MINIREVIEW

The Multigenic Sphingomyelin Synthase Family*

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Sphingomyelin (SM)[3] is a vital component of cellular membranes in organisms ranging from mammals to protozoa. Its production involves the enzymatic transfer of a phosphocholine head group from phosphatidylcholine to ceramide, yielding diacylglycerol in the process. The enzyme catalyzing this reaction, SM synthase, thus occupies a central position in sphingolipid and glycerophospholipid metabolism and has considerable biological potential as a regulator of pro-apoptotic factor ceramide and mitogenic factor diacylglycerol. Recent identification of the enzyme uncovered a multiplicity of SM synthase genes in each organism where SM synthesis is known to occur. This has shed new light on the pathways, reaction mechanism, regulation, phylogenetic distribution, and biological significance of SM synthesis.

Initial Steps in SM Synthesis

The first committed step in SM synthesis is the condensation of L-serine and palmitoyl-CoA. This reaction is catalyzed by serine palmitoyltransferase and yields 3-keto-dihydrosphingosine, which is reduced to dihydrosphingosine. Dihydrosphingosine undergoes N-acylation followed by desaturation to generate ceramide, a central molecule in sphingolipid metabolism (1, 2). These reactions occur on the cytosolic surface of the endoplasmic reticulum (ER) (3). Subsequently, ceramide is delivered to the Golgi apparatus where it is converted to SM or glucosylceramide (GlcCer). GlcCer synthesis is mediated by GlcCer synthase, which catalyzes the transfer of glucose from UDP-glucose to ceramide. This enzyme resides in the *cis* Golgi and has its active site oriented toward the cytosol (4). After translocation to the Golgi lumen, GlcCer is converted to more complex glycosphingolipids.

However, in most mammalian cell types the bulk of ceramide is converted to SM by a SM synthase in the lumen of the *trans* Golgi (5–7). This enzyme, named SMS1, catalyzes the transfer of phosphocholine from phosphatidylcholine (PC) to ceramide, yielding diacylglycerol (DAG) as a side product (Fig. 1) (8, 9). A second SM synthase, SMS2, resides at the plasma membrane (6, 10), but it is unclear whether this enzyme participates in the *de novo* synthesis of SM.

An alternative pathway of SM synthesis has been postulated in which ceramide is first converted to ethanolamine phosphorylceramide (EPC) via transfer of the head group from phosphatidylethanolamine (PE) (11). EPC is then converted to SM by stepwise methylation in a reaction analogous to the *S*-adenosylmethionine-dependent conversion of PE to PC (Fig. 1). Even though this pathway has been demonstrated in isolated membrane fractions from rat brain and liver (12, 13), its precise contribution to the *de novo* synthesis of SM remains to be established.

Biological Significance of SM Synthesis

Several lines of evidence indicate that SM formation is critical for cell growth and survival. Chinese hamster ovary mutant cells with a thermolabile serine palmitoyltransferase, the rate-limiting enzyme in sphingolipid synthesis, die in the absence of exogenously added sphingoid base when shifted to the restrictive temperature (14). The mutant cells could be rescued by added SM, but not by GlcCer, the precursor of complex glycosphingolipids. Moreover, a mouse lymphoid cell line with diminished SM synthase activity ceases growth when cultured under serum-free conditions; growth could be restored by heterologous expression of SMS1 or supplementation with exogenous SM (7). Finally, up- and down-regulation of SM synthase activity has been linked to mitogenic and pro-apoptotic signaling in a variety of mammalian cell types (15–17).

