INFECTION AND IMMUNITY, June 1994, p. 2499–2507
0019-9567/94/$04.00+0
Copyright © 1994, American Society for Microbiology
Vol. 62, No. 6

Molecular Cloning and Characterization of the 78-Kilodalton Glucose-Regulated Protein of *Trypanosoma cruzi*

RANDAL S. TIBBETTS, ISAAC Y. KIM, CHERYL L. OLSON, LISA M. BARTHEL, MARY ANNE SULLIVAN, ANDREA G. WINQUIST, STEPHEN D. MILLER, AND DAVID M. ENGMAN*

Departments of Microbiology-Immunology and Pathology, Northwestern University Medical School, Chicago, Illinois

Received 21 January 1994/Returned for modification 14 February 1994/Accepted 10 March 1994

The protozoan *Trypanosoma cruzi* is the etiologic agent of Chagas' disease, an illness responsible for morbidity and death among millions of Latin Americans. Mice also develop this disease when infected with *T. cruzi* and are a useful model organism for the study of parasite-specific immune responses. To identify immunogenic *T. cruzi* antigens, serum from an infected mouse was used to isolate clones from a *T. cruzi* epimastigote cDNA expression library. One of these clones was found to encode the 78-kDa glucose-regulated protein (grp78), the endoplasmic reticular member of the 70-kDa heat shock protein (hsp70) family. Like the mammalian and yeast grp78s, the *T. cruzi* protein contains an endoplasmic reticular leader peptide and a carboxyl-terminal endoplasmic reticular retention sequence. *T. cruzi* grp78 is encoded by a tandemly arranged family of three genes located on a chromosome of 1.6 Mb. The effects on grp78 expression of heat shock and tunicamycin treatment, the latter of which specifically stimulates mammalian grp78, were investigated. While the level of the grp78 protein remained constant under all circumstances, grp78 mRNA was unaffected by heat shock but induced fivefold by tunicamycin. Finally, we found that grp78 is the most immunogenic of the *T. cruzi* heat shock proteins we have characterized, reacting strongly in immunoblots with sera from infected mice.

Members of the 70-kDa heat shock protein (hsp70) family are among the most highly conserved proteins in nature, demonstrating 50 to 70% amino acid sequence identity in organisms as diverse as bacteria, trypanosomes, yeasts, and humans (reviewed in references 3, 23, and 26). Within the cell, hsp70 family members are found in the cytoplasm and nucleus (hsp70 and hsc70), mitochondrion (mtp70), and endoplasmic reticulum (ER) (grp78/BiP). Belonging to the class of proteins known as molecular chaperones, hsp70s are essential for vital cellular functions such as the translocation of proteins across organellar membranes and the folding and assembly of proteins and multiprotein complexes. In addition, heat shock proteins (hsp's) are essential for the protection of the cell from thermal and chemical stresses (17).

grp78, the ER member of the hsp70 family, was initially identified as a protein whose level increased in response to glucose deprivation (36, 40). Subsequently, the protein has been widely studied, and genes encoding grp78 have been characterized in a number of species, including rats (28) and yeast (32, 38). The functions of grp78 are varied and include the translocation of proteins from the cytoplasm into the ER (39, 47) and the sequestration of immunoglobulin heavy chains and other glycoprotein precursors in the ER matrix until their glycosylation and/or assembly into multiprotein complexes (2). Finally, treatment of cells with agents that inhibit the normal trafficking of proteins through the ER, by interfering with glycosylation or by disrupting normal protein structure, causes an increase in the level of grp78 (reviewed in reference 22).

We have already cloned and characterized the cytoplasmic (8, 9, 33) and mitochondrial (10, 13, 33) hsp70s of *Trypanosoma cruzi*, the protozoan parasite that causes Chagas' disease. These proteins have been of interest because of their immunogenicity in *T. cruzi*-infected humans and mice (8), their unique subcellular locations (10, 33), and their unusual ATPase activities, which are much higher than those of their mammalian counterparts (30, 33). In this report, we describe the cloning, molecular characterization, and immunology of *T. cruzi* grp78. This analysis completes the preliminary cloning and characterization of the *T. cruzi* hsp70 family members that is necessary for our long-term study of the functions of these important proteins in the biology of the parasite and in the interaction of the parasite with its mammalian hosts.

* Corresponding author. Mailing address: Northwestern University, Department of Pathology, 303 E. Chicago Ave., Chicago, IL 60611. Phone: (312) 503-1288. Fax: (312) 503-1265. Electronic mail address: dengman@casbah.acns.nwu.edu.

MATERIALS AND METHODS

***T. cruzi* cultures.** The PBOL strain of *T. cruzi* (19) was used for all experiments. *T. cruzi* epimastigotes were maintained in supplemented liver digest-neutralized tryptose medium as described previously (18). For heat shock experiments, log-phase cells were incubated for 3 h at 26, 37, or 40°C. For glycosylation inhibition experiments, log-phase cells were treated for 10 h with 2 μg of tunicamycin (Sigma, St. Louis, Mo.) per ml.

Immunologic screening of a cDNA library. An epimastigote cDNA expression library of the PBOL strain of *T. cruzi* in λUNIZAP (Stratagene, La Jolla, Calif.) was the kind gift of L. V. Kirchhoff (University of Iowa). A total of 80,000 PFU were immunologically screened as described before (10), using serum from a *T. cruzi*-infected mouse. Twelve clones were plaque purified after three to four rounds of immunoscreening, and plasmids were rescued from the phage by using the in vivo excision protocol provided by the manufacturer.

Nucleic acid analysis. Purification of *T. cruzi* genomic DNA was performed by the proteinase K-phenol method essentially as described before (16). For Southern blot analysis, 5 μg of genomic DNA was digested and analyzed by standard procedures (24). For chromosome blots, *T. cruzi* chromosomes were

prepared, and pulsed-field gradient gel electrophoresis was performed essentially as described previously (12), using a contour-clamped homogeneous electric field DR II apparatus (Bio-Rad, Richmond, Calif.). Chromosome gels were composed of 1.0% agarose in 0.5× Tris-borate-EDTA buffer and were run for 36 h at 190 V with a pulse time of 90 s. For Northern (RNA) blot analysis, RNA was purified by using RNAzol B (Cinna/Biotecx, Friendswood, Tex.) according to the manufacturer's instructions. Five micrograms of RNA was electrophoresed, blotted, and analyzed as described previously (10). Quantitation of RNA levels was performed by scanning laser densitometry of autoradiograms, using a Bio-Rad GS-670 imaging system.

Antisera. Sera from *T. cruzi*-infected mice were obtained at day 100 post-infection as previously described (25). A *T. cruzi* grp78-specific mouse antiserum, generated by immunization with a partial-length recombinant grp78 protein, was generously provided by E. C. Rowland (Ohio University).

Protein analysis. For the preparation of *T. cruzi* protein lysates, 2×10^8 parasites were harvested by centrifugation and lysed by boiling for 10 min in 1 ml of 2× Laemmli sample buffer (21). *Escherichia coli* lysates were prepared in a similar manner. Twenty microliters of lysate per lane was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Immunoblot analysis was performed essentially as described by Towbin et al. (46), using a 1:2,000 dilution of mouse anti-*T. cruzi* grp78 or a 1:500 dilution of *T. cruzi*-infected mouse serum. Blots were developed with alkaline phosphatase-conjugated goat anti-mouse immunoglobulins G plus M (Caltag Laboratories, S. San Francisco, Calif.) followed by 5-bromo-4-chloro-3-indolylphosphate (BCIP) and *p*-nitroblue tetrazolium phosphate (United States Biochemical, Cleveland, Ohio). Quantitation of protein levels was achieved by laser densitometry of the immunoblots.

Generation of hsp fusion proteins. The *T. cruzi* grp78 cDNA was subcloned into a derivative of the glutathione *S*-transferase (GST) expression vector pGEX-2T, and fusion protein was produced as described previously (41). The GST-*T. cruzi* hsp70 and GST-*T. cruzi* mtp70 fusion proteins were produced as described in reference 33, the GST-*T. cruzi* hsp60 fusion protein was produced as described in reference 43, and the GST-*T. cruzi* hsp83 fusion protein was produced after subcloning of a PCR-generated hsp83 coding region based on the sequence of Dragon et al. (4). The identities of all of the above fusion proteins were confirmed by immunoblotting (33, 43, 44).

