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Lipid metabolism in *Giardia*: a post-genomic perspective

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SUMMARY

Giardia lamblia, a protozoan parasite, infects a wide variety of vertebrates, including humans. Studies indicate that this anaerobic protist possesses a limited ability to synthesize lipid molecules *de novo* and depends on supplies from its environment for growth and differentiation. It has been suggested that most lipids and fatty acids are taken up by endocytic and non-endocytic pathways and are used by *Giardia* for energy production and membrane/organelle biosynthesis. The purpose of this article is to provide an update on recent progress in the field of lipid research of this parasite and the validation of lipid metabolic pathways through recent genomic information. Based on current cellular, biochemical and genomic data, a comprehensive pathway has been proposed to facilitate our understanding of lipid and fatty acid metabolism/syntheses in this waterborne pathogen. We envision that the current review will be helpful in identifying targets from the pathways that could be used to design novel therapies to control giardiasis and related diseases.

INTRODUCTION

Although identified by Antoni van Leeuwenhoek more than three centuries ago, *Giardia* has recently occupied a central stage of parasite research. The epidemiological studies conducted over the past few years indicate that a wide range of mammals, including humans and cattle, are infected by this parasite, causing a substantial burden on the global economy (Giangaspero *et al*. 2005). Current knowledge supports the proposal that giardiasis is a zoonotic disease and that contaminated water serves as one of the main sources of infection (Monis *et al.* 2003; Smith *et al.* 2006; Bajer 2008). Various species of *Giardia* are recognised (Thompson, 2009), and efforts have been made over the past few years to enhance the taxonomy using molecular tools (Hunter *et al.* 2005; Xiao *et al.* 2008; Thompson 2009). Based on such tools, 6 species of *Giardia* have been identified to date, representing 6 different assemblages, of which assemblages A and B infect humans and other mammals (Thompson, 2009).

In humans, *Giardia* infection can be symptomatic or asymptomatic. Symptomatic giardiasis can present with fatty diarrhoea, abdominal discomfort, vomiting, malabsorption and/or weight loss (Kamda et al. 2009). In some cases, giardiasis resolves rapidly, but in other cases, it can result in chronic infection (Faubert, 2000). Both cell-mediated and humoral

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immune responses in the host against *Giardia* have been reported, and adaptive responses have been shown to be critical for controlling giardiasis (Faubert, 2000). Non-immune systems such as secretory immunoglobulins also play a role in the severity of the disease (Nayak *et al*. 1987).

This parasite has a simple life cycle, with two morphological forms - i.e., trophozoites and cysts. Following ingestion, cysts pass through the stomach (being exposed to stomach acid), after which trophozoites are released and colonise the small intestine by longitudinal binary fission (Ghosh *et al*. 2001). The *Giardia* trophozoite (12–15 μm long) (Fig. 1, panel A), is non-invasive, and contains a ventral disc made of cytoskeletal proteins that provide support to *Giardia* for attachment to the enterocyte wall (Holberton, 1973, Ghosh *et al.* 2001). The resistant cysts (7–10 μm long) with thick cyst walls (Fig. 1, panel B) are responsible for the transmission of giardiasis *via* contaminated food *or* water. The cyst wall of *Giardia* contains insoluble filamentous materials that consist of glycoprotein, glycolipids, and amino-sugar containing oligo- and polysaccharides (Das and Gillin, 1996; Sener *et al*., 2004; Ratner *et al*., 2008). Three encystation-specific cyst-wall proteins (CWP-1, -2, and -3) are expressed at the time of encystation and concentrated within encystation-specific vesicles (ESVs) before they are targeted to the cyst wall. Besides these three CWPs, a high-cysteine non-variant cyst protein (HCNCp) is present in trophozoites and may participate in cyst production (Davids *et al.* 2006).

Studies conducted in recent years indicate that intestinal lipids and fatty acids influence the growth and encystation of *Giardia* (Farthing *et al*. 1985; Gillin *et al.* 1987, 1988; Lujan *et al*. 1996). Most lipids are taken up by this parasite from its environment and used as required (Kaneda and Goutsu, 1988; Mohareb *et al*. 1991). However, contrary to the earlier notion that *Giardia* is unable to synthesize its own lipids *de novo* (Jarroll *et al*. 1981), results from our laboratory suggest that selective phospholipids can be produced by this parasite *via de novo* and/or remodelling reactions (Gibson *et al.* 1999; Das *et al*. 2001, 2002). The recently established Genome Database (www.giardiadb.org, Morrison *et al*. 2007), which revealed the presence of lipid synthesis and metabolic genes, further validates our observations. The focus of this article is to review progress in the field of lipid research of *Giardia* and to validate lipid metabolic pathways by comparison to genomic sequence information. A possible lipid biosynthesis pathway for *Giardia* has also been proposed.

INTERACTIONS WITH INTESTINAL LIPIDS AND FATTY ACIDS

Because *Giardia* is continuously exposed to bile acids and dietary fats in the small intestine, it was proposed that lipids and fatty acids play important roles in regulating growth, encystation and excystation. Fatty acids from the intestine kill *Giardia in vitro*, whereas mucous and bile salts protect the parasite from being killed by fatty acids and other small intestinal factors (Reiner *et al.* 1986; Das *et al*. 1988). Bile acids were also proposed to facilitate the transport of intestinal lipids into *Giardia* by forming mixed micelles (Das *et al.* 1997). The intestinal factors include aggregated and non-aggregated fats, lipases and secretory immunoglobulins (Farthing *et al.* 1985; Reiner *et al.* 1986). Free fatty acids generated from phospholipids and triglycerides are detrimental to the growth of *Giardia* (Reiner *et al.* 1986; Das *et al.* 1988). Studies suggest that dodecanoic ($C_{12:0}$) acid (also known as lauric acid) possesses an anti-giardial property at a reasonably low concentration (Rayan *et al.* 2005). This medium-chain fatty acid accumulates inside trophozoites and alters membrane permeability and integrity. *Giardia* has the machinery to neutralize the toxic effects of free fatty acids by forming complex with membrane proteins, lipids, and carbohydrates (Das *et al.* 1991; Gibson *et al.* 1999; Touz *et al.* 2005).

