A Stroll Down the CerS Lane

4

Iris D. Zelnik, Batsheva Rozman,
Eden Rosenfeld-Gur, Shifra Ben-Dor,
and Anthony H. Futerman

Abstract

The majority of enzymes in the sphingolipid (SL) biosynthetic pathway have been identified over the past couple of decades. Despite significant work, and despite their crucial and central roles in SL synthesis, significant information is still lacking concerning the enzymes that catalyze the *N*-acylation of sphingoid long chain bases, namely the ceramide synthases (CerS), a family of six mammalian genes originally named longevity assurance (Lass) genes. Each of these six endoplasmic reticulum (ER) membrane-bound enzymes utilizes a relatively restricted sub-set of fatty acyl-CoAs for *N*-acylation, but are far more promiscuous about the use of long chain bases. The reason that mammals and other species have multiple CerS, generating a specific subset of ceramides, is not yet known, but implies an important role for ceramides containing specific fatty acids in cell physiology. In this brief chapter, we will stroll down the CerS lane and discuss what is known, and what is not known, about this important enzyme family.

Keywords

Ceramide synthase · Ceramide · Sphingolipids · *N*-acylation · *N*-acyl transferase

Abbreviations

CerS	(dihydro)ceramide synthases
CK2	casein kinase 2
ER	endoplasmic reticulum
FB1	fumonisin B1
FTY720	fingolimod
FTY720-P	FTY720 phosphate
HEK	human embryonic kidney
IMPs	integral membrane proteins
Lass	longevity assurance
LCB	long chain base
miRs	micro-RNAs
S1P	sphingosine 1-phosphate
SLs	sphingolipids
SPT	serine palmitoyl transferase
TLC	TRAM-Lag-CLN8
TMD	transmembrane domain
TRAM	translocating chain-associating membrane
YFP	yellow fluorescent protein

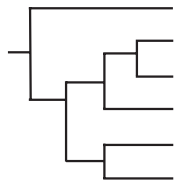
Work in the Futerman laboratory on the CerS is supported by the Israel Science Foundation (1728/15) and by a research grant from the Estate of Emile Mimran.

I. D. Zelnik · B. Rozman · E. Rosenfeld-Gur
A. H. Futerman (✉)
Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot, Israel
e-mail: tony.futerman@weizmann.ac.il

S. Ben-Dor
Life Sciences Core Facilities, Weizmann Institute of Science, Rehovot, Israel

J. Stiban (ed.), *Bioactive Ceramides in Health and Disease*, Advances in Experimental Medicine and Biology 1159, https://doi.org/10.1007/978-3-030-21162-2_4

Table 4.1 Phylogeny of CerS

Phylogeny[a]	Name	Amino acid seq.	Protein size (kD)	Acyl-CoA specificity	Main mRNA tissue distribution[b]
	CerS1	337	39.5	C18	Brain, Skeletal muscle, Lymph nodes, Testis
	CerS2	380	44.9	C22-C24	Bone marrow, Intestine, Kidney, Liver
	CerS3	383	46.2	C26 and higher	Prostate, Skin, Testis
	CerS4	394	46.4	C18-C20	Heart, Leukocytes, Liver, Skin
	CerS5	392	45.7	C16	Heart, Liver, Skeletal muscle, Testis, Kidney
	CerS6	384	44.9	C14-C16	Intestine, Lymph nodes, Spleen, Kidney

CerS phylogeny and primary structure characteristics are shown along with the fatty acyl-CoAs that they each utilize for ceramides synthesis. The four mouse tissues displaying highest mRNA levels for each specific CerS are indicated. The data is based on Refs. [a]Tidhar and Futerman (2013) and [b]Laviad et al. (2008)

4.1 A Stroll Through the CerS Sequence, from N- to C-Terminus

Ceramide, the backbone of all sphingolipids (SLs), is a bioactive second messenger, regulating many vital biological processes (Hannun and Obeid 2018). Ceramide can be produced by two pathways, either by the hydrolysis of complex SLs such as sphingomyelin (SM) (Clarke et al. 2006), or by *de novo* synthesis. The latter begins with the condensation of palmitoyl-CoA and serine by serine palmitoyl transferase (SPT), forming 3-ketosphinganine, which by subsequent reduction generates sphinganine via 3-ketosphinganine reductase. This step is followed by the *N*-acylation of sphinganine via sphinganine *N*-acyl transferase, also known as (dihydro)ceramide synthase (CerS), to form dihydroceramide. Dihydroceramide is converted to ceramide via dihydroceramide desaturase, which forms a *trans* double bond at the 4-5 position. Ceramide is the structural unit of all SLs, and is further used for the synthesis of more complex SLs, by addition of different head groups. The formation of ceramide takes place on the cytosolic leaflet of the endoplasmic reticulum (ER) (Futerman and Riezman 2005).

In mammals, (dihydro)ceramides are synthesized by a family of six CerS, transmembrane proteins located in the ER, with each using fatty acyl-CoAs of defined chain length for ceramide synthesis (Pewzner-Jung et al. 2006) (Table 4.1). Despite their crucial roles in SL synthesis, the three-dimensional structure of CerS is not available, likely due to difficulties in purifying and crystallizing multi-membrane-spanning proteins, which require extraction from the ER membrane in such a way that they retain their enzymatic activity (Lewinson et al. 2008), which is a notoriously difficult proposition. As a result, current information about their membrane topology, substrate binding sites and modes of substrate selectivity is currently inadequate.

Although no 3D structures are currently available, in silico studies, such as use of software to predict the number and topology of transmembrane domains (TMDs), suggest six TMDs (Fig. 4.1a). In a study using 19 different programs (Tidhar et al. 2018), an amino acid residue was considered part of a TMD if at least 10 of the 19 prediction programs identified it as such. Previous data suggested the N- and C- termini of CerS reside on opposite sides of the ER membrane (Mizutani et al. 2005; Laviad et al. 2012), and current data is consistent with the notion that the fourth TMD is unlikely to completely cross the ER membrane (Tidhar et al. 2018) (Fig. 4.1b). Importantly, the majority of the programs gave similar predictions for the first two and last two TMDs although there was little consensus regarding the intermediate TMDs which are likely to contain the active site.

In this chapter, we will walk through the CerS sequence and discuss structural and molecular features of the CerS, strolling from the N- to the C-terminus.

