Glycerol 3-Phosphate Alters *Trypanosoma brucei* Hexokinase Activity in Response to Environmental Change*[^5]*

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**Background:** Mechanisms of regulation of an essential African trypanosome hexokinase are poorly understood.

**Results:** Glycerol 3-phosphate levels increase upon starvation and prevent cellular and recombinant hexokinase from pH-dependent inactivation.

**Conclusion:** Glycerol 3-phosphate prevents pH inactivation-triggered exposure of an inhibitory nucleotide-binding site.

**Significance:** Identifying how an essential parasite enzyme is regulated may reveal new therapeutic avenues.

The African trypanosome, *Trypanosoma brucei*, compartmentalizes some metabolic enzymes within peroxisome-like organelles called glycosomes. The amounts, activities, and types of glycosomal enzymes are modulated coincident with developmental and environmental changes. Pexophagy (fusion of glycosomes with acidic lysosomes) has been proposed to facilitate this glycosome remodeling. Here, we report that, although glycosome-resident enzyme *T. brucei* hexokinase 1 (TbHK1) protein levels are maintained during pexophagy, acidification inactivates the activity. Glycerol 3-phosphate, which is produced *in vivo* by a glycosome-resident glycolytic kinase, mitigated acid inactivation of lysate-derived TbHK activity. Using recombinant TbHK1, we found that glycerol 3-P influenced enzyme activity at pH 6.5 by preventing substrate and product inhibition by ATP and ADP, respectively. Additionally, TbHK1 inhibition by the flavonol quercetin (QCN) was partially reversed by glycerol 3-P at pH 7.4, whereas at pH 6.5, enzyme activity in the presence of QCN was completely maintained by glycerol 3-P. However, glycerol 3-P did not alter the interaction of QCN with TbHK1, as the lone Trp residue (Trp-177) was quenched under all conditions tested. These findings suggest potential novel mechanisms for the regulation of TbHK1, particularly given the acidification of glycosomes that can be induced under a variety of parasite growth conditions.

Regulation of metabolism is a central response of the African trypanosome, *Trypanosoma brucei*, to environmental changes that the parasitic protozoan encounters during its life cycle. One consequence is that parasite metabolic pathways are adapted for nutrients available in either the tsetse fly vector or mammalian host. For example, procyclic form (PF)[^3] parasites residing in the tsetse fly can metabolize both glucose and amino acids, whereas glycolysis is the sole ATP source for bloodstream form (BSF) trypanosomes.

Trypanosomes compartmentalize parts of the glycolytic pathway in an organelle called the glycosome. These organelles contain the first six or seven enzymes of glucose metabolism as well as enzymes involved in ether lipid biosynthesis and fatty acid oxidation. In addition, two enzymes involved in glycerol metabolism, glycerol-3-phosphate dehydrogenase and glycerol kinase, are localized to this compartment (1).

In the glycosome, glucose is converted to fructose 1,6-bisphosphate through the consumption of ATP by both *T. brucei* hexokinase (TbHK) and *T. brucei* phosphofructokinase. These two enzymes are unusual in that they are insensitive to feedback inhibition by their products (2-4). One result of compartmentalization is that ATP production and consumption within the glycosome are balanced, with net ATP synthesized only in the cytosol. It has been suggested by modeling experiments that compartmentalization prevents the unproductive consumption of the net ATP (referred to as “turbo-explosion”) by either the TbHKs or *T. brucei* phosphofructokinase (5). RNAi studies of genes essential to glycosome biosynthesis support this observation, as glucose is toxic to PF *T. brucei* lacking glycosomes, presumably as a result of inappropriate consumption of cytosolic ATP (6), whereas RNAi of TbHK in the glycosome-deficient cells is protective (7).

The turbo-explosion model suggests that compartmentalization protects the cell from inappropriate kinase consumption of ATP. Additional layers of TbHK regulation have been identified recently, including alteration of enzyme activity as a result of changes in the oligomerization state of the enzyme. Trypanosomes express two 98% identical TbHKs (TbHK1 and TbHK2) that have different biochemical properties, including distinct sensitivity to pyrophosphate. Changes in oligomer composition can influence *in vitro* enzyme activity and sensitivity to pyrophosphate (8). As further evidence of TbHK regulation mechanisms, TbHK1 can be inhibited by fatty acids that are found within the glycosome (9).

