

Review Article

Singular Features of Trypanosomatids' Phosphotransferases Involved in Cell Energy Management

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Trypanosomatids are responsible for economically important veterinary affections and severe human diseases. In Africa, *Trypanosoma brucei* causes sleeping sickness or African trypanosomiasis, while in America, *Trypanosoma cruzi* is the etiological agent of Chagas disease. These parasites have complex life cycles which involve a wide variety of environments with very different compositions, physicochemical properties, and availability of metabolites. As the environment changes there is a need to maintain the nucleoside homeostasis, requiring a quick and regulated response. Most of the enzymes required for energy management are phosphotransferases. These enzymes present a nitrogenous group or a phosphate as acceptors, and the most clear examples are arginine kinase, nucleoside diphosphate kinase, and adenylate kinase. *Trypanosoma* and *Leishmania* have the largest number of phosphotransferase isoforms ever found in a single cell; some of them are absent in mammals, suggesting that these enzymes are required in many cellular compartments associated to different biological processes. The presence of such number of phosphotransferases support the hypothesis of the existence of an intracellular enzymatic phosphotransfer network that communicates the spatially separated intracellular ATP consumption and production processes. All these unique features make phosphotransferases a promising start point for rational drug design for the treatment of human trypanosomiasis.

1. Introduction

Protozoan pathogens constitute an important group of parasites with medical and veterinary importance. Among them, *Leishmania spp.* and *Trypanosoma spp.* are examples of mammalian parasites. About half a million people are infected by parasites of the *T. brucei* group in Africa, 11–18 million with *T. cruzi* in the Americas, and 12 million with *Leishmania* in Africa, Asia, Europe, and Americas [1, 2]. The life cycles of *Leishmania* and *T. cruzi* involve an obligatory intracellular stage in mammals, in contrast to the exclusively extracellular parasites of the *T. brucei* group. Both *Leishmania* and *T. cruzi* invade host cells, while *Leishmania* lives inside parasitophorous vacuoles and *T. cruzi* escapes from the vacuole and lives in the cytoplasm of the host cell. In both cases, the parasites have to adhere

to the host cell surface in order to invade the cell and survive under harsh conditions of the host cytoplasm. *Trypanosoma* and *Leishmania* also present an insect stage during its life cycle, all *T. brucei* group organisms are transmitted by tsetse flies of the genus *Glossina*, *T. cruzi* is transmitted by haematophagous insects belonging to the family Reduviidae, and *Leishmania spp.* are transmitted from man to man by different species of sandflies. Therefore, a common feature amongst parasitic protozoan organisms is their ability to adapt their metabolism to a wide range of environmental conditions and selection pressures, which include the availability and quality of carbon sources in the different mammalian and insect hosts [3]. Therefore, enzymes associated in energy metabolism are important candidates to rational designing of trypanocidal therapeutic drugs.

2. A General View of Trypanosomatids' Energy Metabolism

Even though trypanosomes share energy metabolism features with higher eukaryotes, they present unique characteristics which differentiate them from their metazoan host. Furthermore, the exact nature of their energy metabolism varies sensibly not only between trypanosomatid species [3] but also between different life cycle stages of any given specie [4]. It has been considered that these differences evolved from the variable nutrient supply in the particular environments of each trypanosomatid [5]. However, the metabolic disparities among different trypanosomatid species, which share the same host, indicate that metabolite availability alone cannot be the reason for the energy metabolism strategy exploited in each case [6].

To date, the most extensive experimental studies of trypanosome energy metabolism have been conducted in *T. brucei*, more precisely on the mammalian host associated to the bloodstream form and procyclic trypomastigote present in the tsetse fly midgut. Both of these life cycle stages are easily cultured in defined media in vitro.

The completion of the respective genome projects has enabled to in silico deduce a general metabolic pathway map for these trypanosomes; however this strategy alone misses to determine the presence and importance of single metabolic steps in each life cycle stage [5].

