Everybody needs sphingolipids, right! Mining for new drug targets in protozoan sphingolipid biosynthesis

JOHN G. M. MINA* and P. W. DENNY

Department of Biosciences, Lower Mountjoy, Stockton Road, Durham DH1 3LE, UK

(Received 10 April 2017; revised 15 May 2017; accepted 18 May 2017; first published online 22 June 2017)

SUMMARY

Sphingolipids (SLs) are an integral part of all eukaryotic cellular membranes. In addition, they have indispensable functions as signalling molecules controlling a myriad of cellular events. Disruption of either the de novo synthesis or the degradation pathways has been shown to have detrimental effects. The earlier identification of selective inhibitors of fungal SL biosynthesis promised potent broad-spectrum anti-fungal agents, which later encouraged testing some of those agents against protozoan parasites. In this review we focus on the key enzymes of the SL de novo biosynthetic pathway in protozoan parasites of the Apicomplexa and Kinetoplastidae, outlining the divergence and interconnection between host and pathogen metabolism. The druggability of the SL biosynthesis is considered, alongside recent technology advances that will enable the dissection and analyses of this pathway in the parasitic protozoa. The future impact of these advances for the development of new therapeutics for both globally threatening and neglected infectious diseases is potentially profound.

Key words: sphingolipids, ceramide, drug targets, protozoan parasites, apicomplexa, kinetoplastidae.

INTRODUCTION

Protozoan parasites and the global burden of their diseases

Protozoa (kingdom Protista) are single-cell organisms that can be free-living or parasitic in nature (Baron, 1996). Out of more than 50,000 protozoan species that have been described to-date, relatively few have been identified as major contributors to the global burden of human diseases (Kuris, 2012) and animal agriculture (Dubey, 1977). The protozoa represent 19% of all human parasites (83 out of 437 species to-date) and are associated with 30% of parasite-induced human morbidity-mortality (Kuris, 2012).

Of the four groups of infectious protozoa (CDC, 2017), the Mastigophora (flagellates) and Sporozoa contain the Kinetoplastidae and Apicomplexa, respectively. It is to these two phyla that belong many of the causative agents of disease: Mastigophora – the insect vector-borne kinetoplastids Trypanosoma brucei (Human African Trypanosomiasis, HAT), Leishmania spp. (leishmaniasis, cutaneous and visceral) and Trypanosoma cruzi (American trypanosomiasis, Chagas’ disease); Sporozoa – the apicomplexan Toxoplasma gondii (toxoplasmosis), Cryptosporidium spp. (cryptosporidiosis) and Eimeria spp. (coccidiosis in poultry and cattle), Theileria spp. (East Coast Fever in cattle) and Plasmodium spp., including Plasmodium falciparum the causative agent of severe malaria and one of the ‘Big Three’ global infectious diseases alongside HIV and tuberculosis (Torgerson & Macpherson, 2011).

Historically, the diseases caused by some of these parasites have been classified as Neglected Tropical Diseases (NTDs) or Neglected Zoonotic Diseases (King, 2011) and were associated with the classical model of the ‘poverty trap’ covering tropical and sub-tropical regions in Africa, Latin America and the Indian subcontinent (Kuris, 2012). However, with global changes in climate and human demographics and associated practices, the classical models do not promise safe boundaries that might contain and/or stop the further global spread of many of these parasitic diseases (Colwell et al. 2011). The problems associated with these pathogens are further aggravated by the lack of effective vaccines (Dumonteil, 2007; Innes et al. 2011; McAllister, 2014; Black & Mansfield, 2016) and the paucity of reliable drugs (Zofou et al. 2014), in addition to the difficulties of vector or reservoir control (Colwell et al. 2011). Therefore, there is a recognized need to find new therapeutic targets in these causative agents in order to develop effective treatment regimens to avoid potentially catastrophic outbreaks, both in terms of human health and economic impact.

This review presents sphingolipid (SL) biosynthesis and ceramide (CER) homoeostasis as a potential gold mine of tractable drug targets for these protozoan parasites.

State-of-the-art treatment of apicomplexan and kinetoplastid diseases

In general, available treatments for the diseases caused by the Kinetoplastidae and Apicomplexa...
are outdated (if not historic), with relatively few examples that were introduced recently, toxic and require a long treatment regimen, and therefore close monitoring of patients.

The kinetoplastid pathogens in focus here all cause NTDs and as such there are significant problems with the available drug regimens:

*Leishmania spp.* The treatment of leishmaniasis often requires a long course of intravenous pentavalent antimony drugs (e.g. Glucantine and Pentostam), aminosidine (paromomycin) or liposomal amphotericin B (Croft & Coombs, 2003; Center for Food Security and Public Health, 2004; WHO, 2004; Kedzierski et al. 2009). The most recent addition was the orally available miltefosine (Sunder et al. 2002; Verma & Dey, 2004), originally developed as an anti-neoplastic agent. Despite its teratogenic effects (Sunder et al. 2002), due to the lack of other effective medications, it has been registered and is now used in India, Colombia, Guatemala and Germany (Soto & Berman, 2006). Other regimens of treatment include Pentamidine (Bray et al. 2003), allopurinol, dapsone, fluconazole, itraconazole and ketoconazole. However, to-date all available chemotherapeutic agents suffer from being toxic (Chappuis et al. 2007) or inaccessible, both geographically and financially, in endemic areas where public health is under-resourced, poor and underdeveloped. Additionally, the lack of effective vaccines (de Oliveira et al. 2009) and the alarming emergence of resistance to these drugs (Croft et al. 2006), combined with the short-lived prevention resulting from applying measures such as vector and reservoir host control (WHO, 2004; Figueiredo et al. 2012), demand an intensive search for alternative antileishmanials to enable effective treatment and control.

*Trypanosoma brucei.* Another compelling example of the shortcomings of available treatments is HAT (Mina et al. 2009; Buckner et al. 2012), where there is a lack of effective vaccines (Black & Mansfield, 2016) and treatment depends on the stage of the disease. Whilst in the first stage, the drugs used are less toxic, easier to administer and more effective, treatment in the second stage requires drugs that can cross the blood-brain barrier, specifically the arsenates (Gibaud & Jaouen, 2010), making them considerably more toxic and complex to administer (Babokhov et al. 2013). Currently, four drugs are registered for HAT treatment and are provided free of charge to endemic countries through a WHO private partnership with Sanofi–Aventis (Pentamidine, melarsoprol and eflornithine) and Bayer AG (suramin) (Schmidt et al. 2012). Unfortunately, all of them exhibit a broad range of adverse effects. Moreover, treatment regimens are usually highly restrictive, particularly in the second stage of the disease, requiring hospital-based I.V. treatment with continuous monitoring.

*Trypanosoma cruzi.* Despite their toxic side-effects, nifurtimox and benznidazole are the only licenced drugs available for treatment of Chagas’ disease (Carabarin-Lima et al. 2013; Bermudez et al. 2016), with the latter being the first choice due to its lower side effects. Also, benznidazole has been implemented in the treatment of women before pregnancy in order to prevent/reduce vertical transmission (Carabarin-Lima et al. 2013; Murcia et al. 2013). Due to the lack alternatives, efforts have been directed towards implementing different treatment regimens in order to reduce toxicity, e.g. intermittent administration schedules, combination therapy and re-purposing of commercial drugs (Bermudez et al. 2016).

Management of apicomplexan infections is also challenging and faces many of the same shortcomings encountered in the treatment of kinetoplastid infections.

*Toxoplasma gondii.* Treatment regimens for toxoplasmosis patients have essentially remained the same since the 1950s (Eyles & Coleman, 1953). They largely depend on the repurposing of antibacterials (sulfadiazine, spiramycin and clindamycin) and antimalarials (pyrimethamine and atovaquone) (Opremcak et al. 1992; Andrews et al. 2014; Antczak et al. 2016) in combination, therapies that target parasite folic acid synthesis, protein synthesis or oxidative phosphorylation (Greif et al. 2001; Antczak et al. 2016). Most of these chemotherapeutics are not readily bioavailable at the site of infection (e.g. unable to cross the blood-brain barrier); cannot be administered by patients with hypersensitivity to sulphonamides; have suspected teratogenic properties (Montoya & Remington, 2008; Paquet & Yudin, 2013); are threatened by the emergence of resistance (Sims, 2009); or require adjuvant therapies (folinic acid supplement) to minimize toxic side effects (for a detailed review see Antczak et al. 2016).

