A Mammalian Homolog of the Yeast LCB1 Encodes a Component of Serine Palmitoyltransferase, the Enzyme Catalyzing the First Step in Sphingolipid Synthesis

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Serine palmitoyltransferase (SPT; EC 2.3.1.50) catalyzes the initial step dedicated to sphingolipid biosynthesis and is thought to be a key enzyme for regulating cellular sphingolipid content. For SPT activity, the yeast Saccharomyces cerevisiae requires two genes, LCB1 and LCB2. We isolated mammalian LCB1 cDNA homologs from mouse and Chinese hamster ovary (CHO) cells and an LCB2 cDNA homolog from CHO cells. The mammalian LCB1 proteins are predicted to have about 35% amino acid identity to the yeast Lcb1 protein, whereas the CHO LCB2 protein is predicted to have about 40% amino acid identity to the yeast Lcb2 protein. Northern blot analysis of mRNA isolated from various mouse tissues revealed that the tissue distribution of both LCB1 and LCB2 messengers followed a similar pattern. Transfection of an SPT-defective CHO mutant strain with a CHO LCB1-expressing plasmid restored both SPT activity and de novo sphingolipid synthesis to the wild type levels, whereas transfection of the mutant strain with a CHO LCB2-expressing plasmid did not exhibit any recovery effects, indicating that the SPT defect in the mutant cells is specifically complemented by the CHO LCB1 homolog. Furthermore, when the SPT-defective mutant cells were transfected with a plasmid encoding a His6-tagged CHO LCB1 protein, SPT activity bound to a Ni2+-immobilized resin. These results indicate that the CHO LCB1 homolog encodes a component of SPT.

Sphingolipids are ubiquitous constituents of membrane lipids in mammalian cells and are also distributed widely in other animals, plants, and microbes (1). Previous studies with mutant cells defective in sphingolipid biosynthesis have revealed that sphingolipids are essential for the growth of Saccharomyces cerevisiae (2, 3) and Chinese hamster ovary (CHO) cells (4, 5), implying that sphingolipids play crucial roles in eukaryotes. It has also been demonstrated that sphingoid bases and ceramide modulate activities of various enzymes such as protein kinases, protein phosphatases, and phospholipases in cells or in cell-free systems and that these sphingolipids appear to participate in various cellular events including proliferation, differentiation, senescence, apoptosis, and inflammatory responses (for review, see Refs. 6 and 7). Moreover, sphingomyelin and glycosphingolipids have been suggested to be involved in the formation of detergent-resistant membrane subdomains (8–11), where localized signaling events may occur (12–14).

Sphingolipid biosynthesis is initiated by condensation of l-serine with palmitoyl coenzyme A, a reaction catalyzed by serine palmitoyltransferase (SPT; EC 2.3.1.50) to generate 3-ketodihydrosphingosine (for a review of sphingolipid biosynthesis, see Refs. 7 and 15). 3-Ketodihydrosphingosine is reductively converted to dihydrosphingosine, which is N-acetylated and then dehydrogenated to form ceramide. Ceramide is converted to various complex sphingolipids, for instance, sphingomyelin and glycosphingolipids in mammalian cells. The structure of the polar head groups of complex sphingolipids is highly diverse, and more than 300 different types of complex sphingolipids have been reported (16–18). By contrast, the structure of the ceramide moiety is relatively well conserved from mammals to single-cell eukaryotes, although there are heterogeneities in the acyl chain length and the number of the hydroxyl groups (1, 17, 18). Such structural conservation most likely reflects an evolutionary conservation of the biosynthetic pathway for ceramide.

SPT is suggested to be a rate-determining enzyme in the sphingolipid synthetic pathway and, thus, to be a key enzyme for regulating cellular sphingolipid content (15). However, the actual mechanisms for regulating ceramide and complex sphingolipid synthesis remain unknown largely because few biosynthetic enzymes have been purified, and a limited number of genes encoding the enzymes have been isolated. Two S. cerevisiae genes, LCB1 and LCB2, have been isolated by complementation of mutant strains defective in SPT activity (19, 20). That the LCB1 and LCB2 genes are indispensable for SPT activity suggests that SPT is composed of subunits including at least the Lcb1 and Lcb2 proteins (19, 20). Independently, Zhao et al. (21) isolated the SCS1 gene, which is identical to the LCB2, as an allele whose mutations suppressed the hypersensitivity of a yeast mutant to high concentrations of calcium. Recently, a full-length mouse (mLCB2) and partial human (hLCB2) cDNA homologs of LCB2 have been isolated (22). Here we present residues; kb, kilobase(s); ORF, open reading frame; G\textsubscript{A\textsubscript{n}}, N-acetylneuraminyl lactosylceramide; RI, relative intensity.
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evidence for the isolation of mammalian homologs of LCB1 from mouse and CHO cells. In addition, we present biochemical evidence that the CHO LCB1 protein is a component of the SPT enzyme.

