Physical Mapping of a 670-kb Region of Chromosomes XVI and XVII from the Human Protozoan Parasite *Trypanosoma cruzi* Encompassing the Genes for Two Immunodominant Antigens

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As part of the *Trypanosoma cruzi* Genome Initiative, we have mapped a large portion of the chromosomal bands XVI (2.3 Mb) and XVII (2.6 Mb) containing the highly repetitive and immunodominant antigenic gene families *h49* and *jl8*. Restriction mapping of the isolated chromosomal bands and hybridization with chromosome specific gene probes showed that genes *h49* and *jl8* are located in a pair of size-polymorphic homologous chromosomes. To construct the integrated map of the chromosomes harboring the *h49* and *jl8* loci, we used YAC, cosmid, and λ phage overlapping clones, and long range restriction analysis using a variety of probes (i.e., known gene sequences, ESTs, polymorphic repetitive sequences, anonymous sequences, STSs generated from the YAC ends). The total length covered by the YAC contig was approximately 670 kb, and its map agreed and was complementary to the one obtained by long-range restriction fragment analysis. Average genetic marker spacing in a 105 kb region around *h49* and *jl8* genes was estimated to be 6.2 kb/marker. We have detected some polymorphism in the H49/JL8 antigens-encoding chromosomes, affecting also the coding regions. The physical map of this region, together with the isolation of specific chromosome markers, will contribute in the global effort to sequence the nuclear genome of this parasite.

Trypanosoma cruzi is a parasitic protozoan that causes Chagas disease, an ailment without effective drug treatment affecting 16–18 million people in the American continent (Brener 1973). Classical genetic studies on this parasite have been hampered by the existence of many parasite strains with distinct biological and immunological characteristics, the lack of sexual stages, and a high variability in both number and size of chromosomes (Brener 1973; Solari 1995; Cano et al. 1995; Henriksson et al. 1995, 1996; Santos et al. 1997). For this reason, one of the main goals of the recently started *T. cruzi* Genome Initiative is to construct a physical map of the parasite's genome as a resource for DNA sequence analysis (Hanke et al. 1996, 1998; Zingales et al. 1997a; Ferrari et al. 1997; Frohme et al. 1998). A physical map of the *T. cruzi* genome would also facilitate the identification and characterization of genetic loci. Having large chromosome segments that

span these loci cloned in YACs will help to identify genes involved in parasite's survival, pathogenicity, and diagnosis.

Of special interest are the regions harboring genes that encode antigens involved in parasite virulence which might be important for the development of immune therapeutics, drugs, and diagnostic reagents. In *T. cruzi* and other protozoan parasites that infect humans, the occurrence of proteins containing stretches of tandemly arranged amino acid repeats is a commom finding (Frasch et al. 1991). Natural humoral immune responses to many *T. cruzi* antigens appear to be largely directed to epitopes encoded by the repeat units (Frasch et al. 1991; Levin et al. 1991; Umezawa et al. 1999). It has been proposed that repetitive domains may function as ligands for host proteins or immunomodulators (Buscaglia et al. 1999). The repetitive antigens could help the parasite to evade immune response, presenting to the host a large number of nonprotective epitopes (Kemp et al. 1987). The present work focuses on the characterization of a large chro-

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mosomal region harboring genes for the immunodominant repetitive antigens H49 and JL8 as the main markers (Ibanez et al. 1988; Levin et al. 1989; Lafaille et al. 1989; Hoft et al. 1989; Cotrim et al. 1990, 1995). These antigens are high molecular weight proteins located, respectively, along the attachment region between the flagellum and the cell body (Lafaille et al. 1989; Cotrim et al. 1990, 1995) and in the cytoplasm and the peri-nuclear region (Lafaille et al. 1989; Hoft et al. 1995). They are remarkbly immunogenic in man and have been successfully employed in the serological diagnosis of Chagas disease (Levin et al. 1992; Krieger et al. 1992; Carvalho et al. 1993; Paranhos et al. 1994; Pastini et al. 1994; Pereira et al. 1998; Umezawa et al. 1999). The genes *h49* and *jl8* were mapped on two neighboring chromosomal bands of 2.3 and 2.6 Mb (bands XVI and XVII, respectively, in the nomenclature of Cano et al. 1995) of clone CL Brener, the reference strain of the *T. cruzi* Genome Initiative. We present here the long-range restriction analysis and YAC contig assembly of a 670 kb region harboring loci *h49* and *jl8*, which covers 26% of the entire chromosome and includes 21 genetic markers. We also present further evidence supporting the disomic nature of of *H49*/JL8 antigens-encoding chromosomes.

RESULTS AND DISCUSSION

Identification of Markers for Chromosomal Bands XVI and XVII

The chromatin of *T. cruzi* is poorly condensed during nuclear division and chromosomes can not be visualized by conventional cytogenetic methods (Solari 1995). By using pulsed field gel electrophoresis (PFGE) it is possible to separate the chromosome-sized DNA molecules of this protozoan. The molecular karyotype of clone CL Brener consists of 20 chromosomal bands (0.45–3.5 Mb 12 megabase bands (3.5–1.0 Mb), and 8 intermediate bands (0.45–1.0 Mb) (Cano et al. 1995). The distribution of the ethidium bromide fluorescence is not the same for all chromosomal bands indicating that most of the bands contain two or more comigrating chromosomes which are not necessarily homologous (Cano et al. 1995; Henriksson et al. 1996; Santos et al. 1997). Indeed, the existence of homologous chromosomes which differ substantially in size has been suggested (Henriksson et al. 1995, 1996; Cano et al. 1995). For this reason, we refer to chromosomal bands as those bands separated by PFGE and visible after staining with ethidium bromide.