RESULTS

Molecular cloning and sequence analysis of *T. cruzi* grp78. Using sera from mice chronically infected with *T. cruzi*, we set out to molecularly clone immunogenic *T. cruzi* antigens by screening a *T. cruzi* cDNA expression library. Twelve primary clones were isolated. Of these, three clones encode the 24-kDa flagellar calcium-binding protein (11) and two exhibit strong sequence similarity to members of the hsp70 family (see above), with special similarity to the ER member, grp78. One of the grp78 cDNAs, 2,511 bp in size, contains a 1,958-bp open reading frame encoding a protein of 652 amino acids with a predicted molecular mass of 71,451 Da (Fig. 1). Although this cDNA does not contain a spliced leader sequence, which defines the 5′ ends of all trypanosome mRNAs (49), it nevertheless contains a complete grp78 coding sequence (see below). Interestingly, this coding sequence is out of frame with that of the vector-coded fusion moiety, indicating that, in the plated library, the cDNA was expressed not as a phage-encoded fusion protein but via the use of the native ATG start codon. This notion was confirmed by the necessity to subclone the insert into a vector with a reading frame different from that of the phage in order to express grp78 as a true fusion protein (see below). We have observed this phenomenon in a number of *T. cruzi* cDNA clones isolated by immunologic screening in our laboratory.

Alignment of the amino acid sequences of *T. cruzi* grp78, *T. brucei* grp78, rat grp78, and *T. cruzi* mtp70 and hsp70 revealed several regions of sequence conservation, found mainly in the 5′ half of the coding region (Fig. 2). Of note is the fact that the *T. cruzi*, *T. brucei*, and rat grp78 sequences possess hydrophobic ER leaders and terminate in MDDL or KDEL. These tetrapeptides are responsible for the retention of proteins in the ER lumen (1, 29). In contrast, the mtp70 leader is rich in basic and hydroxylated residues, hallmarks of a mitochondrial signal peptide. In addition, besides having common amino- and carboxyl-terminal sequences, *T. cruzi* grp78, *T. brucei* grp78, and rat grp78 are more similar in overall amino acid sequence than either is to *T. cruzi* hsp70 or *T. cruzi* mtp70 (Table 1).

Genomic organization and chromosomal location of the *T. cruzi GRP78* genes. To determine the number and organization of *GRP78* genes in *T. cruzi*, we used the grp78 cDNA as a probe on Southern blots of genomic DNA digested with restriction endonucleases that have one or two sites within the cDNA (Fig. 3). This analysis suggests that there are three *GRP78* genes in *T. cruzi*, organized as a direct tandem array of identical or nearly identical members (schematized in Fig. 3, bottom). Digestion with *Cla*I, which cuts near the 5′ end of the coding region (Fig. 1), gave rise to a unit-length fragment of 4.5 kb and two less intensely hybridizing fragments representing regions flanking the array. Digestion with either *Hin*dIII or *Sal*I, which cut twice within the gene (the second *Hin*dIII site is located just downstream of the 3′ boundary of the cDNA), yielded hybridizing fragments the sum of whose lengths equals 4.5 kb (3.7 + 0.8 [*Hin*dIII]; 4.0 + 0.5 [*Sal*I]), plus one (*Hin*dIII) or two (*Sal*I) flanking fragments. The faintness of the 0.8-kb *Hin*dIII band suggests the presence of polymorphism at this restriction site, which has been observed in other tandemly repeated *T. cruzi* genes (14). Double digestion using combinations of the above enzymes was also performed. When the extent of hybridization of the random-primed cDNA probe to each fragment and the relative intensities of hybridization of the bands in Fig. 3 were considered, it was possible to deduce that there are three *GRP78* genes organized as a simple direct tandem array. Each of the bands in the Southern blot can be assigned to a fragment within the array or flanking it (e.g., the 2.4- and 9.5-kb *Cla*I fragments correspond to the 5′ and 3′ flanking regions, respectively). Hybridization of the grp78 probe to *T. cruzi* chromosomes separated by pulsed-field gradient gel electrophoresis revealed that the *GRP78* array is located on a chromosome of 1.6 Mb (Fig. 4). Consistent with the view that *T. cruzi* is a diploid organism, it is probable that there are two *GRP78* arrays located on comigrating 1.6-Mb sister chromosomes (48).

