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Characterization of a cDNA Clone Encoding the Carboxy-Terminal Domain of a 90-Kilodalton Surface Antigen of *Trypanosoma cruzi* Metacyclic Trypomastigotes

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We have cloned and sequenced a cDNA for a metacyclic trypomastigote-specific glycoprotein with a molecular mass of 90 kDa, termed MTS-gp90. By immunoblotting, antibodies to the MTS-gp90 recombinant protein reacted exclusively with a 90-kDa antigen of metacyclic trypomastigotes. The insert of the MTS-gp90 cDNA clone strongly hybridized with a single 3.0-kb mRNA of metacyclic forms, whereas the hybridization signal with epimastigote mRNA was weak and those with RNAs from other developmental stages were negative, indicating that transcription of the MTS-gp90 gene is developmentally regulated. A series of experiments showed that the MTS-gp90 gene is present in multiple copies in the *Trypanosoma cruzi* genome, arranged in a nontandem manner, and that there are at least 40 copies of the gene per haploid genome. Sequence analysis of recombinant MTS-gp90 revealed 40 to 60% identity at the amino acid level with members of a family of mammalian stage-specific, 85-kDa surface antigens of *T. cruzi*. However, there are considerable differences in the amino acid compositions outside the homology region.

During its life cycle, *Trypanosoma cruzi*, a protozoan parasite that causes Chagas' disease, alternates between different developmental stages in both the triatomine vector and the vertebrate host. In the digestive tract of the insect, the parasite grows as an epimastigote, which later differentiates into the infective metacyclic trypomastigote. Metacyclic forms eliminated in the feces can initiate infection in the mammalian host by invading a variety of cell types. After intracellular replication as an amastigote and then transformation into a trypomastigote, the host cells rupture and the parasites are released into the bloodstream.

Surface molecules of *T. cruzi*, in particular those of the infective trypomastigote stages which may be involved in the process of host cell invasion or may constitute targets for recognition by the host immune system, have been studied intensively (1, 2, 4, 8, 16, 18, 20, 24). Genes encoding surface antigens, such as the 85-kDa antigen and SAPA, which is an antigen with neuraminidase–*trans*-sialidase activity shed during acute *T. cruzi* infection, have been cloned and sequenced (1, 10, 13, 21–23, 28, 29). It should be noted that all of these studies have been performed with tissue culture-derived trypomastigotes, the equivalent of blood trypomastigotes. We have shown previously that metacyclic trypomastigotes bear on the surface a set of molecules that have no counterpart in blood trypomastigotes (30, 34). The stage-specific 90-kDa surface antigen, herein referred to as MTS-gp90, is one such molecule. It is a glycoprotein containing N-linked oligosaccharide side chains of high-mannose type (33), anchored to the membrane through a glycosylphosphatidylinositol moiety (12, 26) and expressed by metacyclic forms of different *T. cruzi* strains (17). The involvement of MTS-gp90 in cell invasion has been suggested by the inhibitory effect of a monoclonal antibody directed to this antigen (3, 33). MTS-gp90 is also of considerable interest immunologically. When inoculated in mice in its purified form, the antigen induces the production of antibodies that react with metacyclic trypomastigotes, stimulates the T-cell response, and protects mice against acute infection by *T. cruzi* provided that antigen is given with alum as the adjuvant (3, 11). In the past few years, we have attempted to clone the gene corresponding to this stage-specific antigen.

Here we report the characterization of a cDNA clone encoding the C-terminal domain of MTS-gp90.

MATERIALS AND METHODS

Parasites. Epimastigotes and metacyclic trypomastigotes of *T. cruzi* G (32) were grown in liver infusion-tryptose medium. Purification of metacyclic trypomastigotes was carried out as described previously (30). Amastigotes and tissue culture-derived trypomastigotes were harvested from Vero cell cultures.