Toxoplasmosis is a representative of the urgent need for new antiprotozoal targets. In addition to the fact that T. gondii is estimated to infect 2–3 billion people worldwide (Welti et al. 2007), its treatment is complicated due to two main factors: (a) the parasite undergoes a complex life cycle with two predominant forms in the human host, namely, tachyzoites (proliferative form) and bradyzoites (encysted form, chronic toxoplasmosis); (b) bradyzoite burden is widespread but usually asymptomatic, although it has been associated with psychiatric disorders (Webster et al. 2013). However, in immunocompromised individuals encysted T. gondii transform into proliferative tachyzoite forms causing symptomatic disease, toxoplasmic encephalitis. As such
**T. gondii** is an opportunistic parasite. Notably, all the above-mentioned drugs act only against the tachyzoite stage with no notable effect against encysted bradyzoites (Antczak et al. 2016). Recent data from our laboratory (Alqaisi et al. 2017) and others (Sonda et al. 2005) have shown that the Aureobasidin A and analogous depsipeptides, known to target yeast SL biosynthesis (Wuts et al. 2015), exhibit activity against bradyzoite *T. gondii*. This class of compounds may offer a potential treatment for chronic toxoplasmosis and, perhaps, some psychiatric disorders; although the mechanism of action is not via inhibition of parasite SL biosynthesis and is yet to be elucidated (Alqaisi et al. 2017).

**Plasmodium falciparum.** Falciparum malaria remains one of the ‘Big Three’, most prevalent and deadly infectious diseases across tropical and subtropical regions, with an estimated 154–289 million cases in 2010 (212 million cases in 2015), and 600,000 (429,000 in 2015) associated deaths; although the actual numbers might be even higher (Biamonte et al. 2013; WHO, 2016).

Similar to *T. gondii*, *Plasmodium* parasite undergoes a complex life cycle with different stages in different organs of the host, rendering treatment challenging: sporozoites and schizonts in the liver, and merozoites, trophozoites and gametocytes in the blood (Dechy-Cabaret & Benoit-Vical, 2012). Artemisinin-based combination therapies (ACTs) are the standard for treating malaria cases with typical partner drugs including lumefantrine and piperaquine, e.g. Coartem™ (Novartis) and Eurartesim™ (Sigma-Tau) (Biamonte et al. 2013). Other regimens include the use of parenteral artesunate (severe malaria) (Dondorp et al. 2010a), primaquine (liver and transmission, gametocyte, stages) (Dondorp, 2013), mefloquine and sulfadoxine/pyrimethamine in combination (effective as single dose antimalarial drug) (Biamonte et al. 2013) and atovaquone/proguanil, Malarone™ (GlaxoSmithKline), as a prophylactic treatment.

However, although combination therapies have now been adopted, resistance against many existing antimalarials has been observed since the 1950s (Bishop, 1951; Hallinan, 1953; Sandosham et al. 1964) and remains a severe threat (Rieckmann & Cheng, 2002; Chiappi et al. 2010; Dondorp et al. 2010b; Newton et al. 2016; Parija, 2016; Menard & Dondorp, 2017; Zhou et al. 2017). This bleak view of the future of available anti-malarial chemotherapeutics makes it imperative to invest more efforts in identifying new potent chemotypes that will offer both efficacy and safety.

**Cryptosporidium spp.** Like *T. gondii*, **Cryptosporidium parvum** and **Cryptosporidium hominis** usually cause a self-limiting disease in healthy individuals but represent a manifest problem in immuno-compromised patients, particularly those with AIDS, where infection leads to acute and protracted life-threatening gastroenteritis (Chen et al. 2002). More recent data have led to a radical reassessment of the impact of cryptosporidiosis, with the number of *Cryptosporidium*-attributable diarrhoea episodes estimated at >75 million in children aged <24 months in sub-Saharan Africa and South Asia where infection is estimated to contribute to >250,000 infant deaths per year (Sow et al. 2016). Current treatment of cryptosporidiosis relies on a single FDA-approved drug, nitazoxanide, which has limited efficacy in those most at risk. More recently, the repurposing of antimalarials, e.g. quinolones and allopurinols, has been proposed (Gamo et al. 2010; Chellan et al. 2017). The distinctive metabolic features of this parasite from other apicomplexan organisms, e.g. no plastid-derived apicoplast and the absence of the citrate cycle and cytochrome-based respiratory chain (Ryan & Hijjawi, 2015), confer several limitations for the identification of targets necessary for the development of anticytosporidial drugs. However, the core metabolic pathways, e.g. energy metabolism and lipid synthesis are still present and exhibit high level of divergence from the mammalian host, thus presenting an opportunity to identify new drug targets that promise effective and selective treatment (Chellan et al. 2017).

**The biological significance of SLs**

SLs are a class of lipids that are ubiquitous in eukaryotic cell membranes, particularly the plasma membrane, as well as in some prokaryotic organisms and viruses (Merrill & Sandhoff, 2002). Since their earliest characterization by Thudichum (1884), they have been a subject of controversy. Initially, they had been considered of structural importance only; however, over the last couple of decades, several reports have revealed their indispensability to a plethora of functions including, but not limited to, the formation of structural domains, polarized cellular trafficking, signal transduction, cell growth, differentiation and apoptosis (Huwiler et al. 2000; Ohanian & Ohanian, 2001; Cuvillier, 2002; Pettus et al. 2002; Buccoliero & Futerman, 2003).

SLs consist structurally of a sphingoid base backbone, e.g. sphingosine (SHP) that can be N-acylated to form CER. To the latter, a variety of head groups: charged, neutral, phosphorylated and/or glycosylated can be attached to form complex SLs, e.g. sphingomyelin (SM), as the primary complex mammalian SL; and inositol phosphorylceramide (IPC) in fungi, plants and numerous protozoa (Fig. 1). These molecules have both polar and non-polar regions giving rise to their amphiphatic character, which accounts for their tendency to aggregate into membranous structures, yet retaining the interfacial ability to interact with various partners, e.g.
involvement of glycosphingolipids (GSLs) in cellular recognition complexes, cell adhesion and the regulation of cell growth (Gurr et al. 2002). Furthermore, the diversity of their chemical structures allows for distinctive roles within cellular metabolism, e.g. the signalling functions of SPH and CER vs sphingosine-1-phosphate (S1P) and ceramide-1-phosphate (C1P) (Merrill & Sandhoff, 2002; Metzler, 2003).

SLs as indispensable structural components

The unique structural features of SLs (the free 3-hydroxy group, the amide functionality and the C4–C5 trans double bond) affect their biophysical properties rendering these molecules different from their glycerolipid counterparts, i.e. SM vs phosphatidylcholine (PC) (Boggs, 1980, 1987; Talbott et al., 2000; Ramstedt & Slotte, 2002). Such interfacial differences give complex SLs, such as SM, the unique ability to form both intra- and intermolecular hydrogen bonds (Bruzik, 1988) that are fine-tuned by the trans double bond (Ramstedt & Slotte, 2002). This ability is reflected in the tendency of SLs to cluster rather than behave like typical ‘fluid’ membrane lipids. Naturally occurring SLs undergo the \( L_\beta \) (gel phase) to \( L_\alpha \) (lamellar phase) transition near the physiological temperature of \( 37 \) °C, in contrast, this transition for naturally occurring glycerolipids is near or below \( 0 \) °C. Additionally, the long saturated alkyl chains of SLs allow them to pack tightly with sterols, stabilized by hydrogen bonding (Ramstedt & Slotte, 2002), to form laterally compact hydrophobic micro-domains commonly known as ‘lipid rafts’ (Futerman & Hannun, 2004). Similar results have been reported with the fungal/plant counterpart of SM, IPC, where it was shown that IPC was able to form sterol containing ordered domains in model systems (Björkbom et al. 2010). These membrane micro-domains can readily segregate from the more disordered and expanded domains of unsaturated
acyl chains of glycerolipids (Merrill & Sandhoff, 2002). They have been proposed to function in a diverse array of processes from polarised trafficking of lipid modified proteins (Brown & London, 1998) and the stabilization of other types of biological structures such as lamellar bodies, to the assembly and activation of signal transduction complexes (Brown & London, 2000; Magee et al. 2002; Pierce, 2002; Vance & Vance, 2002; Hannun & Obeid, 2008). They have also been involved in the formation of detergent-insoluble gel-phase domains (Ramstedt & Slote, 2002) via the extensive hydrogen-bonding network in the head groups of GSLs that have been implicated during the formation of ‘caveolae’ and surface recognition (Merrill & Sandhoff, 2002).

**SLs as indispensable signalling agents**

SLs can also function as bioactive signalling molecules due to their biophysical properties, e.g. the low \( pK_a \) (7–8) of SPH allows it to remain partially uncharged at physiological pH retaining the ability to move across membranes (Merrill & Sandhoff, 2002). Likewise, CER, a neutral species, is able to freely flip flop across membranes (Hannun & Obeid, 2008). Many studies have produced evidence of such signalling functions, e.g. SPH exerts pleiotropic effects on protein kinases; CER mediates many cell-stress responses, including the regulation of apoptosis (Georgopapadakou, 2000); and S1P has crucial roles in cell survival, cell migration and inflammation (Hannun & Obeid, 2008).