Analysis of grp78 mRNA and protein levels during heat shock. Despite being commonly referred to as hsp's, not all members of the hsp70 family are heat inducible. In mammalian cells, two cytoplasmic/nuclear hsp70s exist, one of which is constitutively expressed and the other of which is strictly heat inducible (34). In mammals, grp78 is not appreciably heat inducible, presumably because the *GRP78* genes lack heat shock elements (45), upstream regulatory sequences that confer heat inducibility upon eukaryotic heat shock genes (42, 51). By contrast, yeast grp78 is heat inducible, and a heat shock element is found in the yeast gene encoding grp78, *KAR2* (32). To determine whether *T. cruzi* grp78 is heat inducible, we

```
                                         M L L Q A L L V L S A V V V A V A A P D G T G K V E A P C     29
GGCAAATCAGCCAAACTGAAGTTAACGTCACTTATGCTCCTTCAGGCGCTGCTGGTTCTGTCCGCGGTGGTGGTGGCGGTGGCCGCTCCGGACGGCACCGGGAAGGTGGAGGCGCCATGC  120

V G I D L G T T Y S V V G V W Q K G D V H I I P N D M G N R I T P S V V A F T E     69
GTCGGTATCGATCTCGGCACGACATACTCGGTTGTGGGCGTGTGGCAGAAGGGAGATGTGCACATTATCCCGAACGATATGGGCAACCGTATCACGCCGTCCGTAGTGGCCTTCACGGAG  240
      ClaI
T E R L I G D G A K N Q L P Q N P H N T I Y A I K R L I G R K Y S D T T V Q T D    109
ACTGAGCGTCTCATTGGCGACGGTGCGAAGAACCAACTGCCGCAGAACCCGCACAACACGATCTACGCGATCAAGCGGCTCATTGGACGAAAGTACAGCGACACCACGGTACAGACTGAC  360

K K L L S Y E V V A D K D G K P K V E V E V G G K K K Q F T P E E V S A M V L Q    149
AAGAAGCTGCTGTCGTACGAGGTGGTGGCGGACAAGGACGGTAAGCCTAAGGTCGAGGTGGAGGTTGGCGGTAAGAAGAAGCAGTTCACGCCCGAGGAGGTGAGCGCGATGGTGCTGCAG  480

K M K E I A E T Y L G E K V K N A V V T V P A Y F N E P Q R Q S T K D A G T I A    189
AAGATGAAGGAGATCGCGGAGACGTACCTGGGCGAGAAGGTGAAAAACGCCGTTGTGACGGTTCCCGCGTATTTCAATGAGCCCCAGCGGCAGTCGACGAAGGATGCCGGCACGATCGCG  600
                                                                                                 SalI
G L N V V R I I N E P T A A A I A Y G L N K A G E K N I L V F D L G G G T F D V    229
GGGCTGAATGTTGTGCGCATCATCAACGAGCCGACAGCAGCTGCCATTGCGTACGGTCTGAACAAGGCCGGCGAGAAGAACATCCTTGTGTTTGACCTCGGGGGCGGCACCTTTGATGTG  720

S L L T I D E G F F E V V A T N G D T H L G G E D F D N N M M R Y F V D M L K K    269
TCGCTGCTAACGATCGACGAGGGCTTTTTTGAGGTTGTGGCAACGAACGGGGACACGCATCTCGGCGGCGAGGACTTTGACAACAACATGATGCGCTACTTTGTGGACATGCTGAAGAAG  840

K K N V D V S K D Q K A L A R L R C E A C E A A K R Q L S S H P E A R V E V D S    309
AAGAAGAATGTGGACGTGAGCAAGGACCAAAAGGCGCTGGCGCGCCTGCGGTGCGAGGCGTGCGAGGCGGCGAAGCGGCAGTTGTCGTCGCACCCAGAGGCACGCGTGGAGGTGGACAGT  960

L T E G F D F S E K I T R A K F E E L N M E L F K G T L V P V Q R V L E D A K L    349
CTGACGGAGGGCTTCGACTTTAGTGAGAAGATCACCCGCGCGAAGTTCGAGGAGCTGAATATGGAGCTGTTCAAGGGGACGCTGGTGCCAGTGCAGCGCGTGCTAGAGGACGCGAAGCTG 1080

K K S D I H E I V L V G G S T R V P K V Q Q L I R D F F G G K E P N R G I N P D    389
AAGAAGAGTGACATACACGAGATTGTGCTGGTTGGCGGGTCGACACGCGTGCCAAAGGTGCAGCAACTAATTCGGGACTTCTTTGGCGGTAAGGAGCCGAACCGTGGCATCAACCCCGAC 1200
                                        SalI
E A V A Y G A A V Q A A V L T G E S E V G G R V V L V D V I P L S L G I E T V G    429
GAGGCGGTGGCGTACGGCGCGGCGGTGCAGGCAGCGGTGCTGACCGGAGAGAGCGAGGTCGGTGGGCGCGTTGTGCTGGTGGACGTGATCCCGCTGTCGCTCGGCATCGAGACGGTGGGC 1320

G V M T K L I E R N T Q I P T K K S Q V F S T Y Q D N Q P G V L I Q V Y E G E R    469
GGCGTGATGACGAAGCTGATCGAGCGCAACACGCAGATCCCAACGAAGAAGAGCCAGGTGTTCTCAACGTACCAGGACAACCAGCCGGGAGTGCTGATTCAGGTTTACGAGGGCGAGCGG 1440

Q M T K D N R L L G K F E L S G I P P R P R A V P Q I E V T F D V D E N S I L Q    509
CAGATGACGAAGGACAACCGTCTGCTTGGCAAGTTCGAGCTGTCCGGCATCCCGCCGCGTCCGCGTGCGGTGCCGCAGATTGAGGTAACCTTTGACGTGGACGAAAACAGCATCCTGCAG 1560

V S A V D K S S G K K E E I T I T N D K G R L S E E E I E R M V R E A A E F E G    549
GTGAGCGCCGTGGACAAGTCGTCGGGGAAGAAGGAGGAGATCACCATCACGAATGACAAGGGTCGCCTGAGCGAGGAGGAGATTGAGCGCATGGTGCGCGAGGCTGCGGAGTTTGAGGGC 1680

E D R K V R E R V D A R N S L E S V A Y S L R N Q V N D K E K L G G K L S A D D    589
GAGGACCGCAAGGTGCGCGAGCGCGTGGATGCGCGAAACTCGCTGGAGAGCGTCGCGTACTCGCTGCGCAACCAGGTAAACGACAAGGAGAAGCTTGGTGGGAAACTGAGCGCGGATGAC 1800
                                                                                              HindIII
K S A V E A A V K E A M Q F L D D N P N A D K E E Y D E A R D K L Q S V T N P I    629
AAGAGCGCCGTGGAGGCCGCAGTGAAGGAGGCGATGCAGTTCCTTGACGACAATCCTAACGCTGACAAGGAGGAGTACGACGAGGCGCGCGATAAGCTGCAGAGCGTGACGAACCCGATC 1920

I Q K V Y Q S G G G A D G D E R P E P M D D L *                                  652
ATCCAGAAAGTGTACCAGAGCGGCGGGGGCGCCGATGGGGATGAGCGGCCGGAGCCAATGGACGACCTGTAGGCACGCACGCAGGTCGCGGCCAGGCGCGATAATAGGAGTGCAGCAACC 2040


ACTGCTGTTGCTTTAGGGAGAACGCAATGCTGCCGTATTCTGACCGTAAATAAACTGGTATGGCACCAAGCAGTCGAAGAAGGGGTGAGAAAGAGAGGATTGTACGAACGAACGCGTAGC 2160


CCCTCTGTGTGGCCCAATGTTGTCTTTGTGCGGAGCCGCGAAACTGCTGCTCTGGTTGCCTTTTCCCATCTCCCTCCGACAATGAAAGCCATGCAGGTGGCGTGAAGTTGGCAACGGTAT 2280


GAAGAGAGTGAGGTGGGAAAAGAGGAGGAGGGAAGAAGCGCGGGCAAGTAGACGGACTTCTGAAGCGATACAAGATTTGAGAAGAAGATGTGTAGGGAGAAGAAATGGTGAGAGGCGCCG 2400


CACGCCCCGCATATGTGGTTTTATGTTGTTTTTTATTTTTTTAGCATGTGCGCCAGGTGCGGGGTGTGCGGTCCTTTTTTGTTTCCTTCTGTGCTTTTTAAAAAAAAAAAA               2511
```

FIG. 1. Nucleotide and deduced amino acid sequences of the *T. cruzi* grp78 cDNA (GenBank accession number L23420). The 2,511-bp cDNA contains a single large open reading frame of 1,959 bp encoding a protein of 642 amino acids with a predicted molecular mass of 72 kDa. The putative amino-terminal ER targeting sequence and carboxyl-terminal ER retention sequence are overlined. Restriction sites used for the Southern analysis of Fig. 2 are marked in boldface.

