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Comparative folate metabolism in humans and malaria parasites (part II): activities as yet untargeted or specific to *Plasmodium*

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Abstract

The folate pathway represents a powerful target for combating rapidly dividing systems such as cancer cells, bacteria and malaria parasites. Whereas folate metabolism in mammalian cells and bacteria has been studied extensively, it is understood less well in malaria parasites. In two articles, we attempt to reconstitute the malaria folate pathway based on available information from mammalian and microbial systems, in addition to *Plasmodium*-genome-sequencing projects. In part I, we focused on folate enzymes that are already used clinically as anticancer drug targets or that are under development in drug-discovery programs. In this article, we discuss mammalian folate enzymes that have not yet been exploited as potential drug targets, and enzymes that function in the *de novo* folate-synthesis pathway of the parasite – a particularly attractive area of attack because of its absence from the mammalian host.

Folate enzymes

Folate derivatives (FDs) are important cellular cofactors involved in supplying one-carbon (C1) units for three major metabolic pathways: the biosynthesis of (i) methionine, (ii) purines and (iii) pyrimidines; pathways (ii) and (iii) are essential for DNA generation. A C1 unit is also required for the initiation of protein synthesis in mitochondria through formylation of methionine. Rapidly dividing cells such as tumors, bacteria and malaria parasites rely heavily on the availability of FDs for their growth. Thus, the inhibition of enzymes involved in these processes greatly affects cell division, through inhibition of DNA and protein synthesis. This feature has been exploited for the development of antifolate drugs against cancer cells and microbial infections, including malaria.

Folate is a generic term that comprises nine FDs – folic acid (FA), dihydrofolate (DHF), tetrahydrofolate (THF), 5,10-methenyltetrahydrofolate (5,10-CH^+-THF), 5,10-methylenetetrahydrofolate (5,10-CH_2-THF), 5-methyltetrahydrofolate (5-CH_3-THF), 5-formyltetrahydrofolate (5-CHO-THF), 10-formyltetrahydrofolate (10-CHO-THF) and 5-

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formiminotetrahydrofolate (5-NH=CH-THF) – that are found in mammalian and microbial cells [1]. In this article, we discuss the mammalian folate enzymes that have not yet been exploited as targets for drug discovery and their status in malaria parasites, in addition to enzymes of the *de novo* folate-synthesis pathway, which are not found in mammalian cells. As in part I [1], we have exploited malarial, bacterial and yeast genome information to identify putative malaria candidate enzymes that have not yet been described. The aim of collating this information is to provide a realistic and useful model of the likely composition of the malaria folate pathway and a firmer basis for future evaluation of potential drug targets.

Folate enzymes not yet targeted in cancer studies and their status in *Plasmodium*

The folate enzymes described in this section have not yet been tested as potential targets in tumor cells. Although most of these enzymes have regulatory roles in folate metabolism, some are involved in the synthesis and metabolism of amino acids (methionine, glutamate and histidine), making them good potential targets for drug development. We consider their role in mammalian metabolism and the available evidence of their existence in *Plasmodium*.

Methylenetetrahydrofolate reductase and methionine synthase

Methylenetetrahydrofolate reductase (MTHFR) (EC 1.5.1.20) mediates one of the most important reactions in folate metabolism: synthesizing 5-CH_3-THF from 5,10-CH_2-THF (reaction 13 in Figure 2 of Ref. [1]). Thereafter, the methyl group of 5-CH_3-THF is transferred by methionine synthase (MS) (EC 1.16.1.8) to homocysteine, to generate methionine (reaction 14 in Figure 2 of Ref. [1]). These reactions are the sole source of methionine, which, besides its other roles, functions as the precursor of *S*-adenosylmethionine, the methyl-group donor in ~100 reactions [2]. MS from humans (among other organisms) requires a form of vitamin B12 (cyanocobalamin) as a cofactor to which the methyl group is first transferred from 5-CH_3-THF to form methylcyanocobalamin, before transfer to homocysteine. Deficiency of MTHFR activity, as a result of point mutations in its gene, is associated with a decrease in the remethylation of homocysteine, leading to hyperhomocystemia: a defect with serious and diverse clinical consequences [3,4]. To the best of our knowledge, no attempt has been made to use this enzyme as an antifolate target.