Genes encoding the immunodominant antigens H49 and JL8 were mapped on two neighboring chromosomal bands of 2.3 Mb and 2.6 Mb (bands XVI and XVII, respectively). Densitometric analysis of the DNA content of bands XVI and XVII indicates the presence of at least two comigrating chromosomes per band

which are not necessarily homologous (Cano et al. 1995). To identify additional genetic markers, we have hybridized chromoblots carrying *T. cruzi* chromosomal bands separated by PFGE with a panel of *T. cruzi* cloned sequences (6 repetitive sequences, 42 genes encoding structural proteins and RNAs, 120 ESTs, and 9 STSs). Markers mapped on chromosomal bands XVI and XVII are listed in Table 1. Many genetic markers such as C6, SIRE, E13, minisatellite, B11, SRE, STS–SZ23–14, gp90, gp85, gp82, Tc85, cDNA–Sx23 (gp85-like protein), mucin, and 12 ESTs (TEUF0040, TEUF0078, TEUF0548, TEUF0047, TEUF0052, TEUF0059, TEUF0039, TEUF0051, TEUF131, TEUF133, TEUF0115, TEUF0116) were mapped to chromosomal bands XVI and XVII. This hybridization pattern corroborates the hypothesis that bands XVI and XVII carry size-polymorphic homologous chromosomes (Table 1). The existence of two homologs of a diploid chromosome that differ in size had been reported previously in *T. cruzi* and *Trypanosoma brucei* (Turner et al. 1997; Frohme et al. 1998; Hanke et al. 1998; Melville et al. 1998).

However, other probes hybridized only with either band XVI or band XVII. For instance, some markers such as the spliced leader sequence, trans-sialidase catalytic domain, cDNA–Sx42, TEUF0065, TEUF0101, and TEUF0054 hybridized only with band XVI whereas probes PABP, TEUF0143, TEUF0137, TEUF0132, TEUF0095, TEUF0049, TEUF0206, TEUF0068, TEUF0537, and TEUF0056 hybridized only with band XVII (Table 1). These results could be explained by the presence of nonhomologous chromosomes in both bands XVI and XVII. To test this hypothesis, the chromosomal bands XVI and XVII were digested with *Not*I and hybridized with a *T. cruzi* telomeric sequence (CCCTAA)$_3$ (Chiurillo et al. 1999). In the case where each chromosomal band would contain two or more comigrating heterologous chromosomes, the telomeric probe should hybridize with more than two *Not*I fragments. Although it is difficult to line up the signals for each band, the telomeric probe hybridized with at least four *Not*I restriction fragments per band (not shown). Thus, chromosomal bands XVI and XVII contain comigrating heterologous chromosomes, and the two homologs of a diploid chromosome (*H49*/JL8 antigens-encoding chromosome) that differ in size.

Probes H49 and JL8 Define a Pair of Homologous Chromosomes that Differ in Size

The results above suggest that *h49* and *jl8* loci are located in pair of size-polymorphic homologs chromosomes. We then investigated whether the overall chromosome structure of these homologs is conserved. The chromosomal bands XVI and XVII were separated by PFGE (Fig. 1A), excised from the preparative gels, and incubated with the restriction enzymes *Eco*RI or

Table 1. Summary of Genetic Markers for the Chromosomal Bands XVI and XVII

Marker	GenBank accession no.	Homology or prominent features	Chromosomal band[a]	
			XVI	XVII
h49	L09564	flagellar repetitive antigen	+	+
jl8	AF47956	cytoplasmic repetitive antigen	+	+
Mucin	AF027869	sialic acid acceptor	+	+
gp82	L14824	gp82 surface glycoprotein	+	+
gp90	L11287	gp90 surface glycoprotein	+	+
gp85	J04667	gp85 surface glycoprotein	+	+
Tc-85	AF085686	gp85 surface glycoprotein	+	+
TEUF0040	AA441753	unknown ORF	+	+
TEUF0078	AA426697	γ-1 elongation factor	+	+
TEUF0548	—	unknown ORF	+	+
cDNASX23	—	amastigote gp85-like protein	+	+
B11	U15615	retrotransposon	+	+
SIRE	X75033	interspersed repetitive element	+	+
C6	U16295	interspersed repetitive element	+	+
SRE	M63895	spacer repetitive element	+	+
Satellite	K00393	196-bp minisatellite sequence	+	+
SZ23	—	anonymous marker	+	+
SZ14	—	anonymous marker	+	+
TEUF0047	AA441756	unknown ORF	+	+
TEUF0052	AA676166	histone H3	+	+
TEUF0069	—	unknown ORF	+	+
TEUF0059	AA441765	unknown ORF	+	+
TEUF0039	—	unknown ORF	+	+
TEUF0051	—	unknown ORF	+	+
TEUF0133	AA676182	proteosome 26S subunit	+	+
TEUF0131	AA426657	D18 protein	+	+
TEUF0115	AA433298	unknown ORF	+	+
TEUF0116	AA426656	unknown ORF	+	+
Ts cat.	U01098	trans-sialidase	+	−
SL	K02632	spliced leader sequence	+	−
cDNASX42	—	amastigote gp85-like protein	+	−
TEUF0065	—	unknown ORF	+	−
TEUF0101	AA399705	elongation factor II	+	−
TEUF0054	AA441761	unknown ORF	+	−
TEUF0143	AA433312	unknown ORF	−	+
TEUF0137	AA433310	unknown ORF	−	+
TEUF0132	AA433308	unknown ORF	−	+
TEUF0095	AA441779	unknown ORF	−	+
TEUF0049	AA426691	reverse transcriptase	−	+
TEUF0206	AA426673	unknown ORF	−	+
TEUF0068	AA676169	unknown ORF	−	+
TEUF0537	AA676206	unknown ORF	−	+
TEUF0056	AA441763	tyrosine amino transferase	−	+
PABP	AF044733	poly(A) binding protein	−	+

