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Cloning and Expression of Mitochondrial Heat Shock Protein 70 of *Trypanosoma congolense* and Potential Use as a Diagnostic Antigen

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The ability to use mitochondrial heat shock protein 70 (MTP) of *Trypanosoma congolense* as a diagnostic antigen was examined. One cDNA clone was obtained by immunoscreening of a *T. congolense* procyclic form (PCF) cDNA library with monoclonal antibody (MAb) 10F9. The cDNA clone contained an open reading frame of 1,977 bp encoding a polypeptide consisting of 659 amino acids. Southern blotting analysis indicated that there were at least three copies of the *MTP* gene in the haploid genome. Interference of the MTP RNA resulted in complete inhibition, which indicated that MTP is essential at the PCF stage. Northern and Western blotting analyses revealed that MTP is expressed both in the bloodstream form (BSF) and in PCF. The B-cell epitope recognized by MAb 10F9 was located within 206 amino acids from the C terminus. Depending on the conditions of protein extraction, MTP was cleaved into smaller polypeptides by endogenous proteases. However, the C-terminal epitope of MTP was preserved with a high degree of antigenicity, even after cleavage. Antibody detection by enzyme-linked immunosorbent assay with the truncated recombinant MTP revealed that anti-MTP antibodies exist in experimentally infected mouse sera. Thus, MTP may be useful as an antigen for the serodiagnosis of primary *T. congolense* infection.

Heat shock protein 70 (HSP70) is one of the most highly conserved proteins in eukaryotic and prokaryotic cells. It has been reported that parasite-derived HSP70 plays an important role in the host-parasite interaction (15). An HSP70 of *Toxoplasma gondii* was reported to be highly antigenic and to induce autoantibodies against a host HSP70 during infection. Several studies indicated that this autoimmunity was related to the pathogenicity of *T. gondii* (2, 3). HSP70 of *Trypanosoma cruzi*, the causative agent of Chagas' disease, was reported to be a major target of humoral immunity in human infections. Despite its high degree of evolutionary conservation, antibodies are highly specific for the parasite HSP70 and do not have cross-reactivity to the human HSP70 (6). In general, HSP70 possesses both a highly conserved N-terminal domain and a parasite-specific C-terminal domain. Thus, *T. cruzi* HSP70-specific antibodies are considered to recognize unique amino acid sequences in a C-terminal region. In general, HSP70 is expressed at most of all developmental stages of protozoan parasites and possesses parasite-specific antigenicity. Therefore, HSP70 might be a good candidate as a diagnostic antigen. The diagnosis of trypanosomiasis in mammalian hosts essentially relies on visualization of the parasites in blood. However, parasites are occasionally undetectable because of very low levels of parasitemia. Therefore, detection of antibodies against parasite antigens is required for accurate diagnosis. Most existing antibody detection tests are based on the use of trypanosome extracts as antigens (8), which precludes standardization and specific diagnosis. Recently, a serological method for the detection of *Trypanosoma congolense* with a truncated recombinant HSP70 (Bip homologue) was reported (1). However, since this method showed limited sensitivity for the detection of the organism in cattle with primary infections, more sensitive reagents are required for the accurate detection of primary infections.

African trypanosomes switch their metabolism in response to drastic environmental changes encountered during their life cycle. It is known that the mitochondrion of African trypanosomes in the long slender bloodstream form (BSF) lacks detectable cytochrome activity and that it is missing several key Krebs cycle enzymes. In this developmental stage, the parasite relies almost entirely on glycolysis for energy production. After uptake by the tsetse fly, the procyclic forms (PCFs) in the insect mid gut possess a fully developed mitochondrion and produce ATP by the Krebs cycle and following oxidative phosphorylation in the mitochondrion. Thus, the proteins related to the Krebs cycle and oxidative phosphorylation are developmentally regulated in terms of their enzymatic activities and expression levels (4, 18). A mitochondrial HSP70 (MTP), whose amino acid sequence is distinguishable from those of cytosol HSP70 and Bip, is located in the matrix of a mitochondrion and is required for the translocation and refolding of nucleus-encoded mitochondrial matrix proteins. Because the *MTP* gene of African trypanosomes has not been cloned, the usefulness of recombinant MTP as a diagnostic antigen for African trypanosomiasis has not been clarified. Recently, we reported that monoclonal antibody (MAb) 10F9 recognizes a 76-kDa mitochondrial antigen in *Trypanosoma brucei*, *T. congolense*, and *Trypanosoma evansi* (11). In the present study, we cloned the *MTP* gene of *T. congolense* and clarified that a specific antigenic epitope is located in its C-terminal region. Moreover, we revealed that the C-terminal region of MTP is recognized by sera from mice with primary infection.

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MATERIALS AND METHODS

Cloning and sequencing of *MTP* gene. MAb 10F9, which recognizes a mitochondrial antigen of 76 kDa, was used for immunoscreening (11). A Uni-ZAP cDNA expression library constructed from *T. congolense* PCF mRNA was screened with MAb 10F9 by using the *pico*Blue immunoscreening kit (Stratagene, La Jolla, Calif.). To clone the 5′ region of the *MTP* gene, PCRs with oligonucleotide primers specific for the consensus sequence of the spliced leader RNA (5′-ACGAGGTTTCTGTACTATAT-3′) and the partial cDNA sequence of *MTP* were performed. The nucleic acid sequence was determined with the BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, Calif.).

