Yeast 1,3-β-Glucan Synthase Activity Is Inhibited by Phytosphingosine Localized to the Endoplasmic Reticulum* 

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1,3-β-α-Glucan, a major filamentous component of the cell wall in the budding yeast Saccharomyces cerevisiae, is synthesized by 1,3-β-glucan synthase (GS). Although a yeast gene whose product is required for GS activity in vitro, GNS1, was isolated and characterized, its role in GS function has remained unknown. In the current study we show that Δgns1 cells accumulate a non-competitive and non-proteinous inhibitor(s) in the membrane fraction. Investigations of inhibitory activity on GS revealed that the inhibitor(s) is mainly present in the sphingolipid fraction. It is shown that Δgns1 cells contain phytosphingosine (PHS), an intermediate in the sphingolipid biosynthesis, 30-fold more than wild-type cells do. The membrane fraction isolated from Δsur2 cells contains an increased amount of dihydrosphingosine (DHS) and also exhibits reduced GS activity. Among constituents of the sphingolipid fraction, PHS and DHS show striking inhibition in a non-competitive manner. The intracellular level of DHS is much lower than that of PHS in wild-type cells, suggesting that PHS is the primary inhibitor of GS in vitro. The localization of PHS to the endoplasmic reticulum in wild-type cells coincides with that of the inhibitor(s) in Δgns1 cells. Taken together, our results indicate that PHS is a potent inhibitor of yeast GS in vivo.

In plant and fungi, remodeling of the cell wall is one of the essential processes for cell shape determination. Among cell wall components in the budding yeast Saccharomyces cerevisiae, 1,3-β-α-glucan (glucan) is the main structural component responsible for the rigidity of the cell wall (1). Glucan is synthesized by a specific biosynthetic enzyme, 1,3-β-glucan synthase (GS)1 (EC 2.4.1.34) localized to the plasma membrane. Yeast GS has been extensively studied both genetically and biochemically, revealing spatial and temporal regulation of cell wall synthesis (1, 2). Recent studies of yeast GS revealed that it is composed of at least two subunits: a putative catalytic subunit encoded by two related genes, FKS1 and FKS2, and predicted to be an intrinsic membrane protein with 16-membrane spanning domains (3–5) and a regulatory subunit, a peripheral membrane protein encoded by RHO1 (6–8). Since GTP-bound Rho1p is required not only for cell wall synthesis but also for intracellular actin organization (9), signal transduction leading to Rho1p plays a key role in cell morphogenesis. Another gene, GNS1, was originally isolated as a positive component required for GS activity in vitro (10). GS activity is severely reduced in the membrane fraction of a Δgns1 mutant (10). GNS1 interacts genetically with FKS1: a Δgns1 Δfks1 double mutant grows more slowly and exhibits more reduced GS activity in the membrane fraction than single mutants (10). Although these results suggest that GNS1 is somehow involved in GS activity, the physiological function of GNS1 remained unsolved since the Δgns1 mutant has a normal glucan content (10).

Other lines of evidence revealed that GNS1 is allelic to ELO2, which is involved in fatty acid elongation and sphingolipid synthesis (11, 12). A Δelo2 (Δgns1) mutant is defective primarily in elongation of very long chain fatty acids. Since yeast sphingolipids are structural components of very long chain fatty acids, inability of Δelo2 cells to synthesize very long chain fatty acids results in alteration in the amounts of intermediates in sphingolipid metabolism (11).

In this study, we further investigated the reduced GS activity in the membrane fraction of the Δgns1 mutant. Our results indicate that a PHS accumulation in the Δgns1 mutant causes non-competitive inhibition of GS activity.

EXPERIMENTAL PROCEDURES

Media and Strains—Media for growth of S. cerevisiae and Escherichia coli are as described previously (13). Genetic manipulations and yeast transformations were carried out as described (14). The E. coli strain SCS1 (Stratagene, San Diego, CA) was used for propagation of plasmids used in this study. The yeast strains used in this work were derivatives of YPH500 (MATα ade2 his3 leu2 lys2 trp1 ura3) (14). YOC798 (MATα ade2 his3 leu2 lys2 trp1 ura3 Δgns1::HIS3) was made by transformation of YPH500 with the SphI-Sacl fragment of pYO1929. Transformation of YOC798 with pYO1738 resulted in YOC799. YOC2587 (MATα ade2 his3 leu2 lys2 trp1 ura3 Δgns1::EGR2) was made by transformation of YPH500 with pYO1929 plus the SphI-Sacl fragment of pYO1738.

