A New Type of High Affinity Folic Acid Transporter in the Protozoan Parasite Leishmania and Deletion of Its Gene in Methotrexate-resistant Cells*

The protozoan parasite Leishmania is a folate auxotroph and thus depends on the uptake of folate from the environment to meet its folate requirement. We show here that Leishmania contains several putative pteridine transporter genes. Some of these genes are deleted in methotrexate-resistant Leishmania cells where there is no measurable uptake of methotrexate. Transport studies suggest that Leishmania has more than one active folate transporter, and one of these, named FT5, corresponds to a very high affinity folate transporter ($K_m$, 84 nM). The uptake of both folate and methotrexate was impaired in an FT5 null mutant at low substrate concentrations (50 nM), although transport properties at higher concentrations (1000 nM) were not statistically different between wild-type and the FT5 null mutant. Modulation of the expression of FT5 also changes the susceptibility of Leishmania cells to methotrexate. These results have permitted the characterization of a novel class of folate transporters and suggest that the parasite Leishmania has several gene products possibly transporting folates and related molecules under varying conditions.

The protozoan parasite Leishmania is distributed worldwide and causes a variety of clinical symptoms ranging from self-healing cutaneous lesions to visceral infections that are usually fatal if left untreated (1). The main chemotherapeutic regimen consists of pentavalent antimonials, but resistance to this class is fatal if left untreated (1). The main chemotherapeutic regimen consists of pentavalent antimonials, but resistance to this class is prevalent in several endemic areas (2). New targets are urgently required. One metabolic pathway of antiquated drugs is now prevalent in several endemic areas consists of pentavalent antimonials, but resistance to this class (3). Antifolates have been used successfully as anticancer drugs (methotrexate) or in the treatment of bacterial (trimethoprim) or parasitic infections (pyrimethamine) (4). No effective antifolate chemotherapy has yet been established against infections with the protozoan parasite Leishmania. Nevertheless, many distinct features in the folate metabolism of this organism have been identified that could prove to be useful therapeutic targets (5, 6).

Our understanding of folate metabolism in Leishmania is derived mostly from work carried out on studies related to the mechanisms of resistance to the model antifolate drug methotrexate (MTX). Leishmania is sensitive to MTX, and step by step selection of mutants for MTX resistance has revealed a multiplicity of resistance mechanisms that have been permitted to pinpoint novel biochemical pathways. Amplification of the gene coding for the target DHFR (DHFR in Leishmania is a bifunctional enzyme where it is fused to thymidylate synthase) was shown to contribute to MTX resistance (7–11). A second locus, known as the H locus, was also found to be frequently amplified in MTX-resistant mutants. The gene present on this locus encodes for the pterin reductase PTR1 (12, 13) that can reduce both pterins and folates and produce MTX resistance by bypassing the need for DHFR (14–16). This discovery further linked folate and pterin metabolism in Leishmania. Indeed, it is well established that pterins can have a folate sparing effect permitting Leishmania cells and related parasites to grow in folate-deficient medium (17–21). It remains to be demonstrated, however, whether Leishmania can synthesize folates de novo, but Leishmania promastigotes in culture rely heavily on uptake systems for meeting their folate needs. A folate transport activity has been studied biochemically in Leishmania, but the gene and proteins involved have yet to be characterized at the molecular level. It consists of a saturable active transport system recognizing both folate and MTX with $K_m$ values ranging from 250 to 700 nM depending on the species (22–24). The transport activity is regulated according to cellular growth with maximum activity in the logarithmic phase (25).

Another frequent mechanism of MTX resistance in Leishmania is reduced accumulation of the drug (9, 23, 26, 27). This reduced uptake of MTX is paralleled by a marked decrease in folic uptake, suggesting that the expression of the common folic/MTX transporter is strongly down-regulated in MTX-resistant Leishmania. Given that Leishmania is a folate auxotroph, this brings up the question of how the resistant para-

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site can meet its folate requirements. An answer was found for *Leishmania tarentolae* in which the biotinier transporter *BT1* (24, 28) was found to be overexpressed in all MTX-resistant cells with a markedly reduced folate uptake. Sufficient folates (but not MTX) could be transported through *BT1* to meet the folate requirement of the cell (24). Here we show that *BT1* is part of a large gene family and that some of these genes are consistently deleted in cells in which MTX uptake was abrogated. The molecular and biochemical analyses of one of these genes indicate that it corresponds to a high affinity folate transporter.