5-CH_3-THF is the most prevalent FD in *Plasmodium falciparum* [5] and in human serum. Although radiolabeling studies show that salvage of 5-CH_3-THF by the parasite occurs [6], there is also biochemical evidence of the presence of both MTHFR and MS [6,7]. Thus, significant levels of MTHFR activity were detected in three *Plasmodium* species [6], and MS was partially purified and characterized from extracts of *P. falciparum* [7]. Because *P. falciparum* can be cultured with normal growth rates in methionine-depleted medium [6], it can clearly derive this amino acid from hemoglobin degradation and/or by *de novo* synthesis. However, unlike the host, the parasite is not necessarily dependent upon MTHFR for supply of the 5-CH_3-THF that is required for methionine synthesis because adequate levels of this cofactor are normally present in host plasma. A relative lack of importance of this activity to the parasite would be consistent with the failure of basic local alignment search tool (BLAST) searches, using a wide range of prokaryotic and eukaryotic probes, to detect an MTHFR ortholog in *Plasmodium*, but this apparent absence conflicts with the biochemical data. Moreover, the parasite requires MS, and the failure of similar searches for the gene encoding this activity, using both cobalamin-dependent and -independent enzyme sequences as probes, is perhaps more likely to indicate that the plasmodial enzymes are

highly divergent rather than absent. If so, they might represent valuable targets after they are identified.

10-Formyltetrahydrofolate dehydrogenase

10-Formyltetrahydrofolate dehydrogenase (FTHFD) (EC 1.5.1.6) in mammals consists of two functional domains: a hydrolase that removes the formyl group from 10-CHO-THF, and an NADP($^+$)-dependent dehydrogenase that oxidizes this group to CO_2 [8,9] (reaction 24b in Figure 2 of Ref. [1]). FTHFD is important for the regulation of 10-CHO-THF pools in purine synthesis and for the removal of formate. The hydrolase activity is also found in bacteria (EC 3.5.1.10; also known as 10-formyltetrahydrofolate deformylase), in which it provides the major source of formate for the synthesis of 5′-phosphoribosyl-*N*-formylglycinamide in the purine pathway. This enzyme type has not been reported in malaria parasites, and BLAST searches with bacterial deformylase sequences and the mammalian dehydrogenases provide no evidence of its presence, which, again, is consistent with the dependence of *Plasmodium* on purine salvage.

Glutamate formiminotransferase and formiminotetrahydrofolate cyclodeaminase

Glutamate formiminotransferase (GFT) (EC 2.1.2.5) and formiminotetrahydrofolate cyclodeaminase (FCD) (EC 4.3.1.4), expressed as a single polypeptide in all known organisms, mediate consecutively two important reactions in the metabolism of histidine and glutamate. GFT catalyzes the synthesis of 5-NH=CH-THF and glutamate from THF and formiminoglutamate (FiGlu) (reaction 16 in Figure 2 of Ref. [1]). FiGlu is a product of histidine metabolism; thus, these reactions control both histidine and glutamate levels, in addition to supplying a C1 unit to the folate pathway. The 5-NH=CH-THF produced by GFT is further converted to 5,10-CH^+-THF in the presence of FCD (reaction 17 in Figure 2 of Ref. [1]), which is then converted to either 10-CHO-THF or 5,10-CH_2-THF, both of which are C1 donors. Thus, the role of this part of the pathway is to provide an additional source of such groups. The 3D structure of this enzyme complex has been resolved [10,11]; however, so far, no studies have been devoted to the screening of GFT or FCD inhibitors. It could be that the malaria parasite does not require the extra capacity to provide C1 groups that this complex provides because no gene from any of the *Plasmodium* databases is identified in BLAST searches using either bacterial or vertebrate GFT–FCD probes.

Methenyltetrahydrofolate synthetase

Methenyltetrahydrofolate synthetase (MTHFS), also known as 5-formyltetrahydrofolate cycloligase (EC 6.3.3.2), catalyzes the irreversible ATP-dependent conversion of 5-CHO-THF to 5,10-CH^+-THF (reaction 18 in Figure 2 of Ref. [1]). The reverse reaction, leading to the synthesis of 5-CHO-THF, is carried out by serine hydroxylmethyltransferase (SHMT) (reaction 19 in Figure 2 of Ref. [1]), the same enzyme involved in reaction 9 of this Figure, in which 5,10-CH_2-THF is generated from serine and THF. As mentioned previously, 5-CHO-THF does not seem to have a major biological role in the cell [12], so MTHFS is not considered a good target for drug development. However, 5-CHO-THF, also known as folinic acid or leucovorin, is used as an adjuvant with antifolates to increase their therapeutic index in the treatment of cancer. MTHFS is the sole enzyme that enables the incorporation of leucovorin into the folate pathway; this reaction is, therefore, crucial in cancer therapy for the reduction of toxicity to normal cells of the antifolate inhibitor [13].

