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review

Yeast Sphingolipids – Structure, Biological Importance and Metabolism

Mirela Bauman[1*], *Marko Mesarić*[1] *and Vladimir Marić*[2]

[1] Department of Chemistry and Biochemistry, School of Medicine, University of Zagreb, Šalata 3, HR–10000 Zagreb, Croatia

[2] Department of Biochemical Engineering, Faculty of Food Science and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

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Summary

Sphingolipids, detected and named by J. L. W. Thudicum more than a hundred years ago, have a common long chain sphingoid base. In most mammals this base is sphingosine. In yeasts, phytosphingosine occurs. Complex sphingolipids are made of sphingoid base to which a fatty acid is linked via an amide bond. Yeast sphingolipids are involved in membrane signaling, regulation of cell wall biosynthesis, phospholipid biosynthesis and binding of cell surface glycoproteins. Besides, they are proven to play important roles in signal transduction during the heat stress response, regulation of calcium homeostasis or components in calcium-mediated signaling pathways and in regulation of the cell cycle.

The key reaction in yeasts sphingolipids biosynthesis is condensation of palmitoyl-CoA with serine yielding D*-3-ketosphinganine. This reaction is catalyzed by serine palmitoyltransferase; the mechanism by which yeast cells regulate activity of the enzyme and the concentration of sphingolipids is still being investigated. Little is known about sphingolipids breakdown pathways in yeasts. A form of mammalian sphingomyelinase was found to exist in Saccharomyces cerevisiae. There are no data on the activity of ceramidase in the yeast. Secretory pathway is regarded the main pathway of sphingolipid transport in the cell; Golgi appears to be the branching point in this process.*

Key words: yeast, sphingolipids, degradation, biosynthesis

Introduction

Although sphingolipids were named after their sphinx-like properties more than 120 years ago, they have borne the name all these decades with good reason, because chemical studies of sphingolipids have long failed to produce any substantial information on their role. Studies conducted during the last ten years have contributed to better understanding of this group of compounds, relating them to the transfer of signals that modulate cell growth, differentiation, neoplastic changes, programmed cell death and many other cell functions *(1–4)*. Though intensive, these studies have only partially revealed the role of sphingolipids in biological systems, as briefly illustrated in Table 1.

Despite the fact that sphingolipids are not essential food constituents, recent studies point to their dietary significance, since their breakdown releases bioactive substances in the gastrointestinal tract *(5)*. There are relatively little literature data on the composition and amount of sphingolipids in individual foodstuffs. The foods of plant origin mostly contain cerebrosides, in an amount ranging from < 0.1 μmol/g fresh tissue in apple *(6)* to nearly 2 μmol/g dry matter in lentils *(7)*. Foods of

* Corresponding author: Tel: +385 1 45 66 774, Fax: +385 1 45 90 206, E-mail: mbauman@mamef.mef.hr

Table 1. Some of the biochemical targets of sphingoid bases and complex sphingolipids and their functions in the mammalian cell *(4)*

	Biochemical targets	Cellular physiology
Sphingosine Sphinganine	• inhibition of protein kinase C and phosphatidic acid phosphatase • stimulation of phospholipase D • calcium mobilization	• regulation of growth, differentiation and apoptosis • growth stimulatory at low concentrations • cytotoxic at high concentrations
Ceramide	• activation of protein kinases and phosphatases • inhibition of phospholipase D • down regulation of c-myc • activation of transcription factors	• regulation of growth, differentiation, apoptosis and protein secretion • induction of cellular senescence • mediator of action of vitamin D_3 • induction of heat shock proteins
Sphingomyelin Complex sphingolipids	• modulation of receptor kinases • structural components of membranes • targeting of GPI-anchored proteins	• regulation of cell growth, protein trafficking and sorting • expression of tumor antigens and recognition sites for microorganisms and toxins

animal origin contain sphingomyelin and simple or complex glycosphingolipids. Their amount in pork, veal and chicken ranges from 0.3 to 0.5 μmol/g *(8)*, whereas milk and dairy products contain about 1 μmol/g *(9)*.

Microorganisms might serve as a potential source of lipids, since they can accumulate up to 60 % of lipids in their biomass. Yeasts appear to be particularly suitable for the study of lipid production, because of their relatively fast growth, nontoxicity and lipid content. Moreover, their lipid amount and composition can be considerably influenced by modification of the growth conditions. There are little literature data on yeast sphingolipids, therefore this review is an attempt to unify the present state of the art on the biological role, composition and metabolism of yeast sphingolipids.

The structure of sphingolipids

Sphingolipids, detected and named by J. L. W. Thudicum more than a hundred years ago, are defined as substances having a long chain sphingoid base in common (Fig. 1). In most mammals, the base is D-*erythro*-(2S, 3R)-sphingosine, an amine consisting of 18 C atoms with one double bond at position 4–5 and two hydroxyl groups at positions 1 and 3. Sphinganine (without 4,5-double bond) and phytosphingosine (4-hydroxysphinganine) containing a third hydroxyl group on the fourth position may also be present. Phytosphingosine predominates in plants *(10)*, and yeasts are also abundant in these bases *(11)*. Since they are extremely toxic, free sphingoid bases are found in cells in very low concentrations, mostly as ceramides with amide bound fatty acids (C16 through C24, predominantly saturated or α-hydroxy acids). Complex sphingolipids contain various groups at position 1, *e.g.*, phosphocholine in sphingomyelin or simple and complex carbohydrates such as those found in cerebrosides, gangliosides, sulfatides and inositol phosphosphingolipids.

Quite commonly, sphingolipids are erroneously considered specific for the nervous system because of their names (ceramide, sphingomyelin, ganglioside, etc.) and for having been first detected in the brain. Sphingolipids are actually found in all eukaryotic cells, where they are mostly present in the plasma membrane and related cell membranes, such as Golgi membranes and lysosomes. About 300 different sphingolipids have been isolated and characterized to date.