[a](+) presence or (−) absence of the marker on chromosomal band.

*Bam*HI. After digestion, the restriction fragments were separated by electrophoresis and hybridized to H49 and JL8 probes (Fig. 1B). The restriction patterns of bands XVI and XVII were identical. Probe H49 reacted with two *Eco*RI fragments (20.0 kb and 9.7 kb) and three *Bam*HI fragments (23.0, 10.0, and 5.5 kb) in both chromosomal bands. Probe JL8 hybridized with a 30.0 kb *Eco*RI fragment and a 30.0 kb *Bam*HI fragment in bands XVI and XVII.

We then used rare cutting restriction enzymes, such as *Not*I and *Sf*I, to digest the isolated chromosomal bands (Fig. 1C). Once again, probes H49 and JL8

reacted with the same restriction fragments in both chromosomal bands. Probe H49 hybridized with two *Not*I restriction fragments of about 100 kb and 90 kb indicating the presence of at least two copies of gene *h49*. It is noteworthy that probe JL8 hybridized only to the 100 kb*Not*I restriction fragment, suggesting that gene *jl8* is present in only one copy, located within 100 kb of one of the *h49* genes. As can be seen in Figure 1, the hybridization intensity with the probes JL8 and H49 seems to be similar for each chromosomal band as confirmed by densitometric analysis (not shown). The restriction maps of the isolated chromosomal bands
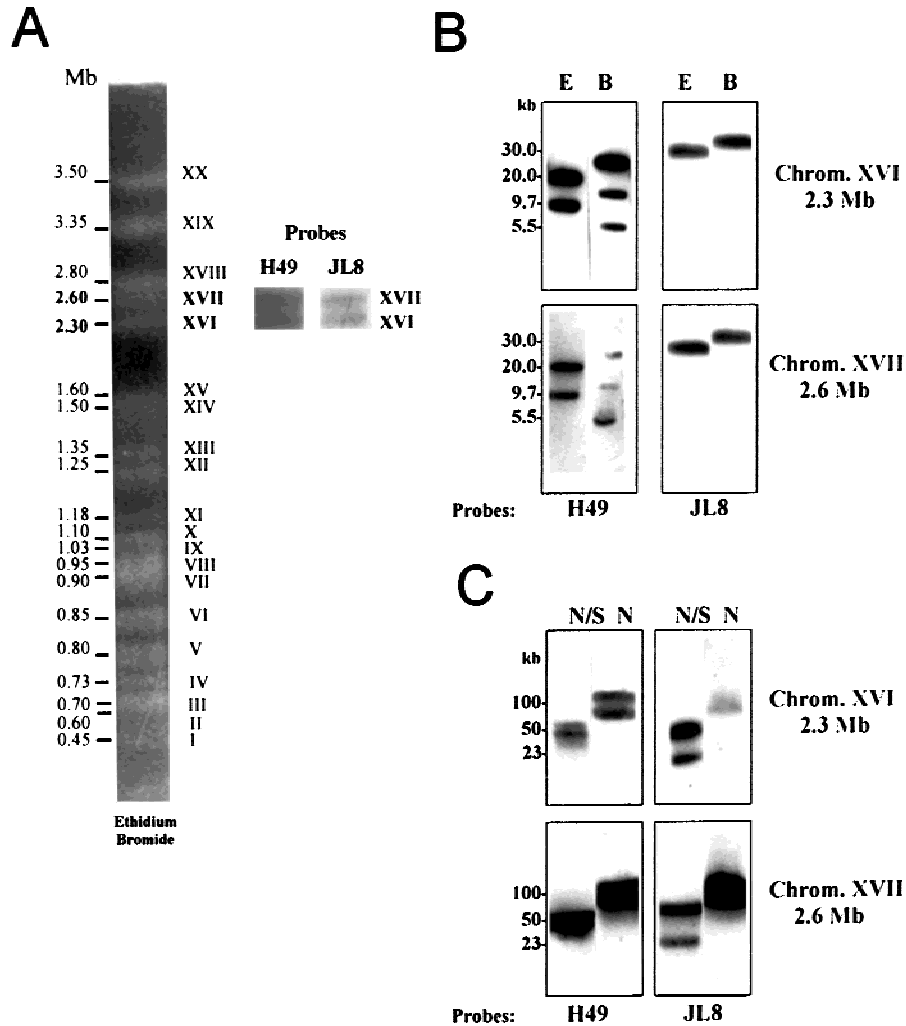
Figure 1 Probes H49 and JL8 define a pair of size-polymorphic homologous chromosomes. (*A*) Separation of *T. cruzi* chromosomal bands by PFGE and staining with ethidium bromide. The arabic and roman numerals indicate the sizes and numbers of the chromosomal bands, respectively. The insert shows the hybridization of probes H49 and JL8 with bands XVI and XVII. (*B*) The chromosomal bands XVI (2.3 Mb) and XVII (2.6 Mb) were isolated from a preparative PFGE gel and digested with *Eco*RI (E) or *Bam*HI (B) restriction enzymes. The restriction fragments were separated by conventional agarose gel electrophoresis, blotted into a nylon membrane, and hybridized with probes H49 and JL8. (C) The chromosomal bands XVI and XVII were isolated from a preparative PFGE gel and digested with *Not*I and *Sfi*I (N/S) or *Not*I (N). Restriction fragments were separated by PFGE (100 V, pulses of 10 sec to 120 sec, 40 hr) and hybridized with probes H49 and JL8.

