Localization of the Phosphoethanolamine Methyltransferase of the Human Malaria Parasite \textit{Plasmodium falciparum} to the Golgi Apparatus*

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Phosphatidylcholine is the most abundant phospholipid in the membranes of \textit{Plasmodium falciparum}, the agent of severe human malaria. The synthesis of this phospholipid occurs via two routes, the CDP-choline pathway, which uses host choline as a precursor, and the plant-like serine decarboxylase-phosphoethanolamine methyltransferase (SDPM) pathway, which uses host serine as a precursor. Although various components of these pathways have been identified, their cellular locations remain unknown. We have previously reported the identification and characterization of the phosphoethanolamine methyltransferase, Pfpmt, of \textit{P. falciparum} and shown that it plays a critical role in the synthesis of phosphatidylcholine via the SDPM pathway. Here we provide the first evidence that the transmethylation step of the SDPM pathway occurs in the parasite Golgi apparatus. We show that the level of Pfpmt protein in the infected erythrocyte is regulated in a stage-specific fashion, with high levels detected during the trophozoite stage at the peak of parasite membrane biogenesis. Confocal microscopy revealed that Pfpmt is not cytoplasmic. Immunoelectron microscopy revealed that Pfpmt localizes to membrane structures that extend from the nuclear membrane but that it only partially co-localizes with the endoplasmic reticulum marker BiP. Using transgenic parasites expressing green fluorescent protein targeted to different cellular compartments, a complete co-localization was detected with Rab6, a marker of the Golgi apparatus. Together these studies provide the first evidence that the transmethylation step of the SDPM pathway of \textit{P. falciparum} occurs in the Golgi apparatus and indicate an important role for this organelle in parasite membrane biogenesis.

Malaria, the world’s most important parasitic disease, is caused by intraerythrocytic protozoan parasites of the genus \textit{Plasmodium}. More than 300 million clinical cases and more than 2 million deaths are reported each year with most deaths mainly caused by \textit{Plasmodium falciparum} (1). Unlike other human pathogens that invade metabolically active host cells, \textit{P. falciparum} invades mature erythrocytes that lack internal organelles and the metabolic pathways necessary for \textit{de novo} lipid synthesis. During its intraerythrocytic life cycle, \textit{P. falciparum} undergoes major metabolic and morphological changes and then divides asexually to produce up to 36 new daughter parasites (2). This rapid growth and multiplication requires active synthesis of new membranes and is fueled by lipid precursors derived from the host.

Phosphatidylcholine is the major phospholipid in \textit{P. falciparum} membranes, representing 50% of parasite phospholipids (for review, see Ref. 3). Pharmacological studies demonstrated that inhibition of phosphatidylcholine biosynthesis is deleterious to parasite intraerythrocytic growth and multiplication, emphasizing the importance of the phospholipid metabolic pathways as possible targets for development of new antimalarial drugs (4–10). Recent studies in \textit{P. falciparum} identified two pathways of biosynthesis of phosphatidylcholine: the CDP-choline and the serine decarboxylase-phosphoethanolamine methyltransferase (SDPM) pathways (for review, see Ref. 3). \textit{The de novo} CDP-choline (Kennedy) pathway uses host choline as a precursor. Choline is first transported into the parasite cytoplasm via host and parasite-specific transporters and is subsequently phosphorylated by a parasite-specific choline kinase into phosphocholine. The phosphocholine formed is then converted into CDP-choline by a CDP-choline cytidylyltransferase. The resulting CDP-choline is further converted into phosphatidylcholine by a parasite-encoded CDP-diacylglycerol-choline phosphotransferase. The SDPM pathway initiates from host serine and consists of five enzymes (8). Three of these enzymes, ethanolamine kinase, CTP:phosphocholine/ethanolamine cytidylyltransferase, and choline phosphotransferase are components of the Kennedy pathways. The other two enzymes, serine decarboxylase and phosphoethanolamine methyltransferase, generate phosphocholine, which then serves as a precursor for phosphatidylcholine biosynthesis. The phosphoethanolamine methyltransferase gene, \textit{PfPMT}, encoding this activity has recently been cloned, and the corresponding enzyme consists of a 266-amino acid

