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Differential Regulation of Two Distinct Families of Glucose Transporter Genes in *Trypanosoma brucei*

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A tandemly arranged multigene family encoding putative hexose transporters in *Trypanosoma brucei* has been characterized. It is composed of two 80% homologous groups of genes called THT1 (six copies) and THT2 (five copies). When *Xenopus* oocytes are microinjected with in vitro-transcribed RNA from a THT1 gene, they express a glucose transporter with properties similar to those of the trypanosome bloodstream-form protein(s). This THT1-encoded transport system for glucose differs from the human erythrocyte-type glucose transporter by its moderate sensitivity to cytochalasin B and its capacity to transport D-fructose. These properties suggest that the trypanosomal transporter may be a good target for antitrypanosomal drugs. mRNA analysis revealed that expression of these genes was life cycle stage dependent. Bloodstream forms express 40-fold more THT1 than THT2. In contrast, procyclic trypanosomes express no detectable THT1 but demonstrate glucose-dependent expression of THT2.

Glucose is the major source of energy for most protozoan parasites. The uptake of this sugar across the plasma membrane is a process of fundamental importance. The well-characterized glucose transport systems of members from the genera *Trypanosoma* (14, 16, 18, 36, 38), *Leishmania* (46, 47), and *Plasmodium* (37) are closely related, as judged by their substrate specificity and drug sensitivity. They act as either facilitative transporters (18, 38) or glucose/H^+ cotransporters (31, 37, 46).

Bloodstream forms of *Trypanosoma brucei* possess a glucose transport system which differs significantly from that present in human erythrocytes. It may therefore provide a potential target for antiparasitic drugs or vaccine development.

African trypanosomes adapt their metabolism to suit the very divergent environments that they encounter at different points in their life cycle (15). Infective bloodstream forms use only glucose (present at a fixed concentration in mammalian blood) as a carbon source. This developmental form transports glucose across the plasma membrane by facilitated diffusion (14, 16), with uptake of glucose representing the rate-limiting step in glycolysis (38). The in vivo estimated K_m for glucose in this process is about 1 mM (14, 16, 36, 38). Procyclic culture forms, homologous to the insect midgut stage, can thrive in the absence of glucose, preferring amino acids (mainly proline) as an energy source. Nevertheless, procyclic forms do express a glucose transport system which differs from that found in the bloodstream forms (31, 38).

In a previous report, we described the cloning and analysis of a developmentally regulated *T. brucei* gene coding for a putative hexose transporter called THT (for trypanosome hexose transporter) (9). Here we report the characterization of a multigene family encoding two highly related proteins, THT1 and THT2. The genes are all located in a single cluster containing six adjacent copies of the THT1 genes and five copies of the THT2 genes. Expression of these genes during the parasite life cycle is discussed; the hexose transporter function of the THT1 gene has been determined by expression in *Xenopus* oocytes.

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MATERIALS AND METHODS

Trypanosomes. The bloodstream forms of *T. brucei brucei* EATRO-164 (kindly provided by R. Brun) were obtained from infected rats as described previously (26). Procyclic forms of *T. brucei brucei* Stib-247 (kindly provided by R. Brun) were cultured at 26°C in a semidefined medium containing 10% fetal calf serum dialyzed by ultrafiltration (Sigma F3260 filter) or undialyzed serum, 3.5 mg of hemin per ml, and 0.5 g of L-proline per liter, with or without 1 g of glucose per liter (3).

Construction and screening of genomic libraries. For cloning of THTg.A, total DNA of EATRO-164 was fully digested by *Eco*RI, and 8- to 25-kb fragments purified by fast protein liquid chromatography were ligated into lambda EMBL3a (Stratagene). The recombinant phage DNA was packaged in vitro (19), and the library was screened by hybridization with a ^{32}P-labeled 2.23-kb cDNA1c fragment contained in clone ptb1c (9); 0.2% of the clones were positive and contained a 17-kb *Eco*RI fragment. A clone called THTg.A was used for subsequent characterization.

The strategy for cloning of THTg.B was the same as that used for cloning of THTg.A except that genomic DNA, partially digested by *Sau*3A, was ligated into lambda Dash II (Stratagene). Among 10^4 plaques, one clone called THTg.B was recognized by ^{32}P-labeled cDNA1c.

Southern and Northern (RNA) blotting analysis. For Southern blot hybridization, 2.5 μg of genomic DNA isolated as described elsewhere (6) was subjected to endonuclease digestion, electrophoresed in a 0.8% agarose gel, blotted onto a Hybond N+ membrane (Amersham), and hybridized with polymerase chain reaction (PCR) fragments, ^{32}P labeled by specific priming, at 65°C in 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.0]–0.1% sodium dodecyl sulfate (SDS). Washings were performed at 65°C in 0.2× SSPE–0.1% SDS before autoradiography. To rehybridize blots, probes were removed from the membranes by washing in boiling water containing 0.5% SDS.

Northern blot analysis was performed with 10 μg of total RNA isolated by the guanidinium thiocyanate-phenol-chloroform method (11). RNA was fractionated on a 1% agarose gel containing 2.2 M formaldehyde, using RNA molecular markers (Bethesda Research Laboratories) as standards. The blot was probed with ^{32}P-labeled PCR fragments at 65°C in 6× SSPE–0.1% SDS and washed at 65°C in 0.2× SSPE–0.1% SDS. After autoradiography, probes were removed from the blots by washing in boiling water containing 0.1% SDS to allow hybridization with other probes. The total amount of RNA from both bloodstream and procyclic forms of trypanosomes was controlled by reprobing blots with a ^{32}P-labeled actin gene (kindly provided by E. Pays).

