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The complexity of sphingolipid biosynthesis in the endoplasmic reticulum☆,☆☆

Rotem Tidhar, Anthony H. Futerman *

Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

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ABSTRACT

Unlike the synthesis of other membrane lipids, sphingolipid synthesis is compartmentalized between the endoplasmic reticulum and the Golgi apparatus. The initial steps of sphingolipid synthesis, from the activity of serine palmitoyltransferase through to dihydroceramide desaturase, take place in the endoplasmic reticulum, but the further metabolism of ceramide to sphingomyelin and complex glycosphingolipids takes place mostly in the Golgi apparatus. Studies over the last decade or so have revealed unexpected levels of complexity in the sphingolipid biosynthetic pathway, mainly due to either the promiscuity of some enzymes towards their substrates, or the tight selectivity of others towards specific substrates. We now discuss two enzymes in this pathway, namely serine palmitoyltransferase (SPT) and ceramide synthase (CerS), and one lipid transport protein, CERT. For SPT and CERT, significant structural information is available, and for CerS, significant information has recently been obtained that sheds light of the roles of the specific ceramide species that are produced by each of the CerS. We consider the mechanisms by which specificity is generated and speculate on the reasons that sphingolipid biosynthesis is so complex. This article is part of a Special Issue entitled: Functional and structural diversity of endoplasmic reticulum.

1. Introduction

The endoplasmic reticulum (ER) is the major site of membrane lipid biosynthesis in mammalian cells. All three major membrane lipid classes, namely glycerolipids, sterols and sphingolipids (SLs), begin their lives in the ER prior to their removal and subsequent transport to the distinct sub-cellular localizations where they reside [1][1]. However, SLs are unique among membrane lipids inasmuch as a number of their biosynthetic steps take place in the Golgi apparatus. This review will focus on SL biosynthesis in the ER, and in particular, on the biosynthesis of dihydroceramide and ceramide, the backbone of all complex SLs [2], and on the transport of ceramide from the ER to the Golgi apparatus.

2. SL structure

Ceramide (Fig. 1) is generated de novo in the ER by a biosynthetic pathway that begins with the condensation of L-serine and palmitoyl-CoA by serine palmitoyltransferase (SPT), generating 3-ketosphinganine [2]. However, over the past few years, a number of other amino acids and fatty acyl CoAs of varying chain lengths have been shown to act as substrates for SPT (see below). 3-Ketosphinganine is subsequently reduced to sphinganine via 3-ketosphinganine reductase. This is followed by *N*-acylation of sphinganine (where the '*N*' signifies that acylation takes place on the free amino group of sphinganine) via sphinganine *N*-acyl transferase to form dihydroceramide, and subsequent reduction of dihydroceramide to ceramide by dihydroceramide desaturase. Sphinganine *N*-acyl transferase can also use sphingosine as substrate (sphinganine and sphingosine differ by the presence of a *trans* double bound in the 4,5 position, which is found in sphingosine but not in sphinganine); evidence is accumulating that this *N*-acyl transferase is relatively promiscuous with respect to the sphingoid long chain base, whereas it is highly specific towards the length of the acyl chain, as discussed in detail below. The *N*-acyl transferase has become known generically as 'ceramide synthase' (CerS) [3]; formally, this enzyme should be known as (dihydro)ceramide synthase, since it converts sphinganine to dihydroceramide in the biosynthetic pathway in the ER but can also convert sphingosine derived from the degradative pathway directly to ceramide; however, the generic name, CerS, has become most well-used.

After formation of ceramide in the ER, most subsequent steps of SL biosynthesis take place in the Golgi apparatus [4]. Interest in the mode of transport of ceramide from the ER to the Golgi apparatus was sparked in the early 2000s by the discovery of the ceramide transfer

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* Corresponding author at: The Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel. Tel.: +972 8 9342704; fax: +972 8 9344112.

E-mail address: tony.futerman@weizmann.ac.il (A.H. Futerman).

[1] We dedicate this review to the memory of Richard (Dick) Pagano (1944–2010), a giant in the field of intracellular lipid transport.

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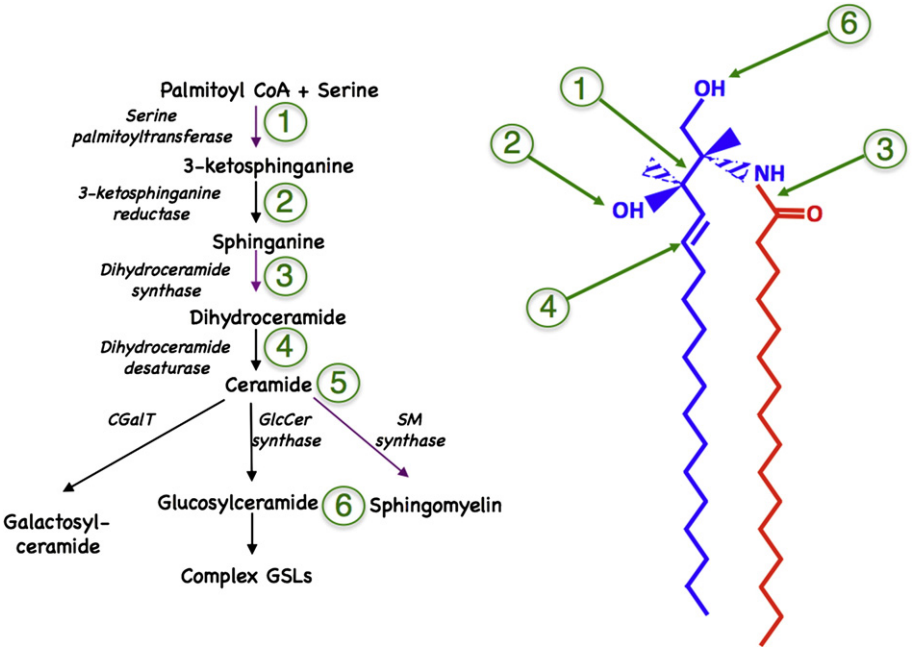

