Cloning and Characterization of *LCB1*, a *Saccharomyces* Gene Required for Biosynthesis of the Long-Chain Base Component of Sphingolipids

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The existence of auxotrophic mutants of *Saccharomyces cerevisiae* having an absolute requirement for the long-chain base (lcb) component of sphingolipids suggests that sphingolipids are crucial for viability and growth. One mutant, termed the *lcb1-1* mutant, lacks the activity of serine palmitoyltransferase, the first enzyme in the pathway for long-chain base synthesis. Here, we present evidence that *LCB1* has been molecularly cloned. The size of the *LCB1* transcript, the direction of transcription, and transcription initiation sites were determined. In addition, the coding region and its 5' and 3' flanking regions were sequenced. Analysis of the DNA sequence revealed a single open reading frame of 1,674 nucleotides, encoding a predicted peptide of 558 amino acids. The hydropathy profile of the predicted peptide suggests a hydrophobic, globular, membrane-associated protein with two potential transmembrane helices. Comparison of the predicted amino acid sequence to known protein sequences revealed homology to 5-aminolevulinic acid synthase and to 2-amino-3-ketobutyrate coenzyme A ligase. These homologies, the similarity of the chemical reactions catalyzed by the three enzymes, and the finding that *LCB1* restores serine palmitoyltransferase activity to an *lcb1*-defective strain indicate that serine palmitoyltransferase or a subunit of the enzyme is the most likely product of *LCB1*. Homology of the *LCB1* predicted protein to the *Escherichia coli* biotin synthetase was also observed, but the biological significance of this observation is not clear. A role for sphingolipids in sporulation is implicated by our finding that diploids homozygous for *lcb1* failed to sporulate.

Sphingolipids are membrane components found in animals (13), higher plants (18), and fungi (5); they are rarely present in procaryotes (20). In spite of much effort, it has been difficult to understand the exact biological role(s) of sphingolipids and their mode of action at the molecular level. In animals, sphingolipids are thought to play a role in such general cellular events as cell-to-cell recognition, regulation of cell growth, and differentiation (13, 19). Sphingolipids have been shown to promote a variety of specific biological activities (for a review, see reference 15). For diseases such as cancer (14), there are changes in the cellular concentration and composition of sphingolipids, but the relationship of these changes to the disease state is unclear. Recently it has been suggested that long-chain bases, such as the sphingolipid precursor sphingosine, and the breakdown products of sphingolipids, lysosphingolipids, may have important biological functions (for a review, see reference 15).

Saccharomyces cerevisiae contains a small and unique set of sphingolipids with the compositions inositol-*p*-ceramide, mannose-inositol-*p*-ceramide, and mannose-(inositol-*p*)₂-ceramide (36, 39). These sphingolipids and those in other fungi and plants all contain the inositol phosphorylceramide moiety and phytosphingosine. Animal sphingolipids lack inositol phosphate and instead contain oligosaccharides or phosphocholine attached to ceramide. Animal sphingolipids usually contain sphingosine instead of phytosphingosine.

To begin to understand the function(s) of sphingolipids in *S. cerevisiae*, we have begun to characterize a mutant strain blocked in sphingolipid biosynthesis (42). Strains carrying

the mutant allele, *lcb1-1*, are absolute auxotrophs and grow only when a long-chain base (*lcb*, phytosphingosine but not sphingosine) is added to the culture medium. The long-chain base requirement is presumably due to a block in the synthesis of phytosphingosine. In this article, we describe the isolation of the *LCB1* gene and the determination of its DNA sequence. We also present preliminary evidence suggesting that *LCB1* codes for serine palmitoyltransferase (3-ketosphinganine synthase [EC 2.3.1.50]), the first enzyme in the sphingolipid long-chain base biosynthetic pathway (37, 40).

MATERIALS AND METHODS

Strains and plasmids. The original *lcb* mutant (*MATa lcb1-1 ino1* [42]), was crossed with strain W303-1B (*MATa ade2-1 can1-100 ura3-1 his3-11,15 trp1-1 leu2-3,112* [29]; obtained from R. J. Rothstein, Columbia University). Progeny from this cross were backcrossed to W303-1B, and several offspring were selected for further study, including strains X2A1B (*MATa lcb1-1 ura3-1 trp1-1 his3-11,15*) and 24D5 (*MATa lcb1-1 ura3-1 trp1-1 leu2-3,112 his3-11,15*). Strain SL1 was derived from strain SJ21R (*MATa ura3-52 leu2-3,112 ade1 MEL1*) by replacement of the *LCB1* allele with a mutant allele that was disrupted by inserting a 1.1-kb *URA3* DNA fragment at the *SalI* site of *LCB1*. The *LCB1::URA3*-disrupted allele was prepared by transferring a 4.3-kb *HindIII-StuI* fragment, carrying *LCB1*, from pLCB (Fig. 1) to pTZ18 (Pharmacia) cleaved with *HindIII* and *SmaI*. The resulting plasmid, pTZ18-*LCB1* (Fig. 1), was cleaved with *SalI* and ligated with a 1.1-kb *URA3* DNA fragment having *SalI* cohesive ends (obtained from pUC-

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FIG. 1. Structures of plasmids. The plasmid pLCB, carrying the *LCB1* allele, was isolated from an *S. cerevisiae* genomic library as described in the text. The approximate location of *LCB1* is shown. Not all restriction endonuclease sites in a given plasmid are indicated. The open arrowhead in pTZ18-LCB1 represents the T7 promoter. DNA sequences are as follows: open box, *S. cerevisiae*; *TRP1* and *URA3*, marker genes for selection in *S. cerevisiae*; *ARS1*, an *S. cerevisiae* autonomous replication sequence; *CEN3* and *CEN4*, centromere for maintenance of a single copy of the vector in yeast; *BLA* and *TET* confer ampicillin and tetracycline resistance in *E. coli*, respectively. Abbreviations for restriction endonucleases are as follows: B, *Bam*HI; C, *Cl*AI; E, *Eco*RI; H, *Hind*III; Ha, *Hpa*I; K, *Kpn*I; N, *Nru*I; P, *Pst*I; S, *Sal*I; Sa, *Sau*3A; Sac, *Sac*I; Sm, *Sma*I; St, *Stu*I; X, *Xba*I.