Trypanosomes. *T. congolense* IL-3000 PCFs were grown in TVM-1 medium as described by Hirumi and Hirumi (10). PCFs were obtained from the culture supernatant by centrifugation at 1,500 × *g* for 10 min at 4°C and washed three times with phosphate-buffered saline. BSFs were grown in male BALB/c mice (age, 8 to 10 weeks; CLEA Japan Inc., Tokyo, Japan). The mice were infected by intraperitoneal injection of BSFs (10^7 parasites/ml, 0.1 ml/mouse). When the mice showed levels of parasitemia greater than 10^8 parasites/ml, the infected blood was collected by cardiac puncture. The trypanosomes were purified by anion-exchange column chromatography (14). Then, the parasites were centrifuged and washed as described above.

Southern blotting analysis. Five micrograms of PCF genomic DNA was digested with restriction enzymes that cut at a single site within the probe-specific sequence (*Eco*RV and *Pst*I) and with restriction enzymes that did not cut within the probe-specific sequence (*Xho*I and *Eco*RI). The DNA was electrophoresed on a 0.8% TAE (Tris-acetate-EDTA)-agarose gel and transferred to a nylon membrane (Hybond-N+; Amersham Biosciences, Little Chalfont, United Kingdom). The blot was probed with a 601-bp fragment (nucleotides 515 to 1,116) labeled by using AlkPhos direct labeling reagents (Amersham Biosciences) under high-stringency conditions. The membrane was washed several times. The results were visualized by using a chemiluminescent substrate (CDP-star; Amersham Biosciences) and the VersaDoc 3000 system (Bio-Rad Laboratories, Hercules, Calif.) according to the recommendations of the manufacturers.

Interference of *MTP* gene RNA of *T. congolense* PCFs. *T. congolense* IL-3000:29-13, which expresses bacteriophage T7 RNA polymerase and the tetracycline repressor, was generated as described previously (12). To construct the RNA interference (RNAi) plasmid used in this experiment, p2T7^{Ti}/*MTP*, we used plasmid p2T7^{Ti}, previously reported by LaCount et al. (13) (see Fig. 4A). The IL-3000:29-13 cells were transiently transfected with p2T7^{Ti}/*MTP* by electroporation (50 μg of DNA/10^7 cells) and grown in TVM-1 medium containing 15 μg of G418 per ml and 10 μg of hygromycin B per ml. At approximately 24 h after transfection, phleomycin (2.5 μg/ml) and tetracycline (1 μg/ml) were added to the medium for the selection and induction of RNA interference.

Northern blotting analysis. Five micrograms of total RNA from two *T. congolense* life cycle stages (BSFs and PCFs) were separated on a 1% agarose gel containing 2.2 M formaldehyde in morpholinepropanesulfonic acid buffer. Then, the RNA was transferred to a nylon membrane (Hybond-N+; Amersham Biosciences). The blot was probed with a 593-bp fragment of the *MTP* gene (nucleotides 736 to 1,329) under high-stringency conditions. The probe was labeled with alkaline phosphatase by using AlkPhos direct labeling reagents (Amersham Biosciences). The blot was also probed with an 18S rRNA-specific probe as a control for standardization of the results. The membrane was washed three times. The results were visualized by using CDP-star (Amersham Biosciences) and the VersaDoc3000 system (Bio-Rad Laboratories) according to the recommendations of the manufacturers. The relative intensities of the bands were determined with Quantity One software (Bio-Rad Laboratories).

Western blotting analysis. Total proteins of *T. congolense* were extracted at different times during the transformation from BSF to PCF (0, 24, 48, and 72 h and 1 week). The cells were washed three times with phosphate-buffered saline and were lysed by the addition of lysis buffer (20 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1% Nonidet P-40, complete protease inhibitor cocktail [Roche Diagnostics GmbH, Mannheim, Germany]) for 30 min at 4°C. Then, the mixture was centrifuged, and the supernatant was collected for use as a protein sample. Protein quantification was performed by use of the BCA Protein Assay Reagent (Pierce Biotechnology, Rockford, Ill.). Eight micrograms of protein was electrophoresed in each lane of a sodium dodecyl sulfate (SDS)–10% polyacrylamide gel and transferred onto a polyvinylidene fluoride membrane (Immobilon-P; Millipore, Billerica, Mass.). The membrane was incubated in 0.05% Tween 20 plus Tris-buffered saline (TBS) containing 1% skim milk and was then incubated with MAb 10F9 for 1 h at room temperature. The membrane was incubated with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG; Amersham Biosciences) for 1 h. The results were visualized by using 3,3-diaminobenzidine and H_2O_2.

