Abstract  
Long-chain bases (LCBs) are the precursors to ceramide and sphingolipids in eukaryotic cells. They are formed by the action of serine palmitoyl-CoA transferase (SPT), a complex of integral membrane proteins located in the endoplasmic reticulum. SPT activity is negatively regulated by Orm proteins to prevent the toxic overaccumulation of LCBs. Here we show that overaccumulation of LCBs in yeast results in their conversion to a hitherto undescribed LCB derivative, an LCB vinyl ether. The LCB vinyl ether is predominantly formed from phytosphingosine (PHS) as revealed by conversion of odd chain length tracers C17-dihydrosphingosine and C17-PHS into the corresponding LCB vinyl ether derivative. PHS vinyl ether formation depends on ongoing acetyl-CoA synthesis, and its levels are elevated when the LCB degradative pathway is blocked by deletion of the major LCB kinase, LCB4, or the LCB phosphate lyase, DPLL. PHS vinyl ether formation thus appears to constitute a shunt for the LCB phosphate- and lyase-dependent degradation of LCBs. Consistent with a role of PHS vinyl ether formation in LCB detoxification, the lipid is efficiently exported from the cells. —Martínez-Montañés, F., M. A. Lone, F.-F. Hsu, and R. Schneiter. Accumulation of long-chain bases in yeast promotes their conversion to a long-chain base vinyl ether. J. Lipid Res. 2016. 57: 2040–2050.

Supplementary key words  ceramide • sphingolipids • Saccharomyces cerevisiae • mass spectrometry

Sphingolipids are an essential class of lipids greatly enriched in the plasma membrane of eukaryotic cells. They have been implicated in the formation and maintenance of lateral membrane domains, important for protein sorting and signaling along the compartments of the secretory pathway. Apart from these structural roles, their biosynthetic precursor and intermediates, such as long-chain bases (LCBs) and ceramide, exert important signaling functions to coordinate complex processes, for example, cell cycle progression, apoptosis, and inflammation. Hence, the synthesis and turnover of these lipids must be precisely controlled (1–3).

Sphingolipid synthesis starts in the endoplasmic reticulum (ER), where serine palmitoyl-CoA transferase (SPT) catalyzes the first step in formation of LCBs (4). Variations in chain length of the condensing acyl-CoA and the incorporation of alternative amino acids can result in the synthesis of a chemically heterogeneous set of sphingoid bases (5, 6). The activity of SPT, the rate-limiting enzyme of the pathway, is negatively regulated by Orm proteins, conserved integral ER membrane proteins whose phosphorylation relieves inhibition of SPT activity (7, 8). Kinases that phosphorylate Orm proteins thus integrate multiple signals to maintain sphingolipid homeostasis, including heat and ER stress, and availability of nutrients (9–13).

The major LCBs in yeast are dihydrosphingosine (DHS) and phytosphingosine (PHS), which upon ceramide formation condense with a CoA-activated C26 very-long-chain fatty acid (14, 15). This reaction is catalyzed by the ER-localized ceramide synthase (CerS). Upon transport to the Golgi apparatus, ceramides are converted to a set of complex sphingolipids: inositol phosphorylceramide, mannosylinositol phosphophorylceramide, and mannosyl-diinositol phosphorylceramide (16–19).

In addition to these biosynthetic routes, complex sphingolipids, ceramide, and LCBs are also subject to degradation. Complex sphingolipids are cleaved by an inositol phosphosphingolipid phospholipase C, Isc1 (20). Ceramide, on the other hand, is degraded through alkaline ceramidases Ydc1 and Ypc1 (21, 22). Phosphorylated LCBs, finally, can be cleaved by a sphingosine-1-phosphate lyase, Dpl1, to ethanolamine phosphate and fatty aldehyde (23). The activity of components of this degradative branch, Isc1, Ydc1, and Ypc1, is controlled by the target of rapamycin complex 1 (24, 25).

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(24). Importantly, the transient intermediates of the pathway, LCB, LCB phosphate, and ceramide, not only act as biosynthetic precursors but also have important signaling functions in stress response (see Fig. 1A for an overview of the pathway) (25, 26).