URA3 cut with *Sal*I [44]) to yield pTZ18-LCB1::URA3. To replace the *LCB1* chromosomal allele with the *URA3*-disrupted allele, 10 μ g of pTZ18-LCB1::URA3 DNA was cleaved with *Xba*I and *Cl*AI, extracted with phenol, phenol-chloroform, and chloroform, and precipitated with ethanol. The DNA was transformed into strain SJ21R with selection for Ura⁺ transformants. Replacement of the *LCB1* chromosomal allele with the *URA3*-disrupted allele was verified by Southern blot analysis (Fig. 2). YIpLCB1-1 was constructed by inserting *TRP1* of *S. cerevisiae*, as a 1.4-kb *Hind*III fragment, into the *Hind*III site of pTZ18-LCB1. YIpLCB1-1 was cleaved at its unique *Bam*HI site (Fig. 1), located on the 3' side of *LCB1*, and the linear DNA was used to transform strain 24D5 with selection for Ura⁺ transformants. Integration at the expected chromosomal location was verified by Southern blotting. Transformants were crossed to strain YPH1 (*MATa ura3-52 lys2-801 ade2-101* [35]).

The plasmid pLCB (Fig. 1) was isolated from an *S. cerevisiae* genomic DNA library carried in a CEN vector. The 6.44-kb vector was pBR322 with a 0.63-kb *Sau*3A *CEN3* DNA fragment inserted into the *Pvu*II site of the vector and a 1.4-kb *TRP1 ARS1* fragment inserted into the *Eco*RI site of the vector. These ligations were done with molecules whose ends were made blunt ended so that the original restriction sites were destroyed. *Sau*3A genomic DNA fragments of 8-kb average size from strain X2180 (*a/a gal2/gal2*) were cloned into the *Bam*HI site of the vector (the library was a gift from ZymoGenetics, Seattle, Wash.). DNA fragments from pLCB were subcloned into YCp50 (32).

Plasmids were propagated in *Escherichia coli* DH5 α .

Media. During the course of this work, several media were used for propagation of *lcb*-defective strains. Only the most

FIG. 2. Southern blot analysis of *LCB1*. Southern blotting was used to show that the *URA3* gene had disrupted the putative *LCB1* gene at the indicated *Sal*I site in strain SL1. Total yeast DNA was isolated and cleaved with the restriction endonucleases *Nru*I and *Stu*I. The cleaved DNA was subjected to Southern blot analysis. The blot was hybridized to the indicated ³²P-labelled 4-kb *Nru*I to *Stu*I DNA fragment. Lanes: 1, DNA from the wild-type strain SJ21R; 2, DNA from the *URA3*-disrupted strain SL1. Abbreviations for restriction endonucleases are as follows: N, *Nru*I; S, *Sal*I; St, *Stu*I.

recent versions of media are given here, as they proved most useful. PYED contained 1% peptone, 1% yeast extract, 2% agar (for plates), 50 mM sodium succinate (pH 5), inositol (50 mg/liter), potassium phosphate monobasic (50 mg/ml), and 2 or 4% glucose. Minimal medium contained 1 $\times$ Difco yeast nitrogen base without amino acids, 50 mM sodium succinate (pH 5), 2% glucose, 1.5% agar (for plates), inositol (50 mg/liter), valine (150 mg/liter), isoleucine (30 mg/liter), threonine (200 mg/liter), and the following supplements at 20 mg/liter: adenine sulfate, arginine-HCl, histidine-HCl, leucine, lysine-HCl, methionine, tryptophan, and uracil. One or more supplements were omitted from minimal medium for selection of yeast transformants. For strains requiring long-chain base, the medium was supplemented with 25 μ M phytosphingosine (Sigma, St. Louis, Mo.). A 10 $\times$ stock solution of phytosphingosine was prepared by adding 0.25 ml of 100 mM phytosphingosine (dissolved in 95% ethanol) to 99.75 ml of a 0.5% solution of tergitol (Sigma).

DNA sequencing. A 2.5-kb *Cl*AI-*Xba*I DNA fragment carrying *LCB1* was subcloned from pTZ18-LCB1 (Fig. 1) into the same restriction sites of the M13 bacteriophage vectors pBluescript KS⁺ and pBluescript KS⁻ (Stratagene, La Jolla, Calif.) to yield pBluescript KS⁺-LCB1 and pBluescript KS⁻-LCB1. The KS⁺-LCB1 DNA was sequenced from a single-stranded template, while the KS⁻-LCB1 DNA was sequenced by using a double-stranded template. Synthetic

oligonucleotide primers were used for dideoxynucleotide sequencing with Sequenase version 2.0 DNA polymerase (U.S. Biochemical, Cleveland, Ohio) essentially as recommended by the supplier.

Primer extension. The 5' ends of the *LCB1* mRNA were mapped by a primer extension method. A synthetic 21-base oligonucleotide, complementary to nucleotides +63 to +43 of the coding region, was used to prime the extension reaction. The primer was labelled at its 5' end by using T4 polynucleotide kinase (Pharmacia) and [γ - 32 P]ATP. The extension reaction was done with avian myeloblastosis virus reverse transcriptase (Pharmacia) as described previously (7). The cDNA products of the extension reaction were separated on a 6% polyacrylamide gel containing 8 M urea. The products of dideoxy sequencing reactions made with the same primer were used as molecular size markers.