Although trypanosomes possess all enzymatic components needed for the glycolytic pathway, the first seven enzymes are contained inside specialized microbodies from the peroxisome class called glycosomes [7]. These are rounded single membrane-bound organelles with a diameter of approximately 300 nm [8]. Many proteins that are localized to the interior of glycosomes contain specific targeting signals called PTS1 and PTS2 [9]. Furthermore the specialized matrix protein import system shares mechanistic similarities with the endoplasmic reticulum/proteasome degradation process which suggests that glycosomes, as well as peroxisomes and glyoxysomes, all share a common evolutionary origin [10, 11]. The key role of glycosomes in trypanosome energy metabolism becomes evident with the fact that the bloodstream form of *T. brucei* depends exclusively on glycolysis for ATP generation. The end metabolite of this pathway of hexose sugars corresponds to excreted pyruvate [12]. Intraglycosomal redox balance is maintained using a glycerol-3-phosphate dehydrogenase shuttle. The reoxidation of the glycolysis-derived NADH coenzyme is accomplished inside this organelle through an NAD-linked glycerol-3-phosphate dehydrogenase which reduces dihydroxyacetone phosphate to glycerol-3-phosphate [7]. In the presence of molecular oxygen, after exportation from the glycosome this molecule is directed to the mitochondria where it becomes reoxidized to dihydroxyacetone phosphate by the cyanide-insensitive trypanosome alternative oxidase [13] which then returns to the glycosome. On the other half of the glycolytic pathway the final product that leaves the glycosome is 3-phosphoglycerate. In the cytosol the remaining three glycolytic steps take place producing pyruvate as end-product, thus the net ATP yield corresponds

to two molecules per glucose. However in anaerobic conditions the yield is halved due to the inability to reoxidize glycerol-3-phosphate, and glycerol becomes an end-product equimolar to pyruvate [5]. The essential role of glycolysis in trypanosome energy metabolism and its particular and divergent strategy of glycosomal confinement, which distinguishes them from other eukaryotes, constitute a clear and plausible target for chemotherapeutic molecules [14]. RNAi-induced down regulation of components of the glycosomal matrix protein import system produces a relocalization of glycolytic enzymes to the cytosol which is accompanied by a lethal phenotype [15, 16]. One of the possible explanations for the essential compartmentalization of glycolytic enzymes relies on the lack of feedback regulation determined for the trypanosome enzymes hexokinase and phosphofructokinase [17–19]. Both of these initial steps of the glycolytic pathway consume ATP that is recovered in later steps as well as the net ATP gain derived from the pathway. In the absence of specific regulation ATP produced by glycolysis would boost the flux through these enzymes above the capacity of the enzymes downstream with lethal accumulation of intermediate metabolites and cellular depletion of ATP. In this sense confinement within a membranous organelle from the final ATP synthesis steps constitutes an alternative regulatory strategy to unregulated enzymes [16].

Returning to the metabolism of bloodstream form *T. brucei*, the end-product of glucose metabolism is pyruvate. It cannot be further metabolized because during this stage pyruvate dehydrogenase, the tricarboxylic acid (TCA) cycle, and the respiratory chain are absent from the mitochondrial compartment. All the members of the order Kinetoplastida are characterized by a single large mitochondrion which contains a unique structure named kinetoplast [12]. This structure is constituted by a gigantic network of concatenated circular DNAs which represent the mitochondrial genome. Among these molecules, those termed maxicircles encode mitochondrial rRNAs and respiratory chain subunits [20]. Evidence for the expendable nature of mitochondrial metabolic pathways during bloodstream stage derive from the findings that *T. equiperdum* and *T. evansi* actually correspond to *T. brucei* mutants which, respectively, contain relics or completely lack kinetoplast DNA [21]. Although ATP synthase is present, it hydrolyzes ATP in order to maintain proton gradient across the inner mitochondrial membrane essential for the translocation of nuclear-encoded proteins into the mitochondrial matrix [22, 23].

On the other hand, *T. brucei* procyclic trypomastigotes contain a complete set of mitochondrial respiratory chain complexes and all the enzymes responsible for the tricarboxylic acid cycle. Despite the suggestive potential aerobic metabolism, glucose catabolism end-products indicate a predominant fermentation activity. Additionally inhibition of respiration and F₀/F₁-ATP synthase has no effect on intracellular ATP concentration [24]. Apart from carbon dioxide, succinate and acetate are the main excreted metabolites [25]. A fraction of the succinate derives from intraglycosomal redox balance maintenance. Glycosomal NADH is reoxidized by a glycosomal malate dehydrogenase which reduces oxaloacetate to malate, and after the subsequent production

of fumarate, another glycosomal reducing reaction yields succinate which is then secreted [26]. The remaining succinate is produced inside the mitochondria through a set of the enzymes relative to the tricarboxylic acid cycle, during the degradation of proline and glutamate [12]. Furthermore mitochondrial pyruvate is not oxidized to carbon dioxide and water [27, 28]. This molecule is decarboxylated, and the resulting acetyl-CoA is converted to acetate yielding an additional molecule of ATP [29, 30]. Acetate represents the essential precursor for lipid biosynthesis in procyclic form of *T. brucei* [31]. The diverse functions of components of the tricarboxylic acid cycle allow concluding that in these organisms there is no cycle [12].