**EXPERIMENTAL PROCEDURES**

*Leishmania Growth*—The *L. tarentolae* cell line *TarII* WT has been described previously (29). *Leishmania* cells were grown in SDM-79 or M199 medium supplemented with 5 or 10% heat-inactivated fetal bovine serum, respectively, and 5 mg/ml hemin. The *L. tarentolae* MTX-resistant mutants *TarII* MTX1000.3 to *TarII* MTX1000.7 have been described previously (26). *L. tarentolae* promastigotes were transfected by electroporation as reported previously (12).

**DNA Manipulations**—Total DNA was isolated using DNAzol reagent (Invitrogen). Southern blotting, hybridization, and washing conditions were performed following standard protocols (30). The probes containing coding sequences of the *FT* gene family were obtained by PCR. The sequences of primers 1–4 (see Fig. 2) are 1, AGTCTAGAGCAGCACTCTTACCTCTGC; 2, ATCGGATCCTGCGCACGGACAGATTAACC; 3, TCAATCAGAGGTGCCGCAGC; and 4, CTTTTGGGCATACACGCGCTTACCTCTGC.

**Generation of the FT Expression Vectors**—The *FT* genes were obtained from a cosmid derived from an *L. tarentolae* genomic DNA library (24), hybridized with a 500-bp *SalI-EcoRI* FT probe that was first isolated from a cosmid using a *BamHI* probe. A 2.3-kb *SphI-PstI* fragment containing the *L. tarentolae* *FT3* was subcloned in the *pSL1180* vector. The resulting construct was then digested by *BamHI* which cuts in the vector multiple cloning site and at the 3′ end of *FT3* after the stop codon. This fragment was then inserted into the *Leishmania* expression vector pSP72aNeo (31). The 3.2-kb *pSL72aNeo* construct was then subcloned into pBluescript II KS (Stratagene) and used in directed cloning. This fragment was subcloned into *pSL1180* vector (32).

**Generation of an FT5-null Mutant**—A 3.2-kb DNA fragment containing the *L. tarentolae* *FT5* gene (2.1-kb) with flanking regions of about 550 bp on each side of the gene was amplified by PCR with primers 1 and 2 (see Fig. 2) containing a *BamHI* and an *XbaI* site, respectively, for directed cloning. This fragment was subcloned into *pH9251* (Stratagene). An EcoRV digest of the resulting construct was made to delete 1.4 kb of the *FT5* coding sequence and replaced by a hygromycin B phosphotransferase expression cassette (HYG) derived from pSPY-HYG (31). This 3.2-kb *BamHI-XbaI* fragment was used for disrupting one *FT5* allele by homologous recombination. An *L. tarentolae* *FT5* null mutant was obtained by selection for loss of heterozygosity (33) by increasing the hygromycin B selection pressure to 400 ng/ml and cloning of the cell pool. *FT5* null mutants were characterized by Southern blot and PCR analyses.

**DNA Sequence Analysis**—DNA sequencing was done on an Applied Biosystems 377 automated sequencer. Analysis of the sequence was performed using the GCG software package (Genetics Computer Group) and Clone Manager 5.0. The nucleotide sequences reported here will appear in the GenBank sequence data base under the accession numbers AY099959 and AY099960.

**Peridine Transport Experiments**—Transport experiments were performed as described previously (24). Tritium-labeled pteridines ([3H]folate (19.6 Ci/mmol) and [3H]MTX (21.2 Ci/mmol) were purchased from Moravek Biochemicals. Transport studies were carried out using various concentrations of radioactive pteridines (10 nM to 5 μM) to determine apparent affinity constant (*Kₐ*). The quantity of accumulated radioactivity was normalized with *Leishmania* cell numbers. To measure folate and MTX transport, the uptake of cells incubated on ice was not subtracted.

**RESULTS**

*Gene Deletion in Methotrexate-resistant L. tarentolae—Leishmania* cells selected for MTX resistance have numerous mutations including transport defects for MTX and folate. Two types of transport defects have been observed. In some mutants, the defect in transport consists of an ~5-fold reduction of both MTX and folate (9, 26), whereas in another class of mutant the uptake of MTX is not measurable (23, 26). The *L. tarentolae* MTX 1000.4 mutant is a representative of the first class of mutants, whereas the mutant MTX 1000.6 has no measurable MTX uptake (Fig. 1A).