gene *jl8*. To investigate whether loci *h49* and *jl8* were located near a telomeric region, agarose blocks containing whole chromosomal *T. cruzi* DNA were incubated with increasing amounts of exonuclease *Bal*31 following digestion with *Sfi*I restriction enzyme (Fig. 3). The loci *h49* were located in two *Sfi*I fragments of 350 kb and 450 kb that were sensitive to the treatment with *Bal*31. On the other hand, JL8 probe reacted with two *Sfi*I fragments (50 and 23 kb, note that there is a *Sif*I restriction site within *jl8* gene) which were not affected by the treatment with *Bal*31 except when large amounts of the enzyme were used. These results suggest that the gene order could be: telomere →*h49*→*h49*→*jl8*. The distance between the telomere and the first copy of gene *h49* was estimated to be ≥200 kb.

The two size-polymorphic homologous chromosomes differ by ~300 kb. The size changes observed in these chromosomes are not merely due to telomere repeat rearrangment events because the telomere repeat arrays of the *T. cruzi* chromosomes are short, ranging in size from 0.5–1.0 kb (Chiurillo et al. 1999). Whether other rearrangements are produced by amplification or deletion or by interchromosomal exchange of large DNA segments must still be determined. It is important to note that the size polymorphism of H49/JL8 antigens-encoding chromosomes was also detected in the parental CL strain maintained in different laboratories.

Isolation and Characterization of YAC Clones Harboring Genes *h49* and *jl8*

A *T. cruzi* YAC library (Ferrari et al. 1997) was screened by PCR and dot blot hybridization using specific oligonucleotides derived from genes *h49* and *jl8*. This strategy identified 6 YAC clones which were sized and characterized for content. Two YACs (insert sizes 200 kb and 400 kb) contained the gene *h49* and 4 YACs
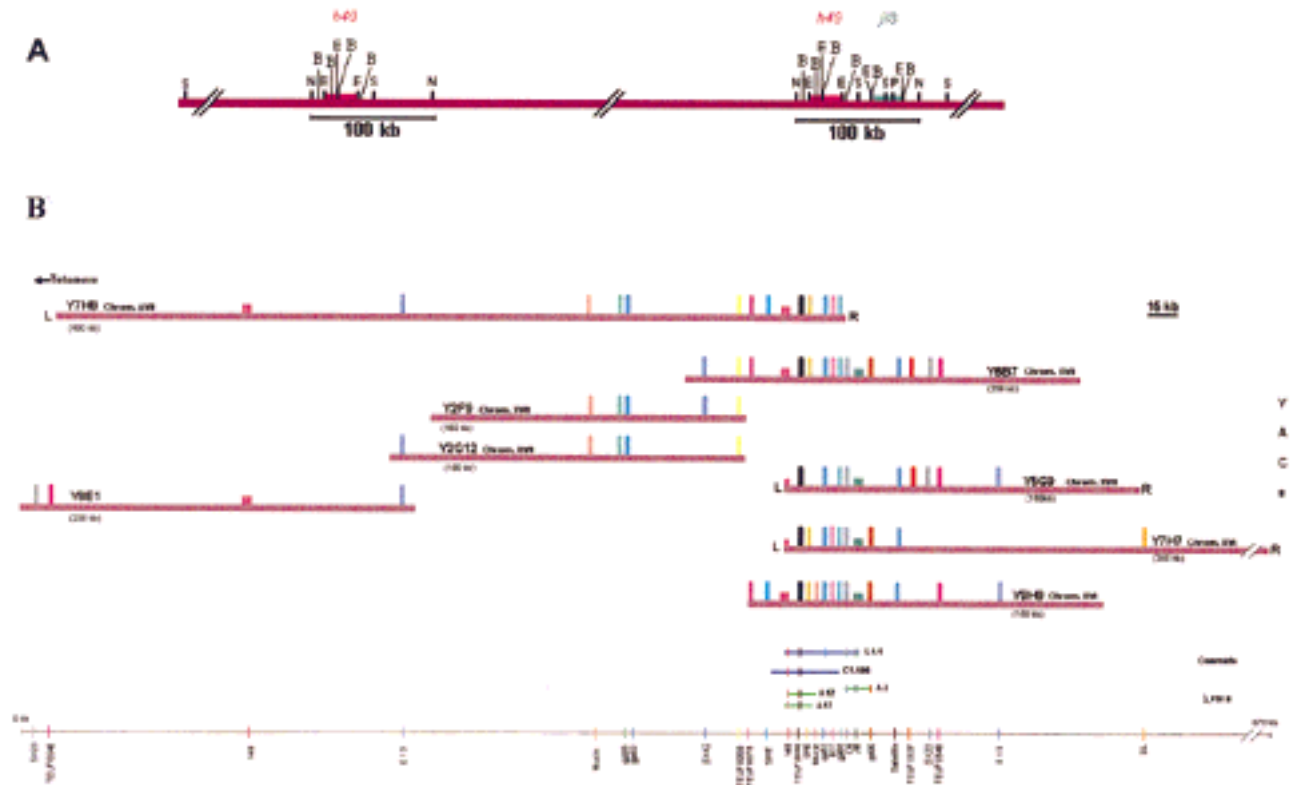
are co-linear with the genomic restriction map (Fig. 2A) obtained by digestion of total chromosomal DNA. Data indicates that the restriction maps of the regions that flank the *h49* and *jl8* genes, covering >400 kb, are identical. This information confirms that these genes are located in a pair of size-polymorphic homologous chromosomes.

