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# Expression of a Novel Marine Viral Single-chain Serine Palmitoyltransferase and Construction of Yeast and Mammalian Single-chain Chimera\*

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The genus *Coccolithovirus* is a recently discovered group of viruses that infect the globally important marine calcifying microalga *Emiliania huxleyi*. Surprisingly, the viral genome contains a cluster of putative sphingolipid biosynthetic genes not found in other viral genus. To address the role of these genes in viral pathogenesis, the ehv050 gene predicted to encode a serine palmitoyltransferase (SPT), the first and rate-limiting enzyme of sphingolipid biosynthesis, was expressed and characterized in *Saccharomyces cerevisiae*. We show that the encoded protein is indeed a fully functional, endoplasmic reticulum-localized, single-chain SPT. In eukaryotes SPT is a heterodimer comprised of long chain base 1 (LCB1) and LCB2 subunits. Sequence alignment and mutational analysis showed that the N-terminal domain of the viral protein most closely resembled the LCB2 subunit and the C-terminal domain most closely resembled the LCB1 subunit. Regardless of whether the viral protein was expressed as a single polypeptide or as two independent domains, it exhibited an unusual preference for myristoyl-CoA rather than palmitoyl-CoA. This preference was reflected by the increased presence of C16-sphingoid bases in yeast cells expressing the viral protein. The occurrence of a single-chain SPT suggested to us that it might be possible to create other fusion SPTs with unique properties. Remarkably, when the two subunits of the yeast SPT were thus expressed, the single-chain chimera was functional and displayed a novel substrate preference. This suggests that expression of other multi-subunit membrane proteins as single-chain chimera could provide a powerful approach to the characterization of integral membrane proteins.

Sphingolipids are essential structural components of membranes, are enriched in lipid rafts, and have been implicated as

important second messengers in many signaling processes. In addition, defects in sphingolipid metabolism are responsible for a variety of diseases. The enzyme serine palmitoyltransferase (SPT)<sup>2</sup> catalyzes the rate-limiting step of sphingolipid synthesis. In both prokaryotes and eukaryotes the intact enzyme contains two polypeptide chains, and there is no evidence for post-translational modification of either chain. The prokaryotic enzyme is a soluble homodimer containing two symmetric active sites (1). In eukaryotes there is substantial evidence that the enzyme is an endoplasmic reticular (ER)-localized heterodimer containing a single catalytic site formed at the interface between the subunits (2–4). It has also been shown that in addition to its ability to catalyze the condensation of serine with palmitoyl-CoA, the eukaryotic enzyme can catalyze the condensation of serine with other acyl-CoAs (5). However, despite the importance of this enzyme, little is known about its structural organization and regulation.

Recently, the sequence of a coccolithoviral genome, EhV-86, was reported (6). This virus infects the marine alga *Emiliania huxleyi*, which plays a significant role in the cycling of carbon in marine environments and is responsible for large algal blooms that reflect sunlight and heat and have been proposed to influence global climate. Viral infection results in attenuation of these blooms and is believed to be important in biogeochemical cycling in the ocean as well as global climate control (7, 8). Remarkably, unlike the sequence of any other virus reported to date, EhV-86 contains a cluster of genes highly homologous to those which in other systems, from yeast to mammals, have been shown to encode enzymes of sphingolipid metabolism. These genes are coordinately expressed within 2 h of infection, suggesting that their role is to alter algal sphingolipid metabolism (9). A particularly surprising feature of the gene cluster is the presence of a single open reading frame (ORF), ehv050, which encodes what appears to be both subunits of a eukaryotic-like SPT. However, the sequence is also homologous to other  $\alpha$ -oxoamine synthases not involved in sphingolipid biosynthesis, leaving open the possibility that this gene might

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<sup>2</sup> The abbreviations used are: SPT, serine palmitoyltransferase; ER, endoplasmic reticulum; HA, hemagglutinin; GFP, green fluorescent protein; vSPT, EhV-86 virally encoded SPT; LCB, long chain base; PHS, phytosphingosine; DHS, dihydrosphingosine; ORF, open reading frame; YPD, yeast extract/peptone/dextrose; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.

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encode an SPT with broad substrate specificity or a new class of  $\alpha$ -oxoamine synthases capable of catalyzing multiple condensation reactions, including some not involved in sphingolipid biosynthesis.

Unfortunately, the inability to experimentally manipulate the genes of the virus or the host makes it difficult to study the products of the putative sphingolipid gene cluster in the native organism. Similarly, proving that ehv050 or any other genes in the cluster are actually involved in sphingolipid synthesis or that sphingolipids are important in the viral life cycle is also not possible. To address these questions we have undertaken the characterization of the putative sphingolipid genes in *Saccharomyces cerevisiae*. We report that the single-chain SPT-like EhV-86 enzyme restores SPT activity and viability to yeast mutants lacking endogenous SPT. We further demonstrate that, unlike the other characterized SPT enzymes, this virally encoded enzyme displays a preference for myristoyl-CoA. We have also expressed both yeast and human SPT as single-chain fusion proteins and show that they are enzymatically active. This suggests that other multisubunit membrane-associated enzymes could be similarly expressed, thus providing a powerful tool for the study membrane proteins.

