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Protean Permeases: Diverse Roles for Membrane Transport Proteins in Kinetoplastid Protozoa

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Abstract

Kinetoplastid parasites such as *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania* species rely upon their insect and vertebrate hosts to provide a plethora of nutrients throughout their life cycles. Nutrients and ions critical for parasite survival are taken up across the parasite plasma membrane by transporters and channels, polytopic membrane proteins that provide substrate-specific pores across the hydrophobic barrier. However, transporters and channels serve a wide range of biological functions beyond uptake of nutrients. This article highlights the diversity of activities that these integral membrane proteins serve and underscores the emerging complexity of their functions.

Keywords

Kinetoplastid parasites; Transporters; Channels Drug sensitivity Organelles Sensing

1. Introduction

Like the sea god Proteus of Greek mythology who could take on the form of many creatures, a diverse array of membrane associated transport proteins in kinetoplastid parasites, though sharing certain similarities, serve remarkably disparate biological functions. A paradigm of the parasitic mode of existence is that the parasite relies upon its hosts, both vertebrate and invertebrate, to provide the invading microorganism with a wide variety of nutrients. These nutrients are typically taken up across the otherwise relatively impermeable plasma membrane by integral membrane proteins called transporters, permeases, or carriers. Transporters are thought to work by an ‘alternating access model’ [1] in which the transport pore is never open to both sides of the membrane at once. Another class of transport protein

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Note Added in Proof

A recently published article (F. Rojas et al., Oligopeptide signaling through TbGPR89 drives trypanosome quorum sensing, *Cell* 176, 2019, 1-12) demonstrates that a novel oligopeptide transporter, TbGPR89, plays a critical role in quorum sensing that allows the developmental transition of long slender (LS) bloodstream form African trypanosomes into short stumpy forms (SF) that are adapted for differentiation into procyclic forms once taken up by the tsetse fly. This permease mediates uptake into the cytosol of LS forms of extracellular oligopeptides, which function as cell density-dependent Stumpy Inducing Factor (SIF), and thus allows the LS to SF transformation to occur in response to SIF.

includes channels [2], which can provide a pore that is open simultaneously to both sides of the membrane and thus allow more rapid flux than transporters.

However, in addition to the role of transport proteins in import of critical solutes, they may serve other roles that are of biological importance to the parasite. Some permeases allow efflux of metabolic end products or xenobiotics that would otherwise build up to toxic levels inside the parasite. Other transporters and channels are not expressed on the parasite surface but on internal organellar membranes and thus support the unique biochemical functions of those subcellular compartments. Various permeases are of considerable therapeutic importance, because they can serve as efficient routes for uptake of drugs, even though the drugs are not their natural substrates. Some transporters can be promising drug targets, as selective inhibition of their transport function by drug-like compounds can be lethal to the parasite. Finally, a limited number of transporters function as signal transduction receptors that allow cells to sense their external environments, and examples of such ‘transceptors’ [3] are likely to exist among the kinetoplastid protozoa. Hence both transporters and channels play broadly important roles in the biology and pharmacology of parasitic protozoa.

While early studies focused upon kinetic characterization of transporters without knowledge of their molecular nature, increasingly sophisticated biochemical and genetic approaches in parasites, including the sequencing of complete genomes, has revolutionized the study of transporters in pathogenic protozoa. Many studies on the kinetoplastid parasites *Trypanosoma brucei*, *Trypanosoma cruzi*, and various *Leishmania* species have identified genes encoding transporters or channels and have thus allowed dissection of these transport proteins at the molecular level. Furthermore, bioinformatic evaluation of the sequenced genomes in the TransportDB 2.0 web site (http://www.membranetransport.org/index_v2_rc1.html) has identified 337 putative or known transporters for *T. brucei* TREU927, 411 for *T. cruzi* CL Brenner, and 270 for *L. major* Friedlin, and the tabulation of all predicted transporters for each species is available at that site. This rapidly evolving area of research provides the opportunity to consider what we have learned about transporters and their biological roles in kinetoplastid parasites as well as what major issues remain to be explored and elucidated. The intention of this review is not to provide a comprehensive summary of membrane transport proteins but rather to focus upon a few selected examples that illustrate the importance of membrane transport proteins for a diverse array of biological processes among the Kinetoplastida.

2. Nutrient transporters

Since a major function of transporters is to allow the influx of nutrients from host to parasite, I will discuss a few of the permeases that mediate uptake of metabolically important solutes. The advent of gene knockout technology, now facilitated by the recent introduction of CRISPR-Cas9 mediated gene editing [4, 5] and other sophisticated molecular genetic methodologies [6], provides a powerful strategy for addressing the functions of individual transporters and their variants. In addition, gene knockout and RNAi approaches allow investigators to determine whether specific permeases are essential for parasite viability, thus identifying those that could be targets for development of novel drugs.