In *L. tarentolae*-resistant cells in which MTX uptake cannot be measured, we have shown that the biotinier transporter *BT1* is overexpressed thereby permitting a small amount of...
folate to enter the cell (24). We had noticed that even at relatively high stringency, a B'T1 probe was hybridizing to fragments other than B'T1 (24), suggesting that the biotin transporter is part of a multigene family. We isolated a cosmid cross-hybridizing to the B'T1 gene, and when we used a 500-bp SalI-EcoRI fragment derived from this cosmid as a probe, we could indeed observe multiple hybridizing bands, indicating that this fragment probe is part of a multigene family (Fig. 1B, lane 1). Some fragments hybridized more strongly than others suggesting that several gene copies could comigrate. This multigene family is not only present in L. tarentolae but also in all other Leishmania species tested (data not shown). Because BT1 can transport folate (24), we hypothesized that one of the hybridizing fragments shown in Fig. 1B could correspond to a folate transporter. The total DNA of various independent L. tarentolae MTX-resistant mutants was hybridized to this 500-bp probe. In the mutants MTX 1000.4 and MTX 1000.5, which have only a partial reduction in folate and MTX transport (Fig. 1A, and not shown), we did not notice any change in the copy number of members of the gene family (Fig. 1B, lanes 2 and 3). Most interestingly in three mutants L. tarentolae MTX 1000.3, MTX 1000.6, and MTX 1000.7, in which MTX uptake was greatly impaired (26), we observed the complete absence of a 3.0-kb SalI-SalI hybridizing band, whereas the intensity of the 2.4- and 6-kb SalI-SalI fragments diminished extensively (Fig. 1B, lanes 4–6). In the latter cases this may correspond to hemizygous deletions, or if the fragments of more than one gene comigrate deletion of one gene will lead only to a decrease in the hybridization signal.

To test whether one of the deleted bands could potentially correspond to a folate transporter, we further characterized cosmids 2B, which contains a 8.6-kb BamHI-BamHI fragment harboring both the 3.0- and 2.4-kb SalI-SalI fragments that appear to be deleted in some of the MTX-resistant mutants (Fig. 2). The nucleotide sequence of the 8.6-kb BamHI-BamHI fragment revealed two open reading frames of about 2 kb each resulting in predicted proteins of 678 and 721 amino acids. These two ORFs were 58% identical to each other and 39% and 40% identical to the Leishmania BT1 (Fig. 3). A search of data banks revealed that the only homologues of the sequenced genes were found in Leishmania and in the related parasite Trypanosoma brucei. At least three homologues were found in T. brucei. One corresponds to the expression site associated gene ESAG10 (34), and the two others consist of ORFs found on chromosomes 1A and VIII of the ongoing T. brucei genome project (GenBank™ accession numbers GB_HTG: TBBCHRIA_05, and AC092212). In Leishmania, in addition to BT1, several other homologous sequences have been identified. In Leishmania donovani there are a series of six putative pteridine transporters (FT1 to FT6; GenBank™ accession numbers AAD52046, AAD52047, AAD52048, AAD52049, AAD52050, and AAD52051) and sequences found in chromosomes 4, 14, and 34 of the Leishmania major genome (GenBank™ accession numbers AL389894; AL358652; GB_HTG: LMFCHR34_14) were similar to the genes sequenced. Sequence comparison and ClustalW analysis indicated that one of the two ORF (GenBank™ accession number AY099959) is closely related to a cluster of L. donovani sequences consisting of FT1, FT3, and FT4 (82% identity with FT3), and the other ORF (GenBank™ accession number AY099960) is highly similar to FT5 (79% identity), and we have thus retained the nomenclature for FT5 and have tentatively given the name FT3 for the other gene product derived from cosmid 2B of L. tarentolae. The L. tarentolae gene corresponding to FT5 contains the 3.0-kb SalI-SalI fragment that is completely absent in the MTX-resistant mutants TarII MTX1000.3, 1000.6, and 1000.7 (Fig. 1B). The FT3 gene contains a 2.4-kb fragment (Fig. 2), and whereas the hybridization intensity of a 2.4-kb fragment decreases in MTX-resistant mutants (Fig. 1B), it is not certain whether this is because of deletion of FT3 or another gene. The family of transporter exemplified by FT5 is predicted to contain 12 putative transmembrane domains as determined by the Kyte-Doolittle hydropathy profiles but otherwise are not closely related to other families of transport proteins. Neither FT3 nor FT5 show sequence similarity with the reduced folate carrier of mammals (35) or folate-binding proteins (36–39), suggesting that if one of these gene products indeed corresponds to a folate transporter, it may constitute a novel type of transport proteins.