### EXPERIMENTAL PROCEDURES

**Yeast Strains, Media, and Growth**—Yeast cells were grown according to standard procedures (10). The yeast strains used have been described previously (11).

**SPT Protein Expression**—The ehv050 ORF was PCR-amplified from EhV-86 viral DNA and cloned into the yeast vector pADH2 under the control of the *ADH1* promoter for expression with an N-terminal  $3 \times$  HA tag. The N- and C-terminal domains of ehv050, tagged with  $3 \times$  HA and Myc, respectively, were cloned under the control of either the *Ga110* (vLCB2) or the *Gal1* (vLCB1) promoter. Briefly, a PCR fragment encoding the N-terminal domain of vSPT (codons 1–472) was generated using the forward primer, 5'-AAGGGCGGCGCTATGTACC-CATACGAT, and the reverse primer, 5'-TTCCGGATCCATG-CGTTTTCTTTGACTC, cleaved with NotI and BamHI (sites underlined), and inserted between the NotI and BglII sites of *pESC-LEU* to generate an HA-tagged vLCB2. A PCR fragment encoding the C-terminal domain of the vSPT (codons 473–870) was generated using the forward primer, 5'-AGACCTCGAGTT-TCACTCCGATGTT, and the reverse primer, 5'-AGGCCAAG-C~~TT~~ATGTACACGGCCGTA, cleaved with XhoI and HindIII (sites underlined), and inserted between the XhoI and HindIII sites of the *pESC-URA* to generate an N-terminal Myc-tagged vLCB1. For construction of GFP-vSPT, a GFP gene flanked by XbaI sites was inserted into an AvrII site introduced into the vSPT gene at codon 2. Intracellular localization was determined by fluorescence microscopy as previously described (12). The yeast Lcb2-Lcb1p fusion was made by fusing the yeast *LCB1* gene to the C terminus of the yeast *LCB2* gene such that the fusion protein contained all of Lcb2p followed by all of Lcb1p except for its N-terminal methionine. The human fusion protein contained all of hLCB2 followed by hLCB1 lacking the first 48 amino acids (hLCB2-LCB1). The human protein was expressed in Ly-B cells as previously described (12).

**Long-chain Base Extraction and Analysis**—Long chain bases (LCBs) were extracted, derivatized for detection, and resolved by high performance liquid chromatography according to published procedures (13).

**Microsome Preparation, Immunoblotting, and SPT Activity Assays**—Yeast and Ly-B microsomal proteins were prepared, resolved using a 4–12% BisTris NuPAGE gel system (Invitrogen), and visualized by immunoblotting as previously described using anti-HA (Roche Applied Science), anti-Myc (Sigma), anti-human LCB1 (Transduction Laboratories), and the previously described yeast anti-LCB1 and anti-LCB2 antibodies (11). Secondary antibodies were purchased from Bio-Rad. SPT was assayed as described previously (11, 12). The SPT inhibitor myriocin was purchased from BIOMOL International L.P.

### RESULTS

**ehv050 Encodes an Active SPT Enzyme**—The EhV-86 genome contains an expressed ORF, ehv050, composed of two tandemly repeated  $\alpha$ -oxoamine synthase domains. Alignments revealed the N- and C-terminal domains to be most closely related to the Lcb2p and Lcb1p subunits, respectively, of SPT (Fig. 1). In addition to the overall homology, the positions of critical catalytic residues in the two subunits of SPT are conserved in the two viral domains. The N-terminal domain of the viral protein contains the lysine residue present in Lcb2p that forms a Schiff's base with the pyridoxal 5'-phosphate cofactor, and the C-terminal domain contains a cysteine in a position analogous to that of the cysteine residue in Lcb1p that has been proposed to lie across the subunit interface from the lysine in Lcb2p. This cysteine is critical for enzymatic activity and when mutated in human SPTLC1 results in the neurodegenerative disease, HSN1 (4, 14, 15). Although the viral protein lacks a region homologous to the N-terminal extensions of yeast and mammalian LCB1s, in which the first of their three membrane spanning domains is located, this extension is not present in other  $\alpha$ -oxoamine synthases and is not required for enzymatic activity (12). Taken together, these results suggest that the ehv050 ORF encodes a novel single-chain SPT (vSPT).

To test this hypothesis, the viral gene was expressed in yeast, and its ability to reverse the LCB requirement of SPT-deficient yeast mutants was determined. As shown in Fig. 2, expression of vSPT rescued the LCB requirement of a yeast *lcb1* $\Delta$  mutant. This rescue was not dependent upon yeast Lcb2p since the vSPT also rescued an *lcb1* $\Delta$ *lcb2* $\Delta$  double mutant. In addition, mutation of the conserved lysine in the Lcb2p-like N-terminal domain or mutation of the conserved cysteine in the Lcb1p-like C-terminal domain resulted in loss of function (data not shown).