Nucleic acid isolation and cloning. Total RNA was extracted from metacyclic trypomastigotes with guanidinium isothiocyanate and purified by centrifugation on a cushion of 5.7 M CsCl (15). Poly(A)$^+$ RNA was prepared by oligo(dT) chromatography, and the first- and the second-strand cDNAs were synthesized from 5 μg of poly(A)$^+$ RNA, using reagents from a cDNA Synthesis System Plus Kit (Amersham). After addition of *Eco*RI adaptors (Amersham), the cDNA was inserted into the *Eco*RI site of a phage lambda gt11 expression vector (Promega Corp., Madison, Wis.). Recombinant bacteriophages were screened for expression of *T. cruzi* antigens as described before (6, 19), using either monoclonal antibody 5E7 or polyclonal monospecific antiserum to MTS-gp90, prepared by immunizing BALB/c mice with antibody affinity-purified antigen and adsorbed with *Escherichia coli* before use. The original cDNA clones were subcloned into M13 bacteriophage and Bluescript plasmids (Stratagene) for sequence analysis and into pGEX expres-

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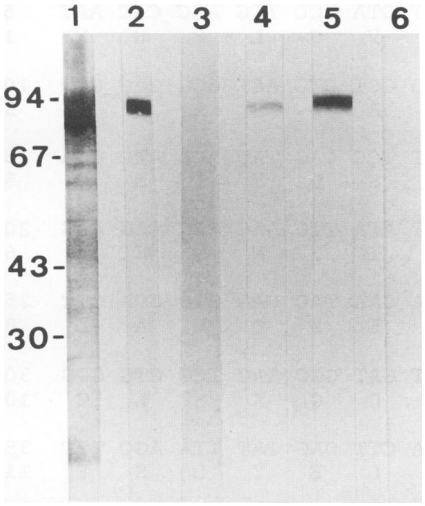

FIG. 1. Identification of *T. cruzi* native protein that shares B-cell epitopes with MTS-gp90 recombinant protein. Extracts of metacyclic trypomastigotes were subjected to Western blotting, and the nitrocellulose strips were probed with the following: rabbit antiserum to metacyclic trypomastigotes (lane 1), rabbit antibodies immunopurified on phages expressing MTS-gp90 antigen (lane 2) or on nonrecombinant lambda gt11 (lane 3), mouse antisera to the purified MTS-gp90 recombinant fusion protein (lane 4), monoclonal antibody 5E7 (lane 5), or mouse antisera to GST (lane 6). Molecular sizes, in kilodaltons, are shown on the left.

sion plasmids (27). DNAs were extracted from parasites and recombinant clones by standard techniques (15).

DNA sequencing. Nucleotide sequences were determined by the dideoxy-nucleotide chain termination method (25), using Sequenase (U.S. Biochemicals, Cleveland, Ohio). Templates of *T. cruzi* cDNA clones were sequenced with specific oligonucleotide primers. Parts of the sequence were confirmed by using overlapping subclones generated by digestion of inserts with appropriate restriction enzymes. The sequences were analyzed and compared by using PCGENE SOFTWARE and the University of Wisconsin Genetics Computer Group programs (9).

Radiolabeling and Southern and Northern transfer. DNA restriction fragments were radiolabeled with [α-^{32}P]deoxynucleotide triphosphate, using a random primer labeling kit from Bethesda Research Laboratories. Northern (RNA) and Southern blot hybridizations with DNA fragments were carried out at 42°C in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt's solution–0.1 mg of ml^{-1} sonicated herring sperm DNA ml^{-1}–0.05 mg of yeast tRNA ml^{-1}–0.1% sodium dodecyl sulfate (SDS). After hybridization, filters were washed in 0.1× SSC–0.1% SDS for 1 h at 65°C (Southern blot) or 50°C (Northern blot).

Antibodies. Monoclonal antibody 5E7 and monospecific antisera to the purified MTS-gp90 were produced as described elsewhere (11, 30). Antisera to whole metacyclic trypomastigotes were prepared by immunizing rabbits with merthiolate-treated metacyclic forms. Antibodies against the MTS-gp90 recombinant protein were purified from rabbit anti-metacyclic serum by the plaque antibody selection method (6, 19). Briefly, recombinant phages were plated at a density of 10^5 per 150-mm LB agar plate and incubated at 42°C for 2 to 3 h. Nitrocellulose filters, soaked in 10 mM isopropylthiogalactoside (IPTG), were layered on the plate, and the plates were incubated for another 2 h. The filters were then incubated with serum diluted 1:50. After washing, bound antibodies were eluted with 5 ml of 0.2 M glycine (pH 2.8) and neutralized with Tris base (19). These antibodies were used to probe Western blots (immunoblots) of parasite extracts.