Here we describe a novel LCB derivative, identified as an LCB vinyl ether. This LCB vinyl ether is generated mainly from PHS in cells that accumulate high levels of LCs either due to deregulated de novo synthesis, a block in the degradative pathway, or uptake of externally provided PHS. Conversion of PHS to the vinyl ether derivative appears to act as a shunt for the catabolic pathway because PHS vinyl ether levels are greatly elevated in mutants that cannot phosphorylate LCBs or in mutants lacking the sphingosine-1-phosphate lyase. Consistent with a potential role in PHS detoxification, the PHS vinyl ether is excreted from cells.

RESULTS AND DISCUSSION

Identification and characterization of a PHS vinyl ether

In the course of analyzing LCB levels by MS in lipid extracts from various yeast mutants, we noticed an uncharacterized peak at m/z 344.3. This lipid was of low abundance in wild-type cells (25 pmol/OD), but its concentration was greatly elevated in elo3 mutant cells (436 pmol/OD; Fig. 1B, C). Elo3 is a component of the ER-associated acyl chain elongase complex required for the synthesis of C26 very long-chain fatty acids (29, 30). elo3 mutant cells make C22 instead of the normal C26 fatty acids. Shorter acyl-CoAs, however, are a poor substrate for CerS, the enzyme that catalyzes the N-acylation of LCs to form ceramide (31, 32). As a consequence, elo3 mutant cells display greatly elevated levels of PHS. While wild-type cells have about 19 pmol/OD of PHS, elo3 mutant cells have up to 451 pmol/OD of PHS. The fact that the relative abundance of the lipid at m/z 344.3 correlated with the abundance of PHS in wild-type and elo3 mutant cells suggested that it might be derived from PHS.

To test this hypothesis, we characterized the structure of this lipid by high-resolution MS. When subjected to ESI in the positive-ion mode, [M + H]+ ions were observed at m/z 344.3164, which corresponds to an elemental composition of C20H39O3N (calculated m/z = 344.3159). In the negative-ion mode, ions at m/z 342.3014, corresponding to an elemental composition of C20H39O3N (calculated m/z = 342.3013), were observed. These results indicate that the compound had an elemental composition of C20H39O3N, representing a 1-O-ethenyl-2-amino-4-octadecene-1,3-diol, that is a PHS derivative containing a vinyl ether at the C1 hydroxyl group of PHS (Fig. 2).

Fragmentation (MS2) of the [M + H]+ ions at m/z 344.3164 gave rise to ions of 326 and 308, arising from consecutive losses of water (Fig. 2A, route a), along with ions of m/z 300 arising from loss of CH3=CHOH, and ions of m/z 282 (500 - H2O), arising from an additional loss of water (supplemental Fig. S1). Two pathways leading to the elimination of CH3=CHOH are proposed. The first pathway involves the participation of the hydrogen of the 3-OH group to...
Fig. 1. Identification of a putative LCB derivative. A: Schematic overview of the yeast sphingolipid biosynthetic and degradative pathways. Key enzymes and lipid intermediates are shown. Mutants used in this study are indicated in bold, and drugs that were used are shown in red. The pathway leading to dihydroceramide is highlighted in orange, the maturation of phytoceramide to the complex sphingolipids is highlighted in green, and the degradative pathway is highlighted in blue. B, C: ESI/MS profile of LCBs present in wild-type (WT; B) and elo3Δ/C mutant cells. Lipid extracts prepared from wild-type and elongase (elo3Δ/C) mutant cells were analyzed by ESI/MS in the positive ion mode using the odd chain-length C17-DHS ($m/z$ 288.3) as internal standard (indicated in blue). The major PHS species present in both strains, C18-PHS ($m/z$ 318.3) is indicated in green. The [M + H]$^+$ ion at $m/z$ 344.3, indicated in red, represents a putative novel LCB derivative.
form a long alkyl chain with a terminal oxetane species of \( m/z \ 300 \) (Fig. 2A, route b), which gave rise to ions of \( m/z \ 282 \) via further loss of water (Fig. 2A, route b1). The second pathway involves the hydrogen of the secondary amino group, leading to the formation of an alkyl chain with a terminal aziridine (Fig. 2A, route c), which undergoes further loss of water to yield ions of \( m/z \ 282 \) (Fig. 2A, route c1). These fragmentation pathways were further supported by MS’ spectrum of the ions of \( m/z \ 282 \) (344 → 282; supplemental Fig. S1B), which is identical to the MS’ spectrum of \( m/z \ 282 \) (344 → 326 → 282; data not shown). The spectrum (supplemental Fig. S1B) also contained ions of \( m/z \ 265 \) and 252 arising from losses of NH\(_3\) (Fig. 2A, route b2, structures highlighted in blue) and HCHO (Fig. 2A, route b3), respectively. The ion of \( m/z \ 264 \) is a hallmark of the sphingosine LCB structure and arises from loss of water from the aziridine precursor ions (Fig. 2A, route c2, structures highlighted in orange) (33, 34). The above fragmentation processes were supported by high resolution MS, from which the deduced elemental composition of the fragment ions are consistent with the suggested structures (data not shown).
The structural assignment was further supported by high resolution LIT MS$^n$ of the corresponding [M – H]$^-$ ions (Fig. 2B). The MS$^2$ spectrum of the [M – H]$^-$ ion at $m/z$ 342 contained ions at $m/z$ 324 and 306 arising from consecutive losses of water, and prominent ions of $m/z$ 255, arising from cleavage of $C_3(OH)$-$C_2(NH_2)$ bond of the LCB, together with ions of $m/z$ 225 arising from cleavage of the $C_3(OH)$-$C_4(OH)$ bond (35) (supplemental Fig. S2A). The cleavage of this latter bond is consistent with the formation of the ions of $m/z$ 116, in which the anionic charge site is located at the oxygen atom attached to C5 of the LCB (Fig. 2B). The presence of the ions of $m/z$ 116 also supports the notion of the presence of the 1-O-ethenyl group in the molecule. Further dissociation of the ions of $m/z$ 255 (342 → 255; supplemental Fig. S2B) gave rise to the terminally conjugated ions of $m/z$ 253 via loss of $H_2$, the prominent ions of $m/z$ 225 arising from loss of $HCHO$, and ions of $m/z$ 237 by loss of water. The MS$^3$ spectrum of the ions of $m/z$ 225 (342 → 255 → 225; supplemental Fig. S2C) are dominated by ions of $m/z$ 223 and 221 representing a terminally conjugated diene and triene, respectively, arising from consecutive losses of $H_2$. The spectrum also contained ions at $m/z$ 197, 183, 169, 155, and so forth, and at $m/z$ 111, 97, 83, arising from cleavages of the C-C bond of the LCB via charge-remote fragmentation (Fig. 2B). These results are consistent with the assignments of the suggested structure of 1-O-ethenyl-2-amino-4-octadecene-1,3-diol.