Trypanosomes in culture universally prefer glucose as carbon source for energy metabolism; however in the digestive environments endured during the insect stages, it is accepted that carbohydrates are only available in limited quantities. Therefore it has been demonstrated that amino acids, such as proline and threonine, can be metabolized for ATP production. This has also been studied in *T. cruzi* epimastigotes which although prefer to use glucose over amino acids as an energy substrate [32]. Under aerobic conditions they produce, in addition to CO₂, considerable amounts of succinate, L-alanine, and acetate [33]. Epimastigotes produce ammonia only after the glucose in the medium has been exhausted [32]. An axenic culture model suggests that *T. cruzi* amastigotes mostly use glycolytic metabolism for ATP production [34]. Amastigotes also ferment glucose to succinate and acetate, but do not seem to excrete ammonia and have little need for the oxidation of amino acids. All these metabolic pathways are summarized in Figure 1.

Maintenance of energy homeostasis requires coordinate regulatory responses according to the surrounding media composition inside the hosts. Most of the enzymes required for energy management, participating in these adaptation processes, are phosphotransferases with a nitrogenous group or a phosphate as acceptors (ECs 2.7.3 and 2.7.4, resp.), such as arginine kinase (AK), nucleoside diphosphate kinase (NDPK), and adenylate kinase (ADK).

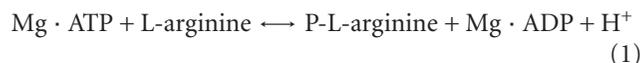
3. Phosphotransferases in Trypanosomatids

Phosphotransferase families related to cell energy management are highly represented in trypanosomatid organisms; for example, since most of the organisms express one to three adenylate kinase isoforms in each cell, *T. brucei* has seven isoforms targeted to different subcellular structures, such as flagellum, glycosome, mitochondrion, and cytoplasm [35, 36]. *L. major* and *T. cruzi* also have six putative adenylate kinase isoforms according to our data and the currently available genome projects [37, 38]. The presence of such number of phosphotransferases and the predicted subcellular localization of each isoform support the hypothesis of the existence of an enzymatic phosphotransfer network that communicates the spatially separated intracellular ATP consumption and production processes [35, 39, 40]. In other organisms, energetic homeostasis is maintained by remodeling this phosphotransfer network. For example, in mammals the lack of muscle creatine kinase is complemented

by glycolytic enzymes and adenylate kinase; in a similar way, the suppression of the adenylate kinase gene produces an upregulation of glycolytic enzymes and creatine kinase [41–43].

Since phosphotransferases participate in a variety of metabolic routes leading to many crucial compounds essential for trypanosomatid organisms, these families of enzymes become interesting targets for drug design.

3.1. Arginine Kinases. Phosphoarginine and phosphocreatine, generally called phosphagens, play a critical role as energy reserve because the high-energy phosphate can be transferred to adenosine diphosphate (ADP) when the renewal of adenosine triphosphate (ATP) is needed. It has been proposed that phosphoarginine supports bursts of cellular activity until metabolic events such as glycogenolysis, glycolysis, and oxidative phosphorylation are switched on [44]. Phosphoarginine synthesis also allows the cells to operate with low ATP levels since it may constitute a usable pool of the high-energy phosphate. Phosphagens act as reservoir, not only of ATP, but also of inorganic phosphate that is mostly returned to the medium by the metabolic consumption of ATP [45]. Arginine kinase (ATP: arginine phosphotransferase; EC: 2.7.3.3) catalyzes the reversible transphosphorylation between N-phospho-L-arginine and ADP [44]:



From an evolutionary viewpoint, arginine kinase was included in a family of conserved proteins with phosphotransferase activity, with creatine kinase as the best known member. Arginine kinase is the most widely distributed phosphagen kinase, which is found in Annelida, Coelenterata, Platyhelminthes, Nemertea, Mollusca, Phoronida, Arthropoda, Echinodermata, Hemichordata, and Chordata [46, 47]. In addition, arginine kinases are considered the most closely related member to the ancestral guanidino kinases [48].

In the last decade, the molecular and biochemical characterizations of arginine kinases in *T. cruzi* and *T. brucei* have been reported [49–53]. Since arginine kinase, an important enzyme involved in the energy supply for the parasite, is absent from mammalian tissues, it becomes a possible target for the future development of chemotherapeutic agents against Chagas' disease and other parasitic diseases caused by related organisms. For this purpose, a rational approach would involve the validation of the enzyme as a therapeutic target and the search for specific enzyme inhibitors. It was also postulated that arginine kinase could be a useful chemotherapeutic target in pesticides development for the control of cockroach proliferation [54].