The glycosomal protein repertoire is altered in response to developmental and environmental changes. For example, as the parasite transits from the short stumpy form (which is found in mammalian blood and is pre-adapted for life in the tsetse fly vec-
tor), glycosomes fuse with lysosomes (10). A process similar to pexophagy, which is the autophagy of peroxisomes in response to metabolic changes found in yeast, has been implicated in the turnover of glycosomal components. Here, we demonstrate that TbHK1, which is sensitive to pH as a result of substrate and product inhibition, is protected by glycerol 3-phosphate, a metabolite that accumulates under some growth conditions. These observations suggest a novel means of regulation of an essential activity in the African trypanosome.

**EXPERIMENTAL PROCEDURES**

*Reagents—* ATP and ADP were purchased from Calbiochem. To confirm purity (reported by the manufacturer to be >95%), both compounds were resolved by HPLC (Waters, Milford, MA) using an isocratic buffering system (100 mM KH$_2$PO$_4$ (pH 6.5) and 1.2% methanol) on a Symmetry C18 column (5 µm, 4.6 × 150 mm) at a flow rate of 1.0 ml/min. Compounds were detected at 259 nm on a dual UV detector and analyzed by Breeze 3.2V software. Antisera to *T. brucei* glycerol kinase (TbGK) (1:100,000), TbHK (which detects both TbHK1 and TbHK2; 1:100,000), *T. brucei* triosephosphate isomerase (1:3500), and *T. brucei* enolase (1:10,000) were the generous gift of Dr. Paul Michels (Laboratory of Biochemistry, Université Catholique de Louvain). Antisera to *T. brucei* PEX11 (1:4,000) was the gift of Dr. Christine Clayton (Center for Molecular Biology Heidelberg, University of Heidelberg). Mouse anti-tubulin antibody (1:50,000; clone B-5-1-2, Sigma) was used as a loading control for Western blot analysis.

*Cell Culture and Transformation—* Both PF and BSF of *T. brucei brucei* strain 427 (29-13 and 90-13, respectively) were used in the cellular studies. PF parasites were grown in SDM-79 (11, 12) to a density of 1 × 10$^7$ cells/ml, collected by centrifugation (10 min, 3000 × g), and stored at −20 °C until used. To lyse cell pellets, parasites were thawed, washed, and resuspended in H$_2$O to a final concentration of 1 × 10$^7$ cells/ml. Buffer (0.1% Triton X-100, 50 mM triethanolamine (pH 7.4), 1 mM PMSE, 20 mg/ml leupeptin, and 100 mg/ml N$^\alpha$-tosyl-L-lysine chloromethyl ketone) was added immediately after lysis. BSF parasites were treated similarly, with cell pellets lysed at 1 × 10$^6$ cells/ml.

*Recombinant Enzyme and Assay Conditions—* For purification of recombinant TbHK1, a previously described protocol (9) was modified to increase yield. Briefly, a starter culture of *Escherichia coli* M15(pREP) harboring pQE30 (Qiagen, Valencia, CA) with TbHK1 cloned in-frame of a His$_6$ tagging sequence was grown in ECPM1 (13). The starter culture was used to inoculate a 5-liter culture in a bioreactor (Biostat B, B. Braun Biotech International, Allentown, PA) at 37 °C and supplemented with O$_2$. The culture was induced with 0.8 mM isopropyl β-D-thiogalactopyranoside when the culture reached an A$_{600}$ between 3 and 5 and allowed to grow for 16 h without O$_2$ supplementation. The cell pellet was collected by centrifugation (20 min, 5000 × g, 4 °C) and resuspended in His buffer (50 mM NaPO$_4$ (pH 8.1), 5 mM glucose, 150 mM NaCl, and 0.1% Tween). Cells were passed through a cell disruptor (Constant Cell Disruption Systems, Sanford, NC) for lysis. After clearing the lysate by centrifugation, the supernatant was run on a 50-ml ProBond column (Invitrogen) at a rate of 5 ml/min on an FPLC system (GE Healthcare) with a step gradient of 5–250 mM imidazole in His buffer. Fractions were screened using hexokinase activity assays and Western blotting, and those containing recombinant TbHK1 were pooled and concentrated. Further purification by ion-exchange chromatography was performed using a HiTrap SP HP column (GE Healthcare) with a gradient elution. At the end of the purification, the protein was determined to be ~99% pure based on SDS-PAGE.

