Topological and Functional Characterization of the ssSPTs, Small Activating Subunits of Serine Palmitoyltransferase*

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The topological and functional organization of the two isoforms of the small subunits of human serine palmitoyltransferase (hssSPTs) that activate the catalytic hLCB1/hLCB2 heterodimer was investigated. A variety of experimental approaches placed the N termini of the ssSPTs in the cytosol, their C termini in the lumen, and showed that they contain a single transmembrane domain. Deletion analysis revealed that the ability to activate the heterodimer is contained in a conserved 33-amino acid core domain that has the same membrane topology as the full-length protein. In combination with analysis of isoform chimera and site-directed mutagenesis, a single amino acid residue in this core (Met25 in ssSPTa and Val25 in ssSPTb) was identified which confers specificity for palmitoyl- or stearoyl-CoA, respectively, in both yeast and mammalian cells. This same residue also determines which isoform is a better activator of a mutant heterodimer, hLCB1*331F/hLCB2a, which has increased basal SPT activity and decreased amino acid substrate selectivity. This suggests that the role of the ssSPTs is to increase SPT activity without compromising substrate specificities. In addition, the observation that the C-terminal domains of ssSPTa and ssSPTb, which are highly conserved within each subfamily but are the most divergent regions between isoform subfamilies, are not required for activation of the heterodimer or for acyl-CoA selectivity suggests that the ssSPTs have additional roles that remain to be discovered.

The α-oxoamine synthases are a family of related proteins almost all of whose members are soluble head-to-tail homodimers with two symmetrical active sites located at the subunit interface (1–3). This is also true for bacterial SPT2

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‡ The abbreviations used are: SPT, serine palmitoyltransferase; 1-deoxySA, 1-deoxyphosphoinosine; EndoH, endoglycosidase H; ER, endoplasmic reticulum; GC, glycosylation cassette; HSAN1, hereditary sensory autonomic neuropathy type I; LCB, long chain base; PHS, phytosphingosine; QC, QuikChange; ssSPT, small subunit of SPT; TMD, transmembrane domain.
To confirm that the central domain is responsible for binding and activation of the heterodimer and to determine which region of the ssSPTs specifies substrate selectivity, we have constructed and analyzed a series of N- and C-terminal deletion mutants and generated ssSPT chimera. In addition, single amino acid substitutions were used to precisely map the residue responsible for the distinct acyl-CoA selectivities conferred by the ssSPT subunits. To more fully characterize this novel family of activator proteins, we also analyzed their membrane topology. The results of these experiments, as well as the fact that coccolithal virus-encoded SPT is a single-chain LCB2/LCB1 heterodimer (19, 20) and our previous success at expressing active yeast and mammalian LCB2-LCB1 fusions, suggested that it might also be possible to express heterotrimeric SPT isoforms as single-chain fusion proteins. Remarkably, not only were single-chain heterotrimers active (21) but they retained the same acyl-CoA preferences as heterotrimers comprised of individual subunits. Taken together, these results suggest that the ssSPTs are essential components of eukaryotic SPT that not only activate the enzyme but contribute to sphingolipid diversity.

**EXPERIMENTAL PROCEDURES**

**Cells and Cell Growth**—The yeast strain TDY9103 (Mata lcb1Δ::KAN lcb2Δ::KAN tsc3Δ::NAT ura3 leu2 his2 his3 trp1Δ::HIS3) lacking all endogenous SPT subunits was constructed using standard methods and used for all heterologous expression studies. The mutant was cultured in medium containing 15 μM phytosphingosine (PHS) and 0.2% tergitol. To assay growth, 0.2 \( A_{600 \text{nm}} \) of exponentially growing cultures were serially diluted 1:5 in a microtiter plate. The cells were transferred to plates and incubated 4–5 days at the indicated temperatures. CHO-Ly-B cells (RIKEN Bioresource Center, Japan) deficient in LCB1 expression (22, 23) were maintained in serum (JRH Biosciences, Lenexa, KS) and penicillin and streptomycin. Cells were serially diluted 1:5 in a microtiter plate. The cells were disrupted by repeated (four times, 1 min each) cycles of vortexing with cooling on ice between. Unbroken cells, beads, and debris were removed by centrifugation (10,000 \( \times \)g, 10 min), and the low speed supernatant was centrifuged at 100,000 \( \times \)g for 40 min. The crude microsomal pellet was homogenized in TEGM and spun at 100,000 \( \times \)g for 40 min to obtain the microsomal pellet. The pellet was homogenized at 5–8 mg/ml in TEGM containing 33% glycerol and stored at −80 °C. Microsomes were prepared from CHO-Ly-B cells as described previously (24).