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‡ The abbreviations used are: GS, 1,3-β-glucan synthase; DHS, dihydrosphingosine; EPI, endoplasmic reticulum; glucan, 1,3-β-glucan; GTP, guanosine 5′-[γ-thio]triphosphate; IPC, inositol phosphoceramide; MIPC, mannosylinositol phosphoceramide; M(IP)C, mannosyl diinositolphosphorylceramide; PHS, phytosphingosine; PCR, polymerase chain reaction; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid.
made by the PCR method described by Sakamoto et al. (15); primers were used to amplify the HHS3 gene of Candida glabrata together with flanking sequences derived from the upstream and downstream regions of SUR2 and IPT1, respectively.

Restriction-modifying enzymes were purchased from TaKaRa (Kyoto, Japan). PHS, ethyro-DHS, trypsin, Sephacryl S-1000, protease inhibitors, and reagents for enzyme assays were obtained from Sigma. Reagents for sphingolipids extraction came from Wako (Osaka, Japan).

DNA Manipulation—Standard molecular biological techniques were used for the construction of plasmids and PCR (13). pUC119-GNS1 containing the full-length of GNS1 was constructed by inserting the 3.0-kilobase Spal-SalI fragment containing GNS1 into pUC119. pYO1929, generated for GNS1 disruption, was made by inserting the BamHI-BamHI fragment of HHS3 from pJ215 into the NruI-EcoRI gap of pUC119-GNS1. YEpU-DPL1 was constructed by inserting the 2.6-kilobase NotI-Hael fragment amplified by PCR with 5’-CCGCTC-GAGCCCGACAGTACGACTTAAAAA-3’ and 5’-CCCGCTCGATATTGTGAGATTGTTT-3’ containing the full-length of DPL1 into pRS326 (14).

In order to insert the SHA epitope at the C terminus of Gns1p, an Nhel site was introduced just before the stop codon of GNS1 as follows. First, pRS331-NcoI was constructed by inserting the NcoI linker at the unique BamHI site of pRS331 (14). Second, pYO1929 was constructed by inserting the SpeI-NcoI fragment containing GNS1 from the NotI-Hael fragment amplified from pUC119-GNS1 into pRS331-NcoI, so that the NcoI-Hael fragment amplified from pUC119-GNS1 was inserted between the SpeI and NcoI sites. These two plasmids were digested with NotI and EcoRI to recover the NcoI-Hael fragment. Finally, the NcoI-Hael fragment containing SHA from pYT11 (17) was inserted into pRS314 at the Nhel site.

Preparation of the Membrane Fraction—Cells were grown at 25 °C in 1 liter of medium in a 2-liter flask rotating in an air incubator (Innova 4330) at 150 rpm until the A600 of the culture reached one. All the following procedures were carried out at 4 °C, unless otherwise stated. The cells were harvested, washed with 1 ml EDTA, and disrupted by vortexing 4 times for 2 min each with 5 ml of glass beads in 20 ml the breaking solution containing 0.5% NaCl, 1 ml EDTA with 1% mercaptoethanol, and 33% glycerol, homogenized with a Dounce homogenizer, and stored at −80 °C.

Solubilization and Purification of GS—Purification of GS was carried out by product entrapment as described previously (6) with some modifications. GS was solubilized from the membrane fraction by adding 0.2 M NaCl, 20 μM GTP-γS, 5 mM dithiothreitol, 0.5% CHAPS, and 0.1% cholesteryl hemisuccinate. This suspension was left on ice for 20 min and centrifuged at 100,000 × g for 30 min. The supernatant was collected by centrifugation at 303 PC tubes (Hitachi). The membrane fraction was collected by centrifugation at 100,000 × g for 30 min in an RP70T rotor (Hitachi) with Himac CP 65 (Hitachi). The resultant pellet was suspended with a membrane buffer containing 50 mM Tri-HCl, pH 7.5, 10 mM EDTA, 1 mM β-mercaptoethanol, and 33% glycerol, homogenized, and stored at −80 °C.