Enzyme assays. Serine palmitoyltransferase (3-ketosphinganine synthase) assays were done by using a modification of the method of Williams et al. (43). For each assay, the following components, in a final volume of 0.2 ml, were used: 0.1 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 8.3), 5 mM dithiothreitol, 2.5 mM EDTA (pH 7.4), 50 mM pyridoxal phosphate, 40 mM palmitoyl-coenzyme A (CoA), 5 mM L-serine, 5 μ Ci of [G - 3 H]L-serine, and 0.2 mg of membrane protein. Each reaction was incubated with shaking for 20 min at 30°C and terminated by the addition of 0.5 ml of 0.5 N NH_4OH containing 5 μ mol of carrier L-serine. To remove the labelled product, 3 ml of $CHCl_3$ - CH_3OH (1:2), 50 μ g of 3-ketosphinganine or sphinganine (in 50 μ l of 95% ethanol), 2 ml of $CHCl_3$, and 4 ml of 0.5 N NH_4OH were added with vigorous shaking. The tubes were centrifuged for 10 min in a clinical centrifuge, and the upper aqueous phase was removed by aspiration and discarded. Any remaining unreacted [3 H]serine was removed from the $CHCl_3$ layer by washing three times with water. A sample of the $CHCl_3$ layer was placed in a scintillation vial and prepared for counting by evaporation over a steam bath. Four milliliters of a scintillation cocktail (2 liters of toluene, 1 liter of Triton X-100, 350 ml of H_2O containing 0.4 mM 2-(4'-*t*-butylphenyl)-5-(4'-biphenyl)-1,3,4-oxadiazole and 0.3 mM 2-(4'-biphenyl)-6-phenylbenzoxazole were added to the vial, and the radioactivity was measured in a Packard PRIAS scintillation counter. A control without enzyme was subtracted from all the assays to calculate the specific activity. One unit of enzyme activity is defined as 1 pmol of product formed per minute.

Membranes were prepared by growing cells to mid-log phase, harvesting, resuspending in buffer (50 mM sodium phosphate, pH 7.0, containing 5 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride), and lysing by vortexing for 3 min (6 $\times$ 30 s) with one-half volume of 0.3- to 0.5-mm glass beads. Unbroken cells and debris were removed by centrifuging the homogenate at 4,000 $\times g$ for 10 min. The resulting supernatant fluid was centrifuged at 100,000 $\times g$ for 1 h at 4°C. The pellet was homogenized in the same buffer with a Potter-Elvehjem tissue grinder. The centrifugation and resuspension steps were repeated with the final sample resuspended in buffer containing 25% glycerol added to improve storage at -20°C. Protein concentration was measured by the biuret method (12) with bovine serum albumin as a standard.

Miscellaneous procedures. *S. cerevisiae* cells were transformed by the lithium acetate procedure (34). Genetic crosses and tetrad analysis were done by standard procedures (34). For Northern (RNA) blotting experiments, strain W303-1B was grown in PYED medium and total RNA was

prepared by the procedure of Carlson and Botstein (6). Poly(A)⁺ RNA was isolated by chromatography on an oligo(dT) cellulose column. Poly(A)⁺ RNA was electrophoresed on a 1.2% agarose gel containing 0.66 M formaldehyde and the RNA was blotted to nitrocellulose. Southern blots were done essentially as described by Maniatis et al. (22). For both blotting procedures, [32 P]dATP-labelled probes were prepared by the method of Feinberg and Volgestein (11).

Nucleotide sequence accession number. The Genbank number assigned to the sequence discussed herein is M63674.

RESULTS

Molecular cloning of *LCB1*. A strain of *S. cerevisiae* requiring the long-chain base DL-erythrodihydrosphingosine was isolated previously (42) and has been shown to lack serine palmitoyltransferase activity (30). Since this strain was not suitable for molecular genetic experiments, it was crossed, as described in Materials and Methods, to give strain X2A1B (*lcb1-1*). We isolated *LCB1* from a genomic library by complementation of the *lcb1* allele. Strain X2A1B was transformed with an *S. cerevisiae* genomic library carried in a vector containing *CEN3* and *ARS1*, for single-copy propagation in yeast cells, and *TRP1*, for selection of Trp⁺ yeast transformants. A genomic library carried in a single-copy vector was used because we did not know if *LCB1* would be lethal when carried on a multicopy vector. Ten thousand Trp⁺ transformants were selected on minimal medium plates containing phytosphingosine but lacking tryptophan. Transformants were pooled and reselected on minimal medium plates lacking both tryptophan and phytosphingosine. About one per 3,500 Trp⁺ colonies was Lcb⁺.

Plasmid DNA was isolated from several Lcb⁺ yeast transformants and transformed into *E. coli* with selection for ampicillin-resistant cells. Plasmid DNA from *E. coli* transformants was isolated and digested with restriction endonucleases. The pattern of restriction fragments indicated that the original Lcb⁺ yeast transformants all contained the same plasmid, which carried an insert of about 8 kb. This plasmid was called pLCB (Fig. 1).

To localize the *LCB1* gene on the 8-kb DNA insert, we subcloned parts of the insert into the *CEN4* vector YCp50 and tested the resulting plasmids for their ability to confer a Lcb⁺ phenotype on strain X2A1B. The experiments localized *LCB1* to a subclone of 4.0 kb (Fig. 1).

Further localization of *LCB1* was achieved by gene disruption. The 4-kb insert was disrupted at the unique *SalI* site by insertion of the *URA3* gene. The plasmid carrying the *URA3* disruption was used to replace the homologous region of the chromosome in strain SJ21R as described in Materials and Methods. A potential *lcb1* disruption strain, SL1 (Lcb⁻ Ura⁺), was obtained. Southern blots were used to verify that the chromosome had been disrupted as expected. Total DNA isolated from SL1 and the nondisrupted parental strain SJ21R was cleaved with the restriction endonucleases *NruI* and *StuI*. The parental strain should show a 4-kb band on a Southern blot when the blot is probed with a 32 P-labelled *NruI*-to-*StuI* DNA probe. Figure 2, lane 1 shows the expected radioactive band of 4 kb. If the *URA3* fragment of 1.1 kb is inserted into the *SalI* site as expected, the Southern blot of the *URA3*-disrupted chromosome in strain SL1 should show two bands that hybridize to the 32 P-probe because *URA3* contains a *StuI* cleavage site. The fragments should be 2.1 and 3 kb in length. The Southern blot (Fig. 2, lane 2) shows the two expected bands of hybridization

indicating that the chromosomal allele of *LCB1* was disrupted by *URA3* in strain SL1.