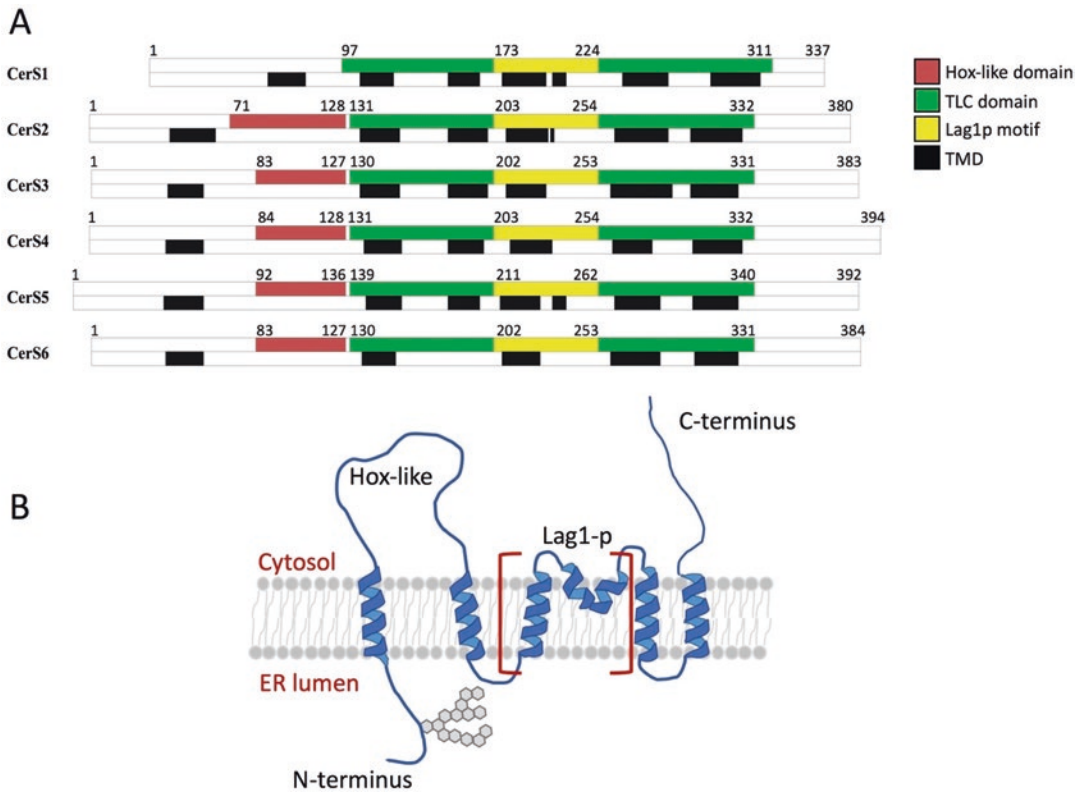


Fig. 4.1 CerS domains and putative topology. (**a**) Schematic representation of human CerS1-6 including the location of three main domains and TMDs. Residues were considered part of a TMD if the majority of the TMD prediction programs (>10/19) predict a TMD. (**b**) Putative topology of the Hox-like containing CerS (i.e. Cer2-6). Red brackets indicate the area where TMD prediction programs are in significant disagreement. Hexagons indicate glycosylation site

4.1.1 The CerS N-Terminus Faces the ER Lumen and Contains a Glycosylation Site

Sequence alignment of the N-termini of the human CerS (Fig. 4.2a) reveals high similarity between CerS2-6 (~50–70%) with substantial identity (~40–50%) (Fig. 4.2b). CerS1, however, shows no similarity to the other five CerS and cannot be aligned with the other CerS in this region (Fig. 4.2a). This is not surprising since phylogeny analysis has shown CerS1 to be in a separate lineage compared to CerS2-6, which forms a separate branch (Pewzner-Jung et al. 2006) (Table 4.1).

A conserved *N*-glycosylation motif (NXT, NXS) is found in CerS2 and CerS4-CerS6 (Fig. 4.2a). CerS2, CerS5 and CerS6 have been shown experimentally to be modified by *N*-glycosylation at Asn-19, Asn-26 and Asn-18, respectively (Mizutani et al. 2005). It was recently shown that, in contrast to CerS3, which is not modified by *N*-glycosylation, CerS4 also undergoes *N*-glycosylation at Asn-19 (Tidhar et al. 2018). Since the initial enzymatic steps of *N*-glycosylation are facilitated by enzymes restricted to the lumen of the ER (Breitling and Aebi 2013), it was inferred that the N-termini of CerS2, and CerS4-6 are located in the ER lumen (Mizutani et al. 2005; Tidhar et al. 2018) (Fig. 4.1b). Due to the high similarity between the CerS in the N-terminal region, with the exception of CerS1, (Fig. 4.2a) it is reasonable to assume that the N-terminus of all of the mammalian CerS reside in the ER lumen. Interestingly, *N*-glycosylation is not essential for the ceramide synthesis activity of CerS; indeed,

A

```
CerS1          MAAAGPAAGPTGPEPMPSYAQLVQRGWGSALAAARG

                              ˅
CerS2          MLQTLYDYFWWERLWLPVNLTWADLEDR-DGRVYAK
CerS3          MFWTFKEWFWLERFWLPPTIKWSDLEDH-DGLVFVK
CerS4          MLSSFNEWFWQDRFWLPPNVTWTELEDR-DGRVYPH
CerS5     MATAAQGPLSLLWGWLWSERFWLPENVSWADLEGPADGYGYPR
CerS6          MAGILAWFWNERFWLPHNVTWADLKNTEEAT-FPQ
                        ◦ * ◦* *** ◦  * ◦*   ◦◦
```

B

	CerS2	CerS3	CerS4	CerS5	CerS6	Identity
CerS2	----	50	55.3	43.6	39.5	CerS2
CerS3	63.2	----	52.6	38.1	34.2	CerS3
CerS4	71.1	68.4	----	46.2	39.5	CerS4
CerS5	56.4	50	56.4	----	38.5	CerS5
CerS6	63.2	50	63.2	61.5	----	CerS6
Similarity	CerS2	CerS3	CerS4	CerS5	CerS6	

Fig. 4.2 Sequence similarity of the N-termini of CerS. (**a**) A comparison of the N-termini of human CerS1-6 by ClustalW multiple alignments. Asterisks (∗) indicate identity in CerS2–6. Circles (°) indicate similarity between CerS2-6. Arrowheads indicate the conserved glycosylation sites. (**b**) Percent identity (light grey) and similarity (dark grey) of human CerS2-6 in the N-terminal region analyzed using EMBOSS Needle

CerS5 activity increases upon de-glycosylation *in vitro* and a possible role for *N*-glycosylation in protein folding was suggested (Tidhar et al. 2018).

4.1.2 The First TMD Targets CerS to the ER

Integral membrane proteins (IMPs) such as CerS are normally inserted into the ER membrane by one of two parallel pathways. The classical co-translational pathway involves targeting by the signal recognition particle followed by membrane insertion through an ER-bound translocon (Rapoport et al. 2004). In rare cases, IMP TMDs are post-translationally inserted into the membrane (Borgese and Fasana 2011).

Since the CerS sequence does not encode a classical signal peptide, we recently studied its mode of insertion into the ER membrane. Chimeric proteins were constructed, composed of the N-terminal sequence of CerS5 fused to yellow fluorescent protein (YFP). We examined two CerS5 sequences, the first including the N-terminus lumenal portion (residues 1-43) and the second consisting of the N-terminus including the first putative TMD (residues 1-66) (Fig. 4.3a). The expression of these constructs clearly showed that the first TMD of CerS5 is essential for insertion of the protein into the ER membrane (Fig. 4.3b). These results were further verified by examining glycosylation of the chimera, confirming that the N-terminus of CerS5$^{(1\text{-}66)}$-YFP chimera indeed resides in the ER lumen (Fig. 4.3c). Since the CerS5$^{(1\text{-}43)}$-YFP chimera failed to co-localize in the ER, along with the fact that expression of a partial CerS sequence was sufficient for targeting of the chimera to the ER, we suggest CerS are inserted into the ER membrane co-translationally via the first TMD.

4.1.3 A Conserved, Enigmatic Homeobox-Like Domain

Approximately two decades ago, the first ceramide synthases, Lag1 and Lac1, were described in yeast (Guillas et al. 2001). The mammalian homolog, *uog1*, now known as CerS1, complemented the lethality of a *lag1*Δ*lac1*Δ double deletion in yeast (Jiang et al. 1998) due to its ability to recover ceramide synthase activity (Venkataraman et al. 2002). An extensive database search revealed that a large