The sphingolipid composition of some yeasts and fungi

Saccharomyces cerevisiae

This yeast has turned out to be a very important organism for the study of sphingolipid metabolism in general. In *Saccharomyces cerevisiae*, sphingolipids make approximately 10 % of total membrane lipids and approximately 40 % of total inositol containing lipids. Their structure is shown in Fig.1. Phytosphingosine is the main long chain amino base of this yeast, whereas 4-hydroxyceramide is the major ceramide. The three main sphingolipid groups in *Sacch. cerevisiae* include inositol phosphorylceramide (IPC), mannosyl inositol phosphorylceramide (MIPC) and mannosyl diinositol phosphorylceramide [$M(IP)_2C$], mostly consisting of phytosphingosine, a long chain amino alcohol with a long chain fatty acid (usually C_{26} – hydroxy fatty acid) bound by amide bond, and a polar head group consisting of myoinositol, phosphate and carbohydrate. The four major, differently hydroxylated species of the sphingolipids found in the *Sacch. cerevisiae* are presented in Table 2 (according to the sequence of elution from the column) *(12)*.

Molecular species 2a and 2b have the same total level of hydroxylation and cannot be separated by standard methods; however, their coexistence is quite unusual. Species 1 is found only when the yeast is grown under anaerobic conditions *(12)*. Preliminary NMR analysis of the polar head group of $M(IP)_2C$ has suggested the following structure of this compound:

Inositol-1-P-(6)mannose(α,1,2)inositol-1-P(1)ceramide

Table 2. The composition of major species of inositolphosphorylceramides in *Saccharomyces cerevisiae*

Species	Composition	
1	$C_{18/20}$ *erythro*-dihydrosphingosines 26:0	long-chain base fatty acid
2a	$C_{18/20}$ *erythro*- dihydrosphingosines OH-26:0	long-chain base fatty acid
2b	$C_{18/20}$ phytosphingosines 26:0	long-chain base fatty acid
3	$C_{18/20}$ phytosphingosines OH-26:0	long-chain base fatty acid
4	$C_{18/20}$ phytosphingosines $(OH)_2$-26:0	long-chain base fatty acid

Histoplasma capsulatum

Histoplasma capsulatum is a pathogenic, dimorphous fungus causing histoplasmosis. It grows in the form of a filamentous mycelium in the soil, and transforms into a yeast form in the tissues of infected animals. Five phosphoinositol sphingolipids have been isolated and identified from the yeast phase of this fungus *(13,14)*. Their structures are shown in Fig. 2.

Ceramides of this fungus mostly consist of C_{18} phytosphingosine and OH-C_{24} fatty acid. Compounds II and III are in fact variants of the inositol phosphoceramides isolated from *Sacch. cerevisiae*. Compound VIII is the main inositol phosphorylceramide in both the yeast and mycelial phase. Compounds V and VI are not found in the mycelial phase, and $M(IP)_2C$, the main inositol phosphorylceramide in *Sacch. cerevisiae*, is absent from both phases. Compounds V, VI and VIII react with antibodies obtained from patients with histoplasmosis.

Neurospora crassa

Several *Neurospora crassa* strains contain inositol phosphoryl ceramides of a composition of (inositol-P)$_2$ ceramide, which make 30–60 % of the lipid-extractable

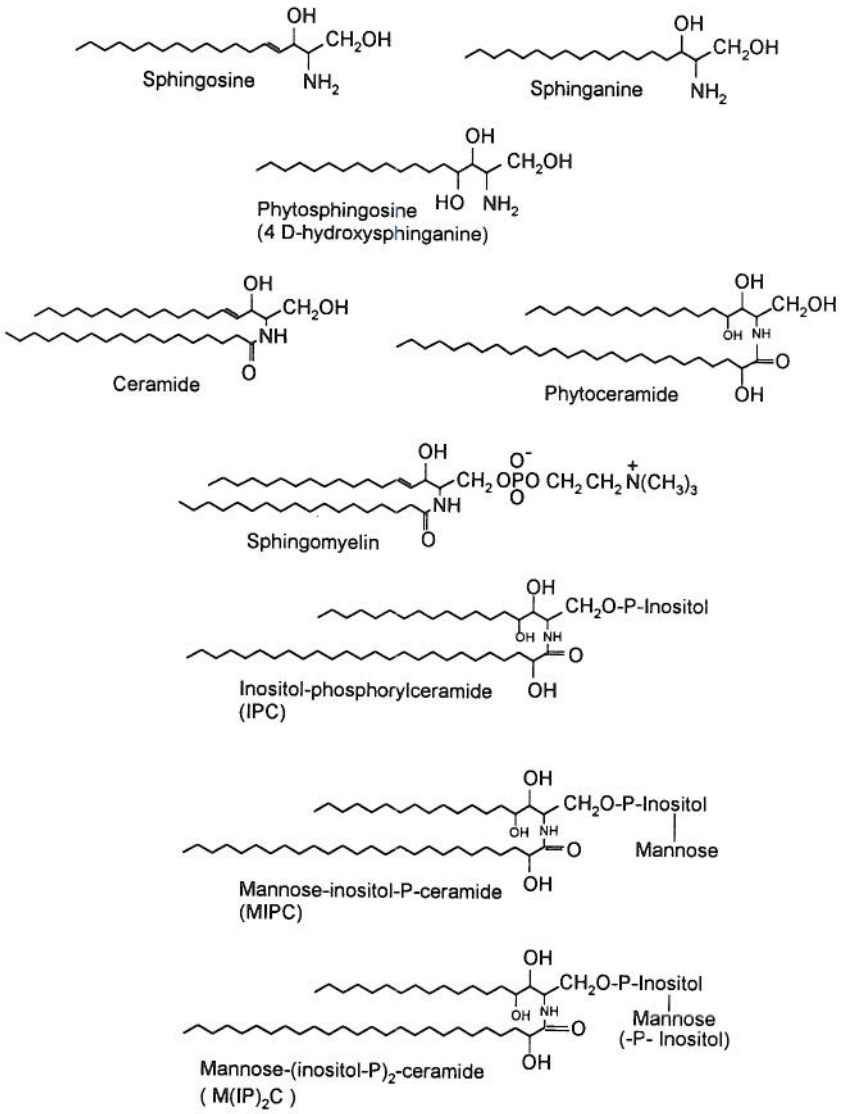

Fig. 1. Structures of some naturally occuring sphingosines, ceramides, and other complex sphingolipids