How SM synthesis contributes to cell growth and survival is unclear, but any of the following scenarios may explain its critical function. (i) SM accumulates in the exoplasmic leaflet of the plasma membrane where its high packing density and affinity for sterols help create a rigid barrier to the extracellular environment. (ii) The SM pool in the plasma membrane acts as a reservoir of lipid signaling molecules, the liberation of which is catalyzed by acidic or neutral SMases in response to a variety of biological stimuli (18, 19). SM metabolites like ceramide, sphingosine, and sphingosine 1-phosphate are emerging as critical regulators of cell proliferation, differentiation, and apoptosis (20, 21). (iii) As SM has a strong, inherent capacity to form microdomains, its production in the *trans* Golgi may affect the lateral organization of other membrane molecules and thus provide a physical basis for sorting events that help establish the compositional and functional differences between the ER, plasma membrane, and Golgi itself (22). (iv) SM synthesis in the *trans* Golgi may create a local pool of DAG, which provides a cue for protein kinase D recruitment and the formation of secretory vesicles (23). (v) By regulating the cellular levels of pro-apoptotic factor ceramide and mitogenic factor DAG in opposite directions, SM synthesis may have a direct impact on cell proliferation and life span (17, 24).

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[3] The abbreviations used are: SM, sphingomyelin; DAG, diacylglycerol; GlcCer, glucosylceramide; EPC, ethanolamine phosphorylceramide; ER, endoplasmic reticulum; IPC, inositol phosphorylceramide; LPP, lipid phosphate phosphatase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SMS, sphingomyelin synthase; SAM, sterile α motif.
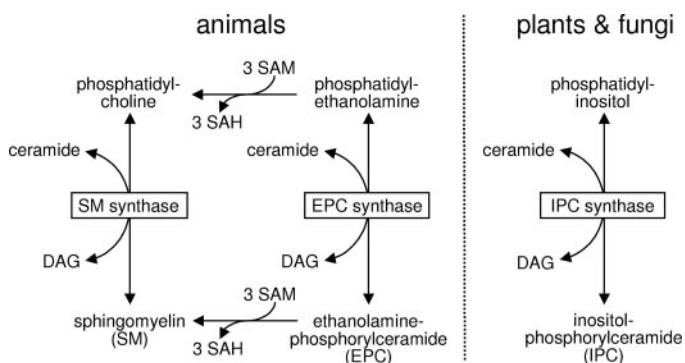
FIGURE 1. **SM synthases and related enzymes interconnect pathways of sphingolipid and glycerolipid metabolism.** The enzymes SM synthase and EPC synthase in animals and IPC synthase in plants and fungi transfer the head groups from PC, PE, and phosphatidylinositol onto ceramide to make SM, EPC, and IPC, respectively. DAG is generated as a side product. Because the reactions are reversible, these enzymes simultaneously control the balance between phosphoglycerolipids and phosphosphingolipids on one hand and between ceramide and DAG on the other. In the same way that PE can be converted into PC by a triple methylation, Muehlenberg *et al.* (11) proposed that EPC has been methylated to form SM. This alternative pathway of SM synthesis has been detected in rat liver and brain microsomes (12, 13). *SAM*, *S*-adenosylmethionine; *SAH*, *S*-adenosylhomocysteine.

SM Synthase Cloning Strategies

Initial studies revealed that mammalian SM synthases are tightly membrane-bound enzymes that readily lose activity upon solubilization with various detergents (8, 9). This severely hampered their identification by classical biochemical approaches. Purification of a soluble SM synthase released by *Pseudomonas aeruginosa* (25) provided no clues on the identity of its mammalian counterparts.

Complementary efforts focused on the isolation of SM synthase mutants by screening Chinese hamster ovary cells for resistance to a SM-directed cytolysin (26). Instead of yielding mutants with a primary defect in SM synthesis, this approach led to the discovery of CERT, a ceramide transfer protein mediating non-vesicular transport of ceramide from the ER to the site of SM synthesis in the *trans* Golgi (27).

Using an expression cloning strategy in yeast, Huitema *et al.* (6) identified a family of integral membrane proteins exhibiting all features previously ascribed to mammalian SM synthases. This approach exploited structural information available for an enzyme catalyzing inositol phosphorylceramide (IPC) synthesis in yeast, a reaction analogous to SM production in which the head group of phosphatidylinositol is transferred to ceramide (Fig. 1). IPC synthesis requires the product of the *AUR1* gene (28), a protein containing the C2 and C3 active site motifs characteristic for members of the lipid phosphate phosphatase (LPP) superfamily (29, 30). BLAST searches for novel sequences encoding integral membrane proteins containing active site motifs common to Aur1p and LPPs identified three families of candidate SM synthase genes with homologues throughout the animal kingdom. Several members of each family were cloned and analyzed for their ability to mediate SM synthesis upon expression in yeast, an organism lacking SM synthase activity. Two of the human proteins tested, SMS1 and SMS2, were active in these assays and localized to the *trans* Golgi and plasma membrane (6), the two principle sites of SM synthesis in mammalian cells (5, 9, 10).

