Isolation and Functional Characterization of the PfNT1 Nucleoside Transporter Gene from Plasmodium falciparum*

(Received for publication, December 8, 1999)

Nicola S. Carter‡, Choukri Ben Mamoun§, Wei Liu‡, Edilene O. Silva§, Scott M. Landfear‡‡, Daniel E. Goldberg§**, and Buddy Ullman‡†‡‡

From the ‡Department of Biochemistry and Molecular Biology and §Department of Molecular Microbiology and Immunology, Oregon Health Sciences University, Portland, Oregon 97201, §Howard Hughes Medical Institute, Departments of Molecular Microbiology and Medicine, Washington University School of Medicine, St. Louis, Missouri 63110, and ‡Department of Pathology, Federal University of Para, Belém, Pará, Brazil

Plasmodium falciparum, the causative agent of the most lethal form of human malaria, is incapable of de novo purine synthesis, and thus, purine acquisition from the host is an indispensable nutritional requirement. This purine salvage process is initiated by the transport of preformed purines into the parasite. We have identified a gene encoding a nucleoside transporter from P. falciparum, PfNT1, and analyzed its function and expression during intraerythrocytic parasite development. PfNT1 predicts a polypeptide of 422 amino acids with 11 transmembrane domains that is homologous to other members of the equilibrative nucleoside transporter family. Southern analysis and BLAST searching of The Institute for Genomic Research (TIGR) malaria data base indicate that PfNT1 is a single copy gene located on chromosome 14. Northern analysis of RNA from intraerythrocytic stages of the parasite demonstrates that PfNT1 is expressed throughout the asexual life cycle but is significantly elevated during the early trophozoite stage. Functional expression of PfNT1 in Xenopus laevis oocytes significantly increases their ability to take up naturally occurring d-adenosine (Kₘ = 13.2 μM) and d-inosine (Kₘ = 253 μM). Significantly, PfNT1, unlike the mammalian nucleoside transporters, also has the capacity to transport the stereoisomer L-adenosine (Kₘ > 500 μM). Inhibition studies with a battery of purine and pyrimidine nucleosides and bases as well as their analogs indicate that PfNT1 exhibits a broad substrate specificity for purine and pyrimidine nucleosides. These data provide compelling evidence that PfNT1 encodes a functional purine/ pyrimidine nucleoside transporter whose expression is strongly developmentally regulated in the asexual stages of the P. falciparum life cycle. Moreover, the unusual ability to transport L-adenosine and the vital contribution of purine transport to parasite survival makes PfNT1 an attractive target for therapeutic evaluation.

Nearly half the world’s population lives in tropical and subtropical areas where malarial parasites are endemic, and 300–500 million people worldwide are afflicted with the disease annually (1). Plasmodium falciparum, the etiologic agent of the most severe form of malaria in humans, is responsible for 1.5–2.7 million deaths per year, >1 million of which occur in children <5 years of age (1). P. falciparum is an obligate intracellular protozoan parasite that undergoes a number of developmental stages in the human host and multiplies asexually in the red blood cell to effect its clinical symptoms and lethal outcome. Chemotherapy for malaria is available but is complicated both by drug toxicity and widespread drug resistance (1). The need for more efficacious and less toxic agents, particularly rational drugs that exploit pathways and targets unique to the parasite, is therefore acute.

A dramatic metabolic discrepancy between P. falciparum and the mammalian host involves purine metabolism. Whereas mammals synthesize purine nucleotides de novo, all protozoan parasites investigated to date (2), including P. falciparum (3), lack an intact purine biosynthetic pathway. Hence, parasites are entirely reliant on their host for preformed purines, and consequently, each parasite genus expresses a unique complement of purine transporters and salvage enzymes that enable purine acquisition from its host. A number of purine salvage enzymes have been described for Plasmodium spp. (2–4), and one, hypoxanthine-guanine-xanthine phosphoribosyltransferase, has been characterized in detail both biochemically (5) and structurally (6).