2.1. Hexose transporters

Traditionally, bloodstream form (BF) African trypanosomes have been considered to be highly dependent upon uptake of large amounts of glucose followed by inefficient metabolism by glycolysis [7, 8]. Two hexose transporter genes have been identified [9–11], THT1 (trypanosome hexose transporter 1) whose mRNA is expressed in BF parasites and THT2, whose mRNA is the sole *THT* transcript detectable in insect stage procyclic forms (PFs). Notably, THT1 is a relatively lower affinity permease with a K_m value for 2-deoxyglucose of ~0.5 mM, while THT2 is a considerably higher affinity permease with a K_m of ~50 μ M [12]. Thus THT1 is a BF selective and THT2 a PF selective permease with different kinetic properties likely adapted to the high glucose levels available to in the mammalian bloodstream compared to the lower glucose availability in the tsetse fly. The open reading frames for both genes were expressed in *Xenopus* oocytes and shown to mediate uptake of radiolabeled glucose [10, 12]. Since BF parasites have been thought to utilize glucose as their sole energy source [8], these two permeases would be expected to play essential roles in BF metabolism [7], and hexose uptake has been investigated as a potential therapeutic target by selective inhibitors of these transporters [13]. The recent discovery that mice infected with African trypanosomes contain a substantial population of parasites in adipose tissue and that these parasites are able to oxidize fatty acids [14], unlike their bloodstream resident neighbors, illustrates that parasite metabolism is more plastic than previously appreciated and that fatty acids could be an energy source for adipose forms of the parasite. Nonetheless, glucose may still be critical for these tissue resident parasites as a source of metabolic precursors or energy that may not be completely fulfilled by fatty acids.

L. mexicana express 3 distinct glucose/hexose transporters, LmxGT1, LmxGT2, and LmxGT3 [15] that exhibit unique biological functions. Thus LmxGT2 is the major hexose transporter expressed during the procyclic insect stage of the life cycle, and its mRNA is strongly downregulated in intracellular amastigotes [15], leading to a pronounced decrease in glucose uptake by amastigotes [16], which are thought to reside in a relatively glucose-poor environment [17], compared to promastigotes. *LmxGT1* mRNA is expressed in both life cycle stages, but the protein is unstable in amastigotes and is strongly downregulated in these intracellular parasites [18]. Strikingly, LmxGT1 is localized to the flagellar membrane [19], where it has been proposed to play a role in sensing extracellular glucose [18]. In contrast, LmxGT3 plays a central role in intracellular amastigotes. A dual *lmxgt1/ lmxgt2* knockout that expresses only LmxGT3 was as infective to macrophages and as virulent in mice as wild type parasites, whereas lines expressing only LmxGT1 or LmxGT2 exhibited much reduced infectivity to macrophages [20]. Additionally, LmxGT3 is localized to the endoplasmic reticulum in intracellular amastigotes, suggesting a possible role in mobilizing glucose from the lumen of that organelle. Since terminal glucose residues are removed from newly synthesized glycoproteins during maturation in the ER of both mammalian cells and *Leishmania* parasites [21], recycling of this luminal glucose into the cytosol could be a critical function for LmxGT3.

A null mutant in which all 3 GTs were deleted by targeted gene replacement was greatly impaired in its ability to survive within macrophages [22] and in mouse infections [20, 23], and LmxGT3 was the one transporter variant that was able to substantially restore

infectivity. Metabolomic analyses in *L. mexicana* by McConville and colleagues [24] suggested that a major function of hexose transporters in intracellular amastigotes is to provide metabolic precursors, through the tricarboxylic acid cycle, for anapleurotic biosynthesis of glutamine. This model could explain, at least in part, the poor viability of glucose transporter null mutants as amastigotes.