EXPERIMENTAL PROCEDURES

Molecular Cloning of mLCB1, cLCB1, and cLCB2—A mouse LCB1 homolog was screened by sequencing a cDNA library for hybridization to a [32P]-labeled 953-bp human cDNA fragment from the expressed sequence tag clone 6H6 (GenBank accession no. T24597) predicted to encode the LCB1 protein. The human sequence was identified by a computer search of the data base of expressed sequence tags of the National Center for Biotechnology Information using the blastn program (23) and the S. cerevisiae Lcb1 protein sequence (19) as a query. The largest mouse cDNA clone, 1.2 kb, lacked a 5′-coding region. The missing 442 bp were obtained by performing the polymerase chain reaction in the presence of a 5′-RACE-ready cDNA, prepared from mouse kidney (CLONTECH), and the LCB1-specific primers 5′-CCCCCTCTTCTTAGAATGACGAGC-3′ and 5′-GCCAGCCGCTCTTCTA-AATC-3′ plus an anchor primer (CLONTECH). The sequence of the mouse LCB1 cDNA was determined by using a cycle sequence protocol (Life Technologies, Inc.).

A cDNA library, made from mRNA of CHO-K1 cells, was prepared by using phageScript™ plasmid system (Life Technologies, Inc.) according to the manufacturer’s protocol. Primers used for amplification of cLCB1 were: primer A, 5′-TTAGTGGCAGCATTATCGTTCCTC-CATGAT-3′ (reverse); primer B, 5′-TTAGTGGCAGCATTATCGTTCCTC-CATGAT-3′ (forward); primer C, 5′-TTAGTGGCAGCATTATCGTTCCTC-CATGAT-3′ (forward). The CHO cDNA library was subjected to polymerase chain reaction by using primers A and B (thermocycling conditions were 94 °C for 45 s, 57 °C for 45 s, and 72 °C for 90 s at 40 cycles). The initial amplified DNA was diluted 20,000-fold, and then the diluent was subjected to a secondary polymerase chain reaction with primers A and C (94 °C for 45 s at 90 cycles). After digestion with EcoRI and SalI, the amplified 0.9-kbp DNA was cloned into pBlueScript SK, and its DNA sequence was determined. Based on the sequence of the cloned 0.9-kbp DNA, a 25-mer oligonucleotide (5′-CCCATCTGTCGAGGATATATCATGGGAGA-GAGTGGCA-3′) was synthesized for the cDNA positive selection system (Life Technologies, Inc.) to isolate LCB1. cDNAs hybridizable to the 25-mer oligonucleotide were retrieved from the CHO-K1 cDNA library, using the procedures described in the Life Technologies, Inc. manual, transferred into Escherichia coli. Bacterial colonies harboring the LCB1 coding sequence were detected by colony hybridization using the cloned 0.9-kbp fragment of LCB1, random probe-labeled with [α-32P]CTP, as a probe. In this way, three bacterial colonies with a 2.7-kbp cDNA corresponding to LCB1 were isolated. The nucleotide sequence of the cloned cDNAs was determined by using an automated DNA sequence (ABI PRISM™ 310 genetic analyzer, Perkin-Elmer Applied Biosystems). The cLCB2 was also isolated by essentially the same procedures as described above with the following exceptions: primer A, 5′-TTAGAATTCAGAACTGATTGAA-3′ (forward); primer B, 5′-TTAGAATTCAGAACTGATTGAA-3′ (reverse); primer C, 5′-TTAGAATTCAGAACTGATTGAA-3′ (forward). The LCB1 cDNA sequence was determined as described previously (24). In some experiments, the cell lysates were incubated with various concentrations of sphingofugin B (a gift from Dr. Shu Kobayashi, Department of Applied Chemistry, Science University of Tokyo, Tokyo) at 4 °C for 10 min before the SPT assay.

Replicates of CHO cell colonies were prepared on polyester discs and assayed in situ for SPT activity as described previously (4).