DNA sequencing and analysis. The 17-kb *Eco*RI fragment and the 13.7-kb *Sau*3A fragments contained in phages THTg.A and THTg.B, respectively, were subcloned into pBluescript (Stratagene) and sequenced by the Sanger dideoxynucleotide chain termination method (34), using modified T_7 DNA polymerase (Pharmacia). Both primers flanking the cloned DNA insert and specific primers were used to sequence most of the subclones.

DNA and amino acid sequences were analyzed by using the DNA Strider program and compared with protein and DNA sequences in the Los Alamos data base.

In vitro transcription. A PCR fragment containing the THT1 coding sequence was synthesized by using oligonucleotides TTCAACAACATGACTGAGCGTCGT and CAA TACCTCACCAGATGAATTAAGCTTCA. The underlined poly(dA-dC)-rich sequence is implicated in the translation initiation of eukaryote mRNA (24). The PCR fragment was introduced into the *Sma*I restriction site localized between the T_7 RNA polymerase promoter and the poly(A) stretch of plasmid BSDP1400H-A_{65} (kindly provided by H. B. Osborne, Rennes, France). Recombinant DNA was linearized with *Eco*RV, recovered by phenol extraction followed by ethanol precipitation, and used for an in vitro transcription of full-length capped transcripts with T_7 RNA polymerase (30). Plasmid was hydrolyzed by RQ1 DNase treatment and then subjected to phenol extraction and ethanol precipitation.

Preparation of oocytes and RNA injection. Ovaries were removed from mature *Xenopus laevis* (13) and suspended in modified Barth medium [MBS; 0.82 mM $MgSO_4$, 0.41 mM $CaCl_2$, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 88 mM NaCl, 2.4 mM $NaHCO_3$, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.6)]. Oocytes were dissociated, and follicle cells were removed by incubation in MBS containing 40 mg of Dispase (neutral protease; Boehringer) per ml for 4 h and in 0.1% collagenase for 2 h at room temperature. After extensive washing in MBS, stage 5 and 6 oocytes were selected and incubated in MBS at 18°C. Oocytes (200 to 300) were microinjected at 20 to 40 nl per oocyte with a solution containing about 10 ng of in vitro-synthesized capped RNA. The oocytes containing injected RNA were incubated for 1 to 3 days at 18°C in MBS, after which healthy oocytes were analyzed for 2-deoxy-D-glucose (2-DOG) uptake.

2-DOG uptake. Oocytes (10 to 20 per group) were incubated for 120 min in 0.6 ml of MBS containing 5 μCi of 2-DOG glucose (Amersham), 2 mM 2-DOG, and eventually the appropriate amount of inhibitor or hexose. Uptake was terminated by washing the oocytes three times in ice-cold MBS containing 250 mM D-glucose. The oocytes were dissolved in 0.2 ml of 2% SDS, and the incorporated radioactivity was assayed by liquid scintillation counting.

Nucleotide sequence accession number. The sequence of the cloned DNA genomic fragments (THTg.A and THTg.B) has been submitted to the EMBL data base under accession number X69091.

RESULTS

Organization of the THT genes. In a previous report, we described the cloning and analysis of a *T. brucei* cDNA (cDNA1c) which is highly homologous to the genes encoding mammalian hexose transporters (9). This cDNA was used to isolate two *T. brucei* genomic clones, THTg.A (17-kb insert) and THTg.B (13.7-kb insert). Restriction enzyme analysis showed that the clones contain an 8-kb overlap, and sequence analysis demonstrated that the region encodes two groups of closely related THT genes (Fig. 1). The first group contains six copies of the THT1 gene; five of these copies were sequenced and found to be at least 98% identical with our published sequence of cDNA1c. The second cloned gene cluster contains 2.5 copies of the THT2 gene which are 80% identical with the cDNA1c sequence. There are spacer regions of about 800 bp between the THT1 genes and of about 1 kb between the THT2 genes.

To determine whether all of the THT genes are tandemly reiterated in the *T. brucei* genome and whether clones THTg.A and THTg.B contain all genomic copies of the gene, detailed restriction mapping of the genomic DNA was undertaken (Fig. 2). A single large fragment of genomic DNA cut with enzymes such as *Sma*I that do not cut in the cloned THT genes hybridizes to THT probes in Southern blots. No smaller fragments that might include nonlinked copies of the gene are observed. Additionally, when restriction enzymes that cut once in all of the cloned genes are used to digest the genomic DNA, only the bands that would be predicted for a single gene cluster are observed on the blots. Digestion with *Bam*HI, *Hae*II, or *Apa*I results in a 2.4-kb band corresponding to the THT1 unit repeat and a 2.6-kb band representing the THT2 unit length plus two bands derived from the 5' end of the first THT1 copy (probe 1) and the 3' end of the last THT2 copy (probe 3'). These observations and results of genomic restriction mapping using 15 different enzymes agree with a clustered organization of the THT genes in a single region.