Although expression of vSPT complemented yeast mutants lacking endogenous SPT activity, growth was generally slower and showed greater temperature dependence. In particular, vSPT did not support growth at 37 °C (Fig. 2). *In vitro* SPT activity measurements revealed that microsomes prepared from vSPT-rescued cells contained  $\sim 1/10$  as much activity as microsomes prepared from wild type yeast (Table 1). Yeast *lcb1* and *lcb2* mutants with reduced SPT activity are temperature-



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drospingosine (C16-DHS) and C16-PHS than in wild type cells (Fig. 3). The high levels of C16-LCBs are especially striking in light of the fact that the endogenous concentration of myristate is much lower than the concentration of palmitate, suggesting that the vSPT has a relative preference for C14-CoA. Indeed, when cells were grown on myristate, the relative abundance of C16-LCBs in the vSPT-rescued cells was even greater (Fig. 3). To more carefully assess the substrate preference of the vSPT, its ability to catalyze the condensation of serine with different acyl-CoAs was determined (Table 1). As predicted by the *in vivo* LCB profiles,

**TABLE 1**  
vSPT prefers C14-CoA as substrate

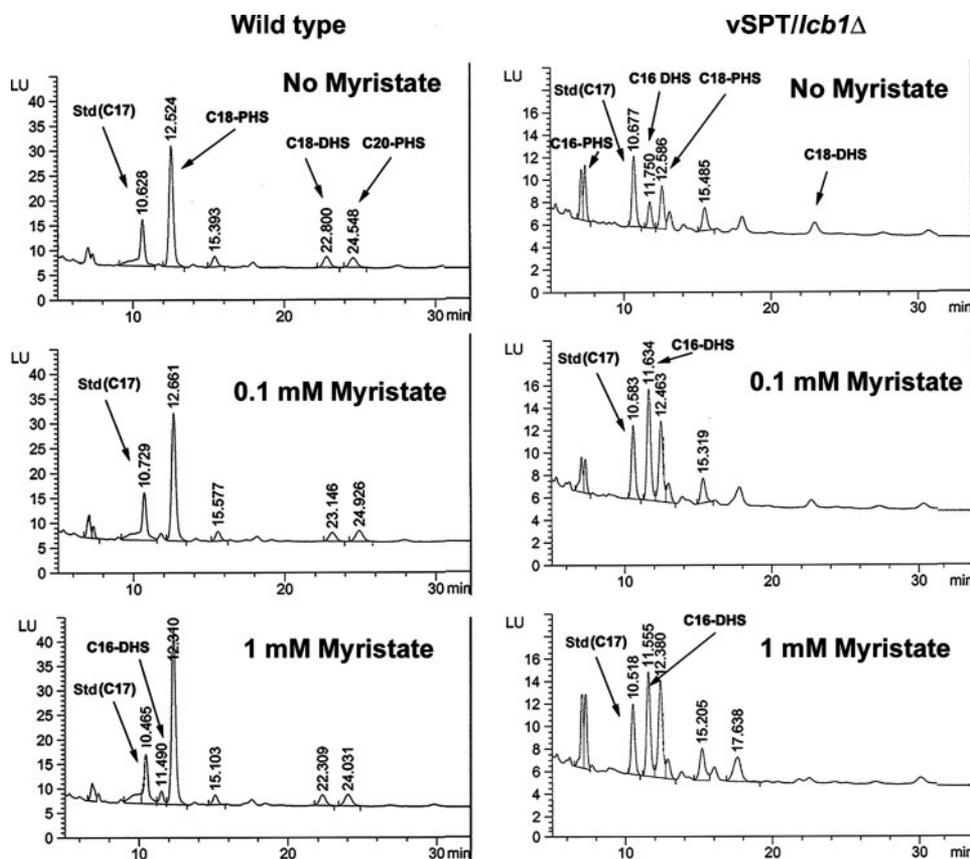
Microsomes were prepared from vSPT-expressing *lcb1Δ* and wild type yeast cells, and SPT activities were assayed using the indicated acyl-CoA substrates. ND, not determined.

| Acyl-CoA chain length | SPT Activity       |           |
|-----------------------|--------------------|-----------|
|                       | Viral SPT          | Yeast SPT |
|                       | <i>pmol/mg/min</i> |           |
| C4                    | 0.10               | ND        |
| C8                    | 0.18               | ND        |
| C10                   | 0.57               | ND        |
| C12                   | 1.10               | 37.25     |
| C14                   | 11.00              | 91.43     |
| C16                   | 7.73               | 129.32    |
| C18                   | 2.82               | 62.42     |
| C16:1                 | 1.39               | ND        |
| C18:1                 | 5.25               | ND        |
| C18:2                 | 1.31               | ND        |

C14-CoA was the preferred *in vitro* substrate. Thus, the vSPT is distinctly different from its yeast counterpart in substrate preference.

In addition to its homology with the subunits of SPT, there is significant homology between the viral protein and other members of the  $\alpha$ -oxoamine synthase family. The greatest homology was with 5-aminolevulinic synthase, encoded by the yeast *HEM1* gene and which catalyzes the committed step of heme biosynthesis, the condensation of succinyl-CoA with glycine. However, vSPT did not complement a *hem1* mutant (Fig. 4A). Moreover, vSPT was sensitive to myriocin, a sphingosine analogue that is a specific and potent inhibitor of yeast and mammalian SPT (17) (Fig. 4B). Thus, although its substrate specificity is somewhat different from previously characterized SPTs, the viral enzyme is a *bona fide* SPT that does not appear to have additional activities.