performed Northern blot analysis of *T. cruzi* epimastigote RNA during heat shock (Fig. 5). Upon incubation of the cells at 37°C, the grp78 mRNA level decreased slightly from that at the physiologic, 26°C culture temperature. Incubation at 40°C caused a threefold decrease in the mRNA level. Rehybridization of the Northern blot with an hsp70 probe revealed a threefold induction of hsp70 mRNA at 37°C but a threefold decrease, similar to that observed for grp78, at 40°C. The decrease in grp78 and hsp70 mRNA levels at 40°C probably results from inhibition of RNA processing known to occur at high temperatures in trypanosomes (27, 54). Thus, the steady-state *T. cruzi* grp78 mRNA level seems to be regulated in a manner similar to that of mammalian grp78 mRNA in that the steady-state mRNA level does not increase following heat shock. Immunoblot analysis was performed to determine whether a posttranscriptional mechanism may upregulate grp78 protein levels upon heat shock. Under the same heat shock conditions used for the Northern analysis, the steady-

```
TCGRP78   M----LLQ----ALLVLSAVVVAV-AAPDGTGKVEAPCVGIDLGTTYSVVGVWQKGDVHIIPNDMGNRITPSVVAFT-ETERLIGDGAKNQLPQNPHNTI   90
TBGRP78   .SRMW.TT----.AVF.TVT.A..S...ESG................................E.............-D.........R...........   95
RRGRP78   .----KFTVVAA...L.C.---R-.EEEDKKEDVGTV...........C...FKN.R.E..A..Q.......Y....P.G......A.....TS..E..V   94
TCHSP70   .-------------------------------TYEGAI.........C.....NER.E..A..Q...T...Y....-D.......A....VAM..T..V   67
TCMTP70   .----FAR----R.RGAGSLAA.S-L.RWQSS..TGDVI.........C.A.MEGDKPRVLE.TE.F.A.......K-GQ.K.V.LA..R.AVT..QS.F   90

TCGRP78   YAIKRLIGRKYSDTTVQTDKKLLSYEVVADKDGKPKVEVEVGGKKKQFTPEEVSAMVLQKMKEIAETYLGEKVKNAVVTVPAYFNEPQRQSTKDAGTIAG  190
TBGRP78   .T.........T.AA..A.........I..R.....Q.M...........I.................................DA............  195
RRGRP78   FDA......TWN.PS..Q.I.F.PFK..EK.TKPYIQVDIG..QT.T.A...I.....T....T..A...K..TH..........DA...A.........  192
TCHSP70   FDA.......F..PV..S.M.HWPFK.ITKG.D..VIQ.QFR.ET.T.N.....S...S.......S...KQ..K..........DS...A.........  167
TCMTP70   F.V......RFE.SNI.H.I.NVP.KIGRSSN.DAW.QDAN.---..YS.SQ.G.F..E....T..NF..R..S.....C.....G....A.........  187

TCGRP78   LNVVRIINEPTAAAIAYGLNKA-G---EKNILVFDLGGGTFDVSLLTIDEGFFEVVATNGDTHLGGEDFDNNMMRYFVDMLKKK-KNVDVSKDQKALARL  285
TBGRP78   ......................-.---........................................................H........-....I..........  290
RRGRP78   ...M...............D.RE.---.......................N.V.................QRV.EH.IKLY...-TGK..R..NR.VQK.  288
TCHSP70   ME.L...............D.V-EDGK.R.V.I.........T.....G.I...K...............RLVSH.T.EF.R.N.GK.LTTS.R..R..  266
TCMTP70   ...I.VV.G.....L....D.T-K---DSM.A.Y........I.V.E.AG.V...K..............LCLSD.ILTEF..S-TGI.L.NERM..Q.I  282

TCGRP78   RCEACEAAKRQLSSHPEARVEVDSLTEGFDFSEKIT----RAKFEELNMELFKGTLVPVQRVLEDAKLKKSDIHEIVLVGGSTRVPKVQQLIRDFFGGKE  381
TBGRP78   .-K..................................----..........D..........................................S.......  385
RRGRP78   .R.-V.K...A...QHQ..I.IE.FF..E....TL.----..........D..RS.MK...K....SD......D..........I..I...VKE..N...  383
TCHSP70   .-T...R...T...AAQ.TI.I.A.FDNV..QAT.-----..R....CGD..R...Q..E...Q...MD.RAV.DV........I...M..VS.......  361
TCMTP70   .-..A.K..CE..TTM.TE.NLPFI.ANQ.GAQHVQMTVS.S...S.AEK.VQRS.G.CKQCIK..AVDLKE.S.V.....M..M...IEAVKQ..-.RD  380

TCGRP78   PNRGINPDEAVAYGAAVQAAVLTGE-SEVGGRVVLVDVIPLSLGIETVGGVMTKLIERNTQIPTKKSQVF-STYQDNQPGVLIQVYEGERQMTKDNRLLG  479
TBGRP78   L........................-............................................-..HA...............L.......  483
RRGRP78   .S...............G..S.--DQDT.DL..L..C..T...............P...VV.......I.-..AS....T.T.K......PL....H...  480
TCHSP70   L.KS..............FI...GK.KQTEGLL.L..T..T.....A.....S..K...T.......I.-...A......H...F....A....CH...  460
TCMTP70   .F..V.......L.G.TLGG..----RRDVKGL..L..T.....V..L...F.RM.PK..T........T.F..AAF..TQ.G.K.FQ...E.AA..QMM.  476

TCGRP78   KFELSGIPPRPRAVPQIEVTFDVDENSILQVSAVDKSSGKKEEITITNDKGRLSEEEIERMVREAAEFEGEDRKVRERVDARNSLESVAYSLRNQ-----  574
TBGRP78   .........AA.G....................M..............................D..............................-----  578
RRGRP78   T.D.T....A..G........EI.V.G..R.T.E..GT.N.NK......QN..TP.......ND.EK.AE..K.LK..I.T..E...Y....K..-----  575
TCHSP70   T.D......A..G.........L.A.G..N...EE.GT..RNQ.V.........KAD.....S...KY.SQ.KEQ...I..K.G..NY.F.VK.T-----  555
TCMTP70   Q.D.V....A..G.........IEP.G.CH.T.K..AT..TQN....-AS.G..K.Q....I.DSESHAES..LK..L.EV..NA.TQ.NTAER.LTEWK  575

TCGRP78   -VNDKEKLGGK--LSADDKSAVEAAVKEAMQFLDDNPNADKEEYDEARDKLQSVTNPIIQKVYQSGGGAD-GDER---------------------PEP  648
TBGRP78   -....D......--.DPN..A...T..A..IR...E.....E....KT.LET..........T...A..G.----K---------------------.Q.  649
RRGRP78   -IG..........--..PE..ETM.K..E.KIEW.ESHQD..I.DFKAKKKE.EEIVQ...S.L.G...PPPT.E.D---------------------TSE  650
TCHSP70   -..-EPNVA..--IEEA..NTITS..E..L.W.NN.QE.S....EHRQKE.ENLCT..MT.M..-..M..G-.GMPGGMPGGMPGGMPGGANPSSSSG.KV  649
TCMTP70   Y.S.A..ENVRTL.R.CR..--------------ME...VT.D.LSA.T....KAVMECGRTE..QAAAGN-SSSS---------------------SGN  639

TCGRP78   MDDL                                                                                                  652
TBGRP78   ....                                                                                                  653
RRGRP78   K.E.                                                                                                  654
TCHSP70   EEVD                                                                                                  653
TCMTP70   T.SSQGEQQQQGDQQKQ                                                                                     656
```

FIG. 2. Alignment of the amino acid sequences of *T. cruzi* grp78 (TCGRP78), *T. brucei* grp78 (TBGRP78), rat grp78 (RRGRP78), *T. cruzi* hsp70 (TCHSP70), and *T. cruzi* mtp70 (TCMTP70). References for these sequences are this paper, 1, 28, 9, and 13, respectively. Amino acid positions identical to those in the *T. cruzi* grp78 sequence are indicated by periods, and computer-generated insertions that maximize alignment are denoted by dashes.

state grp78 protein level did not change following heat shock (Fig. 6), while the steady-state hsp70 level increased 1.5-fold (see also reference 33). Thus, in *T. cruzi* neither the grp78 mRNA level nor the grp78 protein level is induced under conditions of thermal stress.

Analysis of grp78 mRNA and protein levels following treatment with tunicamycin. A feature that distinguishes grp78 from other hsp70 family members is its inducibility by inhibitors of protein glycosylation, such as tunicamycin and the calcium ionophore A23187 (5, 36, 50, 52). To determine whether *T. cruzi* grp78 is inducible by similar stress, we treated *T. cruzi* epimastigotes with tunicamycin and performed analyses similar to those conducted on heat-shocked cells. Treatment of parasites for 10 h with 2 μg of tunicamycin per ml resulted in a 5.4-fold increase in the grp78 mRNA level relative to the control (Fig. 7). The hsp70 mRNA level also increased slightly (1.5-fold) in response to tunicamycin. Despite the increase in grp78 mRNA levels, steady-state grp78 protein levels remained constant, even up to 24 h after tunicamycin addition (Fig. 8). Thus, the steady-state mRNA and protein levels are not coordinately regulated in *T. cruzi*, a phenomenon that, at least among the hsp's, seems to be the rule rather than the exception (10, 33, 43).

TABLE 1. Identity matrix for amino acid sequences of *T. cruzi* (TC) grp78, *T. brucei* (TB) grp78, *Rattus norvegicus* (RN) grp78, *T. cruzi* hsp70, and *T. cruzi* mtp70

Protein	Identity/similarity (%)				
	TCGRP78	TBGRP78	RNGRP78	TCHSP70	TCMTP70
TCGRP78	100/100				
TBGRP78	90/94	100/100			
RNGRP78	64/76	64/76	100/100		
TCHSP70	60/71	60/71	61/72	100/100	
TCMTP70	47/59	46/60	46/60	45/59	100/100

Humoral immunity to grp78 in *T. cruzi*-infected mice. It had been shown previously that *T. cruzi* hsp's are immunogenic in parasite-infected humans and experimental animals (4, 8). Because the grp78 cDNA clone was isolated using serum from a chronically infected mouse, it was likely that the recombinant protein would be detected by the same serum in an immuno-