Multiple evidence indicates that *T. cruzi* arginine kinase is strongly regulated by intra- and extracellular conditions: (1) the arginine kinase protein and the associated-specific activity increase continuously along the epimastigote growth curve, suggesting a correlation between the enzyme activity, and the nutrient availability or parasite density [32];

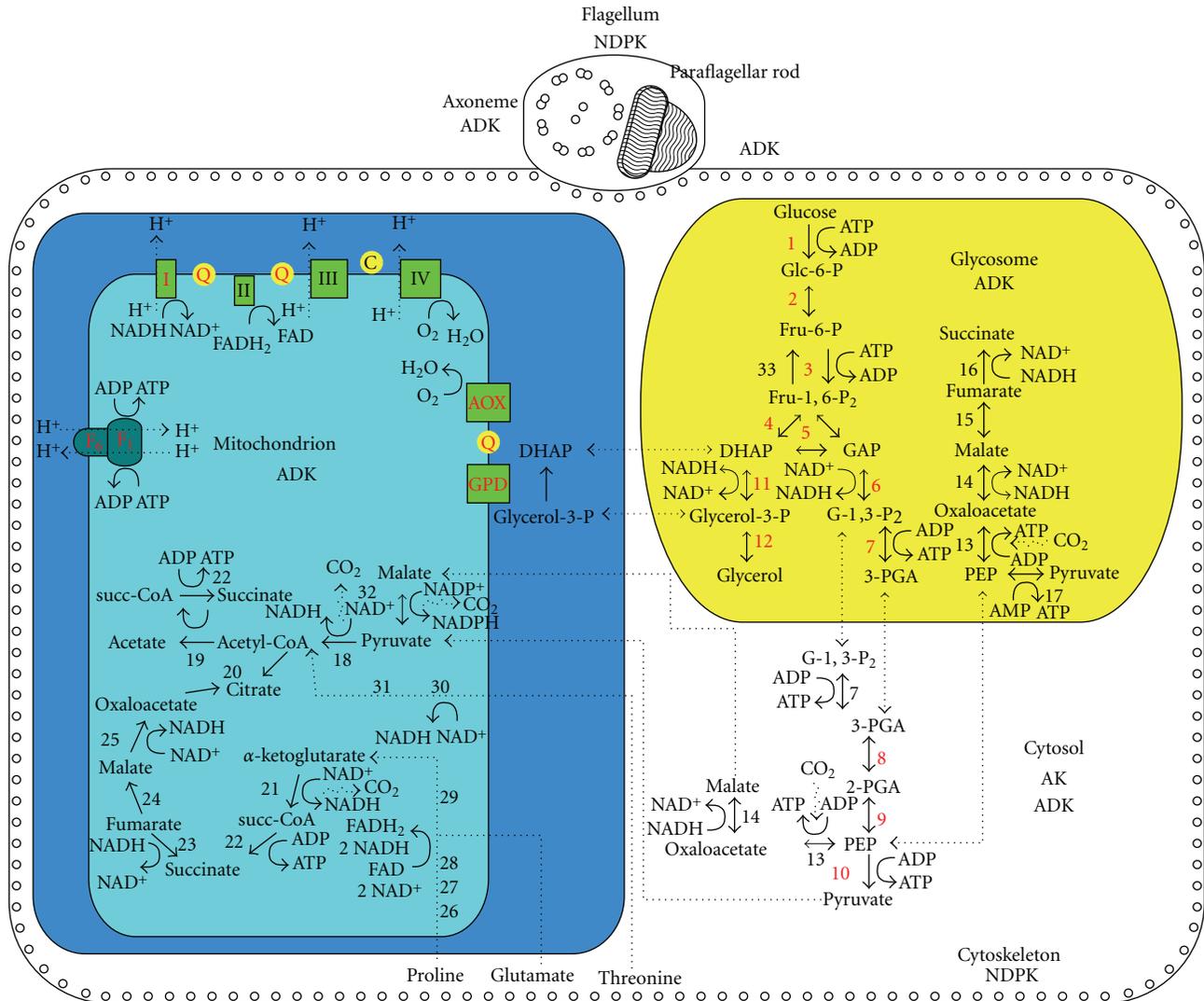


FIGURE 1: Schematic representation of trypanosomatids' energy metabolism. Enzymes are indicated with numbers: 1, hexokinase; 2, glucose-6-phosphate isomerase; 3, phosphofructokinase; 4, aldolase; 5, triosephosphate isomerase; 6, glyceraldehyde-3-phosphate dehydrogenase; 7, phosphoglycerate kinase; 8, phosphoglycerate mutase; 9, enolase; 10, pyruvate kinase; 11, glycerol-3-phosphate dehydrogenase; 12, glycerol kinase; 13, phosphoenolpyruvate carboxykinase; 14, malate dehydrogenase; 15, fumarase; 16, fumarate reductase; 17, pyruvate phosphate dikinase; 18, pyruvate dehydrogenase complex; 19, acetate:succinate CoA transferase; 20, citrate synthase; 21, α -Ketoglutarate dehydrogenase; 22, succinyl-CoA synthetase; 23, succinate dehydrogenase; 24, fumarase; 25, malate dehydrogenase; 26, proline oxidase; 27, Δ' -pyroline-5-carboxylate reductase; 28, glutamate semialdehyde dehydrogenase; 29, glutamate dehydrogenase; 30, threonine dehydrogenase; 31, acetyl-CoA:glycine C-acetyltransferase; 32, NADP-linked decarboxylating malic enzyme; 33, fructose-1,6-bisphosphatase. Enzymes present in bloodstream forms only are indicated in red, procyclic forms enzymes are in black. AOX: alternative oxidase; GPD: FAD-dependent glycerol-3-phosphate dehydrogenase; I: NADH-ubiquinone oxidoreductase (complex I); II: succinate dehydrogenase (complex II); III: cytochrome c reductase (complex III); IV: cytochrome c oxidase (complex IV); c: cytochrome c; Q: ubiquinone; F₀/F₁: F₀/F₁-ATP synthase; Glc-6-P: glucose-6-phosphate; Fru-6-P: fructose-6-phosphate; Fru-1,6-P₂: fructose-1,6-bisphosphate; GAP: glyceraldehyde-3-phosphate; G-1,3-P₂: 1,3-bisphosphoglycerate; 3-PGA: 3-phosphoglycerate; 2-PGA: 2-phosphoglycerate; PEP: phosphoenolpyruvate; DHAP: dihydroxyacetone phosphate; glycerol-3-P: glycerol-3-phosphate; succ-CoA: succinyl-coenzyme A; acetyl-CoA, acetyl-coenzyme A. For a detailed explanation see the text.

(2) the existence of a relationship between the arginine transport rate, arginine kinase activity and the parasite stage and replication capability was recently described, indicating a critical role of arginine kinase as a regulator of energetic reserves and cell growth [55]; (3) the homologous overexpression of *T. cruzi* arginine kinase improves the ability

of the transfectant cells to grow and resist nutritional and pH stress conditions [51]. Arginine kinase would play a role as a stress resistance factor when expressed in organisms that lack this enzyme, such as yeast and bacteria. Recombinant yeast, expressing crab muscle arginine kinase, showed improved resistance under stress challenges that drain cellular energy,