Although MTHFS has not yet been reported in malaria parasites, and BLAST searches with bacterial and plant homologs fail to identify any candidate genes, radiolabeled folinic acid is taken up and processed efficiently by the parasite, providing a much better source of exogenous folate in culture than the usual supplementation with folic acid [14,15]. This is consistent with earlier observations that the *in vitro* activity of some antimalarial antifolate

drugs tested in the presence of 5-CHO-THF is decreased [16,17]. In the apparent absence of an MTHFS-encoding gene, it remains to be determined how 5-CHO-THF is used so efficiently by the parasite. Conceivably, host erythrocytes could convert 5-CHO-THF to other FD forms such as DHF or THF that could be salvaged by the parasite.

Glycine-cleavage system

The glycine-cleavage system (GCV), or the glycine decarboxylase complex (GDC), is a tetrafunctional enzyme complex found in mitochondria that consists of P-protein (glycine decarboxylase; EC 1.4.4.2), L-protein (lipoamide dehydrogenase; EC 1.8.1.4), H-protein (carrier of the lipoamide chain) and T-protein (THF aminomethyltransferase; EC 2.1.2.10). These mediate four consecutive reactions that oxidatively cleave glycine and, with THF, lead to the production of CO_2, NH_3 and 5,10-CH_2-THF with electron transfer to produce NADH (reaction 10 in Figure 2 of Ref. [1]). Thus, this system affects the synthesis of a C1-donor group, as does the SHMT-mediated conversion of serine to glycine (see later). However, several lines of evidence indicate that the primary role of the GCV is to control the metabolism of glycine [18]. On this basis, inhibition of the enzyme system would perturb glycine metabolism, which could lead to the blocking of cell growth.

In *P. falciparum*, genes encoding significantly similar products for all but one of the proteins of the GCV can be identified, and transcripts from them are detected in mature forms from *in vitro* cultures [19]. Genes encoding a *P. falciparum* T-protein (PF13_0345) and an H-protein (PF11_0339) were identified by protein similarities of 48%–56% to relevant eukaryotic orthologs (e.g. yeast, *Arabidopsis thaliana, Anopheles gambiae* and human). However, a gene encoding a product with significant similarity to known P-proteins has yet to be found. Despite this, the existence of a GCV complex in *P. falciparum* is further indicated by the discovery of two different genes that encode products similar to the L-protein, one with a high signal-peptide score that indicates a mitochondrial isoform (PFL1550w), and the other with a strong signature peptide for the apicoplast (PF08_0066): a situation that mirrors the mitochondrial and chloroplast forms seen in plants. The relative importance of the SHMT and GCV enzymes has not been addressed directly in either bacterial or eukaryotic systems. However, the *shmt* gene is present in genomes from which *gcv* genes are absent and it is part of the minimal gene set of genomes with the lowest coding capacity sequenced to date.

Dimethylglycine dehydrogenase and sarcosine dehydrogenase

Dimethylglycine dehydrogenase (DGDH) (EC.1.5.99.2) and sarcosine dehydrogenase (SDH) (EC 1.5.99.1) (reactions 11 and 12 in Figure 2 of Ref. [1]) catalyze the transfer of one C1 unit, for the synthesis of 5,10-CH_2-THF, from dimethylglycine and methylglycine (sarcosine), respectively. Both enzymes use THF as a cofactor, and the product of the first reaction, sarcosine, is the substrate of the second. These enzymes are expressed exclusively in mitochondria, in which they have important roles in choline metabolism and as key components in the glycine–sarcosine cycle that regulates the ratio *S*-adenosylhomocysteine:*S*-adenosylmethionine. This ratio is believed to be important for modulating the plethora of transmethylation reactions that involve *S*-adenosylmethionine as the methyl group donor [2].