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\footnote{The abbreviations used are: SDPM, serine decarboxylation-phosphoethanolamine methylolation; ER, endoplasmic reticulum; GFP, green fluorescence protein; PEF-1 \textit{α}, \textit{P. falciparum} elongation factor-1 \textit{α}; Pfpmt, \textit{P. falciparum} phosphoethanolamine methyltransferase; PIPES, 1,4-piperazinediethanesulfonic acid; RBC, red blood cell; PBS, phosphate-buffered saline.}
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polypeptide that lacks transmembrane regions, contains an S-adenosyl-L-methionine binding domain, and is homologous to plant phosphoethanolamine methyltransferase enzymes (11, 12). Unlike plant phosphoethanolamine methylation using a topoisomerase I-based reaction. The open reading frame of \( P. \) falciparum Pfpmt is a monomeric enzyme that possesses a single catalytic domain responsible for the three-step methylation of phosphoethanolamine to form phosphocholine (8). The substrate specificity of this enzyme was demonstrated biochemically in vitro and genetically using yeast as a surrogate system (13).

Although most genes encoding enzymes of the CDP-choline and SDPM pathways have been identified in \( P. \) falciparum, nothing is known about the cellular location of the enzymes and pathways. Here we report the characterization and cellular localization of Pfpmtn. We show that the transmethylation step of the SDPM pathway occurs in the Golgi apparatus. These studies provide the first cell biological evidence that the transmethylation step of the SDPM pathway of \( P. \) falciparum occurs in the Golgi apparatus and indicate an important role for this organelle in parasite membrane biogenesis.

**EXPERIMENTAL PROCEDURES**

**Parasite Strains and Culture**—The \( P. \) falciparum transgenic strains used in this study were derived from the 3D7 clone (The Netherlands) and are described in Table 1. Parasites were propagated in human RBCs at 2% hematocrit by the method of Trager and Jensen (14) except that the serum component in the culture medium was replaced with 0.5% Albumax (Invitrogen).

**Plasmid Constructions**—To make the Pfpmtn-green fluorescence protein (GFP) fusion construct, the open reading frame of Pfpmtn was PCR-generated from cDNA using primers (forward primer with the added XhoI site in bold and the start codon underlined) and (reverse primer, 5'CTCGAGAACATAAACATTTACT-3'). The fragment was ligated at the XhoI/AvrII sites to create a fusion to GFP cloned in the pHC1-ACP-GFP vector (15). The resulting plasmid contains Pfpmtn-GFP fusion under the regulatory control of the \( P. \) falciparum CAM1 promoter and HSP86 terminator sequences and harbors the Toxoplasma gondii DHFR-TS marker that confers resistance to pyrimethamine. To generate cassettes for the expression of Pfpmtn-GFP fusion proteins, we utilized the Gateway MultiSite™ system to recombine three elements (promoter, gene of interest, and reporter) from pENTR vectors in a desired order and orientation within a destination vector. The destination vector, pCHDR-3/4, and the pENTR vectors, pHHSP86 5' -pENTR-4/1 (as promoter clone bearing HSP86 5' promoter), BiP(s)-pENTR-1/2 (as gene clone bearing the BiP signal peptide), and GFP-pENTR-2/3 and GFP-SDEL-pENTR-2/3 (as reporter clones carrying the GFP and fused GFP-SDEL, respectively), used in this study were generated by van Dooren et al. (16). The \( P. \) falciparum RAB6 gene was cloned into the commercially available pENTR-D/TOPO vector (Invitrogen) using a topoisomerase I-based reaction. The open reading frame of RAB6 was amplified from total cDNA using the forward primer, 5' -CACCAGATCGCTGATGATCCTTTGATAATTACCT-3' (with added CACC for directional cloning in bold and added 8 bp upstream of the start codon underlined), and reverse primer, 5' -ACATATTCTACTAAATATTGTCA-3' (with the stop codon deleted). The PCR product was phenol-extracted and cloned into the pENTR-D/TOPO vector following the manufacturer's instructions to generate Rab6-pENTR-1/2 gene clone. To create the final expression vectors, LR reactions were performed in a mixture containing the pCHDR-3/4 destination vector harboring the hDHFR-positive selectable marker, which confers resistance to WR99210 (17) and pENTR vectors (promoter clone (HSP86 promoter), gene clone (RAB6, BiP) and a reporter clone (GFP, GFP-SDEL)) in the presence of a recombinase enzyme mix following the manufacturer's instructions (Invitrogen).