The intracellular milieu for P. falciparum is unusual in that human erythrocytes lack an intact purine (or pyrimidine) biosynthetic pathway and have limited nucleotide requirements (3). During its intraerythrocytic life cycle, P. falciparum divides rapidly, and the acquisition of purines for nucleic acid synthesis becomes imperative. Accordingly, malaria infection dramatically enhances the ability of erythrocytes to salvage purines (3, 7–9). To salvage preformed purines, P. falciparum must first import these essential nutrients into the parasitized erythrocyte. This is a complex process that requires purine translocation across a number of membranes, including the parasite plasma membrane, the parastichorous vacuolar membrane, and possibly the erythrocyte membrane, depending upon whether purines are salvaged from the erythrocyte or from plasma. Although little is known about purine transport in Plasmodium spp., nucleosides appear to enter parasitized erythrocytes by three or more pathways: (i) by a saturable, high affinity adenosine transport system (8, 9); (ii) by a nonsaturable, anion selective-type channel that also transports L-nucleosides as well as polyls, neutral amino acids, sugars, and cations (10); and (iii) through the tubovesicular membranes
(TVM), an interconnected network extending from the parasitophorous vacuolar membrane to the periphery of the infected erythrocyte (11).

Differenciation among these sundry proposed pathways for nucleoside entry into P. falciparum is hampered by the lack of molecular clones encoding key components of the permeation pathway. Recently, Penny et al. (12) demonstrated that nucleoside and nucleobase uptake capabilities can be conferred upon *Xenopus laevis* oocytes after microinjection of poly(A) RNA isolated from *P. falciparum* asexual stages. We now report the isolation and functional characterization of the first molecular component of the nucleoside permeation pathway of *P. falciparum*. This gene, *PfNT1*, was identified through searching The Institute for Genomic Research (TIGR) malaria data base with the sequence of human and other parasite nucleoside transporters (13–15). Quantitation of poly(A) RNA from various intraerythrocytic stages revealed that *PfNT1* expression is developmentally regulated throughout the intraerythrocytic life cycle, and functional studies in *Xenopus* oocytes confirmed that *PfNT1* is a high affinity, saturable nucleoside transporter exhibiting a broad substrate specificity for purine and pyrimidine nucleosides and their 1-steroisomers. These findings reveal that *PfNT1* is a unique member of the equilibrative nucleoside transporter family and imply that this permease may be a novel therapeutic target for malaria.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Reagents**—Radiolabeled nucleosides, D-[8-3H]ribose and nonradiolabeled 1-adenosine were purchased from Moravek Biochemicals (Brea, CA). D-[6-3H]glucose was purchased from Amersham Pharmacia Biotech. Restriction endonucleases were obtained from New England Biolabs, Inc. (Beverly, MA), Roche Molecular Biochemicals, or Life Technologies, Inc. Oligonucleotides were custom-synthesized by Sigma-Genosys Biotechnologies, Inc. (The Woodlands, TX). All other chemicals, materials, and reagents used in these studies were of the highest grade commercially available and bought from either Aldrich or Sigma.

**Parasite Culture**—*P. falciparum* clones, 3D7 and W2 were maintained as described (16), except that the serum component in the culture medium was replaced with AlbuMAX. 3D7 and W2 are chloroquine-sensitive and chloroquine-resistant field isolates, respectively (17).

**Identification and Isolation of *PfNT1***—*PfNT1* was identified by BLAST searching of TIGR malaria data base for the human equilibrative nucleoside transporter, hENT1 (13), the *Toxoplasma gondii* adenosine transporter, LdNT1 (14), and the *T. cruzi* 2 nucleoside transporter (HydN-2; American Pharmacia Biotech), and probed with oligonucleotide primers *PfNT1* and *PfNT3* used in the Southern blot analysis. Signals were normalized by hybridization to probes corresponding to *P. falciparum* 5 S rRNA: 5'-CAGGGACAAAGGAGTCTAGC-3' (SSS) and 5'-TCCGTTGCTGGTTCCTCC-3' (SSS).

**In Vitro Incorporation of d- and l-Adenosine**—*P. falciparum* clone 3D7 was synchronized twice with sorbitol. The radiolabels D-[2,8-3H]adenosine and l-[2,8-3H]adenosine (50 μCi/ml) were added to the different stages of the parasite culture at 15% parasitemia in 200-μl volumes. Cell pellets were washed twice with 12 ml of cold phosphate-buffered saline and resuspended in 1.0 ml of 5% cold trichloroacetic acid. After precipitation on ice for 30 min and centrifugation at 2900 × *g* for 10 min, cell pellets were lysed for 5 min in 5 volumes of 0.2% saponin prepared in phosphate-buffered saline, and the parasite pellet was then stored at −80°C until use. Total cellular RNA was prepared by the guanidine thiocyanate method (24). RNA was subjected to denaturing agarose electrophoresis, transferred to a nylon membrane (Hybond-N+; Amersham Pharmacia Biotech), and probed with oligonucleotide primers *PfNT5* and *PfNT3* used in the Southern blot analysis. Signals were normalized by hybridization to probes corresponding to *P. falciparum* 5 S rRNA: 5'-CAGGGACAAAGGAGTCTAGC-3' (SSS) and 5'-TCCGTTGCTGGTTCCTCC-3' (SSS).