Consistent with these findings, a subsequent study reported the expression cloning of human SMS1 employing a mouse lymphoid cell line with severely diminished SM synthase activity and susceptible to methyl β-cyclodextrin-induced cell death (7). Moreover, this work provided evidence that SMS1 represents a major SM synthase activity in mammalian cells with a critical role in cell growth.

Structural Organization and Reaction Chemistry of SMS Family Members

Like most LPPs (30, 31), SMS1 and SMS2 have a six times membrane-spanning core domain topology with both termini facing the cytosol and the C2 and C3 active site residues facing the exoplasmic leaflet (Fig. 2*A*) (6), the side of the membrane where SM synthesis is known to occur (5, 10). This strongly suggests that SM synthases adapted an LPP-type reaction chemistry to catalyze the choline phosphotransferase reaction. As outlined in Fig. 3, this reaction is bi-directional and likely proceeds through the following steps: (i) binding of a two-chain choline phospholipid, PC or SM, to a single binding site; (ii) nucleophilic attack on the lipid-phosphate ester bond by the histidine in C3 assisted by the conserved aspartate in this motif; (iii) formation of a choline phosphohistidine intermediate and release of DAG or ceramide, facilitated by the histidine in C2 acting as a base; (iv) nucleophilic attack of the primary hydroxyl of ceramide or DAG on the choline phosphohistidine intermediate assisted by the histidine in C2; (v) release of SM or PC from the active site to allow another round of catalysis.

Consistent with the reported enzymatic characteristics of mammalian SM synthases (10, 32), SMS1 and SMS2 function as bi-directional lipid choline phosphotransferases capable of converting PC and ceramide into SM and DAG and vice versa (6). Directionality of the reaction would be primarily determined by the relative concentrations of the phosphocholine acceptors ceramide and DAG in the membrane. However, because the latter compounds are potent modulators of cell behavior, SMS enzymes are likely subject to additional levels of control. For example, SMS1 contains a predicted SAM (sterile α motif) domain at its N terminus that might provide a means for the enzyme to interact with regulatory proteins. SAM domains can bind to SH2 (Src homology 2) domains or other SAM domains and are often found in proteins involved in signal transduction (33).

Curiously, the mouse SMS1 gene is subject to alternative splicing and gives rise to full-length SMS1 as well as two truncated proteins that contain the N-terminal SAM domain and the first two of the six transmembrane domains (34). It will be of interest to determine whether these truncated SMS1 proteins, which lack an active center, participate in the regulation of SM synthesis.