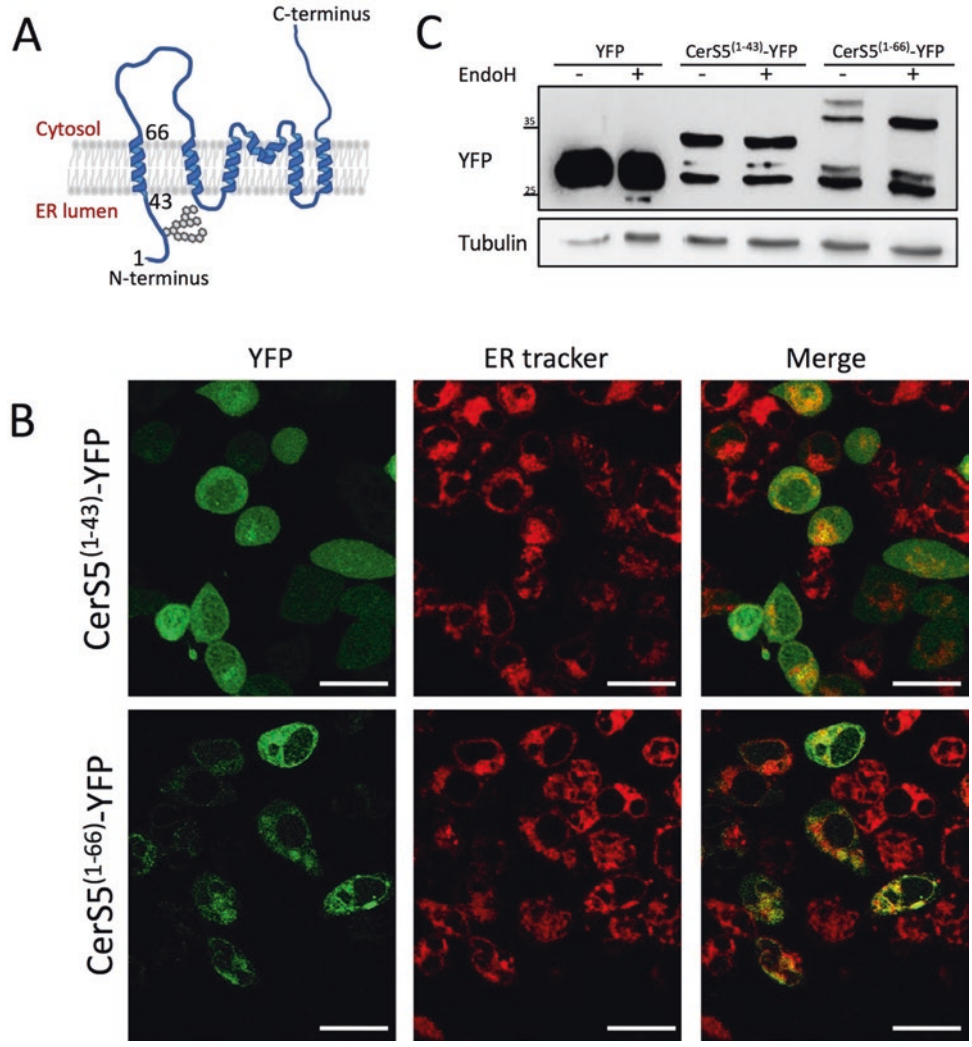


Fig. 4.3 The first TMD is necessary for CerS translocation to the ER membrane. (**a**) The location of residues 1, 43 and 66 in CerS5 are indicated along with the glycosylation site, based on the putative topology designated in Fig. 4.1b. (**b**) Localization of 43 or 66 residues from the N-terminus of CerS5 conjugated to YFP (green) in human embryonic kidney (HEK) cells. ER tracker was used as control (red). Scale bar 20 μm. (**c**) Cell lysates from HEK cells overexpressing the indicated CerS5-YFP construct were incubated with or without endoH prior to Western blotting using an anti-YFP antibody. Molecular weight markers are indicated. Tubulin was used as a loading control

subset of *lag1* homologs in higher organisms contain a Hox-like domain (Venkataraman and Futerman 2002), including all mammalian CerS, with the exception of CerS1. The Hox-like domain is conserved in insects and vertebrates, and in mammals is located after the first TMD in a loop which is predicted to face the cytosol (Venkataraman and Futerman 2002) (Fig.4.1b). The Hox-like domain is homologous to the classic Hox domain derived from homeobox proteins, sequence specific transcription factors important in development (Gehring 1994). These 60-amino-acid long domains are involved in sequence specific recognition and DNA-binding.

Although it has been proposed that the Hox-like domain of CerS may be involved in transcriptional regulation directly linked to ceramide levels, or to rates of ceramide synthesis, there is only one study that supports this suggestion. Schlank, a Drosophila CerS ortholog, downregu-

lates gene expression of *lip3* and *magro* by binding to their promotor regions via the Hox-like domain (Sociale et al. 2018). In the same study it was also shown that CerS2 alters the expression of *lip3*, however no other mammalian CerS were examined. Since the Hox-like domain of CerS2 is the longest of all the Hox-like domains (Fig. 4.1a) and is closest to that of homeobox proteins which act as transcription factors, it will be crucial to study whether other CerS can alter transcription. Additionally, it will be necessary to demonstrate a direct interaction of the CerS with the promotors. The 3D structures of the Hox-like domain of mouse CerS5 and CerS6 have been solved (2CQX and 1X2M, respectively) as part of a larger study of Hox domain structures.

The majority of the Hox-like domain is not required for CerS activity, since upon its deletion in CerS5 and CerS6, there was no alteration in CerS activity assayed *in vitro*. However, a conserved motif of 12 amino acid residues that flanks the end of the Hox-like domain is essential for activity (Mesika et al. 2007). More specifically, two positively charged residues within this 12-residue motif, Lys-134 and Lys-140 in CerS5, are essential for CerS activity. Site-directed mutagenesis of either residue to Ala resulted in 50% loss of activity, whereas mutation of both lead to complete loss of activity. This region is conserved across species, and this therefore suggests an as yet unknown and critical role of the Hox-like domain in CerS structure-function. That being said, even though CerS1 and its yeast homologs, Lag1 and Lac1, lack this domain, they still have CerS activity. Many unresolved questions remain regarding the role of this domain in regulating either CerS activity or in regulating other cellular processes.

4.1.4 The TLC Domain Contains the Active Site and Determines Substrate Specificity

All CerS share a common domain, namely the TRAM-Lag-CLN8 (TLC) domain, a region of ~200 residues also found in other protein families including the translocating chain-associating membrane (TRAM) protein, and CLN8, a protein that is mutated in the human genetic disease, neuronal ceroid lipofuscinoses (Winter and Ponting 2002). However, when carefully examining the TLC domain sequence in CerS, significant differences from that of TRAM and CLN8 become apparent, which suggests that a new classification may be required.

4.1.4.1 The Lag1p Motif May Contain the *N*-Acylation Site

A key example of this distinction is found when examining the Lag1p domain, a stretch of 52 amino acids residues within the TLC domain (Fig. 4.1), and a highly conserved region shared only by the CerS homologs (Jiang et al. 1998). Critical and conserved residues within the Lag1p motif, including two adjacent histidine residues, have been identified as essential for the activity of mouse and human CerS, as well as the yeast homolog, Lag1 (Kageyama-Yahara and Riezman 2006; Spassieva et al. 2006). Although no direct evidence has yet been obtained to support this suggestion, it is generally assumed that these highly conserved histidine residues are involved in catalysis and/or substrate binding (Winter and Ponting 2002). A number of studies demonstrated that *de novo* ceramide formation occurs on the cytosolic leaflet of the ER membrane (Mandon et al. 1992; Hirschberg et al. 1993) (Fig. 4.1b), although these studies, performed before molecular identification of the CerS and using techniques such as proteolytic cleavage of microsomes, cannot be taken as definitive proof that the active site residues of the CerS are located near to the cytosolic leaflet of the ER membrane.

4.1.4.2 Long Chain Base Specificity

Ceramides consist of a sphingoid long chain base (LCB) to which a fatty acid is linked by an amide bond at C-2. The LCB contains two chiral carbon atoms (carbons 2 and 3). Natural SLs occur in the *D*-erythro (2S, 3R) configuration, but three additional stereoisomers exist, *L*-erythro (2R, 3S) (the enantiomer of *D*-erythro-), *D*-threo (2R, 3R) and *L*-threo (2S, 3S). In a study which evaluated the stereospecificity of CerS by *in vitro* analysis

in subcellular fractions as well as in cultured cells, it was shown that the *L*-erythro enantiomers of sphinganine do not act as substrates for the CerS, even though the diastereoisomer, *L*-threo sphinganine, was rapidly metabolized (Venkataraman and Futerman 2001).

SPT, the first enzyme in the SL biosynthesis pathway, determines the chain length of the LCBs. Various LCBs occur in SLs, although for many years the most abundant species in most tissues, d18:1, was considered the main LCB in most tissues. With rapid advances in lipidomics, it is now apparent that there is much more variety in the structure of LCBs than previously appreciated. For instance, in human plasma, d16-LCBs comprise as much as 15% of total circulating sphingoid bases and have been suggested as potential biomarkers for type 2 diabetes (Hornemann et al. 2009; Othman et al. 2012). A study to test the LCB specificity of the CerS isoforms indicated that while CerS2, CerS4, CerS5 and CerS6 all utilize d18-sphinganine more readily than d16-sphinganine, CerS1 displays higher activity towards d16-sphinganine (Russo et al. 2013). The role of d16-ceramides remains unknown. In general, most SL researchers have largely overlooked the role of non-canonical LCBs, but recent work suggests that more studies should be focused on these non-canonical LCBs, not least a systematic analysis of the specificity of the CerS towards LCB variants, so as to determine how their incorporation into ceramides and complex SLs is regulated.