Genetic complementation analysis was used to verify that the *lcb1:URA3* disruption mutation in strain SL1 was allelic to *lcb1-1*. Strain SL1 was crossed to strain 24D5. The resulting diploids had an *Lcb*⁻ phenotype, suggesting allelism of the cloned gene and *lcb1*. Strong support for allelism would be obtained by sporulating these diploids and showing that all tetrads give four *Lcb*⁻ spores. However, such diploids failed to sporulate under a variety of conditions, suggesting that sphingolipids are needed for sporulation. An alternative genetic approach was used to demonstrate allelism. The putative *LCB1* allele, carried on the integrating vector YIpLCB1-1, was integrated into its homologous chromosomal locus. The host strain 24D5 carried the *lcb1-1* mutation. If YIpLCB1-1 did indeed carry the wild-type allele of *lcb1-1*, then the host strain should have this plasmid integrated next to *lcb1-1*. When this strain is crossed to an *LCB1* strain, all progeny should be *Lcb*⁺ since YIpLCB1-1 would be tightly linked to *lcb1-1*. In 14 four-spored tetrads from such a cross, showing 2⁺:2⁻ segregation for the Ade, Ura, and Leu phenotypes, all spores were *Lcb*⁺. We conclude that the *LCB1* gene has been cloned.

Transcript mapping of *LCB1*. The size of the *LCB1* mRNA, its location, and 5' to 3' orientation were determined by Northern blotting. To determine the direction of transcription of *LCB1*, a 4-kb DNA fragment carrying the gene was cloned into pTZ18 to yield pTZ18-*LCB1* and into pTZ19 to yield pTZ19-*LCB1*. These plasmids were propagated in *E. coli* in the presence of a helper M13 phage in order to obtain phage particles carrying single-stranded DNA. DNA was extracted from the phage and used as a template to produce a radioactive probe. The rationale for determining the direction of *LCB1* transcription by using the pTZ18-*LCB1* and pTZ19-*LCB1* single-stranded ³²P-labelled probes is diagrammed in Fig. 3. The rationale is based upon the probes hybridizing to opposite strands of *LCB1*. Only one of the probes should hybridize to mRNA, and this result defines the 5' to 3' direction of mRNA transcription. The pTZ18-*LCB1* probe hybridized strongly to RNAs of 1 and 2 kb. The pTZ19-*LCB1* probe hybridized weakly to RNAs of the same size. The weak hybridization by pTZ19-*LCB1* was traced to minor contamination of the probe by pTZ18-*LCB1*. These data indicate that *LCB1* is transcribed from right to left as shown in Fig. 3.

To determine whether the 1- or the 2-kb mRNA was the *LCB1* transcript, we used various double-stranded ³²P-labelled DNA fragments to probe Northern blots. The most informative probe was the 2.2-kb *Bam*HI-to-*Cla*I fragment shown in Fig. 3. It hybridized only to the 2-kb mRNA. A probe to the left of the *Bam*HI site hybridized only to the 1-kb fragment (data not shown). Since a *Sal*I disruption at the *Sal*I site diagrammed in Fig. 3 created the *Lcb*⁻ phenotype and since the only transcript from this region is the 2-kb transcript, we conclude that the 2-kb transcript is the *LCB1* mRNA.

Strains defective in *lcb1* lack serine palmitoyltransferase activity. To determine whether serine palmitoyltransferase activity was missing in *lcb1*-defective strains, we assayed membranes for the enzyme. The parental strain MC6A contained 54.4 U of enzyme activity per mg of protein, while the *lcb1*-defective strain X2A1B contained 2.5 U per mg of protein, or about 20 times less enzyme activity than the parental strain: this level of activity is at the limit of detection and the actual enzyme activity may be lower. The cloned *LCB1* allele carried in pLCB was able to restore

FIG. 3. Analysis of *LCB1* transcription. The 5' to 3' direction of the *LCB1* transcript and its size were determined by electrophoresing 5 µg of poly(A)⁺ RNA on an agarose gel and subjecting the gel to Northern blot analysis. The filter in lane 1 was hybridized to the single-stranded pTZ18-*LCB1* probe labelled with ³²P. The filter in lane 2 was hybridized to the single-stranded pTZ19-*LCB1* probe labelled with ³²P. The filter in lane 3 was hybridized to the double-stranded *Bam*HI-*Cla*I probe labeled with ³²P. The open arrowhead represents the T7 promoter. BLA confers ampicillin resistance in *E. coli*. *S. cerevisiae* DNA sequences are shown as open boxes, and the thin line represents pBR322 DNA. Abbreviations for restriction endonucleases are as follows: B, *Bam*HI; H, *Hind*III; Sa, *Sau*3A; Sm, *Sma*I; St, *Stu*I.

enzyme activity to about 50% of the wild-type level since three independent transformants of strain X2A1B gave 22.7, 25.6, and 22.8 U of enzyme activity per mg of protein.

DNA sequence analysis of *LCB1*. On the basis of the Northern blot results, the 2.5-kb *Xba*I-*Cla*I fragment of DNA carrying the *LCB1* gene was subcloned and both strands were sequenced completely. The sequence (Fig. 4) was scanned for open reading frames. A single, large open reading frame, encoding 558 amino acids and oriented in the same direction of transcription as the *LCB1* mRNA, was found. This region is assumed to code for the *LCB1* product because it is in the correct 5' to 3' orientation and because a *URA3* disruption of the open reading frame at the unique *Sal*I site created a *Lcb*⁻ phenotype.