**Oocyte Expression**—Stage V-VI oocytes were harvested from *X. laevis* and defolliculated with collagenase (Sigma) as described (15, 25). Defolliculated oocytes were incubated at 16°C in Krebs-Ringer saline buffer (96 mM NaCl, 1 mM KCl, 2.5 mM CaCl2, 1.0 mM MgCl2, 5 mM HEPES, pH 7.5) supplemented with 2.5 mM sodium pyruvate, 0.5 mM theophylline, 50 μg/ml gentamycin, and 1.5% horse serum (Life Technologies). The W2 *PfNT1* coding sequence was subcloned into the BamHI/EcoRI sites of the pOG-1 oocyte expression vector that encompasses the 5′- and 3′-untranslated regions of the *X. laevis* β-globin gene (26). The *PfNT1*- encoding plasmid was linearized with NotI, and capped cRNA was synthesized by the mMessage mMachine™ T7 (Ambion, Inc., Austin, TX). Oocytes were microinjected with 5–20 ng of cRNA 1 day after defolliculation.

**Transport Assays in *X. laevis* Oocytes**—Uptake into oocytes of D-[2,8-3H]adenosine (37.5 Ci/mmol), l-[2,8-3H]adenosine (44 Ci/mmol), D-[2,8-3H]thymidine (24.1 Ci/mmol), l-[2,8-3H]thymidine (24.1 Ci/mmol), l-[5-3H]ribose (20 Ci/mmol), l-[6-3H]glucose (31 Ci/mmol), or [8-14C]hypoxanthine (56 Ci/mmol) was determined 3 days post-injection at ambient temperature in un-supplemented Krebs-Ringer saline, pH 7.5. Kinetic determinations were performed at various concentrations of D-[2,8-3H]adenosine (0.38 Ci/mmol), l-[2,8-3H]thymidine (0.31 Ci/mmol), and l-[1,2,8-3H]adenosine (0.60 Ci/mmol). Inhibition profiles were performed at 5 μM l-[2,8-3H]adenosine (3.8 Ci/mmol). Inhibition profiles were performed at 5 μM l-[2,8-3H]adenosine (3.8 Ci/mmol), 500 μM competitor, except for dipiridamole (10 μM), 4-nitrobenzyl-6-thioinosine (NBMPR) (10 and 50 μM), and furasoxide (10, 100, and 500 μM). Uptake experiments were terminated by the removal of oocytes from radiolabeled buffer and washing each oocyte three times in ice-cold Krebs-Ringer saline. Each oocyte was solubilized in 200 μl of 5% SDS, and incorporation of radiolabel was quantified by liquid scintillation spectrometry.
RESULTS

Isolation and Sequencing of PfNT1—The PfNT1 ORF was identified on contig PNG2538 of chromosome 14 after TBLASTN searching of TIGR malaria database with peptide sequences from human and parasite nucleoside transporters as query sequences (13–15). Genomic and cDNA clones corresponding to the PfNT1 sequence in the database were then isolated. PfNT1 from the W2 isolate differed from the 3D7 sequence in the malaria database by a single C→G transition at position 1154 of the PfNT1 ORF. Thus, amino acid 385 is a Phe in the W2 strain and a Leu in the 3D7 line. This Phe-385→Leu substitution was confirmed by sequencing three independent full-length PfNT1 PCR fragments from the W2 strain. The length of the PfNT1 ORF from the ITG2F6 cDNA clone was identical to the genomic PfNT1 clones from 3D7 and W2. There were two sequence differences observed between the 3D7 and ITG2F6 PfNT1 ORFs, a T→C transition at position 107 and a G→A transition at position 385. These alterations translate into Phe-36→Ser and Val-132→Ile in the PfNT1 clone from the ITG2F6 isolate. The ITG2F6 clone was not characterized further.