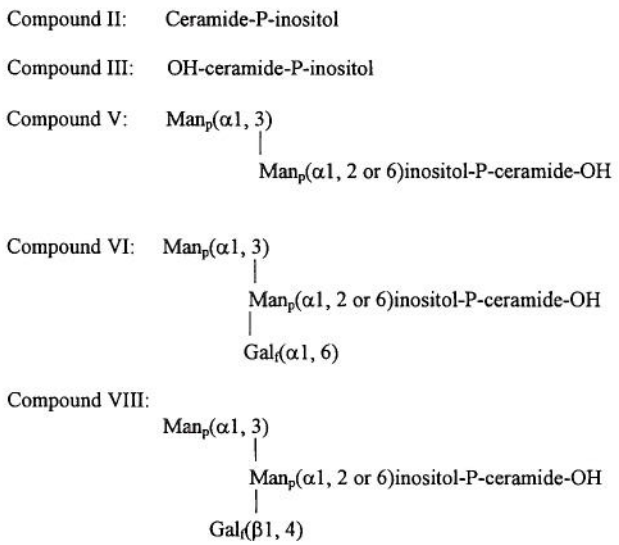

Fig. 2. Phosphoinositol sphingolipids from the yeast phase of *Histoplasma capsulatum*. Man_p stands for mannopyranose and Gal_f for galactofuranose

inositol *(15)*. The compound is sensitive to alkali (1 N KOH, 37 C, 15 hours), thereby producing inositol monophosphates. Some authors *(16)* suggest the following compound structure: inositol-P-inositol-P-ceramide, however, phosphate bonds have not yet been defined in detail. Ceramide mostly consists of C_{18} phytosphingosine and OH-C_{24} fatty acid.

Phytophtora capsici

Phytophtora capsici is a phytopathogenic pepper fungus (*Capsicum annuum*), whose inositol sphingophospholipids induce protective mechanisms of the young pepper cotyledons from necrotic lesions caused by the action of this fungus. The main inositol sphingophospholipid from mycelial phase of this fungus contains C_{16} dihydrosphingosine as a long chain base linked *via* a docosanoyl (22:0) or docosenoyl (22:1) amide bond *(17)*. Inositol and ceramide are linked *via* the phosphate group.

Some differences between individual strains of *P. capsici* have been studied according to the degree of long chain base unsaturation and acyl group nature. The results of these studies have confirmed that inositol sphingophospholipids produced by this pathogen are not glycosylated and that the protective effect against the necrosis of young pepper cotyledons caused by the action of the *P. capsici* neither depends on the C_{16} sphingosine unsaturation nor on the nature of N-acyl side chain *(17)*. The nature of the biospecificity of this fungus sphingolipids remains unclear, because another lipid extracted from this fungus (dihexadecanoyl phosphatidyl choline) also induces protective reaction against *P. capsici* infection in pepper and wheat *(18)*.

Cryptococcus neoformans

Cryptococcus neoformans is an opportunistic fungal pathogen causing infection in humans, and generally located in inositol-rich areas of the central nervous system (CNS). It is present but harmless in the respiratory system of many healthy individuals. Its main characteristics of using inositol as the only carbon source, which has for years been clinically used for identification of cryptococcal infections, and maintaining a constant membrane proportion of phosphatidylinositol, probably play a role in this pathogen localization within the CNS. *C. neoformans* has been demonstrated to contain inositol phosphosphingolipids unique for yeasts, *i.e.* IPC, MIPC and $M(IP)_2C$ *(19)*.

Candida albicans

Candida albicans is a dimorphous pathogenic fungus with the following structure of the major molecular species of cerebroside isolated from the yeast and mycelium form: N-2-hydroxystearoyl-1-*O*-β-glucosyl-9-methyl-C_{18}-sphinga-4,8-dienine *(20)*. Accordingly, the sugar component is a glucose linked to ceramide in β-configuration. The main fatty acid is 2-hydroxystearic acid (62 %). The predominant long chain base has been identified as 9-methyl-C_{18}-sphinga-4,8-dienine *(20)*.

Ceramide synthesis

Biosynthetic pathways in *Sacch. cerevisiae* are shown in Fig. 3. The first reaction in sphingolipid biosynthesis, and at the same time the key reaction along this pathway, is palmitoyl-CoA condensation with serine, whereby D-3-ketosphinganine is formed. This irreversible reaction is catalyzed by serine palmitoyltransferase (SPT) or 3-ketosphinganine synthase. SPT of the *Sacch. cerevisiae* has been cloned and sequenced, allowing the first molecular insight into the enzyme included in the ceramide biosynthesis in general. The *LCB1* and *LCB2* genes are known to encode for Lcb1 and Lcb2 proteins considered to be SPT subunits *(21–23)*. However, additional analysis of SPT subunits is required to confirm it with certainty. Some experiments with yeast cells have shown an enhanced SPT activity to be only achieved with intracellular presence of multiple copies of both *LCB* genes *(22)*. However, the mechanism by which the yeast cell regulates the concentration of sphingolipids, and thus the activity of SPT, has not yet been elucidated.

There is an evidence that two antifungal agents inhibit the activity of serine palmitoyltransferase in *Sacch. cerevisiae* membranes, even when present in nannomolar amounts *(24)*. These two agents are sphingofungin B (2S-amino-3R,4R,5S,14-tetrahydroxyeico-6-enoin acid) and sphingofungin C (2S-amino-5S-acetoxy-3R,4R,14-trihydroxyeico-6-enoin acid). The SPT resistance to sphingofungin B was the greatest when both *LCB* genes were present in multiple copies. Sphingofungin B most likely acts *via* its binding to the SPT catalytic site, indicating that both Lcb proteins are necessary for the formation of the catalytic site *(22)*. Free sphingoid long chain bases do not inhibit the activity of SPT *in vitro* nor reduce the level of activity of this enzyme when added to the culture of cells grown *in vivo (25)*.

The next reaction catalyzed by 3-ketosphinganine reductase, is stereospecific reduction of 3-ketosphinganine (3-ketodihydrosphingosine) with NADPH, whereby an *erythro* isomer of sphinganine (dihydrosphingosine)

is formed *(25)*. The 3-ketosphinganine reductase is encoded by the gene *TSC10 (YBR265w)*; this was confirmed by expressing the gene in *E. coli (26)*.

In mammalian cells, the next step is dihydrosphingosine linkage with fatty acyl-CoA by amide bond, producing dihydroceramide *(27)*. Dihydroceramide dehydrogenation in animals produces ceramide with a long chain sphingosine base which is rapidly converted into complex sphingolipids by the addition of polar components to the 1-hydroxyl group. It has been demonstrated that in animal cells, the introduction of 4-*trans* double bond occurs after the fatty acid linkage [that is at the level of dihydroceramide *(28)*], and if so, then free sphingosine is not an intermediary product from *de novo* synthesis at all but is exclusively formed by the breakdown of complex sphingolipids.