The 5' end of the *LCB1* mRNA was located by primer extension analysis. The major cDNA product of the exten-

CGC	GTA	TTT	TTT	TTT	TGA	GGC	GCC	ATG	ATT	TCT	TAC	ACG	GTT	TCT	TTT	TTT	TTT	CCT	TCT	TTC	CTT	CTT	GCT	TCT	CTG	CTA	ACA	AAT	
				-319				-304					-289					-274					-259						-244
TTT	TCA	CTC	ATT	CTT	TTT	TAT	AGG	GGC	ATA	TTG	CTG	CGG	TTA	ACT	GTA	GTG	AAC	GAA	AGT	AAG	ATT	GAG	AAA	ATA	TAG	TAC	TTA	AGA	AAA
				-229				-214					-199					-184					-169						-154
AGA	AAA	GGA	AAA	ATA	AAA	AAA	ATT	CTT	TTC	AAC	ATC	ATC	GAG	TAG	CAC	AGT	ATA	AGA	GCG	CTC	TAA	CCT	TCT	GCC	TGG	CCT	CCA	ATA	TAC
				-139				-124					-109					-94					-79						-64
ACA	TTT	TGC	TCG	TGT	AGG	GTT	ATT	TAT	CCT	TTT	TTC	TTC	CTT	CCC	ACC	CAA	AAA	AAA	AAA	GCA	ATG	GCA	CAC	ATC	CCA	GAG	GTT	TTA	CCC
				-49				-34					-19					-4		+1			12						27
K	S	I	P	I	P	A	F	I	V	T	T	S	S	Y	L	W	Y	Y	F	N	L	V	L	T	Q	I	P	G	G
AAA	TCA	ATA	CCG	ATT	CCG	GCA	TTT	ATT	GTT	ACC	ACC	TCA	TCG	TAC	CTA	TGG	TAC	TAC	TTC	AAT	CTG	GTG	TTG	ACT	CAA	ATC	CCG	GGA	GGC
				42				57					72					87					102						117
Q	F	I	V	S	Y	I	K	K	S	H	H	D	D	P	Y	R	T	T	V	E	I	B	L	I	L	Y	B	I	I
CAA	TTC	ATC	GTT	TCG	TAC	ATC	AAG	AAA	TCG	CAT	CAT	GAC	GAT	CCA	TAC	AGG	ACC	ACG	GTT	GAG	ATA	GGG	CTT	ATT	TTA	TAC	GGG	ATC	ATC
				132				147					162					177					192						207
Y	Y	L	S	K	P	Q	Q	K	K	S	L	Q	A	Q	K	P	N	L	S	P	Q	E	I	D	A	L	I	E	D
TAT	TAC	TTG	TCC	AAG	CCA	CAA	CAG	AAA	AAG	AGT	CTT	CAA	GCA	CA	AAG	CCC	AAC	CTA	TCG	CCC	CAG	GAG	ATT	GAC	GCG	CTA	ATT	GAG	GAC
				222				237					252					267					282						297
W	E	P	E	P	L	V	D	P	S	A	T	D	E	Q	S	W	R	V	A	K	T	P	V	T	M	E	M	P	I
TGG	GAG	CCC	GAG	CCT	CTA	GTC	GAC	CCT	TCT	GCC	ACC	GAT	GAG	CAA	TCG	TGG	AGG	GTG	GCC	AAA	ACA	CCC	GTC	ACC	ATG	GAA	ATG	CCC	ATT
				312				327					342					357					372						387
Q	N	H	I	T	I	T	R	N	N	L	Q	E	K	Y	T	N	V	F	N	L	A	S	N	N	F	L	Q	L	S
CAG	AAC	CAT	ATT	ACT	ATC	ACC	AGA	AAC	AAC	CTG	CAG	GAG	AAG	TAT	ACC	AAT	GTT	TTC	AAT	TTG	GCC	TGC	AAC	ACT	TTT	TTG	CAA	TTG	TCC
				402				417					432					447					462						477
A	T	E	P	V	K	E	V	V	K	T	T	I	K	N	Y	B	V	B	A	C	G	P	A	G	F	Y	G	N	Q
GCT	ACG	GAG	CCC	GTG	AAA	GAA	GTG	GTC	AAG	ACC	ACT	ATC	AAG	AAT	TAC	GGT	GTG	GGC	GCC	TGT	GGT	CCC	GCC	GGG	TTT	TAC	GGT	AAC	CAG
				492				507					522					537					552						567
D	V	H	Y	T	L	E	Y	D	L	A	Q	F	F	G	T	Q	B	S	V	L	Y	G	Q	D	F	C	A	A	P
GAC	GTT	CAT	TAC	ACG	TTG	GAA	TAT	GAT	TTA	GCA	CAG	TTC	TTT	GGC	ACC	CAA	GGT	TCC	GTT	CTG	TAC	GGG	CAA	GAC	TTT	TGT	GCC	GCA	CCC
				582				597					612					627					642						657
S	V	L	P	A	F	T	K	R	B	D	V	I	V	A	D	D	Q	V	S	L	P	V	Q	N	A	L	Q	L	S
TCT	GTT	CTG	CCT	GCT	TTC	ACA	AAG	CGT	GGT	GAT	GTT	ATC	GTG	GCA	GAC	GAC	CAG	GTG	TCA	TTA	CCA	GTG	CAA	AAT	GCT	CTG	CAA	CTA	AGC
				672				687					702					717					732						747
R	S	T	V	Y	F	N	H	N	D	M	N	S	L	E	C	L	L	N	E	L	T	E	Q	E	K	L	E	K	
AGA	TCC	ACA	GTC	TAC	TAC	TTC	AAC	CAC	AAC	GAT	ATG	AAT	TCG	CTA	GAA	TGT	TTA	TTA	AAC	GAG	TTG	ACC	GAA	CAG	GAG	AAA	CTT	GAG	AAA
				762				777					792					807					822						837
L	P	A	I	P	R	K	F	I	V	T	E	B	I	F	H	N	S	B	D	L	A	P	L	P	E	L	T	K	L
CTG	CCC	GCC	ATT	CCA	AGA	AAA	TTT	ATC	GTC	ACT	GAG	GGT	ATT	TTT	CAC	AAC	TCG	GGC	GAT	TTA	GCT	CCG	TTG	CCT	GAG	TTG	ACT	AAG	CTG
				852				867					882					897					912						927