Epitope mapping of MTP and expression of recombinant protein. Four truncated DNA fragments of the *MTP* gene were amplified by PCR with primers to which the *Eco*RI site was added (see Fig. 7A). These fragments were cloned into the *Eco*RI site of *Escherichia coli* expression vector pRSET (Invitrogen, Carlsbad, Calif.). The vector was introduced into *E. coli* BL21(DE3)pLysS (Invitrogen), and the cells were incubated in SOB medium (2% [wt/vol] Bacto-tryptone, 0.5% [wt/vol] Bacto-yeast extract, 8.6 mM NaCl, 2.5 mM KCl) at 37°C in the presence of isopropyl-β-D-thiogalactopyranoside to express the partial MTP. The truncated recombinant MTP was purified by using the ProBond purification system (Invitrogen).

Detection of anti-MTP IgG in infected mouse sera by enzyme-linked immunosorbent assay (ELISA). Serum samples from 10 female ICR mice (age, 12 weeks; CLEA Japan Inc.) experimentally infected with *T. congolense* IL-3000 and noninfected serum samples from healthy ICR mice were prepared as follows. Four mice were each infected with 1,000 cells of metacyclic forms (MCFs) by intraperitoneal injection, and six mice were each infected with 1,000 cells of MCFs by subcutaneous injection. Infected mice were repeatedly treated with pentamidine (25 μg/dose/mouse) when parasitemia levels increased to $>10^7$ parasites/ml. Each mouse was challenged with 10,000 BSFs at 30 days postinfection (DPI). Serum samples were collected from the tail vein at 30 and 60 DPI.

ELISA. The purified recombinant MTP was diluted with carbonate-bicarbonate buffer (pH 9.6; 10 μg/ml) and dispensed into the wells of flat-bottom 96-well microplates (MaxiSorp; Nalge Nunc International, Rochester, N.Y.) in 100-μl aliquots. After incubation at 4°C for 24 h, unabsorbed MTP was discarded and 350 μl of blocking solution (TBS containing 5% skim milk) was added. After incubation at 37°C for 1 h, the blocking solution was discarded. The plates were washed four times with a washing solution (TBS with 0.05% Tween 20), and 100 μl of diluted serum (diluted 1:100 with TBS) was added to each well. After incubation at room temperature for 2 h, the plates were washed four times with the washing solution, and 100 μl of horseradish peroxidase-conjugated goat anti-mouse IgG antibody (diluted 1:2,000 with TBS) was added to each well. After incubation at room temperature for 3 h, the plates were washed four times with the washing solution. Then, a substrate solution (1 mg of 4-nitrophenylphosphate per ml, 0.97% 2,2′-iminodiethanol, 0.02 mg of $MgCl_2$ per ml, 0.02 mg of NaN_3 per ml) was added to each well in 100-μl aliquots. After incubation at room temperature for 30 min, 25 μl of stop solution (2 M NaOH) was added. Then, the absorbance at 415 nm was read.

Nucleotide sequence accession number. The sequence of the *MTP* gene of *T. congolense* is stored in the DDBJ database under accession no. AB096859.

RESULTS

***MTP* gene cloning and sequence determination.** Immunoscreening of the *T. congolense* PCF cDNA expression library with MAb 10F9 yielded a cDNA clone of 1,987 bp. It showed a high degree of homology to the mitochondrial HSP70 of *T. cruzi* (DDBJ database accession no. M73627) but lacked homology at the 5′ end. By using a partial sequence of this truncated cDNA clone and the consensus sequence of the spliced leader RNA as primers, we obtained full-length *MTP* cDNA from the cDNA library. This cDNA clone was 2,805 bp in length with an open reading frame of 1,977 bp encoding a polypeptide of 659 amino acids. The similarities of the predicted amino acid sequence to the amino acid sequences of the *MTP*s of *T. cruzi*, *Leishmania major*, and humans were 88, 76, and 57%, respectively (Fig. 1). However, the similarity to the Bip homologue (or HSP70) of *T. congolense* was only 46% (Fig. 2). The N-terminal region of MTP contained a sequence highly homologous to the mitochondrial transportation signal peptide present in other trypanosomatid mitochondrial proteins (Fig. 1). Interestingly, there is a glutamine-rich region near the C terminus of MTP (Fig. 1).

Southern blotting analysis. Southern blotting analysis was performed to determine the copy number of the *MTP* gene. Treatment with enzymes that do not cut within the sequence