In yeasts, the next reaction by which phytosphingosine is formed has not yet been carried out *in vitro*. Phytosphingosine lacks the 4,5-double bond found in sphingosine, and has a hydroxyl group at position 4, not found in sphingosine (Fig. 1). *In vivo* studies with the yeast *Hansenula ciferrii (29)* have shown that conversion of the labeled *erythro*-dihydrosphingosine into phytosphingosine occurs. Molecular oxygen seems to be the source of 4-oxygen in phytosphingosine *(30)*. A system of oxydase of various functions has been proposed to catalyze this step. It has not yet been demonstrated for sure whether phytosphingosine is formed from *de novo* synthesis or by degradation of ceramide and complex sphingolipids.

The predominant fungal and plant ceramides contain *N*-α-hydroxyfatty-acyl-phytosphingosine, and are formed by as yet undefined hydroxylation reactions. The most numerous molecular species of ceramides in yeasts is the one containing phytosphingosine and α-OH-hexacosanoide (C:26) acid. Ceramides can be classified according to the degree of hydroxylation. Both the sphingoid and fatty acid groups occur with variable degrees of hydroxylation. The fatty acid bound to the sphingoid base is either non-hydroxylated, monohydroxylated or dihydroxylated *(16)*. In yeast, the first fatty acid group hydroxylation occurs in the endoplasmic reticulum, and the second in Golgi *(31)*, requiring Cu^{2+} and Golgi transporter for copper encoded by CCC2 *(32)*.

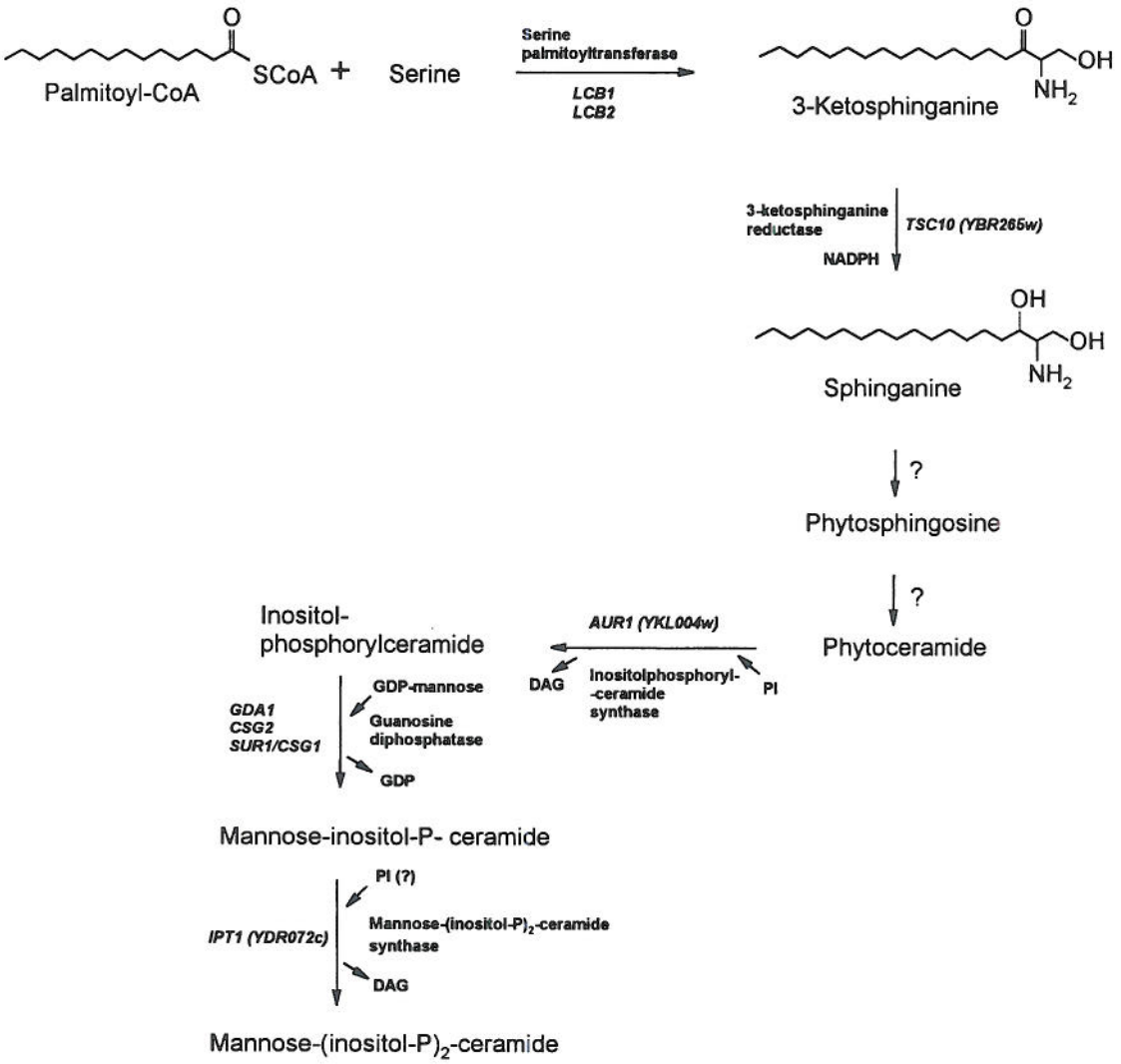

Fig. 3. Sphingolipid biosynthetic pathway in *Sacch. cerevisiae*

The Sur2p protein in *Sacch. cerevisiae* catalyzes hydroxylation of the C-4 sphingoid group of ceramide *(33)*. The substrate (dihydrosphingosine or dihydroceramide) for Sur2p has not yet been identified. As C-4 hydroxylation is not necessary for the synthesis of ceramide or more complex sphingolipids, either dihydrosphingosine or phytosphingosine can serve as a substrate for ceramide synthase, *i.e.* either dihydroceramide or phytoceramide can serve as a substrate for IPC synthase. Another group of authors *(34)* has found that the *Sacch. cerevisiae* gene, *SYR2*, necessary for growth inhibition by syringomycin E is also required for 4-hydroxylation of sphingoid bases. It has been shown that strains mutant in *SYR2* produce sphingolipids missing the hydroxyl group at the C-4 position of the long chain base moiety, that supplying such cells with C-4 hydroxylated long chain base suppresses the syringomycin E-resistant phenotype of *syr2* strains, and that strains that overexpress Syr2p are enriched in 4-hydroxylase activity *(34)*. The research *(34)* has not yet given the answer to the question whether 4-hydroxylation occurs before or after long chain base acylation, *i.e.* if Syr2p converts dihydrosphingosine to phytosphingosine, dihydroceramide to phytoceramide, or both.

