Vacular-type H\textsuperscript{+}-Pyrophosphatases (V-H\textsuperscript{-}PPases) are electrogenic proton pumps found in many organisms of considerable industrial, environmental, and clinical importance. V-H\textsuperscript{-}PPases of several parasites were shown to be associated with acidic vacuoles named acidocalcisomes, which contain polyphosphate and calcium. In this work we functionally characterized a Trypanosoma brucei V-H\textsuperscript{-}PPase gene by using double-stranded RNA interference methodology to produce inducible V-H\textsuperscript{-}PPase-deficient strains of procyclic and bloodstream forms (PFiVP1 and BFiVP1). Acidocalcisomes of these mutated parasites lost acidity and contained 90% less polyphosphate. PFiVP1 did not release calcium after the addition of nigericin, and its total acidity was reduced by 70%. This mutant also failed to stabilize its intracellular pH on exposure to external basic pH >7.4 and recovered from intracellular acidification at a slower rate and to a more acidic final intracellular pH. In the absence of T. brucei V-H\textsuperscript{-}PPase expression, PFiVP1 and BFiVP1 grew at a slower rate with doubling times of 27 h instead of 15 h, and 10 h instead of 7.5 h, respectively. Moreover, BFiVP1 could not grow over 5 × 10\textsuperscript{5} cells/ml corresponding to a cell density reduction of five times for bloodstream form stationary phase growth.

Intracellular acidic vacuoles containing polyphosphate (polyP),\textsuperscript{1} initially called volutin or polyP bodies, have been described in bacteria, algae, yeast, and protozoa (1). In trypanomastids, these polyP vacuoles were called acidocalcisomes (2) and shown to be electron dense and contain large concentrations of P\textsubscript{i}, calcium, magnesium, and other elements (3). Similar organelles have been identified in apicomplexan parasites (4, 5) as well as in the green algae, Chlamydomonas reinhardtii (6) and the slime mold, Dictyostelium discoideum (7). Acidocalcisomes were postulated to play an important role in the regulation of both cytosolic Ca\textsuperscript{2+} concentration and intracellular pH (pHi). For example, polyP hydrolysis (8) and activation of the Na\textsuperscript{+}/H\textsuperscript{+} antiporter (9, 10) were postulated to protect the cells against alkaline pH stress and increase the intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]). In addition, these organelles possess a vacuolar-type H\textsuperscript{+}-translocating pyrophosphatase (V-H\textsuperscript{-}PPase) (11), which is an electrogenic proton pump initially discovered in photosynthetic bacteria and plants (12). It has been shown to be associated with the plasma membrane or vacuoles in plants and with the chromatophore membranes of Rhodospirillum rubrum. The biochemical function of this enzyme in plants and unicellular eukaryotes is to couple hydrolysis of the high energy phosphate bond of PP\textsubscript{i}, with H\textsuperscript{+} translocation from the cytosol to acidify the plant vacuole (tonoplast) or the acidocalcisome, respectively (10, 13). Working on isolated acidocalcisomes, Rodrigues et al. (14) demonstrated that the Trypanosoma brucei H\textsuperscript{-}PPase was able to generate a membrane potential by PP\textsubscript{i}-dependent proton uptake. Moreover, Scott et al. (11, 15) showed that PP\textsubscript{i} could drive Ca\textsuperscript{2+} uptake into the compartments of permeabilized Trypanosoma cruzi epimastigotes (11) or isolated acidocalcisomes (15). However, the physiological importance of the V-H\textsuperscript{-}PPase was investigated less thoroughly because genetic approaches were not used. Studies of V-H\textsuperscript{-}PPase expression in plants have revealed higher expression levels in young growing tissue and during growth under stress (12). Recently, it has been shown that transgenic plants overexpressing V-H\textsuperscript{-}PPase become more resistant to high concentrations of NaCl and water deprivation than the isogenic wild-type strains (16).

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Using RNA interference methodology (17), we have now shown that T. brucei V-H\textsuperscript{-}PPase not only plays roles in the regulation of Ca\textsuperscript{2+}, polyP, and H\textsuperscript{+} content of acidocalcisomes and pH, regulation of procyclins but also is required for normal in vitro growth of bloodstream and procyclic forms.