**Transfection of \( P. \) falciparum**—The 3D7 strain of \( P. \) falciparum cultured in human RBCs was used for transfection studies. About 100 μg of the respective expression plasmid DNA in 400 μl of electroporation buffer was mixed with 100 μl of packed RBCs in a 0.2-cm cuvette. Electroporation was performed using a Gene Pulse II (Bio-Rad) set at 0.31 kV and 950 microfarads (18). Transfected parasites were cultured in Petri dishes for 48 h without drug selection, and thereafter the drug was introduced into the culture medium at 5 and 100 nM final concentrations, respectively, for WR99210 and pyrimethamine. Drug-resistant fluorescent parasites were established after 21 days.

**Antibody Production**—Purification of recombinant Pfpmtn was done as previously described (8). The eluate of the Histagged Pfpmtn, purified by nickel affinity chromatography, was dialyzed in 50 mM NaHCO₃ buffer. The dialyzed protein was incubated with Affi-Gel 15 (Bio-Rad) at 4 °C for 2 h with shaking. The slurry was washed with PBS and glycine (1 μg, pH 12) and incubated at 4 °C for 1 h. The crude anti-serum from the second bleeding of a rabbit injected with two Pfpmtn peptides, NH₂-GA(DG)LDDGWSRKIKDSKRKMQR-COOH, and NH₂-(GC)SS-GGLEATKIKSLDELN-COOH, was added to the mixture and shaken overnight at 4 °C. The antibodies were purified by column chromatography against recombinant Pfpmtn and eluted with 0.1 M glycine. The eluate was collected in 1-ml fractions and neutralized with 150 μl of 1 M Tris, pH 6.8. Immunoblots were performed with the affinity-purified recombinant Pfpmtn, and the column-purified antibody to ascertain antibody specificity.

**Immunoblotting**—To determine the specificity of the anti-Pfpmtn antibodies for the endogenously expressed Pfpmtn protein in \( P. \) falciparum, asynchronous cultures of 3D7 wild-type parasites and transgenic 3D7 parasites, expressing a Pfpmtn-GFP fusion protein, were treated with 0.15% saponin. The

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**TABLE 1**

Wild-type and transgenic \( P. \) falciparum strains used in this study

| Strain | Genetic make-up | Expressed fusion protein | Fusion protein target |
|---|---|---|---|
| 3D7 | Wild-type | None | None |
| 3D7-Pfpmtn-GFP | Transgenic | Pfpmtn-GFP | Golgi |
| 3D7-GFP | Transgenic | GFP | Cytosol |
| 3D7-BiP-GFP-SDEL | Transgenic | GFP-SDEL<sup>+</sup> | ER |
| 3D7-Rab6-GFP | Transgenic | Rab6-GFP | Golgi |

<sup>+</sup> Green fluorescence protein fused to the N-terminal signal peptide and the C-terminal ER retention motif of the \( P. \) falciparum BiP protein.
released parasites were washed in PBS and sonicated, and the soluble fraction was collected by centrifugation. The fraction was mixed with 6× SDS sample buffer and boiled for 3 min. Equal volumes of the samples were fractionated by SDS-polyacrylamide gel electrophoresis, and the proteins were transferred to a nitrocellulose membrane. The blocked blots were incubated with the rabbit anti-Pfpmt antibody (1:50 dilution) or with rabbit pre-immune sera (1:50 dilution) as primary antibodies and then with conjugated goat anti-rabbit antibody (1:2000) as the secondary antibody. Signal generation was performed using an ECL chemiluminescence kit (PerkinElmer Life Sciences). The same blot was stripped and re-probed with the P. falciparum elongation factor-1α antibody (19).