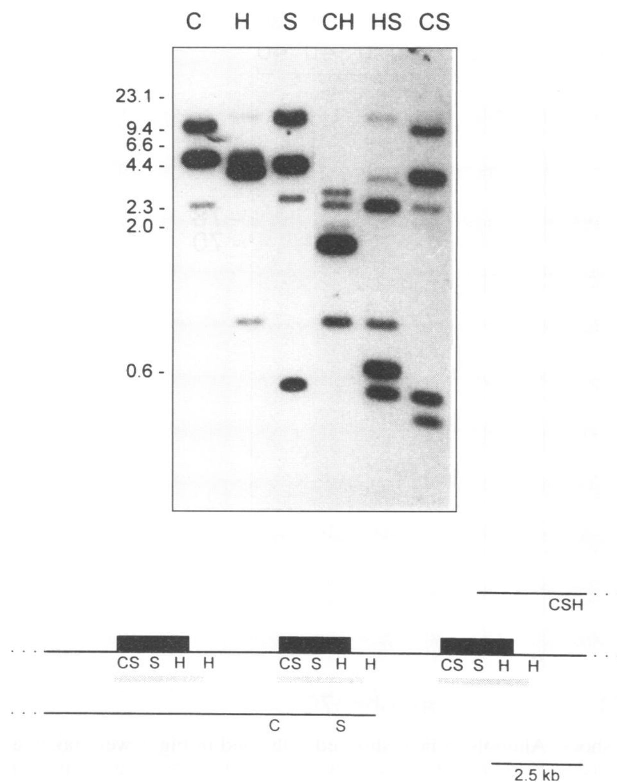

FIG. 3. Organization of the *T. cruzi GRP78* locus. (Top) *T. cruzi* genomic DNA was digested with *Cla*I (C), *Hin*dIII (H), *Sal*I (S), or combinations of these three enzymes and analyzed by Southern blot hybridization, using grp78 cDNA as a probe. The sizes and relative intensities of the resulting bands were used to determine that there are three *GRP78* genes, each approximately 4.5 kb in size, organized as a simple direct tandem array. (Bottom) The locus is depicted schematically. Coding regions are represented as solid boxes, intergenic regions are shown as solid lines, and the regions to which the labeled cDNA probe hybridizes are shown as stippled boxes. The approximate locations of the restriction sites used for the Southern blot are given.

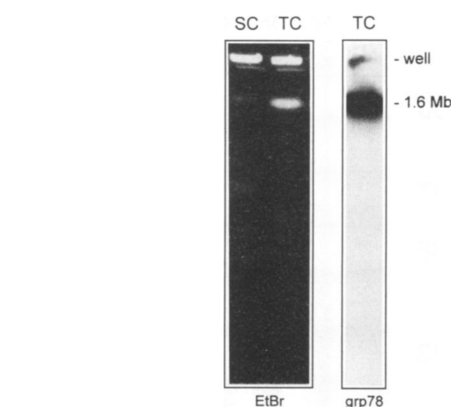

FIG. 4. Chromosomal location of the *GRP78* locus. *T. cruzi* (TC) chromosomes were separated by pulsed-field gradient gel electrophoresis and analyzed by ethidium bromide staining (EtBr) and Southern blot hybridization with the grp78 cDNA probe (grp78). *Saccharomyces cerevisiae* (SC) chromosomes were included as molecular size standards. The *GRP78* array is located on a chromosome of 1.6 Mb in the PBOL strain of *T. cruzi.*

blot assay. To test this, we prepared a bacterial lysate containing the GST-grp78 fusion protein described above, as well as control bacterial and *T. cruzi* epimastigote lysates, for immunoblotting (Fig. 9). Staining with antiserum against recombinant *T. cruzi* grp78 identified both the 78-kDa native protein (anti-grp78, lane T) and the 105-kDa recombinant GST-grp78 fusion protein (and degradation products, lane +). Staining with serum from a mouse chronically infected with *T. cruzi* revealed strong reactivity with both the native and recombinant grp78 proteins, as well as with many other *T. cruzi* antigens of widely varying molecular mass (gels marked CMS). Nonimmune and uninfected mouse sera did not react with these proteins (not shown). Similar reactivity with the GST-grp78 fusion protein has been obtained with a number of other *T. cruzi*-infected mouse sera (not shown), indicating that grp78 is a humoral immunogen during murine *T. cruzi* infection.

To determine the relative intensities of the humoral immune responses to grp78 and the four other major *T. cruzi* hsp's during murine *T. cruzi* infection, we performed immunoblot analysis of a panel of GST-*T. cruzi* hsp fusion proteins with a variety of chagasic mouse sera. A representative blot is shown (Fig. 10). Among all *T. cruzi* hsp fusion proteins, the grp78 fusion is by far the most reactive with chagasic mouse sera. A lower degree of reactivity is also seen with mtp70, but hsp60, hsp70, and hsp83 are conspicuously nonreactive. Failure of the sera to react with hsp70 and hsp83 is surprising, since cDNAs encoding these proteins have been isolated by immunologic screening methods (4, 44). Furthermore, hsp70 and hsp83 are readily precipitated by chagasic human (4, 8) and mouse (7) sera, suggesting that the epitopes of these two proteins that are responsible for their precipitability may be destroyed by solu-

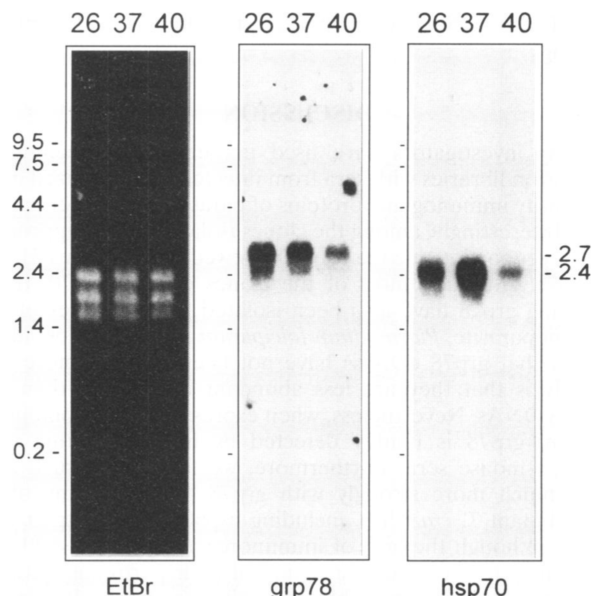

FIG. 5. Analysis of the steady-state *T. cruzi* grp78 mRNA level during heat shock. Five micrograms of total RNA prepared from *T. cruzi* epimastigotes incubated at 26, 37, or 40°C for 3 h was analyzed by Northern blot hybridization, using grp78 and hsp70 cDNAs as probes. The ethidium bromide-stained gel is shown at the left (EtBr) and the positions of molecular size standards (in kilobases) are marked. The steady-state level of the 2.7-kb grp78 mRNA is the same at 26 and 37°C and threefold less at 40°C, while the level of the 2.4-kb hsp70 mRNA is threefold higher at 37°C than at 26°C and threefold lower at 40° than at 26°C.

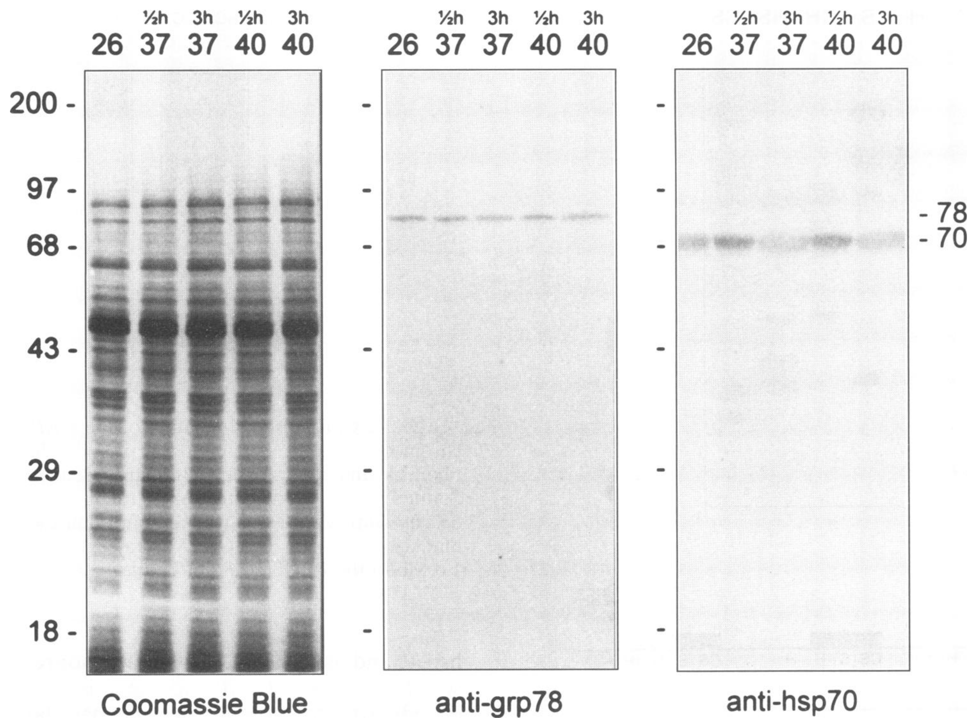

FIG. 6. Analysis of the steady-state *T. cruzi* grp78 protein level during heat shock. Aliquots of heat-shocked cells used in Fig. 5 were taken at 0.5 or 3 h after incubation at elevated temperature, isolated by centrifugation, washed in phosphate-buffered saline, and lysed by solubilization in SDS-sample buffer. The lysates were analyzed by SDS-PAGE and immunoblotting, using grp78-specific and hsp70-specific antisera. A Coomassie blue-stained gel is shown at the left and the positions of molecular size standards (in kilodaltons) are marked. While the steady-state hsp70 level is 1.5-fold higher after heat shock, there is no change in the level of grp78.

bilization in SDS-sample buffer, electrophoresis, and immunoblotting.