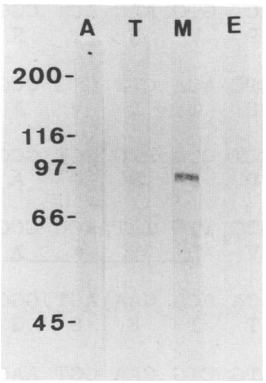

FIG. 2. Reactivity of anti-MTS-gp90 antibodies with different developmental forms of *T. cruzi*. Western blots of extracts of amastigotes (A), tissue culture trypomastigotes (T), metacyclic trypomastigotes (M), and epimastigotes (E) were probed with rabbit antibodies immunopurified on MTS-gp90 recombinant protein. Numbers on the left correspond to molecular sizes, in kilodaltons.

Antibodies against MTS-gp90 recombinant protein were obtained by immunization of mice with the protein fused to *Schistosoma japonicum* glutathione *S*-transferase (GST), prepared as follows. The whole insert and the 762-bp *Bam*HI-*Sma*I fragment of the MTS-gp90 cDNA clone (see Fig. 5) were excised after digestion with *Bam*HI or *Bam*HI-*Sma*I, respectively, and subcloned into plasmid pGEX-3 in frame with the GST gene (27). Recombinant fusion proteins were obtained from IPTG-induced bacterial lysates, as described previously (27), by mild sonication on ice phosphate-buffered saline–1 mM phenylmethylsulfonyl fluoride and centrifugation at 10,000 × *g* for 10 min at 4°C. Fusion protein was extracted from the pellet with 8 M guanidine-HCl–60 mM ethanolamine (pH 9.0) and concentrated. Mice received

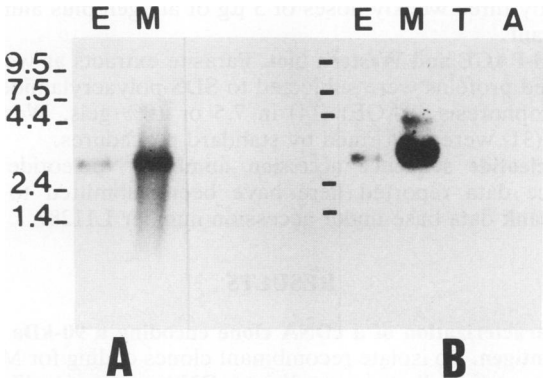

FIG. 3. Identification of *T. cruzi* transcripts complementary to the MTS-gp90 gene. Northern blots of poly(A)$^+$ (A) or total (B) RNA were hybridized with the labeled insert of the MTS-gp90 cDNA clone. Poly(A)$^+$ RNA or total RNA was isolated from epimastigotes (E), metacyclic trypomastigotes (M), tissue culture trypomastigotes (T), and amastigotes (A). Numbers on the left correspond to molecular sizes, in kilobases.

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1     CCT GTG AAC ACT CGG GTT AAT AAT GTG CGC CAT GTA TCC TTG AGC CAC AAC   51
1      P   V   N   T   R   V   N   N   V   R   H   V   S   L   S   H   N   17

52    TTC ACA CTT GTG GCG TCG GTG ACC ATC GAA GAG GCT CCG AGT GCG GAC GCT  102
18     F   T   L   V   A   S   V   T   I   E   E   A   P   S   A   D   A   34

103   CCT CTA ATG GGT GCG ATG CTG GGG GAC ACT AAT TCC CAG TAT ACC ATG GGA  153
35     P   L   M   G   A   M   L   G   D   T   N   S   Q   Y   T   M   G   51

154   GTC TTG TAT ACC GCG GAT AAA GAA TGG GTA ACT ATA TTC AAC GGC AAG AAG  204
52     V   L   Y   T   A   D   K   E   W   V   T   I   F   N   G   K   K   68