To monitor stage-specific expression of Pfpmt, 12 plates of synchronized cultures of 3D7 wild-type parasites were grown to early ring stage (10% parasitemia and 2% hematocrit). The cultures were then pooled, and the medium was removed by centrifugation. The cell pellet was resuspended in fresh medium and mixed thoroughly, and equal volumes of the cell suspension were plated into 12 Petri dishes. Parasites from 4 plates were harvested shortly thereafter as ring stage parasites. For trophozoites and schizont stages, 4 plates each were collected after about 26 and 38 h, respectively. Parasites were extracted from the RBCs by treatment with 0.15% saponin and resuspended in PBS. Sample preparation and immunoblotting were conducted as described above with Pfpmt as the primary antibody.

Analysis of Pfpmt Membrane Association—Asynchronous P. falciparum parasites were extracted from erythrocytes by treatment with 0.07% saponin for 15 min on ice. After centrifugation at 1875 g for 15 min, the pellet was washed in PBS and resuspended in PBS. The parasites were sonicated and spun at 100,000 g for 10 min. Part of the pellet obtained was treated with 1% Triton X-114 for 30 min on ice and then spun at 100,000 g for 10 min to obtain the detergent-solubilized extract in the supernatant and the insoluble fraction in the pellet. The samples were fractionated by SDS-PAGE and transferred on to nitrocellulose membranes followed by immunoblotting.

Microscopy and Immunofluorescence Assays—Live parasitized RBCs were washed in PBS, and the nuclei of the parasites were stained by incubating with Hoechst at 20 μg/ml for 20 min. After washing, the live cells were visualized by confocal microscopy. Immunofluorescence assays were done essentially as described by Rager et al. (20) but with slight modifications. Asynchronous cultures of wild-type and transgenic parasites were washed twice in PBS, smeared onto coverslips, and air-dried. The smears were fixed in 1% formaldehyde in PBS for 30 min at room temperature and then rinsed in wash buffer (0.5% fetal bovine serum, 0.5% normal goat serum, 0.05% saponin) followed by blocking in a PBS solution containing 5% fetal bovine serum, 5% goat serum, and 0.1% saponin. After washing, the coverslips were incubated with either a mixture of affinity-purified rabbit anti-Pfpmt antibodies (1:25 dilution) or anti-PfPP2C (1:100 dilution) and mouse anti-GFP monoclonal antibody (ABGENT) at 1:500 dilution or with a mixture of anti-Pfpmt antibody (1:25 dilution) and mouse monoclonal antibody (Sigma) to the RBC band 3 protein (1:500 dilution) at room temperature with gentle shaking for 1 h. The coverslips were washed and incubated with a mixture of anti-rabbit antibody (1:500 dilution) conjugated to fluorescein isothiocyanate and anti-mouse antibody (1:500 dilution) conjugated to Texas red dye (Molecular Probes) for 20 min at room temperature. Nuclei were stained by incubating the coverslips in PBS containing 3 μg/ml Hoechst stain (Molecular Probes) for 1 h at room temperature. Nuclei were stained by incubating the coverslips in PBS containing 3 μg/ml Hoechst stain (Molecular Probes) for 5 min at room temperature, and after washing, the coverslips were mounted on slides with Antifade (Molecular Probes). Fluorescence microscopy was performed with a Nikon eclipse TE2000-E microscope using filter 96320/HYQ (excitation 480–440/ emission 440) for fluorescein isothiocyanate, 96312/G2EC for Texas red dye and 21307/G2EC for Hoechst.
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**FIGURE 2.** Transmission electron micrographs of ultrathin cryosections of intraerythrocytic ring and trophozoite stages of \textit{P. falciparum} 3D7-Pfpmt-GFP transgenic parasites using anti-GFP and anti-BiP antibodies. \textit{A}, immunogold labeling (18-nm gold particles) of GFP antibodies bound to a ring-stage-infected erythrocyte. \textit{B}, immunogold labeling (18-nm gold particles) of GFP antibodies bound to a trophozoite-stage-infected erythrocyte. \textit{C}, immunogold labeling of GFP (18-nm gold particles) and BiP (12-nm gold particles) antibodies bound to early schizont intraerythrocytic \textit{P. falciparum}. \textit{D}, magnification of the region in panel \textit{C} depicted in the dotted line. \textit{N}, nucleus; \textit{NM}, nuclear membrane; \textit{RBC}, red blood cell; \textit{PPM}, parasite plasma membrane; \textit{FV}, food vacuole; \textit{H}, hemozoin.