To determine the number of the THT1 and THT2 genes, we performed partial digestion analysis with enzymes cutting within the unit repeat. When the large *Eco*RI fragment containing the THT1 repeats was digested with limiting concentrations of *Dra*I (which cuts at least once in each of the THT unit repeats), bands indicating the presence of six THT1 copies were obtained (data not shown). This genomic *Eco*RI fragment corresponds to the fragment contained in clone THTg.A and substantiates the authenticity of this clone. A similar analysis was carried out for the THT2 genes by first cutting the genomic DNA with limiting concentrations of *Cla*I (which cuts in the intergenic region between the repeats) followed by digestion of the DNA with an excess concentration of *Bgl*II (which cuts only in the region before the first THT2 gene). As seen in Fig. 3A, partial digestion creates a ladder of bands detected on a Southern blot when we use a probe that is specific for the region between the THT1 and THT2 repeats and hybridizes with both the THT1- and THT2-containing *Bgl*II fragments (Fig. 3, lane 0). The ladder is consistent with the presence of only five copies of the THT2 genes. Additional copies of THT2 could be present 3' of the fifth copy if those copies did not contain *Cla*I sites. This possibility was eliminated by densitometric analysis of the bands shown in Fig. 2 (probe 2) and by

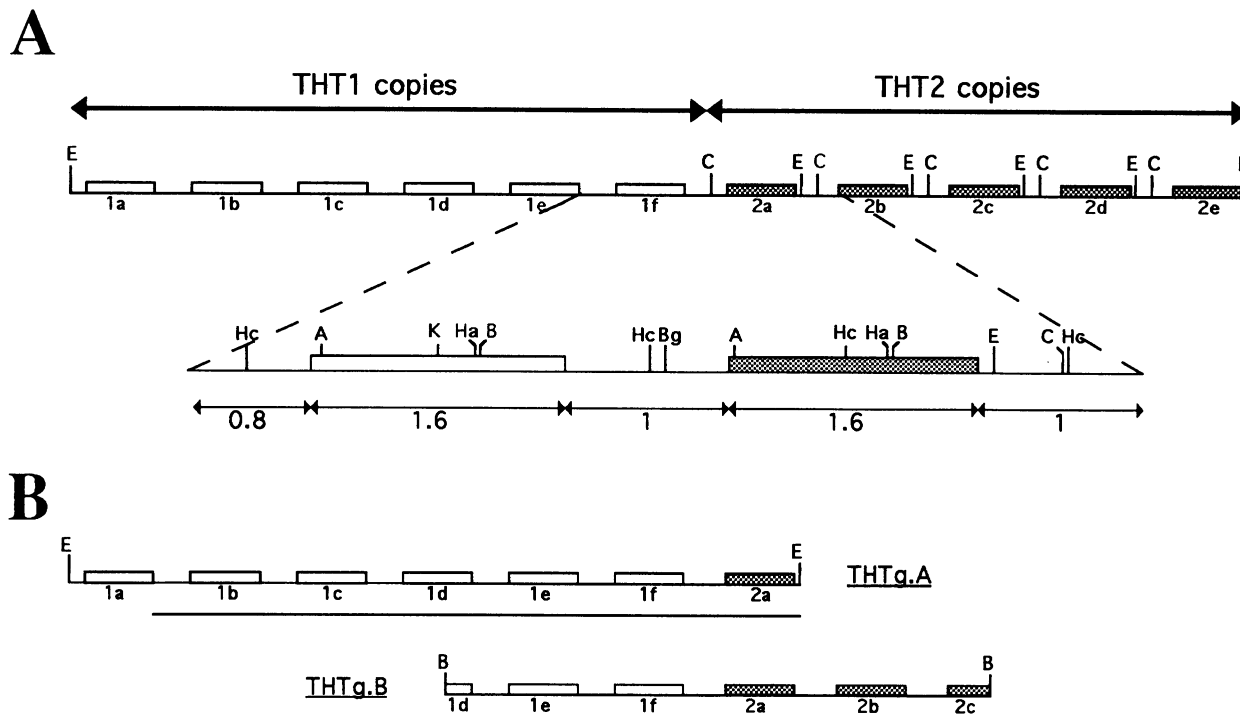

FIG. 1. Physical map of the THT gene family. (A) Organization and restriction endonuclease cleavage sites of THT1a to THT1f copies (white boxes) and THT2a to THT2e copies (grey boxes) are shown; the THT unit repeats and sizes of the intergenic regions are indicated in kilobases below the map. The sequence of the expanded region is shown in Fig. 4. (B) Map showing cloned genomic fragments THTg.A and THTg.B, of which the underlined regions have been sequenced. Abbreviations for restriction sites: A, *Apa*I; B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; Ha, *Hae*II; Hc, *Hinc*II; K, *Kpn*I.

restriction enzyme mapping to detect additional THT2-like genes in the 3′ fragment. In conclusion, the results of the analysis indicate that the *T. brucei* genome contains 6 copies of the THT1 gene, all of which are in clone THTg.A, and 5 copies of the THT2 gene, of which only the first 2.5 are represented in clone THTg.B.

Characterization of THT genes. Figure 4 presents the sequence analysis of the sixth THT1 gene and the first THT2 gene plus the intergenic region associated with both clusters and the region between the two clusters. The homology between the different sequenced copies of the THT1 and THT2 genes is about 98%. In contrast, the homology between a THT1 gene and a THT2 gene is only 80% (Fig. 5). When the deduced amino acid sequences of the THT1 and THT2 genes are analyzed, the hydrophobicity profile suggests that the two proteins have similar structures. The 12 predicted membrane-spanning regions and the similarity of the deduced amino acid sequences with those of known glucose transporters suggest that the THT1 and THT2 genes encode proteins with similar functions. With use of the known structure of the human glucose transporter as a model, the N and C termini of the THT1 and THT2 proteins are probably localized on the cytoplasmic face of the cell membrane, and the first hydrophilic domain forms a large exofacial loop. This first extracellular domain includes the region displaying greatest divergence between the THT1 and THT2 amino acid sequences (positions 63 to 114) (Fig. 5). Interestingly, all of the cysteines in this cysteine-rich region are present in both proteins. The proteins differ from one another significantly at the amino acid level in four other regions, including the third transmembrane region (amino acids 151 to 170), the sixth hydrophilic domain (237 to 244), the sixth hydrophobic domain (250 to 259), and the C terminus (515 to 527).