Fig. 1. The pathway of sphingolipid biosynthesis. The left-hand panel shows the pathway of SL biosynthesis, with enzymes indicated in italics, and the right-hand panel shows the structure of ceramide. Each number in the left-hand panel corresponds to a chemical reaction in the right-hand panel, with the exception of step 5, which refers to the ability of CERT to remove ceramide from the ER and deliver it to the Golgi apparatus. Note that step 6 includes formation of SM, GlcCer or GalCer.

protein, CERT [5,6], which was shown to deliver ceramide from the ER for the synthesis of sphingomyelin (SM) in the lumen of the Golgi apparatus; the current consensus is that glucosylceramide (GlcCer), which is also synthesized in the Golgi apparatus, but on the cytosolic surface [7], utilizes ceramide derived from vesicular transport from the ER.

This review will focus on three critical steps of SL synthesis; the initial step of SL formation by SPT (step *1* in Fig. 1), the *N*-acylation of sphingoid long chain bases (step *3* in Fig. 1), and the removal of ceramide from the ER (step *5* in Fig. 1). Work over the past decade or so has led to many unexpected findings about the complexity of each of these steps. In parallel, lipidomics studies have uncovered many more individual SL structures than previously assumed [2]. We will focus on the major mechanisms by which this diversity is generated in the three steps mentioned above. In the case of SPT and CERT, significant structural information is available which impacts upon our understanding of how this diversity is generated, but much less structural information is available for the CerS.

3. SPT — it's not all in the name

As mentioned above, and as implied by its name, SPT is usually considered to use L-serine and palmitoyl-CoA for the formation of 3-ketosphinganine, even though studies performed nearly 30 years ago suggested that other fatty acyl CoAs could also be utilized by SPT [8]. SPT, the rate-limiting step in SL biosynthesis [9,10], belongs to the α-oxoamine synthase family and is pyridoxal 5′-phosphate-dependent [11]. SPT activity can be modulated by the use of specific inhibitors, such as myriocin [9,10], or by modifying substrate supply by enhancing the rate of amino acid import [12,13].

SPT is a heteromeric protein located in the ER of eukaryotic cells, but located in the cytoplasm of the gram-negative bacterium, *Sphingomonas paucimobilis* [14]; the latter was the source of the enzyme used to determine the first crystal structure of SPT [14]. Structural studies revealed that the pyridoxal 5′-phosphate cofactor binds covalently to a lysine residue (Lys265) and that the active site comprises residues from both SPT subunits [14]. The two mammalian SPT subunits are known as long chain bases 1 and 2 (LCB1/LCB2) or as SPT1 and SPT2 [15,16], with SPT2 found as two isoforms (LCB2a and LCB2b (also known as SPT3)) [17,18]; LCB1 and LCB2 knock-out mice are embryonically lethal while conditional knock-outs are available [19–21]. The SPT heterodimer can also associate with other small subunits; in yeast, Tsc3p is required for maximal SPT activity [22] and in mammals, two subunits, ssSPTa and ssSPTb (small subunits of SPT), substantially enhance SPT activity [23]. Combinations of different subunits confer different specificities towards acyl CoAs [23]. Thus, the complex of LCB1/LCB2a/ssSPTa shows a strong preference for C16-CoA (palmitoyl CoA), whereas LCB1/LCB2b/ssSPTa can also use C14-CoA (myristoyl CoA), and ssSPTb confers specificity towards C18-CoA (stearoyl CoA) [23]. Another study suggested that LCB2b is responsible for the generation of C14- and C16-sphingoid bases, making LCB2b functionally distinct from the LCB2a subunit [24]. In addition, sphingoid long chain bases with a chain length of 16 carbons demonstrate limited N-acyl chain diversity, generated mainly by CerS1 (see below) [25]. Thus, SPT can use at least three different acyl CoAs for formation of 3-ketosphinganine depending on the combination of the subunits.

Serine is not the only amino acid to be used by SPT, as recent studies have suggested that mutant forms of SPT are able to utilize alanine and glycine [26,27]. This was discovered after the unravelling of the molecular basis of hereditary sensory neuropathy type I (HSAN1), an autosomal dominant genetic disorder caused by mutations in SPT [23,28–30]. Initially it was thought that mutant SPT was inactive and that the basis of HSAN1 was haplo-insufficiency [29,31]. However, more recently it was shown experimentally that a mutation causing structural perturbations (C133W) [10] in LCB1 alters the specificity of SPT such that it uses alanine, rather than serine, to generate 1-deoxysphinganine [31,32]. Remarkably, oral administration of L-serine prevented accumulation of deoxysphinganines and alleviated HSAN1 symptoms [33]. Deoxysphinganines are found at low levels in normal individuals [34] suggesting that wild type SPT can use amino acids other than L-serine [35]. Moreover, SPT was shown to form a complex with the Orm family proteins [36]. Orm1 and Orm2 are ER membrane proteins, which play central role in lipid homeostasis. In their absence, long chain bases accumulate [37]. In summary, together with the fact the amino acid and acyl CoA specificity of SPT