205   ACA ACA GAA AGT GGC ACT TGG GAG CCG GGG AAA GAA TAC CAA GTG GCA CTC  255
69     T   T   E   S   G   T   W   E   P   G   K   E   Y   Q   V   A   L   85

256   ATG CTG CAA GGT AAC AAG AGC TCG GTG TAC GTT GAT GGC AAG TCG CTG GGG  306
86     M   L   Q   G   N   K   S   S   V   Y   V   D   G   K   S   L   G  102

307   GAA GAA GAA TTG CCG TTA CAA AGT GAG AGG CCA CTT GAG TAT TTA AGC TTT  357
103    E   E   E   L   P   L   Q   S   E   R   P   L   E   Y   L   S   F  119

358   TGC TTT GGC GGG TGC GGT ATA AAA AAC TTT CCT GTG ACT GTG AAG AAC GTC  408
120    C   F   G   G   C   G   I   K   N   F   P   V   T   V   K   N   V  136

409   TTT CTG TAC AAC CGC CCA CTG AAT CCC ACT GAG ATG ACT GCA ATC AAG GAC  459
137    F   L   Y   N   R   P   L   N   P   T   E   M   T   A   I   K   D  153

460   AGG AAA CCC AAA GAT GAA AAA GGA AGG TGA CAGCTCCATGCGTGAGGGTATGCCTCG  516
154    R   K   P   K   D   E   K   G   R   -                             162

517   GGTGCTGCTGCTGCTGCTGGGGATGTGGGGCATTGCGGCCATTTAATGAGAAATCGCAGTGAGTATT  583
584   TCCTGCCACCAGCGGGAGCTATATGTATCTCTCCATGCACCCTTCGAAATAATTGACTTTTGTGTGG  650
651   ATGTACCTTTTGTTTTATCTGTTTTTATTTTCGATTGGACTACCCCTTTCTGCCCGTGTAAGTCGTT  717
718   ATGTAAAGGGCGTGCTCTCCCTCCCGGGACTGCATCGCGGGCCTTTTTTTCTTTTTTTCTTATATAT  784
785   ATATATTTTTTATATGCTCGTTTTTTTTTAGTGGAATATTTTGAGATTGGCGGGATGTCTTTAATTT  851
852   TTCCGTCGGCCGATGACACGTGCAGTCGGCATTGAGCTCCACGACGATGGATGGTGCGGTGAAGAAG  918
919   GGATTTAAGATGTGCAGCAACCACTGAATGTCCGATGAAATGTGGGTGTTGTGTGGACACCAGGAGG  985
986   CTGAACGGCTGACGCAATATATTTTTACTTCTGCGGGAAGAATATCACGTGTTTTTTTCTTTTCTTT 1052
1053  TTTTTTGCGTCACGATGTTTTTCTTTTTTGTTTTTTTGTTATATTTCTGTGGGTGTGGGAAATGCTT 1119
1120  CAAGGGGACTGTTTTTTTTTTCGTTTTAATTTCGTTTTAATTTCTTTTTTTTGTTTTAAGTTTTTTT 1186
1187  TTTTTGTTTCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA                    1236
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FIG. 4. Nucleotide and predicted amino acid sequences of the insert of MTS-gp90 clone. Potential N-glycosylation and myristoylation sites are in boldface, doubly underlined and underlined, respectively. The region from amino acids 53 to 60 corresponds to one of the neuraminidase motifs.

intraperitoneally a first dose of 10 μg of MTS-gp90 recombinant protein absorbed in alum; this was followed 10 days later by three weekly doses of 5 μg of antigen plus alum as adjuvant.

SDS-PAGE and Western blot. Parasite extracts as well as purified proteins were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) (14) in 7.5 or 10% gels. Western blots (31) were performed by standard procedures.

Nucleotide sequence accession number. Nucleotide sequence data reported here have been submitted to the GenBank data base under accession number L11287.

RESULTS

Characterization of a cDNA clone encoding a 90-kDa surface antigen. To isolate recombinant clones coding for MTS-gp90, a metacyclic trypomastigote cDNA expression library was screened with monoclonal antibody 5E7 and a monospecific antiserum to MTS-gp90. A cDNA clone, encoding a recombinant protein that strongly reacted with both antibodies, was isolated and further characterized.

