A Trk/HKT-Type K\(^+\) Transporter from Trypanosoma brucei\(^*\)
Marc Mosimann,1 Shinobu Goshima,2 Tanja Wenzler,3,4 Alexandra Lüscher,1 Nobuyuki Uozumi,2 and Pascal Mäser1,3,4,*

Institute of Cell Biology, University of Bern, 3012 Bern, Switzerland; Department of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan; Swiss Tropical Institute, 4002 Basel, Switzerland; and University of Basel, 4000 Basel, Switzerland

Received 28 October 2009/Accepted 5 February 2010

The molecular mechanisms of K\(^+\) homeostasis are only poorly understood for protozoan parasites. Trypanosoma brucei subsp. parasites, the causative agents of human sleeping sickness and nagana, are strictly extracellular and need to actively concentrate K\(^+\) from their hosts’ body fluids. The T. brucei genome contains two putative K\(^+\) channel genes, yet the trypanosomes are insensitive to K\(^+\) antagonists and K\(^+\) channel-blocking agents, and they do not spontaneously depolarize in response to high extracellular K\(^+\) concentrations. However, the trypanosomes are extremely sensitive to K\(^+\) ionophores such as valinomycin. Surprisingly, T. brucei possesses a member of the Trk/HKT superfamily of monovalent cation permeases which so far had only been known from bacteria, archaea, fungi, and plants. The protein was named TbHKT1 and functions as a Na\(^+\)-independent K\(^+\) transporter when expressed in Escherichia coli, Saccharomyces cerevisiae, or Xenopus laevis oocytes. In trypanosomes, TbHKT1 is expressed in both the mammalian bloodstream stage and the Tsetse fly midgut stage; however, RNA interference (RNAi)-mediated silencing of TbHKT1 expression did not produce a growth phenotype in either stage. The presence of HKT genes in trypanosomatids adds a further piece to the enigmatic phylogeny of the Trk/HKT superfamily of K\(^+\) transporters. Parsimonal analysis suggests that the transporters were present in the first eukaryotes but subsequently lost in several of the major eukaryotic lineages, in at least four independent events.

Potassium (K\(^+\)) is the most abundant cation in the cytosol of any cell and hence an essential macronutrient for life on earth. Concentrative K\(^+\) uptake across the plasma membrane is energized directly by ATPases and indirectly by the negative membrane potential or by coupling, via symport or antiport, to other transport processes such as H\(^+\) flux. The ancestry of K\(^+\) transporters renders them ideal subjects for phylogenetic comparisons. Indeed, the different kinds of known K\(^+\) transporters—pumps, channels, permeases, symporters, and antiporters—are all found in bacteria (43). Eukaryotes do not appear to have invented further mechanisms of K\(^+\) transport; on the contrary, some families of K\(^+\) transporters were lost over the course of eukaryote evolution, particularly among the metazoa (53).

The Trk/HKT superfamily (TC transporter classification 2.A.38 [43]) consists of bacterial TrkH and KtrB, plant HKT, and fungal Trk transporters (15). These proteins share a topology with 8 transmembrane (TM) domains and, sandwiched between odd- and even-numbered TM domains, 4 shorter hydrophobic helices that resemble the P-loops of K\(^+\) channels (14, 27, 55). In the K\(^+\) channel, these pore-forming loops end in the filter residues glycine-tyrosine-glycine, which coordinate a conserved glycine (48), and these glycines have been shown to determine K\(^+\) selectivity over Na\(^+\) of the transporters (34, 49). Thus, a Trk/HKT monomer with 8 TM domains and 4 P-loops is thought to have a similar pore architecture to a K\(^+\) channel tetramer with two TM domains and one P-loop per subunit. The Trk/HKT transporters are important for cellular K\(^+\) acquisition in microorganisms, since trk null mutant yeast or bacteria exhibit growth phenotypes on media containing low K\(^+\) concentrations (20, 46). The roles of the Trk/HKT transporters in plants are more diverse, including Na\(^+\) distribution (10, 33, 47), osmoregulation (32), and salt tolerance (39). So far, no HKT/Trk transporter has been described from the metazoa or protista.