**Structural Plasticity of the vSPT Enzyme**—Despite the fact that the two  $\alpha$ -oxoamine synthase domains of the ehv050 gene are contained in a single ORE, it is possible that the protein is processed by proteolytic cleavage between the two domains into two polypeptides which then assemble into an active head-to-tail heterodimer, the structure of all previously characterized eukaryotic SPTs. To address this possibility, extracts prepared from yeast cells expressing a functional N-terminally HA-tagged vSPT were analyzed by immunoblotting. A membrane-associated protein of 96 kDa, the predicted molecular



**FIGURE 3. vSPT-rescued mutants accumulate C16-LCBs.** Wild type and the vSPT-rescued *lcb1Δ* yeast were grown in YPD, YPD + 0.1 mM myristate, or YPD + 1.0 mM myristate for several generations before LCB extraction and analysis as described under "Experimental Procedures." The peaks corresponding to the 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate-derivatized C16-PHS, C16-DHS, C18-PHS, C18-DHS, and the C17-DHS internal standard along with their retention times are indicated. LU, luminescence units.

mass for the full-length protein, was easily detected; there was no evidence for the presence of a shorter protein that would have resulted from cleavage between the putative Lcb2p and Lcb1p domains (Fig. 5A). Interestingly however, the full-length protein was resolved into multiple molecular species, which were converted to a single molecular weight component upon treatment with endoglycosidase H. This is consistent with the fact that the viral protein contains several potential *N*-linked glycosylation sites (Fig. 1) and suggested that the protein resides in the ER. The ER localization was confirmed by direct visualization of a GFP-vSPT chimera that showed a pattern of fluorescence typical of ER proteins, including the yeast GFP-Lcb1p (Fig. 5B).

Active SPT could also be reconstituted by separately expressing the N- and C-terminal domains of the vSPT; coexpression of the two domains restored sufficient activity to rescue the yeast SPT-deficient mutants (Fig. 6C). In addition, the reassembled subunits yielded an enzyme with the same preference for myristoyl-CoA as the full-length

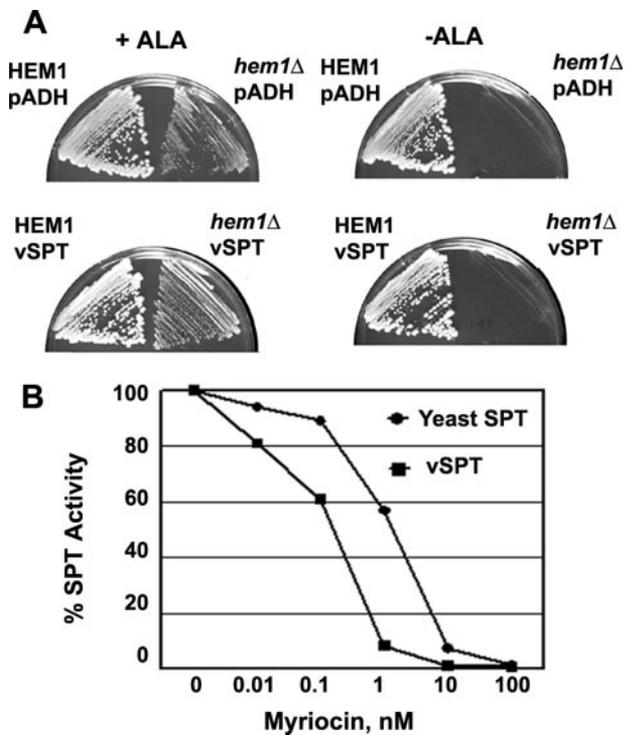


FIGURE 4. vSPT does not rescue a *hem1Δ* mutant and is inhibited by myriocin. *A*, wild type (HEM1) or *hem1Δ* yeast were transformed with pADH1 or a vSPT-expressing pADH1 plasmid, and the transformants were tested for growth on YPD plates with (+ALA) or without (-ALA) aminolevulinic acid. *B*, SPT activity was assayed using microsomes prepared from wild type (yeast SPT) or *lcb1Δ* cells expressing vSPT (vSPT) in the presence of the indicated concentrations of myriocin.

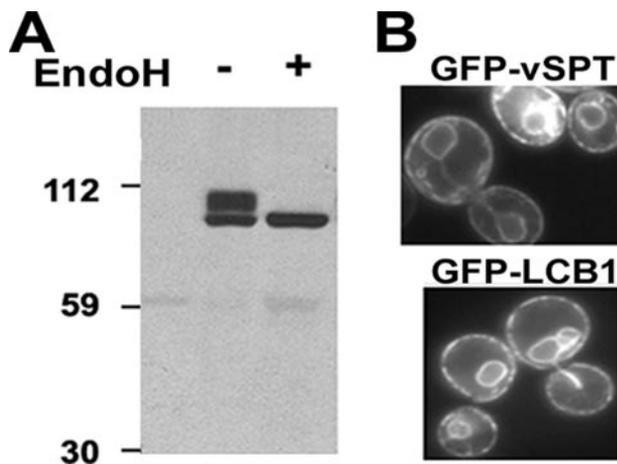


FIGURE 5. vSPT is a single-chain ER-associated protein. *A*, 10  $\mu$ g of microsomal protein from cells expressing 3  $\times$  HA-vSPT were resolved by SDS-PAGE before or after treatment with endoglycosidase H (*EndoH*). vSPT was visualized by immunoblotting as described under "Experimental Procedures." Loss of the higher molecular weight form after endoglycosidase H treatment indicates *N*-glycosylation. *B*, the intracellular localization of GFP-vSPT and GFP-Lcb1p was determined by fluorescence microscopy.