The role of bile and fatty acids in inducing the encystation of *Giardia* was first proposed by Frances D. Gillin. In classic experiments, Gillin and her colleagues showed that a mixture of primary bile acid (glycocholate) and fatty acid (oleic acid or myristic acid) promotes *in vitro* encystation (Gillin *et al.* 1987, 1988). Subsequently, cholesterol and an excess amount of bovine bile, which *Giardia* obtains from the growth medium, were shown to induce encystation (Kane *et al.* 1991; Lujan *et al.* 1996). Interestingly, the homologues of sterol regulatory-element-binding proteins (SREBPs) were identified in *Giardia* and found to regulate the expression of *cwp* genes during encystation (Worgall *et al.* 2004). *Giardia* expresses four genes linked to cholesterol biosynthesis, which are up-regulated during its differentiation into cyst (Hernandez *et al.* 2006). Several proteins of the parasite can undergo post-translational modification by the intermediate of cholesterol (isoprenyl-group) biosynthetic pathway (Lujan *et al.* 1995), and it is possible that these modifications of giardial proteins are important for maintaining membrane integrity and functions.

IMPORT OF LIPIDS AND FATTY ACIDS BY *GIARDIA*

Phospholipids and fatty acids are important constituents of all eukaryotic membranes, including those of *Giardia*. Because of its limited lipid synthesis ability (Das *et al.* 2002), lipids in *Giardia* are acquired from the small intestine of the host, in which the trophozoites are exposed to free and conjugated fatty acids, various sterols, phospholipids and bile acids (Stevens *et al.* 1997). Lujan *et al*. (1994) showed that lipoprotein-like receptor molecules are present in trophozoites, which allow them to internalize serum-lipoproteins through a cytochalasin-D-sensitive pathway. Using fluorescent lipid analogues, we have shown that trophozoites are able to internalize lipids directly from the culture medium and transport them to various locations, including the plasma and nuclear membranes, the cytoplasm and the endoplasmic reticulum (ER). The cellular localisation of each particular lipid analogue studied is distinct. We confirmed that the incorporations of fluorescently-labelled lipid analogues are not dependent on respective fluorophores (i.e., BODIPY or NBD), rather solely on the intrinsic properties (hydrophobicity and hydrophilicity) of lipid probes (Stevens *et al.* 1997; Gibson *et al.* 1999; Das *et al.* 2001). Results indicate that ceramide and phosphatidylglycerol (PG) show preferential localisation at perinuclear membranes, whereas phosphatidylcholine (PC) is incorporated into plasma and flagellar membranes. Palmitic acid (PamA) and sphingomyelin (SM) label the nuclear envelopes and the plasma membrane. Phosphatidylethanolamine (PE) is localised to the plasma membrane and in certain cytoplasmic structures adjacent to the plasma membrane.

As cytoskeletal components (*i.e*., actin filaments and microtubules) are involved in transporting lipid molecules in a range of eukaryotes, we investigated whether the giardial cytoskeleton also participates in the uptake and recycling of fluorescent lipid molecules from plasma- to endo-membranes. In *Giardia*, microtubules constitute numerous structures in trophozoites, including the ventral disc, basal bodies, flagella, paraflagellar rods and median body (Crossley *et al.* 1986; Elmendorf *et al.* 2003). The basal bodies are the major microtubule organizing centre and functional equivalent of the centrosome of higher eukaryotes (Nohynkova *et al.* 2000; Correa *et al.* 2004; Davids *et al.* 2008). A large set of kinesin homologues are present (Iwabe *et al.* 2002; Richardson *et al.* 2006), but, thus far, no putative homologue of myosin has been identified (Elmendorf *et al.* 2003). *Giardia* contains a single copy of the actin gene (Morrison *et al.* 2007), and its protein sequence reveals an ~58% nucleotide identity to other eukaryotic actin sequences (Elmendorf *et al.* 2003).

We have observed that the uptake and inter-organelle transport of fluorescently labelled SM, PC, and PG are interrupted by anti-actin and anti-microtubule agents. Cytochalasin-D, an actin-depolymerising drug, induces the formation of several tubular/vesicular structures and blocks the intracellular trafficking of ceramide and SM (Hernandez *et al*. 2007a; Castillo *et*

al. 2009). Furthermore, vinorelbine (a microtubule depolymerising agent) is effective in significantly lowering the intracellular incorporation of fluorescently labelled ceramide and SM. These observations indicate that both ceramide and SM are taken up by cells through cytoskeletal-dependent processes which require intact actin and microtubule structures. On the contrary, the uptake of PC is not dependent on cytoskeleton, because cytochalasin-D and other microtubule-depolymerising drugs neither alter nor reduce the localisation pattern of PC. Like PC, PamA intake is also not affected by anti-cytoskeleton agents (Castillo *et al.* 2009). We have also observed that anti-microtubule depolymerising agents (*e.g.*, cholchicine, albendazole and nocodazole) blocked the release of PG from the ER/perinuclear regions, suggesting that an intact microtubule structure could be essential not only for uptake and transport, but also for the recycling of PG from the ER to the cytoplasm and plasma membranes (Castillo *et al.* 2009). The results for cytoskeletal-based lipid transport and trafficking experiments (Castillo *et al.* 2009) have been summarized in a model (Fig. 2), which suggests that fluorescently labelled ceramide, SM and PG are mainly taken up by actin-dependent endocytic mechanisms (Hernandez *et al.* 2007a; Castillo *et al.* 2009). It can be postulated that soon after endocytic vesicles are released from the plasma membranes encapsulating lipid molecules, they reach the ER/perinuclear membranes on microtubule rails. Lipids, such as ceramide, SM, PC and PamA, are possibly taken up by the cells through non-endocytic pathways, but, at this stage, it is not clear whether PC localised on the outer cell membrane originates from the ER/perinuclear membranes or from the inner plasma membrane (Fig. 2). Giardial lipid and fatty-acid transport proteins may also participate in translocating lipid molecules that may travel along the microtubules to reach perinuclear membranes. The presence of a fatty-acid binding protein (~8 KD) has been reported in *Giardia* (Hassan *et al.* 2005). The transport of ER/perinuclear PG *via* exocytic vesicles may be regulated by microtubule filaments and not by actin cytoskeleton (Fig. 2), but more, in-depth experiments must be carried out to fully elucidate the lipid transport and trafficking in this organism.