Expression of THT genes. As shown previously (9), mRNA transcripts from THT genes are present at higher levels in the bloodstream-form organisms than in the procyclic forms. THT1 and THT2 transcripts can be differentiated on Northern blots by hybridization with specific probes (Fig. 6). In bloodstream forms, a single 2.45-kb THT2 mRNA is observed, while the THT1 probe detects two transcripts of 2.45 and 2.3 kb. Only the less intense 2.45-kb THT1 band is recognized by a probe specific for the noncoding region downstream of THT1f. This finding suggests that this band corresponds to the transcript containing THT1f. The mRNA corresponding to the first five THT1 copies is contained in the 2.3-kb transcript. The intensity of this 2.3-kb band is about fivefold greater than that of the 2.45-kb THT1f band, suggesting that the steady-state level of the mRNA is equivalent for all of the THT1 copies. A DNA fragment common to THT1 and THT2 used as a probe against total RNA from bloodstream forms hybridized about fourfold more intensely to the 2.3-kb THT1 mRNA than to the 2.45-kb mRNA which contains both THT2 and THT1f. The intensities of the 2.45-kb band of the procyclic forms [PF (−G)] and the trypomastigote forms (BF) probed with a DNA fragment common to THT1 and THT2 (3′ probe) are quite similar, while the intensity of the same bands is about 10-fold higher for the procyclic forms [PF (−G)] when probe 2 is used (Fig. 6). This finding implies that the THT2 transcripts in the bloodstream forms represent only 10% of the signal generated by the 2.45-kb band. Considering that the 2.3-kb THT1

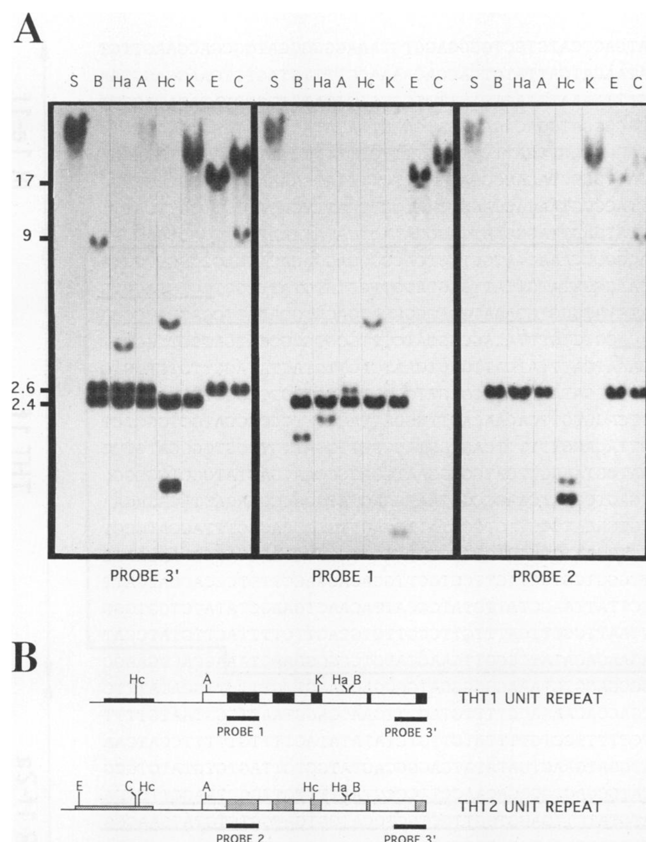

FIG. 2. Southern blot analysis of the THT gene family. (A) Restriction endonuclease digestion products of *T. brucei* EATRO-164 genomic DNA (2.5 μg) were separated, blotted, and hybridized as described in Materials and Methods. The same blot was hybridized successively with three different ^{32}P-labeled probes, specific to THT1 (probe 1), specific to THT2 (probe 2), and common to THT1 and THT2 (probe 3'). Molecular weight markers are DNA fragments from phage lambda digested with *Hin*dIII. Sizes are indicated in kilobases. (B) Schematic representation of the THT1 and THT2 unit repeats. The black regions present in the THT1 unit repeat correspond to those sequences which differ from the grey region localized in the THT2 unit repeat. Stippled boxes represent sequences coding for THT1 and THT2. Probes 1, 2, and 3' are indicated under the coding sequences. Abbreviations for restriction sites: A, *Ava*I; B, *Bam*HI; C, *Cla*I; E, *Eco*RI; Ha, *Hae*II; Hc, *Hin*cII; K, *Kpn*I.