**SL metabolism and the rationale for druggability**

The indispensability of SLs for a myriad of cellular processes and functions, ranging from structural integrity to signalling events, makes it unsurprising that the SL biosynthesis is highly conserved in all eukaryotes where it is, alongside its proposed regulators (Holthuis et al. 2006), an essential pathway (Heung et al. 2006; Sutterwala et al. 2007). This has lead the pathway to be considered vital for protozoan pathogenesis and, therefore, a drug target; e.g. SM synthase activity in fungi, plants and protozoa. The latter comprises first the formation of CER in the ER by the action of ceramide synthase (CerS), and then the formation of complex SLs in the Golgi. These products vary depending on the species, and are formed under the catalysis of what could be generic termed SM synthases: SM synthase in mammals and IPC synthase in fungi, plants and protozoa. It is worth mentioning that another Golgi localized metabolic pathway results in the formation of glycosylated CER species, and also contributes to the regulation CER levels (Holthuis & Menon, 2014) (Fig. 2).

**Protozoan parasites vs host: differences & opportunities**

The cross-species differences encountered in the first, SPT-catalysed, step are mostly minor in terms of the chemical structure of the product; mainly due to the chain length of the acyl-CoA utilised in the reaction, e.g. myristoyl-CoA (in *Leishmania* spp. amongst other odd sphingoid base lengths (Hsu et al. 2007)) and palmitoyl-CoA, with the latter more predominant across the Eukaryota pathogens (Yatsu, 1971; Brady, 1978; Chen et al. 1999; Merrill & Sandhoff, 2002), however here our focus will be on the former pathway. Considering the central position of CER, the druggability of SL metabolism revolves around dys-regulation of ‘Ceramide Homeostasis’ (Young et al. 2012) which in turn leads to ripple effects perturbing the balance between the pro-apoptotic CER and the mitogenic diacylglycerol (DAG), consequently determining cell fate (Fig. 2) – a mechanism that has been associated with resistance to anti-cancer treatments (Ségui et al. 2006) and has been reported in protozoan parasites, e.g. Plasmodium (Pankova-Kholmyansky et al. 2003; Labaied et al. 2004). The characterisation of several key enzymes involved in SL de novo biosynthesis has revealed divergence between mammalian and protozoan species. Thus, attention has been given to the exploitation of the SL biosynthetic pathway (parasite and/or host) for new drug targets or regimens (Sugimoto et al. 2004; Zhang et al. 2005; Denny et al. 2006; Tanaka et al. 2007; Pruett et al. 2008; Mina et al. 2009; Tatematsu et al. 2011; Young et al. 2012).
in mammals, *Plasmodium* and *T. brucei* (Richmond et al. 2010; Botté et al. 2013). Further differences may be apparent with respect to the catalysing enzyme, SPT (vide infra). However, clear divergence is observed in the second and the third steps, both of which represent a cell-fate modulator process. CerSs exhibit differential preferences for the chain length of the acyl-CoA substrate (Park et al. 2014) and its hydroxylation pattern (Layre & Moody, 2013), with 6 isoforms present in humans suggesting different role for each CER species produced (Levy & Futerman, 2010; Figueiredo et al. 2012). To-date, one or, maximum, two genes encoding CerS function have been identified in protozoan parasite species (Koeller & Heise, 2011). However, most interesting is the variation in the complex SL formed in the Golgi, reflecting significant differences in the active site of the SL synthases catalysing the transfer reaction. The divergence of the protozoal complex SL synthases, and the synthetic products, with respect to the mammalian host, may provide opportunities to design selective inhibitors. Previously, this step has been validated as a promising drug target in fungi using aureobasidin A (AbA) (Fig. 2) (Denny et al. 2006).

**Serine palmitoyl transferase (SPT)**

SPTs are members of the pyridoxal 5'-phosphate (PLP)-dependent (Sandmeier et al. 1994) α-oxoamine synthase family and share a conserved motif (T[FL][GTS][K][SAG][FLV][G] around the PLP-binding lysine (in bold) (Young et al. 2012). SPT catalyses the first rate-limiting step in the de novo biosynthesis of SLs (Weiss & Stoffel, 1997; Hojjati et al. 2005) (Fig. 2), a reaction involving the decarboxylative Claisen-like condensation of serine and an acyl-CoA to yield the sphingoid base backbone, 3-ketodihydrosphingosine (3-KDS) (Hanada, 2003; Raman et al. 2009; Lowther et al. 2012). Therefore, SPT represents the ‘Gatekeeper’ of the SL biosynthetic pathway.

All eukaryotic SPTs studied to date are ER-resident and membrane bound with a heterodimeric protein core consisting of two subunits sharing ~20% identity: LCB1 and LCB2, ~53 and ~63
kDa respectively (Hanada, 2003; Denny et al. 2004; Han et al. 2004; Chen et al. 2006). The latter contains the canonical PLP cofactor binding site while the former has been suggested to be important for complex stability (Lowther et al. 2012). In contrast, the orthologous SPT from sphingomonad bacteria is a soluble 45 kDa homodimer (Ikushiro et al. 2001). SPT activity in apicomplexan parasites has been detected and was proposed as a potential drug target (Gerold & Schwarz, 2001; Bisanz et al. 2006; Coppens, 2013), however the enzyme(s) responsible have yet to be further characterized (Mina et al. 2017). In contrast, kinetoplastid parasites have been shown to possess a heterodimeric SPT similar to the mammalian orthologue (Denny et al. 2004). Inhibiting SPT activity (e.g. using myriocin, Fig. 2) results in various effects in different species. Mammalian cells exhibited a loss of viability, with a partial loss of SPT function resulting in a rare SL metabolic disease, Hereditary Sensory Neuropathy type I (HSN1) (Hanada, 2003). In contrast, Saccharomyces cerevisiae were found to be relatively tolerant (Nagiec et al. 1994), and Leishmania major lacking LCB2 were viable but unable to differentiate into infective metacyclic forms (Zhang et al. 2003). However, T. brucei procyclin forms in which SPT expression was reduced were non-viable (Fridberg et al. 2008).

The SPT catalyzed reaction product, 3KDS, is subsequently reduced by 3-ketosphinganine reductase to form sphinganine (dihydrosphingosine). Subsequent minor metabolic differences are encountered across different species; mainly concerning the order of the hydroxylation (in fungi and higher plants) and acylation to produce CERs (Sugimoto et al. 2004).

Ceramide synthase

In all eukaryotic systems studied to date, CerSs are ER-resident integral membrane proteins catalysing the N-acetylation of dihydrosphingosine to produce dihydroceramide, which is then oxidized to form CER, the simplest SL species and a key bioactive molecule in numerous cellular pathways (Lahari & Futerman, 2007).

Mammalian CerSs are orthologues of longevity-assurance genes, LAG1p and LAC1p identified in yeast (Guillas et al. 2001). The eukaryotes studied to date have been found to encode at least two CerSs, with humans expressing six – each generating CER with a defined acyl chain length (C18 to C26) (Pewzner-Jung et al. 2006; Levy & Futerman, 2010). Whilst little is known regarding structure-function relationships or regulation of CerSs, the ubiquitous Lag1 motif has been shown to be important for functionality (Spassieva et al. 2006), likely forming part of the active site.

Experimental evidence (from our laboratory and others) has previously indicated the presence of CerS activity in Leishmania spp (Zhang et al., 2003; Denny et al., 2004, 2006) and in T. cruzi (De Lederkremer et al. 2011). More recently LAG1 orthologues have been identified and functionally and molecularly characterized in the latter parasite (Figueiredo et al. 2012). Other results indirectly suggest the presence of such activity in T. brucei (Putnak et al. 1993; Richmond et al. 2010; Smith & Bütikofer, 2010). Similarly, CerS activity in the Apicomplexa has been inferred (Welti et al. 2007; Zhang et al. 2010; Pratt et al. 2013), but remains unexplored.

Once formed in the ER, CER is transported by CER transfer protein CERT in mammals (Kumagai et al. 2005; Kudo et al. 2010; Rao et al. 2014), to the Golgi apparatus where the synthesis of complex SLs occurs (Ohanian & Ohanian, 2001; Bromley et al. 2003; Bartke & Hannun, 2009; Pata et al. 2010). ER CER concentration is kept under tight control as accumulation of CER here has been shown to result in induction of the mitochondrial apoptotic pathway (Vacaru et al. 2009; Tafesse et al. 2014) via an unknown mechanism (Bockelmann et al. 2015).

Sphingolipid synthase

In the Golgi, CER can be phosphorylated by CER kinase (Rovina et al. 2009), glycosylated by glucosyl or galactosyl CerS (Raas-Rothschild et al. 2004), or acquire a variety of neutral or charged head groups under the catalysis of what could be called generically SLSs, to form various complex phosphorysphingolipids. Phylogenetic analyses have identified at least 4 clades of SLS (Huitema et al. 2004; Denny et al. 2006).

