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 3-ketodihydrosphingosine (SPT; EC 2.3.1.50) to generate serine palmitoyltransferase (SPT) activity bound to a Ni2+-immobilized resin. This paper is available online at http://www.jbc.org
Mammalian LCB1 Gene for Sphingolipid Synthesis

EXPERIMENTAL PROCEDURES

Molecular Cloning of mLCB1, lCB1, and lCB2—A mouse LCB1 homolog was isolated by screening a testis 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 database of expressed sequence tags of the National Center for Biotechnology Information using the tblastn algorithm (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′-CCCTCCTTCATGATAGCAGC-3′ and 5′-GCCAGGCGCTTCTCTA-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 the Bluescript II plasmid system (Life Technologies, Inc.) according to the manufacturer's protocol. Primers used for amplification of lCB1 were: primer A, 5′-TTAGTGCAATACAGTTTCCTC-TGATG-3′ (reverse); primer B, 5′-TTAGGATCAAGAATGTTA-GAGGTGGCA-3′ (forward); primer C, 5′-TTAGAATTCAGAAGTGTAGABB-GCAAGCCAGACC-3′ (forward). The CHO cDNA library was subjected to polymerase chain reaction using by primers A and B (themicycling 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, 57 °C for 45 s, and 72 °C for 90 s at 30 cycles). After digestion with EcoRI and SstI, the amplified 0.9-kbp DNA was cloned into Bluescript II SK, and its DNA sequence was determined.

Based on the sequence of the cloned 0.9-kbp DNA, a 25-mer oligonucleotide (5′-CCCATCATCTTACTCACTACACATCG-3′), which should perfectly match a portion of the lCB1 sequence, was synthesized, biotinylated, and used with the GeneTraper cDNA positive selection system (Life Technologies, Inc.) to isolate LCB1. cDNAs hybridizable to this 25-mer oligonucleotide were retrieved from the CHO-K1 cDNA library, using the procedures described in the Life Technologies, Inc. manual. A cDNA transformed 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 prime-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 sequencing (ABI PRISM 310) genetic analyzer, Perkin-Elmer Applied Biosystems.

lCB2 was also isolated by essentially the same procedures as described above with the following exceptions: primer A, 5′-TTAGGAT-TCTTACAGTTTCAGTTACTACTACAT-3′ (forward); primer B, 5′-TTACGATGGCAAGATCCATCTACACATCTAC-3′ (reverse); primer C, 5′-TTAGGATCAAGAATGTTAGGCAAGAATGTTAGGGA-3′ (reverse). A 1.1-kbp DNA fragment of lCB2 was amplified by polymerase chain reaction, cloned, and its DNA sequence was determined. Then, a 25-mer oligonucleotide sequence (5′-CCGCCAGGTGGTGGATATCAGGAGAGA-3′) was synthesized for the cDNA positive selection system. The previously cloned 1.1-kbp fragment of lCB2 was used for a 32P-labeled probe for the colony hybridization. Eventually, one 2-kbp clone of lCB2 was isolated.

Cell Culture and Transfection—Mutant SPB-1 (4) and its parent CHO-K1 (ATCC CCL 61) cell lines were routinely maintained in Ham's F-12 medium supplemented with 10% newborn calf serum, penicillin G (100 units/ml) and streptomycin sulfate (100 μg/ml) in a 5% CO2 atmosphere in 100% humidity at 33 °C.

pSV-OKneo was constructed by inserting a G418-resistant determinant, from pMC1neo poly(A)+ (Stratagene) into a blunt-ended ClaI site of pSPORT1 (Life Technologies, Inc.), a mammalian expression vector. To construct a lCB1-expressing plasmid, the SalI-NcoI 2.7-kbp fragment of lCB1, which was originally cloned into pSPORT1, was transferred between the SalI and NcoI sites of pSV-OKneo, and the resultant plasmid was designated pSV-lCB1. Similarly, the SalI-NcoI 2.7-kbp fragment of lCB2 was transferred from pSPORT1 to pSV-OKneo, and the resultant lCB2-expressing construct was designated pSV-lCB2. SPB-1 cells were transfected with these expression plasmids by lipofection 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.

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-XhoI fragment of the mouse LCB1, a 1,458-bp Eagl-XhoI 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). A Hewlett-Packard Jetscan II scanner was used to scan the autoradiograms, and the intensity of hybridizing bands was measured using SigmaGel software (Jandel Scientific).

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 cultured in F-12 medium containing 10% serum at 40 °C for 2 days to subconfluence, and then SPT activity of lysates prepared from these cells was assayed 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.