Ceramide hydroxylase, encoded by the *SCS7* gene (therefore being also designated as Scs7p), catalyzes hydroxylation of the very long fatty acid chain *(33)*. The lack of hydroxylation of C4 long chain sphingoid bases diminishes hydroxylation of very long fatty acid chain, catalyzed by means of Scs7p, indicating that hydroxylation of the very long fatty acid chain occurs after the formation of ceramide. It has not yet been clarified whether the majority of free ceramides in the cell is formed by *de novo* synthesis or by sphingolipids breakdown. To answer this question, it should be determined whether free ceramide or IPC is the substrate for Scs7p. Another group of authors *(35)* also reported about α- hydroxylation of sphingolipid-associated very long chain fatty acids. They have shown that disruption of the *FAH1* gene in *Sacch. cerevisiae* leads to the reduction of α-OH 26:0 fatty acids and to the complementary increase in 26:0 fatty acids. It means that Fah1p, which is cytochrome b_5 fusion protein, functions in the α-hydroxylation of very long chain fatty acids.

The *Sacch. cerevisiae* cells do not require hydroxylation catalyzed by Sur2p/Syr2p or Scs7p/Fah1p for the growth or synthesis of sphingolipids. The physiologic role of hydroxylation remains obscure.

The *ELO2* and *ELO3* genes, homologues of the *ELO1* gene in *Sacch. cerevisiae*, encode for the components of the membrane bound fatty acid elongation system, which is responsible for the synthesis of C26 very long chain fatty acids which, in turn, are precursors of the yeast ceramide and sphingolipids *(36)*. Elo2p seems to be involved in fatty acid elongation to the 24 C atoms, and to have the highest catalytic specificity for C20 acyl-CoA. E1o3p is necessary for the conversion of C24:0 fatty acids to C26:0 fatty acids (36). Destruction of any of these two genes (either *ELO2* or *ELO3*) leads to either reduction or loss of C26:0 fatty acids, the end product of the elongation pathway, which in turn entails a reduced ceramide synthesis and very noticeable changes in the sphingolipid composition, with concomitant accumulation of phytosphingosine.

The synthesis of phosphoinositol sphingolipids

In spite of the wide spread of these main membrane constituents, little is known about their biosynthesis and metabolism. Studies on *Sacch. cerevisiae* have proposed the following mechanism of biosynthesis:

Phosphatidylinositol + Ceramide →
Inositol-P-ceramide (IPC) + Diacylglycerol (DAG)

IPC is synthesized in the endoplasmic reticulum, and this reaction is catalyzed by a phosphatidylinositol ceramide phosphoinositol transferase or IPC synthase which is a membrane enzyme. IPC is the first product on the sphingolipid biosynthetic pathway in yeast. The *AUR1(YKL004w)* gene has been shown to compensate for the IPC synthase defect and to restore the activity of IPC synthase in a diploid version of *Sacch. cerevisiae* mutant with a defect in the mentioned enzyme *(37)*. This gene most probably encodes for IPC synthase or a subunit of this enzyme. Mutations in *AUR1* are known to make the yeast resistant to the antifungal agent aureobasidine A, whereas studies have shown the agent to inhibit IPC synthase. This discovery may prove medically important in the struggle against human pathogens.

In Golgi complex, mannosyl inositol phosphorylceramide (MIPC) is formed by IPC mannosylation with GDP-mannose as a carbohydrate donor and in the presence of the guanosine diphosphatase enzyme (encoded by the *GDA1* gene). The *CSG2* and *SUR1/CSG1* genes are necessary for IPC mannosylation *(32,38)*:

IPC + GDP-mannose → MIPC + GDP

Further addition of inositol phosphate to MIPC, proceeding in the Golgi, results in the end product of mannosyl diinositol phosphorylceramide $M(IP)_2C$, the most abundant and most complex sphingolipid in *Sacch. cerevisiae*:

MIPC + Phosphatidylinositol → $M(IP)_2C$ + DAG

Studies have shown the *IPT1 (YDR072c)* gene to be indispensable for the synthesis of $M(IP)_2C$ in *Sacch. cerevisiae (23)*. Likewise IPC synthase, the $M(IP)_2C$ synthase is inhibited by aureobasidin A, even in micromolar amounts. However, the $M(IP)_2C$ synthase is less sensitive to the inhibition than IPC synthase. It has not yet been fully clarified whether phosphatidylinositol is a direct donor of the inositol phosphate group.

The mechanism of inositol phosphoryl ceramide (as well as glycoprotein) mannosylation in the lumen of Golgi vesicles in *Sacch. cerevisiae* begins with the transport of GDP-mannose, synthesized in cytosol, into the Golgi lumen *via* a specific membrane carrier, where sugar is transferred onto the luminal acceptors of mannosyl transferase (which catalyzes MIPC and $M(IP)_2C$ mannosylation), and GDP is converted into GMP by guanosine diphosphatase (GDP-ase). Thus formed GMP leaves the Golgi lumen, allowing the next molecule of

cytosol GDP-mannose to enter it. As in mammalian cells, the transport of nucleotide sugar into the lumen is also related to the exit of monophosphate from the lumen. The *VRG4* gene has been demonstrated to be necessary for the transport of GDP-mannose into the Golgi lumen of *Sacch. cerevisiae (39)*. Since Vrg4p, the protein product of the *VRG4* gene, exhibits homology with other known nucleotide sugar carriers, the *VRG4* gene has been postulated to encode the carrier of GDP-mannose into the Golgi lumen.

GDA1, a gene encoding GDP-ase, has been detected *(40)*. *In situ* GDP-ase has been demonstrated to act as a homodimer, and not to require linking with other membrane proteins such as mannosyl transferase or GDP-mannose carriers for its action *(41)*.