**EXPERIMENTAL PROCEDURES**

**Strains Used—**T. brucei brucei procyclic form host cell line 29–13 and bloodstream form host cell line 90–13 co-expressing the T7 RNA polymerase and the Tet repressor were gifts from G. A.M. Cross (18). Procyclic forms were cultivated to late log-phase in SDM-79 medium (19) supplemented with 10% fetal calf serum. Bloodstream forms were cultured in vitro in modified essential medium supplemented with 10% fetal calf serum (20).
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Cloning of TbVP1—A TBLASTN search of all available sequence databases using the amino acid sequence of V-H'-PPase of T. cruzi (GenBank™ accession number AF159881) (21) yielded a 1.3-kb gene fragment mapping from 20 clones. These clones were derived from a T. brucei GUTat 10.1-sheared DNA library constructed at TIGR (workbench.sdsc.uiuc.edu). Four of the 20 clones were subcloned and sequenced using Bigdye™ Terminator version 1.2 Ready Reaction Cycle sequencing kit (ABI PRISM®). 

To obtain full-length cDNA, rapid amplification of cDNA ends (RACE) using Taq polymerase with 5′-ACGCGTGATGACGGCATGTT-3′ and TbP31 (5′-CAGGACCTCGAGGTCTGCGCTGAGCTCGGC-3′) subcloning and 5′-NdeI primer (5′-GGATCCATATGGCTCTCTTCTGCACGGTG-3′) and specific primers, TbP52 (5′-GGTGGTCGCCAGTCT-3′) and TbP53 (5′-ATAGCTAGTCTGCTGTGAC-3′), for 5′-RACE and oligo dT primer (5′-ACGCGTGATGACGGCATGTT-3′), for 3′-RACE. PCR products were cloned into the TA-cloning pCR™I/II vector (Invitrogen) and sequenced using Bigdye™ Terminator version 3.0 Ready Reaction Cycle sequencing kit (ABI PRISM®).

Sequence Analysis—DNA sequence data were generated at the High Throughput Sequencing and Genotyping Unit of the Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign. Sequence analysis was done using the Biology Workbench 3.0 utility (workbench.sdsc.uiuc.edu), the Wisconsin Package (version 10.0-UNIX, Genetics Computer Group, Madison, WI). The sequence was annotated using the amino acid sequence of V-H (GenBank™ accession number AF159881) (21) yielded a 1.3-kb gene fragment mapping from 20 clones. These clones were derived from a T. brucei GUTat 10.1-sheared DNA library constructed at TIGR (workbench.sdsc.uiuc.edu). The resulting fragment was cloned between the BamHI and HindIII restriction sites of pBluescript SK±. The resulting construct was produced as described previously (26). DNA fragments corresponding to the coding regions of TbVP1 from nucleotides 99 to 416 and from nucleotides 99 to 464 were PCR-amplified. The 317-bp fragment was amplified using the following set of primers: DB/VPI/01 (5′-CCGGGTTCTGGAAGATTGTCGTCGACAGCT-3′) and DB/VPI/02 (5′-CCGGGTATATCCGGCGCTGTCGACAGCT-3′). The 385-bp fragment was amplified with the following two primers, DB/VPI/03 (5′-CCGGGTTAGCTTATGGCATGTCACGACAGCT-3′) and DB/VPI/04 (5′-CCGGGTTATCTAAGGGAAAAATGCAAGCATTT-3′). After precloning in the pGEMT vector (Promega), the resulting fragment was cloned between the BamH1 and HindIII restriction sites of pBluescript SK±. The resulting construct was named pBluescript SK±-VP1 and induced for 5 days with 1 μg/ml tetracycline. Cells were diluted 10-fold when densities reached a minimum of 1×10⁸ cells/ml. They were not allowed to grow beyond 5×10⁸ cells/ml before diluting again.

For stable transfection of bloodstream forms, cells were harvested from a log-phase culture, washed once in ZPFFM and 55 mM glucose and resuspended in ZPFFM containing 2 μM ATP and 5 mM glutathione to a cell density of 2×10⁸ cells/ml (27, 28). 10⁸ cells were electroporated with 10 μg of DNA at 1400 V, 2 μF, and 100 mF atropores using the Eurogentech CellJect machine. Surviving cells were then seeded in a microtitre plate as described by Wirtz et al. (27), and selection was applied the following day by adding phleomycin to a final concentration of 0.5 μg/ml. Two clones were obtained: BVP1/1A1 and BVP1/2A2.