In *P. falciparum*, BLAST searches yield an ambiguous picture because bacterial and eukaryotic probes for both DGDH and SDH give relatively strong (and, often, sole) hits (with probability values up to 2e-13) to the same gene, PF13_0345, the product of which is annotated as having 35% identity to 92% of the mitochondrial precursor of the GCV T-protein described previously. However, BLAST searches identified only the C-terminal half of the PF13_0345 product as having sequence similarity to the DGDH and SDH probes,

indicating that assignment of PF13_0345 as being a gene that encodes a GCV T-protein is likely to be a more reliable prediction. Considering that one of the other key genes of the GCV seems to be missing, however (see earlier), it cannot be excluded that the parasite might lack a functioning GCV and that the product of PF13_0345 functions in the demethylation reactions of modified glycine rather than in C1 transfer from the methylamine group of the H-protein conjugate. These alternatives require biochemical testing.

Enzymes of *de novo* folate synthesis

Apart from that involved in reaction 1 in Figure 2 of Ref. [1], all enzymes of *de novo* folate synthesis (reactions 2-6 of Figure 2 of Ref. [1]) are specific to malaria parasites because the host cannot synthesize folate *de novo*. Therefore, this part of the pathway could offer excellent targets for antimalarial drug development and, indeed, the dihydropteroate synthase (DHPS) (EC 2.5.1.15) activity (reaction 5 of Figure 2 of Ref. [1]) has long been exploited as the target of the sulfa drugs (sulfonamides and sulfones).

GTP cyclohydrolase I

The first enzyme of folate synthesis, GTP cyclohydrolase I (GTPC) (EC 3.5.4.16), catalyzes the conversion of GTP to dihydroneopterin triphosphate (DHNP) (reaction 1 in Figure 2 of Ref. [1]) and has a crucial role in human physiology. DHNP is not used for folate production in mammalian cells but it is the precursor for the synthesis of neopterin and biopterin derivatives. The reduced form of biopterin, tetrahydrobiopterin (THB), is a cofactor for the synthesis of nitric oxide, which is a messenger involved in the regulation of many reactions and the pathology of several diseases. THB is also a cofactor in the hydroxylation of the aromatic amino acids (phenylalanine, tyrosine and tryptophan) [20,21].

GTPC activity has been measured in *Plasmodium* species [22], and the gene characterized from *P. falciparum* [23] and other malarial species (GenBank accession numbers AF043557, AF486639, AF486640, AY582138, AY604168 and AY458431). In *P. falciparum*, transcription peaks during the early trophozoite stage, which is consistent with its role in the synthesis of folate molecules. In other microorganisms, this enzyme controls *de novo* synthesis of folate and regulates the cell cycle [24] and, therefore, might be a good target for drug development. However, no systematic search for GTPC inhibitors has been made so far. Attempts to knock out the gene encoding this protein in *P. falciparum* are in progress, using a transfection system that has been developed specifically to explore the folate pathway [25] and that has been employed successfully to disable DHPS activity [14]. The success of these experiments would provide validation of GTPC as a potentially useful drug target.

Dihydroneopterin aldolase

DHNP, the triphosphate product of the GTPC reaction, is hydrolyzed to the free hydroxyl form; this reaction has been postulated to involve a non-enzymatic loss of pyrophosphate followed by nonspecific phosphatase activity that removes the third phosphate group [26] (reaction 2 of Figure 2 of Ref. [1]). The resulting substrate is converted to 2-amino-4-hydroxy-6-hydroxymethyldihydropterin in the presence of dihydroneopterin aldolase (DHNA) (EC 4.1.2.25) (reaction 3 of Figure 2 of Ref. [1]). Because radiolabeled GTP or guanosine precursors are ultimately converted to folate in the parasite [22], the existence of DHNA as the mediator of a key step along this pathway would seem to be mandatory, yet its identification, at both gene and protein level, remains elusive. Despite considerable efforts both experimentally and *in silico*, we have, as yet, been unable to identify a plausible candidate. At present, the possibilities are (i) that this protein, which is poorly conserved in other organisms, is so divergent in *Plasmodium* that it is unrecognizable, even using

bioinformatics tools that are considerably more sophisticated than BLAST; (ii) that its gene is fragmented into too many small exons for open reading frame (ORF) prediction programs to handle; or (iii) that it is absent from the parasite, which is less likely.