**SPT Assay**—SPT was assayed in 300 μl of 50 mM Tris-HCl, pH 7.5, 1 mM EGTA, 1 mM β-mercaptoethanol) and resuspended in TEGM containing 1 mM PMSF, 2 mg/ml pepstatin A, 1 mg/ml leupeptin, and 1 mg/ml aprotinin. Glass beads were added to the meniscus, and cells were disrupted by repeated (four times, 1 min each) cycles of vortexing with cooling on ice between. Unbroken cells, beads, and debris were removed by centrifugation (10,000 \( \times \)g, 10 min), and the low speed supernatant was centrifuged at 100,000 \( \times \)g for 40 min. The crude microsomal pellet was homogenized in TEGM and spun at 100,000 \( \times \)g for 40 min to obtain the microsomal pellet. The pellet was homogenized at 5–8 mg/ml in TEGM containing 33% glycerol and stored at −80 °C. Microsomes were prepared from CHO-Ly-B cells as described previously (24).

**LCB Extraction and Analysis**—Total LCBs were isolated from 5 \( A_{600 \text{nm}} \) of exponentially growing yeast cells by HCl methanolation. Briefly, washed cells were resuspended in 1 ml of 1 N methanolic HCl (Supelco, Bellefonte, PA), and the tubes
were placed in boiling water for 30 min. After cooling on ice, 1 ml of 0.9% NaCl and 2 ml of hexane-diethyl ether (1:1) were added, and following centrifugation the upper phase was removed and the LCBS extracted from the lower phase by adding 0.25 ml of 10 N NaOH, vortexing, and then adding 2 ml of hexane and vortexing. Following centrifugation, 1.5 ml of the upper phase was dried under N2 and resuspended in 80 ml of MeOH:190 mM triethylamine (2:0.3, v/v), and 20 μl of AccQ reagent (Waters, Milford, MA) was added and allowed to react for 60 min. Derivatized LCBS from 1.0 A_600 nm of yeast cells were analyzed by HPLC as described previously (25).

**Glycosylation Cassette Mobility Shift Assays**—Mobility shift assays of proteins containing the GC cassettes were performed using EndoH according to the protocol provided by New England Biolabs (Beverly, MA).

**Preparation of Rightside-out Vesicles**—Sealed vesicles were prepared as described previously (24) with the exception that following zymolyase 100-T treatment the spheroplasts were purified on a 2 m sorbitol cushion.

### RESULTS

**Membrane Topology of the ssSPTs**—The mammalian ssSPTs are predicted to have two transmembrane domains (TMDs) residing within the conserved core as do other vertebrate ssSPTs (Fig. 1A). However, candidate ssSPTs from invertebrates and plants, identified based on their homologies to the human ssSPTs, only have a single predicted TMD (Fig. 1B). The same is true for the yeast ortholog of the ssSPTs, Tsc3p. To determine whether the mammalian ssSPTs have one or two TMDs, their membrane topology and organization were investigated.

The ssSPTs behave like integral membrane proteins when coexpressed with hLCB1 and hLCB2a in both yeast (Fig. 2A, upper) and mammalian cells (Fig. 2A, lower); in either case, the proteins are solubilized with detergents, but not by salt, bicarbonate, or urea. In addition, a positive split ubiquitin two-hybrid interaction between Cub-ssSPTa or Cub-ssSPTb and hLCB1-Nub, whose C terminus is known to be cytoplasmic (24), demonstrated that their N termini are cytoplasmic (16).