In a series of experiments, we confirmed that the cDNA clone contains sequences encoding MTS-gp90. We used the recombinant protein to affinity purify antibodies from a rabbit antiserum to metacyclic trypomastigotes. By immunoblotting, the purified antibodies specifically recognized a 90-kDa molecule in metacyclic trypomastigote extracts (Fig. 1, lane 2). The parasite antigen also reacted with mouse antiserum generated by immunization with the MTS-gp90 recombinant protein (Fig. 1, lane 4). These data indicate that the B-cell epitopes on the MTS-gp90 recombinant protein are shared by a 90-kDa surface antigen of metacyclic trypomastigotes. Furthermore, the antibodies selected from anti-metacyclic trypomastigote rabbit serum by adsorption to MTS-gp90 recombinant protein recognized on Western blot, a 90-kDa band exclusively in metacyclic trypomastigote extracts, without any detectable reaction to amastigotes, tissue culture-derived trypomastigotes, or epimastigotes (Fig. 2).

That transcription of the MTS-gp90 gene is developmentally regulated was demonstrated by Northern blot analysis. When the insert of the MTS-gp90 cDNA clone was hybridized to a Northern blot containing either poly(A)$^+$ RNAs purified from epimastigotes or metacyclic trypomastigotes (Fig. 3A) or total RNAs isolated from different *T. cruzi* developmental stages (Fig. 3B), the probe reacted essentially

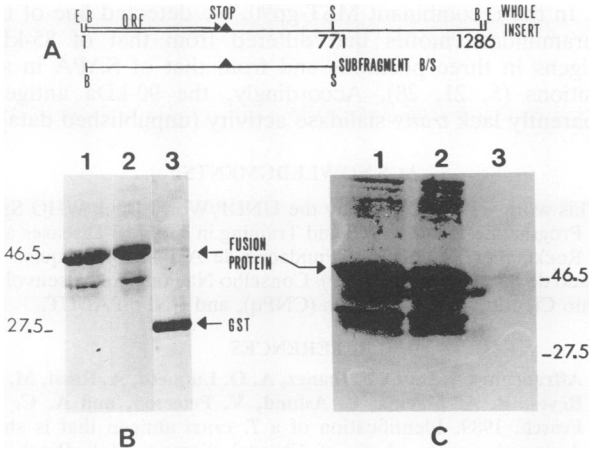

FIG. 5. Subcloning and expression of subfragments of MTS-gp90 cDNA clone in plasmid pGEX-3. (A) Diagrammatic representation of the MTS-gp90 cDNA clone showing the predicted open reading frame (ORF), the first stop codon (▲), and restriction sites for *Eco*RI (E), *Bam*HI (B), and *Sma*I (S). *Eco*RI and *Bam*HI sites are derived from the adaptor used in cDNA cloning. The whole insert and the 762-bp *Bam*HI-*Sma*I subfragment of the cDNA clone were subcloned in frame with the GST gene of plasmid pGEX-3. (B) Coomassie blue-stained SDS-PAGE gel of proteins encoded by recombinant pGEX-3 carrying the whole insert (lane 1) or the *Bam*HI-*Sma*I subfragment (lane 2) and the nonrecombinant pGEX-3 (lane 3). Arrows indicate the GST and MTS-gp90 fusion proteins. (C) Western blot probed with monoclonal antibody 5E7.

with a metacyclic trypomastigote RNA of 3.0 kb, with a weak hybridization signal with epimastigote mRNA that could be due to a small contamination of epimastigote samples by metacyclic trypomastigotes. Hybridization with RNAs of other developmental stages was negative (Fig. 3B), even under mild washing conditions (0.1× SSC at 42°C for 1 h).

Sequence analysis. The nucleotide sequence of the 1,236-bp insert of the MTS-gp90 cDNA clone and the predicted amino acid sequence of the polypeptide are shown in Fig. 4. Translation of the nucleotide sequence revealed the presence of a single open reading frame which could encode a 162-amino-acid protein of about 18 kDa. A noncoding region of 714 bp containing an extensive poly(T) tract, followed by a poly(A) tail of 36 residues, was found at the 3′ end, indicating that the insert of the cDNA clone encodes the C-terminal domain of MTS-gp90. In addition to potential sites for N-glycosylation and N-myristoylation, we found a highly hydrophilic stretch located at the end of the molecule (amino acids 152 to 160).