protein (Fig. 6D). Thus, the separated domains can reassemble and fold into an active SPT enzyme with similar properties to the fusion protein. The data shown in Fig. 6A also suggest that it is the Lcb2p-like domain that is glycosylated since only this domain exhibited endoglycosidase H-sensitive heterogeneity. However, because mutation of the two potential glycosylation sites in the Lcb2p-like domain of the full-length protein did not

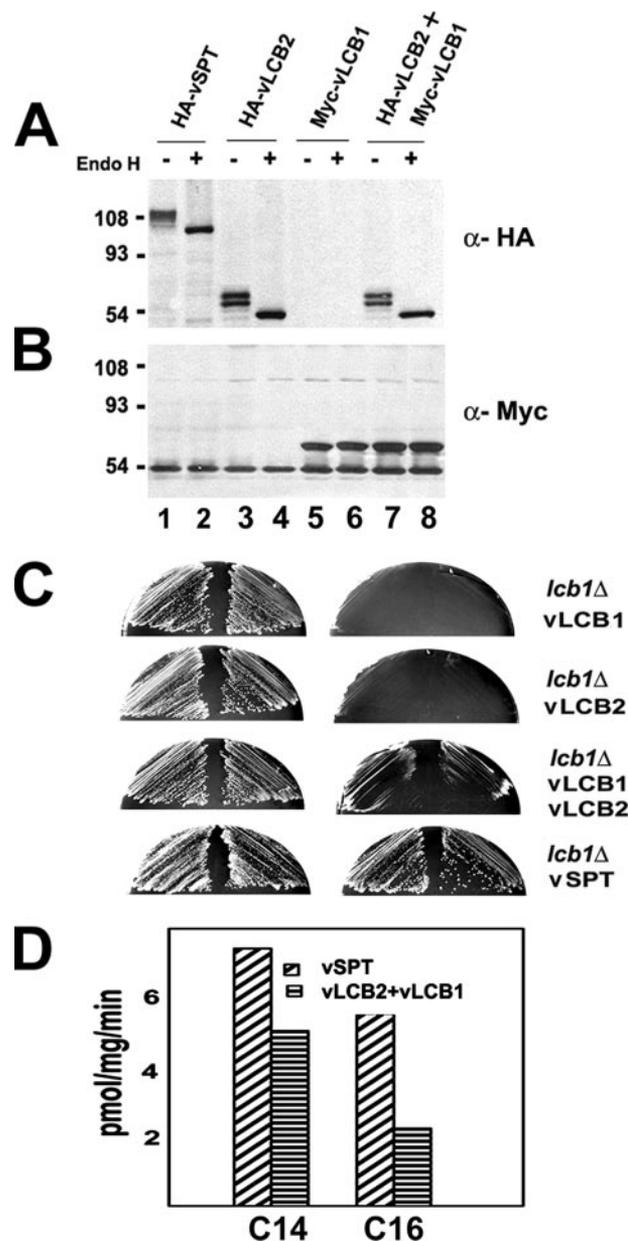


FIGURE 6. Independently expressed domains of the vSPT reassemble into an active protein. *A* and *B*, microsomes prepared from cells expressing full-length HA-vSPT (lanes 1 and 2), the HA-tagged N-terminal domain (HA-vLCB2) of the vSPT (lanes 3 and 4), the Myc-tagged C-terminal domain (Myc-vLCB1) of vSPT (lanes 5 and 6), or both the N- and C-terminal domains (lanes 7 and 8) were resolved by SDS-PAGE after treatment with or without endoglycosidase H (*EndoH*). The tagged proteins were detected with anti-HA (*A*) or anti-Myc (*B*). The approximate 54-kDa protein seen in all lanes (*B*) is an anti-Myc cross-reactive protein unrelated to vSPT. *C*, *lcb1Δ* mutant cells expressing full-length vSPT, vLCB2, vLCB1, or both vLCB2 and vLCB1 were tested for growth with (left) or without (right) PHS at 26 °C. *D*, SPT activity was assayed using microsomes prepared from *lcb1Δ* mutant cells expressing full-length vSPT or vLCB2 and vLCB1 using myristoyl-CoA (C14) or palmitoyl-CoA (C16) as the acyl-CoA substrates.

abolish all glycosylation (data not shown), glycosylation of the full-length vSPT is apparently different from that of the separated domains. Analysis of the coexpressed vSPT subunits also showed that stable expression of the vSPT Lcb2p-like protein does not depend upon the presence of the vSPT Lcb1p-like protein (Fig. 6, *A* and *B*). This is in sharp contrast to the situation in both yeast and mammals where the stability of mono-