RESULTS

A GS Inhibitor(s) Accumulated in the Δgns1 Mutant—To characterize the reduced GS activity in the membrane fraction of Δgns1 cells, we determined the K_{m} and V_{max} values of GS activity. A kinetic analysis of GS activity revealed that the Δgns1 mutant has a decreased K_{m} value (Table I) (27) with several modifications. Cultures were grown at 25 °C in 4 liters medium until the A600 of the cultures reached one. Cell fractions made with glass beads were cleared of debris by centrifugation at 1,000 × g for 5 min. The membrane fraction was prepared by centrifugation at 100,000 × g for 2 h. The pellet was resuspended with 5 ml of STE (10% sucrose in breaking solution with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 2 mg/ml chymostatin, 1 mg/ml leupeptin, 0.5 mg/ml pepstatin, and 0.5 mg/ml aprotinin) and was layered on top of 30 ml of a 20–60% linear sucrose gradient in breaking solution. Samples were centrifuged at 100,000 × g for 2 h at 4 °C in a P28S rotor (Hitachi), and fractions of 3 ml were collected from the top of the gradient. Each fraction was diluted 5-fold by breaking solution and was centrifuged at 100,000 × g for 1 h at 4 °C in an RP70T rotor (Hitachi). The pellet was suspended with the membrane buffer as described above. Plasma membrane ATPase activity (28), Golgi GDPase activity (29), NADPH-cytochrome c reductase activity (30), α,β-mannosidase activity (31), kynurenine activity (32), and invertase activity (19) were assayed in gradient fractions as described previously.

Sphingolipid Extraction—Sphingolipids were extracted from [1H]serine-labeled cells as described (11). Radioactive bands were quantified and visualized with FLA-2000 (Fuji Photo Film) using a tritium screen. Sphingolipids were extracted from non-labeled cells as follows. Cultures each were grown at 25 °C until the A600 of each culture reached one. Cells were washed with 1 ml EDTA, suspended in 15 ml of 0.5 M NaCl, 1 mM EDTA with 1% mercaptoethanol, and 33% glycerol, homogenized, and stored at −80 °C.

Fluorescence Microscopy Procedures—Procedures for immunofluorescence microscopy were as described previously (35). Anti-HA (16B12, Mouse IgG1,κ, clone 16B12/53, Sigma) and anti-Ras antibodies were used as primary antibodies. Cells were observed under the Olympus BX-FLA microscope (Olympus, Tokyo).

| Strain | K_{m} (μM) | V_{max} (nmol/min/mg protein) |
|--------|------------|-----------------------------|
| Wild-type | 0.37 ± 0.12 | 2.92 ± 0.44 |
| Δgns1 | 0.33 ± 0.06 | 0.54 ± 0.01 |

The data presented here are representative of four independent experiments that were performed on three different days. The values were calculated from at least three independent samples, and the standard deviations are shown in parentheses. The data are expressed as the mean ± standard deviation of the mean.
GS decreases, or (iii) an inhibitor of GS accumulates in the membrane fraction of the \( \Delta gns1 \) mutant.

To test the first possibility, we measured the total amounts of Fks1p and Rho1p in \( \Delta gns1 \) cells. Immunoblotting analyses demonstrated that the Fks1p and Rho1p levels in the membrane fraction of \( \Delta gns1 \) cells were indistinguishable from those of wild-type cells (data not shown). In \( \Delta gns1 \) cells, Fks1p and Rho1p exhibited a normal localization pattern (6): both are placed at the growing tip of the bud (data not shown). These results suggested that the amount of GS localized to the plasma membrane was not altered in \( \Delta gns1 \) cells.

We also examined whether or not Gns1p is a component of the GS complex using a strain expressing Gns1p tagged at its C terminus with 3 repeats of the influenza hemagglutinin (3HA) epitope (Gns1:3HAp). A low-copy plasmid expressing Gns1:3HAp (YOC799) was grown at 25 °C and fixed for indirect immunofluorescence microscopy. Cells were stained with anti-HA (a), anti-Kar2p (b, ER marker) antibodies, or 4,6-diamidino-2-phenylindole (c).