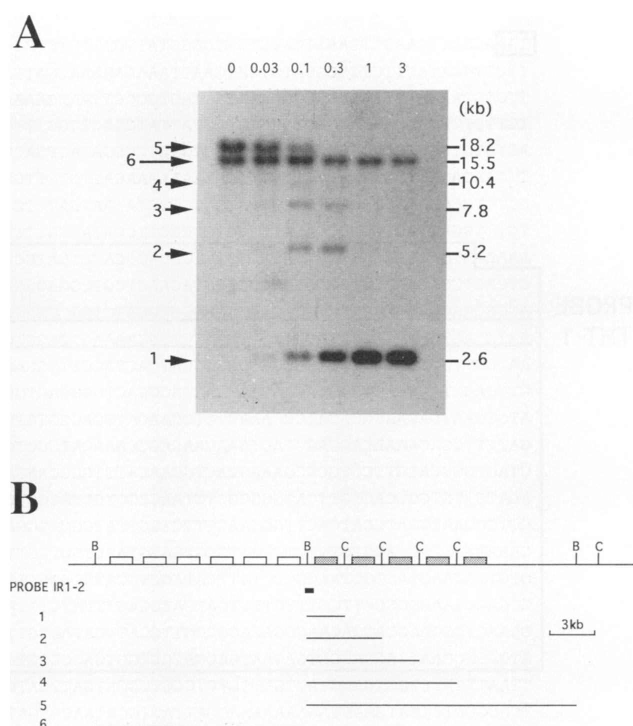

FIG. 3. Determination of THT2 copy number. (A) Genomic DNA (10 μg) was digested first for 1 h with increasing amounts of *Cla*I (0, 0.03, 0.1, 0.3, 1, and 3 U) and then to completion with *Bgl*II. The digests were then separated, blotted, and hybridized with the specific ^{32}P-labeled probe IR1-2 as described in Materials and Methods. The sizes of the different fragments designated 1 to 6 are indicated. (B) Schematic map representing the clustered THT1 (white boxes) and THT2 (grey boxes) genes. The PCR product IR1-2 specific to the THT1f-THT2a intergenic region is shown below by a thick bar. The six different fragments revealed by probe IR1-2 are represented by thin bars. Abbreviations for restriction sites: B, *Bgl*II; C, *Cla*I.

mRNA is about 4-fold more intense than the 2.45-kb mRNA (probe 3'), we estimate that the THT1 genes are expressed at a level about 40-fold higher than the level of THT2 genes in bloodstream forms of *T. brucei*.

Procyclic trypanosomes are usually cultured in medium supplemented with fetal calf serum containing glucose. In the insect, however, they rely predominantly on amino acids, mainly proline, as a carbon source. To determine whether glucose affects the expression of THT genes in procyclic forms, organisms were cultured in a medium supplemented with dialyzed fetal calf serum containing 0.5 mM glucose (0.05 mM, final concentration) and in the same medium containing 5 mM glucose. Northern blot analysis (Fig. 6) indicates that the cells grown in a medium containing 0.05 mM glucose have about a 60-fold-higher level of the THT2 mRNA than do those grown in a medium containing 5 mM glucose and that THT1 mRNA cannot be detected in procyclic forms cultured in either condition (Fig. 6).

We concluded that the THT1 genes are expressed specifically in bloodstream forms of *T. brucei* and that THT1 could encode the principal glucose transporter present in these forms. In contrast, THT2 mRNA is present in both life cycle stages, and the steady-state level in procyclic forms is affected by glucose in the medium. When bloodstream-form trypanosomes are cultured in a medium containing 5 mM glucose, the level of THT2 mRNA remains about 10-fold higher than that observed in the procyclic forms cultured in the same conditions (data not shown). These data suggest that the THT2 genes encode a glucose-related transporter that functions in both bloodstream and procyclic forms of the trypanosomes.

Determination of the THT function. *X. laevis* oocytes have been used extensively for the heterogenous expression of mammalian transport systems, including the insulin-sensitive hexose transporter (41, 42). This system has proved to be an important tool for characterization of the transporters. We have tested the hypothesis that the THT1 genes encode a glucose transporter by injecting in vitro-transcribed THT1 RNA into *Xenopus* oocytes and then measuring the ability of the oocytes to transport 2-DOG. Although the oocytes possess an endogenous glucose transport activity, the uptake of 2-DOG was considerably increased in oocytes injected with 10 ng of THT1 RNA. Indeed, a 180-fold increase