SYNTHESES OF NEW LIPIDS AND FATTY ACIDS

The synthesis and metabolism of phospholipids and fatty acids in *Giardia* was first investigated by Edward Jarroll and his colleagues almost three decades ago (Jarroll *et al.* 1981). Using radioactive acetate, glucose, glycerol, threonine, cholesterol and glycerol-3-phosphate, his group monitored the incorporations, utilisation and subsequent conversions of these lipids into downstream metabolic products. Interestingly, it was reported that none of these radioactive precursors were converted into other lipids, and it was postulated that *Giardia* has little or no ability to synthesize lipids *de novo*. It was thus suggested that *Giardia* obtains most of its phospholipids and fatty acids from bovine serum and bile supplemented to the growth medium or present in dietary lipids which are abundant in the human small intestine (Farthing *et al.* 1985; Gillin *et al.* 1986). This proposal was further supported by Kaneda and Goutsu (1988) and Mohareb *et al.* (1991), who showed that the lipid composition in *Giardia* is similar to that of the growth medium. Thin-layer chromatographic analyses revealed that four phospholipids — *i.e.*, PC, PE, SM, and PG are present in both encysting and non-encysting cells and remain unaltered throughout the process of encystation (Ellis *et al.* 1996).

Recently, we carried out detailed analyses of phospholipids in *Giardia* with the help of electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-qTOF-MS) (Yichoy et al. 2009). The results indicated that PCs and PGs are the major phospholipids in this parasite. Analyses in negative-ion mode revealed that at least 17 different species of PGs are present with various combinations of odd- and even-numbered, carbon-containing fatty acids. Quantitative analyses further elucidated that two PG species containing $C_{18:1}$/$C_{16:0}$ and $C_{18:1}$/$C_{16:0}$ were most abundant, followed by $C_{16:0}$/$C_{16:0}$ and/or $C_{18:0}$/$C_{14:0}$.

Although we detected more species of PCs (19 in positive-ion mode and 6 in negative-ion mode), only one of them (C18:1/C18:1) was abundant. In addition to the PGs and PCs, 6 species of PEs, 3 species of SMs and 2 species of PIs were also detected (Yichoy *et al.* 2009). Interestingly, except for lyso-PCs and PCs, no other phospholipids are present in bile and serum, suggesting that many of these phospholipids (specifically PG and PE) in *Giardia* could be synthesized *de novo via* CDP-DAG and/or fatty acid and head-group remodelling pathways (Das *et al.* 2001). This proposal can be further supported by the finding that radiolabelled fatty acids are directly incorporated into membrane phospholipids (Blair *et al.* 1987; Stevens *et al.* 1997; Gibson *et al.* 1999; Vargas-Villarreal *et al.* 2007), indicating that *Giardia* has the cellular machineries to synthesize new phospholipids. Radiolabelled bases (i.e., choline, inositol, ethanolamine, serine and glycerol) are also incorporated into respective phospholipids of trophozoites when added to the culture medium (Subramanian *et al.* 2000; Das *et al*. unpublished). A schematic diagram of the synthesis of new phospholipids by fatty acid and headgroup exchange reactions (Das *et al.* 2001; Das *et al.* 2002) is shown in Fig. 3. In the future, it will also be interesting to investigate whether some of the phospholipids in *Giardia*, particularly PGs, are synthesized *via* the CDP-DAG *de novo* pathway.

Several studies suggest that sphingolipid (SL) metabolic pathways are critical for encystation process, and that inhibition of their syntheses blocks the production of cysts in culture (Hernandez *et al.* 2008; Sonda *et al.* 2008; Stefanic *et al.* 2010). Only five SL metabolic genes have been annotated in the *Giardia* Genomic Database (www.giardiadb.org), and they are all transcribed differentially between trophozoites and encysting cells. These genes are: (i) giardial serine-palmitoyltransferase-1 and -2 subunit genes (*gspt-1* and *gspt-2)*, (ii) glucosylceramide synthase or glucosylceramide transferase 1 (*gglct-1*), and (iii) two acid sphingomyelinase genes (*gasmase-1* and *-2)*. The enzymatic activities of serine-palmitoyltransferases (gSPTs) and glucosylceramide transferase1 (gGlcT1) were measured and found to be up-regulated during encystation. It was observed that gSPTs (synthesize 3-ketosphinganine—the first rate-limiting step of SL biosynthesis) regulate ceramide endocytosis, which is important because *Giardia* is unable to synthesize ceramide *de novo* (Hernandez *et al.* 2008). On the other hand, gGlcT1 (catalyzes the synthesis of glucosylceramide or GlcCer) is involved in encystation and cyst production by modulating the synthesis of CWPs and ESVs. Inhibition of the synthesis of GlcCer interferes with trophozoite replication and cyst formation (Hernandez *et al.* 2008; Sonda *et al.* 2008). Recently, it has been demonstrated that the inhibition of GlcCer production causes cellular abnormalities, including the formation of enlarged lysosomes, clathrin localization and cell-cycle progression before blocking the overall cyst production (Stefanic *et al.* 2010). Although the function of giardial SMase has yet to be elucidated, it is possible that this enzyme is involved in scavenging ceramide from SM present in the growth medium or in the *milieu* of the small intestine. These results indicate that ceramide and other SLs play important roles in giardial biology and differentiation.