To directly address the location of the C terminus, a glycosylation cassette (GC) containing three consensus NXS(T) glycosylation sites was appended to the C terminus of ssSPTa or ssSPTb and tagged proteins assessed for glycosylation based on their sensitivity to EndoH treatment. The increased electrophoretic mobility of both proteins following EndoH treatment (Fig. 2B) provides strong evidence that their C termini are in the ER lumen. In addition, limited proteolysis of rightside-out vesicles containing N- and C-terminally epitope-tagged ssSPTa or ssSPTb showed that the N termini were accessible to proteinase K in the absence of detergent (Fig. 2C, upper) whereas the C termini were protease-resistant unless the vesicles were detergent-solubilized (Fig. 2C, lower). These results indicate that the ssSPTs, when expressed in yeast, have an odd number of TMDs. To determine the topology of the ssSPTs in mammalian cells, N- and C-terminally GC-tagged ssSPTa or ssSPTb was expressed in CHO-Ly-B cells. EndoH treatment showed that the GC-ssSPTs were not glycosylated whereas the ssSPT-GCs were and that glycosylation was eliminated by mutating the three consensus glycosylation sites in the C-terminal GC from NXS/T to QXS/T (N→Q) (Fig. 2D). These results establish that, as in yeast, the N termini of the ssSPTs are cytoplasmic and the C termini luminal. Given these results and the length of the minimal activating domain (see below), we conclude that, as predicted for their invertebrate counterparts, the human ssSPTs contain a single TMD.

**Activity and Acyl-CoA Selectivity of the ssSPTs Reside in the Conserved Core**—We have previously shown that the human ssSPTs confer distinct acyl-CoA preferences on the SPT heterotrimers (16). Whereas ssSPTa confers a preference for C16-CoA as substrate, heterotrimers containing ssSPTb use both C16- and C18-CoA. Inspection of the alignments in Fig. 1A show that the most highly conserved portion of the candidate members of the ssSPT family is located in a central domain, suggesting that the acyl-CoA preference is determined by the divergent N- or C-terminal domains of the proteins. To test this hypothesis, N- and C-terminal deletion mutants of ssSPTa and ssSPTb were generated and their acyl-CoA preferences determined. The results revealed that deletion of the N-terminal 10 amino acids of ssSPTa or ssSPTb had no effect on the ability of the proteins to activate hLCB1/hLCB2a heterodimers sufficiently to complement growth of yeast lacking endogenous SPT, even at 37 °C where the requirement for SPT activity is relatively high (Fig. 3A). In addition, deletion of the N-terminal 10 amino acids of ssSPTa or ssSPTb yielded stable proteins (Fig. 3B) with activities too low...
Role of ssSPTS in SPT Activation and Acyl-CoA Selectivity