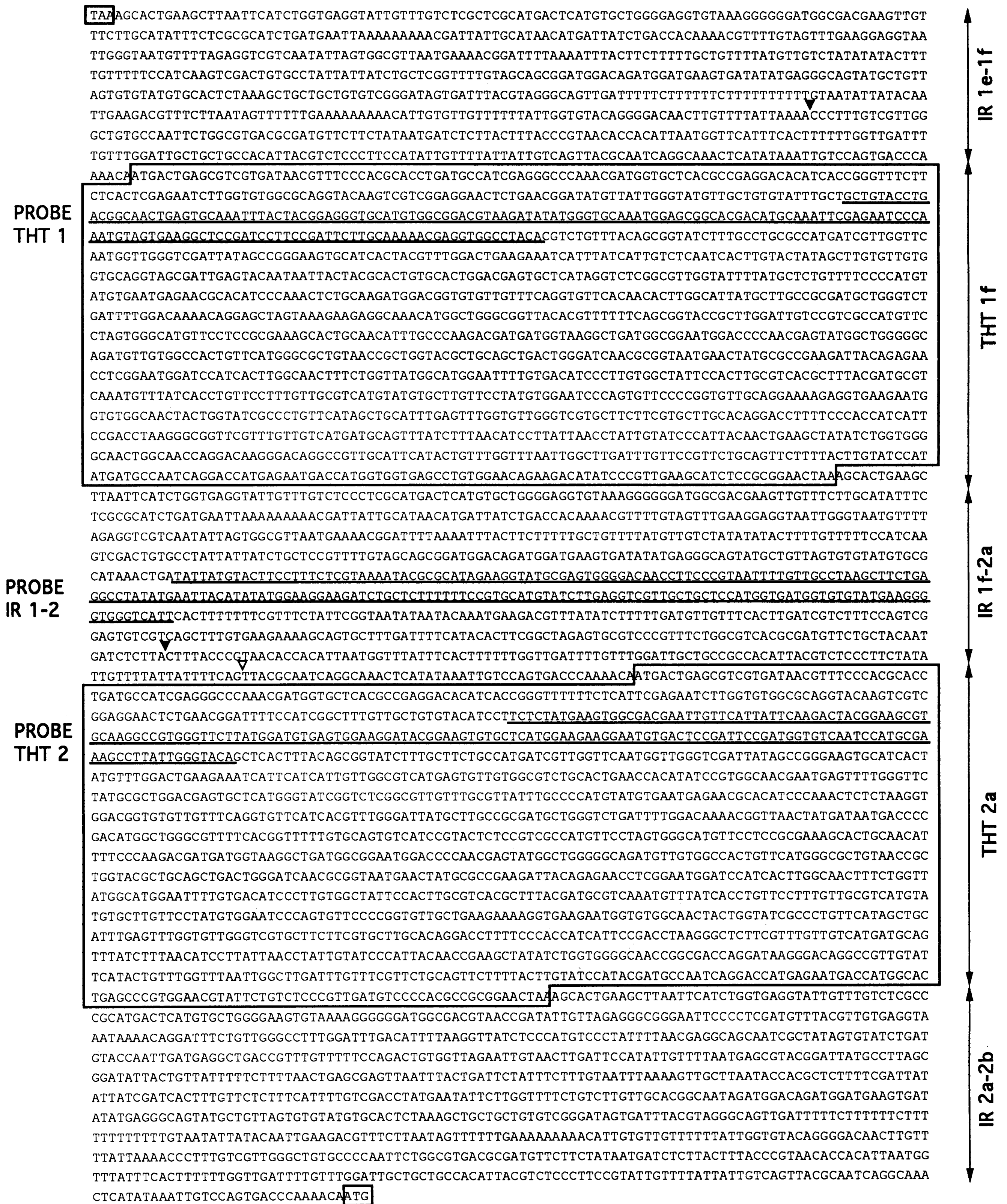

FIG. 4. Nucleotide sequence of the 6-kb genomic region containing the THT1f and THT2a copies which are boxed, separated by the intergenic regions located between THT1e and THT1f (IR1e-1f), THT1f and THT2a (IR1f-2a), and THT2a and THT2b (IR2a-2b). The sequences corresponding to the THT1-, IR1-2-, and THT2-specific probes are underlined. The splice site and polyadenylation sites obtained by cDNA sequence analyses are indicated by open and closed arrowheads, respectively.

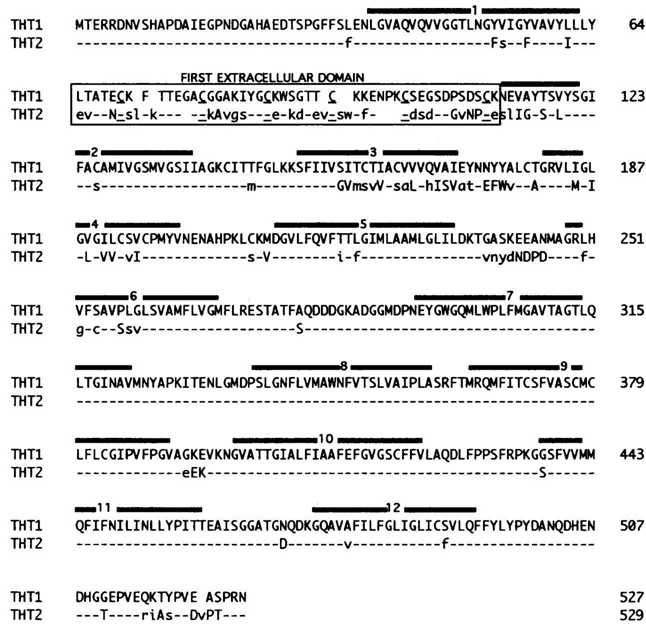

FIG. 5. Amino acid sequence comparison of THT1 and THT2. The THT1 sequence was aligned to maximize identity with the THT2 sequence. In the THT2 sequence, identical amino acids are shown by a dashes, while conservative and nonconservative amino acid substitutions are shown by capital and lowercase letters, respectively. The 12 putative membrane-spanning domains of each protein are overlined, and the cysteine residues present within the boxed first extracellular domain are underlined.

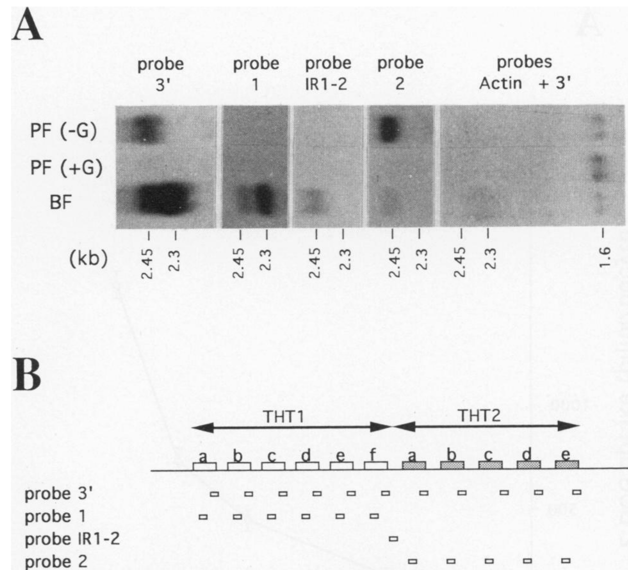

FIG. 6. Expression of the THT genes in the *T. brucei* EATRO-164 bloodstream forms (BF) and *T. brucei* Stib-247 procyclic forms (PF) cultured with glucose at 1 [(+G)] or 0.01 [(−G)] g/liter. The Northern blot was successively hybridized with different ^{32}P-labeled probes (A) common to THT1 and THT2 genes (probe 3′), specific to THT1 genes (probe 1), specific to THT2 genes (probe 2), and specific to the intergenic region located between the THT1 and THT2 clusters (probe IR1-2) (B). The amount of total RNA in each lane was confirmed by hybridization with a ^{32}P-labeled probe specific to the actin gene, which is expressed equally in both forms (5).

in 2-DOG transport was observed 72 h postinjection in cells receiving THT1 RNA (Fig. 7A), while no increase was detected in oocytes injected with water. The uptake of 2-DOG in these injected oocytes was inhibited 95% by a 125-fold excess of D-glucose but not by the same excess of L-glucose, suggesting that transport is stereospecific and carrier mediated (Fig. 7B). Transport of 2-DOG in oocytes injected with mRNA encoding the insulin-sensitive hexose transporter (42) increased linearly for up to 4 days. In our system, this increase seems to be exponential for up to 3 days. This exponential increase could be the result of a latency in THT1 expression due to a momentary sequestration of the mRNA or the proteins in the cell. Alternatively, the transport activity may be cooperative and more active when relatively large amounts of protein are synthesized.