In mammals CER is a substrate for the SLS, SM synthase, to produce SM (Huitema et al. 2004). Whilst in fungi and higher plants phytoceramide is utilized by a different SLS, IPC synthase, to produce IPC as the principal phosphorysphingolipid (Nagiec et al. 1997; Wang et al. 2008). This landscape is significantly divergent when it comes to protozoa.

In the kinetoplastid Leishmania spp and T. cruzi, CER acquires a phosphorylinositol head group from phosphatidylinositol (PI) to produce IPC via IPC synthase (Zhang et al. 2005; Denny et al. 2006; Mina et al. 2010), although there are some reports of SM in T. cruzi (Quiñones et al. 2004) (Fig. 2). Whilst Leishmania encodes a single copy IPC synthase, T. cruzi has two highly related copies (Denny et al. 2006). Further divergence, and possible redundancy, is encountered in T. Brucei, which harbours 4 genes that encode SLSs (Denny et al. 2006; Sutterwala et al. 2008). This enzyme portfolio results in a diverse profile of the complex SL species (SM, IPC and ethanolamine phosphorysphingosine (EPC)) which are developmentally
regulated during the life cycle of the parasite (Sutterwala et al. 2008).

In apicomplexan parasites, previous reports have indicated the presence of glycosyl-ceramide and SM in *P. falciparum* and *T. gondii*, as summarized in Zhang et al. (2010). However, other findings reported the presence of EPC in *T. gondii* (Welti et al. 2007) and, more recently, IPC (Pratt et al. 2013). The latter study also characterized *T. gondii* SLS as demonstrating IPC synthase activity in *vivo* (Pratt et al. 2013).

The divergence of SLS function, with respect to the host, seen in both kinetoplastid and apicomplexan protozoan parasites in intriguing and, perhaps, indicated them as a tractable drug target. In support of this hypothesis, ceramide-analogues with anti-Plasmodium activity have already been identified (Labaied et al. 2004).

In general, SLSs are Golgi-resident transmembrane proteines, presumed to have 6 transmembrane domains with the active site facing the Golgi lumen (Holthuis et al. 2006; Sutterwala et al. 2008). Those orthologues identified in kinetoplastids demonstrated two conserved regions (CGDX2SGHT & HYTVX7VX6YX1Y2X2YH) with respect to the animal SM synthases (Huitema et al. 2004; Denny et al. 2006). These regions contain the so-called the catalytic triad (two Histidines and one Aspartate residues) that mediates a nucleophilic attack on lipid phosphate ester during the transferase/hydrolase activity (Mina et al. 2010). Apicomplexan orthologues form a separate evolutionary clade, yet retain the catalytic triad (Denny et al. 2006; Pratt et al. 2013), as does the fungal orthologue AUR1p (Heidler & Radding, 2000).

Further evidence for the essentiality of these residues was provided when mutation of the active histidine of the triad was shown to deactivate fungal IPC synthase and mammalian SM synthase-related activity (Levine et al. 2000; Vacaru et al. 2009). Furthermore, recently it has been shown that substrate selectivity, and so the diversity of SLS activity, may depend on key residues close to the transferase active residues or on a luminal loop of the protein (Sevova et al. 2010; Kol et al. 2017).

In the Eukaryota SLS’s occupy a central position at the intersection of glycerolipids (PI/PC/PE and DAG) and SLSs ([phyto]ceramide and IPC/SM/EPC). Accordingly, these enzymes act as regulators of a delicate balance between pro-apoptotic CER and pro-mitogenic DAG (Holthuis et al. 2006).

The most significant previous example of SL biosynthesis inhibition as a drug target was reported in fungi. Aureobasidin A (AbA), a depsipeptide, was first reported by Ikai et al. (1991) and soon after its antifungal properties were highlighted (Takesako et al. 1993). The target gene was further characterized (Hashidaokado et al. 1995) revealing its identity to be the IPC synthase (AUR1p). AbA is a specific and potent (low nanomolar) inhibitor of the fungal IPC synthase. This ushered in a new era in the search for anti-fungal chemotherapeutics, positioning IPC synthase as a promising, broad spectrum, anti-fungal drug target (Sugimoto et al. 2004). Other specific inhibitors were later added to the arsenal of fungal IPC synthase inhibitors: kaharefungin (Mandala et al. 1997), rustmicin (Harris et al. 1998; Mandala et al. 1998) and others (Ohnuki et al. 2009). Unfortunately, further development of these inhibitors stalled, either due to physical properties, e.g. aureobasidin A is very sparingly soluble in water (Georgopapadakou, 2000; Sugimoto et al. 2004), or because their highly complex chemical structures rendered chemical synthesis challenging, with the few synthetic efforts reported resulting in compounds with either reduced or no activity (Sugimoto et al. 2004; Aeed et al. 2009). However, recent works have highlighted that semi-synthetic strategies may overcome these barriers (Wuts et al. 2013).

Perhaps reflecting the evolutionary divergence of these enzymes, the protozoan IPC synthase orthologues, from *Leishmania major* and *T. gondii* are not susceptible to AbA inhibition (Denny et al. 2006; Pratt et al. 2013). Some studies have reported the inhibitory effects of AbA and analogues against *T. gondii* in culture (Sonda et al. 2005; Alqaisi et al. 2017), however this is not associated with inhibition of SL biosynthesis. Despite this, the protozoan SLS’s remain tractable drug targets with no functional equivalent in mammalian cells. Surprisingly, at least one SLS isoform from *T. brucei* was acutely sensitive to AbA treatment (Mina et al. 2009), although these findings stirred some controversy due, in part, to the redundancy of *T. brucei* SLSs (4 isoforms) compared with the single copy found, for example, in *L. major* and *T. gondii* (Sutterwala et al. 2008).

### The Enigmatic Nature of SL Druggability

#### Difficulties in pinpointing SL functionality

Investigation and deciphering the functions of each specific SL species remains challenging. This is due to the complexity in SL metabolic interconnexions, their varied biophysical properties (neutral or charged), chain length variation, the hydrophobic nature of the involved enzymes and the presence of multiple pathways that can operate in parallel (Hannun & Obeid, 2008). The interaction with other cellular metabolic pathways (e.g. glycerolipid metabolism) introduces another layer of complexity.

Overall, the signalling effect/role of an individual SL could be defined on spatial-temporal basis with at least five parameters: (a) subcellular localisation, (b) regulation (c) chain length specificity, (d) kinetics of trafficking and (e) mechanism of action. For
example, phosphorylation of 1–3% cytosolic SPH may double the levels of S1P that acts on G protein-coupled receptor (GPCR) to elicit a specific response in a particular cellular locality for certain period of time (Hannun & Obeid, 2008). Such signalling events can be described as a function of cytosolic S1P that is regulated by S1P Kinase, with the signal caused through the interaction of S1P with a GPCR. The elucidation of such complex systems remains challenging and a comprehensive discussion of the issue is beyond the scope of this review. However, an additional layer of significance in terms of the pathogenic protozoa arises when considering the SL signalling network in the case of obligate intracellular parasites, where host SL biosynthesis, and its interaction with parasite de novo synthesis, must be taken into account.

Parasite–host SL interplay

The intimate parasite–host interaction in terms of SL metabolism has been well documented; *L. major* pathogenic amastigotes isolated from mammalian hosts showed normal IPC levels (Zhang et al. 2005) despite lacking LCB2, a functional SPT and the ability to synthesis CER de novo. Alterations in host, macrophage, cell SL biosynthesis upon infection may compensate for this deficiency (Ghosh et al., 2001, 2002). These studies suggest a complex and multifaceted interplay between host and parasite SL metabolism comprising nutritional factors and signalling pathways that could modulate parasite survival and/or host defence (Zhang et al. 2010). Similar observations have been reported in the apicomplexan parasites (Romano et al. 2013). This highlights the striking potential of host and parasite SL modulation as an anti-protozoal target, as is similarly proposed for pathogenic fungi (Zhang et al. 2010; Ramakrishnan et al. 2013).