Trypanosoma brucei subsp. parasites comprise the causative agents of human and livestock trypanosomosis: sleeping sickness and nagana, respectively. The distribution of the parasites is restricted by that of their vector, the blood-sucking tsetse fly (Glossina spp.), to the so-called tsetse belt comprising 36 countries between the Sahara desert and the Kalahari (3, 8). African trypanosomes proliferate extracellularly in the blood, evading the mammalian immune response by antigenic variation. Untreated sleeping sickness is fatal. There is an urgent need for new and better drugs since the current ones, the arsenical melarsoprol in particular, suffer from severe side effects (31). In the mammalian bloodstream, the parasites encounter a rich and steady supply of nutrients, readily imported by specific permeases or endocytosed via receptors (7). Research on trypanosomal nutrient uptake has so far concentrated on transporters of organic substrates: nucleobases, nucleosides, sugars, and amino acids (4, 12, 26, 30, 35, 56). Little is known about how the parasites import inorganic nutrients. The malaria parasite Plasmodium falciparum possesses two putative K\(^+\) channel subunits with 6 TM domains and one P-loop (19, 52). Disruption of an orthologous gene in Plasmodium berghei strongly impaired the development of the malaria...
parasites in the mosquito (18). However, these putative channels have not yet been proven to be permeable to K⁺. The T. brucei genome (6) is annotated to contain two putative K⁺ channels; in addition, a putative ATPase has been identified resembling fungal Na⁺/K⁺-efflux ATPases (5, 45). None of these has been addressed experimentally. Here we present the identification and characterization of TbHKT1 (Tb10.70.2940), a Trk/HKT-type K⁺ transporter from Trypanosoma brucei and representative of a new clade of Trk/HKT genes from kinetoplastid parasites.

MATERIALS AND METHODS

In silico methods. A Trk/HKT-specific profile (hkt hmm) available on request) was made with HMMer (17) from a ClustalW multiple alignment (50) of a homology-reduced set of representative Trk/HKT proteins and validated against the proteomes of Arabidopsis thaliana and Saccharomyces cerevisiae. Parasite predicted proteomes were downloaded from ftp.sanger.ac.uk (T. b. brucei v4.0, Leishmania major, and Trypanosoma cruzi), www.plasmidDB.org (F. felicisparum v.5.5). ftp.dbi.ebi.ac.uk/pub/databases/integr8 (Theileria parva), ftp.ignt.org (Entamoeba histolytica and Trichomonas vaginalis), and www.giardiad.org (Giardia duodenalis v1.1). The obtained hits were run through TMHMM (29) to predict membrane topology and searched against the GenBank nonredundant protein database with Blast (2) to test for eventual relationships to other, non-Trk/HKT sequences. The phylogenetic tree (see Fig. 3) was drawn from a ClustalW alignment with Dendroscope (25). Bootstraping was performed for 1,000 rounds, and results were expressed as percent positives.

Cell lines and media. Monomorphic bloodstream-form T. b. brucei 221 (MiTat 1.2; 221) parasites were cultivated at 37°C, 5% CO₂ in HMI9 medium supplemented with 5% fetal calf serum (9). For RNA interference (RNAi) experiments, bloodstream-form T. b. brucei ‘New York single-marker’ and procyclic T. b. brucei 29-13 were used (54). Transfectants were selected in 0.1 mM/μl puromycin and cultivated in the presence of 1 mg/ml tretamine to induce transcription of the stem-loop RNAi construct (1). The rat L6 myoblast cell line was obtained from ATCC (CRL-1458) and cultivated at 37°C with 5% CO₂ in RPMI 1640 medium supplemented with 2 mM l-glutamine, 5.95 g/liter HEPES, 2 g/liter NaHCO₃, and 10% fetal calf serum. Yeast complementation experiments were performed with trk1- and trk2-deficient S. cerevisiae strain CY162 (20) on arginine/phosphate medium (41), consisting of 8 mM phosphoric acid, 10 mM l-arginine, 2 mM MgSO₄, 0.2 mM CaCl₂, 20 mg/liter histidine, 2% galactose, and 0.3% sucrose, supplemented with the standard vitamins and trace elements and 1.5% agar (because agar contains too much potassium). KCl was added to the desired concentration.