( excitation 540–525/emission 620–660) for rhodamine, and 96310/UV2EC (excitation 360–340/emission 460–450) for 4’,6-diamidino-2-phenylindole.

\textit{Immunoelectron Microscopy}—Immunoelectron microscopy was done as previously described by Rager \textit{et al.} (20) with slight modifications. RBCs parasitized with transgenic \textit{P. falciparum}, expressing the Pfpmt-GFP fusion protein, were pelleted and fixed on ice for 1 h in a solution of 4% paraformaldehyde, 200 mM PIPES, and 0.5 mM MgCl\textsubscript{2}, pH 7.0. After rinsing in PIPES buffer, the cell pellet was embedded in 10% gelatin and fixed overnight at 4 °C. Samples were infiltrated with 20% polyvinylpyrrolidone and 2.3M sucrose in the tissue with antibodies against GFP (Fig. 1B). The non-cytoplasmic nature of the fluorescence pattern was further confirmed by immuno-colocalization studies using antibodies directed against the parasite protein phosphatase 2C (19, 23), which resides in the parasite cytoplasm, and monoclonal antibodies against GDP to detect the Pfpmt-GFP fusion (Fig. 1C). To further localize the fluorescence pattern of Pfpmt-GFP, immunoelectron microscopy was performed using a monoclonal antibody against GFP. As shown in Fig. 2, \textit{A} and \textit{B}, Pfpmt-GFP associates with membranous structures extending from the nuclear membrane, reminiscent of the endoplasmic reticulum (ER) and Golgi apparatus. Because the ER has been associated with the synthesis of lipids in different organisms and the initial step of glycerolipid metabolism catalyzed by the glycerol-3-phosphate acyltransferase, PfGat, also occurs in this organelle (24), we examined the possible ER localization of Pfpmt. Immunocolocalization studies were, therefore, performed using polyclonal antibodies against the ER marker BiP and anti-GFP monoclonal antibodies in the 3D7-Pfpmt-GFP strain. As shown in Fig. 2, \textit{C} and \textit{D}, whereas BiP localization was either on the nuclear membrane or on membrane structures in the immediate surrounding of the nuclear membrane (ER), Pfpmt-GFP localization only partially overlapped with BiP and concentrated in the distal part of the membrane structures extending from the nuclear mem-

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tate and 2% polyvinyl alcohol. Images were viewed on a Zeiss 902 transmission electron microscope.

**RESULTS**

\textit{Pfpmt Is Not a Cytoplasmic Enzyme}—Studies in eukaryotic cells have indicated that the kinases involved in the synthesis of the soluble precursors phosphoethanolamine and phosphocholine from ethanolamine and choline, respectively, are localized in the cytosol (21, 22). This knowledge led to the assumption that the transmethylation step of the SDPM pathway, catalyzed by Pfpmt, could also occur in the parasite cytoplasm. To localize Pfpmt in \textit{P. falciparum}, the GFP was added to the C-terminal portion of the enzyme, and the fusion protein was stably expressed in \textit{P. falciparum}. Analysis of the autofluorescent parasites did not reveal the typical diffusible stain of cytoplasmic proteins (Fig. 1A). Rather, the autofluorescence was limited to specific dots within the parasites, the number of which varied depending on the intraerythrocytic stage, with \~3 dots detected during the ring stage, \~5 dots during the trophozoite stage, and \~10 dots during early schizogony (Fig. 1A). A similar pattern could be detected using antibodies against GFP (Fig. 1B). The non-cytoplasmic nature of the fluorescence pattern was further confirmed by immunocolocalization studies using antibodies directed against the parasite protein phosphatase 2C (19, 23), which resides in the parasite cytoplasm, and monoclonal antibodies against GFP to detect the Pfpmt-GFP fusion (Fig. 1C). To further localize the fluorescence pattern of Pfpmt-GFP, immunoelectron microscopy was performed using a monoclonal antibody against GFP. As shown in Fig. 2, \textit{A} and \textit{B}, Pfpmt-GFP associates with membranous structures extending from the nuclear membrane, reminiscent of the endoplasmic reticulum (ER) and Golgi apparatus. Because the ER has been associated with the synthesis of lipids in different organisms and the initial step of glycerolipid metabolism catalyzed by the glycerol-3-phosphate acyltrans-

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brane. Although antibodies against the Golgi marker ERD2 are available, those antibodies did not recognize the native enzyme in immunoelectron microscopy analyses (not shown) and, thus, could not allow us to determine whether the localization pattern of Pfpmt-GFP reflects a Golgi localization.

