively. Another motif, GT_n ($n = 3$–5), is represented 7 and 4 times in E12 and C6, respectively. Short repetitive motifs are commonly found within large repetitive elements and could be implicated in the recombination and/or gene conversion mechanisms (Rogers, 1985).

However, the most striking structural feature was the presence of a truncated form of the SIRE at one end of C6, E12 and E13 elements. This structural arrangement resembles that described for a retroposon-like element called Ingi or TRS, which is widely distributed in many genomic sites in *T. brucei* (Kimmel *et al*. 1987; Murphy *et al*. 1987). The ends of Ingi/TRS elements consisted of 2 halves of the transposable element RIME (Ribosomal Mobile Element).

It is possible that SIRE and sequences at the end of C6 may have a common origin. A plausible hypothesis is that C6 may have arisen by insertion of its central sequences into a SIRE element. We can speculate that the C6 repetitive unit resulted from recombination (or gene conversion) involving SIRE, followed by gene amplification. During these processes SIRE lost part of its 5′ end. Many examples of retroposons inserting within other repeated sequence are described in the literature. Retroposons, in general, have a tendency to insert each other generating composites which are themselves propagated as single transposons (Rogers, 1985). Our results indicate that the composite nature

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of the C6 repetitive element may reflect the plasticity and capacity of sequence rearrangement of the *T. cruzi* genome.

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