Escherichia coli complementation was performed with the trkD (kap1) trkE trkF trkK trkM, and trkP1 quadruple mutant LB2003 (46) grown in a medium containing 46 mM Na₂HPO₄, 23 mM NaH₂PO₄, 8 mM (NH₄)₂SO₄, 0.4 mM MgSO₄, 6 μg FeSO₄, 1 mg/liter thiamine, 1% glucose, 0.25 mM isopropyl-β-d-thiogalactopyranoside (IPTG), and KCl to the desired concentration.

Drug sensitivity tests. Trypanosomes (10⁶ bloodstream forms per well, respectively, 10⁴ procyclic forms per well) or L6 myoblasts (4 × 10⁶ per well, seeded 24 h prior to the addition of test compounds) were cultivated in 100 μl medium on a 96-well plate containing serial dilutions of test compounds. After 70 h of incubation, 10 μl per well Alamar Blue solution was added (12.5 mg resazurin in 100 ml phosphate-buffered saline) and after another 2 h of incubation, fluorescence was measured with a Spectramax Gemini fluorometer (Molecular Devices, Sunnyvale, CA) at 536 nm excitation and 588 nm emission. Fifty percent inhibitory concentrations (IC₅₀) were determined by nonlinear regression to a sigmoidal dose-response curve with Prism 5 (GraphPad Software, Inc.). Each assay was performed in triplicate and repeated at least 3 times. All chemicals were purchased from Sigma/Fluka.

FACS analysis. Cells (8 × 10⁶ procyclic forms [PCF] or 10⁶ bloodstream forms [BSF]) were incubated with 100 mM K⁺-glutamate, 1.5 mM valinomycin, or both at 27°C (PCF) or 37°C (BSF) for 20 min. Then the cells were brought to a density of 1 × 10⁶/ml, and 1 min before fluorescence-activated cell sorter (FACS) measurement, 400 nM the anionic DIBAC₄(3) bis(1,3-dibutylbarbituric acid trimethine: Molecular Probes) was added and flow cytometry was performed with a Becton Dickinson FACSCalibur (fluorescence window, 505 nm to 530 nm). The lower the membrane potential is (i.e., the more depolarized), the more the anionic fluorescent DIBAC enters the cell. For each measurement, 10,000 cells were analyzed. Data were evaluated with the CellQuest Pro software version 5.2.
from scorpion venom at the highest applicable dose (Table 1). This apparent robustness of the mechanisms underlying K⁺ homeostasis in T. brucei contrasted with the trypanosomes’ extreme susceptibility to the K⁺ ionophores valinomycin and gramicidin (Table 1), the trypanosomes being over 1,000-fold more sensitive than the rat myoblasts. Valinomycin and gramicidin themselves may not be of therapeutic value due to their toxicity. Nevertheless, their trypansomidal potency indicates that K⁺ homeostasis in African trypanosomes, in spite of their insensitivity toward K⁺ analogs and channel blockers, harbors essential and sensitive drug targets.