K	N	K	Y	K	F	R	L	F	V	D	E	T	F	S	I	B	V	L	B	A	T	B	R	G	L	S	E	H	F
AAG	AAC	AAG	TAC	AAG	TTC	AGA	CTA	TTT	GTT	GAC	GAA	ACC	TTC	TCC	ATT	GGT	GTT	CTT	GGC	GCT	ACG	GGC	CGT	GGG	TTG	TCA	GAG	CAC	TTT
				942				957					972					987					1002						1017
N	M	D	R	A	T	A	I	D	I	T	V	B	S	M	A	T	A	L	B	S	T	B	B	F	V	L	B	D	S
AAC	ATG	GAT	CGC	GCA	ACT	GCC	ATT	GAC	ATT	ACC	GTT	GGG	TCC	ATG	GCC	ACC	GCG	TTG	GGG	TCC	ACC	GGT	GGT	TTT	GTC	CTG	GGT	GAC	AGT
				1032				1047					1062					1077					1092						1107
V	M	C	L	H	Q	R	I	B	S	N	A	Y	C	F	A	C	L	P	A	Y	T	V	T	S	V	S	K	V	
GTT	ATG	TGT	TTG	CAC	CAG	CGT	ATT	GGT	TCC	AAT	GCA	TAT	TGT	TTT	TCT	GCC	TGT	TTG	CCG	GCT	TAC	ACC	GTC	ACA	TCC	GTC	TCC	AAA	GTC
				1122				1137					1152					1167					1182						1197
L	K	L	M	D	S	N	N	D	A	V	Q	T	L	Q	K	L	S	K	S	L	H	D	S	F	A	S	D	D	S
TTG	AAA	TTG	ATG	GAC	TCC	AAC	AAC	GAC	GCC	GTC	CAG	ACG	CTG	CAA	AAA	CTA	TCC	AAA	TCT	TTG	CAT	GAT	TCC	TTT	GCA	TCT	GAC	GAC	TCC
				1212				1227					1242					1257					1272						1287
L	R	S	Y	V	I	V	T	S	S	P	V	S	P	V	L	H	L	Q	L	T	P	A	Y	R	S	R	K	F	G
TTG	CGT	TCA	TAC	GTA	ATC	GTC	ACG	TCC	TCT	CCA	GTG	TCT	CCT	GTC	CTA	CAT	CTG	CAA	CTG	ACT	CCC	GCA	TAT	AGG	TCT	CGC	AAG	TTC	GGA
				1302				1317					1332					1347					1362						1377
Y	T	C	E	Q	L	F	E	T	M	S	A	L	Q	K	K	S	Q	T	N	K	F	I	E	P	Y	E	E	E	E
TAC	ACC	TGC	GAA	CAG	CTA	TTC	GAA	ACC	ATG	TCA	GCT	TTG	CAA	AAG	AAG	TCC	CAG	ACA	AAC	AAA	TTC	ATT	GAG	CCA	TAC	GAA	GAG	GAG	GAA
				1392				1407					1422					1437					1452						1467
K	F	L	Q	S	I	V	D	H	A	L	I	N	Y	N	V	L	I	T	R	N	T	I	V	L	K	Q	E	T	L
AAA	TTT	CTG	CAG	TCC	ATA	GTA	GAT	CAT	GCT	CTT	ATT	AAC	TAC	AAC	GTT	CTC	ATC	ACA	AGA	AAC	ACT	ATT	GTT	TTA	AAA	CAG	GAG	ACG	CTA
				1482				1497					1512					1527					1542						1557
P	I	V	P	S	L	K	I	C	C	N	A	A	M	S	P	E	E	L	K	N	A	C	E	S	V	K	Q	S	I
CCA	ATT	GTC	CCT	AGC	TTG	AAA	ATC	TGC	TGT	AAC	GCC	GCC	ATG	TCC	CCA	GAG	GAA	CTC	AAA	AAT	GCT	TGC	GAA	AGT	GTC	AAG	CAG	TCC	ATC
				1572				1587					1602					1617					1632						1647
L	A	C	C	Q	E	S	N	K	Q																				
CTT	GCC	TGT	TGC	CAA	GAA	TCT	AAT	AAA	TAA	AAA	TAG	AAA	GCC	AGT	ATA	TGC	ACA	CGC	ACA	TAT	ATA	TAT	AAA	TAT	TTA	TAC	AAT	AAT	ACA
				1662				1677					1692					1707					1722						1737
AAT	AAT	CGT	AAC	ATC	ATC	TCT	GTC	AAA	TTG	ACG	TGG	TGC	ACG	GCG	CCC	AGA	GAA	TGC	GCT	AAA	AAT	TTT	CGG	ATC	CGA	AAT	TTT	CTT	TCC
				1752				1767					1782					1797					1812						1827
TTT	TAC	CAT	CGA	GGC	AAA	GCA	ACC	TGT	ATT	ATT	TAT	TTG	TTT	ATT	TAT	TAA	TAG	AAA	AGA	AAG	GAG	TAC	TTT	CGT	GGT	ACG	CTT	TCT	TGA
				1842				1857					1872					1887					1902						1917
GCA	TTT	TCG	GTT	TCA	CTA	GGC	AGA	GAA	CTA	ACA	CAA	GAG	ACA	CAG	CAA	ACA	TCA	AAC	AAG	GTT	AAA	ACA	GCA	CAC	CAA	GGC	AAT	ATG	ATG
				1932				1947					1962					1977					1992						2007
CAT	TTT	AGA	AAG	AAA	TCC	AGT	ATC	AGT	AAC	ACG	AGT	GAT	CAT	GAC	GGA	GCG	AAC	CGT	GCC	TCA	GAT	GTC	AAG	ATT	TCT	GAA	GAT	GAC	AAG
				2022

FIG. 5. Hydropathy profile of the LCB1 protein. The hydropathy of the predicted LCB1 peptide was analyzed according to the method of Kyte and Doolittle (17) by using a span setting of nine amino acid residues. Two transmembrane helices, indicated by roman numerals, were predicted by the procedure of Eisenberg et al. (10).