Northern Hybridization Analysis—A Northern blot containing about 2 μg/lane poly(A)+ RNA from mouse tissues (CLONTECH) was hybridized separately with a 32P-labeled 1,308-bp EcoRI-Xhol fragment of the mouse LCB1, a 1,458-bp EcoRI-Xhol fragment of the mouse LCB2, and a 2-kbp fragment of human β-actin cDNA. Stringent hybridization conditions were used as recommended by the manufacturer (CLONTECH).

Metabolic Labeling of Lipids with [14C]Serine in Intact Cells—CHO cells were seeded in 5 ml of F-12 medium containing 10% serum in 60-mm dishes and cultured at 40 °C for 2 days to subconfluence, and then SPT activity of lysates prepared from the cells was assayed with Lipofectin™ reagent (Life Technologies, Inc.) and, after selection for G418 resistance (400 μg/mL), transfectant colonies were purified with cloning cups. As a control, SPB-1 cells were transfected with the vector, pSV-Okneo, and one G418-resistant SPB-1 clone was isolated.

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Total RNA was prepared from CHO cells with an RNA isolation kit (ISOGEN, Nippon gene, Toyama, Japan). After electrophoresis in an agarose gel, RNA was blotted onto a Nylon membrane (Hybond N, Amersham). The membrane was hybridized with the 32P-labeled 0.9-kbp fragment of LCB1 under stringent conditions, and hybridizing LCB1 mRNA was detected by autoradiography.

Assay of SPT Activity in Cell Lysates and in Replicated Colonies—CHO cells were cultivated in F-12 medium containing 10% serum at 40 °C for 2 days to subconfluence, and then SPT activity of lysates prepared from the cells was assayed with Lipofectin™ reagent (Life Technologies, Inc.) and, after selection for G418 resistance (400 μg/mL), transfectant colonies were purified with cloning cups. As a control, SPB-1 cells were transfected with the vector, pSV-Okneo, and one G418-resistant SPB-1 clone was isolated.

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Fig. 1. Comparison of LCB1 protein sequences. LCB1 protein sequences from CHO (CLCB1), mouse (MLCB1), and S. cerevisiae (YLCB1) were compared using the CLUSTAL algorithm (35). Identical amino acids are indicated by shading. The transmembrane domain shown in CLCB1 and MLCB1 (underlined) was predicted by the ALOM method (29). The ALOM method does not detect transmembrane domains in YLCB1, but transmembrane domains predicted by another algorithm (36) (underlined) are shown in YLCB1.

mg of protein) were incubated in 0.5 ml of a solubilization buffer consisting of 0.1 M sodium phosphate buffer (pH 8.0), 0.1 M NaCl, 10 mM imidazole, and 1% sucrose monolaurate (Mitsubishi Kasei Shoku- hin Inc., Tokyo) for 10 min. After centrifugation (105,000 × g, 30 min) of the supernatant fluid, the resin was incubated with 50 μl of Ni2+-nitrilotriacetic acid agarose resin (Qiagen, Hilden) in a microcentrifuge tube for 1 h with gentle shaking. The resin was pelleted by centrifuging for 1 min at 2,000 × g, and the supernatant fluid was stored as an unabsorbed fraction. After washing twice with 1 ml of the solubilization buffer, the resin was incubated with 0.3 ml of an elution buffer consisting of 0.1 M sodium phosphate buffer (pH 8.0), 50 mM NaCl, 10 mM imidazole, and 1% sucrose monolaurate for 10 min with gentle shaking. After precipitating the resin, the supernatant fluid was recovered as an absorbed fraction. The recovered fractions were assayed for SPT activity.

Protein Determination—Protein concentrations were determined by the method of Lowry et al. (27) using bovine serum albumin as the standard.