A comprehensive analysis of fatty acids by Ellis *et al.* (1996) revealed that major fatty acids in *Giardia* were $C_{16:0}$ followed by $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$. Small amounts of $C_{14:0}$, $C_{15:0}$, $C_{17:0}$, $C_{18:3}$, $C_{19:0}$, $C_{20:0}$, $C_{22:0}$, $C_{24:0}$, $C_{26:0}$, and $C_{28:0}$ were also detected. Interestingly, no dramatic differences were observed between the fatty acid content of non-encysting and encysting *Giardia*. The authors also determined the fatty acid compositions of low-bile (1% bile-containing growth medium) and high-bile (10% bile-containing encystation medium) and noticed that major fatty acids (i.e., $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$)were present in both media, although some quantitative differences were recorded (Ellis *et al.* 1996). A detailed analysis of fatty acids by gas chromatography-mass spectrometry (GC-MS) showed that $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ are indeed the major fatty acids in *Giardia* and that they remained essentially unaltered during the transition from vegetative forms to encysting (0–48 h) and mature cysts

(Yichoy *et al.* 2009). Traces of shorter-chain fatty acids—*i.e.*, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{15:0}$ — were also detected. It is interesting that $C_{12:0}$ and $C_{14:0}$ were found to be present in the adult bovine serum and bovine bile, the major sources of lipids in giardial growth and encystation medium (Yichoy *et al.* 2009).

Our results (Yichoy *et al.*, 2009) and those of Ellis *et al.* (1996) further supported the proposal that very long-chain fatty acids (i.e., $C_{20:0}$, $C_{20:1}$, $C_{21:0}$, $C_{22:0}$, $C_{23:0}$, $C_{24:0}$, and $C_{24:1}$) that are present in *Giardia* are not taken up from bile and serum and thus could be generated by the action of fatty acid elongase activity, because a similarity (BLAST) search of the giardial genome predicted the presence of fatty-acid elongase 1 gene (*gelo*) (Yichoy *et al.* 2009). Free and esterified cholesterols were found to be the major neutral lipids in non-encysting and encysting stages. In addition, the presence of cholesterylesters and small quantities of ergosterol and glycerides were reported (Ellis *et al.* 1996). However, the GC-MS analyses (Yichoy *et al.* 2009) suggest that cholesterol is the only sterol present in trophozoites, encysting cells and cysts, and that it is obtained directly from the growth medium.

LIPID SYNTHESIS AND METABOLIC GENES

Giardia is polyploid, and its genome is very much like the eukaryotic genome that includes linear chromosomes flanked by telomere sequences (TAGGG). The five chromosomes, ranging in size from 1.6 to 3.8 Mb, are constituted of 1.34×10^8 bp, which predicts up to 8–12 copies of each chromosome compared with the haploid genome (Yu *et al.* 2002). The trophozoite stage of the parasite has two morphologically indistinguishable nuclei that both replicate at approximately the same time and that are transcriptionally active. Each nucleus contains approximately the same copy numbers of ribosomal RNA genes on a single chromosome (chromosome 1), which indicates that this chromosome is present in both nuclei and contains the same complement of DNA (Adam, 2001). To understand the biology of the organism as well as to identify new drug targets, the *Giardia* Genome Project was initiated (www.GiardiaDB.org) in 1998 by Mitchell Sogin and his colleagues at the Marine Biological Laboratory, Woods Hole, MA, with the support from the National Institutes of Health, USA (McArthur *et al.* 2000; Morrison *et al.* 2007). This genome project (Morrison *et al.* 2007) has assisted in identifying several putative homologues of lipid synthesis and metabolic genes in assemblages A (isolate WB), B (isolate GS) and C (isolate P 15) of *Giardia*. Table 1 demonstrates the classes of phospholipid syntheses/metabolic genes that were annotated in GenBank (http://www.ncbi.nlm.nih.gov/genbank/). These classes represent the putative genes encoding phosphatidylinositol synthase (PIS), phosphatidylglycerolphosphate synthase (PGPS), phosphatidylserine synthase (PSS) and decarboxylase (PSD). The presence of these genes in the database, together with our earlier lipidomic study that PG, PE, and PI are not obtained from the growth medium (Yichoy *et al.* 2009), supports the hypothesis that *Giardia* has the ability to synthesize selective phospholipids *de novo*. We have observed that *Giardia* has a strong PSD activity and that it converts [^{14}C]-PS to [^{14}C]-PE instantly (Das *et al.* unpublished). It is likely that *Giardia* utilises the product of the *gpss* gene to synthesize PS from PC and PE, respectively. In mammalian cells, two *pss* genes are present—*pss-1* and *pss-2*. While *pss-1* facilitates the formation of PS from PC, *pss-2* converts PE to PS (Kent 1995; Dowhan 1997). Because *Giardia* is considered an early diverging eukaryote (Sogin *et al.* 1989), its *pss* gene may encode an enzyme that functions both as gPSS1 and gPSS2. Similarly, PG may be synthesized from CDP-DAG in a reaction catalyzed by PGPS, encoded by the *gpgps* gene expressed throughout its life cycle (Yichoy *et al.* 2009).

The genome database (Morrison *et al.* 2007) also suggests the presence of several classes of phospholipid-transport (PLT) ATPases or flippases (FLIPs) that allow the parasite to

internalize phospholipids (particularly amino-phospholipids, which include PC and PE) from the environment in the small intestine. As the database suggests, there are several flippase genes in the WB, GS and P15 isolates of *Giardia* (Table 1). In an unpublished observation, we found that all of these flippase genes in the WB isolate are active and expressed differentially in trophozoites and encysting stages of the parasite's life cycle (K. Y. Aguilera and S. Das, unpublished). Although presently the reason for the existence of so many flippases is not known, it can be presumed that *Giardia* has evolved an efficient mechanism to internalize amino-phospholipids, particularly PC, from the intestinal environment.

Several phosphatidylinositol kinase (PIK) - and phosphatidylinositol phosphatase (PIP)-related lipid signalling genes were also annotated in the genome database for *Giardia* (Table 1), and many of them were shown to be involved in regulating the growth and encystation. An example is the giardial target of rapamycin (TOR), which is an analogue of the FAKB-rapamycin associated protein (FRAP)/TOR of eukaryotes expressed in dividing parasites and is not inhibited by rapamycin (Morrison *et al.* 2007). The bioinformatic analyses of three giardial PIKs genes (*gpiks*) [two *gpi3ks* (*gpi3k-1* and *gpi3k-2*), and one *gpi4k*] were also carried out (Cox *et al.* 2006; Hernandez *et al.* 2007*b*). The analyses revealed that giardial PI3Ks, unlike higher eukaryotes, contain only catalytic (p110) but not regulatory subunits (p85) (Hernandez *et al.* 2007*b*). Transcriptional analyses demonstrated that *gpiks* are expressed in *Giardia* and are differentially regulated during encystation. In addition, two PI3K inhibitors, wortmannin and LY 294002, have been shown to inhibit the replication of trophozoites in culture, supporting the notion that the activities of PIKs could be linked to the growth and encystation of *Giardia* (Cox *et al.* 2006; Hernandez *et al.* 2007*b*). Thus, signal-transducing phospholipid molecules are synthesized in *Giardia* and participate in cell growth and differentiation.