Cell viability being a rather crude indicator, we used the fluorescent bis-oxonol dye DiBAC₄(3) to monitor by FACS analysis (fluorescence-aided cell sorting) the immediate effect of externally added K⁺ on the membrane potential in trypanosomes. DiBAC₄(3) serves as a qualitative potentiometric probe since the influx of the dye is inversely proportional to the polarization of the target cell. Bloodstream-form T. brucei trypanosomes exhibited a higher fluorescence after addition of bis-oxonol (400 nM) than procyclic forms, indicating a less negative membrane potential (Ψₑ) in the bloodstream forms (green curves in Fig. 1). This is in agreement with previous studies reporting a membrane potential of ~152 mV for procyclics (11) and ~82 mV for bloodstream forms (37). The trypanosomes (bloodstream as well as procyclic forms) did not spontaneously depolarize in the presence of high (100 mM, added in the form of glutamate salt in order to prevent potential artifacts from Cl⁻ currents) extracellular K⁺ concentrations (Fig. 1, pink curves). The effect of exogenous K⁺ on Ψₑ in T. brucei has been controversial (11, 37). Our results indicate that in T. brucei, bloodstream as well as procyclic forms, the plasma membrane is not immediately penetrable for K⁺. Addition of the K⁺ ionophore valinomycin (1 μM) alone did not affect Ψₑ (Fig. 1, blue curves), indicating that K⁺ is at electrochemical equilibrium at physiological external [K⁺]. Only upon addition of 100 mM K⁺ in combination with 1 μM valinomycin, did the cells depolarize within minutes (Fig. 1, orange curves). This was harmful to the cells, and they died after 50 min of incubation (not shown). Thus, although K⁺ is in equilibrium at resting membrane potential, there do not seem to be open K⁺ channels through which the cation could readily pass the T. brucei plasma membrane.

A new clade of Trk/HKT genes from trypanosomatids. The T. brucei genome (6) encodes two putative K⁺ channels, Tb09.160.3380 and Tb927.1.4450. A further gene product, Tb927.10.1670, is annotated as a putative K⁺ channel in the TriTryp database (TriTrypDB.org), but while it has a predicted K⁺ channel tetramerization domain (Pfam profile PF02214), it is not predicted to possess transmembrane domains by TMHMM (29). In order to identify further K⁺ transporters from T. brucei, we screened the predicted proteome (version 4, 9192 proteins) with hidden Markov model-based profiles for various cation transporter families. The profiles were made with HMMer (16) from homology-reduced sets of representative transporters and validated against the predicted proteomes of Arabidopsis thaliana and S. cerevisiae. Based on these searches, African trypanosomes do not possess cyclic-nucleotide-gated cation channels, KUP/HAK K⁺ permeases (TC 2.A.72) or K⁺:H⁺ antiporters (TC 2.A.37), transporter families which are ubiquitous in plants (21). The only search that returned a significant hit (E-value of 1.5e⁻⁵) from T. b. brucei was with the Trk/HKT-specific profile; the identified protein, Tb10.70.2940, was named TbHKT1. The gene product Tb10.70.2940 has recently been renamed to Tb927.10.4300 (TriTrypDB.org). The similarity of TbHKT1 to known Trk/HKT proteins is rather low at the level of primary amino acid sequence; the founding member of the HKT family, TaHKT2:1 from wheat (44), was used as a reference in Table 2. The similarity becomes more evident when comparing hydropathy profiles (Fig. 2). TbHKT1 has an extended hydrophilic N terminus of 100 amino acids without similarity to any known sequence, followed by the typical topology of HKT/Trk proteins: four P-loop like domains, each between two predicted TM domains and each followed by a glycine residue (Fig. 2), the presumed K⁺ selectivity filter. TbHKT1 has orthologues in other trypanosomatids: two almost identical alleles in T. cruzi, one in Trypanosoma vivax and in various Leishmania spp. (Table 2);
only *Trypanosoma congoense* appears to lack a *TbHKTI* orthologue. The respective genomic regions are syntenic in all trypanosomatids (TriTrypDB.org). These trypanosomatid HKTs form a new, distinct branch on the phylogenetic tree of the Trk/HKT superfamily, equally distant to the plant HKT and fungal Trk proteins (Fig. 3). There is no evidence for horizontal transfer of plant HKT genes to trypanosomes, as has been suggested for metabolic enzymes (22). In *E. coli* and other bacteria, the K⁺/H¹⁺ translocator TrkH requires the NAD-binding subunit TrkA (36) and the ATP-binding subunit TrkE/SapD (23). However, HMMer searches with TrkA- and TrkE/SapD-specific profiles did not provide any evidence for such accessory proteins in *T. brucei* (not shown).