(Fig. 4) beginning with the first ATG codon at position +1 and ending with the stop codon TAA at position +1675. Assuming that this ATG codon is the true translation initiation site, the product of the open reading frame is a protein of 558 amino acids with a molecular weight of 62,232.

Because serine palmitoyltransferase activity is present in the membrane fraction of lysed cells, we expected the LCB1 protein to be membrane associated. The hydrophobicity of the deduced protein sequence was therefore examined (Fig. 5). The protein is moderately hydrophobic; 42.2% of the 558 amino acid residues are nonpolar, 36.9% are polar, and 20.9% are charged. According to the theory of Kyte and Doolittle (17), the grand average hydropathy score for the LCB1 product is -1.39 , a value that places the LCB1 peptide in the same class as globular proteins. A globular, rather than integral membrane, protein is also predicted by the procedure of Eisenberg et al. (10). In addition, this analysis predicts two very hydrophobic, membrane-associated helices which are designated by roman numerals in Fig. 5. Helix I spans amino acid residues 12 to 32 and has the sequence $\text{IIPAFIVTTSSYLWYYFNLV}$, while helix II spans residues 344 to 373 and has the sequence $\text{ATAIDITY GSMATALGSTGGFVLG}$.

Residue 80 is a potential glycosylation site of the type Asn-X-Ser where the sugar would be N linked to the Asn residue (23).

DISCUSSION

We have described the isolation and characterization of an *S. cerevisiae* gene, termed *LCB1*. Several results suggest that *LCB1* codes for serine palmitoyltransferase. The cloned gene complements a mutant lacking serine palmitoyltransferase activity, by allowing growth in the absence of exoge-

nous long-chain base. In addition, the cloned gene restores serine palmitoyltransferase activity to the *lcb1-1* mutant strain. Finally, the predicted amino acid sequence of the LCB1 protein shows high similarity to the enzymes 5-aminolevulinic acid synthase (ALA synthase) and 2-amino-3-

	10	20	30	40	50	
EKBL#81				FICGTQDSHKEQKLA		#97
LCB1#150	LASNFLQLFATEPVKEVVKTTIKNYGVGACGPAGFYGNQDVHYTLEYDLA					#200
ALSM#193	WCSNDYLGISRHPVLQAIETLKNHAGAGGTRNISGTSKFHVLEQELA					#243
ALSC#243	WCSNDYLGISRHPVCGAVMDTKLQHGAGAGGTRNISGTSKFHVLEKELA					#293
ALSY#118	WCSNKYLALSKHPEVLDAHKTIDKYGCGAGGTRNIAGHNIPITLNLEAELA					#168

	10	20	30	40	50	
EKBL#98	AFLGMEDAILYSSCFDANGGLFETLLG--AEDAIISDALNHASIIDGVRLC					#146
LCB1#201	QFFGTQGSVLYGQDFCAAPSVLPFTK--RGDVI VADDQVSLPVQNALQLS					#249
ALSM#244	ELHGKDSALLFSSCFVANDSTLFTLAKLLPGCEIYSDAGNHASMIQGI RNS					#294
ALSC#294	DLHGKAALLFSSCFVANDSTLFTLAKMLPGCEIYSDSGNHASMIQGI RNS					#344
ALSY#169	TLHKKEGALVFSSCYVANDAVLSLLGKQKDLVIFSDLNHASMIQGIKHA					#219

	10	20	30	40	50	
EBIO#170				QMVTGEGVFSMDGDS		#184
EKBL#147	KAKRYRYANNDHGELEARLKEARERG-----ARH-VLIATDGLFSMDGVI					#190
LCB1#250	RSTVYFYFNHNDMNSLECLLNELTEQEKLEKLPAPRKFIVTEGIFHNSGDL					#300
ALSM#295	GAAKFVFRHNDPGHLKLL-----EKSDPKTPKIVAFETVHSDGAI					#336
ALSC#345	RVPKHI FRHNDVNHLELL-----KKSDPSTPKIVAFETVHSDGAV					#386
ALSY#220	NVKKHIFKHNDLNELEQLL-----QSPKSVPKLI AFESVYSMAGSV					#261

	10	20	30	40	50	
EBIO#185	APLAEIQQVTQQHNGWLMVDDAHGTGVIGEQGRG					#218
EKBL#191	ANLKGVCADLADY					#203
LCB1#301	APLPETLTKLNKYKFRFVDETFISIGVLTATGRGL					#335
ALSM#337	CPLEELCDVAHQYGALT FVDEVHAGVLYGARGAGI					#371
ALSC#387	CPLEELCDVAHEHGAIT FVDEVHAGVLYGARGGGI					#421
ALSY#262	ADIEKICDLADKYGALT FLDVHAGVLYGPHGAGVAEHCD FESHASGIAT					#312

	10	20	30	40	50	
LCB1#336	-----SEH--FNMDRATAIDITVGSMTALGSTGGFVLGDSVNCILHQRI G					#378
ALSM#372	-----GER--DGIMHKLDIISGTLGKAFGCVGGYI ASRDLVDHVRYS					#412
ALSC#422	-----GDR--DGVHMKMDIISGTLGKAFACVGGYISSTSAIDTVRSY					#462
ALSY#313	PKTNDKGA---KTVMDRVDMITGTLGKSGVSGVGGYGAASRKLIDWFRSF					#363