We note that the structure of the proposed PHS vinyl ether has the same elemental composition as C2-dihydroceramide and is thus isobaric with C2-dihydroceramide. The fragmentation pattern of the PHS vinyl ether in both positive and negative ion mode, however, is clearly distinct from that of C2-dihydroceramide (supplemental Fig. S3). Fragmentation of acetylated LCBs typically results in a characteristic loss of $m/z$ 42, corresponding to the loss of a ketene (supplemental Fig. S3). This is not observed upon fragmentation of PHS vinyl ether, which instead loses a fragment of $m/z$ 44, corresponding to a vinyl alcohol. Thus,
Wild-type cells incubated with 10 μM PHS during 30 min displayed high levels of PHS vinyl ether. In these cells, levels of PHS vinyl ether were 1.7-fold higher than free PHS levels (Fig. 3B). Cells incubated with DHS, however, displayed only low levels of the DHS vinyl ether, supporting the conclusion that PHS is the preferred substrate for formation of the LCB vinyl ether.

To distinguish between the conversion of internally synthesized LCBs and that of externally added LCBs to the vinyl ether, we challenged cells with a synthetic, odd chain length LCB tracer. We have previously shown that these C17-LCBs are efficiently taken up and incorporated into ceramide and complex sphingolipids (28). Wild-type cells converted C17-PHS efficiently to the C17-PHS vinyl ether, whereas C17-DHS was only inefficiently transformed to the C17-DHS vinyl ether (Fig. 3B). Other LCBs, such as sphingosine, or the stereoisomer of the natural DHS, L-threo-DHS, were also only very inefficiently converted to the corresponding vinyl ether derivatives. Deoxysphinganine, on the other hand, was not converted to the vinyl ether, which is consistent with the fact that the vinyl ether group is bound to the C1 hydroxyl group, which is missing in deoxysphinganine. Taken together, these results thus show

PHS is efficiently converted to PHS vinyl ether

To confirm this structural assignment and to test whether DHS could also be converted to a DHS vinyl ether, we analyzed the formation of the LCB vinyl ether in cells that cannot form PHS due to a deletion of the Sur2 hydroxylase, which converts DHS into PHS (36). Compared with eloΔ mutant cells, the eloΔ sur2Δ double mutant had greatly elevated levels of DHS. Despite these elevated DHS levels, the eloΔ sur2Δ double mutant produces only very low levels of the corresponding DHS vinyl ether. PHS, accumulating in the eloΔ single mutant, however, is efficiently converted to the PHS vinyl ether as eloΔ mutant cells display about equal levels of both PHS and PHS vinyl ether (Fig. 3A). We thus conclude that PHS rather than DHS is the preferred substrate for formation of the LCB vinyl ether.