A Single Amino Acid in the Core Domain Determines Acyl-CoA Selectivity of ssSPTa and ssSPTb—The deletion studies demonstrated that the acyl-CoA preference of the ssSPTS resides in a central core. To map the acyl-CoA-specifying region more precisely, ssSPT chimera were constructed and tested for their ability to confer preference for C-16 and C18-CoAs. Accordingly, a chimera (aba) in which residues Glu27 to Pro54 of ssSPTa were replaced with residues Glu27 to Pro54 of ssSPTb or a chimera (ab) in which residues Glu27 to Gln68 of ssSPTa were replaced by residues Glu27 to Pro54 of ssSPTb was expressed in yeast, along with hLCB1 and hLCB2a. Microsomal SPT assays showed that both chimeric heterotrimers preferred palmitoyl-CoA as a substrate (Fig. 5). In combination with the analysis of the N-terminal deletions, this places the region responsible for the acyl-CoA selectivity of ssSPTa between residues Ser11 and Glu27. The conclusion that the acyl-CoA selectivity does not lie in the C-terminal region of ssSPTb was confirmed by analysis of a chimera (ba) in which residues Pro54 to Asn76 of ssSPTb was replaced by residues Pro54 to Gln68 of ssSPTa, and hLCB1 and hLCB2a were extracted on ice with an equal volume of TEGM or TEGM containing 1 M NaCl, 5 M urea, 0.2 M Na2CO3, 0.4% Nonidet P-40 or 2% Triton X-100 for 60 min. The samples were subjected to centrifugation at 100,000 g for 30 min, and equal proportions of the supernatants and pellets were resolved by SDS-PAGE. The hssSPTs are integral ER membrane proteins with their N termini in the cytosol and C termini in the lumen. A, microsomes prepared from yeast (upper) or CHO-Ly-B cells (lower) expressing HA-hssSPTa, hLCB1 and hLCB2a were extracted on ice with an equal volume of TEGM or TEGM containing 1 M NaCl, 5 M urea, 0.2 M Na2CO3, 0.4% Nonidet P-40 or 2% Triton X-100 for 60 min. The samples were subjected to centrifugation at 100,000 g for 30 min, and equal proportions of the supernatants and pellets were resolved by SDS-PAGE. HA-hssSPTa was detected by immunoblotting with anti-HA antibody. B, ssSPTa and ssSPTb containing an N-terminal HA tag and a C-terminal GC cassette were expressed in yeast along with hLCB1 and hLCB2a. Microsomal protein (12.5 μg with or without EndoH treatment) was resolved by SDS-PAGE, and ssSPTa was detected by immunoblotting. C, sealed microsomal vesicles were prepared from yeast expressing N- or C-terminally HA-tagged ssSPTa or ssSPTb, hLCB1, and hLCB2a. The microsomes were mock digested or digested with proteinase K (PK) in the absence or presence of Triton X-100. The luminal ER protein, Kar2p, served as a control for vesicle integrity. D, the N- or C-terminally GC-tagged ssSPTs were expressed along with hLCB1 and hLCB2a in CHO-Ly-B cells and assessed for glycosylation as described above. GC cassettes in which the asparagines in the consensus glycosylation sites were mutated to glutamates (N→Q) were used as controls.

FIGURE 2. The hssSPTs are integral ER membrane proteins with their N termini in the cytosol and C termini in the lumen. A, microsomes prepared from yeast (upper) or CHO-Ly-B cells (lower) expressing HA-hssSPTa, hLCB1 and hLCB2a were extracted on ice with an equal volume of TEGM or TEGM containing 1 M NaCl, 5 M urea, 0.2 M Na2CO3, 0.4% Nonidet P-40 or 2% Triton X-100 for 60 min. The samples were subjected to centrifugation at 100,000 g for 30 min, and equal proportions of the supernatants and pellets were resolved by SDS-PAGE. HA-hssSPTa was detected by immunoblotting with anti-HA antibody. B, ssSPTa and ssSPTb containing an N-terminal HA tag and a C-terminal GC cassette were expressed in yeast along with hLCB1 and hLCB2a. Microsomal protein (12.5 μg with or without EndoH treatment) was resolved by SDS-PAGE, and ssSPTa was detected by immunoblotting. C, sealed microsomal vesicles were prepared from yeast expressing N- or C-terminally HA-tagged ssSPTa or ssSPTb, hLCB1, and hLCB2a. The microsomes were mock digested or digested with proteinase K (PK) in the absence or presence of Triton X-100. The luminal ER protein, Kar2p, served as a control for vesicle integrity. D, the N- or C-terminally GC-tagged ssSPTs were expressed along with hLCB1 and hLCB2a in CHO-Ly-B cells and assessed for glycosylation as described above. GC cassettes in which the asparagines in the consensus glycosylation sites were mutated to glutamates (N→Q) were used as controls.
ssSPTa; like the full-length ssSPTb, this chimera conferred a preference for stearoyl-CoA (Fig. 5).