DISCUSSION

Many investigators have used immunologic screening of expression libraries with sera from infected or immunized mice to identify immunogenic proteins of parasites and other pathogens. Interestingly, among the clones isolated by this approach, a high proportion encode hsp's (reviewed in references 31, 35, and 37). Although most of the clones encode hsp70, those encoding grp78 have also been isolated from libraries of the malaria parasite, *Plasmodium falciparum* (20). The most likely reason that grp78 cDNAs have not been isolated more frequently is that they are less abundant in libraries than are hsp70 cDNAs. Nevertheless, when expressed as a recombinant protein, grp78 is readily detected by immunoblotting with infected mouse sera. Furthermore, we found that such sera react much more strongly with grp78 than with any other recombinant *T. cruzi* hsp, including hsp60, hsp70, mtp70, and hsp83, although the lack of immunoreactivity with hsp70 and hsp83 may result from destruction of their pertinent epitopes by processing for immunoblot analysis (see above). It remains to be determined whether chagasic human sera react with grp78 as strongly as do chagasic mouse sera.

The molecular characterization of *T. cruzi* grp78 cDNA and the analysis of gene organization and chromosomal location revealed the protein to be similar to other *T. cruzi* hsp's in many respects. Although we did not formally prove that the encoded protein is located in the ER of the cell, the presence of ER targeting and retention sequences in the protein indicate that it is. One unusual aspect of the retention sequence,

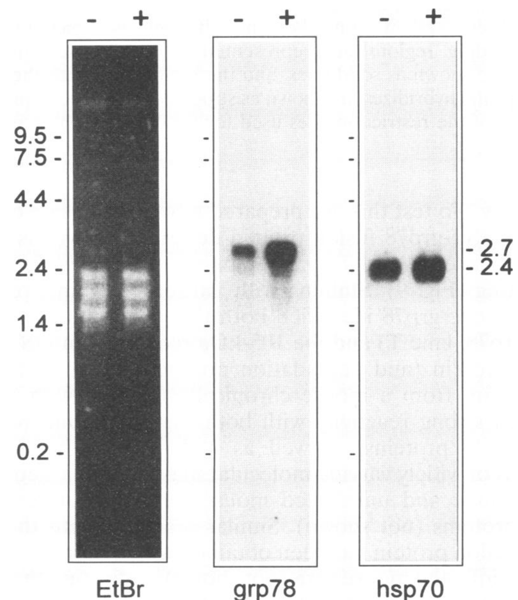

FIG. 7. Analysis of the steady-state *T. cruzi* grp78 mRNA level in epimastigotes incubated with the glycosylation inhibitor tunicamycin. Five micrograms of total RNA prepared from *T. cruzi* epimastigotes cultured for 10 h in the presence (+) or absence (−) of 2 μg of tunicamycin per ml was analyzed by Northern blot hybridization, using grp78 and hsp70 cDNAs as probes. The ethidium bromide-stained gel is shown at the left (EtBr). The steady-state level of the 2.7-kb grp78 mRNA is 5.4-fold higher in the treated cells than in the control cells, while that of the 2.4-kb hsp70 mRNA is 1.5-fold higher.

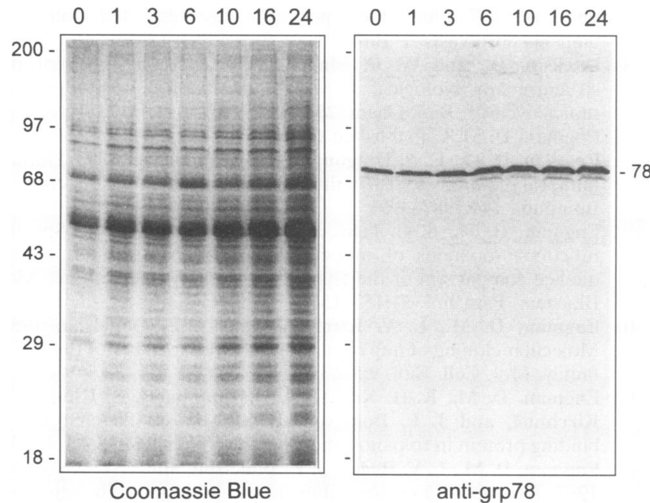

FIG. 8. Analysis of the steady-state *T. cruzi* grp78 protein level in epimastigotes incubated with the glycosylation inhibitor tunicamycin. *T. cruzi* epimastigotes were cultured in the presence of 2 μg of tunicamycin per ml for increasing lengths of time (top, in hours). At each time point, an aliquot of the cells was taken, pelleted, washed with phosphate-buffered saline, and suspended at a density of 2×10^8 per ml in SDS-sample buffer. The lysates were analyzed by SDS-PAGE and immunoblotting, using a grp78-specific antiserum. A Coomassie blue-stained gel is shown at the left, and the positions of molecular size standards (in kilodaltons) are marked. After normalization to the total protein loaded per lane, no change in the steady-state grp78 protein level was found after tunicamycin treatment.

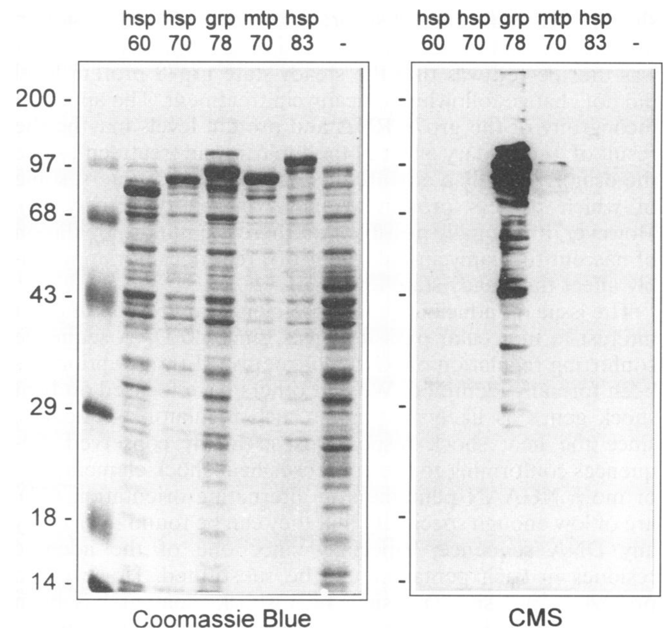

FIG. 10. Relative reactivity of chagasic mouse serum with *T. cruzi* hsp fusion proteins. Lysates of *E. coli* expressing GST-*T. cruzi* hsp60, GST-*T. cruzi* hsp70, GST-*T. cruzi* grp78, GST-*T. cruzi* mtp70, GST-*T. cruzi* hsp83, or no GST fusion protein (−) were analyzed by SDS-PAGE and immunoblotting with a representative chagasic mouse serum (CMS) sample. A Coomassie blue-stained gel containing molecular size standards (marked, in kilodaltons) is shown at the left. This and other chagasic mouse sera react strongly with the grp78 fusion protein, weakly with the mtp70 fusion protein, and little, if at all, with the other fusion proteins. Normal mouse serum does not react with these proteins (not shown).