Hydroxymethyldihydropterin pyrophosphokinase and dihydropteroate synthase

Hydroxymethyldihydropterin pyrophosphokinase (PPPK, or HPPK) (EC 2.7.6.3) catalyzes the diphosphorylation of 2-amino-4-hydroxy-6-hydroxymethyldihydropterin (reaction 4 in Figure 2 of Ref. [1]). The resulting compound then condenses with *p*-aminobenzoate (PABA) to generate dihydropteroate, mediated by DHPS (reaction 5 in Figure 2 of Ref. [1]). PABA can be obtained either by *de novo* synthesis through the shikimate pathway or by salvage from the host plasma. PPPK and DHPS occur as a bifunctional protein in *Plasmodium* [27,28], other protozoa and plants. Together with dihydrofolate reductase (DHFR), DHPS is a longstanding target of choice for antimalarial antifolates. The sulfa drugs are structural analogs of PABA and their inhibition of DHPS functions in potent synergy with anti-DHFR inhibitors, justifying their use in the antimalarial antifolate combinations typified by sulfadoxine–pyrimethamine (SP) and Lapdap® [1]. Similar to the situation for the *dhfr* gene [1], a small number of mutations in *dhps* (principally in codons 437, 540 and 581) contributes to the observed clinical resistance of parasites to SP [29,30]. Although DHPS is a good target for further drug development, there have been few studies on the identification of new antimalarial DHPS inhibitors. Several compounds are being analyzed as potent anti-DHFR agents but the development of these compounds will require their use in combination with other inhibitors to increase potency further and to help retard the onset of clinical resistance. Novel anti-DHPS inhibitors could fulfill this role.

Dihydrofolate synthase

Dihydrofolate synthase (DHFS) (EC 6.3.2.12) catalyzes the conversion of dihydropteroate to dihydrofolate by addition of a single L-glutamate moiety; this reaction (reaction 6 of Figure 2 of Ref. [1]) is the final step in *de novo* folate synthesis. The gene encoding *P. falciparum* DHFS has been characterized [23,31] and is expressed as a bifunctional protein that also exhibits folylpolyglutamate synthase (FPGS) activity, which adds further glutamate residues to the molecule [1]. So far, no attempt has been made to target DHFS activity in the malaria parasite but this protein could provide a powerful target for new drugs. Unlike the bifunctional molecules DHFR–TS (thymidylate synthase) and PPPK–DHPS, in which discrete domains carry the individual activities, both the DHFS and FPGS activities are likely to be mediated by residues that are distributed throughout the entire molecule [31]. Thus, successful inhibitors are likely to block both activities, and investigations of the inhibition of DHFS activity are currently underway.

Concluding remarks

In the two parts of this review (this article and Ref. [1]), we have systematically examined the enzymes that catalyze reactions of the folate pathway to the extent that this pathway is understood in other organisms, in particular the human host of the malaria parasite *P. falciparum*. With regard to both anticancer and antimalarial agents, we have categorized actual and potential drug targets in terms of their current clinical or experimental status. From this comprehensive analysis, we argue that, of the 23 enzyme activities described from mammalian systems, only six [GTPC, DHFR, TS, SHMT, FPGS and methionyl-tRNA formyltransferase (MTFT)] can be ascribed to the malaria parasite with confidence. Another three (PPPK, DHPS and DHFS) are found only in the parasite and, based on biochemical evidence, three more are thought to be present (DHNA, MTHFR and MS) but have not yet been identified at the gene or protein level. A further two enzyme activities (the T- and L-proteins of the GCV) seem to be part of an incomplete pathway (Table 1).

Thus, we can conclude that the most promising novel targets at present, based purely on their firm assignment, would be TS, DHFS–FPGS, SHMT, GTPC, PPPK and, possibly, MTFT. The sequence relationship (expressed as amino acid identities) of those with human orthologs decreases in the order TS (50%)>SHMT (43%)>GTPC (17%)>FPGS (16%)>MTFT (11%) [23]. By comparison, DHFR has an identity of 25% with its host ortholog and provides a paradigm for the successful development of inhibitors with marked differential binding to the host and parasite molecules. These values are, admittedly, only crude indicators of potential use because, ideally, such comparisons should be between the regions of the molecule to which the inhibitors bind.