To identify precisely the residues responsible for conferring acyl-CoA selectivity, we focused on residues 18–25, all of which differ between ssSPTa and ssSPTb. Accordingly, a series of mutant ssSPTs in which nonconserved residues were exchanged between ssSPTa and ssSPTb was generated and expressed in yeast along with hLCB1 and hLCB2a. Extracts prepared from these cells were analyzed for the presence of C20-PHS (eluting at 22.5 min), the defining characteristic of heterotrimers containing ssSPTb (compare Fig. 6, A and B).

Substitution of TALYM in ssSPTa with the corresponding residues (SCCAV) from ssSPTb resulted in a clear increase in the presence of C20-PHS (Fig. 6 C, left). Conversely, the reciprocal substitution in which SCCAV was replaced with TALYM in ssSPTb eliminated the accumulation of C20-PHS (Fig. 6 C, right). Thus, acyl-CoA selectivity is specified within this nonconserved pentapeptide. Swapping the second two amino acids (AL to CC or CC to AL) did not alter the amount of C20-PHS synthesized in cells expressing either mutant ssSPT isoform (Fig. 6 D). However, swapping the last two amino acids (YM to AV or AV to YM) resulted in an increase in C20-PHS in cells expressing the mutant ssSPTa isoform and a decrease in C20-PHS in cells expressing the mutant ssSPTb isoform (Fig. 6 E). Remarkably, acyl-CoA preference could be completely reversed by swapping only Met25 of ssSPTa with Val25 of ssSPTb; heterotrimers containing the ssSPTaM25V mutant synthesized C20-PHS at levels comparable with heterotrimers containing wild-type ssSPTb whereas heterotrimers containing the ssSPTbV25M mutant accumulated no C20-PHS (Fig. 6 F).
Thus, a single amino acid is responsible for the difference in acyl-CoA substrate preference of the two SPT isoforms. The effects of mutating residue 25 of the SPTs on acyl-CoA selectivity were also seen in in vitro SPT assays using microsomes from cells expressing the mutant subunits (Fig. 7A). One explanation for these results is that the size of the side chain helps to determine the selectivity of the heterotrimer for acyl-CoAs of various chain lengths. Indeed, this appears to be the case; a heterotrimer containing SPTa in which Met25 has been replaced by glycine is capable of efficiently condensing C20-CoA with serine (Fig. 7B).

The Single Amino Acid Residue Also Controls Acyl-CoA Selectivity in Mammalian Cells—Single-chain heterotrimeric SPTs (hLCB2-ssSPT-hLCB1) have been used to study the effects of HSAN1 mutations (which increase the ability of SPT to condense alanine with acyl-CoAs thereby generating 1-deoxySA (26)) on amino acid substrate preference (21). The single-chain SPT results in stoichiometric expression of the three subunits and ensures that every heterodimer in transfected CHO-Ly-B cells, which lack endogenous hLCB1, contains the same wild-

FIGURE 4. hsSPTs lacking the second predicted TMD retain their native membrane topology. A, sealed microsomal vesicles were prepared from yeast expressing hLCB1, hLCB2a, and N-terminally HA-tagged ssSPTa with either the C-terminal 21 or 25 amino acids replaced by a Myc tag. The microsomes were mock digested or digested with proteinase K (PK) in the absence or presence of Triton X-100. The luminal ER protein, Kar2p, served as a control for vesicle integrity. B, the C-terminal 21 or 25 amino acids of ssSPTa were replaced with a GC cassette. After expression in yeast, glycosylation was assessed as described in Fig. 2.

FIGURE 5. ssSPT chimera reveal that residues 11–26 specify acyl-CoA selectivity. A, schematic represents ssSPT chimera. B, microsomes were prepared from lcb1Δlcb2Δtsc3Δ mutant yeast expressing hLCB1, hLCB2a, and either ssSPTa (a), ssSPTb (b), a chimera that contains the first 27 residues of ssSPTa and the last 49 residues of ssSPTb (ab), or a chimera that contains the first 54 residues of ssSPTb and the last 14 residues of ssSPTa (ba), or a chimera in which residues 27–54 of ssSPTa were replaced by residues 27–54 from ssSPTb (aba). SPT activities were measured using C16- and C18-CoA as substrate.