Restriction mapping studies indicated that genes *h49* and *jl8* were located on a same 100 kb *Not*I restriction fragment and an additional copy of gene *h49* was mapped on a 90 kb *Not*I fragment (Figs. 1, 2A). This additional copy of gene *h49* was located ~370 kb from

Figure 2 Megarestriction map and YAC contig of the region containing the loci *h49* and *j18*. (*A*) Megarestriction map of the region spanning the loci *h49* and *j18*. As described in legend of Figure 1, *T. cruzi* chromosomal DNA and isolated chromosomal bands XVI and XVII were digested with different restriction enzymes, separated by conventional agarose gel electrophoresis or by PFGE, blotted into nylon membrane, and hybridized with probes H49 and JL8. Restriction enzymes: S, *Sfl*; N, *Notl*; E, *Eco*RI; B, *Bam*HI; P, *Pac*I. Red and green boxes indicate the location of genes *h49* and *jl8*, respectively. To facilitate comprehension, the multiple *Hin*dIII restriction sites within the repetitive region of gene *h49*, and *Hae*III restriction sites within the repetitive region of gene *jl8* were not represented. (*B*) Results of YAC contiging based on marker content of clones. A subset of 8 YACs, 2 cosmids, and 3 λ phages is shown. Marker ascertainment was carried out by PCR and dot blot hybridization. The exact order of markers has not been established, and the intermarker distances are not to scale. There are several discrepancies in the marker content of the map: markers SRE, gp85, gp90, mucin, and B11 are not contained in several YACs. Cosmids and λ phages containing genes *h49* and *jl8* are shown below the YACs. The YAC ends (L, left end; R, right end) were determined by inverse PCR (Materials and Methods).

(insert sizes 180–300 kb) contained the genes *h49* and *jl8*.

To test whether the YAC clones harboring genes *h49* and *jl8* are mitotically stable in long term cultures, they were grown for 75 generations and examined by Southern blot analysis. No rearrangements were observed suggesting the YAC clones are propagated faithfully during mitosis. This observation was further supported by restriction analysis of *h49* and *jl8* loci after several rounds of propagation. The hybridization profiles obtained with these probes were identical to those obtained with *T. cruzi* genomic DNA (see below). These data suggest that the YAC clones carry authentic *h49* and *jl8* loci and are stable. Finally, to check whether more than one YAC recombinant was present in a single yeast colony, YAC clones were separated by PFGE and hybridized with ^{32}P-labeled pBR322 sequences (present in the YAC cloning vector) or *T. cruzi* total genomic DNA. This assay confirmed that all colonies tested contained a single YAC recombinant.

Establishment of YAC Contig and Colinearity with the Genomic Restriction Map

To determine whether the physical maps of YACs are colinear with the genomic map, YAC DNAs were digested with *Eco*RI and hybridized with H49 and JL8 probes (Fig. 4). Clones Y7H8, Y9H8, Y8E1, and Y6B7 contained two *Eco*RI fragments of 20 kb and 9.7 kb hybridizing with the H49 probe, while clones Y7H7 and Y5G9 presented only the 20 kb *Eco*RI fragment. Clones Y7H7, Y5G9, Y9H8, and Y6B7 presented an additional *Eco*RI fragment of 30 kb that hybridized with the JL8 probe. It is interesting to note that the *T. cruzi* YAC library was constructed with fragments obtained by partial digestion of *T. cruzi* genomic DNA with *Eco*RI, and the genes *h49* and *jl8* are flanked by *Eco*RI restriction sites (Fig. 2A). This could explain the presence of several clones which contain only the 20 kb *Eco*RI fragment recognized by the H49 probe.