PfNT1 encodes a polypeptide of 422 amino acids that exhibits significant homology with other members of the equilibrative nucleoside transporter family, including human hENT1 (13) and hENT2 (27, 28), L. donovani LdNT1 (14), and T. gondii TgAT (15) (Fig. 1). PfNT1 by pairwise alignment showed amino acid identities of 36, 31, 34, and 38% with hENT1, hENT2, LdNT1, and TgAT, respectively. However, a multiple sequence alignment revealed only 17 amino acids conserved among the 5 proteins (Fig. 1). No significant sequence similarity to members of the mammalian Na+ dependent nucleoside transporter family (29, 30) or to bacterial nucleoside transporters (31, 32) was observed. Hydropathy plots predicted 11 hydrophobic membrane-spanning domains with a large hydrophilic loop between transmembrane (TM) domains 6 and 7 (Fig. 2). There are 5 potential N-glycosylation sites at Asn227, Asn270, Asn275, Asn295, and Asn304 (Fig. 2). Only Asn270, located between TM segments 7 and 8, is predicted to be within an extracellular loop (Fig. 2).

Molecular Characterization of the PfNT1 Locus—High-stringency Southern blot analysis of P. falciparum genomic DNA digested with restriction endonucleases that cut within (TaqI, NsiI, HindIII, EcoRV, ClaI) the PfNT1 ORF indicated that
PfNT1 is a single copy gene (Fig. 3). This result is consistent with the BLAST search data, which, thus far, reveal the presence of only one PfNT1 gene within the malarial genome. Low stringency Southern blots, however, did reveal other weakly hybridizing bands within P. falciparum genomic DNA (data not shown).

Northern blot analysis with total RNA isolated from different asexual stages of the P. falciparum life cycle suggested that PfNT1 is expressed as a 1.7-kb transcript in all intraerythrocytic stages but is significantly elevated in the early trophozoite stage of the parasite (Fig. 4A). This stage-specific expression was confirmed by reprobing the same blot with the P. falciparum elongation factor 1α (PfEF-1α) and PfEF-1β) that also exhibit elevated expression in the early trophozoite stage (33). To verify that each lane contained equivalent amounts of RNA, the Northern blot was normalized to the 28S rRNA (Fig. 4B).

The elevation of the PfNT1 transcript in the early trophozoite stage correlated with a marked increase in the incorporation of D-[2,8-3H]adenosine into the nucleic acids of P. falciparum parasitized erythrocytes harboring predominantly ring and early trophozoite parasites (Fig. 4B).

Functional Characterization of PfNT1—X. laevis oocytes are highly efficient at translating RNA after microinjection, express only modest levels of nucleoside transport activity, and consequently have been used to study a number of nucleoside systems and uptake mechanisms of nonparasitic origin and by parameters that influence the metabolism and distribution of the nucleoside. The ability of PfNT1 cRNA to induce the uptake of the D-stereoisomers of naturally occurring nucleosides was also evaluated, because previous studies in parasitized erythrocytes implied that adenosine transport was not entirely stereospecific (8, 9). As demonstrated in Fig. 5B, PfNT1 was also capable of transporting both L-adenosine and L-thymidine into oocytes. Rates of incorporation of 0.3 and 0.23 pmol/oocyte/h were obtained for the purine and pyrimidine L-nucleosides, respectively, at concentrations of 1 μM. L-Nucleoside uptake into water-injected control oocytes was negligible. Kinetic analysis of D-adenosine and D-inosine transport activity in oocytes indicated that PfNT1 exhibited Michaelis-Menten kinetics with an apparent Km value of 13.2 ± 4.3 μM for D-adenosine (Fig. 6A) and an apparent Km value of 253 ± 33 μM for D-inosine (Fig. 6B). Km values of 5.7 μM and 103 μM have been reported for adenosine entry into Plasmodium yoelii-(8) and P. falciparum-infected red blood cells (9), respectively. The Km values reported for adenosine uptake into parasitized erythrocytes are complicated by the multiplicity of membrane systems and uptake mechanisms of nonparasitic origin and by parameters that influence the metabolism and distribution of the nucleoside.