Perspective

Classically dissecting the role and locale of critical enzymatic steps in SL biosynthesis and assessing the effect on the parasite fitness and virulence could turn into an overwhelmingly challenging task aggravated by: the complexity of the metabolic pathway itself; the ability of the parasite to salvage (Coppens, 2013), hijack and remodel host SL; and developmental regulation during the parasitic life cycle, which adds another layer of intricacy rendering the deconvolution of any observed effects difficult to interpret. Fortunately, many of those problems can be now overcome with advances in technology. High resolution localization studies in protozoan parasites can benefit greatly from new microscopic techniques such as Airy-scan (Huff, 2015), super-resolution microscopy (Florentinio et al. 2014) and upcoming technologies, e.g. phase-modulation nanoscopy (Pal, 2015; Ward & Pal, 2017), which can elucidate spatial arrangement of proteins of interest within the parasite to reveal potential interaction partners and shed light on mechanistic features. Similarly, new advances in chemical probes, and SL analogues in particular, such as bifunctional lipid technology (Haberkant & Holthuis, 2014) coupled with high throughput proteomic (Ramaprasad et al. 2015), could identify different interaction partners that would help map the biosynthetic pathway and its critical interactions. The effects of these probes on the parasite (and host) cell can now be comprehensively evaluated by monitoring the transcriptome, proteome, metabolomics (Watson, 2010) and lipidome (Marechal et al. 2011). Such studies could reveal multiple windows of opportunity to exploit as potential drug targets. The targets identified in this way can now be rapidly genetically validated in the parasitic protozoa by applying modern gene editing technologies, such as CRISPR/Cas9 (Sugi et al. 2016). Compared with the classical methodologies, this tool enables fast and efficient application for single gene (Serpeloni et al. 2016), and systematic genome-wide knockout generation (Sidik et al. 2016). Additionally, the development of novel orthogonal approach for conditional knockout strategies, e.g. tetracycline-induced gene disruption Tet-system (Meissner et al. 2002), rapamycin-induced Cre recombinase-assisted gene excision (Andenmatten et al. 2013; Collins et al. 2013; Jimenez-Ruiz et al. 2014), has allowed testing of essential gene functionality, in *Leishmania* spp. (Duncan et al. 2016) and *T. gondii* (Pieperhoff et al. 2015).

Aside from the increase ability to robustly validate targets such as SL biosynthesis, global collaboration between academia and pharmaceutical partners is expediting the process of drug discovery of new anti-protozoal drugs. For example, within the sphere of targeting SL biosynthesis in the protozoa, we have managed several projects with industrial partners, MRCT and Tres Cantos Open Lab Foundation (https://www.openlabfoundation.org, an initiative of GlaxoSmithKline), in the pursuit of identifying new compound scaffolds active against the *Leishmania* spp IPC synthase utilising yeast (Norcliffe et al. 2014) as a vehicle for drug discovery (Denny & Steel, 2015). The generated results and techniques could readily be translated to other disease targets. Other global initiatives include Open Innovation Drug Discovery, Eli Lilly, which is focused on cancer, cardiovascular disease, endocrine disorders, neuroscience and tuberculosis. The Centers for Therapeutic Innovation, facilitates Pfizer and academic researchers to work together in order to develop new biologics programs and WIPO Re:Search, provide participant researchers with access to patents and expertise related to drug
discovery for 19 NTDs, malaria and tuberculosis (Sheridan, 2011).

Finally, SL biosynthesis represents a gold mine for new drug targets alongside at least two axes, de novo synthesis and salvage and remodelling. On one hand, the protozoan de novo SL biosynthetic pathway comprises three key steps, and considering their divergence compared with the mammalian host, identifying specific inhibitors for those could open an opportunity for anti-protozoal drugs with synergistic effects and lower incidences of resistance. On the other hand, the nature of obligate intracellular parasites dictates that further efforts should be directed towards the catabolic/salvage pathway where parasite–host dependencies could be exploited in order to identify additional key steps, or host enzymes, where inhibitors would exert further synergism with the de novo inhibitors.

To summarize, the landscape of anti-protozoan drug discovery requires immediate attention: with the re-evaluation of knowledge gained, the application of recent technologies; and the support of coordinated global discovery efforts. The multifaceted effects of SLs as a dynamic matrix of interaction (spatial and temporal) and function makes SL biosynthesis highly alluring for drug intervention, after all, everybody needs SLs, right?

ACKNOWLEDGMENTS

We thank Dr Ehmk Pohl for helpful discussions.

FINANCIAL SUPPORT

JGM and PWD are supported by the Biotechnology and Biological Research Council (BB/M024156/1 and NPRONET awards).

REFERENCES

Aeed, P. A., Young, C. L., Nagiec, M. M. and Elhammer, A. P. (2009). Inhibition of inositol phosphorylceramide synthase by the cyclic peptide aureobasidin A. Antimicrobial Agents and Chemotherapy 53, 496–504.

Alqaisi, A. Q. I., Mbekani, A. J., Llorens, M. B., Elhammer, A. P. and Denny, P. W. (2017). The antifungal Aureobasidin A and an analogue against the protozoan parasite Toxoplasma gondii but do not inhibit sphingolipid biosynthesis. Parasitology, 1–8: doi: 10.1017/S0031182017000506.

Andenmatten, N., Egarter, S., Jackson, A. J., Jullien, N., Herman, J. P. and Meissner, M. (2013). Conditional genome engineering in Toxoplasma gondii uncovers alternative invasion mechanisms. Nature Methods 10, 125–127.

Andrews, K. T., Fisher, G. and Skinner-Adams, T. S. (2014). Drug repurposing and human parasitic protozoan diseases. International Journal for Parasitology: Drugs and Drug Resistance 4, 95–111.

Antczak, M., Dzitko, K. and Długoszka, H. (2016). Human toxoplasmosis–searching for novel chemotherapeutics. Biomedicine & Pharmacotherapy 82, 677–684.

Babokhov, P., Sanyalou, O. O., Oyibo, W. A., Fagbenro-Beyioku, A. F. and Iriemenam, N. C. (2013). A current analysis of chemotherapy strategies for the treatment of human African trypanosomiasis. Pathogens and Global Health 107, 242–252.

Baron, E. J. (1996). Classification. In Medical Microbiology, 4th edn. (ed. Baron, S. J.). University of Texas Medical Branch at Galveston, Galveston, TX, USA.

Bartke, N. and Hannun, Y. A. (2009). Bioactive sphingolipids: metabolism and function. Journal of Lipid Research 50, S91–S96.

Bermudez, J., Davies, C., Simonazzi, A., Pablo Real, J. and Palma, S. (2016). Current drug therapy and pharmaceutical challenges for Chagas disease. Acta Tropica 156, 1–16.

Biamonte, M. A., Wanner, J. and Le Rock, K. G. (2013). Recent advances in malaria drug discovery. Bioorganic & Medicinal Chemistry Letters 23, 2829–2843.

Bisanz, C., Bastien, O., Grando, D., Jouhet, J., Marechal, E. and Cesbron-Delauw, M. F. (2006). Toxoplasma gondii acetyl-lipid metabolism: de novo synthesis from apicoplast-generated fatty acids versus scavenging of host cell precursors. Biochemical Journal 394, 197–205.

Bishop, A. (1951). Drug-resistance in malaria. British Medical Bulletin 8, 47–50.

Björkholm, A., Ohvo-Rekilä, H., Kankaanpää, P., Nyholm, T. K. M., Westerlund, B. and Slotte, J. P. (2010). Characterization of membrane properties of inositol phosphorylceramide. Biochimica et Biophysica Acta (BBA) – Biomembranes 1798, 453–460.

Black, S. J. and Mansfield, J. M. (2010). Prospects for vaccination against pathogenic African trypanosomes. Parasite Immunology 38, 735–743.

Bockelmann, S., Mina, J., Jain, A., Ehring, K., Korneev, S. and Holthuis, J. C. M. (2015). Molecular dissection of ceramide-induced apoptotic signaling in trypanosomes. PLoS Pathogens 11, e1005077.

Boggs, J. M. (1980). Intermembrane hydrogen-bonding between lipids – influence on organization and function of lipids in membranes. Canadian Journal of Biochemistry 58, 755–770.

Boggs, J. M. (1987). Lipid intermembrane hydrogen-bonding – influence on structural organization and membrane-function. Biochimica et Biophysica Acta 906, 353–404.

Botté, C. Y., Yamayo-Botté, Y., Rupasinghe, T. W. T., Mullin, K. A., MacRae, J. I., Spurch, T. P., Kalanon, M., Shears, M. J., Coppell, R. L., Crellin, P. K., Marechál, E., McConnville, M. J. and McFadden, G. I. (2013). Atypical lipid composition in the purified relict plastid (apicoplast) of malaria parasites. Proceedings of the National Academy of Sciences of the United States of America 110, 7506–7511.

Brady, R. O. (1978). Sphingolipidoses. Annual Review of Biochemistry 47, 687–713.

Bray, P. G., Barrett, M. P., Ward, S. A. and de Koning, H. P. (2003). Pentagonide uptake and resistance in pathogenic protozoa: past, present and future. Trends in Parasitology 19, 212–229.

Bromley, P. E., Li, Y. N. O., Murphy, S. M., Sumner, C. M. and Lynch, D. V. (2003). Complex sphingolipid synthesis in plants: characterization of inositolphosphorylceramide synthase activity in bean microsomes. Archives of Biochemistry and Biophysics 417, 219–226.

Brown, D. A. and London, E. (1998). Functions of lipid rafts in biological membranes. Annual Review of Cell and Developmental Biology 14, 111–136.