To test whether conversion of PHS to the vinyl ether derivative is a general reaction of cells to high levels of PHS, we challenged wild-type cells with externally added LCBs.

Fig. 4. Elevated levels of PHS provoke the accumulation of PHS vinyl ether. A: Mutants that accumulate high levels of PHS also display high levels of the vinyl ether derivative. Lipids were extracted from cells of the indicated genotypes and LCB levels were quantified by ESI/MS. For the myriocin treatment, ormlΔ ormlΔ double mutant cells were incubated with 1 μg/ml myriocin for 4 h. B: Inhibition of SPT activity by myriocin leads to a simultaneous decrease in free PHS and PHS vinyl ether levels. eloΔ mutant cells were treated with 5 μg/ml myriocin for 4 h, and LCB levels were quantified by ESI/MS. C: Pharmacologic and genetic disruption of ceramide synthesis results in elevated levels of PHS vinyl ether. Wild-type cells were treated with 200 μM of fumonisin B1, an inhibitor of CerS, for 4 h, and PHS vinyl ether levels were quantified by ESI/MS. lagΔ locΔ double mutants lacking two components of the CerS have elevated levels of PHS vinyl ether. D: Conditional mutants in fatty acid elongation display elevated levels of C18- and C20-chain length PHS vinyl ethers. Cells that express the essential 3-hydroxyacyl-CoA dehydratase, PHS1, from a tetracycling repressible promoter (TET-PHS1) were cultivated in rich media, in which the promoter is only weakly active, lipids were extracted, and LCB levels quantified by ESI/MS. Values represent means ± SD of three independent determinations. Asterisks denote statistical significance (* P < 0.05; ** P < 0.001; *** P < 0.0001).
phosphatase that dephosphorylates exogenously imported LCB phosphates, and this activity is necessary for the incorporation of exogenous LCBs into sphingolipids (21, 37–39). Levels of free PHS and those of PHS vinyl ether were reduced to wild-type concentrations upon deletion of Lcb3 in the orm1/H9004 orm2/H9004 double mutant (Fig. 4A). Similarly, upon inhibition of SPT activity by myriocin, both PHS and PHS vinyl ether levels were significantly reduced in both orm1/H9004 orm2/H9004 and elo3/H9004 mutant cells (Fig. 4A, B).

Consistent with the notion that levels of the vinyl ether parallel those of free PHS, wild-type cells treated with the CerS inhibitor fumonisin B1, which results in increased PHS levels, displayed a slight but significant increase in PHS vinyl ether levels (28) (Fig. 4C). Deletion of the catalytic components of the CerS, Lag1 and Lac1, on the other hand, resulted in very high levels of PHS vinyl ether, consistent with the fact that lag1/H9004 lac1/H9004 double mutant cells have high levels of free PHS (14) (Fig. 4C). Repression of the phosphatase that dephosphorylates exogenously imported LCB phosphates, and this activity is necessary for the incorporation of exogenous LCBs into sphingolipids (21, 37–39). Levels of free PHS and those of PHS vinyl ether were reduced to wild-type concentrations upon deletion of Lcb3 in the orm1/H9004 orm2/H9004 double mutant (Fig. 4A). Similarly, upon inhibition of SPT activity by myriocin, both PHS and PHS vinyl ether levels were significantly reduced in both orm1/H9004 orm2/H9004 and elo3/H9004 mutant cells (Fig. 4A, B).

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essential component of the acyl chain elongase, PHS1, which codes for a 3-hydroxyacyl-CoA dehydratase, through a tetracycling regulatable promoter (TET-PHS1), again resulted in high levels of both the C18-PHS and the C20-PHS vinyl ether (30, 40, 41) (Fig. 4D). It is interesting to note that bg/delta lcb4/delta double mutant cells and TET-PHS1 cells have previously been shown to accumulate abnormally polar ceramides, as shown by TLC analysis of lipids labeled with $^{3}$H]DHS or $^{14}$C]serine (14, 15, 41). Given the very high levels of PHS vinyl ether produced in these cells, it is possible that the lipids identified as ceramide in these studies actually was the PHS vinyl ether. The presence of the vinyl ether group renders PHS so hydrophobic that it migrates shield this LCB derivative from phosphorylation and subsequent degradation through the lyase pathway.