2.2. Heme transporters

Heme is a metalloporphyrin prosthetic group for a variety of proteins involved in oxygen transport, redox reactions, and signal transduction. Most kinetoplastid protozoa are devoid of the heme biosynthetic pathway and are thus dependent upon their hosts to provide this critical cofactor. A homology search of the *L. amazonensis* genome, employing the prototypical heme transporter from *Caenorhabditis elegans* HRG-4, identified a weakly homologous open reading frame designated LHR-1 [25]. This protein was able to restore heme uptake to a *S. cerevisiae* mutant deficient in heme transport, its overexpression in *L. amazonensis* promastigotes increased uptake and accumulation of a fluorescent heme analog, and the protein targeted to the plasma membrane and endocytic vesicles of the parasite. It was not possible to delete both alleles of the gene, suggesting that this permease, which is divergent from the human ortholog HRG1 [26], is likely essential for both promastigotes and amastigotes and could thus serve as a potential drug target. In *T. brucei*, BFs import heme along with hemoglobin using the haptoglobin-hemoglobin receptor, but PFs do not express that receptor and instead express an LHR-1 ortholog, TbHRG, that is localized to the flagellar membrane and has been proposed to function as a heme sensor during infections of the tsetse fly [27]. In contrast, TbHRG is expressed in the endocytic vesicles of BF trypanosomes, where it is essential for BF viability, probably by mediating uptake of heme from the digested hemoglobin within the endocytic system into the cytosol of the parasite [28]. The functional disparity and distinct localizations of HRG proteins among different kinetoplastid parasites and between separate developmental stages underscore the pronounced plasticity of this transporter family within these related protozoa.

2.3. Fe²⁺ transporters

Iron is a critical transition metal for organisms, because there are many proteins that require iron as an essential cofactor. Iron is limiting in the parasitophorous vacuoles in which *Leishmania* amastigotes reside, due to its export across the vacuole membrane by host Nramp1 transporters. A homology search using the sequence of the *Arabidopsis thaliana* iron transporter IRT1 identified an ortholog in *L. amazonensis* that was named LIT1 [29], and this transporter promoted the uptake of radiolabeled Fe²⁺ in promastigotes. It was normally expressed at very low levels in promastigotes, but was induced in the plasma membrane following development of the parasites to intracellular amastigotes in the iron-poor environment of the phagolysosome. *lit1* null mutants were unable to survive inside macrophages and were essentially avirulent following infection of BALB/c mice, confirming that this iron transporter is essential for import of Fe²⁺ from host macrophages. Subsequent studies demonstrated that influx of Fe²⁺ through this transporter served as a trigger of promastigote differentiation into amastigotes within macrophages [30]. The increased intracellular Fe²⁺ generated by induction of LIT1 increased the activity of the iron-containing enzyme superoxide dismutase, which then converted intracellular superoxide to

H₂O₂, a differentiation signal in these parasites as well as in other eukaryotes. Iron is also a cofactor for various mitochondrial proteins, so that cytosolic Fe²⁺ must permeate the mitochondrial inner membrane to support mitochondrial functions. A homology search employing the sequence of the yeast mitochondrial iron transporter *mrs.3* identified the *LMIT1* gene in *L. amazonensis* [31]. Deletion of one allele (*LMIT1/ lmit1*) was possible, but null mutants could not be obtained, suggesting essentiality of the gene in promastigotes. Notably, *LMIT1/ lmit1* mutants could develop into metacyclic promastigotes, but they could not sustain infections of macrophages *in vitro* nor could they induce cutaneous lesions in mice. These studies establish the critical role of iron and its transporters in viability, development, and virulence in *Leishmania* parasites and suggest that both LIT1 and LMIT1 might serve as targets for development of novel antileishmanials. They also underscore the requirement for multiple transporters to shuttle the critical Fe²⁺ cofactor into and within parasites. Of interest, *in silico* studies on ion transporters and channels in the genomes of 37 unicellular eukaryotes found that clustering of predicted transporters for iron and other transition metals were especially useful in distinguishing extracellular from obligate intracellular protists [32]. These comparative omics studies suggest that acquisition of iron is a selective force in the evolution of unicellular parasites and highlight the importance of this metal in the infectious life style.

Despite the requirement for iron, over accumulation of this transition metal is toxic to cells due to its ability to generate reactive hydroxide free radicals via the Fenton reaction. Thus cells typically develop mechanisms to sequester or export excess iron to avoid toxicity. Major facilitator superfamily proteins of the nodulin-like family mediate iron sequestration in plants and yeast [33], suggesting that similar proteins might be involved in iron homeostasis in *Leishmania* parasites. Indeed, an iron-responsive gene was identified recently in *L. amazonensis* that encodes a member of the nodulin-like family and is expressed in the plasma membrane of promastigotes and intracellular amastigotes [34]. This protein, designated *Leishmania* iron regulator 1 or LIR1, mediates efflux of [⁵⁵Fe²⁺] preloaded into the parasites but does not affect iron uptake. Knockout of the *LIR1* gene resulted in parasites that survived very poorly inside primary murine macrophages and that were almost avirulent in mice, generating a parasite load that was decreased by a factor of 10⁶ compared to wild type infections. The absence of an orthologous gene in humans suggests that this novel permease could be a target of therapeutic importance.