For pH measurements, we used a Titrino (Metrohm) with a combination electrode and a Sigma 724 (Metrohm). The pH was measured in triplicate.

After harvesting and washing, 10⁷ cells/ml (50 μg of protein) were incubated with 150 μM Neutral Red in culture medium for 30 min. After three washes in Ringer’s solution (155 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 2 mM NaHPO₄, 10 mM Hepes, pH 7.2, 10 mM glucose, and 0.5 mg/ml bovine serum albumin), 5×10⁷ cells were counted and 0.5 ml of water/gluconic acid (50:49:1) and the amount of dye incorporated was determined spectrophotometrically at 540 nm (31).

APTS- and PP-driven Proton Transport in Permeabilized Procyelic Trypanosomes—Cells (0.2 mg of protein/equiv) were incubated with 2×10⁷ cells in a buffer containing 2 mM MgSO₄, 50 μM EGTA, 10 mM K-Hepes, pH 7.2, 65 mM KCl, and 125 mM sucrose. Acidine Orange
was used at 3 \( \mu M \), PP at 0.1 mM, ATP at 1 mM, and digitonin at 16 \( \mu M \).

The spectrophotometer Cary 100 (Varian) was used to follow Acridine Orange uptake as described previously (14).

Analysis of PolyP
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PolyP levels were determined as described by Ruiz et al. (8) using the recombinant exopolyphosphatase from Saccharomyces cerevisiae. PFiVP1 procyclic trypomastigotes were induced with 1 \( \mu g/ml \) tetracycline during 4 days. 2 \( \times 10^7 \) cells then were washed twice with Dulbecco’s phosphate-buffered saline, and long and short chain polyP was quantified.

Subcellular Fractionation
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Subcellular fractionation was performed according to Rodrigues et al. (14). Procyclic cells were lysed in a lysis buffer (125 mM sucrose, 50 mM KCl, 4 mM MgCl2, 0.5 mM EDTA, 20 mM Na-Hepes, pH 7.4, 5 mM dithiothreitol) containing a protease inhibitor mixture with final concentrations of 1 \( \mu M \) chemostatin, 1 \( \mu M \) leupeptin, 1 \( \mu M \) pepstatin, and 10 \( \mu M \) phenylmethylsulfonyl fluoride. After lysis and removal of silicon carbide by centrifugation at 144,110 \( g \) for 5 min, the extract was centrifuged again at 580,110 \( g \) for 10 min. 5.76 ml of supernatant were mixed with 6.12 ml of Percoll and 6.12 ml of 0.5 M sucrose before centrifugation at 69,500 \( g \) for 50 min.

RESULTS AND DISCUSSION

Cloning and Sequence Analysis of T. brucei V-H\(^+\)-PPase—To screen for genes encoding plant-like V-H\(^+\)-PPases in T. brucei, the amino acid sequence of V-H\(^+\)-PPase from T. cruzi, TcPPase (21), was used to search all available sequence databases using TBLASTN. This search yielded a 1.3-kb gene fragment, which closely resembled the region encompassed by nucleotides encoding amino acid residues 10–445 of TcPPase. On the basis of this information, we cloned TbVP1 by using the reverse transcriptase-PCR technique. This 1.2-kb cDNA enabled appropriate gene-specific primers to be designed for the generation of 5’-end and 3’-end DNA fragments by using the RACE method (22) and the reconstruction of a full-length cDNA. The nucleotide sequence of 3510 bp revealed an open reading frame of 2481 bp (nucleotides 136–2616) that encodes a 826-amino acid protein with a relative molecular mass of 85.9 kDa and a calculated pI of 5.08.

To assess the copy number of TbVP1 in the nuclear genome of T. brucei, Southern blots were probed with a 1.2-kb cDNA fragment (nucleotides 168–1387) (data not shown). Under stringent conditions, the blot contained a single band except when predicted internal restriction sites (PstI and SacI) were present. These data together with the reverse transcriptase-PCR, 5’-RACE, and 3’-RACE were consistent with a single copy number gene in the T. brucei genome.