Brown, D. A. and London, E. (2000). Structure and function of sphingolipid–cholesterol-rich membrane rafts. Journal of Biological Chemistry 275, 17221–17224.

Bruzik, K. S. (1988). Conformation of the polar headgroup of sphingomyelin and its analogues. Biochimica et Biophysica Acta (BBA) – Biomembranes 939, 315–326.

Buccoliero, R. and Futerman, A. H. (2003). The roles of ceramide and complex sphingolipids in neuronal cell function. Pharmacological Research 47, 409–419.

Buckner, F. S., Waters, N. C. and Avery, V. M. (2012). Recent highlights in anti-protozoan drug development and resistance research. International Journal for Parasitology: Drugs and Drug Resistance 2, 230–235.

Carabarin-Lima, A., González-Vázquez, M. C., Rodríguez-Morales, O., Baylón-Pacheco, L., Rosales-Encina, J. L., Reyes-López, P. A. and Arce-Fonseca, M. (2013). Chagas disease (American trypanosomiasis) in Mexico: an update. Acta Tropica 127, 126–135.

CDC (2017). About Parasites. Centers for Disease Control and Prevention, Atlanta, GA, USA. https://www.cdc.gov/parasites/about.html

Center for Food Security and Public Health, C. o. V. M., Iowa State University, Ames, Iowa 50011 (2004). Leishmaniasis (cutaneous and visceral). Center for Food Security and Public Health, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011.

Chappuis, F., Sundar, S., Hailu, A., Ghalib, H., Rijal, S., Page, J., Reiling, R., Alvar, J. and Boelaert, M. (2007). Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nature Reviews Microbiology, 5, 873–882.

Chellam, P., Salder, P. J. and Land, K. M. (2017). Recent developments in drug discovery against the protozoal parasites Cryptosporidium and Toxoplasma. Bioorganic & Medicinal Chemistry Letters 27, 1491–1501.

Chen, C.-S., Patterson, M. C., Whealeey, C. L., O’Brien, J. F. and Pagano, R. E. (1999). Broad screening test for sphingolipid-storage diseases. Lancet 354, 901–905.
Chen, M., Han, G., Dietrich, C. R., Dunn, T. M. and Cahoon, E. B. (2006). The essential nature of sphingolipids in plants as revealed by the functional identification and characterization of the Arabidopsis LCB1 subunit of serine palmitoyltransferase. *Plant Cell* 18, 3576–3593.

Chen, X.-M., Keithly, J. S., Paya, C. V. and LaRussu, N. F. (2002). Cryptosporidiosis. *New England Journal of Medicine* 346, 1723–1731.

Chinappi, M., Via, A., Marcattili, P. and Tramontano, A. (2010). On the mechanism of chloroquine resistance in Plasmodium falciparum. *Proc Natl Acad Sci USA* 107, 4566–4571.

Collins, C. R., Das, S., Wong, E. H., Andenmatten, N., Stallmach, R., Hackett, F., Herman, J. P., Muller, S., Meissner, M. and Blackman, M. J. (2013). Robust inducible CRE recombination activity in the human malaria parasite *Plasmodium falciparum* enables efficient gene deletion within a single asexual erythrocytic growth cycle. *Molecular Microbiology* 88, 687–701.

Colwell, D. D., Dantas-Torres, F. and Otranto, D. (2011). Vector-borne parasitic zoonoses: emerging scenarios and new perspectives. *Veterinary Parasitology* 182, 14–21.

Coppen, O. W. (2012). Sphingolipid-free *Leishmania* are defective in membrane trafficking, differentiation and infectivity. *Molecular Microbiology* 82, 111–126.

Croft, S. L., Sundar, S. and Fairlamb, A. H. (2002). Sphingosine in apoptosis signaling. *Biochimica et Biophysica Acta* (BBA) – *Molecular and Cell Biology of Lipids* 1585, 153–162.

Dechy-Cros, V. and Benito-Viel, F. (2012). Effects of antimarial molecules on the gametocyte stage of *Plasmodium falciparum*: the debate. *Journal of Medicinal Chemistry* 55, 10128–10144.

De Lederkremer, R. M., Agusti, R. and Docampo, R. (2011). *Parasitology International* 60, 125–128.

Dondorp, A. M., Young, S., White, L., Nguon, C., Day, N. P., Socheat, D. and von Seidlein, L. (2008). Sphingolipid synthesis is necessary for kinetoplast segregation and cytokinesis in *Trypanosoma brucei*. *Journal of Cell Science* 121, 522–535.

Futterman, A. H. and Hannun, Y. A. (2004). The complex life of simple sphingolipids. *EMBO Reports* 5, 777–782.

Gamo, F.-J., Sanz, L. M., Vidal, J., de Cozar, C., Alvarez, E., Lavandera, J.-L., Vanderwall, D. E., Green, D. V. S., Kumar, V., Hasan, S., Brown, J. R., Peishoff, C. E., Cardon, L. R. and Garcia-Bustos, J. F. (2010). Thousands of chemical starting points for antimalarial lead identification. *Nature* 465, 28–39.

Georgopapadakou, N. H. (2009). Antifungals targeted to sphingolipid synthesis: focus on isositol phosphorylceramide synthase. *Expert Opinion on Investigational Drugs* 9, 1787–1796.

Gerold, P. and Schwarz, R. T. (2001). Biosynthesis of glycosphingolipids de novo by the human malaria parasite *Plasmodium falciparum*. *Molecular and Biochemical Parasitology* 112, 29–37.

Ghosh, S., Bhattacharyya, S., Das, S., Raha, S., Maulik, N., Das, D. K., Roy, S. and Majumdar, S. (2001). Generation of ceramide in murine macrophages infected with *Leishmania donovani* alters macrophage signaling events and impairs the parasitic survival. *Molecular and Cellular Biochemistry* 223, 47–60.

Ghosh, S., Bhattacharyya, S., Sirkar, M., Sa, G. S., Das, T., Majumdar, D., Roy, S. and Majumdar, S. (2002). *Leishmania donovani* suppresses activated protein 1 and NF-kappaB activation in host macrophages via ceramide generation. Involvement of extracellular signal-regulated kinase. *Infection and Immunity* 70, 6828–6838.

Gibaud, S. and Jaouen, G. (2010). Arsenic-based drugs: from Fowler’s solution to modern anticancer chemotherapy. In *Medicinal Organometallic Chemistry* (ed. Jaouen, G. and Metzler-Nolte, N.), pp. 1–26. Springer Berlin Heidelberg, Berlin, Heidelberg.

Greif, G., Harder, A. and Haberkorn, A. (2001). Chemotherapeutic approaches to protozoa: Coccidiae – current level of knowledge and outlook. *Parasitology Research* 87, 973–975.

Guillas, I., Kirchman, P. A., Obar, R., Pfeiffer, M., Jiang, J. C., Jazwinski, S. M. and Conzelmann, A. (2001). C26-CoA-dependent ceramide synthesis of *Saccharomyces cerevisiae* is operated by Lsg1p and Lcg1p. *Embo Journal* 20, 2655–2665.

Gurr, M. I., Harwood, J. L. and Frayn, K. N. (2002). *Lipid Biochemistry: An Introduction*, 5th edn. Blackwell Science Ltd, Oxford, UK.

Haberkant, P. and Holthus, J. C. M. (2014). Fat & fabulous: Bisphospholipid functions in the spotlight. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1841, 1022–1030.

Hallinan, T. C. (1953). Drug resistance in malaria. *British Medical Journal* 2, 135–136.

Han, G., Gable, K., Yan, L., Natrajan, M., Krishnamurthy, J., Gupta, S. D., Borovitskaya, A., Harmon, J. M. and Dunn, T. M. (2004). The topology of the Lec1p subunit of yeast serum palmitoyltransferase. *Journal of Biological Chemistry* 279, 53707–53716.

Hanada, K. (2003). Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. *Biochim Biophys Acta* 1632, 16–30.

Hannun, Y. A. and Obeid, L. M. (2008). Principles of bioactive lipid signalling: lessons from sphingolipids. *Nature Reviews Molecular Cell Biology* 9, 139–150.

Harris, G. H., Shafiee, A., Cabello, M. A., Curott, J. E., Genilloud, O., Goklen, K. E., Kurtz, M. B., Rosenbach, M., Salmon, P. M., Thornton, R. A., Zink, D. L. and Mandalia, S. M. (1998). Inhibition of fungal sphingolipid biosynthesis by rustecin, galbonolide B and their new 21-hydroxy analogs. *Journal of Antibiotics* 51, 837–844.

Hashidaiokado, T., Ogawa, A., Endo, M., Takesako, K. and Kato, I. (1995). Cloning and characterization of a gene conferring resistance to the antifungal antibiotic aureobasidin-A (R106-3) in yeast. *FASEB Journal* 9, A1371–A1371.