2.4. Pyruvate transporters

The pyruvate transporters of *T. brucei* are of interest, because unlike most nutrient transporters that facilitate import of their solutes, the physiological function of the pyruvate transporters is to export pyruvate that accumulates from the high glycolytic flux of BF parasites. It is important to note that as facilitative transporters, they can import pyruvate in uptake assays if the external concentration of this solute is artificially raised, as the direction of flux is determined by the concentration gradient of pyruvate. BFs do not metabolize glucose beyond the glycolytic pathway and thus generate abundant quantities of the end product pyruvate. Inhibition of trypanosome pyruvate transporters with the compound UK5099 leads to rapid lysis of BF parasites [35–37] from the resulting acidification and osmotic imbalance of the cytosol. A pyruvate transporter gene from *T. brucei*, *TbPT0*, was

cloned by genetic complementation of a yeast mutant deficient in pyruvate uptake and shown to confer high affinity uptake of labeled pyruvate upon this complemented line [38]. The protein localizes to the plasma membrane of BF trypanosomes, and inhibition of its expression by RNAi arrests growth of the parasites. Of interest, the 5 closely related TbPTs are unrelated to the sequences of vertebrate monocarboxylate transporters but are similar to the nodulin-like transporters from plants and thus represent potential unique targets for drug development.

2.5. Other nutrient transporters

A variety of transporters for other nutrients have been studied in some detail in various kinetoplastid parasites, including those for amino acids, calcium, folates, magnesium, *myo*inositol, polyamines, purines, sugar nucleotides, and others. Space limitations prevent summaries of this substantial body of work, but the Table lists some of these permeases, and readers are referred to a more comprehensive review article [39] for an overview of other transport proteins.

3.0. Organellar transporters

Organelles are intracellular membrane bound bodies that serve a variety of functions, and include mitochondria [40], endoplasmic reticulum [20], endosomes and lysosomes [28], Golgi apparatus [41], acidocalcisomes [42], and others. These organelles need to control flux of many solutes across their limiting membranes to carry out their myriad biological functions. Studies have been carried out on a plethora of such organellar transporters in the Kinetoplastida, but the discussion here will be limited to acidocalcisomes, storage vesicles that illustrate the need for specific solute transporters to carry out flux into and out of this membrane bound body. In addition, examples of transporters in the endoplasmic reticulum, mitochondria, and endosomal membranes have been noted above.

Acidocalcisomes exist widely in both prokaryotes and eukaryotes and serve as acidic storage reservoirs for Ca^{2+} , polyphosphates, various metal ions, and basic amino acids [42]. Specific acidocalcisomes transporters that have been studied at the biochemical level in kinetoplastid parasites, such as *T. cruzi*, *T. brucei*, and *Leishmania* species, include a Ca^{2+} -ATPase, a vacuolar H^{+} -ATPase, a H^{+} -pyrophosphatase, $\text{Na}^{+}/\text{H}^{+}$ and $\text{Ca}^{2+}/\text{H}^{+}$ exchangers, phosphate transporters, transporters for Mg^{2+} , K^{+} , Na^{+} , Zn^{2+} , and Fe^{2+} , an aquaporin that is dedicated to water transport and probably volume regulation, and a chloride channel. Recently many of these permeases have been identified in the proteome of the *T. brucei* PF acidocalcisome and localized to the acidocalcisomes by immunofluorescence microscopy [43]. Collectively, these proteins are involved in concentrating H^{+} , Ca^{2+} , phosphate, and metal ions in the lumen of this organelle. Intriguingly, an inositol 1,4,5-triphosphate (IP_3) receptor from *T. brucei*, a calcium channel that is gated by IP_3 , was identified in the acidocalcisome membrane, and it releases Ca^{2+} from this organelle upon binding of IP_3 [44]. Depletion of this IP_3 receptor by RNAi resulted in defects in BF trypanosome growth in culture and greatly reduced infectivity in mice, suggesting that Ca^{2+} signaling via the acidocalcisomes is required for normal parasite growth and infection. Of interest, no IP_3 receptor was detected in the endoplasmic reticulum membrane, the site for IP_3 receptors and calcium mobilization

in most other eukaryotes, implying that acidocalcisomes play especially central roles in Ca^{2+} signaling in trypanosomes and other kinetoplastid parasites.

4.0. Drug transporters

Drugs frequently enter cells as unnatural substrates of transporters for biologically important solutes. Indeed, several transporters have been identified in kinetoplastid parasites that mediate uptake of drugs. The existence of these transporters can make the parasites selectively sensitive to the drugs, and genetic alteration of these transporters can result in resistant parasites [45].