**FT5 Is a High Affinity Folate Transporter**—To test whether FT3 or FT5 were capable of transporting folates, we subcloned the two genes in Leishmania expression vectors and transfected them into L. tarentolae MTX 1000.6 in which folate and MTX uptake are greatly impaired (Fig. 1A and Fig. 4). The steady-state accumulation of [3H]MTX and [3H]folic acid were thus measured in these transfectants. Under the conditions tested, we could not observe any increase in either folate or MTX uptake in the FT3 transfectant (data not shown). However, the FT5 transfectant exhibited a clear increase in folate and MTX transport (Fig. 4). The level of accumulation in the TarII MTX1000.6–FT5 transfectant is only about 10% that of wild-type cells, suggesting that FT5 is not the sole folate transporter. The apparent Km of this FT5 transporter for folate and methotrexate was calculated using a double-reciprocal Lineweaver-Burk plot and found to be lower than the conventional Km measured for folate and methotrexate uptake in Leishmania wild-type cells (Table 1). This may suggest that FT5 corresponds to a high affinity folate transporter. To test this hypothesis further, we generated an L. tarentolae FT5 null mutant. To generate an FT5 null mutant, we made a targeting construct to replace most of the coding region of FT5 by an hygromycin phosphotransferase (HYG) expression cassette (Fig. 2). This cassette, containing ~550 bp of flanking intergenic sequences on each side of the deleted FT5 gene necessary for homologous recombination, was electroporated in L. tarentolae wild-type cells. Transfectants growing in the presence of hygromycin B were analyzed by Southern blots. In wild-type cells, an FT5 probe recognizes an 8.6-kb BamHI-BamHI fragment (Fig. 5A, lane 1). Because FT5 is part of a gene family, other hybridizing bands are also observed. The integration of the HYG cassette also introduces a unique EcoRI site (Fig. 2). A BamHI-EcoRI digest would lead to new 3.1- and 5.2-kb frag-
ments being hybridized by the probe. This is exactly what we have observed by analyzing one cloned transfectant (Fig. 5A, lane 2). *Leishmania* is a diploid organism, and thus for a null mutant, two alleles need to be disrupted. Loss of heterozygocity can be achieved by increasing the concentration of the selective drug hygromycin B (24, 33). As expected, the hybridization intensity of the 3.1- and 5.2-kb bands corresponding to the inactivated *FT5* increased in a cloned transfectant subjected to high hygromycin B selection (Fig. 5A, lane 3), suggesting that the second allele was inactivated. Nonetheless an 8.6-kb fragment at the size of the wild-type *FT5* allele hybridized to an *FT5* probe, suggesting either that another homologous gene

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**FIG. 3.** Predicted amino acid sequences of the two putative *L. tarentolae* folate transporters FT3 and FT5. The sequence of the transporters available under GenBank™ accession number AY099959 and AY099960 were compared with the *L. mexicana* bipterin transporter BT1 (24). Identical amino acids are in black, and conservative amino acids are shaded. FT3 and FT5 are 58% identical, and FT3 and FT5 are 39 and 40% identical to BT1, respectively.
was comigrating with FT5 or that the Leishmania recombinant strain became triploid for FT5. To discriminate between these two possibilities, we carried out PCRs using primers 3 and 4 (Fig. 2) that can distinguish between an intact copy of FT5 and a disrupted one. PCR amplification of the wild-type allele should lead to a 2.1-kb fragment, whereas the deleted gene with the HYG cassette should give rise to a 1.9-kb PCR fragment. PCR amplification of the wild-type DNA with primers 3 and 4 indeed lead to a 2.1-kb PCR fragment (Fig. 5B, lane 1), whereas the heterozygous parasites gave, as expected, 2.1- and 1.9-kb fragments representing the wild-type and deleted allele, respectively (Fig. 5B, lane 2). Finally, a single 1.9-kb fragment was amplified in the heterozygous FT5 mutant submitted to loss of heterozygocity, demonstrating the disruption of both FT5 alleles and the generation of an FT5 null mutant.