**TABLE 2. Predicted Trk/HKT proteins from trypanosomatids***

| Species          | Protein | TriTryp accession no. | Length (aa) | % similarity to TaHKT2;1* |
|------------------|---------|------------------------|-------------|--------------------------|
| *T. b. brucei*   | TbHKT1  | Tb927.10.4300           | 599         | 29                       |
| *T. vivax*       | TbHKT1  | TVY486_1004280          | 610         | 26                       |
| *T. cruzi*       | TbHKT1  | Tc00.1047053511469.60   | 592         | 33                       |
| *L. major*       | LmjHKT1 | LmjF35.0080             | 579         | 31                       |
| *L. braziliensis*| LbrHKT1 | LbrM34_V2.0120          | 580         | 31                       |
| *L. infantum*    | LinHKT1 | LinJ35_V3.0080          | 579         | 30                       |

*Percent similarity to TaHKT2;1 from wheat (*Triticum aestivum*) was measured by Needleman-Wunsch global alignments with Blosum62.*

**FIG. 2. TbHKT1 carries the hallmarks of Trk/HKT transporters.** (A) Schematic representation of the membrane topology of KcsA-type K⁺ channels (top) and Trk/HKT transporters (bottom). Transmembrane domains are depicted in light blue, P-loops in dark blue, and K⁺ selectivity filters in red. (B) Kyte-Doolittle hydrophobicity, colored according to the same scheme, of TbHKT1, wheat HKT1 (TaHKT2;1), and *Enterococcus hirae* KtrB.

**FIG. 3. Phylogenetic tree of the Trk/HKT superfamily with representative members.** Bootstrap numbers of the main branches are shown in percent positives of 1,000 rounds. Protein abbreviations and GenBank accession numbers are as follows: *T. cruzi* TcHKT1 (71424637), *L. braziliensis* LbhKT1 (154344371), *L. infantum* LiHKT1 (146100295), *L. major* LmHKT1 (72546739), *Pyrococcus furiosus* PfTrk (18894843), *Archaeoglobus fulgidus* AfTrkK (11498445), *Salinibacter ruber* SrTrkH (83816848), *Halobacterium* HaloTrk (15791310), *Arabidopsis thaliana* AtHKT1;1 (7716474), *Oryza sativa* OsHKT2;1 (14588581), *Triticum aestivum* TaHKT2;1 (567062), *Eucalyptus camaldulensis* EcHKT1;1 (9719299), *Leptospira interrogans* TrkH (24193699), *Vibrio alginolyticus* KtrB (3395637), *Curvularia lunata* HKT (G53610), *Baclulus anrubus* TrkH (229604742), *Candida albicans* CaTrk1 (809790), *Neurosponia crassa* NcTrk1 (3724137), *S. cerevisiae* Trk1 (72015), and *Schizosaccharomyces pombe* Trk1 (1182049).

TrkE/SapD (23). However, HMMer searches with TrkA- and TrkE/SapD-specific profiles did not provide any evidence for such accessory proteins in *T. brucei* (not shown).

**Functional characterization of TbHKTI in heterologous expression systems.** *TbHKTI* was functionally expressed in *E. coli*, *S. cerevisiae*, and in *Xenopus laevis* oocytes. For expression in *E. coli*, we used the potassium transport-defective quadruple mutant L2003, which lacks the Trk/HKT superfamily genes TrkG and TrkH, the Kup gene TrkD, and the ATPase Kdp (46). LB2003 cells need ≥20 mM K⁺ in the medium to grow. When *TbHKTI* was expressed in *E. coli* LB2003, the cells grew at K⁺ concentrations as low as 2 mM (Fig. 4A). Similar results were obtained when *TbHKTI* was expressed, under the control of a Gal1 promoter, in *S. cerevisiae* strain CY162, a K⁺ uptake-defective double mutant that lacks the Trk/HKT genes Trk1 and Trk2 (20) and is unable to grow on potassium concentration below 20 mM. Expression of *TbHKTI* allowed the CY162 mutant to grow on 1 mM KCl while control transformants with the empty vector required at least 20 mM KCl for growth (Fig. 4B). As some of the plant HKT proteins function as K⁺/Na⁺ symporters (42), *TbHKTI*-mediated K⁺ transport was tested for Na⁺ dependency. Yeast CY162 mutants cells were still able to grow on 1 mM KCl in the absence of Na⁺ (Fig. 4B), indicating that *TbHKTI* is not a K⁺:Na⁺ symporter. To better address the substrate specificity of *TbHKTI*, the gene was...
expressed in *Xenopus* oocytes. A voltage ramp from −150 to 50 mV produced currents only in the presence of external K⁺; when K⁺ was substituted with Na⁺, only small currents were recorded (Fig. 5A). *Arabidopsis* AtHKT1 (51) was used as a positive control for Na⁺/H¹ exchange (Fig. 5B). At 120 mM external K⁺, the currents mediated by TbHKT1 were independent of Na⁺ (Fig. 5C). Thus, based on the heterologous expression in bacteria, yeast, and oocytes, TbHKT1 is a Na⁺/H¹-independent K⁺ transporter. It is interesting to note that in the only multicellular eukaryotes known to possess Trk/HKT genes, the vascular plants, the majority of the transporters function in Na⁺/H¹ homeostasis (39). Mediating root-to-shoot transport of Na⁺, they are important for salt tolerance and—with a few exceptions (42)—affect K⁺ homeostasis only indirectly (21). The function of TbHKT1 in trypanosomes remains to be elucidated.