Hexokinase assays were performed in triplicate using a coupled reaction to measure enzyme activity as described previously (9, 14). In short, the coupled assay uses Glc-6-P dehydrogenase to convert Glc-6-P generated by hexokinase to 6-phosphogluconate with coincident reduction of NADP to NADPH, which is monitored spectrophotometrically at 340 nm. Reagent final concentrations in the 200-µl reactions were buffered with the appropriate Good’s buffer (50 mM triethanolamine, MOPS, or PIPES) and included 3.3 mM MgCl$_2$, 20 mM glucose, 5.25 mM ATP, 0.75 mM NADP, and 0.1 unit of Glc-6-P dehydrogenase. Kinetic analyses were performed using KaleidaGraph 4.1 (Synergy Software, Reading, PA) with the Michaelis-Menten curve fitting algorithm.

Glycerol 3-P levels were measured enzymatically following perchloric acid extraction of cells (3 volumes/per volume of cells of HClO$_4$ on ice) as described (15, 16). For TbHK assays, glycerol 3-P (20 mM) was added with the other assay components. This concentration is similar to that reported in parasites (5) and found here (see Fig. 2A).

*Enzyme Activity in Parasite Lysates—* For hexokinase assays using PBS-treated cells, parasites were pelleted, resuspended in either PBS or PBS supplemented with 100 mM glycerol, incubated for 15 min at room temperature, and then lysed as described above. Cell equivalents (1 × 10$^5$ or 4 × 10$^5$ for PF and BSF, respectively) were mixed with all hexokinase assay components, and the hexokinase reaction was monitored spectrophotometrically.

*Microscopy and Glycosome Visualization—* To stain cells, 1 × 10$^6$ PF cells were pelleted (5 min, 800 × g) and resuspended in SDM-79 or PBS containing either acridine orange (5 µg/ml; Alfa Aesar, Ward Hill, MA) or LysoTracker Red DND-99 (Invitrogen). Cells were incubated under standard growth conditions (28 °C, 5% CO$_2$) for 15 min, pelleted, washed, and then applied to slides after the addition of VECTASHIELD (Vector Laboratories, Burlingame, CA). Images were captured by epifluorescence microscopy (Axiovert 200M, Carl Zeiss MicroImaging, Inc., Thornwood, NY).

*Tryptophan Quenching Assay of TbHK1—* Quercetin (QCN; varying concentrations) and glycerol 3-P (20 mM) were added individually and in combination to a solution (3 ml) of 0.1 M MOPS (pH 6.5). A scanning spectrofluorometer (QM-Y, Photon Technology International, Birmingham, NJ) was used to monitor emission from 300 to 550 nm after excitation of the lone Trp residue (Trp-177) on TbHK1 at 280 nm (17). Once background emission was acquired, ~1 µg of TbHK1 was added to the solution, and a second emission scan was performed. The area under the emission curve from 370 to 380 nm was integrated using software supplied with the spectrophotometer. The values were converted to Trp emission lost and plotted versus concentration of QCN using KaleidaGraph 4.1 to determine IC$_{50}$ values.
RESULTS

Starvation Alters Cellular pH and Glycosome-resident Protein Abundance—PF trypanosomes can adapt their metabolism to conditions of low glucose, presumably by up-regulation of amino acid metabolism (18, 19). Because of this dynamic metabolism, PF parasites can grow normally in the near absence of glucose. However, complete elimination of nutrients by incubation in PBS triggers association of glycosomes with the acidic (pH 4.8 (20)) lysosomal compartment in PF cells, a process similar to pexophagy in yeast (10).

With acridine orange as a marker for acidic compartments, incubation of live cells in PBS changed the distribution of cellular and glycosomal pH. A, PF cells were incubated (28.5 °C, 5% CO2) with acridine orange in SDM-79 (left) or PBS for 15 min (right) prior to visualization. Acidification is indicated by Texas Red localization, whereas signal in the FITC channel indicates neutral or basic compartments. B, PF cells expressing glycosomally targeted eYFP were incubated with LysoTracker Red DND-99 for 15 min in either SDM-79 or PBS. All images were captured using a Zeiss Axiovert 200M microscope. Aldo, aldolase; PTS, peroxisomal targeting sequence. C, incubation of PF parasites in PBS led to altered abundance of some glycosome-resident proteins, although its impact on TbHK protein and enzyme activity was minimal. Whole cell lysates from parasites maintained in SDM-79 (5 × 10^6 (lane 1) or 1 × 10^7 (lane 2) cells) or incubated in PBS for 15 min (5 × 10^6 (lane 3) or 1 × 10^7 (lane 4) cells) were probed with antisera raised against glycosome-resident proteins (TbGK, TbHK, and T. brucei triose-phosphate isomerase (TbTIM)), a glycosomal membrane protein (T. brucei PEX11 (TbPEX11)) (29), and a cytosolic protein (T. brucei enolase (TbENO)). Tub, tubulin.
Parasites incubated in standard medium typically had three to five small acidic compartments, whereas incubation in PBS for 15 min led to widespread acidification of the cell (Fig. 2).