GS was purified from the membrane fraction prepared from \( \Delta gns1 \) cells expressing Gns1:3HAp (YOC799) as described under “Experimental Procedures.” A series of fractions were analyzed with anti-HA (16B12) and anti-Rho1p antibodies (6). C, localization of Gns1:3HAp on the ER. \( \Delta gns1 \) cells expressing Gns1:3HAp (YOC799) were grown at 25 °C and fixed for indirect immunofluorescence microscopy. Cells were stained with anti-HA (a), anti-Kar2p (b, ER marker) antibodies, or 4,6-diamidino-2-phenylindole (c).

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Although we did not rule out the possibility that Gns1p activates GS, we obtained strong evidence suggesting that the \( \Delta gns1 \) mutant contains a GS inhibitor(s). We solubilized the membrane fractions of wild-type and \( \Delta gns1 \) mutant cells with detergents and treated with trypsin and heat (“the trypsin-digested detergent fraction”). GS activity of the detergent fraction of wild-type cells was measured in the presence of the trypsin-digested detergent fraction. E, kinetic assay of GS inhibition by the detergent fraction of the \( \Delta gns1 \) mutant. In the presence of 10 \( \mu \)l of the wild-type detergent fraction, various volumes (\( I \)) of the trypsin-digested detergent fraction of the \( \Delta gns1 \) mutant were added. GS activity was measured in the presence of 0.2 or 0.3 mM UDP-glucose.

Subcellular Distribution of Inhibitor(s) in the \( \Delta gns1 \) Cells—We analyzed the subcellular distribution of the inhibitor(s) in \( \Delta gns1 \) cells. Because inhibitory activity was mainly...
recovered from the membrane fraction (data not shown), the membrane fraction of Δgns1 cells was further fractionated on a 20–60% linear sucrose gradient by centrifugation. We first determined the distributions of marker enzymes to check organelle distributions along the gradient. ER membranes were distributed in lower density fractions, while the plasma membrane was found in higher density fractions both in wild-type and Δgns1 fractions (Fig. 3A).

Each fraction was solubilized with detergents, followed by treatment with trypsin and heat, and was subjected to the inhibition assay. Fractions around the peak of the plasma membrane exhibited little difference in the inhibitory activity between the wild-type and Δgns1 strains. In contrast, lighter fractions of Δgns1 prominently inhibited GS activity (Fig. 3C). These results suggested that the inhibitor(s) of the Δgns1 mutant is concentrated not in the plasma membrane, but in lighter fractions containing the peak of ER.

PHS Inhibits GS Activity—The Δgns1 mutant is defective in elongation of very long chain fatty acids and synthesis of sphingolipids with very long chain fatty acids (11). Since the inhibitor(s) accumulated in Δgns1 cells is non-proteinous and mainly localized to the membrane fraction, we hypothesized that the inhibitor(s) is a lipid. To test this idea, we examined whether the total lipid extracted from Δgns1 cells has an inhibitory activity. It was found that the total lipid extracted from the Δgns1 mutant strikingly inhibited GS activity (Fig. 4), while that extracted from wild-type cells had less inhibitory activity. Furthermore, Fig. 4 shows that the sphingolipid prepared from the total lipid of the Δgns1 mutant by mild alkaline had the same inhibitory activity. These results suggested that the inhibitor(s) accumulated in the Δgns1 cells is mainly present in the sphingolipid fraction.

In order to identify which sphingolipid(s) inhibits GS activity, we resolved the sphingolipid fraction from [3H]serine-labeled cells by TLC (11). As previously reported (11), the amounts of ceramide, inositol phosphoceramide (IPC), mannosylinositol phosphoceramide (MIPC), and mannosyl diinositol-phosphorylceramide (M(IP)2C) decreased in the Δgns1 mutant (Fig. 5A), while both PHS and DHS contents increased 30-fold as compared with those in wild-type cells. Measurements of GS activity in the presence of each sphingolipid demonstrated that the PHS fraction from Δgns1 cells strikingly inhibited GS activity (Fig. 5B). The DHS fraction prepared from Δgns1 cells also inhibited GS activity to some extent.

Mutations defective in sphingolipid biosynthesis have been widely studied in S. cerevisiae (Ref. 36; Fig. 6A). We constructed Δsur2 and Δipt1 mutants, both of which affect sphingolipid biosynthesis (37–39), and measured their sphingolipid compositions and GS activities. Δsur2 cells contained an increased amount of DHS, while Δipt1 cells contained normal levels of PHS and DHS (Fig. 6B). GS activity was specifically reduced in the membrane fraction isolated from Δsur2 cells (Fig. 6C). These results also suggested that DHS has GS inhibitory activity.