RESULTS

Cloning of Mammalian LCB1 Homologs—A computer search for sequences with similarity to the yeast Lcb1 protein identified a human expressed sequence tag (GenBank accession no. T24597). Most of the base sequence in this expressed sequence tag was determined and then used to identify a 1.2-kbp cDNA in a mouse testis cDNA library by nucleotide sequence analysis. The mouse cDNA was cloned using the RACE technique yielding a 1.7-kbp mouse cDNA (designated mLCB1; GenBank accession no. AF003823). Because the size of mLCB1 mRNA was estimated to be about 2.7 kb by Northern blot analysis (see below), the 1.7-kbp clone could not represent a full-length mRNA. However, the 1.7-kbp mouse cDNA appears to encode a protein of 473 amino acids with a molecular mass of 52,519 Da (Fig. 1). The ACCATGG sequence around the first ATG codon of the ORF corresponds to a Kozak sequence (28) for efficient translational initiation, and there is a poly(A) attachment signal (AATAAA) 33 bases upstream of the poly(A) tail. The size of mRNA for cLCB1 was estimated by Northern blot analysis to be about 2.7 kb (data not shown). Thus, the 2.7-kbp cLCB1 is suggested to encode a complete ORF. mLCB1 also encodes a homologous 1,419-bp ORF, and there is 95% amino acid identity between the predicted cLCB1 and mLCB1 proteins (Fig. 1). The putative products of cLCB1 and mLCB1 have about 35% amino acid identity to the yeast Lcb1 protein (Fig. 1), suggesting that these mammalian cDNAs are homologs of the yeast LCB1 gene. The ALOM algorithm (29) predicts that the cLCB1 and mLCB1 proteins have one transmembrane domain near their amino terminus (Fig. 1), and another algorithm for predicting protein localization sites (29) predicts that these mammalian LCB1 proteins are located in the endoplasmic reticulum membrane. These predictions are in agreement with SPT being a membrane-bound enzyme enriched in the endoplasmic reticulum (30).

Isolation of an LCB2 cDNA Homolog from CHO Cells—In yeast cells, both the LCB1 and LCB2 genes are necessary for expression of SPT activity (19, 20). A mouse LCB2 homolog (mLCB2) has been described recently (22). We isolated an LCB2 homolog from a CHO cell cDNA library by procedures similar to those used to isolate cLCB1. The 2,044-bp cDNA (designated mLCB2; GenBank accession no. AF004830) contains a 1,680-bp ORF that potentially encodes a polypeptide consisting of 560 amino acid residues with a molecular mass of 62,882 Da. The cLCB2 protein is 98% identical to the mLCB2 protein and 43% identical to the yeast Lcb2 protein (Fig. 2), suggesting that the cLCB2 is a CHO LCB2 homolog. The cLCB2 and mLCB2 proteins are predicted to have one transmembrane domain (Fig. 2) and to be located in the endoplasmic reticulum membrane.

Level of Expression of LCB1 and LCB2 mRNAs in Mouse Tissues—The distribution of the LCB1 mRNA in mouse tissue was analyzed and compared with the LCB2 mRNA (22). Northern blot analysis of mRNA isolated from various mouse tissues revealed a 2.7-kbp LCB1-specific mRNA in all tested tissues (Fig. 3). The highest level of mRNA, measured relative to the

Table 1. Sequence Analysis of mLCB1 and cLCB1 Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Sequence Characteristics</th>
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<tbody>
<tr>
<td>mLCB1</td>
<td>AF003823</td>
<td>Complete ORF, 1,728 bp</td>
</tr>
<tr>
<td>cLCB1</td>
<td>AF004831</td>
<td>Complete ORF, 1,716 bp</td>
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</tbody>
</table>
The level of the 2-kb β-actin mRNA, was found in kidney followed by brain and liver. Two LCB2-specific messengers were detected. The 2-kb mRNA, whose size corresponds to the cloned 1.89-kb cDNA (22), was found in all tissues, whereas the 6.5-kb mRNA was present in high level in some tissues but was undetectable in others (Fig. 3). The highest level of the LCB2 mRNA was also found in kidney and brain.

Complementation of the SPT Deficiency of SPB-1 Cells by Expression of cLCB1—We previously isolated an SPT-defective CHO mutant strain, SPB-1 (4). The SPT activity in SPB-1 cells is thermolabile, and thus sphingolipid synthesis stops almost completely at nonpermissive temperatures (4, 5). To determine if expression of cLCB1 or cLCB2 complemented the SPT defect in SPB-1 cells, we constructed plasmids pSV-cLCB1 and pSV-cLCB2, in which cLCB1 and cLCB2, respectively, were cloned into pSV-OKneo, a mammalian expression vector with a G418-resistant determinant. SPB-1 cells were transfected with these plasmids, and G418-resistant clones were isolated. Colonies of the drug-resistant cells were replicated on polyester discs, and in situ SPT assays using replicated colonies were carried out to determine the population of colonies having SPT activity. When transfected with plasmid pSV-cLCB1, about 50% of the population of G418-resistant SPB-1 colonies had SPT activity. In contrast, no colonies of G418-resistant SPB-1 cells transfected with pSV-cLCB2 showed any recovery of SPT activity. These results demonstrate specific complementation of the SPT defect of SPB-1 cells by the cLCB1 and eliminate the possibility that the recovery of SPT activity in SPB-1 cells transfected with pSV-cLCB1 cells is caused by spontaneous reversion events.