Heidler, S. A. and Radding, J. A. (2000). Isosteric phosphoryl transferases from human pathogenic fungi. *Biochimica et Biophysica Acta - Molecular Basis of Disease* 1500, 145–152.

Heung, L. J., Luben-Teluca, C. and Del Poeta, M. (2006). Role of sphingolipids in microbial pathogenesis. *Infection and Immunity* 74, 28–39.
Druggability of the protozoan sphingolipid biosynthesis

Hojati, M. R., Li, Z. and Jiang, X.-C. (2005). Serine palmitoyl-CoA transferase (SPT) deficiency and sphingolipid levels in mice. Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids 1737, 46–54.

Holtzhu, J. C. M. and Menon, A. K. (2014). Lipid landscapes and pipelines in membrane homeostasis. Nature 510, 48–57.

Holtzhu, J. C. M. and Tafesse, F. G. and Ternes, P. (2006). The multicentric sphingomyelin synthase family. Journal of Biological Chemistry 281, 29421–29427.

Hsu, F.-F., Turk, J., Zhang, K. and Beverley, S. M. (2007). Characterization of Inositol Phosphorylceramides from Leishmania major by Tandem Mass Spectrometry with Electrospray Ionization. Journal of the American Society for Mass Spectrometry 18, 1591–1604.

Huff, J. (2015). The Airyscan detector from ZEISS: confocal imaging with improved signal-to-noise ratio and super-resolution. Nature Methods 12, ii-i.

Huijtema, K. van den Dikkenberg, J., Brouwers, J. and Holtzhu, J. C. M. (2004). Identification of a family of animal sphingomyelin synthases. Embryo Journal 23, 33–44.

Humiston, R., Rutledge, T., Pfleischfeder, J. and Sandhoff, K. (2000). Physiology and pathophysiology of sphingolipid metabolism and signaling. Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids 1485, 63–99.

Ibáñez, K., Takesako, K., Shiomi, K., Moriguchi, M., Umeda, Y., Yamaoka, K. and Naganuma, H. (1991). Structure of avenacosidin-A. Journal of Antibiotics 44, 925–933.

Ishikuro, H., Hayashi, H. and Kagamiyama, H. (2001). A water-soluble homodimeric serine palmitoyltransferase from Sphingomonas paucimobilis YZ293ST strain. Purification, characterization, cloning, and over-production. Biochimica et Biophysica Acta 1449, 1834–1852.

Innes, E. A., Bartley, P. M., Rocchi, M., Benavidas-Silvan, J., Burrells, A., Hotchkiss, E., Chianini, F., Canton, G. and Katzer, F. (2011). Developing vaccines to control protozoan parasites in ruminants: dead or alive? Veterinary Parasitology 180, 155–163.

Jimenez-Ruiz, E., Wong, E. H., Pai, G. S. and Meissner, M. (2014). Advantages and disadvantages of conditional systems for characterization of essential genes in Toxoplasma gondii. Parasitology 141, 1390–1398.

Kedzierski, L., Sakhanianandewarre, A., Curtis, J. M., Andrews, P. C., Junk, P. C. and Kedzierska, K. (2009). Leishmaniasis: current treatment and prospects for new drugs and vaccines. Current Medicinal Chemistry 16, 599–614.

King, L. (2011). The Causes and Impacts of Neglected Tropical and Zoonotic Diseases: Opportunities for Integrated Intervention Strategies. National Academies Press, Washington, DC, USA.

Koeller, C. M. and Heise, N. (2011). The sphingolipid biosynthetic pathway is a potential target for chemotherapy against Chagas disease. Enzyme Research 2011, 13.

Kol, M., Panatala, R., Nordmann, M., Swart, L., Van Suijlekom, L., Holthuis, J. C. M., Tafesse, F. G. and Ternes, P. (2004). Identiﬁcation of the CERT domain in Leishmania species. Journal of Lipid Research 45, 962–973.

Kol, M., Nordmann, M., Matsumura, T., Kobayashi, S., Hanada, K., Wakatsuki, S. and Kato, R. (2010). Crystal structures of the CERT START domain with inhibitors provide insights into the mechanism of ceramide transfer. Journal of Molecular Biology 396, 245–251.

Kumagai, K., Yasuda, S., Okemoto, K., Nishijima, M., Kobayashi, S. and Hanada, K. (2005). CERT mediates intermembrane transfer of a novel inhibitor of sphingolipid synthesis in a phylum of eukaryotic protozoan parasites. Journal of Biological Chemistry, in press. doi: 10.1074/jbc.M111.792374.

Montoya, J. G. and Remington, J. S. (2008). Management of Toxoplasma gondii infection during pregnancy. Clinical Infectious Disease 47, 554–566.

Murcia, L., Carrilero, B., Munoz-Davila, M. J., Thomas, M. C., Lopez, M. C. and Segovia, M. (2013). Risk factors and primary prevention of congenital Chagas disease in a nonendemic country. Clinical Infectious Diseases 56, 496–502.

Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1994). The LC52 gene of Saccharomyces cerevisiae and the related LC51 gene encode subunits of serine palmitoyltransferase, the initial enzyme in sphingolipid biosynthesis. Proceedings of the National Academy of Sciences of the United States of America 91, 7899–7902.

Nagiec, M. M., Nagiec, E. E., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1997). Membrane and Cellular Biochemistry 131, 554–566.

Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1997). Membrane and Cellular Biochemistry 131, 554–566.

Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1997). Membrane and Cellular Biochemistry 131, 554–566.

Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1997). Membrane and Cellular Biochemistry 131, 554–566.

Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1997). Membrane and Cellular Biochemistry 131, 554–566.

Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1997). Membrane and Cellular Biochemistry 131, 554–566.

Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1997). Membrane and Cellular Biochemistry 131, 554–566.

Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L. and Dickson, R. C. (1997). Membrane and Cellular Biochemistry 131, 554–566.
Pankova-Kholmyansky, I., Dagan, A., Gold, D., Zaslavsky, Z., Skutelsky, E., Gatt, S. and Flescher, E. (2003). Ceramide mediates growth inhibition of the Plasmodium falciparum parasite. Cellular and Molecular Life Science 60, 577–587.

Paquet, C. and Yudin, M. H. (2011). Toxoplasmosis in pregnancy: prevention, screening, and treatment. Journal of Obstetrics and Gynaecology Canada 35, 78–81.

Pariraj, S. C. (2016). Drug resistance in malaria: a predicament. Tropical Parasitology 6, 1.

Park, J. W., Park, W. J. and Futterman, A. H. (2014). Ceramide synthases as potential targets for therapeutic intervention in human diseases. Biochim Biophys Acta 1841, 671–681.

Pata, M. O., Hannun, Y. A. and Ng, C. K.-Y. (2010). Plant sphingolipids: decoding the mysteries of the Sphin. Biochimica et Biophysica Acta 1805, 185–192.

Panaïk, P. K., Field, M. C., Meon, A. K., Cross, G. A., Yee, M. C. and Butikofer, P. (1993). Molecular species analysis of phospholipids from Trypanosoma brucei bloodstream and procyclic forms. Molecular and Biochemical Parasitology 58, 97–105.

Pernaute, B. J., Chaffant, C. E. and Hannun, Y. A. (2002). Ceramide in apoptosis: an overview and current perspectives. Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids 1585, 114–125.

Pewzner-Jung, Y., Ben-Dor, S. and Futterman, A. H. (2006). When do Langes (longevity assurance genes) become CerS (ceramide synthases)? Insights into the regulation of ceramide synthesis. Journal of Biological Chemistry 281, 25001–25005.

Pieperhoff, M. S., Pall, G. S., Jimenez-Ruiz, E., Das, S., Melatti, C., Gow, M., Wong, E. H., Heng, J., Muller, S., Blackman, M. J. and Meissner, M. (2015). Conditional U1 gene silencing in Toxoplasma gondii. PloS ONE 10, e013924.

Pierce, S. K. (2002). Lipid rafts and B-cell activation. Nature Reviews Immunology 2, 96–105.

Pratt, S., Wansadhipathi-Kannangara, N. K., Bruce, C. R., Mina, J. (2016). Novel transcript features. Antimicrobial Drug Resistance: Clinical and Pharmacological Aspects (ed. Maders, D. L.), pp. 1121–1126. Humana Press, Totowa, NJ.

Smith, T. K. and Butikofer, P. (2010). Lipid metabolism in Trypanosoma brucei. Molecular and Biochemical Parasitology 172, 66–79.

Sonda, S., Sala, G., Ghidoni, R., Hempill, A. and Pieters, J. (2005). Inhibitory effect of Asero-biaudens on Toxoplasma gondii. Antimicrobial Agents and Chemotherapy 49, 1794–1801.

Soto, J. and Berman, J. (2006). Treatment of New World cutaneous leishmaniasis with miltefosine. Transactions of the Royal Society of Tropical Medicine and Hygiene 100, 834–840.