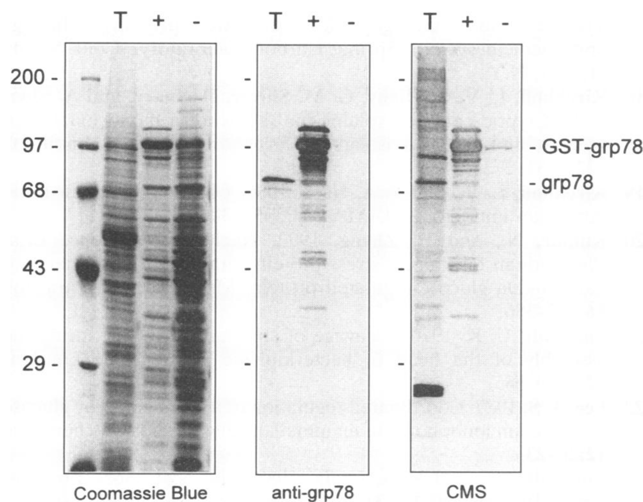

FIG. 9. A humoral immune response to grp78 is generated in *T. cruzi*-infected mice. The grp78 cDNA insert was subcloned into the GST expression vector pGEX-2T and expressed as a GST fusion protein of approximately 100 kDa. Lysates of *T. cruzi* epimastigotes (T) and *E. coli*-expressing (+) or nonexpressing (−) GST-grp78 were analyzed by SDS-PAGE and immunoblotting with grp78-specific antiserum (anti-grp78) or chagasic mouse serum (CMS). A Coomassie blue-stained gel containing molecular size standards (marked, in kilodaltons) is shown at the left. The positions of grp78 in lanes T and GST-grp78 in lanes + are indicated. Antibodies reactive with both native grp78 and GST-grp78 are produced in the *T. cruzi*-infected mouse. Normal mouse serum does not react with these proteins (not shown).

MDDL, is that it diverges slightly from the mammalian XDEL consensus, a finding also true of the grp78 of the African trypanosome, *T. brucei* (1). Like the other *T. cruzi* heat shock genes (4, 15, 33, 43), the *GRP78* genes are organized as an array of identical or nearly identical genes, most likely located on each of two sister chromosomes of 1.6 Mb. However, one aspect of the *GRP78* genes that is distinct from the other *T. cruzi* heat shock genes is that they have unusually large intergenic regions. The unit lengths of the tandemly arranged *HSP60*, *HSP70*, *MTP70*, and *HSP83* genes are about 400 to 700 bp larger than their respective coding regions and approximately the same size as their respective mRNAs. The unit length of each *GRP78* gene is 4.5 kb, 2.5 kb larger than the coding region, which suggested to us that an additional coding region might be present within this 4.5-kb fragment. To investigate the possibility, we amplified and cloned the 2.5-kb intergenic fragment from *T. cruzi* genomic DNA, using primers specific for the 5′ and 3′ ends of the cDNA and directed away from the coding sequence. After confirming that this was indeed an "intergenic" fragment by partial sequencing, we used the fragment as a probe on Northern blots of *T. cruzi* epimastigote RNA but detected no product. Thus, only the 2.7-kb grp78 mRNA appears to be expressed at detectable levels from the 4.5-kb genomic region. The significance of the large intergenic sequence is not known.

One of the distinguishing features of hsp's is that their expression is regulated by heat and/or other stresses. We found that the steady-state *T. cruzi* grp78 mRNA level is induced by tunicamycin treatment but is not appreciably affected by heat

shock. This result was not surprising, since grp78 expression in mammalian cells is regulated in a similar manner (45). What was unexpected was that the steady-state grp78 protein level did not change following tunicamycin treatment. The apparent incongruity of the grp78 RNA and protein levels may be the result of a secondary effect of the tunicamycin treatment, since the drug is actually a mixture of several distinct isomers, some of which possess protein synthesis inhibitory activity (6). However, it is equally possible that there is a minor population of nascent tunicamycin-induced grp78 that does not appreciably affect the steady-state level.

The issue of inducible gene expression is, at present, of great interest to molecular parasitologists, since no DNA sequence conferring regulation of RNA polymerase II transcription has been formally identified. We and others have focused on heat shock genes as likely to possess such regulatory sequences, since the heat shock response is so highly conserved. Sequences conforming to the consensus heat shock element, two or more NGAAN pentamers in alternating orientation (53), are of low enough specificity that they can be found in virtually any DNA sequence, especially since one of the adenine residues in each pentamer can be substituted. However, in protozoan parasites, no such heat shock element has been successfully used to confer heat inducibility on a test sequence. It is of interest, then, that there are other sequences that are found among the heat shock genes of *T. cruzi*. A region containing repeats of the trinucleotide TTA is specifically found in the 3′-nontranslated regions of *T. cruzi HSP60*, *HSP70*, *MTP70*, and *HSP83*, all of which are heat inducible, but is absent from the *GRP78* gene. If the TTA repeat plays a role in heat-inducible expression, then the absence of this sequence from the *GRP78* gene would explain the failure of the grp78 mRNA level to increase upon heat shock. The work presented in this paper completes our preliminary molecular characterization of the major hsp's of *T. cruzi* and will allow us to address the issues of protein localization, function, and immunity that are certain to be critical for the biology of this ancient eukaryotic organism.

ACKNOWLEDGMENTS

We thank E. M. Roland (Ohio University) for the grp78-specific antiserum and C. R. Waltenbaugh and his coworkers (Northwestern University) for their generous assistance with tissue culture.

This work was supported in part by funds from the National Institutes of Health and the Lucille P. Markey Charitable Trust. D.M.E. is an American Federation for Clinical Research/Merck, Sharp and Dohme M.D.-Ph.D. Fellow. R.S.T. was supported by a Northwestern University Cellular and Molecular Basis of Disease (National Institutes of Health) Training Grant.