4.1.4.3 Acyl-CoA Specificity Is Determined by the Last Loop of the TLC Domain

The first mammalian CerS was identified when the CerS1 gene (*uog1*) was over-expressed in cultured cells, resulting in an increase in C18-ceramide synthesis and in C18-ceramide (and C18-SL) levels (Venkataraman et al. 2002). Later, it was demonstrated that overexpression of each CerS led to an increase in a specific subset of ceramides containing a unique fatty acid composition (Table 4.1). Thus, CerS1 uses mostly C18 acyl-CoA (C18-CoA) (Venkataraman et al. 2002), CerS4 uses C18- and C20-CoAs (Riebeling et al. 2003), CerS5 and CerS6 use mostly C16-CoA (Mizutani et al. 2005; Lahiri and Futerman 2005), CerS3 uses very-long chain acyl-CoAs (C26 and higher) (Mizutani et al. 2006); CerS2 can utilize a wider range of fatty acyl-CoAs, from C20 to C24 (Laviad et al. 2008). In order to investigate CerS acyl-CoA specificity, chimeric proteins, which combined sequences from CerS2 and CerS5, were generated (Tidhar et al. 2012). A chimeric CerS5/2 protein containing the first 158 residues and the last 83 residues of CerS2 displayed specificity toward C16-CoA similar to that of CerS5. Likewise, a chimeric CerS2/5 protein containing the first 150 residues and the last 79 residues of CerS5 displayed specificity toward C22-CoA similar to that of CerS2. These and additional results demonstrate that a 150-residue region within the TLC domain is sufficient for determining CerS acyl-CoA specificity.

More recently, the region involved in determining specificity was narrowed-down significantly to an 11-amino acid sequence in a loop putatively located between the last two TMDs of the CerS (Tidhar et al. 2018). The specificity of a chimeric protein based on the backbone of CerS5 (which produces C16-ceramide), but containing 11 residues from CerS2 (which synthesizes C22–C24-ceramides), was altered such that it generated C22–C24 ceramides. Moreover, chimeric CerS4 proteins with either CerS2 or CerS5 sequences in the same region, displayed a significant elevation in activity towards C24:1-CoA and C16-CoA respectively. Structurally, it was suggested that this short loop may restrict the movement of adjacent transmembrane domains, which may cause a conformational change in the membrane. Further examination of this loop indicated that the loop in CerS5 and CerS6 consists of 15 and 16 residues, in CerS1 and CerS4, 21 and 20 residues respectively, and in CerS2 and CerS3, only 11 and 9 residues (Fig. 4.1a). Hence, CerS that utilize the longest acyl-CoAs have the shortest number of residues in this loop. This finding led to the hypothesis that CerS which utilize shorter acyl-CoAs may have a longer and more flexible loop, permitting transmembrane flexibil-

ity and proximity, which constricts them to using shorter length acyl-CoAs (Tidhar et al. 2018).

4.1.4.4 CerS2 Contains a S1P Receptor-Like Motif

A sphingosine 1-phosphate (S1P) receptor-like motif has been identified within the TLC domain of CerS2. S1P, a bioactive SL, binds this motif and thus regulates the activity of CerS2. S1P can therefore be classified as a low affinity, non-competitive inhibitor of CerS2. Two charged residues (Arg-230 and Arg-325 in both human and mouse CerS2) were reported to regulate this inhibition (Laviad et al. 2008), perhaps suggesting an important interplay between two SLs that could be relevant to the regulation of SL metabolism, and relevant to the opposing functions that these lipids play in signaling pathways such as cell proliferation, migration, and survival.

4.1.5 The CerS C-Terminus Faces the Cytosol and Contains Phosphorylation Sites

The sequence of CerS1 indicates that there are several putative phosphorylation sites, and when tested *in vivo*, CerS1 was phosphorylated by protein kinase C (Sridevi et al. 2009). Furthermore, in an additional study, CerS2-6 were phosphorylated at the C-terminus (Sassa et al. 2016). Most of the phosphorylated residues were part of a consensus motif for phosphorylation by casein kinase 2 (CK2), and treatment of cells with the CK2-specific inhibitor, CX-4945, lowered the phosphorylation levels of CerS2, 4, 5, and 6. Phosphorylation of CerS2 was especially important for its catalytic activity, acting mainly by increasing its V_{max}. Dephosphorylation of endogenous CerS in the mouse brain led to reduced activity toward the CerS2 substrates, C22:0/C24:0-CoAs. This suggests that the phosphorylation of CerS may be a key regulatory point in the control of the distribution and levels of SLs of various acyl-chain lengths. Interestingly, no potential phosphorylation sites were predicted in the C-terminal region of CerS1. Since CK2 is located in the cytoplasm (Ahmad et al. 2008), these findings are consistent with an earlier report suggesting that the C-terminus of CerS6 faces the cytosol (Mizutani et al. 2005) (Fig. 4.1b).

4.2 CerS Regulation

In addition to using different length acyl-CoAs as substrates, CerS differ in their tissue distribution, which presumably regulates the formation of ceramides and complex SLs with defined acyl chain lengths in these different tissues. CerS mRNA distribution in mouse tissues shows significant variability (Laviad et al. 2008) (Table 4.1). Unfortunately, high quality antibodies against CerS are for the most part not commercially available, and therefore systematic studies of CerS protein distribution have not been performed. Such studies are critical because there is little correlation between CerS mRNA levels and the SL distribution within specific tissues, as shown over 10 years ago (Laviad et al. 2008). qPCR analysis demonstrated that CerS2 mRNA was found at the highest level of all CerS and has the broadest tissue distribution. CerS2 displays wide acyl-CoA specificity, showing no activity using C16:0-CoA and very low activity using C18:0, rather using longer acyl-chain-CoAs (C20–C24) for ceramide synthesis. Although CerS2 mRNA levels are high, this does not always correspond to CerS2 activity levels, suggesting post translational regulation of CerS. This regulation remains poorly understood, although since the study in 2008, various modes of post-translational regulation of CerS activity have been demonstrated experimentally, such as phosphorylation, glycosylation, and homo- and hetero-dimerization. While glycosylation and phosphorylation have been shown to take place at the N- and C-termini of the various CerS (see Sects. 4.1.1 and 4.1.5), there is no empiric data defining the localization of the dimerization sites or inhibition sites of CerS (with the exception of S1P inhibition of CerS2, see Sect. 4.1.4.4). Below, we summarize what is currently known about the mechanisms of CerS regulation.

4.2.1 CerS Dimerization

It has been proposed that CerS exist as hetero-complexes and can form both homo- and heterodimers. Upon over-expression of CerS2 with either CerS5 or CerS6, dimers are formed and CerS2 activity is up-regulated (Laviad et al. 2012). Moreover, it was shown that both CerS2 and CerS5 activity was inhibited when co-expressed with a catalytically-inactive CerS5, implying that activity of one member of a heterodimer depends upon, and can be modulated by the activity of the other members. This supports a potentially rapid and reversible mode of regulation of ceramide synthesis. To further examine this, the activity of each CerS was assayed upon co-expression with other CerS (Table 4.2). CerS1 and CerS2 activities were up-regulated by CerS4-6 while CerS3 activity was unaffected upon co-expression of other CerS. CerS4 activity was upregulated by CerS2-3. When co-expressing CerS5 with CerS6, an elevation in activity was observed. However, since CerS5 and CerS6 utilize the same acyl-CoA, it is not possible to distinguish between their activities. While CerS5 activity was not influenced by other CerS, CerS6 was up-regulated upon co-expression with CerS3 and CerS4. These results indicate that most CerS can be regulated by dimerization.