Of these parasite activities, TS, DHFR, SHMT, FPGS and MTFT have been considered in detail in the context of their mammalian counterparts [1]. However, all of the reactions, except the first, involved in *de novo* folate synthesis are absent from host cells. In bacterial systems, in which such synthesis is the norm, mutations or deletions of genes in this pathway generally result in nonviability. Moreover, crystal structures exist for all of the enzymes involved. GTPC is regarded as being a potentially good target for rational drug design, although there are concerns that the well-conserved active site would be too similar to that of human GTPC for effective discrimination [32]. Inhibitors of *Escherichia coli* DHNA have been investigated [33] but, as described previously, this enzyme is still unidentified in *Plasmodium*. PPPK is also seen as being a particularly attractive target [32] and inhibitors based on binding to its two substrate pockets have been described [34]. In addition to the clinically validated sulfa drugs, high-potency pterin analogs have been developed against DHPS [35], and inhibitors of DHFS have been investigated in *E. coli* [36] and *Neisseria* [37]. Again, with the latter enzyme, careful product design would be required to achieve sufficient selectivity with respect to human FPGS because of the likely similarities in active-site geometries. Overall, however, this area of the folate pathway should represent potentially fertile ground for antimalarial drug discovery. A counterargument would be that, unlike many bacteria, the parasite can salvage folate from external sources. This is known to antagonize DHPS inhibitors strongly when tested *in vitro* [17,38] but the extent to which salvage from the host can meet the needs of the parasite *in vivo* is still uncertain. Reinforcing the view that this area of folate metabolism should be investigated more thoroughly, recent transfection studies indicate that blockage of biosynthesis cannot be compensated for completely by salvage of exogenous folate and, thus, an element of *de novo* synthesis, possibly located in a separate cellular compartment, seems to be essential for parasite growth [14]. Moreover, by reducing the uptake of exogenous folate by including a transport inhibitor such as probenecid [39,40], the effectiveness of the traditional antifolates can be much enhanced and this represents a promising area for further exploration. We also note that, after GTPC, all subsequent enzymes in the *de novo* pathway use pterin derivatives as substrates but efforts to test pterin analogs as potential inhibitors of the different steps in folate synthesis are relatively rare [41], possibly owing to the generally poor solubility of these compounds.

Although determining the genome sequences of *P. falciparum* and the other *Plasmodium* species has been useful – for example, indicating the likely presence of an MTFT activity that might be exploited [1] – further functional studies are required before the picture of the folate-pathway components can be considered complete. However, it seems clear that the principal role of this pathway in these parasites is geared to the production of thymidine because, of the additional roles seen in mammals and other organisms, purine synthesis is absent and amino acids are derived mostly from hemoglobin degradation and salvage from the host. The enzymes that are involved in thymidine biosynthesis are well defined and it is here that short-term to medium-term efforts should be applied to validate these targets and assess their potential use.

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Table 1

Folate-associated enzymes in *Plasmodium*[a]

Enzyme	EC number	Gene locus in *Plasmodium falciparum*[b]	GenBank accession *number*[c]	Reaction in Figure 2 of Ref. [1]
GTPC	3.5.4.16	PFL1155w	AF043557	1
PPPK–DHPS	2.7.6.3–2.5.1.15	PF08_0095	Z31584	4 and 5
DHFS–FPGS	6.3.2.12–6.3.2.17	PF13_0140	AF161264	6 and 7
DHFR–TS	1.5.1.3–2.1.1.45	PFD0830w	J03028	8 and 15
SHMT	2.1.2.1	PFL1720w	AF195023	9
MTFT	2.1.2.9	MAL13P1.67	CAD52276	21
MTHFR	1.5.1.20	?[d]	None	13
MS	1.16.1.8	?[d]	None	14
GCV T-protein	2.1.2.10	PF13_0345	CAD52774	10
GCV H-protein	None	PF11_0339	AAN35923	10
GCV L-protein 1	1.8.1.4	PFL1550w	AAN36396	10
GCV L-protein 2	1.8.1.4	PF08_0066	CAD51214	10

[a] Table lists enzymes for which there is definite or reasonable evidence from experimental and/or sequence analysis.

[b] 3D7 clone (see http://plasmodb.org).

[c] Multiple accession numbers for different strains of *P. falciparum* and different *Plasmodium* species are not shown.

[d] Activities reported (see main text) but genes not yet identified.