An early example for identification of a drug transporter was the uptake of the arsenical melarsoprol [46] and diamidine drugs such as pentamidine [47] by the P2 adenosine/adanine transporter of *T. brucei*. The gene for this permease, designated *TbAT1*, was cloned by complementation of a mutant strain of *S. cerevisiae* that was defective in purine biogenesis [48]. The *TbAT1* gene from a melarsen oxide-resistant strain of *T. brucei* contained multiple point mutations, 6 of which resulted in changes in amino acid sequence, and this mutant permease was unable to promote uptake of adenosine or induce sensitivity to melarsen oxide in yeast. Subsequent work demonstrated that no single mutation impairs sensitivity of trypanosomes to melarsoprol, pentamidine, or diminazine, although several combinations of mutations resulted in strong impairment [49]. Molecular modeling of the TbAT1 structure suggests that the mutated amino acids are distant from the substrate binding site, consistent with the requirement for multiple such mutations to impair activity by structural alterations. The model also suggested potential binding modes for these drugs in the substrate binding site of the permease, explaining how these diverse drugs are accommodated by a purine permease.

Studies using radiolabeled pentamidine showed that only half of the uptake activity could be inhibited by adenine or adenosine, suggesting that there was another pentamidine transporter in addition to TbAT1. Using RNAi studies to identify drug resistance determinants, a second route for uptake of diamidines and melarsoprol in trypanosomes was determined to be the aquaglyceroporin TbAQP2 [50, 51]. Modeling of TbAQP2 structure suggests ligand binding sites for pentamidine and melarsoprol with the channel pore and indicates that the aperture is wide enough to accommodate these drugs [45]. However, another study suggests that pentamidine is not a transport substrate for TbAQP2 but is rather a high affinity inhibitor of flux through this channel [52]. The role of TbAQP2 in pentamidine uptake was proposed to be as a receptor for pentamidine that allows its import via endocytosis.

Miltefosine, hexadecylphosphocholine, is the only current orally bioavailable drug for treatment of visceral leishmaniasis [53]. A miltefosine transporter gene, *LdMT*, was cloned from *L. donovani* by functional complementation of a miltefosine resistant line [54]. *LdMT* is a P-type ATPase and aminophospholipid translocase that flips such lipids across the plasma membrane. Expression of *LdMT* in miltefosine-resistant parasites restored drug sensitivity and uptake of [^{14}C]miltefosine, confirming that it is a miltefosine transporter. Furthermore, the drug-resistant line contained inactivating point mutations in both alleles of *LdMT*. Subsequent work by the same group identified another subunit of the transporter,

LdRos3 [55], that associates with LdMT and is necessary for trafficking of the heterodimer from the endoplasmic reticulum to the cell surface. In this case, the drug is structurally related to the natural substrates, aminophospholipids, explaining the utilization of this uptake pathway.

Eflornithine is a drug employed against African trypanosomiasis that inhibits the parasite ornithine decarboxylase and prevents synthesis of essential polyamines [56]. An eflornithine-resistant line was generated by exposing trypanosomes to increasing concentrations of the drug, and this line was impaired for uptake of the labeled drug [57]. Since eflornithine is an amino acid analog, its uptake on an amino acid transporter was suspected. PCR amplification of various hypothetical amino acid transporter genes from wild type and mutant parasites indicated that only the *TbAAT6* gene was absent from the mutants. Induction of RNAi against *TbAAT6* made the resulting cell line resistant to eflornithine, and complementation of the resistant mutant with the cloned *TbAAT6* gene restored drug sensitivity to this line, confirming that this amino acid transporter mediates uptake of the drug.

The large ATP Binding Cassette or ABC family that has 42 members in *L. major* [58], includes transporters that are responsible for export of drugs or xenobiotics. These exporters can reduce sensitivity of kinetoplastid parasites to therapeutic agents either by amplification of the gene encoding the transporter or by mutation of the coding sequence. Early studies identified the ABC transporter PGPA from *L. major*, subsequently designated ABCC3, as a determinant of resistance against therapeutically important arsenicals and antimonials [59]. PGPA transports metal-thiol conjugates and is located in vesicles close to the flagellar pocket [60], suggesting that it confers drug resistance by sequestering these metals intracellularly and allowing their subsequent export via vesicular trafficking. Additional studies have identified other ABC family members, ABCG2 [61] and the ABC half-transporter ABCC14 [62] that forms homodimers, as alternate determinants of antimonial resistance. ABCG2 is located in the plasma membrane and vesicles close to the flagellar pocket, while ABCC14 traffics to the mitochondrion and plasma membrane, indicating potential roles for both sequestration and export of cytotoxic metals as thiol conjugates.