FIGURE 6. Residue 25 is responsible for the difference in acyl-CoA preference of ssSPTa and ssSPTb. Total LCBs prepared from lcb1Δlcb2Δtsc3Δ mutant yeast expressing hLCB1, hLCB2a and the indicated wild-type or mutant ssSPT subunit were analyzed by HPLC as described under “Experimental Procedures.” A and B, complete elution profiles containing all of the C18 and C20 LCBs. C–F, only the regions of the elution profiles containing C18-dihydrosphinganine (DHS), C20-PHS, and C18-anhydrous PHS since the C20-PHS, eluting at 22.5 min, is diagnostic for the presence of an active ssSPTb subunit.

FIGURE 7. In vitro SPT activity and acyl-CoA selectivity of ssSPT residue 25 mutants. A, microsomes prepared from lcb1Δlcb2Δtsc3Δ mutant yeast cells expressing hLCB1, hLCB2a, and wild-type or mutant ssSPTs were assayed for SPT activity using either C16- or C18-CoA as described in Fig. 3. B, microsomes prepared from cells expressing hLCB1, hLCB2a, and the indicated ssSPTa mutants were assayed for SPT activity using C14-, C16-, C18-, C20-, or C22-CoA as substrate.
type or mutant isoform of ssSPT regardless of their endogenous levels of expression. To determine whether residue 25 of ssSPT also controls acyl-CoA preference in mammalian cells, single-chain fusions containing either ssSPTa or ssSPTb were constructed, tested in yeast for acyl-CoA selectivity, and then transfected into CHO-Ly-B cells. Microsomes prepared from yeast cells lacking endogenous SPT but expressing hLCB2a-ssSPT-hLCB1 fusion proteins had SPT activities comparable with those expressing the individual subunits (Fig. 8A). More importantly, the fusion SPTs displayed the same acyl-CoA preferences as did heterotrimers of the individual subunits in vitro (Fig. 8A) and in vivo (Fig. 8B). Furthermore, the single amino acid residues responsible for acyl-CoA preference in heterotrimers containing individual subunits also controlled preference in the fusion proteins (Fig. 8B). Not surprisingly, similar results were obtained when the fusion SPTs were expressed in CHO-Ly-B cells (Fig. 8C).

Residue 25 of the ssSPTs Also Influences Their Ability to Activate the hLCB1S331F/hLCB2 HSAN1 Mutant Heterodimer—In the course of these studies, we identified a spontaneous mutation in hLCB1 (S331F) that results in elevated basal activity of the hLCB1/hLCB2a heterodimer. Whereas yeast lacking endogenous SPT and expressing the wild-type hLCB1/hLCB2a heterodimer fail to grow at 26 °C (Figs. 3A and 9A), the hLCB1S331F/hLCB2a heterodimer supports robust growth at 26 °C (Fig. 9A). In addition, in vitro enzyme assays revealed that the hLCB1S331F/hLCB2 heterodimer had several-fold higher SPT activity than the wild-type heterodimer (data not shown). It was therefore of interest to determine whether the mutant heterodimers would be responsive to the ssSPTs.