The ability of PfNT1 cRNA to induce the uptake of the L-stereoisomers of naturally occurring nucleosides was also evaluated, because previous studies in parasitized erythrocytes implied that adenosine transport was not entirely stereospecific (8, 9). As demonstrated in Fig. 5B, PfNT1 was also capable of transporting both L-adenosine and L-thymidine into oocytes. Rates of incorporation of 0.3 and 0.23 pmol/oocyte/h were obtained for the purine and pyrimidine L-nucleosides, respectively, at concentrations of 1 μM. L-Nucleoside uptake into water-injected control oocytes was negligible. Kinetic analysis of L-adenosine transport activity in PfNT1-injected oocytes suggests that L-adenosine is a low affinity ligand for PfNT1, since uptake is linear at concentrations up to 500 μM (Fig. 7). However, L-adenosine, unlike its D-isomer, is not incorporated into the nucleic acids of intact parasites (Fig. 4B), confirming previous observations made by Upston and Gero (9).

Competition studies established that 5 μM D-adenosine transport was inhibited by a spectrum of naturally occurring purine and pyrimidine nucleosides (Fig. 6A). Transport of D-adenosine (5 μM) was inhibited 88, 87, 79, 82, and 55% by a 100-fold excess of nonradioiodinated adenosine, inosine, guanosine, thymidine, or uridine, respectively. Transport of D-adenosine was only marginally affected by a 100-fold excess of either xanthosine (10%) or cytidine (8%). PfNT1-induced D-adenosine transport was also not inhibited to any significant degree by the purine or pyrimidine nucleobases, hypoxanthine, adenine, guanine, xanthine, cytosine, and uracil. In a separate experiment, no incorporation of 1 μM hypoxanthine could be
detected in PfNT1 cRNA-injected oocytes (data not shown). Additional characterization of PfNT1-mediated adenosine transport revealed that uptake was inhibited by 10 μM dipiridamole (85%) but only marginally, if at all, affected by 10 or 50 μM NBMPR (Fig. 8B). No inhibition was noted with 500 μM antimalarial drug chloroquine, whereas nucleoside analogs such as adenine arabinoside, formycin B, and tubercidin had variable effects, presumably because of different affinities for the transporter (Fig. 8B).

Since the L-stereoisomers of nucleosides are recognized by both PfNT1 and the “new permeation” pathway described by Kirk et al. (10), a number of new permeation pathway ligands were evaluated as potential substrates for PfNT1 by assessing their abilities to impede d-adenosine transport into oocytes. The new permeation pathway substrates, d-ribose, d-glucose, L-alanine, and L-leucine (10), at concentrations of 500 μM only modestly (<30%) impeded d-adenosine (5 μM) uptake capability by PfNT1, as did L-adenosine (Fig. 8B). In contrast to L-adenosine, no uptake of 1 μM d-ribose or d-glucose was detected in PfNT1 cRNA-injected oocytes (data not shown). Finally, furadone, a potent inhibitor of the new permeation pathway (10, 35, 36), at concentrations that inhibit the new permeation pathway (10–100 μM), did not significantly affect the ability of PfNT1 to transport 5 μM d-adenosine in oocytes, although uptake was inhibited by higher concentrations of 500 μM (Fig. 8B).


discussion

The identification of the molecular components of the purine translocation pathways in P. falciparum is a key step toward understanding how these vital nutrients are transported into the parasite. Searches of the malarial genome sequencing project with both human and parasite nucleoside transporter sequences revealed an ORF, designated PfNT1, whose translation product resembles members of the equilibrative nucleoside transporter family (13–15, 27, 28, 34, 37). The isolation and sequencing of both PfNT1 genomic and cDNA clones from the W2 and ITG2F6 strains, respectively, demonstrated that they were virtually identical to PfNT1 of the 3D7 strain in the malaria database. The genomic clone from the W2 strain that was characterized in detail in this study differed from the 3D7 by a single nucleotide transversion that changed PheE85 within TM segment 11 to a Leu residue. This sequence divergence was not PCR-induced since this LeuE85 was confirmed by sequencing three independently amplified PCR fragments. Moreover, the corresponding amino acids in the human hENT1 (13) and hENT2 (27, 28), the L. donovani LdNT1 (14), and the T. gondii TgAT (15) transporters are also Leu residues (see Fig. 1). The ITG2F6 PfNT1 ORF determined from a single cDNA-derived PCR clone also differed in two positions from the 3D7 sequence. Whether these differences were PCR-induced was not substantiated, since this cDNA was not characterized further. The equivalence of both the cDNA and genomic sequences indicates that PfNT1 lacks intervening sequences.