Sow, S. O., Msukhe, K., Narra, D., Blackwelder, W. C., Wu, Y., Farag, T. H., Panchalingam, S., Sur, D., Zaidi, A. K. M., Faruque, A. S. G., Saha, D., Huet, D., Ganesan, S. M., Huynh, M.-H., Wang, T., Raj, A., Thiru, P., Sehgal, P. J. P., Carruthers, V. B., Niles, J. C. and Lourido, S. (2016). A genome-wide CRISPR screen in toxoplasma identifies essential apicomplexan genes. Cell 166, 1423–1435.e1412.

Simms, P. F. G. (2009). Drug Resistance in Toxoplasma gondii. In Antimicrobial Drug Resistance: Clinical and Pharmacological Aspects (ed. Maders, D. L.), pp. 1121–1126. Humana Press, Totowa, NJ.

Rovina, P., Schanzer, A., Graf, C., Mechtcheriakova, D., Jaritz, M. and Bornancin, F. (2009). Subcellular localization of ceramide kinase and ceramide kinase-like protein requires interplay of their Pleckstrin Homology domain-containing N-terminal regions together with C-terminal domains. Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids 1791, 1023–1030.

Ryan, U. and Hijjawi, N. (2015). New developments in Cryptosporidium research. International Journal for Parasitology 45, 367–373.

Sandmeier, E., Hale, T. I. and Christen, P. (1994). Multiple evolutionary origin of pyrido-5-phosphate-dependent amino acid decarboxylases. European Journal of Biochemistry 221, 997–1002.

Sandosham, A. A., Eyles, D. E. and Montgomery, R. (1964). Drug-resistance in falciparum malaria in South-East Asia. Medicinal Journal of Malaysia 18, 172–185, 611–630.

Schmitz, T. J., Khalid, S. A., Romanja, A. J., Alves, T. M., Biavatti, M. W., Brun, R., Da Costa, F. B., de Castro, S. L., Ferreira, V. F., de Lacerda, M. V. G., Lago, J. H. G., Leon, L. L., Lopes, N. P., Amorim, R. C. D., Niehues, M., Ogunbve, I. B., Piment, A. M., Wang, X. and Bornancin, F. (2014). Ceramide sphingolipids: beyond the enzymatic potential therapeutic targets. Biochimica et Biophysica Acta (BBA) – Biomembranes 1758, 2104–2120.

Serpeloni, M., Jimenez-Ruiz, E., Vidal, N. M., Kroeker, C., Andematten, N., Lemgruber, L., Moring, P., Pall, G. S., Meissner, M. and Avila, A. (2012). UAP56 is a conserved crucial component of a divergent miRNA export pathway in Toxoplasma gondii. Molecular Microbiology 102, 672–689.

Sevova, E. S., Goren, M. A., Schwartz, K. H., Hsu, F. F., Turk, J., Fox, B. G. and Bangs, J. D. (2010). Cell-free synthesis and functional characterization of sphingolipid synthases from parasitic trypanosomatid protozoa. Journal of Biological Chemistry 285, 20850–20857.

Sheridan, C. (2011). Industry continues dabbling with open innovation models. Nature Biotechnology 29, 1063–1065.

Sidik, S. M., Huet, D., Ganesan, S. M., Huynh, M.-H., Wang, T., Nasamu, A. S., Thiru, P., Sehgal, P. J. P., Carruthers, V. B., Niles, J. C. and Lourido, S. (2016). A genome-wide CRISPR screen in toxoplasma identifies essential apicomplexan genes. Cell 166, 1423–1435.e1412.

Sims, P. F. G. (2009). Drug Resistance in Toxoplasma gondii. In Antimicrobial Drug Resistance: Clinical and Pharmacological Aspects (ed. Maders, D. L.), pp. 1121–1126. Humana Press, Totowa, NJ.

Smith, T. K. and Butikofer, P. (2010). Lipid metabolism in Trypanosoma brucei. Molecular and Biochemical Parasitology 172, 66–79.

Sonda, S., Sala, G., Ghidoni, R., Hempill, A. and Pieters, J. (2005). Inhibitory effect of Asero-biaudens on Toxoplasma gondii. Antimicrobial Agents and Chemotherapy 49, 1794–1801.

Reilly, C. E., Berkeley, L. Y., Livio, S., Tennant, S. M., Guadagni, M., Sullards, M. C., Liotta, D. C. and Merrill, A. H. (1978). Phosphorous vacuole. Host Golgi through the rerouting of selected Rab vesicles to the parasitophorous vacuole. Molecular Biology of the Cell 24, 1974–1997.

Romano, J. D., Sonda, S., Bergbower, E., Smith, M. E. and Oppenheim, I. (2013). Toxoplasma gondii salvages sphingolipids from the host Golgi through the rerouting of selected Rab vesicles to the parasitophorous vacuole. Molecular Biology of the Cell 24, 1974–1997.

Rovina, P., Schanzer, A., Graf, C., Mechtcheriakova, D., Jaritz, M. and Bornancin, F. (2009). Subcellular localization of ceramide kinase and ceramide kinase-like protein requires interplay of their Pleckstrin Homology domain-containing N-terminal regions together with C-terminal domains. Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids 1791, 1023–1030.

Ryan, U. and Hijjawi, N. (2015). New developments in Cryptosporidium research. International Journal for Parasitology 45, 367–373.
and Membranes

185

SMSr controls ceramide homeostasis in the ER.

Hermansson, M., Brouwers, J., Somerharju, P., Rabouille, C. and

Brain

Thudichum, J. L. W.

therapeutic target for the inhibition of hepatitis B virus replication.

Mizokami, M.

Tatematsu, K., Tanaka, Y., Sugiyama, M., Sudoh, M. and

487

(2007). Inhibition of Leishmania (Leishmania) amazonensis growth and

Tanaka, A. K., Valero, V. B., Takahashi, H. K. and Straus, A. H.



Sphingomyelin synthase-related protein SMSr is a suppressor of
ceramide-induced mitochondrial apoptosis. Journal of Cell Science 127,

445–454.

Takesako, K., Kuroda, H., Inoue, T., Haruna, F., Yoshikawa, Y.,

Kato, I., Uchida, K., Hiratani, T. and Yamaguchi, H. (1993). Biological properties of aureobasidin-A, a cyclic depsipeptide antifungal antibiotic.

Journal of Antibiotics 46, 1414–1420.

Talbott, C. M., Vorobyov, I., Borchman, D., Taylor, R. G., DuPré, D.

B. and Yappert, M. C.

Talbott, C. M., Vorobyov, I., Borchman, D., Taylor, K. G., DuPré, D.

–

296.

Veterinary Parasitology

1746.

127

(1993).

Roth, M., Roberts, C. W., Botte, C., Marechal, E. and McLeod, R.

(2004). Possible mechanism of miltefosine-

mediated death of

Toxoplasma gondii

but not growth in Leishmania.

Weiss, B. and Stoffel, W. (1997). Human and murine serine-palmitoyl-CoA transferase. European Journal of Biochemistry 249, 239–247.

Welti, R., Mui, E., Sparks, A., Wernimont, S., Isaac, G., Kirisits, M.,

Roth, M., Roberts, C. W., Botte, C., Marechal, E. and McLeod, R.

(2007). Lipidomic analysis of Toxoplasma gondii infection reveals unusual polar lipids. Biochemistry 46, 13882–13890.

WHO (2004). The World Health Report 2004. Changing History. WHO, Geneva.

WHO (2016). Malaria – Fact Sheet (Dec 2016). WHO, Media Centre, vol.

2017.

Wuts, P. G. M., Simons, L. J., Metzger, B. P., Sterling, R. C.,

Sightom, J. L. and Elhammer, A. P.

Zofou, D., Nyasa, R. B., Nsagha, D. S., Ntie-Kang, F., Meriki, H. D.,

Assob, J. C. N. and Kuete, V.

Zhang, K., Hsu, F.-F., Scott, D. A., Docompo, R., Turk, J. and

Beverley, S. M. (2003). Sphingolipids are essential for differentiation but not growth in Leishmania. Embryo Journal 22, 6016–6026.

Zhang, K., Hsu, F.-F., Scott, D. A., Docompo, R., Turk, J. and

Beverley, S. M. (2005). Leishmania salvage and remodelling of host sphingolipids in amastigote survival and acidocalcisome biogenesis. Molecular Microbiology 55, 1566–1578.

Zhang, K., Rang, J. D. and Beverley, S. M. (2010). Sphingolipids in parasitic protozoa. Advances in Experimental Medicine and Biology 688, 238–248.

Zhou, L. J., Xia, J., Wei, H. X., Liu, X. J. and Peng, H. J. (2017). Risk of drug resistance in Plasmodium falciparum malaria therapy-a systematic review and meta-analysis. Parasitology Research 116, 781–788.