FIGURE 2. Glycerol 3-P is synthesized by parasites incubated in PBS, and the metabolite impacts pH-based inactivation of TbHK activity. A, parasites cultured with or without glycerol (Gly) in SDM-79 or PBS were lysed, metabolites were acid-extracted, and glycerol 3-P (Gly3P) levels were measured. Glycosome volume and number per cell were based on values determined previously (30). BSF lysate (4 × 10⁵ cell eq; B) or PF lysate (1 × 10⁵ cell eq; C) was assayed for hexokinase activity at pH 7.4 (black bars) and pH 6.5 (white bars) with or without the addition of glycerol 3-P, glycerol 2-P (Gly2P), or glycerol (20 mM each).

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FIGURE 3. Recombinant TbHK1 inhibition under acidic conditions can be prevented by glycerol 3-P, which accumulates in PBS-treated cells. A, pH profile of TbHK1 (150 ng) activity in the presence (white bars) or absence (black bars) of glycerol 3-P (Gly3P). Three different Good’s buffers with a buffering capacity in the range of pH values tested were used for the assay: MOPS, PIPES, and triethanolamine (TEA). B, TbHK1 activity at pH 7.4 or 6.5 with or without glycerol 3-P, glycerol 2-P (Gly2P), or glycerol (20 mM each).
A. Cellular pH was also monitored using LysoTracker-stained PF parasites expressing enhanced yellow fluorescent protein (eYFP) targeted to glycosomes by a peroxisomal targeting sequence 2 from aldolase (Fig. 1B) (21). Cells in standard growth medium had limited LysoTracker staining and numerous glycosomes. Following incubation in PBS, however, LysoTracker distribution was widespread, and many of the cells lacked detectable glycosomal eYFP signal. The rapid loss of signal is most likely the result of acidification, as eYFP has an apparent pKₐ of 7.1 and is quenched under acidic conditions (22).

To assess further the consequence of association of glycosomes with acidic compartments, parasites were grown under standard conditions or incubated in PBS, and cell lysates were probed with antisera to glyosome-resident proteins and cytosolic proteins. Although incubation in PBS led to a modest increase in TbHK signal (likely a mixture of TbHK1 and TbHK2, as the antiserum does not discriminate) (Fig. 1C), the fate of other glycosomally targeted proteins varied. TbGK abundance was not altered (data not shown), whereas T. brucei triose-phosphate isomerase levels were markedly reduced (Fig. 1C). The impact of incubation in PBS was not systemic, as the cytosol-resident glycolytic enzyme T. brucei enolase and the glycosomal membrane protein T. brucei PEX11 were not affected.

B. TbHK Activity Is pH-sensitive, but Glycerol 3-P Prevents Inactivation—Glycosomes are dynamic organelles that are subject to changes in enzyme and metabolite content, depending on both the environment in which the cell resides and the life cycle stage. For example, incubation of cells in glycerol led to rapid accumulation of glycerol 3-P (Fig. 2A) (5). Although this process occurred in cells incubated in either SDM-79 or PBS, the dramatic increase (~130-fold) in glycerol 3-P in cells incubated in PBS suggests that the lone cellular glycerol kinase, a glycosome-resident protein (5), is competent for activity under acidic conditions (Fig. 2A). These findings led us to assess the consequences of glycerol 3-P on TbHK activity.