For further analysis of SPB-1 cells transfected with pSV-cLCB1, one stable transformant designated SPB-1/cLCB1 was chosen. As a control, a G418-resistant isolate of SPB-1 cells transfected with the pSV-OKneo vector was also isolated. After cells were cultivated at the nonpermissive temperature (40 °C) for 2 days to inactivate the endogenous SPT activity of SPB-1 cells, lysates were prepared from the cells for SPT assay. SPT activity in SPB-1 cells was less than 5% of that in the parental CHO-K1 cells (Table I) as described previously (4). In contrast, SPT activity in SPB-1/cLCB1 cells was more than 90% of that in CHO-K1 cells, whereas the vector-transfected control cells exhibited no recovery of SPT activity (Table I). Sensitivity of SPT activity to sphingofungin B, a potent inhibitor of SPT (31), was identical between CHO-K1 and SPB-1/cLCB1 cells (the dose producing 50% inhibition of SPT activity was about 10 nM in both cell types; data not shown), indicating that SPB-1/cLCB1 cells produce SPT activity that behaves like the wild type.

Recovery of Sphingolipid Synthesis in SPB-1/cLCB1 Cells—To monitor de novo sphingolipid synthesis, cells were incubated with [1-14C]serine for 2 h at the nonpermissive temperature (40 °C), and metabolically labeled lipids were analyzed. Although SPB-1 cells made no appreciable sphingolipids, SPB-1/cLCB1 cells synthesized sphingolipids (sphingomyelin, ceramide, and sphingosine) as shown by thin-layer chromatography (Fig. 2) and by using specific radiolabeled reagents (data not shown).
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TABLE I

<table>
<thead>
<tr>
<th>Strain</th>
<th>KDS produced pmol/mg protein/10 min</th>
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<tbody>
<tr>
<td>CHO-K1</td>
<td>548 ± 5</td>
</tr>
<tr>
<td>SPB-1</td>
<td>&lt;20</td>
</tr>
<tr>
<td>SPB-1/cLCB1</td>
<td>501 ± 21</td>
</tr>
<tr>
<td>SPB-1/pSV-OK</td>
<td>&lt;20</td>
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</tbody>
</table>

Table I: cLCB1 restores SPT activity to SPB-1 mutant CHO cells

After CHO cells were cultivated in F-12 medium containing 10% serum at 40 °C for 2 days, cells lysates were prepared by sonication. The cell lysates were incubated in the SPT assay medium at 40 °C for 10 min, and the 3-ketodihydrosphingosine (KDS) produced was determined as described under "Experimental Procedures." SPB-1/pSV-OK is a G418-resistant SPB-1 clone transfected with a vector, pSV-OKneo. The data shown are the mean values ± S.D. from triplicate experiments.

G₃⁻ ganglioside, glucosylceramide, and ceramide) as well as wild type CHO-K1 cells (Fig. 4), in agreement with the recovery of SPT activity in SPB-1/cLCB1 cells (Table I). There was no difference in the metabolic labeling of phosphatidylserine or phosphatidylethanolamine with [14C]serine among these cell types (Fig. 4), suggesting a specific effect of the transfected cLCB1 gene on sphingolipid metabolism.

Recovery of sphingolipid synthesis in SPB-1/cLCB1 cells was confirmed further by showing that the content of sphingomyelin, the most abundant sphingolipid, was restored in SPB-1/cLCB1 cells. When cells were cultured in a sphingolipid-deficient medium at 40 °C for 2 days, sphingomyelin in SPB-1 cells amounted to 3.9 ± 0.4% (n = 3) of the total phospholipids, whereas in CHO-K1 cells amounted to 9.6 ± 1.2% (n = 3) (Table II). Sphingomyelin in SPB-1/cLCB1 cells amounted to 9.3 ± 0.8% (n = 3) of the total phospholipids (Table II), indicating full recovery of the sphingomyelin level in SPB1/cLCB1 cells. The content of G₃⁻ ganglioside in SPB-1/cLCB1 cells was also restored to the wild type level (not shown).