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

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. After washing twice with 2 ml of F-12 medium, the cell monolayers were incubated in 1.3 ml of F-12 medium containing 1% Nutridoma-SPT (Boehringer Mannheim) and 24 kilohectoquaters of 1-L-[14C]-serine (Amersham) at 40 °C for 2 h. The monolayers were washed twice with 2 ml of cold phosphate-buffered saline, harvested by scraping, and precipitated by centrifugation at 4 °C. The cell pellets were suspended in 0.9 ml of cold phosphate-buffered saline, and 0.1 ml and 0.8 ml of the suspension were used for protein determination and lipid extraction, respectively. Lipids extracted from the cells by the method of Bligh and Dyer (25) were separated on TLC plates (Silica Gel 60, Merck) with a solvent system of methylecetate, propanol-1, chloroform, methanol, and 0.25% KCI (25/25/25/10/9 v/v). Radioactive lipids on the TLC plates were visualized, and their relative radioactivity was determined by using a BAS2000 Image Analyzer (Fuji).

Phospholipid Composition—CHO cells (1.5–3.5 x 106 cells) were seeded in a 150-mm culture dish containing 15 ml of 10% serum and F-12 medium and cultured at 33 °C for 1 day. Then, after washing twice with 10 ml of cold phosphate-buffered saline, the cell monolayers were cultivated in 25 ml of Nutridoma-BO containing gentamicin (10 μg/ml), a sphingolipid-deficient medium, at 40 °C for 2 additional days. After washing twice with 10 ml of cold phosphate-buffered saline, the cell monolayers were harvested by scraping, phospholipids were extracted from the harvested cells (25), and the extracted phospholipids were separated by TLC with a solvent of chloroform/methanol/acetic acid (65/25/10 v/v). The phosphorous content of the phospholipids was determined by the method of Rouser et al. (26).

Hisα-tagged lCB1 Protein—lCB1 cloned into pSPORT1 was digested with BsrGI and self-ligated to delete the Ncol site in the 3′-untranslated region. The resultant plasmid, digested with Ncol and SstI, was ligated with annealed oligonucleotides (5′-TCGACATGGAATGTTA-GAGGTGGCA-3′) synthesized for the Hisα-tag sequence. The Sall-NcoI fragment of the tagged lCB1 was transferred from pSPORT1 to pSV-OKneo, and the resultant construct was designated pSV-HTCBB1. The fusion protein encoded by pSV-HTCBB1 has the sequence Met-Ala-His-His-His-His-His before the first methionine of the wild type lCB1 protein. SPB-1 cells were transfected with pSV-HTCBB1, and G418-resistant transformants were selected as by an in situ SPT assay of the drug-resistant colonies, transformants having SPT were identified, and one purified clone was designated SPB-1-HTCBB1.

All manipulations were done at 4 °C or on ice unless noted otherwise. After CHO cells were cultured at 40 °C for 2 days, membranes were prepared from the cells as described previously (11). The membranes (1

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.
mg of protein) were incubated in 0.5 ml of a solubilization buffer consisting of 0.1 M sodium phosphate buffer (pH 8.0), 50 mM 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 N\textsuperscript{32}P-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. The resin was pelleted by centrifuging for 1 h with gentle shaking. 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

Molecular 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. AF004831), for further analysis. cLCB1 contains a 1,419-bp ORF predicted 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 the 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
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 levels in some tissues but was undetectable in others. The highest levels of LCB2 mRNA were 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 L-[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,
TABLE I

| Strain            | KDS produced (pmol/mg protein/10 min) |
|-------------------|---------------------------------------|
| CHO-K1            | 548 ± 5                               |
| SPB-1             | <20                                   |
| SPB-1/cLCB1       | 501 ± 21                              |
| SPB-1/pSV-OK      | <20                                   |

G\textsubscript{M3} 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 of SPT activity in SPB-1/cLCB1 cells (Table I). There was no wild type CHO-K1 cells (Fig. 4), in agreement with the recovery of SPT activity in SPB-1/cLCB1 cells (Table I).

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 G\textsubscript{M3} 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 nonpermissive temperature was partially restored by transfection with pSV-cLCB1 but not with the vector, and was still slower than that of wild type CHO-K1 cells.2 Because pSV-cLCB1 restored SPT activity and sphingolipid synthesis to the wild type level, the partial restoration of growth suggests that SPB-1 cells may contain an additional mutation(s), unrelated to sphingolipid synthesis, which affects growth.