**Pfpmt Expression and Localization**—To further confirm the localization of Pfpmt, we have raised polyclonal antibodies against the enzyme. These antibodies were affinity-purified and used in immunoblot studies to monitor protein expression in *P. falciparum*. A single band of ~30 kDa was detected in the 3D7 strain, and two bands, one corresponding to the native Pfpmt and a second corresponding to the Pfpmt-GFP fusion, could be detected in the 3D7-Pfpmt-GFP transgenic parasites (Fig. 3A). No bands could be detected using the preimmune serum in this strain (Fig. 3A). As a positive control, antibodies against the *P. falciparum* elongation factor PFEF-1α (19) revealed a single band in both strains. To determine the stage(s) during which Pfpmt protein is expressed, immunoblot analyses were performed on protein extracts isolated from highly synchronized cultures at the ring, trophozoite, and schizont stages of the parasite intraerythrocytic development and using an equal number of parasites. As shown in Fig. 3B, Pfpmt expression was detected in all the stages, but high levels were detected during the trophozoite stage during which active synthesis of parasite lipids occurs. To determine whether Pfpmt is a soluble or membrane-associated protein, protein extracts from parasite-soluble and membrane fractions were prepared and analyzed by immunoblotting using anti-Pfpmt antibodies. Pfpmt associated only with the soluble fraction (Fig. 3C). As a control, the parasite purine transporter PfNT1 associated with the membrane fraction and could be solubilized using Triton X-114 (Fig. 3C), consistent with published studies (20, 24).

Pfpmt monospecific antibodies were then used in immunofluorescence analyses to localize the native enzyme. A pattern similar to that of Pfpmt-GFP was detected at the three stages of parasite intraerythrocytic development (Fig. 4). To identify the exact cellular localization of the native enzyme, we have generated three different transgenic parasites expressing either GFP alone targeted to the cytoplasm, GFP containing a C-terminal SDEL retention signal and, thus, targeted to the ER, or GFP fused to the Golgi apparatus marker Rab6 (25, 26) (Fig. 5). Co-localization studies using anti-Pfpmt affinity-purified polyclonal antibodies and an anti-GFP monoclonal antibody confirmed that the native Pfpmt is not cytoplasmic (Fig. 5A), showing only partial overlap with BiP (Fig. 5B), but revealed a complete co-localization with Rab6-GFP (Fig. 5C), thus demonstrating that Pfpmt resides in the Golgi apparatus of the parasite.

**DISCUSSION**

Although significant progress has been made in the past few years in the understanding of the metabolic pathways involved in the synthesis of the major phospholipid, phosphatidylcholine, of the human malaria parasites, little is known about the cellular sites where these pathways operate. Evidence for the role of the ER in the biosynthesis of parasite phospholipids came from the detailed localization of the glycerol-3-phosphate acyltransferase of *P. falciparum*, PfGat, to this organelle (24). This enzyme catalyzes the acylation of the precursor glycerol 3-phosphate at the sn-1 position. This is the first step in a series of reactions that lead to the formation of the fatty acid donors and phospholipid precursors, diacylglycerol and CDP-diacylglycerol.