Phylogenetic Distribution of SMS Family Members

Consistent with the presence of SM in many organisms, SMS homologues are found throughout the animal kingdom, from mammals and nematodes to protozoa like the malaria parasite *Plasmodium falciparum*. In fact, each organism capable of SM production displays a multiplicity of SMS genes in its genome (Table 1; Fig. 2*B*). Hence, two orthologous SMS sequences have
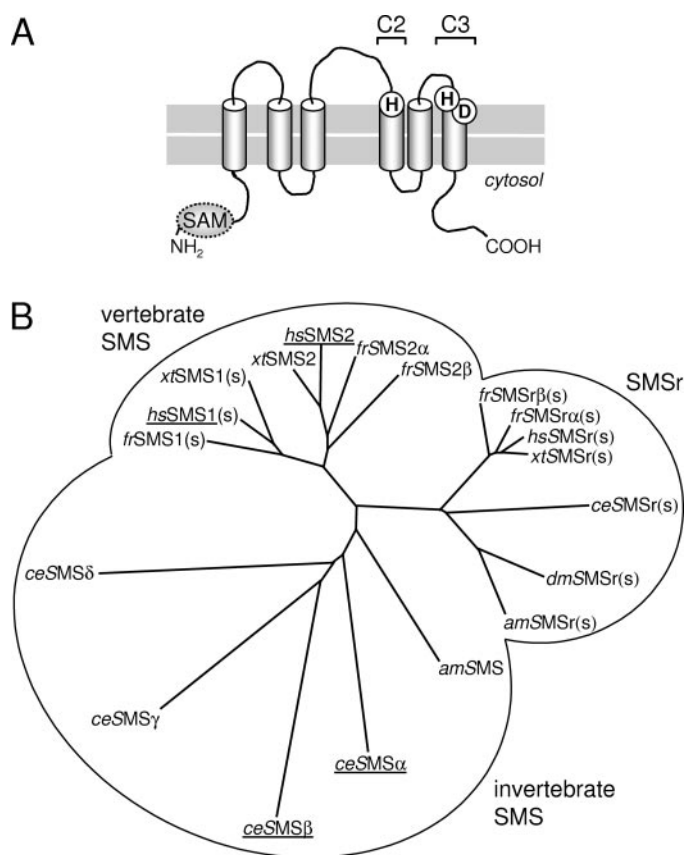
FIGURE 2. **SM synthases comprise a family of integral membrane proteins whose members occur throughout the animal kingdom.** *A*, like most LPPs, SM synthases have six predicted transmembrane helices, cytosolic N- and C-terminal tails, and two putative active site motifs (C2 and C3) with conserved histidine and aspartic acid residues that are located at the exoplasmic side of the membrane (6). The N terminus of several family members contains a SAM domain that may be involved in protein-protein interactions. *B*, a phylogenetic tree showing that SMS family members can be divided into three clusters. Proteins from both the vertebrate and invertebrate SMS clusters were found to possess SM synthase activity (*underlined*) (6). In contrast, none of the proteins from the SMSr cluster show SM synthase activity. Instead, SMSr proteins are prime candidate EPC synthases. SAM domains (*s*) are present in vertebrate SMS1 and in SMSr proteins but not in vertebrate SMS2 or invertebrate SMS proteins. Protein sequences were aligned with ClustalX (36). After alignment, the N- and C-terminal cytosolic tails (including SAM domains) were removed manually, and a phylogenetic tree was constructed with ClustalX. Swiss-Prot/TrEMBL or NCBI accession numbers of SMS proteins are: *Homo sapiens*: *hs*SMS1, Q86VZ5; *hs*SMS2, Q8NHU3; *hs*SMSr, Q96LT4; *Xenopus tropicalis* (western clawed frog): *xt*SMS1, Q640R5; *xt*SMS2, Q5M7L7; *xt*SMSr, CAJ81494; *Apis mellifera* (honey bee): *am*SMS, XP_392299; *am*SMSr, XP_396152; *Drosophila melanogaster*: *dm*SMSr, Q9VS60; *Caenorhabditis elegans*: *ce*SMSα, Q9XTV2; *ce*SMSβ, Q20735; *ce*SMSγ, Q965Q4; *ce*SMSδ, Q9TYV2; *ce*SMSr, Q20696. SMS protein sequences from *Fugu rubripes* (puffer fish) have been provided freely by the Fugu Genome Consortium for use in this publication only and their accession numbers are: *fr*SMS1, SINFRUP00000154157; *fr*SMS2α, SINFRUP00000163409; *fr*SMS2β, SINFRUP00000155834; *fr*SMSrα, SINFRUP00000164184; *fr*SMSrβ, SINFRUP00000164390.

been identified in *P. falciparum,* and the nematode *Caenorhabditis elegans* contains five SMS homologues, at least two of which function as SM synthases (6).