TbHK activity from PF or BSF lysates was sensitive to the pH of the assay; at pH 6.5, enzyme activity was nearly abolished (Fig. 2, B and C). Performing similar assays in the presence of glycerol 3-P or glycerol 2-P, but not glycerol, modulated pH inactivation of the enzyme. Purified recombinant TbHK1 was also sensitive to pH, with ~50% reduction in activity at pH 7.0 and nearly complete loss of activity at pH 6.5. Like lysate-derived TbHK activity, glycerol 3-P modulated pH inactivation of the recombinant protein (Fig. 3A), even maintaining detectable enzyme activity at pH 4.0 (data not shown). The pH of the reaction mixture remained unchanged after glycerol 3-P addition, ruling out the possibility of glycerol 3-P buffering the reaction. Glycerol 2-P also prevented inactivation, whereas glycerol did not (Fig. 3B).

Glycerol 3-P had no impact on TbHK1 oligomerization (a previously characterized mechanism for regulation of activity...
(8), indicating that the maintenance of enzyme activity was not related to a change in the nature of the hexamer (supplemental Fig. 1). Although glycerol 3-P could be serving as a molecular crowding agent, leading to a reduced impact of pH, substituting glycerol or PEG (data not shown) did not prevent inactivation, suggesting that, if the observed results were due to crowding, it was a specific response to glycerophosphate compounds.

To ensure that the protection of the recombinant enzyme was not an artifact of the reporter assay, Glc-6-P dehydrogenase was incubated at pH 6.0 with Glc-6-P as substrate, thereby fore-going the need for hexokinase to produce Glc-6-P from ATP and glucose. Glc-6-P dehydrogenase activity was unaffected by the acidic pH, readily converting Glc-6-P to 6-phosphogluconate at the acidic pH values tested here, in the presence or absence of ATP and glucose (supplemental Fig. 2). Therefore, the observations are not a result of altering the reporter enzyme activity.

ATP and ADP Are Inhibitory under Acidic Conditions—The finding that glycerol 3-P or glycerol 2-P altered recombinant TbHK1 activity led us to consider whether the change in pH was somehow altering the interaction of the protein with other phosphoryl-bearing compounds, including the substrate ATP. At pH 7.4, a broad range of ATP concentrations supported TbHK1 activity, with a negative consequence on activity only at very low (<0.1 mM) and very high (>5 mM) concentrations (Fig. 4A). At pH 6.5, however, the enzyme was more sensitive to the substrate, with optimal activity at ~1 mM and inhibition at higher concentrations. The affinity for ATP did not change dramatically, with an apparent $K_m$ of 0.22 ± 0.17 mM at pH 6.5 compared with 0.28 mM at pH 7.4 (9). The addition of glycerol 3-P only slightly increased the apparent affinity to 0.16 ± 0.07 mM.

Supplementing the low concentration of ATP (1 mM) needed to fuel the TbHK1 reaction at pH 6.5 with ADP inhibited the enzyme (Fig. 4B). ADP is a mixed inhibitor of TbHK1, with an IC$_{50}$ of 4.2 mM (Fig. 4C). The impact of ADP on the assay at pH 7.4 was reduced, yielding an IC$_{50}$ of ~10 mM. Similar experiments using AMP in place of ADP did not inhibit the enzyme. Introduction of additional MgCl$_2$ into the reaction did not alter the results, indicating that the inhibition was not a result of the nucleotide-depleting Mg$^{2+}$ required for enzyme function.

QCN Inhibition, but Not Binding, Is Relieved by Glycerol 3-P at pH 6.5—To characterize further the interaction of glycerol 3-P with TbHK1, we employed the fluorescent probe QCN, which binds TbHK1 and quenches fluorescence from the lone Trp residue (Trp-177) that lies near the enzyme hinge adjacent to the catalytic base, Asp-214 (17). Under standard conditions (pH 7.4), QCN quenched 50% of Trp-177 emission at 35

![Graph](image-url)