While this paper was in preparation, a search of GenBank and the T. brucei genome project databases using the full-
length cDNA of TbVP1 indicated the existence in chromosome IV (GenBank™ accession number AC09781, nucleotides 161180–163660) of a predicted amino acid sequence potentially encoding for a V-H⁺-PPase-like protein. The open reading frame was identical to the full-length cDNA. When the cDNA sequence was compared with the genomic sequence from chromosome IV, the predicted translation initiation site of TbVP1 was preceded by 102 bp of 5’-untranslated sequence. The polyadenylation site of TbVP1 was preceded by 874 bp of 3’-untranslated sequence.

Sequence and Structural Analysis of TbVP1—The HMMTOP software (www.enzim.hu/hmmtop) revealed the presence of the 15 potential transmembrane domains that we oriented as described previously (Fig. 1) (32). We have no experimental evidence for the membrane orientation of TbVP1. However, the N-terminal extremity analyzed by the sequence analysis PSORT (psort.nibb.ac.jp), may correspond to a 26-amino acid signal peptide with a predictable cleavage site at positions 26–27. This last characteristic was previously reported by Hill et al. (21) for the T. cruzi V-H⁺-PPase and was suggested to play a role in enzyme sorting.

The sequence comparison analysis shown in Fig. 1 confirmed the presence in TbVP1 of the five “tool box” sequences characteristic of the V-H⁺-PPases (33). In particular, the cytosolic loop III containing the putative PPi binding motif (D/E)X7KXE is conserved (34).

Localization of TbVP1 in Procyclic and Bloodstream Form Trypanosomes—In a first step toward the characterization of TbVP1, we analyzed its subcellular location in bloodstream and procyclic trypomastigote forms. An histidine-tagged recombinant protein (A259 to D377), comprising loop III and the two adjacent regions, was expressed in E. coli, purified by Ni²⁺HisBind™ chromatography and used to immunize mice. Indirect immunofluorescence using this antiserum as the probe revealed vesicular-like structures of varying sizes in procyclic and bloodstream forms indicating expression in both stages. These structures are mainly distributed in the central region, with some additional vesicles at the anterior region of the bloodstream forms (Fig. 2A). A similar distribution of acidocalcisomes in T. brucei was previously reported (14). To further analyze the subcellular location of TbVP1, we obtained procyclic acidocalcisomes from a high density Percoll gradient fraction of trypanosomes as described previously (14). We observed by immunofluorescence that 80% of the purified high density vesicles were recognized by the anti-TbVP1 mouse antiserum...
indicating an association of this V-H\textsuperscript{+}-PPase with acidocalcisomes (data not shown). TbVP1 was also detected by Western blots of this high density fraction (Fig. 2B). No reaction was detected when the antisera was pre-adsorbed with the His-tagged TbVP1L3 protein (Fig. 2B). The recognized polypeptide had an apparent molecular mass of 55 kDa. A size discrepancy between the expected (80 kDa) and the observed molecular mass was already reported for the V-H\textsuperscript{+}-PPases of plants (35, 36) and trypanosomatids (21). It could be attributed either to the usual anomalous migration of hydrophobic proteins on SDS gels (37) or to partial degradation. In agreement with these results, Fig. 2B shows that the 55-kDa protein band is absent in a mutant deficient in V-H\textsuperscript{+}-PPase (see below). We conclude that TbVP1 is associated with vesicles of size, distribution, and density that correspond to the acidocalcisomal compartment.

**Characterization of the H\textsuperscript{+}-translocating Activity of TbVP1—PP\textsubscript{i}—driven H\textsuperscript{+} transport activity was shown previously to be associated with acidocalcisomes (14). Using RNA interference methodology to silence TbVP1 expression, we tested whether in the absence of TbVP1 acidocalcisomes failed to display PP\textsubscript{i}-dependent H\textsuperscript{+}-translocating activity. Because TbVP1 is expressed and associated with acidocalcisomes in both procyclic and bloodstream trypomastigote forms, the gene-silencing experiment was performed initially on the former stages. After 5 days of double-stranded RNA expression, TbVP1 protein in the acidocalcisomes completely disappeared (Fig. 3A, photograph b), indicating high stability and slow turnover of the protein. Nevertheless, the presence of acidocalcisomes was confirmed by electron microscopy (data not shown).