Of all the mammalian CerS, CerS2 activity is the most significantly up-regulated. Based on *in vitro* studies, CerS2 is less active enzymatically, requiring a longer reaction time and more protein to obtain similar levels of enzymatic activity to the other CerS (as discussed in (Tidhar et al. 2018)). This is somewhat surprising, since C22-C24:1-SLs are found at high levels in many tissues, even though enzyme activity, at least when assayed *in vitro*, is much lower than the other CerS. We suggest that CerS2 might be rapidly regulated by dimerization *in vivo* to up-regulate its activity. Thus, in cases where large quantities of C22-C24:1-ceramides are required, dimerization could play an important role in the activation of CerS2 and the generation of very-long-chain SLs. Studies are currently ongoing to determine dimerization sites and to determine whether formation of higher complexes, such as trimers, quatromers and even complexes of higher orders, may also exist. In fact, IMPs, which span the membrane multiple times, are often organized in functional complexes and form homo- or hetero-oligomeric assemblies (Cymer and Schneider 2012).

Table 4.2 Dimerization affects enzymatic activity of CerS1-6

CerS	1	2	3	4	5	6
1 (C18)	–	1.4 ± 1.0	4.8 ± 2.8	8.5 ± 0.3***	9.3 ± 1.3***	9 ± 1.1***
2 (C24:1)	0.8 ± 0.8	–	5.1 ± 4.7	8 ± 2*	11.8 ± 8.7***	16.3 ± 9.1***
3 (C26)	0.7 ± 0.2	0.9 ± 0.7	–	0.7 ± 0.7	1 ± 0.6	0.9 ± 0.3
4 (C20)	1.2 ± 0.8	5.5 ± 3.3*	9.8 ± 7.9*	–	6.1 ± 7.0	6.9 ± 6.4
5 (C16)	1.1 ± 1.2	0.4 ± 0.4	1.8 ± 1.1	1.2 ± 1.6	–	2.2 ± 0.5
6 (C16)	1.2 ± 0.8	1.9 ± 0.7	3.5 ± 0.5*	3 ± 0.7*	3.2 ± 0.3*	–

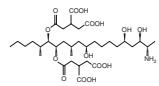
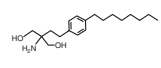
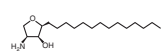
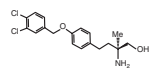
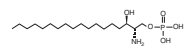
CerS activity was assayed using the indicated acyl-CoA for each of the human CerS upon co-expression with other CerS. Data is represented as fold-change compared to the activity of the monomeric CerS ± SD. ∗, $p < 0.05$; ∗∗∗, $p < 0.001$

4.2.2 CerS Inhibition

Over the years, a variety of inhibitors of the CerS have been described (Table 4.3). The first inhibitor identified, Fumonisin B1 (FB1), was initially described in 1988 (Bezuidenhout et al. 1988) after two decades of research aimed to explain the high incidence of esophageal cancer in certain villages in South Africa (Merrill et al. 1996). Fumonisins are a class of mycotoxins produced by fungi that are common contaminants of maize (*Zea mays*), sorghum and related grains throughout the world. FB1, the most prevalent species, was shown to act as a natural, potent competitive inhibitor of the CerS enzymes, inhibiting the *N*-acylation of both sphinganine and sphingosine (Wang et al. 1991; Merrill et al. 1993). Inhibition of ceramide biosynthesis by FB1 differentially affects the relative formation of different SLs downstream to ceramide production (Merrill et al. 1993) and causes sphinganine to accumulate (Wang et al. 1991), thus increasing the formation of sphinganine l-phosphate and cleavage of the sphingoid base backbone to fatty aldehydes and ethanolamine l-phosphate (Smith and Merrill 1995). FB1, as well as its hydrolyzed form, HFB1, can act as LCB substrates and be *N*-acylated by different CerS to form acylated metabolites of various chain lengths (Humpf et al. 1998; Seiferlein et al. 2007). Interestingly, HFB1 acts as a better substrate of CerS compared to the non-hydrolyzed form (Harrer et al. 2013). This might be due to the lack of the tricarboxylic acid moieties in HFB1, which may allow better access to the active site. Surprisingly, the acylation of HFB1 does not detoxify hydrolyzed fumonisins (Seiferlein et al. 2007). Moreover, the acylated FB1 is significantly more cytotoxic than the non-acylated precursor, suggesting an important contribution to the cytotoxicity of FB1 (Harrer et al. 2013).

The sphingosine analog, FTY720, is a multi-target inhibitor, affecting the activity of S1P lyase (Bandhuvula et al. 2005), cytosolic phospholipase A2 (Payne et al. 2007) as well as CerS (Lahiri et al. 2009). It acts as an uncompetitive inhibitor towards the sphinganine substrate of CerS, suggesting there may be two sphinganine binding sites that act allosterically with respect to one another, or that CerS dimers interact allosterically (Lahiri et al. 2009). *In vivo*, FTY720 is rapidly phosphorylated by sphingosine kinase 2 (Billich et al. 2003; Kharel et al. 2005) to form FTY720 phosphate (FTY720-P), an analog of S1P. FTY720-P binds the S1P receptor (Mandala et al. 2002; Brinkmann et al. 2002) and induces a variety of occurrences including T-lymphocyte

Table 4.3 CerS inhibitors

Name	Chemical formula	Structure	CerS inhibited	Mode of inhibition	Acylated by CerS	References
FB1	$C_{34}H_{59}NO_{15}$		CerS1-6	Competitive	+	Wang (1991) and Merrill (1993)
FTY720	$C_{19}H_{33}NO_2$		CerS1, CerS2, CerS4, CerS5	Noncompetitive toward acyl-CoA and uncompetitive inhibition toward LCB	–	Lahiri (2009)
Jaspine B	$C_{18}H_{37}NO_2$		CerS1-2, CerS4-6	Competitive	+	Cingolani (2017)
P053	$C_{18}H_{21}Cl_2NO_2$		CerS1	Non competitive	N/A	Turner (2018)
S1P	$C_{18}H_{38}NO_5P$		CerS2	Non competitive	N/A	Laviad (2008)

A list of known CerS inhibitors including their chemical formula, structure, inhibition mode as well as their ability to be acylated by CerS. FB1, fumonisin B1; FTY720, Fingolimod; S1P, sphingosine1-phosphate

migration (Zhang and Schluesener 2007; Kihara and Igarashi 2008). While S1P only inhibits the activity of CerS2, FTY720 inhibits most CerS.

A variety of ceramide species are implicated in numerous pathologies including cystic fibrosis (Grassmé et al. 2013), cardiovascular pathologies (Yu et al. 2015; Laaksonen et al. 2016), cancer (Saddoughi and Ogretmen 2013; Jensen et al. 2014) and epilepsy (Mosbech et al. 2014), hence, a number of laboratories are attempting to identify inhibitors of individual CerS. Recently, a non-phosphorylatable analog of FTY720 was characterized (Turner et al. 2018). Unlike FTY720, P053 selectively downregulated CerS1 activity resulting in a reduction of C18-ceramide levels in cultured cells and mouse skeletal muscle, making it the first potent, isoform-selective CerS inhibitor. Although CerS1 is highly expressed in the brain (Table 4.1), P053 failed to show any effect on brain ceramide levels in mice, presumably due to its inability to cross the brain blood barrier, or possibly due to a lower rate of SL turnover in the brain.

An additional sphinganine analog, Jaspine B, a cyclic anhydrophytosphingosine naturally found in marine sponges, also acts as a significant inhibitor of all CerS studied (Cingolani et al. 2017). This inhibition results in the accumulation of free LCBs and alteration of the cellular lipidome. Interestingly, Jaspine B was shown to induce bulk cellular vacuolation and cell death in a non-apoptotic and non-autophagic manner. This vacuolation process was shown to occur in additional cancer cell models, suggesting the generality of this effect.

4.2.3 Transcriptional Regulation of CerS

As discussed above, mRNA levels do not always correspond to levels of CerS activity (Laviad et al. 2008), and moreover, little is known about the regulation of mRNA levels *in vivo*. In a preliminary study, we examined the role of micro-RNAs (miRs) on CerS expression. miRs are small non-coding RNAs that bind to target mRNAs and act as gene repressors, regulating expression in cells and tissues. miR-124, which is known to have a significant impact on neuronal differentiation (Maiorano and Mallamaci 2010), as well as being a tumor-suppressor (Izzotti et al. 2009), was predicted to target CerS2 by homology to a 3′-untranslated region. Indeed, when expressing miR-124 in HEK cells, down-regulation of CerS2 mRNA and protein levels was observed (Fig. 4.4a, b). This effect was specific to CerS2 and was not observed with any of the other CerS. The specificity of miR-124 suggests CerS2 has a unique site which allows this negative regulation.