To confirm the predicted coding region further, the whole insert and a 762-bp *Bam*HI-*Sma*I subfragment of the MTS-gp90 cDNA clone (Fig. 5) were subcloned in frame with the GST gene of plasmid pGEX-3 (27). Both constructions produced a protein of an expected size of about 46.5 kDa (Fig. 5), corresponding to the GST peptide (27.5 kDa) fused to the recombinant peptide (19.0 kDa), the latter containing additional amino acids encoded by nucleotides derived from the adaptor used in the cDNA cloning.

Comparison of sequences of recombinant MTS-gp90 and surface antigens of mammalian-stage trypomastigotes. The homology of recombinant MTS-gp90 with the 85-kDa antigens SA85-1 (13), Tt43c1 (28), and TSA-1 (10) was 41 to 62% (Fig. 6), and that with SAPA (5) was around 36%. Homologous regions, located in a domain of about 200 amino acids before the carboxy terminus of the 85-kDa antigens, were separated by nonhomologous sequences displaying significant amino acid differences.

Genomic organization. To identify genomic sequences corresponding to the MTS-gp90 gene, a Southern blot of *T. cruzi* genomic DNA digested with several restriction enzymes was probed with the insert of the MTS-gp90 cDNA clone (Fig. 7). The probe hybridized to multiple genomic bands, suggesting the existence of a family of related sequences arranged in a nontandem manner. The copy number of MTS-gp90-related sequences was determined by quantitative dot blot hybridization assays (data not shown). We estimated that 40 copies per haploid genome are present, consistent with the results of Southern blot hybridization.

DISCUSSION

In this work, we describe the characterization of a cDNA clone encoding the carboxy-terminal domain of MTS-gp90, a stage-specific 90-kDa surface antigen of *T. cruzi* metacyclic trypomastigotes. To our knowledge, this is the first report on a gene encoding a metacyclic trypomastigote-specific antigen.

We have several lines of evidence that this cDNA clone encodes a portion of MTS-gp90. First, both monoclonal antibody 5E7 and monospecific antisera directed to MTS-

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MTSgp90 ----PVNTRVNNVRHVSLSHNFTLVASVTIEEAPSADAPLMGAMLGDTNSQYTMGVLYTADKEWVTIF  64
SA85-1  RAVWSVNIPDGNVRHISLSHNFTLVASVIIEEAPSGNTPLLTAVLVDAGPEYFMRLSYTADNKWMTML 581
Tt34c1  RVLWSVNTRDNNLRHVFLSHDFTVVATVIIQNVPSGKTSLLTATLANTESNYTMGLSYTADNKWETIF 621
TSA-1   RATWPVNSRWDIKQYGFVDYNFTIVAMATIHQVPSESTPLLGASLRGNKRTKLIGLSYGAGGKWETVY 639
        .. ..** . .  ..  ....**.** ..*...**....*..* * .. .   . . *.*. .* *.

MTSgp90 NGKK--TTESGTWEPGKEYQVALMLQ-GNKSSVYVDGKSLGEEELPL-QSERPLEYLSFCFGGCGI-- 126
SA85-1  KDEKKPTTESRPWEAGKEHQVALMLQ-GNKASVYVDGELLGEEEVPLT-GEKPLEIFAFCFGACKIDG 647
Tt34c1  KGDKKPTTESRPWEPKKEYQVALMLQ-GKKASVYIDGRSLGEGEALLT-DEKSLEFVHFCFGACV--- 684
TSA-1   DGTK--TVQGGTWEPGREYQVALMLQDGNKGFVYVDGVLVGNPAMLPTPEERWTEFSHFYFGG----- 700
        ...*  *... .**. .*.******* *.*. **.**  .*. .     .*.  *    *.**.