To assess the ability of the ssSPT subunits to activate the mutant hLCB1S331F/hLCB2a heterodimer, various combinations of mutant and wild-type subunits were expressed in yeast as single-chain fusions. Not surprisingly, expression of hLCB2a-ssSPTb-hLCB1S331F allowed growth at both 26 °C and 37 °C, presumably reflecting the ability of ssSPTb to activate the mutant heterodimer (Fig. 9B). However, whereas ssSPTa activates the wild-type enzyme to a greater extent than ssSPTb (Fig. 9D and Ref. 16), the opposite is seen for the mutant; yeast expressing hLCB2a-ssSPTa-hLCB1S331F barely grow at 26 °C and fail to grow at 37 °C (Fig. 9B). Strikingly, introducing the Met to Val substitution into the ssSPTa component of hLCB2a-ssSPTa-hLCB1S331F increased the ability of the fusion SPT to support growth, whereas introduction of Val to Met in the ssSPTb component of hLCB2a-ssSPTb-hLCB1S331F resulted in decreased growth (Fig. 9B). These results were confirmed in vivo and in vitro by comparison of the LCB profiles (Fig. 9C) and microsomal SPT activities (Fig. 9D) from cells expressing the four single-chain fusion SPTs. Rottthier et al. have shown that the LCB1S331F mutation results in the human hereditary sensory neuropathy, HSAN1 (27). Thus, residue 25 of the ssSPTs also results in differential activation of heterodimers containing a disease-causing mutation. It seems unlikely that our identification of a spontaneous hLCB1 mutation that increases basal heterodimeric SPT activity and their identification of the same mutation in HSAN1 patients are simply coincidence. Indeed, the counterparts of two recently identified HSAN1 mutations in hLCB2a (G382A and I504F) (28) were originally identified in yeast Lcb2p based on their ability to elevate basal activity of the Lcb1p/Lcb2p heterodimer (29).

**DISCUSSION**

Recent studies from our laboratory and others have demonstrated that SPT is a far more complex enzyme than was previ-
The purpose of the present study was to identify the regions responsible for activation of the heterodimer and acyl-CoA selectivity. The data in Fig. 4 show that the longest C-terminal deletions retain their native topology; although exactly which residues comprise the TMD is unclear, given that the average membrane spanning domain contains ~20 amino acids, the majority of the minimal activating core must reside within the membrane. Consequently, it is likely that the ssSPTs interact with one or more of the TMDs of LCB1. Topological analysis of yeast Lcb1p identified three TMDs, the most N-terminal of which can be deleted without altering targeting or reducing enzymatic activity (24). Similarly, the N-terminal TMD of mammalian LCB1 can be deleted without apparent functional consequence (24). Thus, it seems that the ssSPTs interact with one or more of the distal TMDs. Interestingly, a mutation in hLCB1, S331F, increases the basal activity of the heterodimer. Because this disease-causing mutation in mammalian SPT results in increased condensation of alanine with palmitoyl-CoA (30), it appears that the penalty for increasing SPT activity of the heterodimer through mutation in one of the catalytic subunits is loss of amino acid substrate specificity. This is consistent with the observation that mutations in hLCB2a corresponding to those we identified in Lcb2p that increased the activity of the yeast heterodimer also result in relaxed amino acid selectivity (28). We therefore hypothesize that a major role of the ssSPTs is to increase enzymatic activity while allowing the enzyme to maintain high amino acid selectivity.

4 A. N. Kimberlin, S. Majumder, M. Chen, G. Han, J. M. Stone, T. M. Dunn, and E. B. Cahoon, unpublished data.

5 S. D. Gupta, D. Bacikova, J. M. Harmon, and T. M. Dunn, unpublished data.
Role of ssSPTs in SPT Activation and Acyl-CoA Selectivity

Because of the decreased response of the hLCB1S331F-containing mutant heterodimer to ssSPTb but not ssSPTa in which Met87 is replaced with valine, it is tempting to speculate that mutation of residue Ser331 of hLCB1 to the bulkier phenyalanine results in a steric clash with Met87 of ssSPTa. In addition, whereas hLCB1S331F-containing mutant heterotrimers synthesize 1-deoxySA when expressed in mammalian cells (30), unlike other HSAN1-causing mutations in hLCB1, such as C133W and V144D, heterotrimers containing hLCB1S331F do not synthesize 1-deoxySA in yeast (data not shown). One possible explanation for this difference is that the structural organization of mammalian SPT is not entirely recapitulated in yeast. Alternatively, there may be post-translational modifications in mammalian cells affecting the catalytic site that do not occur in yeast. This question will need to be resolved in future experiments.

The results presented here show that the N- and C-terminal domains are not essential for function when human heterotrimers are expressed in yeast. However, the high degree of conservation in the C-terminal domains of the ssSPTa and ssSPTb subfamilies is strongly suggestive of a regulatory role in higher eukaryotes. Experiments are in progress to determine whether this is the case.

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