REFERENCES

1. **Bangs, J. D., U. Uyetake, M. J. Brickman, A. E. Balber, and J. C. Boothroyd.** 1993. Molecular cloning and cellular localization of a BiP homologue in *Trypanosoma brucei*: divergent ER retention signals in a lower eukaryote. J. Cell. Sci. **105:**1101–1113.
2. **Bole, D. G., L. M. Hendershot, and J. F. Kearney.** 1994. Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. J. Cell Biol. **102:**1558–1566.
3. **Craig, E. A., B. D. Gambill, and R. J. Nelson.** 1993. Heat shock proteins: molecular chaperones of protein biogenesis. Microbiol. Rev. **57:**402–414.
4. **Dragon, E. A., S. R. Sias, E. A. Kato, and J. D. Gabe.** 1987. The genome of *Trypanosoma cruzi* contains a constitutively expressed, tandemly arranged multicopy gene homologous to a major heat shock protein. Mol. Cell. Biol. **7:**1271–1275.
5. **Drummond, I. A. S., A. S. Lee, E. Resendez, and R. A. Steinhardt.** 1987. Depletion of intracellular calcium stores by calcium ionophore A23187 induces the genes for glucose-regulated proteins in hamster fibroblasts. J. Biol. Chem. **262:**12801–12805.
6. **Ducksin, D., and W. C. Mahoney.** 1982. Relationship of the structure and biological activity of the natural homologues of tunicamycin. J. Biol. Chem. **257:**3105–3109.
7. **Engman, D. M.** Unpublished data.
8. **Engman, D. M., E. A. Dragon, and J. E. Donelson.** 1990. Human humoral immunity to hsp70 during *Trypanosoma cruzi* infection. J. Immunol. **144:**3987–3991.
9. **Engman, D. M., S. C. Fehr, and J. E. Donelson.** 1992. Specific functional domains of mitochondrial hsp70s suggested by sequence comparison of the trypanosome and yeast proteins. Mol. Biochem. Parasitol. **51:**153–155.
10. **Engman, D. M., L. V. Kirchhoff, and J. E. Donelson.** 1989. Molecular cloning of mtp70, a mitochondrial member of the hsp70 family. Mol. Cell. Biol. **9:**5163–5168.
11. **Engman, D. M., K.-H. Krause, J. H. Blumin, K. S. Kim, L. V. Kirchhoff, and J. E. Donelson.** 1989. A novel flagellar Ca^{2+}-binding protein in trypanosomes. J. Biol. Chem. **264:**18627–18631.
12. **Engman, D. M., L. V. Reddy, J. E. Donelson, and L. V. Kirchhoff.** 1987. *Trypanosoma cruzi* exhibits inter- and intra-strain heterogeneity in molecular karyotype and chromosomal gene location. Mol. Biochem. Parasitol. **22:**115–123.
13. **Engman, D. M., S. R. Sias, J. D. Gabe, J. E. Donelson, and E. A. Dragon.** 1989. Comparison of *HSP70* genes from two strains of *Trypanosoma cruzi*. Mol. Biochem. Parasitol. **37:**285–287.
14. **Giambiagi-de Marval, M., K. Gottesdiener, E. Rondinelli, and L. H. Van der Ploeg.** 1993. Predicted amino acid sequence and genomic organization of *Trypanosoma cruzi* hsp 60 genes. Mol. Biochem. Parasitol. **58:**25–31.
15. **Gonzalez, A., T. J. Lerner, M. Huecas, B. Sosa-Pineda, N. Nogueira, and P. M. Lizardi.** 1985. Apparent generation of a segmented mRNA from two separate tandem gene families in *Trypanosoma cruzi*. Nucleic Acids Res. **13:**5789–5804.
16. **Gross-Bellard, M., P. Oudet, and P. Chambon.** 1973. Isolation of high-molecular-weight DNA from mammalian cells. Eur. J. Biochem. **36:**32–38.
17. **Hahn, G. M., and G. C. Li.** 1990. Thermotolerance, thermoresistance, and thermosensitization, p. 79–100. *In* R. I. Morimoto, A. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Kirchhoff, L. V., S. Hieny, G. M. Shiver, D. Snary, and A. Sher.** 1984. Cryptic epitope explains the failure of a monoclonal antibody to bind to certain isolates of *Trypanosoma cruzi*. J. Immunol. **133:**2731–2735.
19. **Kirchhoff, L. V., and F. A. Neva.** 1985. Chagas' disease in Latin American immigrants. JAMA **254:**3058–3060.
20. **Kumar, N., and H. Zheng.** 1992. Nucleotide sequence of a Plasmodium falciparum stress protein with similarity to mammalian 78-kDa glucose-regulated protein. Mol. Biochem. Parasitol. **56:**353–356.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
22. **Lee, A. S.** 1987. Coordinated regulation of a set of genes by glucose and calcium ionophores in mammalian cells. Trends Biochem. Sci. **12:**20–23.
23. **Lindquist, S., and E. A. Craig.** 1988. The heat shock proteins. Annu. Rev. Genet. **22:**631–677.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **McCormick, T. S., and E. C. Rowland.** 1989. *Trypanosoma cruzi*: cross-reactive anti-heart autoantibodies produced during infection in mice. Exp. Parasitol. **69:**393–401.
26. **Morimoto, R. I., A. Tissieres, and C. Georgopoulos.** 1990. Stress proteins in biology and medicine. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Muhich, M. L., and J. C. Boothroyd.** 1988. Polycistronic transcripts in trypanosomes and their accumulation during heat shock: evidence for a precursor role. Mol. Cell. Biol. **8:**3837–3846.
28. **Munro, S., and H. R. B. Pelham.** 1986. An hsp-70 like protein in

the ER: identity with the 78kd glucose-regulated protein and immunoglobulin heavy chain binding protein. Cell **46:**291–300.
29. **Munro, S., and H. R. B. Pelham.** 1987. A C-terminal signal prevents secretion of lumenal ER proteins. Cell **48:**899–907.
30. **Nadeau, K., M. A. Sullivan, M. Bradley, D. M. Engman, and C. T. Walsh.** 1992. 83 kDa heat shock proteins of trypanosomes are potent peptide-stimulated ATPases. Protein Sci. **1:**970–979.
31. **Newport, G., J. Culpepper, and N. Agabian.** 1988. Parasite heat-shock proteins. Parasitol. Today **4:**306–312.
32. **Normington, K., K. Kohno, Y. Kozutsumi, M. J. Gething, and J. Sambrook.** 1989. *S. cerevisiae* encodes an essential protein homologous in sequence and function to mammalian BiP. Cell **57:**1223–1236.
33. **Olson, C. L., K. C. Nadeau, M. A. Sullivan, A. G. Winquist, J. E. Donelson, C. T. Walsh, and D. M. Engman.** 1994. Molecular and biochemical comparison of the 70 kDa heat shock proteins of *Trypanosoma cruzi*. J. Biol. Chem. **269:**3868–3874.
34. **Pelham, H. R. B.** 1990. Functions of the hsp70 protein family: an overview, p. 287–299. *In* R. I. Morimoto, A. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
35. **Polla, B. S.** 1991. Heat shock proteins in host-parasite interactions. Immunol. Today **12:**38–41.
36. **Pouyssegur, J., R. P. C. Shiu, and I. Pastan.** 1977. Induction of two transformation-sensitive membrane polypeptides in normal fibroblasts by a block in glycoprotein synthesis or glucose deprivation. Cell **11:**941–947.
37. **Rondinelli, E.** Conservation and variation of heat shock proteins in *Trypanosoma cruzi*. Parasitol. Today, in press.
38. **Rose, M., L. M. Misra, and J. P. Vogel.** 1989. KAR2, a karygamy gene, is a yeast homolog of mammalian BiP/Grp78. Cell **57:**1211–1221.
39. **Sanders, S., K. Whitfield, J. Vogel, M. Rose, and R. Schekman.** 1992. Sec61p and BiP directly facilitate polypeptide translocation into the ER. Cell **69:**353–366.
40. **Shiu, R. P. C., J. Pouyssegur, and I. Pastan.** 1977. Glucose depletion accounts for the induction of two transformation-sensitive membrane proteins in Rous sarcoma virus-transformed chick embryo fibroblasts. Proc. Natl. Acad. Sci. USA **74:**3840–3844.
41. **Smith, D. B., and K. S. Johnson.** 1988. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene **67:**31–40.
42. **Sorger, P. K.** 1991. Heat shock factor and the heat shock response. Cell **65:**363–366.
43. **Sullivan, M. A., C. L. Olson, A. G. Winquist, and D. M. Engman.** Expression and localization of *Trypanosoma cruzi* hsp60. Submitted for publication.
44. **Tibbetts, R. S.** Unpublished data.
45. **Ting, J., and A. S. Lee.** 1988. The human gene encoding the 78,000-dalton glucose-regulated protein and its pseudogene: structure, conservation and regulation. DNA **7:**275–286.
46. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
47. **Vogel, J. P., L. M. Misra, and M. D. Rose.** 1990. Loss of BiP/Grp78 function blocks translocation of secretory proteins in yeast. J. Cell Biol. **110:**1885–1895.
48. **Wagner, W., and M. So.** 1990. Genomic variation of *Trypanosoma cruzi*: involvement of multicopy genes. Infect. Immun. **58:**3217–3224.
49. **Walder, J. A., P. S. Eder, D. M. Engman, S. T. Brentano, R. Y. Walder, D. S. Knutzon, D. M. Dorfman, and J. E. Donelson.** 1986. The 35-nucleotide spliced leader sequence is common to all trypanosome messenger RNA's. Science **233:**569–571.
50. **Welch, W. J., J. I. Garrels, G. P. Thomas, J. J.-C. Lin, and J. R. Feramisco.** 1983. Biochemical characterization of the mammalian stress proteins and identification of two stress proteins as glucose- and Ca^{2+}-ionophore-regulated proteins. J. Biol. Chem. **258:**7102–7111.
51. **Wu, C., V. Zimarino, C. Tsai, B. Walker, and S. Wilson.** 1990. Transcriptional regulation of heat shock genes, p. 429–442. *In* R. I. Morimoto, A. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
52. **Wu, F. S., Y.-C. Park, D. Roufa, and A. Martonosi.** 1981. Selective stimulation of the synthesis of an 80,000-dalton protein by calcium ionophores. J. Biol. Chem. **256:**5309–5312.
53. **Xiao, H., O. Perisic, and J. T. Lis.** 1991. Cooperative binding of Drosophila heat shock factor to arrays of a conserved 5 bp unit. Cell **64:**585–593.
54. **Zwierzynski, T. A., G. Widmer, and G. A. Buck.** 1989. In vitro 3′ end processing and poly(A) tailing of RNA in *Trypanosoma cruzi*. Nucleic Acids Res. **17:**4647–4660.