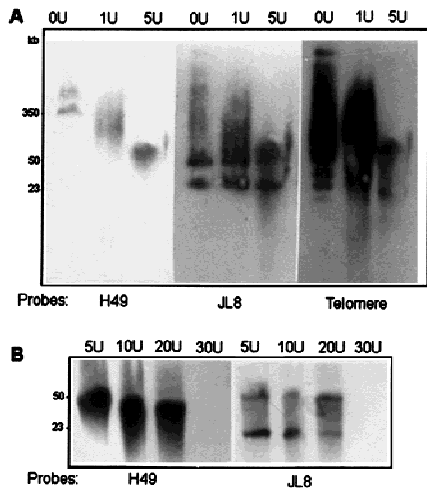As shown above, genomic restriction mapping indicated that genes *h49* and *jl8* were located in one 100

Figure 3 Nuclease *Bal*31 sensitivity of genes *h49* and *jl8*. Agarose blocks containing whole chromosomal *T. cruzi* DNA were incubated with increasing amounts of exonuclease *Bal*31 (1–5 units in panel A, and 5–30 units in panel B) during 6 h at 30°C following digestion with *Sfi*I restriction enzyme. DNA restriction fragments were resolved by PFGE and hybridized with probes H49 and JL8, and a *T. cruzi* telomere sequence.

kb *Not*I restriction fragment, and an additional copy of gene *h49* was located in a *Not*I restriction fragment of ~90 kb. The distance between the copies of the *h49* genes should be ~300 kb and according to the genomic map, YAC clones harboring two copies of the gene *h49* must generated two *Not*I restriction fragments recognized by probe H49. This fact was demonstrated for the clone Y7H8 (data not shown).

To determine the overlapping sections of the YACs and their localization with regard to the genomic restriction map, EST and STS markers, previously assigned to chromosomal bands XVI and XVII, were hybridized with YAC clones in a dot blot hybridization and/or detected by PCR (Table 1). The resulting contig comprises 8 overlapping YAC clones (Fig. 2B) that were aligned based on the presence or absence of 21 DNA markers: 6 repetitive DNA polymorphic sequences (SIRE, C6, SRE, retrotransposon B11, E13, 196-bp minisatellite sequence), 10 genes for known *T. cruzi* proteins and structural RNA (H49, JL8, gp90, gp82, gp85, trans-sialidase, cDNA Sx23, cDNA Sx42, mucin, spliced leader sequence), and 5 ESTs (TEUF0548, TEUF0068, TEUF0078, TEUF0040, TEUF0537). Subsequent screening of the *T. cruzi* YAC library with EST TEUF0068, a specific marker for chromosomal band XVII, allowed us to isolate two clones (Y2F9, Y2G12) that covered the region localized between the two copies of gene *h49*. To further confirm the distal position of clones Y7H8 and Y7H7 at the two extremes of the contig, and the relative positions of YACs, we used YAC left arm primers to amplify by PCR the insert sequences immediately adjacent to the YAC vector.The amplimer of the left arm of Y7H8 hybridized with clone Y8E1 but not

with other YACs, while the left amplimer of clone Y7H7 reacted with clones Y5G9, Y9H8, and Y6B7. The contiging of YACs was also confirmed by comparison of the restriction site maps of the recombinant clones with that of *T. cruzi* genomic DNA (not shown).

As expected, markers belonging to repetitive DNA or multigene families encoding surface glycoproteins mapped at different sites of the YAC contig. For instance, copies of the polymorphic repetitive sequence E13 (10,000 copies/haploid genome, Requena et al. 1996) were found on both sides of the YAC contig. Genes encoding *T. cruzi* surface glycoproteins (gp90, gp85, gp82, cDNA Sx23, cDNA Sx42, mucin) are part of a large polymorphic group (Colli 1993; Salazar et al. 1996; DiNoia et al. 1998; Freitas et al. 1998) and they were mapped in different YACs. The repertoire of surface glycoprotein related genes is very large, gross calculations placing the figure in hundreds of copies (Peterson et al. 1989; Khan et al. 1991; Franco et al. 1993; Araya et al. 1994; DiNoia et al. 1998; Freitas et al. 1998). Our results are in agreement with previous data showing the presence of many clusters of genes encoding *T. cruzi* surface glycoproteins spread throughout the genome (Cano et al. 1995; Salazar et al. 1996; Chiurillo et al. 1999).