which were transient pH reduction and starvation [56, 57]. *T. cruzi* epimastigotes treated with hydrogen peroxide presented a time-dependent increase in arginine kinase expression, up to 10-fold, when compared with untreated parasites. Among other oxidative stress-generating compounds tested, only nifurtimox produced more than 2-fold increase in arginine kinase expression [52]. Moreover, parasites over-expressing arginine kinase showed significantly increased survival capability during hydrogen peroxide exposure. These findings suggest the participation of arginine kinase in oxidative stress response systems. It is important to remark that the insect stage of the *T. cruzi* life cycle is frequently exposed to nutritional and pH stress conditions, depending on the feeding status of the vector. For example, the pH of excreted material of the *T. cruzi* vector *T. infestans* varies between 5.7 and 8.9, accordingly with the time after feeding [58]. All these data suggest that arginine kinase is involved in the adaptation of the parasite to environmental changes and stress conditions. Recently, the crystal structure of ligand-free TcAK was determined by molecular replacement methods and refined at 1.9 Å resolution [59]. This information could be a new relevant tool for rational trypanocidal drug design.

Until today no outstanding arginine kinase inhibitors have been found. Only a few compounds have been reported which present a partial inhibition of arginine kinase. For example, the trypanocidal action of green tea (*Camellia sinensis*) catechins against two different developmental stages of *T. cruzi* was demonstrated by Paveto et al. [60]. Furthermore, recombinant *T. cruzi* arginine kinase was 50% inhibited by nanomolar concentrations of these polyphenols (catechin gallate or galocatechin gallate). *In silico* docking studies indicated that the flavonoid rutin is an arginine kinase noncompetitive inhibitor and interacts mainly by a hydrophobic force forming an intermolecular complex with the enzyme [61]. Arginine kinase was also inhibited by the arginine analogs, agmatine, canavanine, nitroarginine, and homoarginine [62]. In addition, canavanine and homoarginine also produce a significant inhibition of the epimastigote growth in culture.

3.2. Nucleoside Diphosphate Kinases. Nucleoside diphosphate kinases (EC: 2.7.4.6) are enzymes involved in the intracellular nucleotide maintenance that catalyze the reversible transference of high energy phosphates from a nucleoside triphosphate donor to a nucleoside diphosphate acceptor as follows [63]:



Although the high energy phosphate is mainly supplied by ATP, these enzymes have broad spectrum substrate specificity and are able to use other ribo- and deoxyribonucleotides having preference for GTP formation [64].