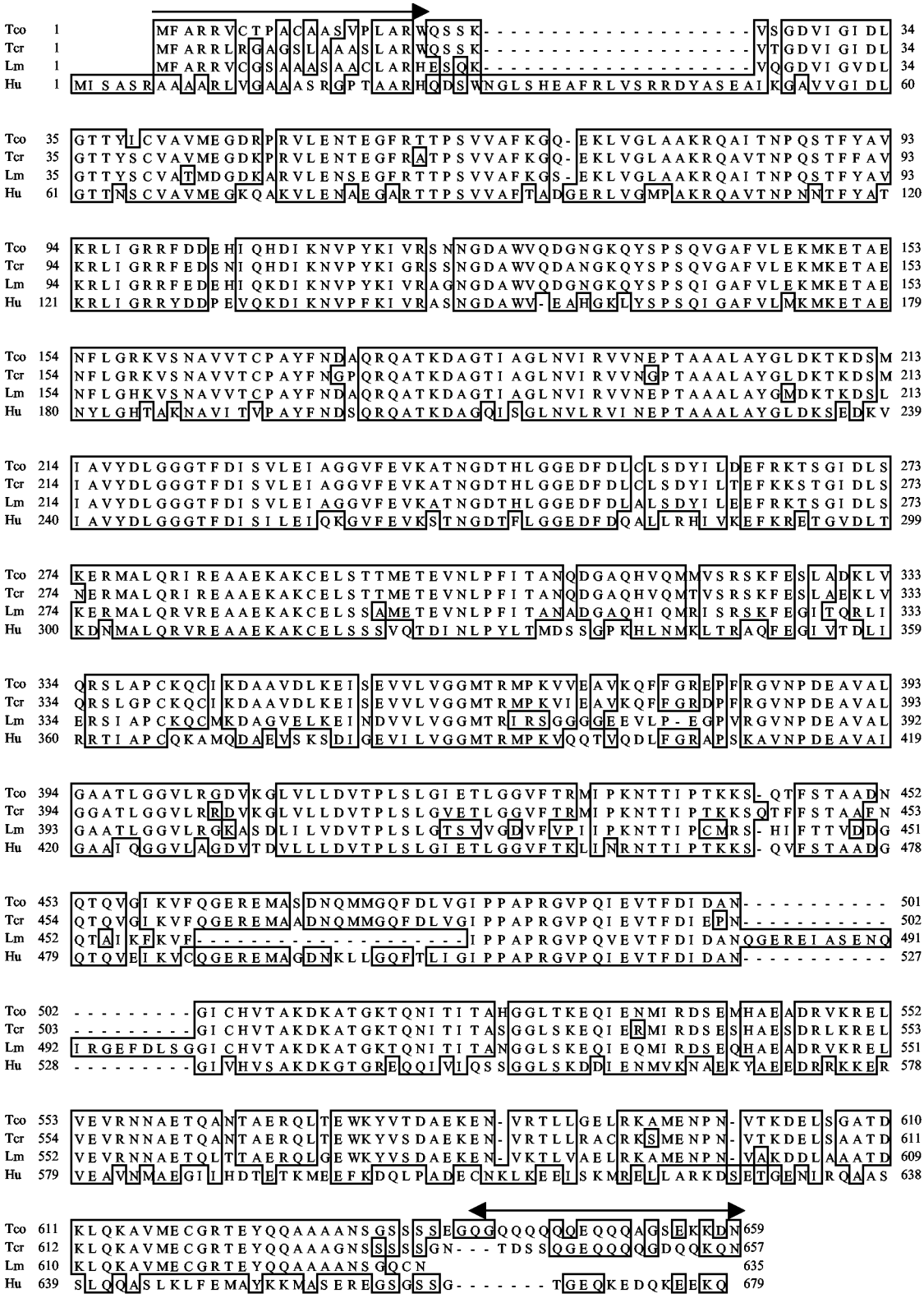

FIG. 1. Comparison of predicted amino acid sequences of *MTP* genes. Tco, Tcr, Lm, and Hu, MTP genes of *T. congolense*, *T. cruzi*, *L. major*, and humans, respectively; arrow, mitochondrial transportation signal peptide; double-headed arrow, glutamine-rich region in MTP.