It has previously been shown that the facilitative glucose transport system in the bloodstream forms of *T. brucei* can also transport D-fructose and D-mannose (14) and is inhibited by phloretin and cytochalasin B (36). The effects of these hexoses and drugs on the uptake of 2-DOG by oocytes injected with THT1 RNA were tested to confirm that the THT1-stimulated transport has the same biochemical characteristics as those measured in *T. brucei* (Fig. 7B). The encoded protein takes up D-mannose with the same efficiency as D-glucose, resulting in 92 and 94% competitive inhibition, respectively, of 2-DOG transport into the injected oocytes. Competitive inhibition of transport by D-fructose is less efficient (80%). Phloretin (0.3 mM) and cytochalasin B (0.3 mM) were effective inhibitors of THT1 transport (89 and 53%, respectively). In contrast, the transport of 2-DOG was not inhibited by either 1 mM ouabain or 1 μM FCCP, suggesting that the transporter is neither an H^+ or an Na^+ cotransporter. These results argue that THT1 is a glucose transporter, similar to that observed in bloodstream-form trypanosomes which has been characterized as a facilitative transporter.

DISCUSSION

We have cloned and characterized a multigene family composed of 11 related genes arranged in a single 40-kb genomic DNA fragment. This gene family is divided into two groups of highly conserved genes, with at least 98% homology within each group. These genes, called THT1 and THT2 (9), code for proteins belonging to the facilitative glucose transporter family.

The THT1 and THT2 proteins are highly homologous except for four distinct areas, including the first predicted extracellular domain. While this loop has the greatest divergence in comparison with THT1, THT2, and LTP, a putative hexose transporter cloned from *Leishmania enriettii* (10), the cysteine residues spaced by five to nine amino acids are conserved. This putative exofacial cysteine-rich region is specific to these parasite transporters and may confer a distinct property on these proteins.

We have demonstrated that the injection of *Xenopus* oocytes with in vitro-synthesized RNA encoding THT1 results in the expression of a functional glucose transporter with the properties of the trypanosome enzyme(s). Two arguments lead us to suppose that THT2 has the same function: (i) induction of THT2 mRNA expression in the procyclic forms by glucose depletion (Fig. 6) and (ii) the homology between the amino acids of the trypanosomal glucose transporters THT1 and THT2 and the same pre-