NDPKs are ubiquitous and widely studied enzymes, and they can be divided in two groups according to the primary structure [65]. Group I is composed of canonical NDPKs, which are broadly studied and found in prokaryotes and eukaryotes. They form homotetramers in prokaryotes and

homo-hexamers in eukaryotes [66]. The monomers have molecular mass between 15 and 18 KDa and are highly conserved during evolution; for example, there is about 40% identity between NDPKs from *Escherichia coli* and humans [64]. In contrast, group II is formed by divergent NDPKs that are present only in eukaryotes. Proteins included in this group are still poorly characterized and contain one or more NDPK's canonical domains and N-terminal or C-terminal extensions. Some domains such as DM10 domains are present in single copy in this type of NDPKs at the N-terminus. These domains are also contained in other unrelated proteins which have three repeats of this domain. There are only a few studies made on these proteins, in one of them they could associate DM10 domains to protein-axoneme connection [67]. However, the function of NDPK's DM10 domain remains unexplored. Thioredoxin domains are also found in this group of NDPKs, and it was postulated its participation in regulation of NDPK activity by a redox mechanism [68].

NDPKs are also involved in numerous and diverse biological processes. Because of the phosphohistidine enzyme formation in the transference reaction, the phosphate can also be transferred to different acceptors such as other proteins in serine or threonine residues and was postulated to participate in protein G activation by GTP supplement, so NDPKs are implicated in transduction pathways [69–74] playing many functions in, for example, development, proliferation, differentiation, and apoptosis [65, 72, 75–79]. Interestingly, it was also observed that some NDPKs have several roles in DNA processing. Human beings have ten NDPK isoforms (NM23-H1 to 10), five of which were found to interact to nucleic acids. NM23-H2 is considered to be a transcription factor because of its capacity to bind to specific DNA sequences, and NM23-H1, H5, H7, and H8 had 3'-5' exonuclease activity *in vitro*. All these characteristics together with others, as recognition, cleavage, and structural modification of DNA molecules, allow the association between NDPKs and DNA repair mechanisms [77, 80–82].

In the context of studying the biological function of an enzyme, the subcellular localization may be a critical point to establish. Being multifunctional enzymes, NDPKs have been found in numerous subcellular compartments such as cytoplasm, nucleus, mitochondria, intermembrane space, plasmatic membrane, and flagellum from different organisms [82–88]. Considering that NDPKs are involved in phosphotransfer networks their subcellular distribution might be related to an efficient energy distribution inside the cells [39].

Trypanosomatid's NDPKs are of particular interest due to its inability to synthesize purines *de novo* relying on NDPKs for nucleotide recycling [89]. For this reason these enzymes are considered potential therapeutic targets for trypanosomiasis such as Chagas disease. *T. cruzi* has four putative isoforms of NDPK, TcNDPK1–4. TcNDPK1 is the unique canonical isoform, while TcNDPK2, 3, and 4 correspond to group II variants. Isoforms 2 and 3 have one DM10 domain preceding the catalytic region, and variant 4 has unknown N- and C-terminal extensions. The orthologous

genes of these enzymes are also present in the genomes of the related parasites *T. brucei* and *L. major*, except for the absence of TcNDPK4 in the latter [90]. The first report of NDPK activity in trypanosomatids was published in 1995, where Ulloa et al. detected activity in different subcellular fractions including membranes and purified a soluble NDPK from *T. cruzi* epimastigotes with biochemical properties similar to canonical enzymes, probably corresponding to TcNDPK1 [91]. TcNDPK1 has a molecular mass of 16 KDa and like eukaryotic NDPKs forms homohexamers [92]. In addition, it is expressed in trypomastigote and amastigote stages [90]. This is an interesting enzyme because it showed not only phosphotransference activity but also DNase activity with similar rates to commercial nucleases [93]. This new activity was extensively characterized in NM23-H2, the human orthologous of TcNDPK1, and a Lys inside the catalytic site seems to be responsible for it [94]. As *T. cruzi* genomic DNA is also susceptible to TcNDPK1 nuclease activity, it evidences that TcNDPK1 could act at nuclear level, for example, being component of programmed cell death machinery in trypanosomatid organisms [86, 95]. Reinforcing this idea, *T. brucei*-related NDPK was localized mainly in the nucleus of the parasites [96]; conversely it was also identified as a secreted protein [97]. Other results were obtained for members of Leishmania genus; in *L. major* this isoform was associated to microsomal fractions, and in *L. amazonensis* it is secreted and involved in macrophage infection [98, 99]. In *T. cruzi* it is still not clear the localization of the unique canonical isoform. It could be possible that the enzyme has several positions inside the cell and can move from one to another in response to stimuli. In this context a regulation by compartmentalization or phosphorylation is expected as was reported for *T. brucei* [100].