Taken together, these results indicate that the levels of the vinyl ether consistently parallel those of the free PHS under the various conditions tested here, indicating that the half-life of the two lipids are similar, arguing thus against the possibility that the vinyl ether acts as an inert storage form for the free LCB. In addition, the fact that LCBs of different chain length are converted to the respective vinyl ether indicates that the converting enzymes do not discriminate between these chain length variants of PHS.

**PHS vinyl ether formation acts in parallel to the degradative pathway**

LCBs enter the degradative pathway by first being phosphorylated by either Lcb4 or Lcb5, the two LCB kinases in yeast. Lcb4 is the major kinase located in the ER, whereas Lcb5 has only minor activity in exponentially growing cells (42, 43). The resulting LCB-phosphates (LCB-P) can then either be dephosphorylated by Lcb3/Ysr3 or they are cleaved by the LCB-P lyase, Dpl1, to ethanolamine phosphate and 1-hexadecanal (23). To test whether formation of the vinyl ether depends on prior phosphorylation of the LCB or whether it acts in parallel to this catabolic pathway, we analyzed LCB levels in double mutants of elo3 with either lcb/lcb or lcb/lcb. Deletion of these LCB kinases in the elo3 mutant background resulted in slightly elevated levels of DHS and PHS (Fig. 5A). Deletion of the main LCB kinase, Lcb4, in the elo3 mutant background, however, resulted in a dramatic accumulation of PHS vinyl ether, suggesting that a block in the catabolic pathway shuts PHS toward the formation of the vinyl ether derivative. Deletion of the minor kinase activity, Lcb5, on the other hand, did not significantly increase vinyl ether levels (Fig. 5A).

Given that blocking the degradative pathway through deletion of the major LCB kinase Lcb4 resulted in greatly elevated levels of PHS vinyl ether, we examined whether deletion of the lyase would lead to a similar increase in PHS vinyl ether levels. Deletion of Dpl1 in an elo3 mutant background again gave rise to slightly elevated levels of DHS and PHS; PHS vinyl ether levels, however, were not significantly increased. Upon overexpression of the LCB phosphate phosphatase, Lcb3, however, PHS vinyl ether levels became significantly elevated (Fig. 5B). Overexpression of Lcb3 is expected to reduce the efficiency of the degradative pathway and hence to further increase levels of free LCBs. The fact that this result in accumulation of PHS vinyl ether is thus consistent with a shunt function of the pathway, which diverges free PHS into formation of the vinyl ether.

Taken together, these data indicate that vinyl ether synthesis is independent of the degradative pathway and that it acts in parallel to the catabolic pathway, possibly as a shunt for the degradative pathway under conditions of great excess of free intracellular LCBs. In addition, this shunt is possibly important to prevent the detrimental accumulation of LCB-phosphate (44, 45). On the other hand, the presence of the vinyl group on the C1 hydroxyl shield this LCB derivative from phosphorylation and subsequent degradation through the lyase pathway.