The availability of an FT5 null mutant has enabled further studies on its role in folate transport. We would have expected that less folate would enter the cells in the mutant. When we used 1 µM of either folate or MTX, we did indeed observe a reduction in the uptake of both substrates, but this was not statistically different from wild-type cells (Fig. 6, A and B). In contrast, at a lower substrate concentration of 50 nM, there was a clear reduction in the uptake of both folate and MTX by the FT5 null mutant compared with wild-type cells (Fig. 6, C and D). This further supports other results suggesting that FT5 is a high affinity folate transporter (Table I).

**FT5 and Its Role in Antifolate Resistance**—The mutant TarII MTX 1000.6 is highly resistant to MTX and is associated with a marked reduction in the uptake of the drug. FT5 is deleted in this mutant (Fig. 1B), and re-introduction of FT5 in this mutant increases the transport of both folate and MTX (Fig. 4). Because MTX uptake is increased in the MTX 1000.6-FT5 transfectant, we would expect a concomitant increase in the MTX sensitivity of the mutant. The FT5 transfectant was indeed found to be much more sensitive to MTX than the mutant, but the reversion was not to wild-type levels (Fig. 7A), suggesting that further mechanisms of resistance possibly including other transport mutations have occurred in this mutant. In the case of the FT5 null mutant, we could have expect a decrease in MTX susceptibility. However, when cells were grown in SDM-79, a medium with a high concentration of folates (15.4 µM), we could not observe a difference in MTX susceptibility between wild-type cells and the FT5 null mutants (Fig. 7B). Because the role of FT5 appears to be more important under limiting concentrations of folate (Fig. 6), we grew the wild-type cells and the FT5 null mutant in M199, a medium much poorer in folates (22.7 nM). As observed previously, the IC50 value of MTX in Leishmania cells is highly dependent on the folate concentration of the medium (5, 40), and indeed, L. tarentolae wild-type cells were 60 times more sensitive to MTX than in M199 (15.4 µM) and 40 times more sensitive to MTX than in SDM-79 (22.7 nM). As observed previously, the IC50 value of MTX in Leishmania cells is highly dependent on the folate concentration of the medium (5, 40), and indeed, L. tarentolae wild-type cells were 60 times more sensitive to MTX than in M199 than in SDM-79 (Fig. 7, B and C). Interestingly, the FT5 null mutant was hypersensitive to MTX in M199 medium with an IC50 of 10 nM (Fig. 7C). To prove that the effect observed was due to a difference in folate concentration, we added folate acid to M199 medium to reach the concentration found in SDM-79. Under these conditions of high folate concentration, we did not observe a significant difference in MTX susceptibility between the wild-type cell and the FT5 null mutant (not shown).

**DISCUSSION**

In recent years, a number of genes involved in pterin and folate metabolism have been isolated in Leishmania (5, 6). The bioppterin transporter BT1 was recently characterized with regard to both substrate affinity and specificity (24) and its role in parasite physiology (41, 42). The BT1 gene appears important for intracellular parasite survival (43). BT1 appears to be the sole bioppterin transporter in Leishmania but can also transport folate although with a much lower affinity than the Leishmania folate transporter (24). Through this work we have pinpointed a novel large gene family, with at least one member transporting folate. At least nine hybridizing Sall/Sall fragments of various intensities could be observed (Fig. 1B). Data bank searches indicated that, in addition to BT1, six complete homologous open reading frames were found in L. donovani, and to date three L. major chromosomes (chromosome 4, 14, and 34) whose sequences are currently being completed also carry sequences that are related to the folate transporter gene family. It is not known yet whether all these genes are active or if some correspond to pseudogenes. Most strikingly, some members of this gene family appear to be deleted in MTX-resistant Leishmania cells having no measurable MTX uptake (Fig. 1B, lanes 4–6). We assume that one of these genes could likely correspond to a folate transporter. It is nonetheless worth noting that two mutants, with a 5-fold decrease in MTX uptake, appear to have an intact set of FT gene homologues (Fig. 1B,
lanes 2 and 3). Thus, in mutants TarII MTX1000.4 and 1000.5, the transport defect could be related to either point mutations or to the modulation of RNA expression in one of the FT genes. It is also possible that the transport defect in these two mutants was not related to this newly described FT gene family.