As shown in Table 1, only five SL metabolic genes have been annotated in the *Giardia* genomic database, including the genes that encode serine-palmitoyltransferases 1 & 2 (*gspt-1 and -2*)—glucosylceramide transferase or GlcT-1 (*gglct-1*), and two separate acid sphingomyelinase enzymes (*gasmases*). All five genes are reported to be expressed differentially between the two different stages of the life cycle of *Giardia*, suggesting that SL pathways could be involved in modulating the growth and differentiation of this waterborne pathogen (Hernandez *et al.* 2008).

With regard to fatty acids (FAs), genomic information for *Giardia* infers the presence of 9 fatty-acid transport, synthesis and metabolic genes (Table 1). Three 1-acyl-sn-glycerol-3-phosphate acyltransferases (AGPATs) have been annotated, suggesting that *Giardia* might use the products of these genes to import fatty acids from its surrounding environment. Additional FA genes annotated are putative lysophosphatidic acid acyltransferase (*glaat*), elongase 1 (*gelo*), several long-chain fatty-acid (LCFA)-CoA ligases—LCFA-CoA ligase (*glcfal*), LCFA-CoA ligase 4 (*glcfal4*), and three different forms of LCFA-CoA ligase 5 (*glcfal5*)—and acetyl-CoA/pyruvate carboxylase (*gacpc*). The presence of these FA genes further indicates that a very basic but essential FA metabolism is present in *Giardia*, which is linked to transferring fatty acids across the membranes, forming reactive fatty-acid species (fatty acyl-CoA), acylating lysophosphatidic acid (LPA) to form phosphatidic acid (PA) and elongating and ligating fatty-acid chains (Table 1). *Giardia* contains two isoforms of secreted and cytoplasmic phospholipase B enzymes (*gplb*) which are responsible for the simultaneous removal of *Sn1* and *Sn2* fatty acids from a phospholipid (Morgan *et al.* 2004).

PROPOSED PATHWAY AND FUTURE PERSPECTIVES

Based on biochemical, cell biology, and genomic information, we have inferred a comprehensive pathway describing the synthesis and metabolism of phospholipids, neutral lipids FAs and SLs in *Giardia* (Fig. 4). The model reveals that a vibrant and metabolically active trophozoite synthesizes putative gPLTs (or gFLIPs) that allow the parasite to import PC and lyso-PC from the external environment by facilitated diffusion and to convert these molecules into various downstream lipids. For example, PC can be converted to PS by the enzyme encoded by *gpss*. Table 1 also indicates that both *gpsd* and *gpss* are present in *Giardia*, and that the parasite has the ability to synthesize PS from PE and PE from PS by base-exchange reactions. PG is synthesized *de novo*, as proposed earlier (Yichoy *et al.* 2009), and PC may serve as a major precursor. It is likely that a novel PC-to-PG remodelling enzyme may exist and that the parasite uses this enzyme to synthesize PG directly from PC. Nevertheless, such an enzyme has yet to be identified and characterized. The gene *gpgps* (encoding PGPS) was identified and shown to express in non-encysting and encysting cells (Yichoy *et al.* 2009). However, it is not known whether this gene participates in the synthesis of new PG *via* the CDP-DAG (*de novo*) pathway.

As shown in Table 1, and also reported earlier (Morgan *et al.* 2004), *Giardia* has the genes that encode phospholipase B (PLB). The hydrolysis of Sn_1 and Sn_2 FAs from PC by PLB produces lyso-PC and soluble glycerophosphorylcholine. The presence of lyso-phosphatidic acid acyltransferase (LPAAT) gene (*glpaat*) in the genomic database suggests that this parasite also has the ability to convert lyso-PA to PA.

The pathway also proposes that most FAs can be taken up by simple and facilitated diffusion (Gibson *et al.* 1999). Once internalized, FAs undergo elongation and/or desaturation reactions. The presence of a giardial FA desaturase was reported earlier by Ellis *et al.* (1996); the gene (*gelo*) that is likely to encode elongase was annotated in the database (Table 1). Diacylglycerol (DAG) or other neutral lipids present in the medium (Yichoy *et al.* 2009) can also be obtained by membrane diffusion and/or *via* transport proteins. Intracellular DAG can form triacylglycerol (TAG) by *agpat* gene products, and diacylglycerol (DAG) can be activated by cytidine diphosphate (CDP) to produce CDP-DAG, which then can be used as a precursor to synthesize PI. Newly synthesized PI can be utilized to generate PIP, PIP_2, and PIP_3 by giardial PIKs for cellular signalling (Cox *et al.* 2006; Hernandez *et al.* 2007*b*).

As mentioned, *Giardia* expresses *gspt*, *gglct1*, and *gsmase* genes, indicating a limited SL synthesis/metabolic pathway. It is possible that PalmA obtained from the growth medium is converted to Palm-CoA by acyl-CoA ligase and then is used by the parasite to synthesize 3-ketosphinganine by the action of serine-palmitoyltransferase enzymes encoded by *gspts*. We have proposed earlier (Hernandez et al., 2008) that 3-ketosphinganine regulates ceramide uptake in *Giardia* by controlling its endocytic machinery, and this is important because ceramide is not synthesized by this parasite *de novo*. The newly acquired ceramide is then used by *Giardia* as precursors to synthesize GlcCer by glucosylceramide synthase (encoded by *gglct1*) which may serve as a key regulator of encystation and cyst production (Hernandez *et al*. 2008). Taken together, it has been proposed that ceramide uptake and GlcCer synthesis is important for the encystation of *Giardia* (Hernandez *et al*. 2008; Sonda *et al.* 2008; Stefanic *et al.* 2010).