The described YAC contig represents a genuine reconstruction of a region of the genome, despite missing sequences in several YACs. The hybridization data revealed several minor differences in the gene content of the size polymorphic H49/JL8 antigens-encoding chromosomes. The presence or absence of chromosome specific markers such as the ESTs TEUF0068 and TEUF0537 for band XVII or the spliced leader sequence for band XVI, allowed us to define whether the YAC clone was derived from band XVI or XVII. For instance, clones Y7H8, Y6B7, Y2F9, and Y2G12 were derived from band XVII, whereas clones Y7H7 and Y5G9 were derived from band XVI. This result indicates the existence of some sequence polymorphism in the H49/JL8
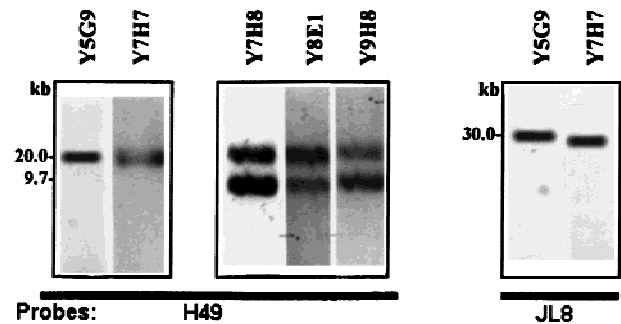

Figure 4 Presence of *h49* and *jl8* genes on YAC clones. YAC clones containing genes *h49* and *jl8* (Y5G9 and Y7H7) or gene *h49* (Y7H8, Y8E1 and Y9H8) were digested with *Eco*RI, and the restriction products were resolved by gel electrophoresis, blotted, and hybridized to probes H49 and JL8.

antigens-encoding chromosomes. In this case, the chromosome size polymorphism could affect the coding regions and would not be solely confined to repeated sequence, as observed in *T. brucei* (Melville et al. 1998).

We have also found that some polymorphic repetitive sequences (SRE, SIRE, B11, E13) and genes encoding surface glycoproteins (mucin, gp90, Sx42, Sx23) appear to be absent in several YACs, confirming polymorphism between bands XVI and XVII. *T. cruzi* reproduces asexually by binary fission and sexual reproduction has never been demonstrated in this parasite (Solari 1995; Tibayrenc et al. 1986). The loss of markers on chromosomal homologs could be common in *T. cruzi*, possibly as a consequence of a high frequency of mitotic recombination events; in this kind of recombination chromosome pairing may not be very precise.

Interestingly, many genetic markers were concentrated in a region of about 105 kb including loci *h49* and *jl8* (Fig. 2B). Further confirmation of this arrangement was obtained by analysis of recombinant cosmids (insert sizes ~30 kb) harboring *h49* and/or *jl8* genes, and λphage recombinant clones (insert sizes 15–20 kb) containing *h49* or *jl8* genes (Fig. 2B). Data indicates that the majority of the gene markers are clustered in 1/6 of the whole contig, with an average estimated marker spacing of 1 marker/6.2 kb in the 105 kb region. This might be due either to an unequal gene distribution or to the absence, by chance, of markers for the remainder of the contig. In the latter case, the cloned material present in the YAC contig shown in Figure 2B may prove useful for the isolation of those still unknown genes/markers.

Recently, Andersson et al. (1998) reported the complete sequence of a 93.4 kb contig from *T. cruzi* chromosome 3. This contig contains a strand-switch region where genes appear to be organized in two long clusters harboring multiple genes on the same strand, with the two clusters oriented in opposite directions. We currently are trying to determine the transcription direction of genes in the region around the *h49* and *jl8* loci. The mapping of *T. cruzi* chromosomes indicates the complexity of the genomic DNA in this organism. We have demonstrated the existence of some sequence polymorphism in the H49/JL8 antigens-encoding chromosomes. Thus, the construction of a clone map prior or concomitantly to DNA sequencing is an essential step for the identification of homologous regions and determination of rearrangements.

METHODS

Separation of *T. cruzi* Chromosomal DNA by PFGE

Epimastigotes from *T. cruzi* clone CL Brener (Zingales et al. 1997b) were grown to late logarithmic phase (5–10 × 10⁶/ml) and collected by centrifugation. Cells were resuspended in phosphate-buffered saline and mixed with an equal volume of 1% low-melting point agarose. Approximately 1×10^8 cells were used for each gel plug and incubated in a solution containing 0.5 M EDTA (pH 8.0), 1% sodium lauroylsarcosinate, and 1 mg/ml proteinase K at 50°C for 48 hr and stored at 4°C in 0.5 M EDTA (pH 8.0).

Chromosomal bands were separated on agarose gels in a Gene Navigator apparatus (Pharmacia) using a hexagonal electrode array (Cano et al. 1995). PFGE was carried out in 1.2% agarose gels in 0.5 × TBE (45 mM Tris45 mM boric acid, 1 mM EDTA [pH 8.3] at 13°C for 132 hr. Separation was carried out with 5 phases of homogeneous pulses with interpolation at 80 V: phase 1, pulse time 90 sec (run time 30 hr); phase 2200 sec (30 hr); phase 3350 sec (24 hr); phase 4500 sec (24 hr) and phase 5800 sec (24 hr) (Cano et al. 1995). Gels were stained with ethidium bromide (0.5 µg/ml), photographed, and transferred onto nylon filters.