SPB-1 cells show a marked growth retardation at the non-permissive temperatures compared with the parental CHO-K1 cells (5). Growth of SPB-1 cells at the non-permissive temperature was partially restored by transfection with pSV-cLCB1, SPB-1/cLCB1, or CHO-K1 cells absorbed to the resin. These results demonstrate that the His₆-tagged cLCB1 protein is a component of SPT.

The cLCB1 Protein Is a Component of SPT—To prove that the cLCB1 protein is a component of SPT, we constructed a plasmid, pSV-HT-cLCB1, expressing a His₆-tagged cLCB1 protein, and we determined whether SPT in an SPB-1 transfectant producing the tagged cLCB1 protein bound to a Ni²⁺-immobilized resin that has a strong affinity to neighboring histidine residues (32). An in situ SPT assay of G418-resistant transformants of SPB-1 cells transfected with pSV-HT-cLCB1 showed that about 50% of the drug-resistant colonies had SPT activity, suggesting that the His₆-tagged cLCB1 protein functioned as well as the wild type cLCB1 protein. One transformant having SPT activity was purified and named SPB-1-HT-cLCB1.

Membranes prepared from SPB-1-HT-cLCB1, SPB-1/cLCB1, and CHO-K1 cells were solubilized with a non-ionic detergent so that about 90% of the SPT activity was recovered in the supernatant fluid after high speed centrifugation. The supernatant fluid was incubated with a Ni²⁺-immobilized resin, and unadsorbed proteins were withdrawn. After washing the resin, adsorbed proteins were eluted with a high concentration of imidazole, a chelator of Ni²⁺. About half of the SPT activity from SPB-1-HT-cLCB1 cells adsorbed to the Ni²⁺-immobilized resin, whereas none of the SPT activity from SPB-1/cLCB1 cells or CHO-K1 cells absorbed to the resin. These results demonstrate that the His₆-tagged cLCB1 protein is a component of SPT. That a portion of the SPT activity from SPB-1-HT-cLCB1 cells did not bind to the Ni²⁺-immobilized resin was probably because of a limited accessibility of the tag sequence to the immobilized metal ions inasmuch as a partial population of the solubilized SPT molecules might be oriented inside detergent micelles.

**DISCUSSION**

We report the molecular cloning of a mouse (mLCB1) and a CHO (cLCB1) cDNA homolog of the yeast LCB1 gene, shown


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Previously, it was found that the specific activity among rat tissues was detected in lung, kidney, brain, and liver (expected for mRNAs encoding subunits of the same enzyme, messengers follow a similar pattern in mouse (Fig. 3), but the level becomes low when calculated as percent of distribution of the total activity recovered.

Table III

<table>
<thead>
<tr>
<th>Strain</th>
<th>SPT activity distribution</th>
<th>Unabsorbed</th>
<th>Adsorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPB-1/HtLCB1</td>
<td></td>
<td>58</td>
<td>42</td>
</tr>
<tr>
<td>SPB-1/cLCB1</td>
<td>&gt;90</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>CHO-K1</td>
<td>&gt;90</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
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</table>

SPT activity in SPB-1 cells expressing a His6-tagged cLCB1 protein binds to an affinity matrix

Membranes prepared from the indicated strains were treated with a non-ionic detergent, and the solubilized sample was incubated with a Ni2+-nitritrolic acid agarose resin as described under “Experimental Procedures.” The resin was precipitated, and the supernatant fluid was recovered as the unabsorbed fraction. After washing the resin, proteins specifically bound were eluted with a buffer containing 0.1 M imidazole for SPT activity. Distribution of SPT activity to each fraction is shown as percent of distribution of the total activity recovered.

SPT catalyzes the initial step dedicated to sphingolipid biosynthesis and is suggested to be rate-determining for de novo sphingolipid synthesis (for review, see Ref. 15). However, little is known about the regulation of SPT activity. Our finding that the tissue distribution of both LCB1 and LCB2 proteins is related to a pyridoxal phosphate binding motif found in the yeast Lcb1 protein (Fig. 1). Such strong conservation, despite a long phylogenetic distance between S. cerevisiae and mammals, leads us to speculate that this region of the LCB1 protein is probably a functional homo-

**Acknowledgments**—We thank Dr. Juan L. Iovana (INSERM) for plasmid 6H6 and Dr. Debra J. Wolgemuth (Columbia University) for the mouse cDNA library.

**References**

24. MMP: “Experimental Procedures.”
A Mammalian Homolog of the Yeast LCBI Encodes a Component of Serine Palmitoyltransferase, the Enzyme Catalyzing the First Step in Sphingolipid Synthesis

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