Procyclic trypomastigotes permeabilized with digitonin were shown to accumulate and retain the weak base Acridine Orange after the addition of 0.1 mM PP\textsubscript{i}. The resultant H\textsuperscript{+} gradient collapsed completely after the addition of 1 mM nigericin, a H\textsuperscript{+}/K\textsuperscript{+} ionophore, or the alkalinizing agent NH\textsubscript{4}Cl, confirming that the Acridine Orange absorbance decrease was attributed to its accumulation in an acidic compartment (Fig. 3B) as previously demonstrated (14). In the induced PFIVP1 parasites, the establishment of the proton gradient across the acidocalcisomal membrane and the corresponding increase in acidity did not occur after PP\textsubscript{i} addition (Fig. 3C). Therefore, the absence of the proton-translocating process associated with the V-H\textsuperscript{+}-PPase catalytic activity was confirmed with the absence of the TbVP1 enzyme. By this genetic analysis, we confirmed that we were characterizing the acidocalcisomal V-H\textsuperscript{+}-PPase. Interestingly, digitonin-permeabilized PFIVP1 parasites were able to accumulate Acridine Orange when ATP was added to the reaction medium (Fig. 3C). This accumulation was inhibited when concanamycin A was added prior to the beginning of the reaction (Fig. 3C, dashed line) (14). Moreover, the addition of 40 mM NaCl induced partial release of Acridine Orange accumulated after ATP addition, and subsequent addition of ADP stimulated this release (Fig. 3C). Also, as previously shown (38), the hydrophobic antioxidant 3,5-dibutyl-4-hydroxytoluene inhibited the Na\textsuperscript{+}/H\textsuperscript{+} exchanger activity (data not shown). These data suggest the presence on these mutated acidocalcisomes of a functional H\textsuperscript{+}-ATPase pump and a Na\textsuperscript{+}/H\textsuperscript{+} exchanger, which were shown to be important for acidification and calcium uptake and release from acidocalcisomes (9, 38). In permeabilized procyclic trypomastigotes of T. brucei (14) and in isolated acidocalcisomes of T. cruzi (15), the H\textsuperscript{+}-translocating pyrophosphatase activity is sufficient for the acidification of acidocalcisomes. However, in vivo acidification by the V-H\textsuperscript{+}-ATPase pump in the absence of the H\textsuperscript{+}-translocating PPase activity had never been tested. Therefore, we evaluated the state of this acidic compartment in the induced PFIVP1 and the bloodstream RNA interference mutant (BFIVP1) strains.

**Effects of the Inhibition of TbVP1 Expression on the Acidity and PolyP Content of Procyclic and Bloodstream Form Acidocalcisomes—**The BFIVP1 strain was obtained as described under “Experimental Procedures.” After 4 days of double-stranded RNA expression, TbVP1 protein completely disappeared as occurred with PFIVP1 (Fig. 3A) from the acidocalcisomes of BFIVP1 (Fig. 4A). Concomitantly, in both forms, the amount of accumulated Acridine Orange in acidocalcisomes was drastically reduced (Figs. 4B and 5A). Although some vesicles located around the nucleus (Figs. 4B and 5A) were associated with the perinuclear acidic compartment (39), others corresponded to less acidic acidocalcisomes. These results suggest that TbVP1 was essential to maintain acidocalcisomal acidity and consequently that the H\textsuperscript{+}-ATPase alone could not fulfill that function. In this regard, we have previously reported (40) that only very high concentrations (>5 \mu M) of bafilomycin A\textsubscript{1}, a specific inhibitor of the V-H\textsuperscript{+}-ATPase when used at nanomolar concentrations, can release Acridine Orange from acidocalcisomes of permeabilized trypanosomes. We also reported that neither bafilomycin A\textsubscript{1} nor concanamycin A inhibit PP\textsubscript{i}-driven Acridine Orange transport into acidocalcisomes (14).

In T. cruzi, acidocalcisomal alkalinization by NH\textsubscript{4}Cl was followed by progressive decrease in the levels of both short and long chain poly(P) (8). A loss of acidity in the acidocalcisomes of

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**Fig. 5.** H\textsuperscript{+} and polyphosphate content of PFIVP1 acidocalcisomes. A, accumulation of Acridine Orange in acidic vacuoles of PFIVP1 procyclic trypomastigotes non-induced (a) and tetracycline-induced (b). B, PolyP content of non-induced and induced T. brucei mutants. The average means ± S.D. from three replicates of each condition are shown.
induced PFiVP1 and BFiVP1 parasites might have affected the polyP content of the cells. The results shown in Fig. 5B indicated a 10-fold reduction in short and long chain polyP in the acidocalcisomes of the tetracycline-induced PFiVP1. DAPI staining, which was shown to allow the detection of polyphosphate in vesicles (8), did not reveal an accumulation of polyphosphate in the BFiVP1 cells (Fig. 4C).