Isolation of Chromosomal Bands XVI and XVII

Plugs containing whole *T. cruzi* chromosomal DNA were incubated for 1 hr in TE buffer (10 mM Tris-HCl [pH 8.0], 5 mM EDTA) containing 1 mM phenylmethylsufonyl fluoride and washed twice in TE buffer. Chromosomal bands XVI and XVII were separated by PFGE as described above and excised from the preparative agarose gels. Agarose gel slices were washed twice in TE buffer, twice with the restriction endonuclease buffer, and incubated for 1 hr at 4°C with the restriction endonuclease buffer. The restriction enzyme was added (50 units per slice) followed by incubation for 12 hr at the appropriate temperature. In order to check for chromosomal integrity during manipulations, a chromosomal band was manipulated exactly as described above, but without restriction enzyme.

Screening of a *T. cruzi* YAC Library and Analysis of YAC clones

The YAC library was constructed using DNA of epimastigotes of clone CL Brener in pYAC4 (Ferrari et al. 1997). The library consists of 2,770 individual YACs with a mean insert size of 365 kb, encompassing ~10 genome equivalents. The library was screened by two different methods. The first involved PCR on DNA pools using primers derived from genes *h49* and *jl8* and subsequent hybridization of specific pool blots. The second method utilized direct hybridization of DNA probes labeled with ³²P to YAC library grid blots as described previously (Ferrari et al. 1997).

Cell plugs (100 µl) were prepared in low melting point agarose from yeasts grown in overnight cultures at 30°C on YPD liquid medium. The plugs were incubated at 30°C for 3 hr in SCEM buffer (1 M sorbitol, 50, mM EDTA, 0.1 M sodium citrate, and 7 mM β-mercaptoethanol) with zymolyase (10 units/plug) at 37°C. They were then washed and treated with 1 ml PKB [0.1 M NaCl, 50 mM EDTA (pH 8.0), 1% *N*-laurylsarcosine] with 1 mg/ml of proteinase K at 50°C overnight and finally washed several times with 0.5× TBE to remove proteinase K. Yeast chromosomes were separated by electrophoresis on 1% low-melting agarose gels in 0.5 × TBE using a contour-clamped homogenous electric field (CHEF DRIII, BioRad) apparatus with pulse times of 60–90 sec for 18 hr at 12°C, 220 V. After migration, the gels were stained with ethidium bromide and transferred to nylon membranes. Membranes were hybridized overnight with radiolabeled probes at 42°C in 50% formamide, 5× Denhart's solution, 5×

SSC ($1\times$ SSC = 0.15 M NaCl, 0.015 M sodium citrate), 50 µg/ml yeast tRNA, 100 µg/ml sonicated herring sperm DNA and 0.1% SDS. After hybridization, filters were washed in $0.1\times$ SSC, 0.1% SDS at 55°C for 1 hr.

YAC DNA embedded in agarose was digested with restriction enzymes, separated on agarose gels, and transferred to nylon membranes. Radiolabed probes were used for hybridization as described above. For PCR assays, plugs containing YAC DNA were treated with agarase (Epicentre Technologies, France); the DNA was diluted in water, boiled for 10 min, and stored at $-70°$C. PCR reactions were carried out in 50 µl reaction volume mixtures containing 200 µM of each deoxyribonucleotide triphosphate, $1\times$ *Taq* polymerase buffer (Perkin Elmer, CA), and 1.25 units of Taq polymerase. The DNA was denatured at 94°C, and 30 cycles of amplification were performed with denaturation at 94°C for 1 min, annealing temperature specific to each primer for 1 min, and extension at 72°C for 30 sec. A final extension was performed at 72°C for 6 min. The products were separated by electrophoresis on agarose gels and visualized by ethidium bromide staining. DNA fragments were transferred to nylon membranes and hybridized with specific probes.

Vector end PCR was performed as described by Ochman et al. (1988). DNA was digested with *Hae*III and digested fragments were ligated. PCR was perfomed on the ligated fragments using primers Y5U and Y5R (Joslyn et al. 1991), specific for the left arm of vector pYAC-4, thus amplifying the YAC end fragments. The PCR products were isolated, radiolabeled, and hybridized against the other YACs.

Screening of *T. cruzi* Cosmid and Lambda Phage Libraries

A chromosome-specific cosmid library was constructed using DNA fragments from the chromosomal bands XVI and XVII purified from PFGE preparative gels. The chromosomal bands were partially digested with *Bam*HI and inserted into the *Bam*HI cloning site of cosmid Lawrist 4. Ligation, packaging, and transfection were carried out as described by Hanke et al. (1996). A *T. cruzi* (clone CL Brener) genomic library constructed in lambda phage *Fix*II vector was kindly provided by E. Rondinelli (UFRJ, Rio de Janeiro, Brasil) through the *T. cruzi* genome network. Cosmid and lambda libraries were screened by hybridization with H49 and JL8 probes.

DNA Probes

ESTs derived from an epimastigote cDNA library (clone CL Brener) were obtained as cDNA fragments by PCR (Brandão et al. 1997). All partially sequenced cDNAs (ESTs) are identified by TEUF number originating in the sequencing laboratory (Table 1). For other cloned *T. cruzi* DNA sequences (Table 1), the inserts of recombinant plasmids were excised by digestion with appropriate restriction enzymes, purified by gel electrophoresis, and radiolabeled with ^{32}P. Hybridization of the probes with the chromoblots was carried out as described previously (Cano et al. 1995).

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