In addition to SMS1 and SMS2, the human genome contains a third, SMS-related (SMSr) gene of unknown function. This gene is highly conserved. SMSr homologues occur in vertebrates, nematodes, and insects, including the fruit fly *Drosophila melanogaster* (Fig. 2*B*). Strikingly, *Drosophila* lacks SMS1 and SMS2 homologues and does not synthesize SM. Instead, this organism produces the SM analogue EPC (35). Although
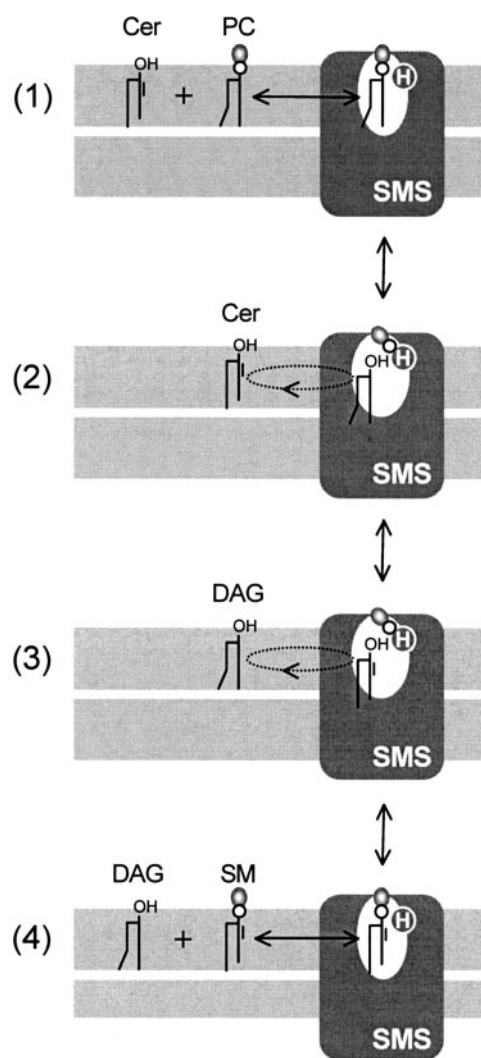

FIGURE 3. **Putative reaction mechanism of SMS-mediated SM synthesis.** As the simplest model, we propose an LPP-like reaction mechanism that involves a single lipid binding site and proceeds via formation of a choline phosphohistidine intermediate along the following steps. *1*, PC binds to the enzyme; *2*, the phosphocholine head group is transferred to a conserved histidine residue in the enzyme's active site; *3*, while the head group stays bound to the enzyme, DAG is replaced by ceramide; *4*, the phosphocholine head group is transferred to ceramide forming SM, which is then released from the enzyme. All steps in this reaction mechanism are reversible, thus satisfying the experimental observation that SM and DAG also can be converted to PC and ceramide (6).

TABLE 1

Phylogenetic distribution of SM, EPC, IPC, and SMS family members

Phylum	Species	Phosphosphingolipid	SMS genes
Vertebrata	*R. norvegicus*	SM, EPC (11,12)[a]	3
	X. tropicalis	SM (38)	3
	D. rerio	ND[b]	4
	F. rubripus	ND	5
Nematoda	*C. elegans*	SM (39)	5
Arthropoda	*A. mellifera*	SM, EPC (40)	2
	D. melanogaster	EPC (35)	1
Apicomplexa	*P. falciparum*	SM (41)	2
Fungi	*S. cerevisiae*	IPC (1)	0
Viridiplantae	*A. thalania*	IPC (42)	0

[a] Numbers in parentheses are references.
[b] ND, not determined.