We foresee that the current review not only contributes to our understanding of the lipid pathways in *Giardia* but also should assist researchers in identifying unique targets for developing effective therapies in the future. One of these targets might be the enzymes of PG biosynthesis, because PG appears to be the major phospholipid in *Giardia* (Gibson *et al.*

1999; Yichoy *et al.* 2009). Lipid transport and lipid-based cell signalling could be another important area for future investigation. As mentioned above, *Giardia* has evolved mechanisms to import exogenous lipids and cholesterol by receptor-mediated endocytosis (Lujan *et al.* 1994) and traffic *via* clathrin-mediated and actin/microtubule-dependent pathways (Hernandez *et al*. 2007a). Therefore, the identification of lipoprotein-like receptors and the study of lipid transport vesicles in *Giardia* should open a new research area that might lead to the discovery of unique pathways and mechanisms of lipid sorting and targeting. At present, it is not fully understood how extracellular signals regulate the growth and differentiation of *Giardia*. Future investigation may suggest that PI3K-based signalling is associated with this phenomenon and drives the process of encystation and excystation. Finally, it would also be fascinating to investigate whether giardial lipids and lipid metabolic enzymes are involved in host-parasite interactions.

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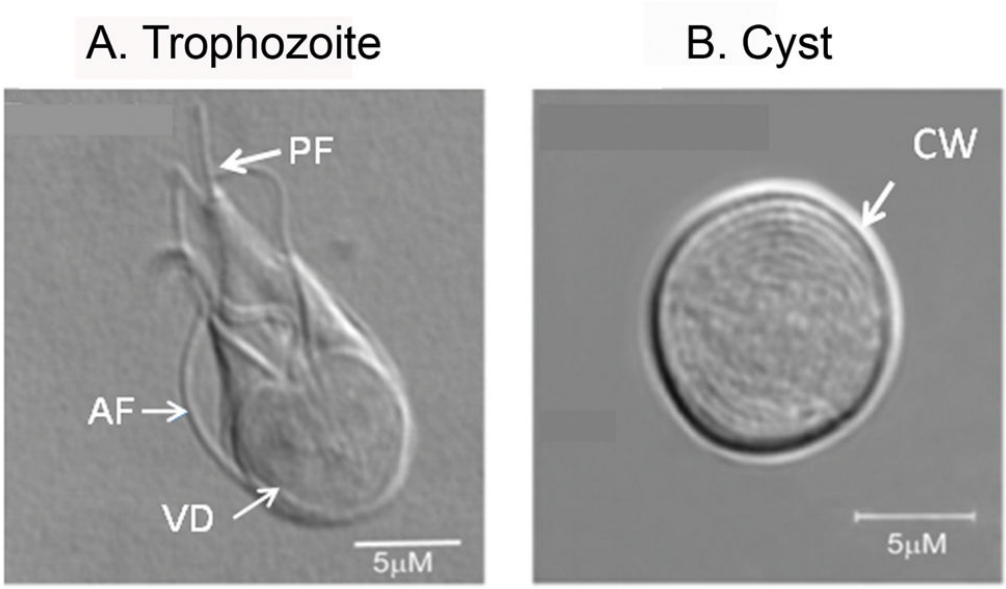

Fig. 1.

Direct interference contrast (DIC) microscopy pictures of *Giardia* trophozoite (panel A) and water-resistant cyst (panel B) cultured in the laboratory. The trophozoites (12–15 μm long) contain two nuclei (not visible in the picture) and a ventral disc (VD) made of cytoskeletal proteins that provide support to *Giardia* for attachment to the intestinal cell wall. The water-resistant cysts (7–10 μm long) with thick cyst walls (CW) are responsible for the transmission of giardiasis via contaminated water. VD, ventral disc; AF, anterior flagellum; PF, posterior flagellum; CW, cyst wall. Bar: 5 μM.

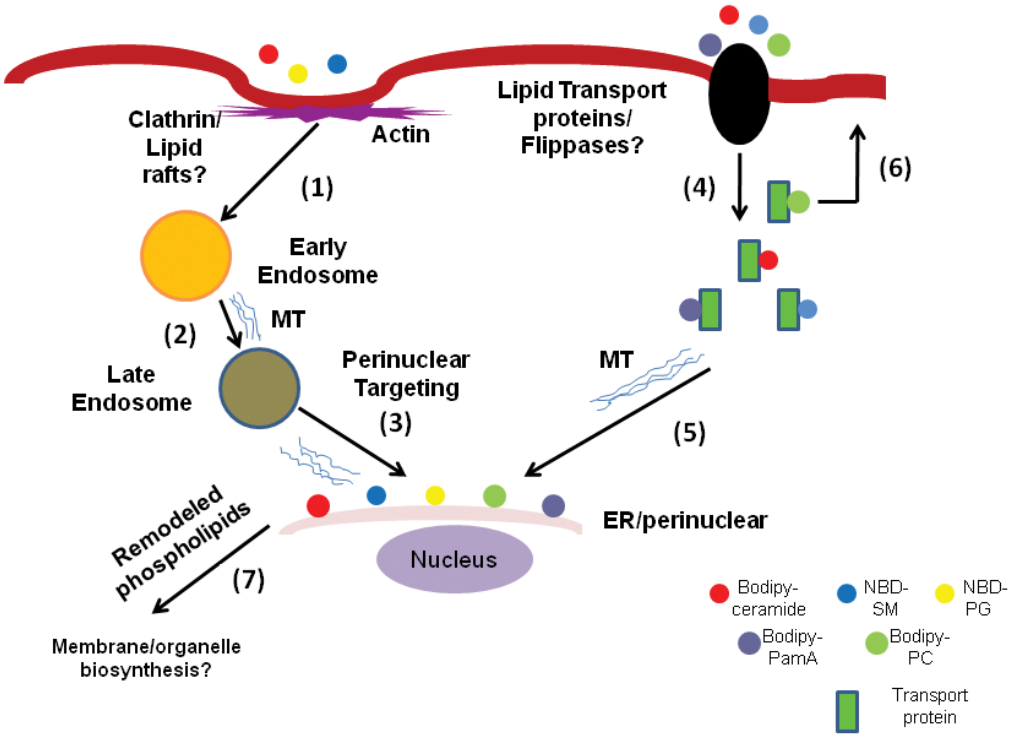

Fig. 2.