In the absence of guanosine diphosphatase, reduction in the GMP concentration leads to a decreased entry of GDP-mannose in the Golgi lumen, and thus to a significantly reduced mannosylation in the Golgi complex *(40)*. This was demonstrated by *in vitro* experiments *(42)* with a wild type *Sacch. cerevisiae* and *gda1* null mutants (lacking GDP-ase), in which the rate of GDP-mannose transport into Golgi vesicles of *gda1* null mutants was found to be five times lower compared to the wild type vesicles. Accumulation of GDP relative to intraluminal GMP in the vesicles of *gda1* mutants (following GDP-mannose transport) was also observed, whereas the reduced amount of GDP-mannose available in null mutants was found to cause decreased macromolecule mannosylation within the Golgi complex. In the same experiments *(42)*, GDP and GMP concentrations inside the vesicle lumen of wild type and *gda1* mutants were measured and they were found to be by far lower than the concentration levels required for inhibition of the α1,2-mannosyl transferase activity, indicating that the reduced transport of mannose to the vesicles of *gda1* null mutants is not due to the GDP inhibition of mannosyl transferase.

Regulation of sphingolipid biosynthesis

Since IPC synthase catalyzes the key step in the biosynthesis of the yeast phosphoinositol sphingolipids, the regulation of this enzyme would logically be expected to play an important role in the regulation of the sphingolipid composition and functioning of yeast membranes. Various detergents have been investigated for their ability to release the activity of IPC synthase from the *Sacch. cerevisiae* membranes. Triton X-100 was found to be most efficient, having released more than 90 % of the enzyme activity *(43)*. The same studies have also shown the activity of IPC synthase in phosphatidylinositol/ceramide/Triton X-100 mixed mycelium to depend on the surface concentrations of phosphatidylinositol and ceramide. The maximal enzyme activity was measured at 30 °C and pH = 7.0, in the presence of 5 mM Triton X-100, 1 mM manganese ions and 5 mM magnesium ions. These studies detected the basic enzymatic properties of IPC synthase in one mixed mycelium; however, detailed information on the regulation of this enzyme has not yet become available.

The activity of IPC synthase in *Sacch. cerevisiae* has been shown to be influenced by the phase of cell growth, addition of inositol to the growth medium, and mutations affecting the regulation of phospholipid biosynthesis *(44)*. The highest relative activity of IPC synthase (90–100 %) occurs in the mid- to late exponential phase, whereas a substantial activity decrease (even seven times) was observed during the stationary phase. The addition of inositol to the growth medium results in a two-fold increase of the IPC synthase specific activity. It has been postulated that the activity of IPC synthase might be regulated by the same mechanism that controls the biosynthesis of phospholipids in yeasts as well as the many enzymes of this pathway.

Studies have shown that inositol does not exert any direct influence on the activity of IPC synthase, and the regulation of IPC synthase has been postulated to occur at the genetic level *(44)*. The increase in the activity of IPC synthase in the wild type cells with the addition of inositol to the growth medium has been found to depend on the *INO4* regulatory gene *(44)*. However, definite evidence for the genetic regulation of IPC synthase by inositol requires *in vivo* analysis of IPC synthase mRNA.

The regulation of the IPC synthase activity with the addition of inositol suggests that the synthesis of phosphoinositol sphingolipids may be regulated coordinatingly with the synthesis of phosphatidylinositol *via* inositol synthesis control. The control of the IPC synthase activity by inositol may further have a physiologic role, since the amount of phosphatidylinositol in yeast membranes is increased in the cells grown in the presence of exogenous inositol. Accordingly, the increased concentrations of phosphatidylinositol and IPC synthase may be a way in which the *Sacch. cerevisiae* regulates the synthesis of its phosphoinositol sphingolipids *(44)*.

Sphingoid bases exert an effect on IPC synthase in *Sacch. cerevisiae* opposite to that of inositol, as demonstrated by some authors *(45)* during *Sacch. cerevisiae* growth on a medium to which fumonisin B_1 was added. Fumonisin B_1 (a mycotoxin produced by the fungus *Fusarium moniliforme*) inhibits the synthesis of sphingolipids in yeast by inhibiting the ceramide synthase enzyme, which results in a decreased cellular concentration of ceramide with simultaneous accumulation of free sphinganine and phytosphingosine. Thus formed free sphingoid bases further inhibit the key enzymes of lipid biosynthesis in yeast, including IPC synthase.

Studies of the influence of inositol on the composition of the *C. neoformans* membrane lipids have shown that, as differentiated from the non-pathogenic *Sacch. cerevisiae* yeast, neither the phosphatidylinositol composition of *C. neoformans* nor the synthesis of methylated phospholipids change with the addition of exogenous inositol to the growth medium. It seems therefore that *C. neoformans* possesses a metabolic mechanism for the maintenance of a constant lipid content irrespective of inositol in its environment *(19)*. Some authors propose that the metabolism of sphingolipids in *Sacch. cerevisiae* is either regulated by Ca^{2+} and/or is required for Ca^{2+} homeostasis *(46)*.

Two novel genes in *Sacch. cerevisiae* (*LBP1* and *LBP2*) encoding sphingoid base 1- phosphate phosphatase considered as a key regulator of sphingolipid metabolism and stress response have been cloned and characterized

(47). LBP1 regulates the levels of phosphorylated sphingoid bases and ceramide. *LBP1* was cloned from a yeast mutant that accumulated phosphorylated long chain sphingoid bases and directed sphingoid base intermediates from sphingolipid pathways to glycerophospholipid biosynthesis. The deletion of this gene results in the accumulation of phosphorylated long chain sphingoid bases and lower ceramide levels. *LBP2* seems to play a major role when yeast is grown under stressful conditions *(47)*. Yeast has been observed to have dramatically enhanced survival upon severe heat shock when both genes (*LBP1* and *LBP2*) are deleted.