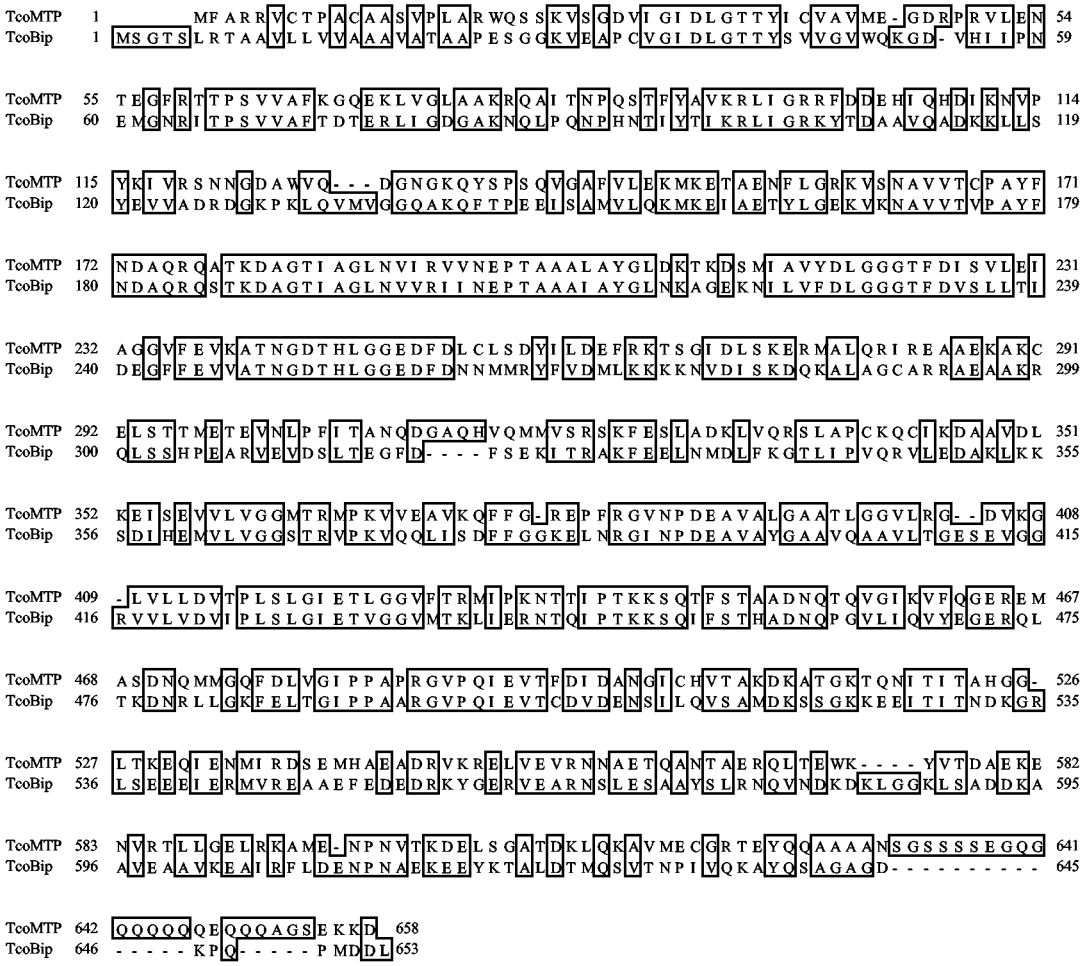

FIG. 2. Comparison of predicted amino acid sequences of *MTP* gene and *Bip* (*HSP70*) gene of *T. congolense* (Tco).

consistent with the probe produced three bands (Fig. 3, lanes 1 and 2), and five or six bands were observed after treatment with enzymes that cut at a single position within the probe sequence (Fig. 3, lanes 3 and 4). These results indicate that there are at least three copies of the *MTP* gene in the haploid genome and that two of the genes are tandemly arranged.

Interference of *MTP* gene RNA in *T. congolense* PCFs. *MTP* gene expression was transiently knocked out to determine its function in *T. congolense* PCFs. *T. congolense* IL-3000:29-13 cells were transfected with p2T7^{Ti}/*MTP* by electroporation. As controls, vectors with the α-tubulin (DDBJ database accession no. AH001117) or the enhanced green fluorescent protein (DDBJ database accession no. AF323988) gene or without an insert were prepared. Cells transfected with p2T7^{Ti}/α-tubulin were microscopically examined for the FAT phenotype due to the loss of a normal cytoskeletal structure in order to determine that the RNA interference system was working correctly (data not shown) (13). Cells transfected with the control vectors showed normal growth. In 48 h the number of cells increased more than 3.3-fold compared with the initial number of cells and gradually decreased after that. Cells that interfered with the expression of *MTP* were not able to increase in number at all (Fig. 4B).

Quantitative change of MTP expression during transformation from BSF to PCF in vitro. Northern blotting analysis was carried out to determine changes in the sizes and the quantities of the transcripts between BSFs and PCFs. The probe for MTP mRNA recognized a 2.4-kb transcript in the total RNA (Fig. 5), which indicated that there were no qualitative and quantitative changes in mRNA expression between BSFs and PCFs.

Western blotting analysis was performed to compare the protein expression levels between the two developmental stages of the parasite. MAb 10F9 recognized a single band of 70 kDa, which corresponded to the predicted size of the protein in BSFs. The relative intensities of the bands of 70 kDa were almost the same between the two life cycle stages, supporting the results of Northern blotting analysis. Interestingly, the number of bands increased after the transformation from BSF to PCF in vitro (Fig. 6). The sizes of the newly detected bands were 66, 55, 41, and 39 kDa. We speculated that these bands were due to proteolytic cleavage of the 70-kDa polypeptide. In order to confirm the instability of MTP, the following experiment was performed. A PCF extract was incubated with or without protease inhibitors at 4, 27, and 37°C for 30 to 120 min and was then subjected to Western blotting analysis. As a result, even in the presence of protease inhibitors, the small