EPC is widely spread among animals, no EPC synthase has been identified to date. SM and EPC synthesis involve a similar reaction mechanism (12, 13). Because SMS1, SMS2, and SMSr are
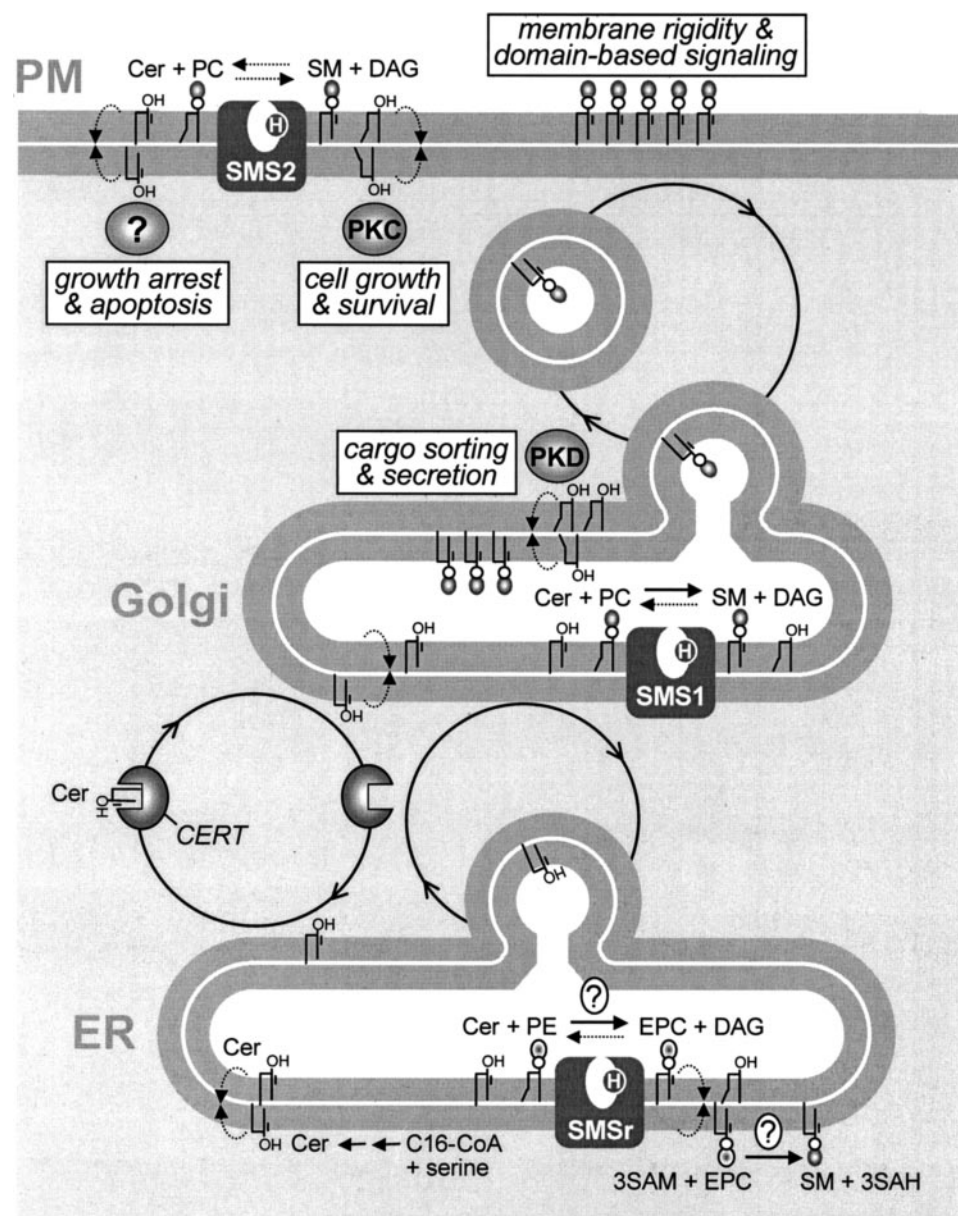
FIGURE 4. **SMS-mediated pathways of SM synthesis and their potential impact on fundamental cellular processes.** Initial steps of SM synthesis involve the formation of ceramide from fatty acyl-CoA and serine on the surface of the ER. Part of the newly synthesized ceramide is flipped to the ER lumen where it is converted to EPC by EPC synthase (12, 13), an enzyme for which SMSr is a prime candidate (6). Methylation of EPC could then give rise to significant amounts of SM already in the ER. The major part of ceramide is exported to the Golgi by both vesicular and monomeric transport involving ceramide transfer protein CERT (27). SMS1 active in the Golgi lumen consumes ceramide for SM synthesis. DAG generated in this reaction stimulates protein kinase D (*PKD*) recruitment (23) and facilitates the budding of exocytic vesicles by satisfying the geometrical constraints of the emerging vesicle neck. SM cycles in vesicles between the Golgi and plasma membrane (*PM*) where it contributes to bilayer rigidity and the formation of microdomains that act as sorting and signaling platforms (37). At the plasma membrane, SMS2 regulates the balance between pro-apoptotic signaling molecule ceramide and pro-mitogenic signaling molecule DAG by reversibly interconverting PC and SM. *PKC*, protein kinase C.