PfNT1 shares a number of conserved residues and has an overall predicted membrane topology consistent with other transporters within the equilibrative nucleoside transporter family (13–15, 27, 28, 34, 37). Of the 17 amino acids conserved among the five nucleoside transporters aligned in Fig. 1, 6 were glycine, 5 were aromatic residues, and virtually all were located within predicted membrane-spanning domains. Six of the conserved amino acids were located within TM segments 7 and 8, implying that these two membrane-spanning domains may be critical for nucleoside recognition, permeation, or both. Despite the sequence divergence among human and parasite nucleoside transporters, the number and location of the TM segments and the large hydrophilic loop between TMs 6 and 7, features common to all these transporter proteins (see Figs. 1 and 2), provide compelling evidence that PfNT1 is a member of the equilibrative nucleoside transporter family.

Functional characterization of PfNT1 within the Xenopus oocyte system suggests that it has a broad substrate specificity, mediating the Na+-independent uptake of both purine and pyrimidine nucleosides but not nucleobases (Figs. 5–8). D-Adenosine thus far appears to be the preferred substrate, with a K_m value one order of magnitude lower than that for d-inosine (Fig. 6). The substrate specificity of PfNT1 is similar to that of hENT1 (13), the nucleoside transporter of the human erythrocyte (13, 38), with two notable exceptions. First, PfNT1 exhibits
low sensitivity toward NBMPR, to which hENT1 is extraordinarily sensitive (38), although PfNT1 is inhibited by another hENT1 inhibitor, dipyridamole (38) (Fig. 8B). Second, PfNT1 exhibits a novel stereo-promiscuity, transporting both D- and L-stereoisomers of purine and pyrimidine nucleosides (Fig. 5), whereas hENT1 is stereospecific for D-nucleosides (8, 9, 39). The substrate specificity and inhibition profile ascribed to PfNT1 is most akin to the *T. gondii* TgAT transporter, since TgAT is a nucleoside transporter of broad specificity that shares a common susceptibility to dipyridamole and resistance to NBMPR (15). The stereospecificity of TgAT, however, has not been ascertained. Indeed, the ability of PfNT1 to recognize L-adenosine and other L-nucleosides is, thus far, peculiar to the malaria parasite, since L-adenosine is poorly transported, if at all, by uninfected erythrocytes, a number of cultured mammalian cells, or a variety of other protozoan parasites, including *Crithidia luciliae*, *Trichomonas vaginalis*, and *Giardia intestinalis* (39). Although L-adenosine is a ligand for PfNT1, it is apparently not incorporated into nucleic acids in *P. falciparum* (Fig. 4B) (9).

The uptake of nutrients from the human host into *P. falciparum* requires transport across a multiplicity of membranes and appears to involve a variety of complex and novel components, including transporters (12, 40), channels (10), ducts (41), and the TVM (11), several of which have been implicated in nucleoside permeation. The role of each of these pathways in nucleoside permeation has been difficult to dissect, because they operate simultaneously in intact parasites and because of the heretofore lack of molecular reagents and heterologous expression systems to functionally evaluate individual components of the nucleoside transport pathway. These nucleoside translocation mechanisms include the new permeation path-
way proposed by Kirk et al. (10). This pathway exhibits non-saturable and nonspecific channel-like activity that is inhibited by the anion transport inhibitor furosemide and is capable of recognizing D- and L-nucleosides as well as certain amino acids, sugars, cations, and vitamins (10, 35, 36). However, PfNT1 is biochemically distinct from this pathway, despite the fact that both recognize L-nucleosides, because the transporter is clearly saturable, does not transport neutral substrates of the channel such as D-glucose, D-ribose, and neutral amino acids, and is not inhibited by furosemide at concentrations of 10–100 μM that inhibit the channel (10, 35, 36). A second nucleoside permeation pathway recently described for P. falciparum is the TVM (11). However, nucleoside uptake by the TVM exhibits distinct biochemical properties from PfNT1, and the membraneous network of the TVM appears to develop in the later intraerythrocytic stages, whereas PfNT1 is maximally expressed in the early trophozoite stage (Fig. 4A). Finally, a duct for macromolecules that bypasses the erythrocyte membrane of infected red blood cells has been proposed (41). PfNT1 can be distinguished from this nonendocytic pathway both by its specificity for small molecules and its ability to function outside the erythrocyte.