Sphingolipid degradation

Sphingolipid degradation in mammalian cells has been relatively well investigated. However, there is little information on the sphingolipids breakdown pathways in yeasts. Sphingomyelinases which hydrolyze sphingolipids, whereby ceramides are produced, participate in the signal transduction pathways in the cells of mammals. As the yeast contains many homologues of the mammalian signal proteins, it would be expected to contain an enzyme homologous to mammalian sphingomyelinase. Studies have shown that *Sacch. cerevisiae* contains a form of sphingomyelinase found in mammals, whose activity was recorded in the *Sacch. cerevisiae* cell membrane *(48)*. Some of the properties of this enzyme are similar to the properties of neutral sphingomyelinase found in mammalian cells. First of all, the enzyme is active at both neutral and acid pH, requires divalent cations (magnesium or manganese), it is insensitive to dithiothreitol, and uses sphingolipid substrate with a bulky substitute at position 2 *(48)*. It seems that the enzyme has bimodal pH optima. Sphingomyelinase activity was observed in yeast membrane fractions as well as in intact yeast cells.

It has been shown that the sphingolipids containing phytosphingosine or inositol are not substrates for yeast sphingomyelinase *(48)*. This means that, if the yeast sphingomyelinase does not use endogenous sphingolipids as its substrate, then it may elicit an effect on exogenous sphingolipids, as in the case of bacterial sphingomyelinase. As *Sacch. cerevisiae* uses a variety of energy sources from its natural surrounding, it could be proposed that in this case yeast sphingomyelinase does not play a role in intracellular signalling (like in mammalian cells) but in meeting the cell requirements of nutrients. Alternatively, the enzyme may use some minor, as yet unidentified yeast phospholipids (*e.g.*, glycosphingolipids) as a substrate. In any case, the substrate specificity of this enzyme remains unknown.

Sphingosine and phytosphingosine can inhibit the yeast growth. The intracellular formation of these products from ceramide, a sphingomyelin breakdown product, would thus depend on the expression of ceramidase activity. However, there are no data on the activity of ceramidase in yeast.

Intracellular transport of sphinoglipids

The majority of the *Sacch. cerevisiae* phosphoinositol sphingolipids is mostly (80–100 %) located in the plasma membrane, where they make 7–8 % of total membrane weight or about 30% of its total phospholipid content. Therefore, they can serve as plasma membrane chemical markers *(49)*. $M(IP)_2C$ and MIPC are mostly found in the plasma membrane, although they are also present in Golgi, vacuoles, secretory vesicles and, in a considerable amount, in microsomes. IPC, acting as an intermediary in the biosynthetic pathway, also migrates toward the plasma membrane; however, it is found as the major sphingolipid in the membranes of vacuoles and Golgi. The occurrence of IPC in Golgi can be explained by the fact that IPC serves as a substrate for MIPC synthesis in this organelle. However, its role within the vacuoles is completely unknown *(50)*.

It has been demonstrated that the secretory pathway of proteins can be regarded the main pathway of sphingolipid transport *(51,52)*. The transport of sphingolipids from endoplasmic reticulum to Golgi proceeds along the protein secretory pathway *(53)*, and it is believed that the same pathway is used for the transport of sphingolipids from Golgi to the plasma membrane. Golgi appears to be the branching point in the intracellular transport of sphingolipids. Most of the mannosylated sphingolipids migrate toward cellular periphery, while the sphingolipids without the sugar group, especially IPC, migrate to vacuoles.

When the cells of a certain *Sacch. cerevisiae* strain with an ergosterol biosynthesis defect (so-called *erg6* mutant) are treated with brefeldin A (a toxin affecting the Golgi integrity), it leads to a decreased production of IPC and MIPC due to the impact on the IPC transport between endoplasmic reticulum and Golgi, whereas the toxin has no impact on the production of $M(IP)_2C$ *(53)*.

Retrograde migration of sphingolipids from the plasma membrane toward internal membranes, *e.g.*, *via* endocytosis, has only been demonstrated in mammalian cells *(54)*. In yeasts, it has been exclusively demonstrated for phospholipids *(55)*. Lipid transport from vacuoles to Golgi is a hypothetical issue.

Biological importance of sphingolipids in yeasts

The role of sphingolipids in cellular growth and in yeast metabolism in general has not yet been fully elucidated. Recent *in vitro* studies have shown certain inositol phosphoryl ceramides to be required for the normal physiologic activity of H^+-ATPase *(56)* (the plasma membrane enzyme pumping the protons outside the cell, thus forming the electrochemical gradient used for the transport of various nutrients into the cell *(16)*). Besides their involvement in the regulation of plasma membrane H^+-ATPase activity, yeast sphingolipids are also included in membrane signalling *(57)*, regulation of cell wall biosynthesis, regulation of phospholipid biosynthesis *(58)*, and binding of the cell surface glycoproteins *(59)*. In *Sacch. cerevisiae*, sphingolipids and their intermediates play important roles in signal transduction during the heat stress response, regulation of calcium homeostasis or components in calcium-mediated signaling pathways and in the regulation of the cell cycle *(60)*.

Some *Sacch. cerevisiae* proteins, *e.g.*, Cwp2 protein, bind to the plasma membrane by two different types of phosphoinositol containing lipids. Glycosyl phosphati-

dylinositol (GPI) is such a lipid, whereas the other type of lipids contains ceramide instead of diacylglycerol. It has been demonstrated that GPI are first bound by covalent bond to protein, whereafter its structure changes through the substitution of ceramide for diacylglycerol *(16)*. The GPI group synthesis and linking to the protein carboxy end occur in endoplasmic reticulum, followed by vesicular transport to the Golgi apparatus and then to the plasma membrane *(61)*. The GPI-anchored proteins are necessary for the *Sacch. cerevisiae* cell survival *(62)*. These proteins are known to appear as the main component of the cell wall *(63)*.

Sphingolipids, perhaps the ceramide itself, are indispensable for the normal transport rate of the GPI-anchored proteins (including Cwp2 protein) from endoplasmic reticulum to Golgi apparatus *(64)*. Cwp2 (cell wall protein 2) is the main mannoprotein component of the outer layer of the yeast cell wall *(65)*, which plays an important role in the cell protection from the stress induced by low pH. In the absence of sphingolipids, the transport of Cwp2 to cell wall is impaired, resulting in the cell wall inability to protect the cells from the stress induced by low pH. However, the protective role of Cwp2 protein is also evident in the presence of sphingolipids, since a strain with multiple copies of *CWP2* gene shows better low pH survival than the cells with a single copy of the gene which, in turn, show better survival than the cells lacking the *CWP2* gene *(64)*.