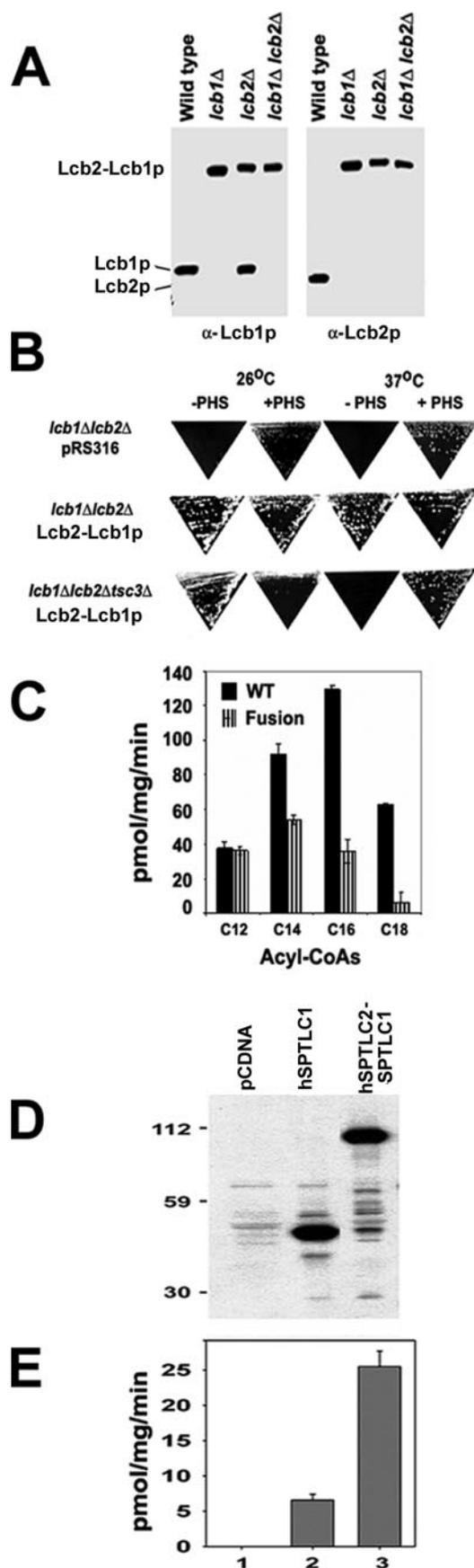


FIGURE 7. Yeast and human fusion SPTs are enzymatically active. *A*, 10  $\mu$ g of microsomal protein from wild type yeast cells or cells expressing the yeast Lcb2-Lcb1p fusion but lacking endogenous Lcb1p, lacking endogenous

meric Lcb2p is strictly dependent upon the presence of Lcb1p (3, 11, 12).

*Characterization of a Yeast Single-chain SPT Provides Additional Evidence for Structural Plasticity of the SPT Enzymes*—The discovery of this novel viral fusion SPT enzyme composed of two separable domains suggested that fusion of the two subunits of the yeast and other eukaryotic SPTs would yield active enzymes. To test this possibility, full-length yeast Lcb1p was fused to the C terminus of Lcb2p to create a yeast Lcb2-Lcb1p fusion protein. Expression of this fusion protein provided sufficient SPT activity to complement the LCB-requiring phenotype of a yeast *lcb1Δ lcb2Δ* mutant (Fig. 7*B*). Unlike the vSPT, the yeast fusion protein was dependent on Tsc3p for full activity, demonstrating that like the native heterodimer the yeast fusion protein retains its interaction with Tsc3p. SPT activity assays showed that the yeast Lcb2-Lcb1p fusion protein, which was expressed to the same extent as the individual subunits (Fig. 7*A*), had  $\sim$ 30% as much activity as the native Lcb2p/Lcb1p heterodimer when assayed with C16-CoA as the substrate (Fig. 7*C*). However, with C14-CoA as the substrate the activity of the fusion protein was  $\sim$ 60% that of the native enzyme, and with C12-CoA as substrate, the fusion protein and the native protein had equivalent activities. Thus, it appears that like vSPT, the yeast fusion protein has an altered substrate preference. In addition, a human hSPTLC2-SPTLC1 fusion protein was active when expressed in a Chinese hamster ovary cell line lacking endogenous SPT activity (Fig. 7, *D* and *E*), demonstrating that the ability to construct an active SPT chimera is a general property of this protein.

The data presented thus far are consistent with the conclusion that the fusion proteins fold as monomers with single active sites formed at the interface between the two domains. However, the possibility that two full-length fusion proteins associate to form a head-to-tail homodimer, creating an enzyme with two active sites, cannot be excluded. Two lines of evidence argue that this is not the case. First, coexpression of a yeast Lcb2-Lcb1p fusion protein containing an inactivating mutation at the lysine residue of the Lcb2p domain with a yeast Lcb2-Lcb1p fusion protein containing an inactivating mutation at the cysteine residue of the Lcb1p domain did not rescue an *lcb1Δ lcb2Δ* mutant (data not shown). If the active form of the enzyme were a head-to-tail homodimer, then coexpression of these mutants should have yielded a population of dimers, 50% of which contained one active site and one mutated site. Because of the robust activity of the yeast Lcb2-Lcb1p fusion protein (40 pmol/mg/min), even this reduced level of activity should have been sufficient to restore growth. Second, if the