MTSgp90 ----------KNFPVTVKNVFLYNRPLNPTEMTAIKDRKPKDEKGR                      162
SA85-1  DEEESSPKEIGKKPRVTVTNVFLYNRPLNSTEMRAIKDRIPVPTRAP                      694
Tt34c1  ---QESSPTAAQKTKVTVTNVFLYNRPLNSTEMRAIKDRIPIPKRGP                      728
TSA-1   -------DEGDSGSDATLTDVFLYNRPLSVGELKMIKEVEDKKEKGS                      740
                      .*...********. .*.  **.     ....
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FIG. 6. Comparison of sequences of recombinant MTS-gp90 with members of the 85-kDa family SA85-1 (13), TSA-1 (10), and Tt34c1 (28). Asterisks and dots are used to indicate identical and similar residues, respectively.

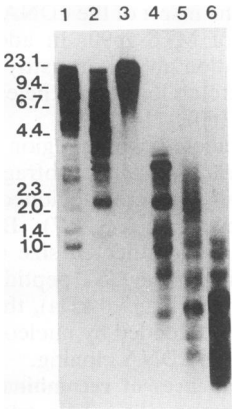

FIG. 7. Hybridization of the insert of the MTS-gp90 clone with *T. cruzi* genomic DNA digested with several restriction enzymes. A Southern blot of epimastigote genomic DNA (10 μg) was probed with a labeled insert of the cDNA clone. Lanes: 1, *Eco*RI; 2, *Bam*HI; 3, *Hin*dIII; 4, *Mbo*I; 5, *Rsa*I; 6, *Hae*III. Numbers on the left correspond to the molecular sizes, in kilobases.

gp90 recognized the recombinant protein encoded by the cDNA clone. Second, anti-metacyclic rabbit antibodies affinity purified on the MTS-gp90 recombinant protein reacted with a 90-kDa antigen of metacyclic forms, but not with other developmental stages. Furthermore, mouse antisera to the purified recombinant antigen recognized the native 90-kDa antigen. Third, transcripts complementary to the MTS-gp90 gene were detected in metacyclic forms, but not in amastigotes, tissue culture-derived trypomastigotes, or epimastigotes.

MTS-gp90 is synthesized from a 75-kDa precursor polypeptide, which is N-glycosylated to give the mature glycoprotein (33). Assuming a molecular mass of 75 kDa for the nonglycosylated protein, the portion encoded by the cDNA clone would correspond to at least 25% of the protein.

We have not found in MTS-gp90 recombinant protein the hydrophobic carboxy-terminal extension, which is a putative site for the addition of a glycosylphosphatidylinositol-membrane anchor (7) and is present in 85-kDa and SAPA antigens (5). Schenkman et al. (26) have shown that metacyclic 90-kDa surface antigens have a glycosylphosphatidylinositol anchor, but we are now left with the possibility that some 90-kDa antigens may be devoid of such an anchor.

Sequences represented by the MTS-gp90 cDNA clone are present in no fewer than 40 copies in the *T. cruzi* genome, arranged in a nontandem manner, indicating that the gene encoding the MTS-gp90 is a member of a multigene family. By sequence analysis, the recombinant MTS-gp90 was found to bear considerable homology (41 to 62%) to *T. cruzi* surface antigens of the 85-kDa antigen family. Members of this family include the TSA-1 antigen containing a repeated nonapeptide sequence (10) and antigens lacking repeats such as SA85-1 (13) and Tt34c1 (28). Despite this homology, we think that the MTS-gp90 antigen should be considered a member of a gene family distinct from that of the 85-kDa gene on the basis of its stage-specific expression and the amino acid differences outside the homology region.

As pointed out by Campetella et al. (5), the 85-kDa antigens and SAPA all have up to four copies of partially or completely conserved sequences encoding the bacterial neuraminidase motif Ser-X-Asp-X-Gly-X-Thr-Trp, but only SAPA appears to have neuraminidase–*trans*-sialidase activity. In the recombinant MST-gp90, we detected one of the neuraminidase motifs that differed from that of 85-kDa antigens in three positions and from that of SAPA in six positions (5, 21, 28). Accordingly, the 90-kDa antigens apparently lack *trans*-sialidase activity (unpublished data).

ACKNOWLEDGMENTS

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