Since most mammalian miRs silence their target genes by preventing translation rather than altering transcript levels, it is somewhat surprising that mRNA levels of CerS2 were altered, which may imply a possible mechanism of silencing by destabilization of the CerS2 mRNA via cleavage of the poly-A tail, which targets the mRNA for degradation. miR-124 is crucial for cell fate in differentiation to either neuronal or glial cells (Maiorano and Mallamaci 2010) and CerS2 expression is negatively correlated to miR-124 expression in these two cell types (Becker et al. 2008), reinforcing our finding and the importance of this regulation *in vivo*. Unexpectedly, despite the reduction in mRNA levels, CerS2 *in vitro* enzymatic activity was elevated twofold subsequent to miR-124 regulation (Fig. 4.4c). miR-124 is known to downregulate the activity of sphingosine kinase 1 (Xia et al. 2012), the key enzyme in S1P synthesis, and S1P has been shown to inhibit CerS2 (Sect. 4.1.4.4). The broad tissue distribution of CerS2, along with studies from CerS2 null mice (Ben-David et al. 2011; Silva et al. 2012; Park et al. 2013), suggest that CerS2 requires tight regulation, and maintenance of its activity might be crucial for cell viability.

4.3 Summary and Conclusions

CerS are essential enzymes in the SL biosynthetic pathway. Studying SLs, their metabolism, and their biological and physiological signifi-

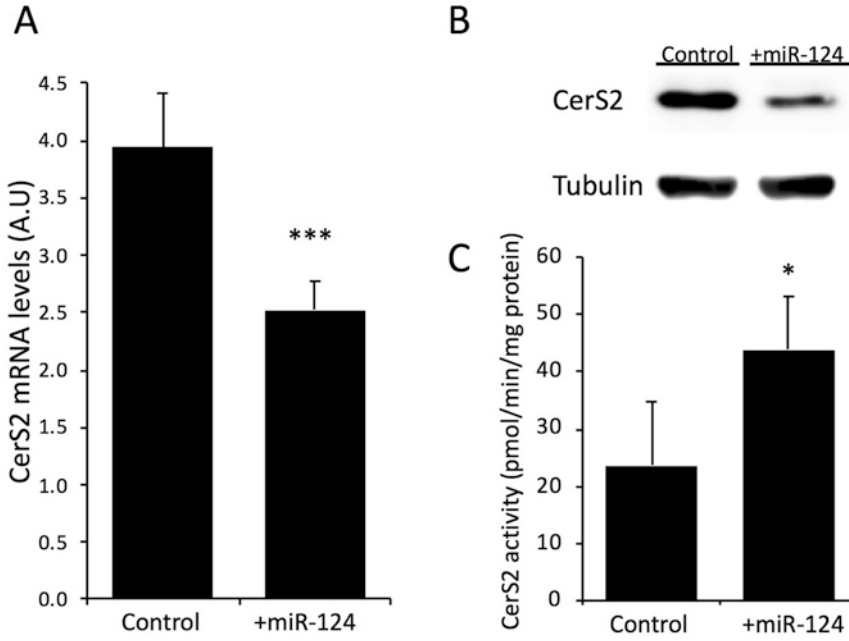


Fig. 4.4 CerS2 is regulated by miR-124. (**a**) CerS2 mRNA levels in HEK cells transfected with miR-124 or a vector control. The results were normalized to TBP levels. Values are means ± SD n = 4. ∗∗∗, $p < 0.001$. (**b**) CerS2 expression was ascertained by Western blotting using an anti-CerS2 antibody. An anti-tubulin antibody was used as a loading control. (**c**) *In vitro* assay of CerS2 activity in HEK cells transfected with miR-124 or an empty vector. Values are means ± SD n = 3. ∗, $p < 0.05$

cance is crucial for answering key questions regarding many pathologies. However, studies examining the structural features of CerS are few and far between. In this chapter, we have documented what is known about CerS structure and function, and have attempted to compile a global picture of the known domains, motifs, topology as well as regulating mechanisms of the CerS enzymes. Although different CerS act on distinct substrates, they all share the same enzymatic activity and exhibit high sequence similarity as well as shared domains. This implies structural similarity within this enzyme family. Resolving the three-dimensional structure of the CerS enzymes is imperative in order to develop a more thorough understanding of CerS activity and regulation. Recent studies have made it clear that a substantial and basic understanding of the CerS enzymatic properties is key to cracking the enigma of the significance of ceramides and other SLs in metabolism and pathology.

References

Ahmad KA, Wang G, Unger G, Slaton J, Ahmed K (2008) Protein kinase CK2 – a key suppressor of apoptosis. Adv Enzym Regul 48:179–187. https://doi.org/10.1016/j.advenzreg.2008.04.002

Bandhuvula P, Tam YY, Oskouian B, Saba JD (2005) The immune modulator FTY720 inhibits sphingosine-1-phosphate lyase activity. J Biol Chem 280:33697–33700. https://doi.org/10.1074/jbc.C500294200

Becker I, Wang-Eckhardt L, Yaghootfam A, Gieselmann V, Eckhardt M (2008) Differential expression of (dihydro)ceramide synthases in mouse brain: oligodendrocyte-specific expression of CerS2/Lass2. Histochem Cell Biol 129:233–241. https://doi.org/10.1007/s00418-007-0344-0

Ben-David O, Pewzner-Jung Y, Brenner O, Laviad EL, Kogot-Levin A, Weissberg I, Biton IE, Pienik R, Wang E, Kelly S, Alroy J, Raas-Rothschild A, Friedman A, Brügger B, Merrill AH Jr, Futerman AH (2011) Encephalopathy caused by ablation of very long acyl chain ceramide synthesis may be largely due to reduced galactosylceramide levels. J Biol Chem 286:30022–30033. https://doi.org/10.1074/jbc.M111.261206

Bezuidenhout SC, Gelderblom WCA, Gorst-Allman CP, Horak RM, Marasas WFO, Spiteller G, Vleggaar R (1988) Structure elucidation of the fumonisins, mycotoxins from Fusarium moniliforme. J Chem Soc Chem Commun 0:743–745. doi: https://doi.org/10.1039/c39880000743

Billich A, Bornancin F, Dévay P, Mechtcheriakova D, Urtz N, Baumruker T (2003) Phosphorylation of the immunomodulatory drug FTY720 by sphingosine kinases. J Biol Chem 278:47408–47415. https://doi.org/10.1074/jbc.M307687200

Borgese N, Fasana E (2011) Targeting pathways of C-tail-anchored proteins. Biochim Biophys Acta 1808:937–946. https://doi.org/10.1016/j.bbamem.2010.07.010

Breitling J, Aebi M (2013) N-linked protein glycosylation in the endoplasmic reticulum. Cold Spring Harb Perspect Biol 5:a013359–a013359. https://doi.org/10.1101/cshperspect.a013359

Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR (2002) The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 277:21453–21457. https://doi.org/10.1074/jbc.C200176200

Cingolani F, Simbari F, Abad JL, Casasampere M, Fabrias G, Futerman AH, Casas J (2017) Jaspine B induces non apoptotic cell death in gastric cancer cells independently of its inhibition of ceramide synthase. J Lipid Res:jlr.M072611. https://doi.org/10.1194/jlr.M072611

Clarke CJ, Snook CF, Tani M, Matmati N, Marchesini N, Hannun YA (2006) The extended family of neutral sphingomyelinases. Biochemistry 45:11247–11256. https://doi.org/10.1021/bi061307z

Cymer F, Schneider D (2012) Oligomerization of polytopic α-helical membrane proteins: causes and consequences. Biol Chem Hoppe Seyler 393:1215–1230. https://doi.org/10.1515/hsz-2012-0231