Lcb2p, or lacking both Lcb1p and Lcb2p were fractionated by SDS-PAGE, and the subunits were visualized by immunoblotting using anti-Lcb1p (*left*) or anti-Lcb2p (*right*). *B*, yeast *lcb1Δ lcb2Δ* (*rows 1 and 2*) or *lcb1Δ lcb2Δ tsc3Δ* (*row 3*) mutant cells containing empty vector (*row 1*) or vector expressing yeast Lcb2-Lcb1p (*rows 2 and 3*) were assayed for growth at 26 or 37  $^{\circ}$ C in the presence or absence of PHS. *C*, SPT activities in microsomes prepared from the wild type (WT) and the Lcb2-Lcb1p-rescued *lcb1Δ lcb2Δ* mutant (*Fusion*) were assayed using acyl-CoA substrates of the indicated chain length. *D*, Ly-B Chinese hamster ovary cells (24) were transfected with vector (*lane 1*), hSPTLC1 (*lane 2*), or hSPTLC1-SPTLC2 (*lane 3*), and the expressed protein was detected by immunoblotting using anti-hSPTLC1. *E*, Microsomes prepared from transfected cells were assayed for SPT activity as in *C*.

Lcb1p domain of the yeast Lcb2-Lcb1p fusion protein were able to associate with the Lcb2p domain of a second fusion protein, then it should also be able to associate with, and thereby stabilize an Lcb2p monomer. However, the immunoblot clearly shows that there is no Lcb2p monomer stabilized in the *lcb1Δ* mutant expressing the fusion protein (Fig. 7A). Thus, we conclude that the active form of SPT fusion proteins is a folded monomer.

## DISCUSSION

Sequencing of the *Coccolithovirus* genome identified a putative SPT-encoding gene in which both subunits of the classical enzyme are contained in a single ORF (6). Similar fusion proteins are found in EST libraries from *E. huxleyi*,<sup>3</sup> and *Acanthamoeba histolytica* (18). For all three single-chain proteins, the N-terminal domain most closely resembles the Lcb2p subunit of traditional SPTs, and the C-terminal domain most closely resembles the Lcb1p subunit. However, in the absence of genomic sequence from either organism it is unclear that these are the only SPTs present or how the genes that encode them are organized. In addition, since the domains of vSPT are no more homologous to those of the putative SPT from the host than they are to the individual subunits of yeast, *Arabidopsis*, or mammalian SPTs, the phylogenetic relationship between the viral and host SPT fusion proteins is not apparent.

The unique preference of vSPT for C14-CoA suggests that during infection it might function as a serine myristoyltransferase. However, because little is known about the sphingolipid composition of *E. huxleyi*, it is also possible that there may be as yet unidentified substrates that are condensed with serine. Although when measured 24 h after infection, the ratio of C16:C18 LCBs was greater in infected cells than has been seen in other organisms, this increased ratio was also observed in uninfected cells. Thus, despite the relatively low homology between the viral and host enzymes, there is no evidence that this translates into an altered profile of LCBs. Resolution of the question of whether expression of vSPT and other virally encoded sphingolipid genes alter LCB or complex sphingolipid composition will require more extensive sphingolipid profiling over the entire course of EhV-86 infection.

When proteins are found as multidomain "chimera," it has been suggested that elsewhere in nature there will be examples where the individual domains are contained in separate proteins that heterodimerize (19). Thus, the presence of naturally occurring single-chain and heterodimeric SPTs is not entirely surprising. Structural plasticity in the  $\alpha$ -oxoamine synthases was also suggested by studies on murine erythroid 5-aminolevulinic synthase, a homodimeric  $\alpha$ -oxoamine synthase with significant homology to SPT, which is active in at least three circularly permuted configurations (20–22). In addition, we have previously shown that the Lcb1p subunit of yeast and mammalian SPT can tolerate insertion of a 53-amino acid domain from yeast invertase at multiple positions without loss of activity (12). These results led us to consider the possibility that yeast and/or mammalian SPT, expressed as a single-chain fusion, would be active. Our results clearly demonstrate that

this is the case. In addition, the yeast fusion protein localizes to the same intracellular organelle, and its maximum activity depends on the presence of Tsc3p, indicating that the single-chain protein retains many important properties of the native heterodimer. A yeast Lcb1-Lcb2p chimera also showed some activity (data not shown). Thus, an active enzyme can be formed in at least three different ways, demonstrating a high degree of flexibility in the formation of its active site. It should be pointed out that although the vSPT is not proteolytically cleaved to distinct domains in yeast, we cannot exclude the possibility that this or other posttranslational modifications that could alter substrate specificity occur in the algal host. In addition, although there is no Tsc3p-like protein encoded within the viral genome, it is possible that the viral genome encodes a functional ortholog or that the host expresses such a protein.

One property of the single-chain yeast protein significantly different from that of the native heterodimer was its relative preference for shorter chain acyl-CoA substrates. Unlike the native heterodimer, maximum activity was obtained with C14-CoA, and the enzyme was just as active with C12-CoA as with C16-CoA. Although the altered substrate preference of the single-chain yeast SPT is comparable with the preference of the viral enzyme, it is not clear to what extent this reflects an intrinsic property of single-chain SPTs since when the viral protein was expressed as individual domains it retained its preference for C14-CoAs. Nonetheless, altered preference and high activity of the yeast single-chain protein offers a novel approach for altering the cellular levels of the shorter LCBs and their metabolic products, including LCB-Ph and ceramides, which have been implicated in numerous signaling pathways.