TcNDPK2, a longer NDPK isoform from *T. cruzi*, is a protein of 37 KDa whose first 88 amino acids correspond to the DM10 domain. It is expressed in the three major stages of *T. cruzi* life cycle, and apparently it has distinct regulation from TcNDPK1 because it is inhibited at high substrate concentration [90]. Using immunofluorescence and biochemical techniques we recently demonstrated that TcNDPK2 isoform is a microtubules-associated enzyme mainly localized in the cytoskeleton and flagellum (Miranda et al., unpublished results). TcNDPK2-like NDPKs are conserved in a wide range of eukaryotes with motile axoneme, from unicellular to superior organisms. For example, *T. brucei* orthologous genes codify for an NDPK found in parasite's flagella [101, 102], and in humans the related protein (NM23-H7) is also expressed in flagella-containing cells such as spermatozooids [103]. These are interesting results because they suggest a common possible function for TcNDPK2-like enzymes such as GTP supplying for tubulin polymerization and thus being involved in microtubule dynamics. DM10 domains present in this type of NDPK have not been investigated till the moment. However, we recently demonstrate, by expression of truncated and fusion variants of TcNDPK2 in *T. cruzi*, that the DM10 domain is sufficient and necessary for cytoskeleton delivery of the enzyme (Miranda et al., unpublished results). In addition, it is possible that DM10 domains were implicated in flagella-targeting machinery, a poorly understood

process yet. Importantly, trypanosomatids are considered model organisms for the study of human illness based on cilia and flagella disorders, since NM23-H7 is associated to cone rod dystrophy, a progressive retinal disorder, then the study of TcNDPK2-related enzymes opens new insights in this interesting field.

There is an increasing amount of information about NDPKs that evidence their participation in many diverse biological processes. NDPKs seem to be key metabolic enzymes, thus further studies need to be made in trypanosomatids to understand how they work, to understand their role in metabolism and pathogenesis.

3.3. Adenylate Kinases. One of the enzymes related to cell energy management is adenylate kinases, which are ATP:AMP phosphotransferases. These enzymes are involved in the homeostasis of adenine nucleotides by interconversion of the adenine nucleotide pool, which includes ATP synthesis from ADP and an increase in the ATP energetic potential. They catalize reversible phosphotransfer between ADP, ATP, and AMP molecules, which have been implicated in processing metabolic signals associated with cellular energy utilization [104–106]:



Recent evidence indicates that adenylate kinases facilitate intracellular energetic communication. In typical mammalian cells the loss of adenylate kinase function can be complemented by activation of creatine kinase phosphotransfer [40, 107]. Furthermore a similar role can be attributed to creatine and adenylate kinases, being both implicated in the renewal of ATP from ADP and a phosphorylated compound. In some organisms they participate in muscle contraction, metabolic sensing of K^+ -ATP channels [106–108], and cell motility. In unicellular organisms such as *Tetrahymena* and *Paramecium*, adenylate kinases are involved in the ATP-regenerating system required for ciliary and flagellar movement.

They can be considered key enzymes in life support as they are present in almost all living organisms; they are distributed from bacteria to vertebrates.

The tridimensional structure of adenylate kinases can be decomposed into three subdomains, based on the functional roles and induced fit movements: the NMP-bind and LID domains, the moving parts, and the CORE domain that is unaffected by substrate binding. The “long” and “short” adenylate kinases classification is based on the differences in the LID domain; LID is an 11-residue segment in the short type, whereas that in the long type consists of 38 residues and the difference leads to drastic changes in the conformation of the LID domain. They are small globular proteins that suffer conformational changes when they interact with their substrate [109]; moreover, in most of the cases they are found as monomers [110].

Adenylate kinases isolated from prokaryotes belong to the long type [111]. Similarly the ADK localized in the chloroplast is also the long type [112]. Considering the chloroplast ADKs and the mitochondrial ADKs to have

TABLE 1: Characteristics of trypanosomatids' phosphotransferases. Summary of the main features of arginine kinases (AKs), adenylate kinases (ADKs), and nucleoside diphosphate kinases (NDPKs) isoforms from trypanosomatids. Predicted subcellular localizations are indicated with a question mark. The existence or not of human equivalents (human), N- or C-terminal extensions (N-t and C-t), peroxisomal targeting signal (PTS-1), and DM10 motifs are also detailed.