Whether PfNT1 is the primary saturable adenosine transport mechanism that is observed in intact P. falciparum parasites is unknown. Analysis of nucleoside transport in both P. falciparum-infected human (7, 9) and P. yoelii-infected mouse (8) erythrocytes, although complicated by the multiplicity of membranes of both mammalian and parasite origin, indicates that infection with malaria parasites induces an NBMPR-insensitive, stereo-nonspecific adenosine transport activity that parallels the properties of PfNT1 in the heterologous oocyte expression system. As yet, no other closely related sequences have been uncovered in the TIGR P. falciparum data base, although low stringency Southern blot analysis suggested that other sequences in the P. falciparum genome exhibit limited homology to at least some portion of PfNT1 (data not shown).

By contrast, many other protozoan parasites express multiple nucleoside transporters: L. donovani express two nucleoside transporters of nonoverlapping substrate specificity, an adenosine-pyrimidine transporter and an inosine-guanosine transporter (14, 43); T. brucei possess a multiplicity of nucleoside transporters, one of which transports a spectrum of purine nucleosides (34, 44) and the other of which recognizes adenosine, adenine, and several antityranosomal drugs (37, 44, 45); and T. gondii, a related apicomplexan parasite, express at least two nucleoside transporters, one that recognizes adenosine and other purine nucleosides and a second distinct inosine transporter that remains to be characterized (15, 46). Whether PfNT1 is the only mechanism for purine (or even nucleoside) translocation into P. falciparum awaits the construction and phenotypic characterization of Δpft1 knockout parasites by targeted gene replacement techniques (17, 47). Analogous tar-

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**Fig. 6.** PfNT1-mediated transport kinetics. Panels A and B, uptake of [2, 8-3H]adenosine (specific activity 0.375 Ci mmol−1) or [2, 8-3H]inosine (specific activity 0.075 Ci mmol−1) by PfNT1-injected oocytes (10 ng/oocyte) was determined over a 60-min time period for a range of substrate concentrations (either 5–110 μM for D-adenosine or 0.5–2 mM for D-inosine). The rate of uptake was determined at each substrate concentration by linear regression analysis. The results, represented as a Hanes analysis, are expressed as either adenosine (μM) versus adenosine (μM)/rate of uptake (pmol/min/oocyte) or inosine (μM) versus inosine (μM)/rate of uptake (pmol/min/oocyte).

**Fig. 7.** PfNT1-mediated uptake of L-adenosine. Uptake of L-[2, 8-3H]adenosine (specific activity 0.088 Ci mmol−1) by PfNT1-injected oocytes (10 ng/oocyte) was determined over a 60-min time period for a range of substrate concentrations (0.01–0.5 mM). The rate of L-adenosine uptake was determined at each substrate concentration by linear regression analysis. The results are expressed as L-adenosine (μM) versus mean rate of uptake (pmol/min/oocyte).

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*N. S. Carter, M. E. Drew, M. A. Sanchez, S. M. Beverley, S. M. Landfear, and B. Ullman, manuscript in preparation.*
there is evidence for the insertion of malarial proteins into the plasma membrane of the parasitized erythrocyte (48–50). The cellular milieu of PNT1 is also important from a therapeutic perspective. Although PNT1 remains to be validated therapeutically, the unique substrate specificity of the malarial nucleoside transporter implies that it might be a novel target for therapeutic manipulation of \textit{P. falciparum} and other types of malaria. Indeed, several L-isomers of toxic nucleoside analogs have demonstrable antimarial activity against \textit{P. falciparum in vitro} (42). The PNT1 clone, the first molecular component of the complex nucleoside permeation pathway to be identified, isolated, and characterized, as well as a functional heterologous expression system for PNT1 facilitates the prerequisite pharmacological studies that ultimately may validate PNT1 as a chemotherapeutic target for the treatment of malaria.

\textbf{Acknowledgments—}We are grateful to Anna Oksm and Ilya Gluzman for help with parasite cultures and to Rachel Dresbeck for critical reading of the manuscript. Preliminary sequence data for \textit{P. falciparum} chromosome 14 was obtained from The Insitute for Genomic Research website. Sequencing of chromosome 14 was part of the International Malaria Genome Sequencing Project and was supported by awards from the Burroughs Welcome Fund and the U. S. Department of Defense.

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Nicola S. Carter, Choukri Ben Mamoun, Wei Liu, Edilene O. Silva, Scott M. Landfear, Daniel E. Goldberg and Buddy Ullman

*J. Biol. Chem.* 2000, 275:10683-10691.
doi: 10.1074/jbc.275.14.10683

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