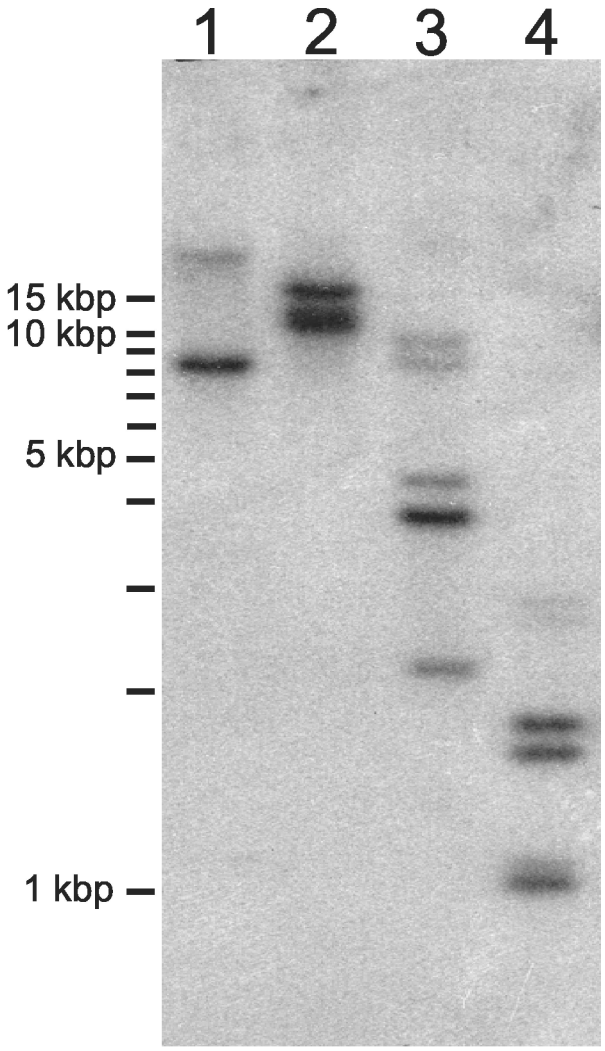

FIG. 3. Southern blotting analysis for determination of the copy number of the *MTP* gene. Five micrograms of genomic DNA digested with *Xho*I (lane 1), *Eco*RI (lane 2), *Eco*RV (lane 3), or *Pst*I (lane 4) was electrophoresed on a 0.8% TAE gel. The DNA was transferred to a nylon membrane and probed with the 601-bp fragment of the *MTP* gene.

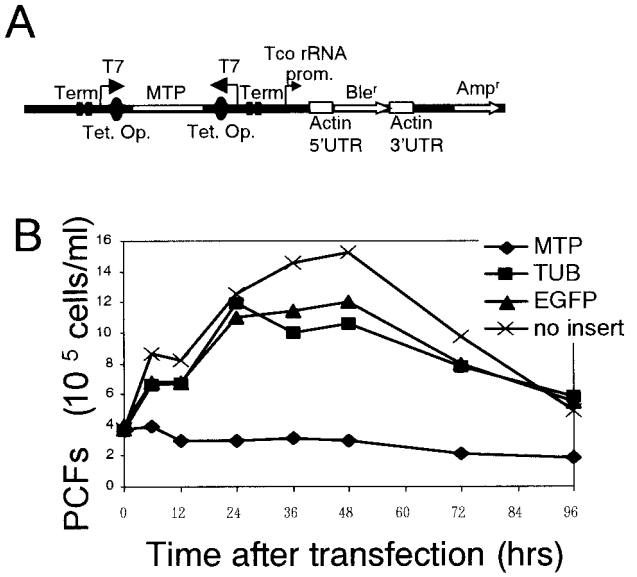

FIG. 4. Transfection of RNAi plasmids into *T. congolense* IL-3000: 29-13 cells. (A) Schematic diagram of plasmid p2T7^{Ti}/*MTP*. T7, Tet. Op., prom., UTR, Ble^r, and Amp^r, T7 promoter, tetracycline operator, promoter, untranslated region, phleomycin resistance gene, and ampicillin resistance gene, respectively. (B) Time course of growth of parasites transfected with p2T7^{Ti}/*MTP* (MTP) compared to those of parasites transfected with p2T7^{Ti}/*TUB* (TUB), p2T7^{Ti}/*GFP* (EGFP), and p2T7^{Ti} (no insert).

fragments were observed after 2 h of incubation at all temperatures tested. However, the epitope of MTP recognized by MAb 10F9 was conserved even after incubation at 37°C for 2 h without protease inhibitors (data not shown).

Epitope mapping of MTP. Four truncated versions of MTP, named ΔMTP1, ΔMTP2, ΔMTP3, and ΔMTP4, were expressed in *E. coli* (Fig. 7A). Western blotting analysis with MAb 10F9 showed that the MAb recognized ΔMTP4, which was a C-terminal polypeptide consisting of 206 amino acids (Fig. 7B). Therefore, ΔMTP4 was purified for use as an antigen in the antibody capture ELISA detection system.

Detection of anti-ΔMTP4 antibodies in infected mouse sera. To evaluate whether ΔMTP4 can be a suitable diagnostic antigen for the detection of *T. congolense* infection, purified ΔMTP4 was used as an antibody capture antigen for ELISA and Western blotting. As shown in Fig. 8, the absorbances of all serum samples from mice experimentally infected with *T. congolense* were significantly higher than those of serum samples from healthy mice ($P < 0.01$). The anti-ΔMTP4 antibody was not detected by Western blotting analysis (data not shown), probably due to the lower sensitivity of Western blotting compared with that of the ELISA.

DISCUSSION

We have cloned the *T. congolense MTP* gene and determined its entire nucleic acid sequence. Southern blotting analysis demonstrated that the haploid genome contains at least three copies of the *MTP* gene and that two of the genes may be closely arranged on the same chromosome. The open reading frame encodes a polypeptide of 659 amino acids that is highly homologous to a series of MTPs in eukaryotes and DnaK of *E. coli* (5, 7, 17). The N-terminal region of MTP contained a sequence highly homologous to the mitochondrial transportation signal sequences demonstrated in other trypanosomatid mitochondrial proteins (Fig. 1). The transportation signal sequence for some mitochondrial matrix proteins in trypanosomes appears to be relatively short (7 to 9 amino acids or less, according to a recent report [9]), but the length of the signal peptide of *T. congolense* MTP is as long as that of *T. brucei* MTP (15 to 20 amino acids). Interestingly, there is a glutamine-rich region near the C terminus of MTP that is not present in *L. major* and human MTPs. Although the function and biological importance of the glutamine-rich region are not yet understood, the region is unique in *T. congolense*. Therefore, the region may be the parasite-specific epitope in MTP.