**PHS vinyl ether formation depends on ongoing acetyl-CoA synthesis**

To examine how the PHS vinyl ether is synthesized, we hypothesized that PHS may first be acetylated and the ketone group may subsequently be reduced, first to the hydroxyl...
duced by at least three major pathways in yeast: through the mitochondrial pyruvate dehydrogenase (PDH) complex, through peroxisomal β-oxidation, and through the two acetyl-CoA synthetases, Acs1 and Acs2 (46–48). Under aerobic conditions and on glucose-containing media, ACS2 is essential and ACS1 and the β-oxidation genes, e.g., POT1, are repressed, and the PDH genes are not essential (46, 47, 49). To examine the requirement of acetyl-CoA for the conversion of PHS into PHS vinyl ether, we challenged wild-type and acs2 mutant cells with externally provided PHS and monitored the appearance of the vinyl ether. Wild-type and acs2 mutant cells carrying a plasmid borne copy of ACS2 (acsΔ + pACS2) displayed an essentially equimolar ratio between PHS and PHS vinyl ether (Fig. 6B). The acs2 mutant rescued by a temperature-sensitive (ts) allele of ACS2 (pACS2-t) and the acsΔ acs2Δ double mutant carrying the same ts allele of ACS2, however, displayed significantly decreased levels of PHS vinyl ether compared with free PHS. These data thus indicate that normal acetyl-CoA levels are required for the efficient conversion of free PHS into PHS vinyl ether and hence that vinyl ether formation may proceed through the formation of an acetylated LCB intermediate. It is interesting to note that acetylated LCBs are found in certain microorganisms, such as *Wickerhamomyces ciferrii* (50). However, deletion of the acyltransferases implied in the formation of the W. ciferrii acetylated LCBs, SL11 and AT2, in *Saccharomyces cerevisiae* did not affect PHS vinyl ether synthesis (data not shown). In mammals, on the other hand, 3-O-acetyl-sphingosine is present in so-called fast migrating forms of cerebrosides. They appear during myelination and may play critical functions in myelin structure and function (51). The possibility that PHS vinyl ether synthesis occurs through an acetylated intermediate renders its synthesis analogous to that of the mammalian ether containing glycerophospholipids, which occurs through the exchange of sn-1-bound acyl chain by dihydroxyacetone phosphate by an alkyl group. The resulting alkyl ether can then be further reduced to a vinyl ether, as typically found in the plasmalogens (52).

**The PHS vinyl ether is excreted into the culture medium**

Given that a PHS vinyl ether is considerably more hydrophobic than PHS itself, we wondered whether synthesis of the vinyl ether derivative might be a means to detoxify the cells from the detrimental effects of high PHS levels. We thus analyzed PHS and PHS vinyl ether levels in the cell pellet and the culture supernatant of elongase double mutant cells, *acs1Δ-1 dpl23*, which have high levels of free PHS and PHS vinyl ether (Fig. 7A). *TSG13* encodes for the enoyl reductase that catalyzes the last step in each very long-chain fatty acid elongation cycle (53). Interestingly, PHS and PHS vinyl ether levels present in the cell pellet were similar to the levels of these two lipids present in the culture supernatant, indicating that both of these LCBs can be exported by the cells. PHS vinyl ether levels, however, far exceeded PHS levels in both the cell pellet and the culture supernatant, PHS vinyl ether were comparable, it is conceivable that export of these lipids occurs by passive, energy-independent transport pathways. PHS export, on the other hand, has previously been described to be ATP dependent and to rely on Rsb1, a seven transmembrane protein, whose overexpression rescues the LCB sensitivity of *dpl1Δ* mutant cells (54, 55). To examine the energy requirement of the export of the vinyl ether, we challenged *dpl1Δ* mutant cells overexpressing RSB1 with C17-PHS for 20 min, switched cells to medium containing 2-deoxyglucose and NaN3/NaF to deplete ATP levels for 15 min, and then analyzed C17-PHS and C17-PHS vinyl ether levels in the cell pellet and the culture supernatant. Levels of both C17-PHS and that of the C17-PHS vinyl ether in the extracellular medium dropped significantly upon energy depletion of the cells, consistent with the notion that the export of both of these LCBs is dependent on an active, energy-requiring process (Fig. 7B).

Taken together, the data presented here indicate that free PHS is efficiently converted to a PHS vinyl ether derivative. This conversion is dependent on acetyl-CoA levels, and both free PHS and PHS vinyl ether are efficiently excreted by the cells. Based on these observations, we propose that the synthesis of the vinyl ether containing LCB may act to reduce levels of endogenous free LCBs and thus relieve cells of the growth inhibition of these LCBs. In this model, PHS vinyl ether synthesis and its export may thus act as a detoxification pathway to reduce levels of endogenous PHS (Fig. 8). If this were correct, one would predict that unlike free PHS, the PHS vinyl ether would not be taken up by the cells. A prediction that can be tested once a synthetic PHS vinyl ether will be available. In any case, conversion of free PHS into the vinyl ether derivative provides an additional means to regulate and fine tune the levels of free LCBs and thus to sustain cell proliferation under adverse conditions.

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![Fig. 8](http://doc.rero.ch)

**Fig. 8.** Different fates of LCBs. Scheme summarizing the conversions of LCBs into ceramide, the vinyl ether derivative, and the phosphorylation-dependent catabolic pathway. The open questions of whether the LCB vinyl ether can be converted back into a free LCB and whether it can be efficiently taken up by the cells are indicated.
REFERENCES