5.0 Ion channels

Ion channels have been studied in a wide range of eukaryotes, where they mediate rapid flux of specific ions across the membrane and are involved in diverse physiological functions, such as setting and altering membrane potential, neuronal transmission, sensing, and signal transduction [2]. However, functional studies on channels in kinetoplastid parasites have been limited to date. The few examples of ion channels that have been studied in some detail are summarized below, but other orthologs of putative ion channels in these parasites remain to be interrogated.

5.1. *T. cruzi* cation channel

The first kinetoplastid ion channel that was expressed and characterized was the TcCat cation channel from *T. cruzi* [63]. Unlike most eukaryotic channels, it was possible to achieve recombinant expression of this 2 transmembrane segment protein in bacteria and to

reconstitute the channel in artificial liposomes. Electrophysiological characterization demonstrated a weak selectivity of ~ 2.5 for flux of K^+ over Na^+ , as opposed to typical K^+ channels that are highly ion selective, and the protein has thus been designated as a cation channel. Remarkably, this channel undergoes rapid changes in cellular localization with alterations in environmental conditions and in different life cycle stages. TbCat was localized to both the cell surface and internal sites of epimastigotes, but in trypomastigotes, it was targeted to the surface of the flagellum. When trypomastigotes were subjected to acidic conditions, TbCat migrated to the tip of the flagellum. In intracellular amastigotes, it exhibited a restricted internal localization, and this internal localization could be important for sequestering channel activity in the high K^+ environment of the host cell cytosol. However, when amastigotes were released from host cells into a low K^+ environment, the channel redistributed to the parasite surface. Under conditions of hyperosmotic stress, TbCat translocated almost completely to the plasma membrane in epimastigotes, but it disappeared from the cell surface in trypomastigotes and was released into the supernatant. This behavior suggests potential roles for this channel in the osmotic stress response.

5.2. *T. brucei* potassium channels

Two potassium channels, TbK1 and TbK2, were initially identified by screening the predicted *T. brucei* proteome for ion channel profiles from the Pfam database [64]. Both proteins were predicted to have 6 transmembrane domains and a conserved potassium channel signature motif (TTGFG and TIGYG, respectively) in the loop between transmembrane segments 5 and 6. Expression of each channel in *Xenopus* oocytes did not lead to significant transmembrane currents, but coexpression of both channels induced robust K^+ -selective currents. This result suggests that the two proteins must form hetero-oligomers to be active. Addition of increasing concentrations of K^+ to wild type BF cells resulted in membrane depolarization, but induction of RNAi against either channel abrogated membrane depolarization. These results imply that these channels are involved in establishing membrane potential in trypanosomes, similar to the function of many K^+ channels in higher eukaryotes. RNAi experiments also demonstrated that both channels were essential for growth of BF, but not PF, trypanosomes. Hence, these potassium channels could serve as novel antitrypanosomal drug targets, a strategy supported by drug targeting approaches employing mammalian potassium channels [65].

The same group subsequently discovered another K^+ channel by screening the *T. brucei* predicted proteome with the specific Pfam profile for inward rectifier potassium channels, IRKs [66]. This protein, called TbIRK, has a divergent consensus selectivity filter, GGYVG, instead of the classical TxGYG motif. Electrophysiological characterization of TbIRK in *Xenopus* oocytes nonetheless established that the channel generates large currents in the presence of K^+ ions and has a ~ 7 -fold selectivity for permeation of K^+ over Na^+ , and mutations in the divergent filter sequence reduced the selectivity for K^+ . Remarkably, epitope tagged TbIRK localized to acidocalcisomes, indicating a likely function associated with this organelle, but the specific role of this channel in these vesicular bodies is not known.

6.0. Transporters and channels with likely sensory functions

One notable difference between kinetoplastid parasites and most other eukaryotes is the absence in these protozoa of genes coding for many of the larger families of membrane receptors involved in sensing and signal transduction, including G-protein coupled receptors, heterotrimeric G-proteins, and receptor tyrosine kinases. This observation suggests that other surface proteins are likely to play enhanced roles in sensing the environment, a process that must be central to the ability of these protozoa to adapt to the dramatically different environments they encounter during their life cycles. One intriguing possibility is that transporters and channels may serve important sensory functions. Such activity could manifest itself in several distinct ways. Permeases may allow selective influx of solutes that induce physiological changes once they have entered the cytosol. Activation of ion channels may allow mobilization or influx of second messengers, such as Ca^{2+} . Finally, transporters themselves may act as signal transduction ‘transceptors’ [3] by binding to ligands and sending a secondary signal to the cytosol, a receptor-like mode of action that has been recognized for multiple transporters or transporter-like proteins in various eukaryotes.