**FIGURE 5.** Glycerol 3-P relieves QCN-based enzyme inhibition but does not alter binding of the fluorescent probe. A, Trp-177 emission was quenched by QCN at pH 6.5 in the absence (upper) or presence (lower) of glycerol 3-P. Increasing concentrations of QCN were incubated with recombinant TbHK1 in a solution of 100 mM MOPS (pH 6.5). Trp-177 was excited at 280 nm, followed by an emission scan from 300 to 550 nm. The percentage of Trp emission lost upon the addition of QCN was calculated from the emission peak between 370 and 380 nm. B, TbHK activity in the presence or absence of glycerol 3-P (Gly3P) with QCN (10 or 100 μM). (Please note that activity was not detected at pH 6.5 in the absence of glycerol 3-P; hence, the hatched bar is not visible.)
At pH 6.5, quenching was slightly enhanced by the presence of glycerol 3-P, with 50% quenching at 43 and 50/110 and 50/110 M QCN with or without glycerol 3-P, respectively (Fig. 5A). Although glycerol 3-P had only a modest impact on QCN binding (slightly enhancing its quenching impact), it altered enzyme inhibition by the compound. At pH 7.4, 10/110 M QCN inhibited TbHK1 by ~50%. Inclusion of glycerol 3-P in the reaction prevented the inhibition, but higher QCN concentrations (100/110 M) could overcome this rescue (Fig. 5B). At pH 6.5, glycerol 3-P prevented QCN inhibition, even at 100/110 M.

**DISCUSSION**

*T. brucei* alternates between a tsetse fly vector and mammalian host, utilizing changes in environmental nutrient availability as part of a complex pathway to regulate development. These changes include activation of a “cryptic” mitochondrion in the BSF to a metabolically active organelle in the PF parasite. Additionally, glycosome contents are altered in a developmentally coordinated fashion. For example, BSF parasites metabo-lize glucose to 3-phosphoglycerate within the glycosome, followed by further processing in the cytosol to ultimately yield pyruvate. However, PF parasites process glucose to 1,3-bisphosphoglycerate in the glycosome, indicating that the parasites have stage-specific differences in glycosome content.

Changes in glycosome content occur through glycosomal autophagy (10). During differentiation, glycosomes co-localize with lysosomes, which occurs quickly (and involves a high percentage of glycosomes) during the transition from short stumpy form parasites (the mammalian blood form pre-adapted for life in the insect) to PF parasites (10). This process resembles pexophagy, the mechanism for peroxisome turnover in meth-ylo-trophic yeast that occurs when nutritional conditions change (23).

Here, we have found that a change in protein environment (a change in pH) impacts the response of TbHK1 to both substrate (ATP) and product (ADP), heretofore unrecognized as regulators of enzyme activity (Fig. 6). Product inhibition by ADP is not competitive with substrate, indicating that a second nucleotide-binding site has been exposed in response to the acidic conditions of the assay. The change in protein structure is subtle, as the enzyme remains catalytically competent, and the probe QCN continues to bind the protein and quench Trp-177 fluorescence under acidic conditions. However, the flavonol fails to inhibit the enzyme, suggesting that the subtle change in structure has moved the QCN-binding site sufficiently far from the active site to allow activity (Fig. 6).

We expect total TbHK activity to differ between BSF and PF parasites, as BSF parasites rely exclusively on glycolysis for ATP production. Reflecting metabolic differences in the life stages, glycolytic proteins make up ~90% of the protein content in BSF glycosomes, whereas glycolytic proteins make up only ~40–50% of the protein content in PF glycosomes (24–26). Nevertheless, TbHKs are important to both life stages, as TbHK1 and TbHK2 have been shown to be essential to BSF parasites (27, 28), whereas *TbHK2*−/− PF parasites are viable, but the knock-out has a pleiotropic impact on the cells. Viable *TbHK1*−/− PF cells have not yet been isolated despite repeated attempts under different growth conditions, suggesting that *TbHK1* is also essential to PF cells.

The importance of the interaction of glycerol 3-P with TbHKs and the subsequent regulation of enzyme activity can be inferred from parasites that lack glycosomes due to genetic manipulation of the *PEX14* gene, which is involved in peroxi-somal and glycosomal protein import. As a result of the *PEX14* deficiency, glycosome biogenesis is blocked, yielding parasites that grow normally in the absence of glucose. However, the addition of glucose to cultures of *PEX14*-deficient parasites is

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4 M. T. Morris, unpublished data.
toxic (6). Glucose toxicity has been proposed to result from the lack of appropriate compartmentalization of TbHKs and *T. brucei* phosphofructokinase, leading to uncontrolled consumption of ATP (6). Interestingly, RNAi of TbGK was found to rescue the parasites from glucose sensitivity in the *PEX14*-deficient background (5). This observation suggests that the absence of TbGK leads to a reduction in cellular glycerol 3-P, which therefore allows pH inactivation of TbHK activity. Without sufficient glycerol 3-P to maintain TbHK activity, the cell is protected from the lethal “runaway” activity of the enzyme.

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