It is commonplace to study structure-function relationships by rearranging protein domains or swapping domains from one protein to another. In addition, expression of the heterodimeric gonadotropin hormones as single-chain chimera has allowed the folding of these hormones to be investigated in great detail (23). The data presented here demonstrate that the same approach can be applied to the study of multisubunit membrane-associated proteins. Characterization and purification of such proteins has proven difficult in part because of subunit dissociation during solubilization. If, as is the case with SPT, expression of individual subunits as a single-chain chimera does not substantially alter the essential properties of the enzyme, preservation of quaternary structure should be less difficult. Finally, our results demonstrate that, as has been shown to be the case for mammalian and plant genes, expression and characterization of novel sphingolipid genes from marine organisms in appropriate yeast mutants can provide important insights into the role of these genes in their native hosts.

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## REFERENCES

- Ikushiro, H., Hayashi, H., and Kagamiyama, H. (2001) *J. Biol. Chem.* **276**, 18249–18256

<sup>3</sup> B. Read, unpublished data.

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- Hanada, K. (2003) *Biochim. Biophys. Acta* **1632**, 16–30
- Hanada, K., and Nishijima, M. (2003) *Methods Mol. Biol.* **228**, 163–174
- Gable, K., Han, G., Monaghan, E., Bacikova, D., Natarajan, M., Williams, R., and Dunn, T. M. (2002) *J. Biol. Chem.* **277**, 10194–10200
- Merrill, A. H., Jr., and Williams, R. D. (1984) *J. Lipid Res.* **25**, 185–188
- Wilson, W. H., Schroeder, D. C., Allen, M. J., Holden, M. T., Parkhill, J., Barrell, B. G., Churcher, C., Hamlin, N., Mungall, K., Norbertczak, H., Quail, M. A., Price, C., Rabinowitsch, E., Walker, D., Craigon, M., Roy, D., and Ghazal, P. (2005) *Science* **309**, 1090–1092
- Fuhrman, J. A. (1999) *Nature* **399**, 541–548
- Wilson, W. H., Tarran, G. A., Schroeder, D., Cox, M., Oke, J., and Malin, G. (2002) *Journal of the Marine Biological Association of the United Kingdom* **82**, 369–377
- Allen, M. J., Forster, T., Schroeder, D. C., Hall, M., Roy, D., Ghazal, P., and Wilson, W. H. (2006) *J. Virol.* **80**, 7699–7705
- Sherman, F., Fink, G. R., and Hicks, J. B. (1986) *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor
- Gable, K., Slife, H., Bacikova, D., Monaghan, E., and Dunn, T. M. (2000) *J. Biol. Chem.* **275**, 7597–7603
- Han, G., Gable, K., Yan, L., Natarajan, M., Krishnamurthy, J., Gupta, S. D., Borovitskaya, A., Harmon, J. M., and Dunn, T. M. (2004) *J. Biol. Chem.* **279**, 53707–53716
- Lester, R. L., and Dickson, R. C. (2001) *Anal. Biochem.* **298**, 283–292
- Bejaoui, K., Uchida, Y., Yasuda, S., Ho, M., Nishijima, M., Brown, R. H., Jr., Holleran, W. M., and Hanada, K. (2002) *J. Clin. Investig.* **110**, 1301–1308
- Dawkins, J. L., Hulme, D. J., Brahmabhatt, S. B., Auer-Grumbach, M., and Nicholson, G. A. (2001) *Nat. Genet.* **27**, 309–312
- Beeler, T., Bacikova, D., Gable, K., Hopkins, L., Johnson, C., Slife, H., and Dunn, T. (1998) *J. Biol. Chem.* **273**, 30688–30694
- Miyake, Y., Kozutsumi, Y., Nakamura, S., Fujita, T., and Kawasaki, T. (1995) *Biochem. Biophys. Res. Commun.* **211**, 396–403
- Hofer, M., and Duchene, M. (2005) *Exp. Parasitol.* **110**, 178–183
- Marcotte, E. M., Pellegrini, M., Ng, H. L., Rice, D. W., Yeates, T. O., and Eisenberg, D. (1999) *Science* **285**, 751–753
- Cheltsov, A. V., Barber, M. J., and Ferreira, G. C. (2001) *J. Biol. Chem.* **276**, 19141–19149
- Ferreira, G. C., and Cheltsov, A. V. (2002) *Cell. Mol. Biol. (Noisy-le-Grand)* **48**, 11–16
- Cheltsov, A. V., Guida, W. C., and Ferreira, G. C. (2003) *J. Biol. Chem.* **278**, 27945–27955
- Garcia-Campayo, V., Jablonka-Shariff, A., and Boime, I. (2004) *J. Biol. Chem.* **279**, 44286–44293
- Hanada, K., Nishijima, M., and Akamatsu, Y. (1990) *J. Biol. Chem.* **265**, 22137–22142