A clear example of transporters that mediate environmental sensing by transporting solutes into African trypanosomes, but presumably not as transceptors, involves PAD1 and PAD2 (proteins associated with differentiation) that are expressed on stumpy form (SF) parasites [67], a non-replicating life cycle form that emerges when parasitemia reaches higher levels. Upon uptake of the blood meal by a tsetse fly, the SFs differentiate into PF parasites and are thus developmental forms that are committed to transformation into the insect stage upon sensing of appropriate environmental signals within the tsetse fly. Physiological prompts that stimulate the SF to PF differentiation include citrate and *cis*-aconitate. PAD1 and PAD2 were initially identified from transcripts that were differentially regulated in a differentiation defective line compared to the parental parasites. The corresponding open reading frames predicted proteins with 14 transmembrane domains and sequence similarity to major facilitator superfamily and nodulin-like permeases, suggesting potential transporter activity. Expression of both proteins in *Xenopus* oocytes stimulated uptake of [^{14}C]citrate, confirming their ability to mediate import of this metabolite and differentiation signal. Induction of RNAi against PAD1 and PAD2 impaired differentiation of SF to PF parasites in vitro, establishing their critical role in this transformation. The relay of the citrate/*cis*-aconitate signal by PAD proteins constituted the first molecular pathway to link environmental sensing to life cycle stage differentiation in trypanosomes.

The recognition that cilia and flagella in many organisms are sensory organelles [68] raises the possibility that flagellar membrane associated transporters could function in sensory capacities, possibly as transceptors. Promastigotes of the *lmxgt1* null mutant of the *L. mexicana* flagellar glucose transporter were viable but exhibited catastrophic population collapse upon reaching high cell density and exhausting glucose from the medium [18]. In contrast, either wild type or add-back strains entered stationary phase. These results raise the possibility that LmxGT1 senses glucose in the medium and is important for the transition from logarithmic to stationary phase. In addition, LmxGT1 is upregulated ~50-fold in glucose deficient compared to glucose replete medium, indicating a substrate regulation of expression that is exhibited by various transceptors.

Similarly, the LdAAT3 arginine transporter is localized at least partially to the flagellar membrane [69]. Starvation of promastigotes for arginine upregulates LdAAT3 expression and also modulates expression of a cohort of other proteins and transporters, constituting an arginine deprivation response (ADR) pathway. Notably, this ADR is dependent upon a mitogen-activated protein kinase, suggesting that binding of ligand to this permease may control a signal transduction cascade. Sensing of arginine within the parasitophorous vacuole may be critical for amastigotes, as arginine is an essential amino acid, but it is also depleted by host macrophages to fuel synthesis of NO when nitric oxide synthase is induced. It is possible that salvaging of arginine from the parasitophorous vacuole by LdAAT3 also blunts the ability of the host macrophage to generate NO and thus attenuates the host immune response.

Several other flagellar transporters or channels have been identified that could be implicated in sensing. The TbHrg heme transporter is localized to the flagellar membrane of BF parasites [27], as are the LmAQP1 [70] and TbAQP2 [71] aquaporins. *Leishmania* promastigotes and axenic amastigotes overexpressing the wild type LmAQP1, but not a non-functional mutant, exhibited increased ability to recover from hypoosmotic shock, compared to parasites transfected with either a non-functional mutant or the vector control. In addition, promastigotes overexpressing the wild type LmAQP1 were able to migrate up an osmotic gradient more rapidly than negative controls. These observations imply that LmAQP1 plays a role in sensing osmotic conditions in the extracellular medium. A putative Ca²⁺ channel has been localized to the flagellar membrane of BF trypanosomes [72, 73] and may play a role in Ca²⁺ mediated signaling.

7.0. Concluding remarks

Transporters and channels play key roles in parasite biology by importing nutrients, exporting metabolites, drugs, and xenobiotics, supporting the functions of diverse intracellular organelles, mediating uptake of drugs and conferring drug sensitivity and resistance, establishing physiological properties such as membrane potential and responses to osmolarity, and in sensing and responding to changes in the environment. Continued research on this fascinating cohort of proteins is likely to reveal further unanticipated complexity in their diverse biological functions.

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Highlights

- Kinetoplastid parasites express hundreds of membrane transport proteins.
- Transporters are critical to the parasitic mode of existence.
- Transporters have a wide range of biological functions in addition to nutrient uptake.
- Transporters and channels have a diversity of subcellular locations.
- Some transporters play roles in environmental sensing.

A. Membrane Transport Proteins

B. Leishmania Cell Organelles

Fig.