indicates that most mammalian cell types contain two distinct SM synthases: SMS1 in the *trans* Golgi and SMS2 predominantly at the plasma membrane. Because SMS1 is located proximal to SMS2 with respect to receiving newly synthesized ceramide from the ER, one may anticipate that SMS1 is primarily responsible for generating the bulk of cellular SM.

In fact, cells seem to do everything to prevent newly synthesized ceramide from reaching the plasma membrane. This transport block, which may serve to avoid mixing of the metabolic pool of ceramide with the signaling pool of ceramide at the plasma membrane, is accomplished by the combined actions of GlcCer synthase on the Golgi surface, SMS1 in the Golgi lumen, and ceramide transfer protein CERT in the cytosol with the latter ensuring that no ceramide formed on the ER surface can escape the metabolic trap in the Golgi. If SMS2 does not contribute significantly to the *de novo* SM synthesis, what role does it have?

Like SMS1, SMS2 is a phosphocholine transferase that uses PC or SM as donors and ceramide or DAG as acceptors in any combination (6). In the ample presence of PC and SM at the plasma membrane, the reaction catalyzed by SMS2 will in the first instance be driven by the relative concentrations of ceramide and DAG. Consequently, an exciting prospect is that SMS2 serves to balance the local pools of DAG and ceramide generated by phospholipases C and SMases in its vicinity and hence plays a role in attenuating mitogenic and pro-apoptotic lipid signaling at the plasma membrane. However, given the opposing effects of ceramide and DAG on cell proliferation and life span, it's not very hard to imagine that cells developed mechanisms to control the SMS-mediated interconversion of these lipids beyond their relative concentrations in the membrane.

structurally related and share conserved sequence motifs with putative active site residues (6), SMSr proteins are prime candidates for the elusive EPC synthase.

SM Synthase Multiplicity and Its Biological Implications

Fig. 4 summarizes the concepts discussed in this review concerning the pathways of SM synthesis and their potential impact on fundamental cellular processes. The uniform tissue distribution of SMS1 and SMS2 transcripts in mammals (6, 34)

Outlook

The co-existence of multiple SM synthases in animal cells raises a number of important issues with great potential for future investigation. If SM formation is required for cell growth and survival, then what part do SMS1 and SMS2 play in these

processes? Are these enzymes functionally redundant or do they serve unique biological roles because of their association with different cellular organelles?

How significant is the site of SM synthesis for membrane trafficking and the compartmental organization of cells? For example, what would happen if SMS1 would be relocated from the Golgi to the ER? Does SMSr indeed represent the elusive EPC synthase? Where is this enzyme localized? Does EPC methylation, as postulated more then 30 years ago (11), contribute to the *de novo* SM synthesis? If so, can cells or animals survive with EPC methylation as the only pathway for SM production?

Given that some animals, including fruit flies, produce EPC and no SM, can EPC synthesis functionally substitute for SM synthesis in mammalian cells if it would occur at the same location? Why is the SMS family in nematodes and in some vertebrates, including puffer fish, so elaborate? Does each SMS family member in these organisms act as SM synthase, or do some catalyze a related reaction? How do cells regulate SM synthesis in accordance with their needs? More specifically, what are the mechanisms by which cells control the rate and directionality of the reactions catalyzed by SMS proteins?

Only a few years ago, many of these questions would have seemed farfetched, if not impossible to tackle. However, with the identification of a multigenic SM synthase family in animals, a unique toolbox has been uncovered, the contents of which can now be used to dissect the pathways, biological roles, and regulation of SM synthesis in molecular detail.

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