Since DPL1 encodes a possible long-chain base-phosphate lyase that catabolizes sphingolipids (Ref. 40 and Fig. 6A), it is likely that overexpression of DPL1 results in reduced intracellular levels of PHS and DHS (41). In order to test this possi-

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**Fig. 3. Intracellular distribution of GS inhibitory activity in Δgns1 cells.**

**A**, marker enzyme distribution. PM ATPase (plasma membrane marker; open triangles), GDPase (Golgi marker; open squares), and NADPH:cytochrome c reductase (ER marker; closed circles) activities were determined by respective enzymatic assays. **B**, protein concentration (open squares) and sucrose density (open circles). **C**, inhibition of GS activity with the trypsin-digested detergent fraction. Trypsin-digested and heat-treated detergent fractions were added to the detergent fraction of wild-type cells and the GS activity in each sample was measured.
ability, we introduced multiple copies of DPL1 to the Δgns1 strain. We found that DPL1 overexpression in Δgns1 cells caused a 50% decrease in the amounts of PHS and DHS (Fig. 7A) as well as a reduction in GS inhibition (Fig. 7B). This result further supported the idea that an accumulation of PHS or DHS or both causes reduced GS activity in the membrane fraction.

To examine whether PHS and/or DHS inhibit GS activity, we isolated sphingolipids from wild-type cells, and directly measured their effects on the activity of purified GS. Ceramide, IPC, MIPC, and M(IP)_2C did not affect GS activity in the concentration ranges examined. In contrast, PHS and DHS highly inhibited GS activity. The IC₅₀ of PHS was about 0.5 mg/ml (Fig. 8A), which is approximately in the same range as the physiological concentration in the Δgns1 mutant but notably higher than that in wild-type strain (data not shown). Fig. 8B shows that PHS inhibited GS activity in a non-competitive fashion. DHS also inhibited GS activity non-competitively (Fig. 8A and data not shown), but the intracellular level of DHS was much lower than that of PHS in wild-type cells (Figs. 5A and 7A), suggesting that in vivo PHS is the primary GS inhibitor. Taken together, PHS, the intermediate of sphingolipids that accumulated in the Δgns1 mutant, was judged to be a potent intrinsic inhibitor to GS.

PHS Is Localized to the ER—In order to investigate the localization of PHS, we measured the PHS contents in purified organelle membranes. The quality of the organelle preparation was monitored by marker enzyme distributions (see “Experimental Procedures”). It was found that each organelle preparation contained only small amounts of other organelles (Table II). 250 μg of sphingolipids extracted from each organelle membrane was subjected to TLC (Fig. 9A). As previously reported (24, 42), IPC was mainly localized to the Golgi membrane,
while MIPC and M(IP)_{2}C were concentrated in the plasma membrane. Lipid particles contained neither MIPC nor M(IP)_{2}C. Measurements of the fluorescent intensity of the PHS fraction demonstrated that this sphingolipid was largely localized to the microsomal membrane fraction (Fig. 9B). The localization pattern of PHS in wild-type cells is the same as that of Gns1p (Ref. 12 and Fig. 1C) and was consistent with the distribution of the inhibitor accumulated in Δgns1 mutant cells.

FIG. 7. Decrease of intracellular PHS and GS inhibition by DPL1 overexpression. A, PHS is decreased in Δgns1 cells by DPL1 overexpression. Wild-type cells were transformed with pRS326, while Δgns1 cells were transformed with both pRS326 and YEpU-DPL1. Cells were labeled with [3H]serine for 6 h and sphingolipids were extracted. Samples containing an equal scintillation count of tritium were applied. Radioactive bands were quantified with FLA-2000 (Fuji Photo Film) using a tritium screen. Closed bars, DHS; open bars, PHS. B, GS inhibition is diminished by DPL1 overexpression. GS inhibitory effect was measured as described in the legend of Fig. 2. The data represent the means and standard deviations of three experiments.