**Functional characterization of TbHKT1 in trypanosomes.** TbHKT1 is expressed in both procyclic and bloodstream-form *T. b. brucei* trypanosomes (Fig. 6A), albeit at low levels as the Northern blot signals were only obtained with 32P-radiolabeled probes, not with digoxigenin-labeled probes (not shown). Furthermore, in situ tagging (38) of TbHKT1 with a hemagglutinin tag did not yield an immunofluorescence signal above background, neither in bloodstream-form nor in procyclic trypanosomes (not shown). To investigate the function of TbHKT1 in trypanosomes, the expression of TbHKT1 was downregulated...
by RNAi-mediated gene silencing. Bloodstream-form *T. b. brucei* NYSM and procyclic-form *T. b. brucei* 29-13 were stably transformed with a stem-loop construct of a *TbHKT1* fragment under the control of the Tet operator. Forty-eight hours after addition of tetracycline (1 mg/ml) to the medium, the *TbHKT1* mRNA steady-state levels were reduced substantially in procyclic trypanosomes. However, the *TbHKT1* knockdown trypanosomes did not show any growth phenotype when cultivated in standard medium plus tetracycline over 10 days (Fig. 6B), indicating that expression of *TbHKT1* is not required under these conditions or that residual *TbHKT1* expression after RNAi was sufficient to maintain viability. When the K⁺ concentration in the medium was lowered from 5 mM to 0.5 mM, the trypanosomes (bloodstream forms) proliferated more slowly; but again, there was no significant difference in growth rate between *TbHKT1*-RNAi cells incubated with (population-doubling time of 27.3 h) or without (population-doubling time of 24.6 h) tetracycline. We were not able, after 10 independent attempts to disrupt the genes with antibiotic resistance markers, to create homozygous *tbtkt1−/−* knockout trypanosomes. Thus, the question of whether, and under which conditions, *TbHKT1* is essential for *T. brucei* remains unresolved.

Repeated loss of *Trk/HKT* genes from eukaryotes. A number of genomes from endoparasitic protozoa have been fully sequenced. Besides the trypanosomatids (Table 2), we screened the predicted proteomes of *Plasmodium falciparum*, *Theileria parva*, *Entamoeba histolytica*, *Giardia duodenalis*, and *Trichomonas vaginalis* for the presence of *Trk/HKT* proteins with the same profile that was used to identify *TbHKT1*. Additional representatives of the major eukaryote groups were included as well: the ciliates *Tetrahymena thermophila* and *Paramecium tetraurelia*, the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*, the amoebozoan *Dictyostelium discoideum*, and *Trichoplax adhaerens* as an additional metazoan. No complete genomes are available yet from the rhizaria (foraminifers, radiolarians, etc.). When the presence of *Trk/HKT* genes is overlaid on the phylogeny of the analyzed organisms, the emerging picture is enigmatic at first sight since the distribution of *Trk/HKT* genes among eukaryotes is not coherent (Fig. 7). While the genes occur in kinetoplastids, amoebozoa, fungi, and vascular plants, they are absent from *Chlamydomonas* (53), *Giardia*, and *Trichomonas*, as well as from all available chromalveolate and metazoan genomes. However, the broad occurrence of *Trk/HKT* genes in bacteria and archaea indicates that the primordial eukaryotes possessed *Trk/HKT*-type K⁺ transporters. Thus, the parsimonial interpretation of Fig. 7 is multiple independent gene loss, at least four times among the major eukaryote lineages: within the chromalveolates, the green algae and the diatoms, in contrast to all other excavates analyzed, have retained an *HKT* orthologue suggests that these *K⁺* transporters fulfill an indispensable function at some point in the life cycle of a trypanosomatid parasite.

