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Review

Molecular mechanisms and regulation of ceramide transport

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Abstract

De novo biosynthesis of sphingolipids begins in the endoplasmic reticulum (ER) and continues in the Golgi apparatus and plasma membrane. A crucial step in sphingolipid biosynthesis is the transport of ceramide by vesicular and non-vesicular mechanisms from its site of synthesis in the ER to the Golgi apparatus. The recent discovery of the ceramide transport protein CERT has revealed a novel pathway for the delivery of ceramide to the Golgi apparatus for sphingomyelin (SM) synthesis. In addition to a ceramide-binding START domain, CERT has FFAT (referring to two phenylalanines [*FF*] in an acidic tract) and pleckstrin homology (PH) domains that recognize the ER integral membrane protein VAMP-associated protein (VAP) and Golgi-associated PtdIns 4-phosphate, respectively. Mechanisms for vectorial transport involving dual-organellar targeting and sites of deposition of ceramide in the Golgi apparatus are proposed. Similar Golgi–ER targeting motifs are also present in the oxysterol-binding protein (OSBP), which regulates ceramide transport and SM synthesis in an oxysterol-dependent manner. Consequently, this emerges as a potential mechanism for integration of sphingolipid and cholesterol metabolism. The identification of organellar targeting motifs in other related lipid-binding/transport proteins indicate that concepts learned from the study of ceramide transport can be applied to other lipid transport processes.

Keywords: Ceramide; Hydroxycholesterol; Lipid transport; CERT; Endoplasmic reticulum; Golgi apparatus

1. Introduction

In general, lipid biosynthetic pathways involve different organelles, thereby necessitating the transport of metabolic intermediates and end products for the completion of synthesis and production of cellular membranes, respectively. The properties of lipids that are essential for membrane structure also render free movement across the cytoplasmic space thermodynamically unfavorable. This restriction requires that lipid transport occur by bulk membrane fusion events and/or lipid-binding proteins. Clearly, this transport machinery is efficient and highly selective since lipid biosynthetic pathways are tightly coordinated and organelles exhibit distinct lipid compositions. Thus, compartmentalization of lipid synthesis, restricted lipid movement and the preservation of membrane

Abbreviations: ARF, ADP-ribosylation factor; CERT, ceramide transport protein; CGlcT, ceramide glucosyltransferase; CHO, Chinese hamster ovary; DAG, diacylglycerol; D609, tricyclodecan-9-yl xanthate; HPA-12, *N*-(3-hydroxyl-1-hydroxylmethyl-3-phenylpropyl)dodecanamide; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FFAT, two phenyalanines in an acidic tract; FAPP, phosphatidylinositol 4-phosphate adapter protein; GFP, green fluorescent protein; GlcCer, glucosylceramide; GPBP, Good-pasture binding protein; GPI, glycosylphosphatidylinositol; IPC, inositol phosphorylceramide; KDS, 3-ketosphinganine; LAG, longevity assurance gene; MCS, membrane contact sites; OSBP, oxysterol-binding protein; ORP, OSBP-related protein; PA, phosphatidic acid; PCTP, phosphatidylcholine transfer protein; PH, pleckstrin homology; PITP, phosphatidylinositol transfer protein; PM, plasma membrane; PtdCho, phosphatidylcholine; PtdIns, phosphatidylinositol; SM, sphingomyelin; SMS, sphingomyelin synthase; StAR, steroidogenic acute regulatory protein; StarD4, START domain protein 4; START, StAR-related lipid transfer; SPT, serine palmitoyltransferase; SRD6, sterol regulatory defective 6; SREBP, sterol-regulation element binding protein; TGN, *trans*-Golgi network; VAP, vesicle-associated membrane protein-associated protein; 25-OH, 25-hydroxycholesterol

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compositions implies the existence of multiple discrete intracellular lipid transport pathways.