DISCUSSION

It was previously found that the membrane fraction of Δgns1 cells exhibits reduced GS activity (10). In this study, several lines of evidence indicated that an accumulation of PHS in the Δgns1 mutant causes non-competitive inhibition of GS activity. First, Δgns1 cells accumulated a non-competitive and non-proteinous inhibitor(s) in the membrane fraction. Second, the Δgns1 mutant accumulated PHS, which was discovered to inhibit GS non-competitively. Among the six sphingolipids examined, PHS was clearly the most potent GS inhibitor. Third, the localization of PHS to the ER was identical to that of the inhibitor accumulated in Δgns1 cells. Fourth, DPL1 overexpression partially lowered the level of PHS accumulated in the Δgns1 cells, resulting in reduced inhibition of GS. Thus, our results are consistent with the idea that PHS negatively regulates GS activity.

PHS inhibits GS activity non-competitively in several possible ways. First, PHS may repress the interaction between Fks1p and Rho1p. For instance, PHS may form a microdomain around Fks1p to inhibit the interaction with the prenylated form of Rho1p required for GS activity (43). It has been reported that the particular lipid fraction isolated from mammalian cells contains glycosylphosphatidylinositol-anchored proteins (44–46), but does not include prenylated proteins (47). Likewise, a particular lipid microdomain containing PHS possibly excludes prenylated Rho1p, preventing its interaction with Fks1p. Alternatively, PHS may alter the environment of lipid bilayer, causing inactivation of GS. The physicochemical state of lipid bilayer plays an important role in the activities of a number of enzymes (48).

Since wild-type cells contain PHS on the ER membrane, it is likely that PHS inhibits GS activity on the ER. Many membrane-bound proteins localized to the plasma membrane are synthesized on the ER and transported through the secretory pathway (49). Fks1p, a putative catalytic subunit of GS, accumulated in intracellular organelles when vesicular transport was blocked by sec mutations, suggesting that Fks1p is transported along this pathway to the plasma membrane after its synthesis on the ER. Furthermore, the GS activity was reduced in the membrane fraction of sec12-1, sec16-2, and sec21-1 cells, all of which are defective in transport from the ER to the Golgi at the restrictive temperature (50, 51). Furthermore, the reduction in GS activity in the sec mutants is diminished by overexpression of DPL1. These results raise a possibility that a basal level of PHS residing in the ER functions to prevent nascent GS from being activated in the ER. If this is the case,
Characterization of yeast subcellular fractions by specific activities of marker enzymes

| Marker enzyme                  | Nuc | MS  | Golgi | Mit | Vac | LP  | SV  | PM |
|--------------------------------|-----|-----|-------|-----|-----|-----|-----|-----|
| NADPH:cytochrome c reductase   | 1.1 | 4.4 | 1.0   | 1.7 | 1.4 | 1.5 | 0.6 | 1.4 |
| GDPase                         | 14  | 13  | 51    | 5   | 18  | 6   | 27  | 12  |
| Kynurenine hydroxylase         | 0.01| 0.01| 0.00  | 0.17| 0.01| 0.00| 0.00| 0.00|
| α-1,3-Mannosidase              | 6   | 5   | 5     | 6   | 35  | 2   | 15  | 1   |
| Invertase                      | 0.0 | 0.0 | 0.1   | 0.0 | 0.0 | 0.2 | 3.2 | 0.0 |
| PM ATPase                      | 0.1 | 0.8 | 0.1   | 0.2 | 0.6 | 0.6 | 2.5 | 5.0 |

The following abbreviations are used in the table: Nuc, nucleus; MS, microsomes; Mit, mitochondria; Vac, vacuoles; LP, lipid particles; SV, secretory vesicles; PM, plasma membrane.

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it is the first example suggesting that a sphingolipid is involved in the inactivation of an enzyme at a specific organelle. In the case of many yeast enzymes that remain inactive until transported to their specific organelles, activation of enzymes is brought about by a modification or cleavage of their precursors. N-Glycosylation is required for activation of invertase (52, 53) and exo-β,1,3-glucanase (54), both of which are then translocated to the plasma membrane. The N-linked oligosaccharide has been shown to affect the activities of these enzymes by stabilizing protein-protein interactions or by altering the affinity to their substrates. Peptide cleavage is required for activation of vacuolar enzymes such as proteinase A (55) and carboxypeptidase Y (56, 57). Further study will be necessary to test whether sphingolipid in fact affects activities of nascent GS transferred through the ER.
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Yeast 1,3-β-Glucan Synthase Activity Is Inhibited by Phytosphingosine Localized to the Endoplasmic Reticulum

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