Lipid import and trafficking by *Giardia*. The figure shows that BODIPY-ceramide, NBD-SM, and NBD-PG could be imported by actin-dependent endocytic pathways and targeted to ER/perinuclear membranes (steps 1–3) (Hernandez *et al.* 2008; Castillo *et al.* 2009). Membrane lipids and fatty acids like ceramide, SM, PC, and PalmA can also be taken up by a flippase-dependent, non-vesicular mechanism and migrated intracellularly. Lipid-binding proteins and microtubule filaments may participate in this process (steps 4–5). Membrane phospholipids like PC, which is mostly localised in the plasma membrane (Das *et al.* 2001), can be flipped back to the plasma membrane (step 6), although the mechanism of this outward movement is not known. It is possible that the internalised lipids are remodelled at the ER/perinuclear regions (step 7) and utilised by the parasite for the synthesis of membranes and organelles. SM, sphingomyelin; PG, phosphatidylglycerol; PalmA, palmitic acid; PC, phosphatidylcholine; MT, microtubule.

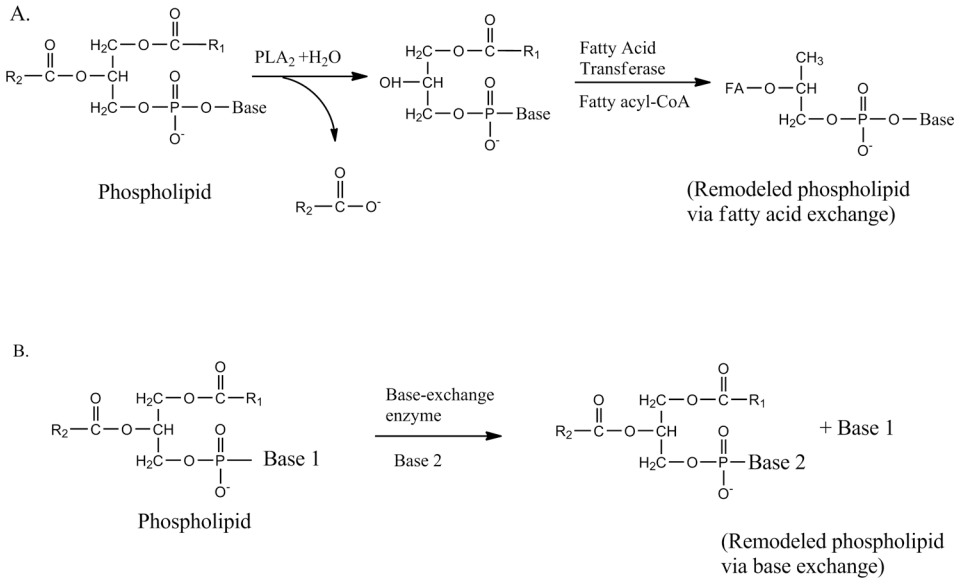

Fig. 3.

Generation of new lipids by fatty acid and headgroup remodelling reactions. Panel (A) shows fatty acid remodelling by deacylation/reacylation reaction (the Lands cycle), in which phospholipase A_2 and fatty acyl CoA transferase enzymes are involved. Panel B indicates the generation of new lipids by headgroup or base-exchange reactions (Das *et al.* 2001; Das *et al.* 2002).

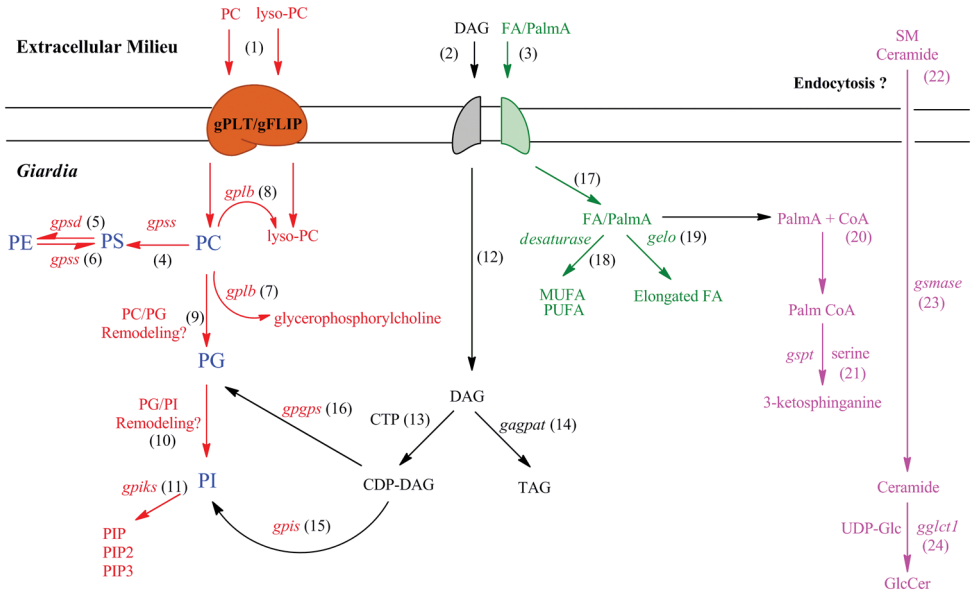

Fig. 4.