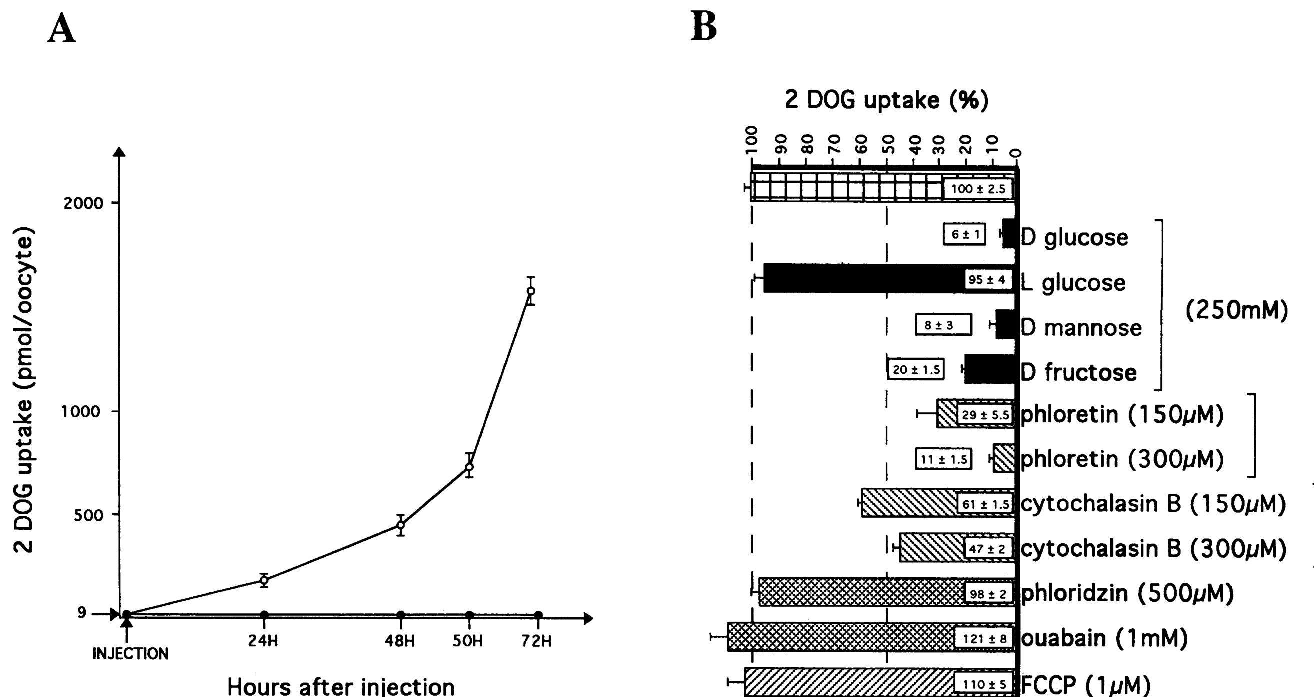

FIG. 7. Functional characterization of hexose uptake in *Xenopus* oocytes injected with RNA encoding THT1. (A) Dependence of expression of THT1 in oocytes on time after RNA injection. Oocytes, injected with 10 ng of THT1 RNA (○) or H_2O (●), were incubated for the times indicated, and 2-DOG uptake was determined as described in Materials and Methods. Each point represents the mean ± standard error of three groups of 10 oocytes, each corresponding to three distinct series of injected oocytes. (B) Effects of hexoses and drugs on glucose uptake by injected *Xenopus* oocytes. Assays were performed 2 or 3 days after injection with RNA. 2-DOG uptake of injected oocytes, treated at the indicated inhibitor concentration, was determined as described in Materials and Methods. The velocity of 2-DOG uptake by the oocytes was calculated and expressed as a percentage of the value for the control without inhibitor. Each value represents the mean ± standard error of three to four groups of 10 injected oocytes. The following potential inhibitors of the three different eukaryotic glucose transport systems were tested: facilitated diffusion (▨; phloretin, cytochalasin B, and phloridzin), Na^+/glucose symport (▩; phloridzin and ouabain), and H^+/glucose symport (▧; FCCP).

dicted topological features of the two proteins. Expression of THT2 mRNA in *Xenopus* oocytes is necessary to determine the protein function. The *Xenopus* oocyte system will be important for further study of THT structure and function.

A facilitative glucose transporter has been characterized in the bloodstream forms of *T. brucei* (18, 38). While there is general agreement on the properties of the transporter system in bloodstream-form organisms, a controversy exists about the nature of the transporter found in the procyclic forms. Parsons and Nielsen (31) reported that the glucose transport system is coupled to an active H^+ cotransporter, while Ter Kuile and Opperdoes (38) have presented data showing that a facilitative transport system is present. Nevertheless, kinetic studies of glucose transport in bloodstream and procyclic forms indicate that the K_m for glucose or its analogs in this carrier-mediated process is in the range of 1 mM in bloodstream forms (14, 16, 36, 38) and 38 μM in procyclic forms (31). These results suggest the presence of two different transporters differentially expressed between bloodstream and procyclic forms. The transporter encoded by THT1 has the characteristics of the glucose transporter previously demonstrated in the bloodstream forms: (i) THT1 genes are expressed at least 500-fold more in the bloodstream forms than in the procyclic forms cultured in the same conditions; (ii) bloodstream forms contain about 40-fold more stable mRNA encoding THT1 than THT2; and (iii) the transporter expressed in oocytes injected with THT1 mRNA has the characteristics of the *T. brucei* bloodstream-form transporter(s) (hexose specificity and drug sensibility) (14, 36) (Fig. 7). The putative THT2 transporter could represent the procyclic isoform, since the THT2 genes are expressed about 10-fold more in procyclic forms cultured without glucose than in the bloodstream forms.

The human glucose transporter expressed in erythrocytes transports D-fructose with 1,000-fold less efficiency than D-glucose (14) and is 93% inhibited by 5 μM cytochalasin B (22), while THT1 transports fructose and is only 53% inhibited by 300 μM cytochalasin B. We have confirmed the previously observed differences between the human erythrocyte and the *T. brucei* bloodstream-form glucose transporters. The glucose transport system of *T. brucei* could be a target for chemotherapeutic intervention, since the two transporters differ in hexose specificity and drug sensitivity. Glucose transporters from both humans and trypanosomes have now been expressed successfully in oocytes (42; this report). This system may now be adapted to study new compounds with specific activity against the trypanosomal transporters. Additionally, the parasite and host glucose transporters have important amino acid differences in their exofacial loops. The immunogenicity of the specific epitopes of this glucose transporter will be tested to determine its potential as an eventual vaccine.

Northern blot analysis demonstrated that the expression of THT1 and THT2 genes is differentially regulated during trypanosome development. Firstly, THT1 and THT2 RNAs

are not expressed at the same level; in the bloodstream-form organisms, THT1 RNA is about 40-fold more abundant than THT2, and only THT2 RNA is observed in the procyclic forms. Second, expression of the THT genes is stage regulated, with THT1 and THT2 genes being expressed at a higher level in the bloodstream forms. Third, glucose starvation of procyclic forms results in about a 60-fold increase in the level of the THT2 steady-state mRNA, while expression of THT1 is not affected. The differential expression of trypanosomal stage-regulated genes is well documented (8, 17, 20, 25, 27, 33, 35, 39, 43–45), but there are few examples of trypanosomal genes whose expression is regulated by specific environmental agents.

Analysis of genes coding for tubulin (21), calmodulin (40), actin (4), fructose biphosphate aldolase (43), procyclin (12), and variant-specific surface antigen (1, 23) indicates that most of the clustered genes in *T. brucei* are transcribed in a polycistronic fashion, and their expression is regulated post-transcriptionally (17, 32, 39, 43). Northern blot analysis using THT1- and THT2-specific probes detected a 5-kb mRNA corresponding to a doublet of THT precursor mRNA probably derived from polycistronic transcription (data not shown). The THT genes may be transcribed as a single polycistronic transcript, although the presence of multiple promoters cannot be ruled out. Nuclear run-on transcription experiments will allow us to address this question.

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