In trypanosomes, Ca\(^{2+}\)/H\(^{+}\) uptake by acidocalcisomes is mediated by a Ca\(^{2+}\)-ATPase acting as a Ca\(^{2+}\)/H\(^{+}\)-exchanging ATPase (14), and polyP because of its high calcium chelation capacity appears to play a central role in the regulation of cellular Ca\(^{2+}\) (8, 41). Furthermore, a correlation between the short and long chain polyP content in the acidocalcisomes and changes in the pH, was suggested by Ruiz et al. (8). Therefore, we first analyzed the calcium content of acidocalcisomes and its mobilization and next studied the pH regulation of the induced PFiVP1 parasites.

\(\text{Ca}^{2+}\) and \(\text{pH}\) Homeostasis in the Induced PFiVP1 Trypanosomes—In a first step, we analyzed the intracellular calcium storage in the acidocalcisomes. This study was performed in intact cells loaded with the fluorescent \(\text{Ca}^{2+}\) indicator Fura-2. In the induced PFiVP1 strain, the starting \([\text{Ca}^{2+}]\) was identical to that of the non-induced strain. We have previously described that in \(T.\ brucei\), the combination ionomycin + nigericin is able to release \(\text{Ca}^{2+}\) from the acidocalcisomes (29). Ionomycin binds essentially no \(\text{Ca}^{2+}\) below pH 7.0, and it cannot move \(\text{Ca}^{2+}\) out of acidic compartments because of the competition of protons at the inside face of the membrane (42). In the absence of nigericin or NH\(_4\)Cl, ionomycin releases a relatively small amount of \(\text{Ca}^{2+}\) only from neutral or alkaline compartments (Fig. 6A), but significantly more \(\text{Ca}^{2+}\) is released after the addition of either nigericin (Fig. 6B) or NH\(_4\)Cl (Fig. 6C). The addition of nigericin did not raise the \([\text{Ca}^{2+}]\) in the induced parasites (Fig. 6A). Moreover, a subsequent addi-
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...been transferred. Cells were induced for 3 days before starting the experiment. Similar results were obtained with different clones and with different mutant constructions (S. Luo and R. Docampo, unpublished results).

...stimulating hydrolysis of the remaining polyP, NH₄Cl might release considerable amounts of calcium (1). By stimulating hydrolysis of the remaining polyP, NH₄Cl might release bound calcium. In contrast, nigericin would not release this bound calcium. In contrast, nigericin would not release this bound calcium. In contrast, nigericin would not release this bound calcium. In contrast, nigericin would not release this bound calcium.

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FIG. 7. Effects of inhibition of TbVP1 expression on the growth of PFVP1 and BFVP1. Growth of non-induced (gray lines) and induced (black lines) cultures of PFVP1 (A) and BFVP1 (B). Cumulative cell numbers reflect normalization for dilution during cultivation. A, PFVP1 cells were maintained in the exponential phase of growth, and tetracycline (1 μg/ml) was added at t = 0 (black line). B, BFVP1 non-induced (gray line) and induced (black line and square) cultures were transferred where indicated by the arrows. The black diamond growth curve corresponds to the induced BFVP1 culture, which has not been transferred. Cells were induced for 3 days before starting the experiment. Similar results were obtained with different clones and with different mutant constructions (S. Luo and R. Docampo, unpublished results).

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In conclusion, TbVP1 expression inhibition induced a loss of functional acidocalcisomes and a lower capacity of PFVP1 cells to adapt to environmental pH stress. Because the cells were maintained under exponential growth conditions to perform the RNA interference experiments, no fundamental changes in the culture medium occurred during growth. In their life cycle, trypanosomes encounter many environmental changes including pH variations to which they must adapt in order to survive and multiply (45). In this regard, polyP has been shown to be important to support the survival of bacteria in the stationary phase (46). Functional acidocalcisomes may be essential for these adaptations. We are currently studying the effect of non-functional acidocalcisomes on the survival of procyclic and bloodstream forms in their insect and mammalian hosts, respectively.

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