Futerman AH, Riezman H (2005) The ins and outs of sphingolipid synthesis. Trends Cell Biol 15:312–318. https://doi.org/10.1016/j.tcb.2005.04.006

Gehring W (1994) Homeodomain proteins. Annu Rev Biochem 63:487–526. https://doi.org/10.1146/annurev.biochem.63.1.487

Grassmé H, Riethmüller J, Gulbins E (2013) Ceramide in cystic fibrosis. Handb Exp Pharmacol 216:265–274. https://doi.org/10.1007/978-3-7091-1511-4_13

Guillas I, Kirchman PA, Chuard R, Pfefferli M, Jiang JC, Jazwinski SM, Conzelmann A (2001) C26-CoA-dependent ceramide synthesis of Saccharomyces cerevisiae is operated by Lag1p and Lac1p. EMBO J 20:2655–2665. https://doi.org/10.1093/emboj/20.11.2655

Hannun YA, Obeid LM (2018) Sphingolipids and their metabolism in physiology and disease. Nat Publ Group 19:175–191. https://doi.org/10.1038/nrm.2017.107

Harrer H, Laviad EL, Humpf HU, Futerman AH (2013) Identification of N-acyl-fumonisin B1 as new cytotoxic metabolites of fumonisin mycotoxins. Mol Nutr Food Res 57:516–522. https://doi.org/10.1002/mnfr.201200465

Hirschberg K, Rodger J, Futerman AH (1993) The long-chain sphingoid base of sphingolipids is acylated at the cytosolic surface of the endoplasmic reticulum in rat liver. Biochem J 290:751–757

Hornemann T, Penno A, Rütti MF, Ernst D, Kivrak-Pfiffner F, Rohrer L, Eckardstein v A (2009) The SPTLC3 subunit of serine palmitoyltransferase generates short chain sphingoid bases. J Biol Chem 284:26322–26330. https://doi.org/10.1074/jbc.M109.023192

Humpf HU, Schmelz EM, Meredith FI, Vesper H, Vales TR, Wang E, Menaldino DS, Liotta DC, Merrill AH (1998) Acylation of naturally occurring and synthetic 1-deoxysphinganines by ceramide synthase. Formation of N-palmitoyl-aminopentol produces a toxic metabolite of hydrolyzed fumonisin, AP1, and a new category of ceramide synthase inhibitor. J Biol Chem 273:19060–19064

Izzotti A, Calin GA, Arrigo P, Steele VE, Croce CM, De Flora S (2009) Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke. FASEB J 23:806–812. https://doi.org/10.1096/fj.08-121384

Jensen SA, Calvert AE, Volpert G, Kouri FM, Hurley LA, Luciano JP, Wu Y, Chalastanis A, Futerman AH, Stegh AH (2014) Bcl2L13 is a ceramide synthase inhibitor in glioblastoma. Proc Natl Acad Sci U S A 111:5682–5687. https://doi.org/10.1073/pnas.1316700111

Jiang JC, Kirchman PA, Zagulski M, Hunt J, Jazwinski SM (1998) Homologs of the yeast longevity gene LAG1 in Caenorhabditis elegans and human. Genome Res 8:1259–1272

Kageyama-Yahara N, Riezman H (2006) Transmembrane topology of ceramide synthase in yeast. Biochem J 398:585–593. https://doi.org/10.1042/BJ20060697

Kharel Y, Lee S, Snyder AH, Sheasley-O'neill SL, Morris MA, Setiady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KSK, Engelhard VH, Macdonald TL, Pearson-White S, Lynch KR (2005) Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. J Biol Chem 280:36865–36872. https://doi.org/10.1074/jbc.M506293200

Kihara A, Igarashi Y (2008) Production and release of sphingosine 1-phosphate and the phosphorylated form of the immunomodulator FTY720. Biochim Biophys Acta 1781:496–502. https://doi.org/10.1016/j.bbalip.2008.05.003

Laaksonen R, Ekroos K, Sysi-Aho M, Hilvo M, Vihervaara T, Kauhanen D, Suoniemi M, Hurme R, März W, Scharnagl H, Stojakovic T, Vlachopoulou E, Lokki M-L, Nieminen MS, Klingenberg R, Matter CM, Hornemann T, Jüni P, Rodondi N, Räber L, Windecker S, Gencer B, Pedersen ER, Tell GS, Nygård O, Mach F, Sinisalo J, Lüscher TF (2016) Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol. Eur Heart J 37:1967–1976. https://doi.org/10.1093/eurheartj/ehw148

Lahiri S, Futerman AH (2005) LASS5 is a bona fide dihydroceramide synthase that selectively utilizes palmitoyl-CoA as acyl donor. J Biol Chem 280:33735–33738. https://doi.org/10.1074/jbc.M506485200

Lahiri S, Park H, Laviad EL, Lu X, Bittman R, Futerman AH (2009) Ceramide synthesis is modulated by the sphingosine analog FTY720 via a mixture of uncompetitive and noncompetitive inhibition in an Acyl-CoA chain length-dependent manner. J Biol Chem 284:16090–16098. https://doi.org/10.1074/jbc.M807438200

Laviad EL, Albee L, Pankova-Kholmyansky I, Epstein S, Park H, Merrill AH, Futerman AH (2008) Characterization of ceramide synthase 2: tissue distribution, substrate specificity, and inhibition by sphingosine 1-phosphate. J Biol Chem 283:5677–5684. https://doi.org/10.1074/jbc.M707386200

Laviad EL, Kelly S, Merrill AH Jr, Futerman AH (2012) Modulation of ceramide synthase activity via dimerization. J Biol Chem 287:21025–21033. https://doi.org/10.1074/jbc.M112.363580

Lewinson O, Lee AT, Rees DC (2008) The funnel approach to the precrystallization production of membrane proteins. J Mol Biol 377:62–73. https://doi.org/10.1016/j.jmb.2007.12.059

Maiorano NA, Mallamaci A (2010) The pro-differentiating role of miR-124: indicating the road to become a neuron. RNA Biol 7:528–533

Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thornton R, Shei G-J, Card D, Keohane C, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H (2002) Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 296:346–349. https://doi.org/10.1126/science.1070238

Mandon EC, Ehses I, Rother J, van Echten G, Sandhoff K (1992) Subcellular localization and membrane topology of serine palmitoyltransferase, 3-dehydrosphinganine reductase, and sphinganine N-acyltransferase in mouse liver. J Biol Chem 267:11144–11148

Merrill AH Jr, Liotta DC, Riley RT (1996) Fumonisins: fungal toxins that shed light on sphingolipid function. Trends Cell Biol 6:218–223. https://doi.org/10.1016/0962-8924(96)10021-0

Merrill AH, van Echten G, Wang E, Sandhoff K (1993) Fumonisin B1 inhibits sphingosine (sphinganine) N-acyltransferase and de novo sphingolipid biosynthesis in cultured neurons in situ. J Biol Chem 268:27299–27306

Mesika A, Ben-Dor S, Laviad EL, Futerman AH (2007) A new functional motif in Hox domain-containing ceramide synthases: identification of a novel region flanking the Hox and TLC domains essential for activity. J Biol Chem 282:27366–27373. https://doi.org/10.1074/jbc.M703487200

Mizutani Y, Kihara A, Igarashi Y (2005) Mammalian Lass6 and its related family members regulate synthesis of specific ceramides. Biochem J 390:263–271. https://doi.org/10.1042/BJ20050291

Mizutani Y, Kihara A, Igarashi Y (2006) LASS3 (longevity assurance homologue 3) is a mainly testis-specific (dihydro)ceramide synthase with relatively broad substrate specificity. Biochem J 398:531–538. https://doi.org/10.1042/BJ20060379

Mosbech M-B, Olsen ASB, Neess D, Ben-David O, Klitten LL, Larsen J, Sabers A, Vissing J, Nielsen JE, Hasholt L, Klein AD, Tsoory MM, Hjalgrim H, Tommerup N, Futerman AH, Møller RS, Færgeman NJ (2014) Reduced ceramide synthase 2 activity causes progressive myoclonic epilepsy. Ann Clin Transl Neurol 1:88–98. https://doi.org/10.1002/acn3.28