A number of technical advances have overcome previous limitations for studying intracellular lipid trafficking. Improved methodologies for accurate quantitative analysis of membrane lipid composition have provided a framework for elucidating which lipids are targeted to different membranes [1–7]. Genomics has facilitated the identification of lipid biosynthetic enzymes and entire pathways leading to a better understanding of intracellular sites of lipid synthesis. Finally, modifications to lipids to improve water solubility and fluorophore-conjugation has improved delivery and visualization in cells [8–10], although these modifications alter physical properties, making it difficult in some instances to extrapolate results to the natural compounds. These advances had, by far, the greatest impact on the study of sphingolipid metabolism. Entire sphingolipid biosynthetic pathways have been identified in numerous organisms, leading to the elucidation of intracellular sites of synthesis and prediction of transport pathways for metabolic intermediates and products [11–16]. The visualization of these intracellular sites and monitoring of intermembrane movement was facilitated by the generation of fluorescent-tagged sphingolipids [17,18] and short *N*-acyl-chain analogs with improved solubility [19,20] (reviewed in [21]). Collectively, these studies showed that sphingolipid synthesis begins in the ER and continues in the Golgi apparatus and plasma membrane (PM) [22] by continuous and uninterrupted pathways dependent on specific intracellular lipid transport mechanisms. An early and critical step in sphingolipid synthesis involves the transport of ceramide, the precursor for glycosphingolipids and sphingomyelin (SM), from its site of synthesis in the ER to the Golgi apparatus. This review will focus on recent advances in our understanding of the molecular mechanisms and regulation of ER-to-Golgi ceramide transport. The reader is referred to other reviews for a broader description of sphingolipid transport in yeast [23] and mammalian cells [24].

2. Synthesis of ceramide, sphingomyelin and glucosylceramide

Johann L. W. Thudichum originally named sphingolipids after the Greek Sphinx, owing to the enigmatic chemical properties of the sphingosine backbone [25]. On a functional level, this enigma has been lifted to reveal an ever-expanding role for sphingolipids in signal transduction, cell survival and death pathways, and in the formation of membrane microdomains, termed rafts. In order for these functions to be exerted in different intracellular compartments, sphingolipids must be generated locally or transported to the site of action [26,27]. De novo synthesis of sphingolipids begins in the ER with the serine palmitoyltransferase (SPT)-catalyzed condensation of serine and palmitoyl-CoA to generate 3-ketosphinganine (KDS) [12,28]. SPT is the rate-limiting enzyme in this biosynthetic pathway and is regulated by transcriptional [29–33] and post-transcriptional mechanisms [34–37].

Next, a series of three reactions, reduction of KDS to sphinganine, *N*-acylation to produce dihydroceramide and desaturation to generate a 4-trans double bond, produce ceramide on the cytosolic surface of the ER [12–14]. Until recently, the intracellular localization and topology of these latter enzymes has been based solely on kinetic evidence. Mammalian longevity assurance gene (LAG) family members have now been shown to regulate acyl-CoA-dependent dihydroceramide synthesis in the ER [38–40]. However, it is not known if these proteins represent bona fide dihydroceramide synthase enzymes or specify acyl chain selectivity for another dihydroceramide synthase. Mammalian Δ4-desaturases have also been identified and shown to prefer dihydroceramide as a substrate compared to sphinganine [41–43]. Interestingly, the Δ4-desaturases also exhibit C-4 hydroxylase activity to generate phytoceramide. While the yeast KDS reductase has been identified [44], the mammalian homologue remains elusive.

The conversion of ceramide to glucosylceramide (GlcCer) or SM requires efficient transport to the Golgi apparatus where two enzymes catalyze the addition of polar head groups to the primary hydroxyl group of ceramide. The glucosylation of ceramide is catalyzed by ceramide glucosyltransferase (CGlcT) on the cytosolic surface of the early Golgi apparatus [45,46]. CGlcT has also been identified in pre-Golgi compartments [45], including the ER [47], and thus ER-to-Golgi ceramide transport may not be necessary for GlcCer synthesis. Following synthesis on the cytosolic surface, GlcCer is translocated to the luminal surface of the Golgi apparatus where it is converted to lactosylceramide and complex glycosphingolipids (e.g. gangliosides) [48–51].

In contrast, SM synthesis is dependent on the translocation of ceramide from the cytoplasmic to the luminal surface of Golgi membranes [52,53]. There, SM is synthesized by phosphatidylcholine:ceramide cholinephosphotransferase, also referred to as SM synthase (SMS), by the transfer of the phosphorylcholine moiety from phosphatidylcholine (PtdCho) onto the primary hydroxyl of ceramide, generating SM and diacylglycerol (DAG). Since SMS also catalyzes the reverse reaction [15,54,55], it has been suggested to function as a molecular switch between ceramide-dependent cell death pathways and pro-survival DAG-dependent pathways. Two SMS genes in mammals encode integral membrane enzymes expressed in the Golgi apparatus (SMS1) and PM (SMS2) [15]. Since Golgi-localized SMS1 is proximal to ER-derived ceramide relative to SMS2, SMS1 appears to be the principal enzyme involved in de novo SM synthesis. SMS1 was shown to co-localize with the *trans*-Golgi cisternae marker sialyltransferase by immunofluorescence microscopy [15]. However, previous cell fractionation studies demonstrated