Diverse functions of membrane transport proteins in kinetoplastid parasites. A. Membrane transport proteins can serve different biological roles as importers of nutrients, exporters of metabolites, drugs, or xenobiotics, channels for ions or other solutes, drug transporters, or sensors of solutes. B. Transporters and channels can be located in various surface or internal membranes of distinct organelles. For illustration, the image displayed is that of a *Leishmania* promastigote, but the principle is the same regardless of the species or life cycle stage under consideration. Examples of specific transport proteins discussed in the text

include the GT1 glucose transporter in the flagellum, nucleoside sugar transporters in the Golgi apparatus, the GT3 glucose transporter in the endoplasmic reticulum, amino acid transporters in the plasma membrane, the IP3 receptor in acidocalcisomes, the ADP/ATP exchanger in the inner mitochondrial membrane, and the TbHERG heme transporter in endosomes of *T. brucei*.

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Table.
Examples of other kinetoplastid transporters.

Some additional transporters not discussed in the text are listed along with a relevant reference.

Transporter	Species	Substrates	Reference
Amino Acids			
LdAAP7	<i>L. donovani</i>	Lysine	Inbar, E. <i>et al.</i> (2012) Amino Acids 42:347-360
LdAAP24	<i>L. donovani</i>	Proline/Alanine	Inbar, E. <i>et al.</i> (2013) Biochem J 449:555-566
Calcium			
MCUC	<i>T. cruzi</i>	Ca ²⁺ into mitochondrion	Ramakrishnan, S. and Docampo, R. (2018) Genes 9:304-322
Folates/Biopterin			
FTs	<i>L. major</i>	Folates, S-adenosyl methionine	Vickers, T.J.M. and Beverley, S.M. (2011) Essays Biochem 51:63-80
BT1	<i>L. major</i>	Biopterin	
Magnesium ions			
MGT1, MGT2	<i>L. major</i>	Mg ²⁺	Zhu, Y. <i>et al.</i> (2009) Int J Parasitol 39:713-723
Mitochondrial Carriers			
Mitochondrial carriers	<i>T. brucei</i>	Various metabolites	Colosante, C. (2009) Mol Biochem Parasitol 167:104-117
TbMCP5	<i>T. brucei</i>	ADP/ATP	Peña-Díaz, P. <i>et al.</i> (2012) J Biol Chem 287:41861-41874
myo-Inositol			
LdMIT	<i>L. donovani</i>		Drew, M.E. <i>et al.</i> (1995) Mol Cell Biol 15:5508-5515
TbMIT	<i>T. brucei</i>		Gonzalez-Salgado, A. <i>et al.</i> (2012) J Biol Chem 275:20935-209287:13313-13323
Polyamines			
LmPOT1	<i>L. major</i>	Putrescine/Spermidine	Hasne, M.-P. and Ullman, B. (2005) J Biol Chem 280:15188-15194
TcPOT1	<i>T. cruzi</i>	Putrescine/Cadaverine	Hasne, M.-P. <i>et al.</i> (2010) Mol Micro 76:78-91
Purines			
LdNT1	<i>L. donovani</i>	Adenosine and pyrimidine nucleosides	Vasudevan, G. <i>et al.</i> (1998) Proc Natl Acad Sci USA 95:9873-9878
LdNT2	<i>L. donovani</i>	Guanosine/Inosine	Carter, N.S. <i>et al.</i> (2000) J Biol Chem 275:20935-20941
LmaNT3, LmaNT4	<i>L. major</i>	Purine nucleobases	Sanchez, M.A. <i>et al.</i> (2004) Mol Membr Biol 21:11-18; Ortiz, D. <i>et al.</i> (2009) J Biol Chem 284:16164-16169
TbNT2-12	<i>T. brucei</i>	Purine nucleosides and nucleobases	Landfear, S.M. <i>et al.</i> (2004) Eukar Cell 3:245-254; deKoning, H.P. <i>et al.</i> (2005) FEMS Microbiol Revs 29:987-1020
TbNBT1/TbNT8	<i>T. brucei</i>	Purine nucleobases	Burchmore, R.J.S. <i>et al.</i> (2003) J Biol Chem 278:23502-23507; Henriques, C. <i>et al.</i> (2003) Mol Biochem Parasitol 130:101-110
Sugar-nucleotides			
LPG2	<i>L. major</i>	GDP-Man	Ma, D. <i>et al.</i> (1997) J Biol Chem 272:3799-3805
LPG5	<i>L. major</i>	GDP-Gal	Capul, A.A. <i>et al.</i> (2007) J Biol Chem 282:14006-14017