Genetic and biochemical studies in yeast and mammalian cells have revealed two major routes for the synthesis of phosphatidycholine; (i) the *de novo* CDP-choline (Kennedy) pathway, which initiates from choline and (ii) the phosphatidylethanolamine transmethylation pathway that converts phosphatidylethanolamine into phosphatidylcholine via three consecutive methylation reactions catalyzed by one or two phospholipid methyltransferases (for review, see Refs. 27 and 28). In most mammalian cells the *de novo* pathway is the primary route of phosphatidylcholine biosynthesis. In mammalian hepatocytes as well as in yeast cells, in addition to the *de novo* synthesis from choline, phosphatidylcholine biosynthesis can also be derived from the transmethylation of phosphatidylethanolamine. In plant cells, however, no significant methylation of phosphatidylethanolamine occurs. Instead, these cells can methylate phosphoethanolamine into monomethylphosphoethanolamine, dimethylphosphoethanolamine, and phosphocholine (11, 12, 29). The phosphocholine, thus, formed is subsequently used for phosphatidylcholine biosynthesis via the
CDP-choline pathway. Interestingly, metabolic studies in *P. falciparum* and the molecular identification of the phosphoethanolamine methyltransferase Pfpmt have demonstrated that this parasite uses a plant-like pathway for synthesis of phosphatidylcholine (8). This pathway initiates from serine, which is either transported from host plasma or obtained from active degradation of host hemoglobin. Serine is first decarboxylated into ethanolamine by a parasite-specific serine decarboxylase. The gene encoding this activity has not yet been identified, and no homologs of known plant serine decarboxylases could be found in the finished genomes of *Plasmodium* species. The ethanalamine obtained via the serine decarboxylation reaction is rapidly phosphorylated by a parasite-specific ethanolamine kinase to yield phosphoethanolamine, which then serves as a substrate for the Pfpmt transmethylation reaction to form phosphocholine. The final two metabolic enzymes of the SDPM pathway that catalyze the formation of phosphatidylcholine from phosphocholine are also components of the CDP-choline pathway. The importance of these enzymes and pathways in parasite development and survival remains to be elucidated.

Here we demonstrated using various microscopic analyses that Pfpmt resides in the Golgi apparatus of the parasite. The ultimate demonstration was obtained using a transgenic parasite expressing GFP fused to Rab6 and targeted to the Golgi apparatus. These parasites along with other transgenic parasites expressing GFP in other parasite localizations will help our efforts to map the cellular locations of the parasite lipid metabolic pathways. Thus far, of the five genes of the SDPM pathway involved in the synthesis of phosphatidylcholine from host serine, only the serine decarboxylase gene remains to be identified. Genetic and biochemical studies to reveal the molecular iden-

**FIGURE 4. Immunofluorescence microscopy of Pfpmt in *P. falciparum*-infected red blood cells.** Uninfected (U) or erythrocytes infected with 3D7 parasites at ring (R), trophozoite (T), and schizont (S) stages of parasite intraerythrocytic development were fixed and prepared as described under “Experimental Procedures.” Parasites were examined by microscopy using illumination at 546 nm to visualize Pfpmt (green) conjugated to the fluorescein isothiocyanate-conjugated anti-rabbit secondary antibody or at 488 nm to visualize Band 3 complexed with the Texas Red-conjugated anti-mouse secondary antibody (red). DNA was counterstained with Hoechst (blue). DIC, differential interference contrast images of parasitized erythrocytes.

**FIGURE 5. Immunofluorescence microscopy of Pfpmt in transgenic *P. falciparum*-infected red blood cells expressing GFP in the cytoplasm, ER, and Golgi apparatus.** Erythrocytes infected with transgenic parasites expressing GFP in the cytoplasm (GFP) (panel A), the ER (GFP-SDEL) (panel B), and Golgi apparatus (Rab6-GFP) (panel C) at different stages of parasite intraerythrocytic development were fixed and prepared as described under “Experimental Procedures.” Parasites were examined by microscopy using illumination at 546 nm to visualize Pfpmt (green) conjugated to the fluorescein isothiocyanate-conjugated anti-rabbit secondary antibody or at 488 nm to visualize GFP complexed with the Texas Red-conjugated anti-mouse secondary antibody (red). DNA was counterstained with Hoechst (blue). DIC, differential interference contrast images of parasitized erythrocytes. R, ring; T, trophozoite; S, schizont.
tity of this gene and its product are under way. The localization of Pfpmt and glycerol-3-phosphate acyltransferase of *P. falciparum* represents an important step toward understanding the temporal and special organization of the parasite lipid machineries and indicates that the Golgi apparatus and ER are crucial organelles in this essential process.

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