ACKNOWLEDGMENTS

We thank Gaby Schumann-Burkard for help with FACS, Evert Bakker for the *E. coli* strain LB2003 and plasmid pPAB404, George Cross for the *T. b. brucei* NYSM line, and Rick Gaber for *S. cerevisiae* CY162.

This work was supported by the Swiss National Science Foundation.

REFERENCES

1. Allemann, N., and A. Schneider. 2000. ATP production in isolated mitochondria of procyclic Trypanosoma brucei. Mol. Biochem. Parasitol. 111: 87–94.
2. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403–410.
3. Barrett, M. P., D. W. Boykin, R. Brun, and R. R. Tidwell. 2007. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. Br. J. Pharmacol. 152:1155–1171.
4. Barrett, M. P., E. Tetaud, A. Seyfang, F. Brinza, and T. Bultz. 1998. Trypanosome glucose transporters. Mol. Biochem. Parasitol. 91:195–205.
5. Benito, B., B. García-de-Blas, and A. Rodríguez-Navarro. 2002. Potassium- or sodium-efflux ATPase, a key enzyme in the evolution of fungi. Microbiology 148:933–941.
6. Berriman, M., E. Ghedin, C. Hertz-Fowler, G. Blandin, H. Renaud, D. C. Bartholomeu, N. J. Lennard, E. Caler, N. E. Hamlin, B. Haas, U. Bohme, L. Hannich, M. A. Askett, J. Shalloom, L. Marcello, L. Hou, B. Wickstead, U. C. Alsmark, C. Arrowsmith, R. J. Atkin, A. J. Barron, F. Brinza, K. Brooks, M. Carrington, I. Chernevach, T. J. Chillingworth, C. Churcher, L. N. Clark,
51. Uozumi, N., E. J. Kim, F. Rubio, T. Yamaguchi, S. Muto, A. Tsuboi, E. P. Bakker, T. Nakamura, and J. I. Schroeder. 2000. The Arabidopsis HKT1 gene homolog mediates inward Na" current in Xenopus laevis oocytes and Na(+) uptake in Saccharomyces cerevisiae. Plant Physiol. 122:1249–1259.

52. Waller, K. L., S. M. McBride, K. Kim, and T. V. McDonald. 2008. Characterization of two putative potassium channels in Plasmodium falciparum. Malar. J. 7:19.

53. Ward, J. M., P. Maser, and J. I. Schroeder. 2009. Plant ion channels: gene families, physiology, and functional genomics analyses. Annu. Rev. Physiol. 71:59–82.

54. Wirtz, E., S. Leal, C. Ochatt, and G. A. Cross. 1999. A tightly regulated inducible expression system for conditional gene knock-outs and dominant-negative genetics in Trypanosoma brucei. Mol. Biochem. Parasitol. 99:89–101.

55. Zeng, G. F., M. Pypaert, and C. L. Slayman. 2004. Epitope tagging of the yeast K(+) carrier Trk2p demonstrates folding that is consistent with a channel-like structure. J. Biol. Chem. 279:3003–3013.

56. Zilberstein, D. 1993. Transport of nutrients and ions across membranes of trypanosomatid parasites. Adv. Parasitol. 32:261–291.