One of the striking characteristics of trypanosomes is the flexibility of their energy metabolism in response to the vari-

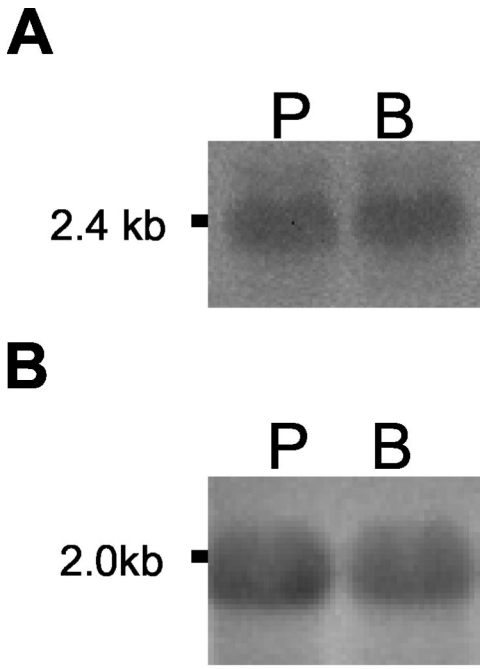

FIG. 5. Northern blotting analysis of *MTP* transcript. Five micrograms of total RNA from PCFs (lanes P) and BSFs (lanes B) was electrophoresed and transferred to a nylon membrane. (A) Comparison of *MTP* transcripts of PCFs and BSFs. The membrane was probed with the 593-bp fragment of the *MTP* gene. (B) The membrane was probed with the 590-bp fragment of 18S rRNA gene as a control.

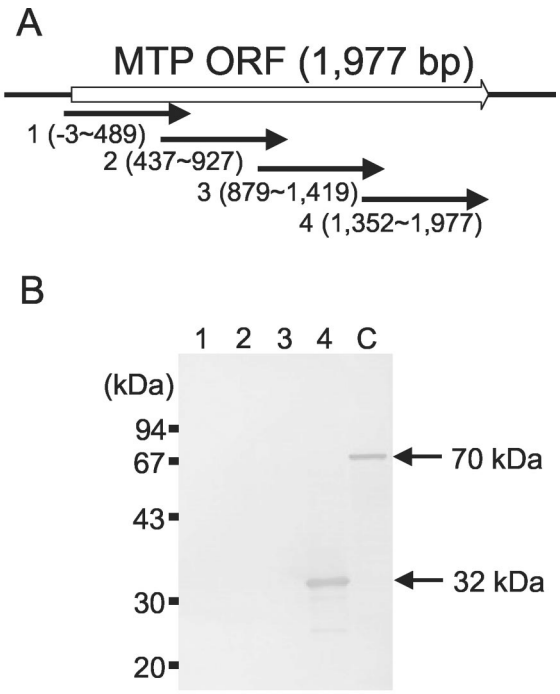

FIG. 7. Epitope mapping of MTP. (A) Schematic diagram of the locations of four DNA fragments inserted and expressed for determination of the epitope. 1, 2, 3, and 4, ΔMTP1, ΔMTP2, ΔMTP3, and ΔMTP4, respectively. The numbers in parentheses indicate the nucleic acid positions in the *MTP* gene sequence. (B) Western blotting analysis of recombinant ΔMTPs. ΔMTPs (lanes 1, 2, 3, and 4, ΔMTP1, ΔMTP2, ΔMTP3, and ΔMTP4, respectively) were electrophoresed on an SDS–10% polyacrylamide gel and transferred to a nitrocellulose membrane. Immunoblotting was performed with MAb 10F9. Whole trypanosome extract was electrophoresed in lane C. ORF, open reading frame.

able environmental changes encountered during their life cycle. It has been reported that the levels of expression of ATP synthase subunits α, β, and 9, which are nucleus-encoded mitochondrial proteins, are regulated during the life cycle by differential mRNA stability. The levels of expression of the genes for these proteins are lower in BSFs than in PCFs, reflecting the switching of the metabolic system (4, 18). Since MTP is also one of the mitochondrial matrix proteins, the MTP expression level might be lower in BSFs if the level of expression of the *MTP* gene is regulated during the life cycle. Northern blotting and Western blotting analyses were carried out to determine the level of MTP expression in the BSF stage. The