Proposed lipid metabolic pathways in *Giardia*. The model proposes that PC and lyso-PC, which are abundant in the growth medium, can be taken up by *Giardia* with the help of gPLT or gFLIP (step 1). Diacylglycerol (DAG) and FA are internalised by specific transporter(s) from the growth medium (steps 2 & 3). Internalised PC can be converted to PS with the help of gPSS1-like enzyme encoded by putative *gpss* gene (step 4). *Giardia* expresses *psd* gene (Yichoy *et al.* 2009), and its possible encoded product (gPSD) may facilitate PE synthesis from PS (step 5). The putative *gpss* can also encode gPSS2-like enzyme for the synthesis of PS from PE (step 6). Because PG is the major phospholipid in *Giardia* and is not present in the growth media (Yichoy *et al.* 2009), it is likely that *Giardia* has the ability to synthesize PG not only by CDP-DAG pathway (step 16) but also by the headgroup remodelling reaction shown in step 9. Similarly, PI is synthesized from PC by base or headgroup exchange reactions from PG (step 10). However, the presence of these two pathways—i.e., PC -> PG and PG -> PI—is yet to be elucidated in *Giardia* or other eukaryotic cells. PI can be converted to various phosphinositides facilitated by *gpiks* (step 11) as mentioned before (Cox *et al.* 2006; Hernandez *et al.* 2007b). Giardial *plb* and *lpl* gene may be responsible for synthesizing glycerophosphorylcholine from PC and lyso-PC, respectively (steps 7 & 8). Diacylglycerol (DAG) obtained from the growth medium can be converted to CDP-DAG (step 13) and serves as a precursor for TAG (step 14), PG (step 15), and PI (step 16). Exogenous FAs can produce unsaturated FAs (MUFA and PUFA) and elongated FAs as depicted in steps 18 & 19. Exogenous PalmA can be used as a precursor to synthesize 3-ketosphinganine with the help of *gspts* (steps 20–21). Similarly, both ceramide and SM can be acquired from the growth medium (step 22) by endocytic and non-endocytic pathways. Exogenously obtained SM can be hydrolyzed by *gsmases* to produce ceramide (step 23), and ceramide can be used to synthesize GlcCer (step 24). PC, phosphatidylcholine; lyso-PC, lyso-phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; PIP, phosphatidylinositol phosphate; PIP2, phosphatidylinositol bisphosphate; PIP3, phosphatidylinositol triphosphate; DAG, diacylglycerol; TAG, triacylglycerol; CTP, cytidine triphosphate; FA, fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SM, sphingomyelin; GlcCer, glucosylceramide. For better understanding, phospholipid pathways are shown in red, neutral lipids (DAG and TAG) in black, FAs in green, and SLs in pink.

Table-1

Lipids and Fatty Acid Metabolic Genes Annotated in GS, WB and P15 Isolates in *Giardia*

Classification	Homologues	NCBI Accession Number	GiardiaDB ORF		
		50803 (WB)	50581 (GS)	50803 (WB	P15
Phospholipid	PI Synthase/CDP-DAG-inositol 3-phosphatidyltransferase (gPIS)	XP_001707169	831	9829	1650
	PI transfer protein alpha isoform (PITPα)	XP_001705528	3968	4197	2564
	PGP synthase/CDP-DAG-glycerol-3-phosphate-3-phosphatidyltransferase (gPGPS)	XP_001707005	4006	7529	1650
	PS decarboxylase (gPSD)	XP_001707910	1294	16495	5211
	PS synthase (gPSS)	XP_001707737	928	17427	660
	Phospholipid-transporting ATPase IA, putative (gPLTATPase 1A)	XP_001704967, XP_001710085	177, 3570	8182, 16958, 10019	2350, 2846, 1870
	Phospholipid-transporting ATPase IIB, putative (gPLTATPase IIB)	XP_001704293, XP_001707954	900, 2201	137725, 101810, 38104	2493, 1733
Fatty Acid	1-acyl-sn-glycerol-3-phosphate acyl transferase (gAGPAT4)	XP_001704656		7126	
	gAGPAT3	XP_001704595		2692	
	gAGPAT2	XP_001707326		12109	
	Lysophosphatidic acid acyltransferase, putative (gLPAAT)	XP_001707002	4004	14403	1652
	Fatty acid elongase 1 (gFAELO)	XP_001708101	2228	92729	536
	Long chain fatty acid CoA ligase 5 (gLCFACL5)	XP_001705891, XP_001705009, XP_001706424, XP_001707853	2104, 2579, 2829, 3493	9062, 2118, 15063, 17170	2620, 2964, 109, 5157
	Long chain fatty acid CoA ligase 4 (gLCFACL5)	XP_001708520	3754	30476	1330
	Long chain fatty acid CoA ligase, putative (gLCFACL)	XP_001709411		113892	4443
	Acetyl-CoA carboxylase (gACC)/pyruvate carboxylase fusion protein, putative	XP_001705655	1829	113021	184
Sterol	Lecithin-cholesterol acyl transferase, putative (gLCCAT)	XP_001705338, XP_001706263	1681, 4525	5746, 16286	297, 1123
Neutral Lipid	Phospholipase B (gPLB)	XP_001704922, XP_001709220	128, 380	93548, 17277	2398, 711, 1744
Sphingolipids	Ceramide glucosyltransferase (gGlcT1)	XP_001704299	2206	11642	1728
	Acid sphingomyelinase-like phosphodiesterase 3b (gASMase)	XP_001709364, XP_001705202	370, 4397	16737, 8360	757, 809
	Serine palmitoyltransferase-1(gSPT1)	XP_001707207	798	23015	2116
	Serine palmitoyltransferase-2 (gSPT2)	XP_001704960	1960	14374	1863
Signaling Lipids	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (gPI3Pase)	XP_001709198	1218	16728	1550
	Type II inositol-1,4,5-trisphosphate 5-phosphatase precursor (gITP5Pase)	XP_001705945	3898	14787	3004

Classification	Homologues	NCBI Accession Number	GiardiaDB ORF		
		50803 (WB)	50581 (GS)	50803 (WB	P15
	Inositol 5-phosphatase 4 (gI5Pase)	XP_001709238	146	9077	363
	Phosphoinositide-3-kinase, class 3 (gPI3K)	XP_001708644	2073	17406	3357
	PI-3-kinase, catalytic, alpha polypeptide (gPI3Kα)	XP_001709235	144	14855	361
	Phosphatidylinositol-4-phosphate 5-kinase, putative (gPI4P5K)	XP_001709292, XP_001705538 XP_001707008, XP_001705604, XP_001709854, XP_001710017	649, 950, 1909, 3977, 4008, 4088	14628, 24712, 7261, 2622, 13606, 11897	2573, 606, 2900, 1648, 4395, 2048
	PI-4-kinase (gPI4K)	XP_001706660	1085	16558	1075
	Phosphatidylinositol-glycan biosynthesis, class O protein (gPIG)	XP_001709629	1892	14975	2782