To test whether one of the deleted genes in the mutant TarII MTX1000.6 corresponds to the folate transporter, we carried out gene transfection and gene deletion events. Our work on FT5 has shown that it corresponds to a high affinity (Fig. 6 and Table I) but relatively low capacity (Fig. 4) folate transporter. FT5 is therefore not the primary folate transporter of Leishmania, and possibly another related gene could correspond to this transporter. We currently think that the SaI-SalI band at 2.4 kb (Fig. 1B) represents at least a doublet, one band corresponding to FT3 (Fig. 2) and the other possibly to the main folate transporter. Work is in progress to clone this gene part of the family. Leishmania is a pteridine auxotroph, and pterins such as biopterin have long been known to be essential growth factors (6) although their exact role remains unclear. One role appears to be in parasite differentiation (41). Due to the importance of pteridines in Leishmania growth, it is understandable that this parasite has evolved a number of redundancies in pteridine transporters. BT1 preferentially transports biopterin; FT5 corresponds to a high affinity folate transporter, whereas at least one more transporter of folic acid is present as indicated by the observed folate uptake in the FT5 null mutant (Fig. 6). Leishmania cycles between sand flies and host macrophages, and through various steps of its life cycle, the parasite may encounter a folate-poor environment, thereby needing a high affinity folate transporter such as FT5. Leishmania and possibly the African trypanosome appear to have several more genes related to the BT1-FT family. Some of these products could have the ability to transport different types of pteridines, or perhaps multiple genes are required because differential expression is required at discrete periods during the parasite life cycle. The generation of gene-specific probes should permit us to study the expression of individual genes along the Leishmania life cycle. The uptake of folate is indeed known to be dependent on the growth phase of the parasite (22, 25). FT5 would be the first non-mammalian folate transporter characterized in such detail. In addition to mammalian systems (reviewed in Refs. 44–46), folate transport activities have been reported in Xenopus (47) and in bacteria (48, 49), but the gene products responsible for these activities have not yet been isolated in non-mammalian systems.

The FT5 gene is deleted in L. tarentolae mutants selected for MTX resistance, suggesting that it may be implicated in resistance. As expected, transfection of this gene in the L. tarentolae MTX1000.6-resistant mutant partially reverts resistance because increased MTX uptake will sensitize cells to this inhibitor (Fig. 7A). We could have expected that a null mutation of FT5 would lead to resistance. However, this was not observed in SDM-79 (Fig. 7B), and under limited folate concentration it led to hypersensitivity (Fig. 7C). This is nonetheless consistent with the idea that there may be other genes involved in resistance.
with FT5 being a high affinity folate transporter. Indeed, in folate-rich medium most folates enter the cell independently of FT5, whereas in folate-poor medium the role of FT5 is much more important. Because MTX susceptibility was modulated very effectively by folate concentration in *Leishmania* (5, 40), it does make sense that an FT5 null mutant becomes more sensitive to MTX in a folate-poor environment. Indeed, because less folates enter the cells, it cannot compete as effectively with MTX. The mutants were selected in the folate-rich SDM-79 medium, and thus the deletion of FT5 should be more neutral in term of MTX susceptibility (Fig. 7B). We think that FT5 was possibly codeleted with another member of the PT gene family, and the deletion of both genes contributes to the resistance phenotype in TarII MTX1000.6. Indeed, in addition to FT5 a number of other fragments hybridizing to an FT probe are deleted (Fig. 1B). DNA deletion in *Leishmania* occurs exclusively by homologous recombination (50, 51), and this recombination could have occurred between the conserved sequence of two members of the FT family that are physically linked.

In conclusion, we have described a novel gene product in *Leishmania* that corresponds to a high affinity folic acid transporter. Moreover, several related transporters are also present in *Leishmania* that could potentially transport various pteridine substrates under a variety of conditions. *Leishmania* is a successful parasite that is a pteridine auxotroph and has thus needed to develop a complex pathway of pteridine transporters and salvage enzymes. FT5 is part of a novel family of 12 putative transmembrane domain proteins, and the uniqueness of the pteridine transporters family in *Leishmania* opens the possibility to generate specific inhibitors.

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![Fig. 7](image_url)

**Fig. 7.** Effects of modulation in the expression of FT5 on methotrexate susceptibility. Susceptibility of *L. tarentolae* MTX1000.6 cells transfected with FT5 in SDM-79 medium (A). Susceptibility of the FT5 null mutant to methotrexate in the folate-rich medium SDM-79 (B) and in M199 medium (C). ■, TarII MTX1000.6; □, TarII MTX1000.6 transfected with FT5; ●, TarII wild-type cell; ○, TarII FT5 null mutant.
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