Othman A, Rütti MF, Ernst D, Saely CH, Rein P, Drexel H, Porretta-Serapiglia C, Lauria G, Bianchi R, Eckardstein v A, Hornemann T (2012) Plasma deoxysphingolipids: a novel class of biomarkers for the metabolic syndrome? Diabetologia 55:421–431. https://doi.org/10.1007/s00125-011-2384-1

Park J-W, Park W-J, Kuperman Y, Boura-Halfon S, Pewzner-Jung Y, Futerman AH (2013) Ablation of very long acyl chain sphingolipids causes hepatic insulin resistance in mice due to altered detergent-resistant membranes. Hepatology 57:525–532. https://doi.org/10.1002/hep.26015

Payne SG, Oskeritzian CA, Griffiths R, Subramanian P, Barbour SE, Chalfant CE, Milstien S, Spiegel S (2007) The immunosuppressant drug FTY720 inhibits cytosolic phospholipase A2 independently of sphingosine-1-phosphate receptors. Blood 109:1077–1085. https://doi.org/10.1182/blood-2006-03-011437

Pewzner-Jung Y, Ben-Dor S, Futerman AH (2006) When do lasses (longevity assurance genes) become CerS (ceramide synthases)? J Biol Chem 281:25001–25005. https://doi.org/10.1074/jbc.R600010200

Rapoport TA, Goder V, Heinrich SU, Matlack KES (2004) Membrane-protein integration and the role of the translocation channel. Trends Cell Biol 14:568–575. https://doi.org/10.1016/j.tcb.2004.09.002

Riebeling C, Allegood JC, Wang E, Merrill AH, Futerman AH (2003) Two mammalian longevity assurance gene (LAG1) family members, trh1 and trh4, regulate dihydroceramide synthesis using different fatty acyl-CoA donors. J Biol Chem 278:43452–43459. https://doi.org/10.1074/jbc.M307104200

Russo SB, Tidhar R, Futerman AH, Cowart LA (2013) Myristate-derived d16:0 sphingolipids constitute a cardiac sphingolipid pool with distinct synthetic routes and functional properties. J Biol Chem 288:13397–13409. https://doi.org/10.1074/jbc.M112.428185

Saddoughi SA, Ogretmen B (2013) Diverse functions of ceramide in cancer cell death and proliferation. Adv Cancer Res 117:37–58. https://doi.org/10.1016/B978-0-12-394274-6.00002-9

Sassa T, Hirayama T, Kihara A (2016) Enzyme activities of the ceramide synthases CERS2-6 are regulated by phosphorylation in the C-terminal region. J Biol Chem 291:7477–7487. https://doi.org/10.1074/jbc.M115.695858

Seiferlein M, Humpf HU, Voss KA, Sullards MC, Allegood JC, Wang E, Merrill AH (2007) Hydrolyzed

fumonisins HFB1 and HFB2 are acylated in vitro and in vivo by ceramide synthase to form cytotoxic N-acyl-metabolites. Mol Nutr Food Res 51:1120–1130. https://doi.org/10.1002/mnfr.200700118

Silva LC, Ben David O, Pewzner-Jung Y, Laviad EL, Stiban J, Bandyopadhyay S, Merrill AH, Prieto M, Futerman AH (2012) Ablation of ceramide synthase 2 strongly affects biophysical properties of membranes. J Lipid Res 53:430–436. https://doi.org/10.1194/jlr.M022715

Smith ER, Merrill AH (1995) Differential roles of de novo sphingolipid biosynthesis and turnover in the "burst" of free sphingosine and sphinganine, and their 1-phosphates and N-acyl-derivatives, that occurs upon changing the medium of cells in culture. J Biol Chem 270:18749–18758

Sociale M, Wulf A-L, Breiden B, Klee K, Thielisch M, Eckardt F, Sellin J, Bülow MH, Löbbert S, Weinstock N, Voelzmann A, Schultze J, Sandhoff K, Bauer R (2018) Ceramide synthase schlank is a transcriptional regulator adapting gene expression to energy requirements. Cell Rep 22:967–978. https://doi.org/10.1016/j.celrep.2017.12.090

Spassieva S, Seo J-G, Jiang JC, Bielawski J, Alvarez-Vasquez F, Jazwinski SM, Hannun YA, Obeid LM (2006) Necessary role for the Lag1p motif in (dihydro) ceramide synthase activity. J Biol Chem 281:33931–33938. https://doi.org/10.1074/jbc.M608092200

Sridevi P, Alexander H, Laviad EL, Pewzner-Jung Y, Hannink M, Futerman AH, Alexander S (2009) Ceramide synthase 1 is regulated by proteasomal mediated turnover. Biochim Biophys Acta 1793:1218–1227. https://doi.org/10.1016/j.bbamcr.2009.04.006

Tidhar R, Futerman AH (2013) The complexity of sphingolipid biosynthesis in the endoplasmic reticulum. Biochim Biophys Acta 1833:2511–2518. https://doi.org/10.1016/j.bbamcr.2013.04.010

Tidhar R, Ben-Dor S, Wang E, Kelly S, Merrill AH, Futerman AH (2012) Acyl chain specificity of ceramide synthases is determined within a region of 150 residues in the Tram-Lag-CLN8 (TLC) domain. J Biol Chem 287:3197–3206. https://doi.org/10.1074/jbc.M111.280271

Tidhar R, Zelnik ID, Volpert G, Ben-Dor S, Kelly S, Merrill AH, Futerman AH (2018) Eleven residues determine the acyl chain specificity of ceramide synthases. J Biol Chem 293:9912–9921. https://doi.org/10.1074/jbc.RA118.001936

Turner N, Lim XY, Toop HD, Osborne B, Brandon AE, Taylor EN, Fiveash CE, Govindaraju H, Teo JD, McEwen HP, Couttas TA, Butler SM, Das A, Kowalski GM, Bruce CR, Hoehn KL, Fath T, Schmitz-Peiffer C, Cooney GJ, Montgomery MK, Morris JC, Don AS (2018) A selective inhibitor of ceramide synthase 1 reveals a novel role in fat metabolism. Nat Commun 9:3165. https://doi.org/10.1038/s41467-018-05613-7

Venkataraman K, Futerman AH (2001) Comparison of the metabolism of L-erythro- and L-threo-sphinganines and ceramides in cultured cells and in subcellular fractions. Biochim Biophys Acta 1530:219–226

Venkataraman K, Futerman AH (2002) Do longevity assurance genes containing Hox domains regulate cell development via ceramide synthesis? FEBS Lett 528:3–4

Venkataraman K, Riebeling C, Bodennec J, Riezman H, Allegood JC, Sullards MC, Merrill AH Jr, Futerman AH (2002) Upstream of growth and differentiation factor 1 (uog1), a mammalian homolog of the yeast longevity assurance gene 1 (LAG1), regulates N-stearoyl-sphinganine (C18-(dihydro)ceramide) synthesis in a fumonisin B 1-independent manner in mammalian cells. J Biol Chem 277:35642–35649. https://doi.org/10.1074/jbc.M205211200

Wang E, Norred WP, Bacon CW, Riley RT, Merrill AH (1991) Inhibition of sphingolipid biosynthesis by fumonisins. Implications for diseases associated with Fusarium moniliforme. J Biol Chem 266:14486–14490

Winter E, Ponting CP (2002) TRAM, LAG1 and CLN8: members of a novel family of lipid-sensing domains? Trends Biochem Sci 27:381–383

Xia J, Wu Z, Yu C, He W, Zheng H, He Y, Jian W, Chen L, Zhang L, Li W (2012) miR-124 inhibits cell proliferation in gastric cancer through down-regulation of SPHK1. J Pathol 227:470–480. https://doi.org/10.1002/path.4030

Yu J, Pan W, Shi R, Yang T, Li Y, Yu G, Bai Y, Schuchman EH, He X, Zhang G (2015) Ceramide is upregulated and associated with mortality in patients with chronic heart failure. Can J Cardiol 31:357–363. https://doi.org/10.1016/j.cjca.2014.12.007

Zhang Z, Schluesener HJ (2007) FTY720: a most promising immunosuppressant modulating immune cell functions. Mini Rev Med Chem 7:845–850