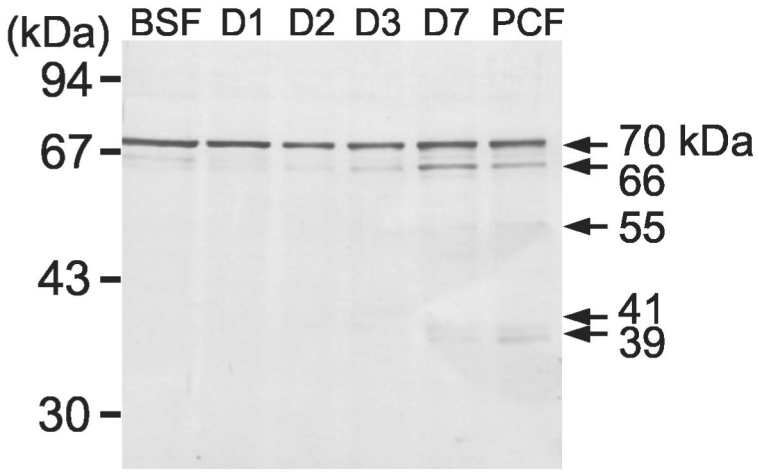

FIG. 6. Western blotting analysis of the quantitative change in MTP expression during parasite transformation. Protein samples were collected at the indicated times after the initiation of the in vitro culture (day 0 [BSF], day 1 [D1], day 2 [D2], day 3 [D3], day 7 [D7], and day 14 [PCF]). Eight micrograms of protein samples was electrophoresed on an SDS–10% polyacrylamide gel and transferred to a nitrocellulose membrane. Immunoblotting was performed with MAb 10F9.

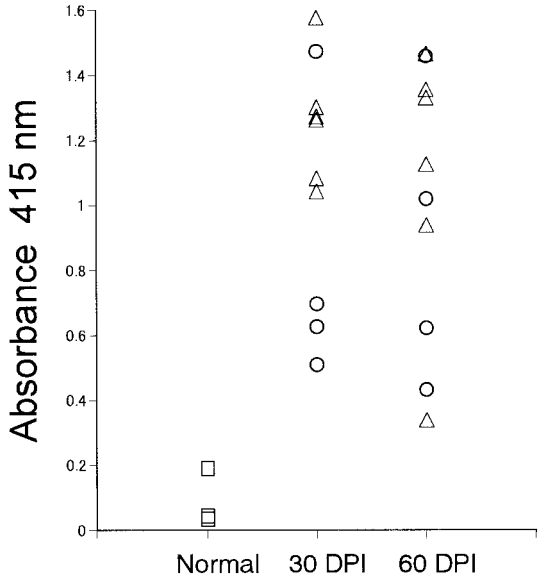

FIG. 8. Detection of anti-ΔMTP4 IgG in infected mouse sera by ELISA. Four mice were each infected with 1,000 MCFs by the intraperitoneal route (circles), and six mice were each infected with 1,000 MCFs via the subcutaneous route (triangles). When parasitemia levels increased to $>10^7$ parasites/ml, the mice were treated two to seven times with pentamidine (25 μg/dose/mouse). At 30 DPI, each mouse was challenged with 10,000 MCFs via the same route by which primary infection was initiated. Negative control sera were obtained from three mice (squares).

results showed that there were no significant differences in transcription and translation levels between BSFs and PCFs. This indicates that MTP is equally required by both PCFs and BSFs, although cytochrome-mediated respiration and oxidative phosphorylation are not active in the latter form. Since the level of MTP expression in BSFs is the same level as that in PCFs, host immune responses could be directed to MTP.

Specific antibodies against MTP might not effectively inhibit the MTP function because MTP is not accessible to the antibody. However, if MTP could induce antibody production in the infected host, it would be a candidate as a diagnostic antigen. Therefore, we examined an antigenic epitope of MTP and anti-MTP antibody production in hosts with primary infection. The result of epitope mapping showed that there was a highly antigenic epitope in the C-terminal region of MTP. This antigenicity might have some relation to the unique glutamine-rich region located near the C terminus (Fig. 1). The stability of the epitope should therefore be considered if MTP is used for serological diagnosis, because it has been reported that the HSP70 of *T. cruzi* is specifically cleaved by its own intracellular proteases according to the conditions and time of incubation of the protein samples (16). By Western blotting analysis we discovered that MTP was also rapidly cleaved by endogenous proteases. However, the C-terminal epitope was not destroyed after a long incubation time, which suggests that this epitope is highly stable and capable of eliciting antibodies during *T. congolense* infection. In order to detect anti-MTP antibodies, an ELISA with the recombinant ΔMTP4, which contains the C-terminal epitope, was carried out. The result indicated that *T. congolense*-infected mice could produce ΔMTP4-specific antibodies during infection. The average level of antibody to ΔMTP4 seemed to be lower at 60 DPI than at 30 DPI, but in six mice the antibody levels increased from 30 to 60 DPI, while the antibody levels decreased in the other four mice. The mice were infected with *T. congolense* MCFs by either the intraperitoneal or the subcutaneous route. Subcutaneous injection of MCFs mimics natural infection by a tsetse fly bite, while intraperitoneal injection is commonly used to induce experimental infection. However, the site of injection did not make a difference in the antibody response pattern. Although we could not clearly demonstrate in this study how early during the infection the antibody to ΔMTP4 became detectable, the result clearly indicated that ΔMTP4 was useful for detection of the primary stage of *T. congolense* infection by ELISA. As the sera used in the test were obtained from experimentally infected mice, further evaluation of ΔMTP4 with sera from naturally infected animals is required.

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