The cLCB1 Protein Is a Component of SPT—To prove that the cLCB1 protein is a component of SPT, we constructed a plasmid, pSV-HTcLCB1, expressing a His\textsubscript{6}-tagged cLCB1 protein, and determined whether SPT in an SPB-1 transfectant producing the tagged cLCB1 protein bound to a Ni\textsuperscript{2+}-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 His\textsubscript{6}-tagged cLCB1 protein functioned as well as the wild type cLCB1 protein. One transformant having SPT activity was purified and named SPB-1/HTcLCB1.

Membranes prepared from SPB-1/HTcLCB1, 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 Ni\textsuperscript{2+}-immobilized resin, and unadsorbed proteins were withdrawn. After washing the resin, adsorbed proteins were eluted with a high concentration of imidazole, a chelator of Ni\textsuperscript{2+}. About half of the SPT activity from SPB-1/HTcLCB1 cells adsorbed to the Ni\textsuperscript{2+}-immobilized resin, whereas none of the SPT activity from SPB-1/cLCB1 cells or CHO-K1 cells adsorbed to the resin. These results demonstrate that the His\textsubscript{6}-tagged cLCB1 protein is a component of SPT.

That a portion of the SPT activity from SPB-1/HTcLCB1 cells did not bind to the Ni\textsuperscript{2+}-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
 Both SPT specific activity among rat tissues was found in lung specific activity found in these tissues in rat (33). The highest level in testis correlates well with the relative level of SPT LC2.

Messengers follow a similar pattern in mouse (Fig. 3), as expected for mRNAs encoding subunits of the same enzyme, indicating that the tagged cLC2 protein specifically associates with 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 homology.

The recovery of SPT activity in SPB-1 cells after transfection with the cLC2-expressing plasmid but not with the mLC2-expressing plasmid suggests specific complementation of the SPT defect in SPB-1 cells by the cLC2. Possibly, the endogenous LCB1 protein in SPB-1 cells is thermolabile, and in SPB-1/cLC2 cells the LCB1 protein derived from pSV-cLC2 complexes with the endogenous LCB2 protein to form a functional SPT complex, thereby restoring SPT activity to the wild type level. The nature of the genetic defect in SPB-1 cells leading to loss of SPT activity is not known. It does not appear to affect transcription because there was no difference in the size (2.7 kb) of the LCB1 messenger or in its expression level between SPB-1 and CHO-K1 cells even after culture at the non-permissive temperature, as judged by Northern blot analysis.

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 messengers follows a similar pattern in mouse (Fig. 3), as expected for mRNAs encoding subunits of the same enzyme, suggests that transcription of the two genes is coordinately controlled in vivo.

The high relative level of LCB1 messengers (Fig. 3) in mouse kidney, brain, and liver (LCB1 mRNA only) as well as the low level in testis correlates well with the relative level of SPT specific activity found in these tissues in rat (33). The highest SPT specific activity among rat tissues was found in lung (33). Both LCB1 messengers are also present at a high level in lung relative to total poly(A)+ RNA (relative intensity (RI) value, Fig. 3), but the level becomes low when calculated relative to the 2-kb actin mRNA (actin-normalized RI value, Fig. 3), whose concentration is high in this tissue. The nature of the relationship between the level of LCB1 messenger and the specific activity of SPT in various cells and tissues requires further study.

There is high amino acid conservation between the mammalian LCB1 proteins and between residues 150–449 of the yeast Lcb1 protein (Fig. 1). Such high conservation, despite a long phylogenetic distance between S. cerevisiae and mammals, leads us to speculate that this region of the LCB1 protein may be necessary for SPT catalytic activity, for regulation of SPT activity, or for self-interaction or interaction with other subunits.

That the cLC2 and mLC2 proteins are predicted to have about 40% amino acid identity to the yeast Lcb1 protein (Fig. 2) suggests their functional resemblance. The mammalian LCB2 proteins contain a motif, P77TFTKSFG80 (Fig. 2) which is related to a pyridoxal phosphate binding motif found in the E. coli 2-amino-3-ketobutyrate coenzyme A ligase (34). The underlined Lys77 of the mammalian LCB2 proteins is predicted to correspond to a lysine residue that forms a Schiff base with pyridoxal phosphate in the 2-amino-3-ketobutyrate coenzyme A ligase (34). This motif is conserved perfectly among the predicted yeast, mouse, and CHO LCB2 proteins (Fig. 2), implying that these proteins are a catalytic component of SPT. However, it remains to be shown biochemically that these mammalian LCB2 proteins are a component of the SPT enzyme. The availability of both the mammalian LCB1 and LCB2 cDNA should enable us not only to address this issue but also to elucidate the mechanism(s) for regulating SPT activity in mammalian cells. Purification of SPT may be facilitated by using the His6-tagged cLC2 protein, and examination of purified SPT should allow identification of all components of SPT.

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