The fact that in yeast, the cell's inability to synthesize sphingolipids leads to an increase in cell density, loss of cell's ability to divide, and ultimately to cell death, indicates that sphingolipids are really indispensable for cell viability *(31)*. The viability function of sphingolipids in yeast cells lies in their main, polar head group (phosphoinositol), according to which they actually differ from the mammalian sphingolipids (phosphocholine). Another important feature of yeast sphingolipids is that they contribute to membrane fluidity and charge *(16)*.

Although sphingolipids have been demonstrated to be necessary for the *Sacch. cerevisiae* growth, a yeast mutant, the so-called SLC strain (Sphingolipid Compensation), capable of growth without the production of sphingolipids owing to two mutations, has been discovered. The first mutation is deletion of the *LCB1* gene encoding serine palmitoyltransferase (an enzyme catalyzing the first step in the synthesis of sphingoid bases), which produces the need of a long chain sphingoid base such as phytosphingosine. The second mutation is point mutation on the *SLC* gene which enables the yeast cell to suppress or avoid *lcb1* defect, allowing the growth without exogenous sphingoid bases *(66)*. This mutation results in a suppressor gene, *SLC1-1* whose product, Slc1 protein has been shown to have fatty acyltransferase activity *(67)*. When the SLC strain is grown on a medium lacking exogenous long chain sphingoid bases, the yeast grows but makes no detectable sphingolipid. The suppressor gene stimulates the cells for the production of new glycerophospholipids, whose structure resembles that of inositol sphingolipids found in wild type *Sacch. cerevisiae (66)*. These novel lipids have the same polar head groups and the C26 fatty acid moiety as normal sphingolipids. Instead of ceramide, however, the lipid moiety consists of diacylglycerol esterified with one C26 and one medium chain fatty acid. These suppressor lipids probably structurally mimic sphingolipids compensating thereby for some sphingolipid functions necessary for growth. It has been found that a single amino acid change in the predicted Slc1p is responsible for suppression of the Lcb^- phenotype and production of the suppressor lipids *(67)*; however, the precise role of Slc1 protein in the synthesis of the suppressor lipids is still obscure.

When growing SLC mutant on a medium containing phytosphingosine, it normally produces sphingolipids. Lcb1 mutation blocks the first step in the synthesis of sphingolipids, so that accumulation of toxic sphingolipid intermediates does not occur when the cells are grown on a medium lacking the long chain base. So, has the gene encoding for the enzyme found further along the sphingolipid biosynthetic pathway, *e.g.*, the gene for ceramide synthase, been mutated, the strain should still be capable of growth, however, without the production of sphingolipids due to the *SLC1-1* suppressor gene, and should not accumulate toxic intermediates because of *lcb1* mutation *(37)*. It has not been completely clarified how new lipids compensate for the lack of sphingolipids and allow the growth. Some authors suggest that one of the roles of suppressor lipids may include return of the transport of GPI-anchored proteins from endoplasmic reticulum to Golgi to the level allowing growth under non-stress conditions *(56)*. Besides, they may be used instead of ceramides to reconstitute GPI-anchored proteins, one or more of them being necessary for the growth.

The importance of sphingolipid backbones (phytosphingosine, phytoceramide) in the stress response has been demonstrated in experiments with *SLC* cells. When exposed to extreme pH and temperature, these cells cannot grow unless phytosphingosine has been added to the growth medium *(56)*. In relation to this, ceramide and other sphingolipid intermediates have been shown to act as signal molecules in response to heat-induced cell stress in *Sacch. cerevisiae (68)*. Soon after the temperature has been raised from 25 to 37 °C (heat shock), accumulation of the trehalose disaccharide protecting the proteins and biological membranes from heat denaturation occurs *(69,70)*. Ceramide and other sphingolipid metabolites (dihydrosphingosine, phytosphingosine) accumulate during heat shock and activate transcription of the *TPS2* gene (encoding for the trehalose synthase subunit), which leads to the accumulation of trehalose *(68)*. Ceramide produced in response to heat shock is formed by *de novo* synthesis, not by the breakdown of previously synthesized sphingolipids.

Conclusions and perspectives

Although insufficient, the present knowledge about the role of sphingolipids in the physiology of both normal and diseased states in humans indicates that, according to their bioactivity, these substances play an important role in maintaining health, *i.e.* in the prevention of particular disorders *(71)*. Unfortunately, these studies have been limited by the inability to obtain adequate amounts of sphingolipids, pointing to the need of ensur-

ing an economical source of these substances. Yeasts may be one of the sources of sphingolipids; however, little is known about the yeast sphingolipids, their metabolism and regulation. Further studies are needed in the field of yeast sphingolipids to allow us to produce, by modifying growth conditions, *i.e* by genetic engineering, adequate amounts of sphingolipids to be used in dietary studies or as an additive to human and animal food.

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Sfingolipidi kvasaca – struktura, biološka važnost i metabolizam

Sažetak

Sfingolipidi, koje je prije više od stotinu godina otkrio i imenovao J. L. W. Thudicum, imaju zajedničku dugolančanu sfingoidnu bazu. Kod većine sisavaca dolazi sfingozin, a u kvascima fitosfingozin. Složeni su sfingolipidi izgrađeni od sfingoidne baze povezane amidnom vezom s masnom kiselinom. Sfingolipidi kvasaca sudjeluju u membranskom signaliziranju, regulaciji biosinteze stanične stijenke i biosinteze fosfolipida te u vezanju glikoproteina na površini stanice. Osim toga, dokazano je da imaju važnu ulogu u signalnoj transdukciji za vrijeme odgovora stanice na stres izazvan toplinom, zatim u regulaciji homeostaze kalcija ili spojeva u signalnim putevima kojima upravlja kalcij te u regulaciji staničnog ciklusa.

Ključna reakcija na biosintetskom putu sfingolipida u kvascima je kondenzacija palmitoil-CoA sa serinom pri čemu nastaje D*-3-ketosfinganin. Tu reakciju katalizira serin palmitoiltransferaza, a još se uvijek istražuje mehanizam kojim stanice kvasca reguliraju aktivnost tog enzima i koncentraciju sfingolipida. Malo se zna i o putevima razgradnje sfingolipida u kvascima. Jedan je oblik sfingomijelinaze sisavac pronađen u Saccharomyces cerevisiae. Za sada nema podataka niti o aktivnosti ceramidaze u kvascima. Sekrecijski put proteina drži se transportnim putem sfingolipida u stanici; čini se da Golgijev aparat predstavlja točku grananja u tom procesu.*