Enzyme	Organism	Isoform	Localization	Human	Features
AK	<i>T. cruzi/T. brucei</i>	1	Cytosol	No	Canonical
		2	?	No	N-t
	<i>T. brucei</i>	3	?	No	C-t
ADK	<i>T. cruzi/T. brucei/L. major</i>	1	Flagellum	Yes	N-t
		2	Reservosome (?)	Yes	—
		3	Glycosome	Yes	PTS-1
		4	Flagellum	Yes	N-t
		5	Cytosol	Yes	Canonical
		6	Mitochondria	Yes	—
	<i>T. brucei</i>	7 (A)	Flagellum	Yes	N-t
NDPK	<i>T. cruzi/T. brucei/L. major</i>	1	Nucleus (?)	Yes	Canonical
		2	Cytoskeleton	Yes	DM10
		3	?	Yes	DM10
	<i>T. cruzi/T. brucei</i>	4	?	Yes	Putative

a prokaryotic origin in view of the endosymbiont hypothesis, the classification can be subdivided into the eukaryotic short type and the prokaryotic long type. As always there are exceptions to this classification. For example, the cytosolic ADK from *S. cerevisiae* is the long type [113]. Another interesting case is the long type ADK from *Giardia* [114]. *Giardia* belongs to the most primitive group in the Eukaryota, it does not have mitochondria. Consequently it is highly impossible, and *Giardia*'s ADK has derived from mitochondria. Lastly the third exception is the short type ADK from the bacteria, *Micrococcus* [115]. Gathering all the information many authors postulate that the long and short types of adenylate kinase have diverged before the appearance of eukaryotes; this hypothesis could perfectly explain why both types of ADKs are found in prokaryotes and eukaryotes [116].

Their function can be related to their subcellular localization. They have been found in the cytoplasm, mitochondrial matrix, chloroplasts [117], and hydrogenosomes [118], structures that can be directly related to energy consumption and generation places. There is absolutely no doubt that adenylate kinases in those structures are responsible for the interconversion of the adenosine nucleotides. On the other hand essential functions have been related to adenylate kinases; for example, null growth is observed in *E. coli* [119] and *S. pombe* [120], lacking in their adenylate kinases. On the other hand they can be linked to consumption sites of ATP. For example, in *Paramecium* it has been proposed that they would be involved in the fast interconversion of ADP to ATP in the process of cilia reactivation [121].

In a few words adenylate kinases can be considered as key enzymes in cell energetic with the ability of doubling the ATP energy potential. They are a key sensor in cell energetic status sensing; thanks to their catalytic activity small variations in the nucleotide pool of ATP and ADP can be reflected as big changes in the AMP pool, increasing in

this way the sensibility and response of the AMP responding mechanisms [107]. Lastly they can be linked to cellular energetic communication; under highly demanding energy process in some subcellular structures such as nucleus or flagella, fast relocalization of organelles involved in energy synthesis has been observed [39, 40]. These movements have been considered as mechanisms for reducing the distances between energy consumption and generating places. Even so these mechanisms would not be enough, diffusion processes are slow, so it has been proposed that the energy transport is catalyzed enzymatically. Key enzymes in the phosphotransferase network would be adenylate kinases [122].

In parasitic protozoa adenylate kinases have been detected and characterized. In *L. donovani* a long LID domain adenylate kinase has been cloned and characterized [123]. The unicellular malaria parasite, *P. falciparum* (Apicomplexa), presents two adenylate kinase isoforms [124].

In other parasitic protozoa a larger number of isoforms have been characterized. *L. major* has six putative adenylate kinase isoforms according to the genome project data. In other trypanosomatids, *T. brucei* and *Phytomonas spp*, adenylate kinases have been detected in diverse organelles, microbodies, glycosomes [125]. Recently two flagellar, cytoplasmic and mitochondrial associated adenylate kinases were characterized in *T. brucei* [35, 36]. A large number of isoforms, six in total, have also been described in *T. cruzi*, with possible flagellar, glycosomal, mitochondrial, and cytoplasmic subcellular localization [37]. The data presented reveals an enormous variability within organisms, which are reflection of their adaptation to their life cycle. In the case of *T. cruzi* the high number of adenylate kinases can be attributed to the complex life cycle it goes through which involves distinct environments (insect vector gut, mammalian blood, and mammalian host cell cytoplasm) and consequently variable nutritional conditions. Another possible explanation to the highly unusual number of

adenylate kinase can be that they are not only in different subcellular localization but that they are stage specific.

A summary of the main characteristics from each phosphotransferase mentioned in the text is presented in Table 1.

It is evident that hosts and parasites have a large number of differences in terms of their energy metabolism. Trypanosomatids' enzymes completely absent in mammals, different isoforms in subcellular localization, structure, and number are some of these divergences. Phosphotransferases are mainly involved in crucial processes such as the maintenance of the ATP balance in the cell. Slight disruptions on this equilibrium are usually lethal for all living organisms; in consequence we consider that trypanosomatids' phosphotransferases are promising targets for rational drug design.

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