	10	20	30	40	50	
LCB1#379	SNAYCFSACLPAITVTSVSKVLKMDSNDAV					#410
ALSM#413	AAGFI FTSLPPMMLSGALESVRLKKEEGQA					#444
ALSC#463	AAGFI FTSLPPMLLAGALESVRTLSAEGQV					#494
ALSY#364	APGFI FTTLPPSMAGATAAIRYQRCHIDL R					#391

FIG. 6. Comparison of the deduced amino acid sequence of LCB1 to other proteins. The protein sequences of LCB1 and the mouse (ALSM), chicken (ALSC), and yeast (ALSY) 5-aminolevulinic acid synthases were compared by using the procedure of Pearson and Lipman (28) and aligned for maximum similarity. The 2-amino-3-ketobutyrate CoA ligase (EKBL) (1) and the biotin synthetase (EBIO) (27) sequences were identified and aligned by using the FASTA algorithm as described in the text. Colons represent identity between residues, while dots indicate conservative replacements by similar residues. Insertions made during the alignment optimization process are indicated by dashes.

SERINE PALMITOYLTRANSFERASE:

5-AMINOLEVULINIC ACID SYNTHASE:

2-AMINO-3-KETOBUTYRATE LIGASE:

FIG. 7. Comparison of the reactions catalyzed by serine palmitoyltransferase, ALA synthase, and 2-amino-3-ketobutyrate CoA ligase.

ketobutyrate CoA ligase (Fig. 6). These two enzymes and serine palmitoyltransferase catalyze very similar chemical reactions, and all use the cofactor pyridoxal phosphate (Fig. 7). The similarity of the amino acid sequences and the reactions catalyzed by these three enzymes argues that the product of *LCB1* is most likely serine palmitoyltransferase or a catalytic subunit of the enzyme, rather than a regulatory protein that regulates transcription of *LCB1* or the activity of serine palmitoyltransferase.

ALA synthase catalyzes the first step in heme biosynthesis. Molecular cloning techniques have revealed the primary structure, regulation, and mechanisms of synthesis and transport of ALA synthase. For example, the nucleotide sequence of cDNAs encoding the human (2), rat (38, 45), and chicken (3, 4, 21) hepatic enzymes and the mouse (33) and chicken (31) erythroid isozymes along with the complete nucleotide sequences of ALA synthase genes from *S. cerevisiae* (*HEM1*) (41) and *Bradyrhizobium japonicum* (*hemA*) (24) have revealed structural similarities between the predicted primary sequences of these enzymes.

Because the first homology search with the entire *LCB1* peptide could have missed proteins having small regions of amino acid similarity, a second homology search was performed with small, overlapping portions of *LCB1*. Two sets of contiguous 30-residue peptides were used in the second search, with one set overlapping the other by 15 residues. Many members of the two sets of peptides showed high sequence similarity to numerous proteins in the translated GENBANK data base by the FASTA algorithm: the ALA synthase similarities shown in Fig. 6 did not have particularly high similarity scores and would not have been seen as significant without further analysis. To identify statistically and biologically significant similarities, we searched for proteins having similarity to two or more of the 30-residue peptides, since this approach would identify larger regions of similarity. This strategy revealed the ALA synthase results shown in Fig. 6, and in addition, the similarities to the *E. coli* enzymes 2-amino-3-ketobutyrate CoA ligase (EKBL, Fig. 6) and biotin synthetase (EBIO, Fig. 6).

The similarity of the *LCB1* protein to 2-amino-3-ketobutyrate CoA ligase seemed particularly significant since the ligase catalyzes a reaction (Fig. 7) that is very similar to those catalyzed by serine palmitoyltransferase and ALA synthase. In addition, the *E. coli* 2-amino-3-ketobutyrate CoA ligase uses pyridoxal phosphate as a cofactor (26), as do serine palmitoyltransferase and ALA synthase. 2-Amino-3-

ketobutyrate CoA ligase catalyzes the second reaction in the threonine utilization pathway in both procaryotes (16) and eucaryotes (8).

The region of amino acid sequence similarity shown in Fig. 6 most likely defines the catalytic domain(s) of serine palmitoyltransferase, ALA synthase, and 2-amino-3-ketobutyrate CoA ligase. However, this suggestion awaits further analysis since the catalytic site(s) of none of these enzymes has been determined.

The similarity of the *LCB1* peptide to biotin synthetase, the product of *bioB*, seems statistically significant because it covered a region of 49 amino acids (Fig. 6) in which 40.8% of the residues were identical and another 44.8% were similar. The functional significance of this similarity is not obvious since biotin synthetase catalyzes the addition of sulfur to *d*-dethiobiotin to yield biotin (see reference 27 for references) by a reaction not yet identified but seemingly different than that catalyzed by serine palmitoyltransferase.

What might be the function of the N-terminal portion of *LCB1*, the portion that lacks similarity to those of other proteins? One possible function is to direct the protein to its proper cellular location. The mature form of serine palmitoyltransferase is found in the microsomal membrane fraction of animal cells, but its exact cellular location is unknown (25). Preliminary data (30) indicate that the *S. cerevisiae* serine palmitoyltransferase is a membrane-bound enzyme. The predicted *LCB1* protein has two potential membrane-spanning helices (Fig. 5) that could tether the protein to the membrane. One of these helices, I in Fig. 5, is in the N-terminal domain that shows no amino acid similarity to ALA synthase, so this region might target the protein to a specific membrane location.

Analysis of the 5' noncoding region of *LCB1* revealed several putative TATA boxes, including TATTTAT at position -43, AATATA at position -70, TATAA at position -103, and TAAAAAAAATT at position -140. Since nothing is known about the regulation of *LCB1* or any other eucaryotic gene required for sphingolipid biosynthesis, mutational analysis of the 5' upstream region may reveal the presence of novel *cis*-acting elements that regulate transcription.

We have described the initial characterization of *LCB1* and its predicted protein product. The gene most likely codes for serine palmitoyltransferase. An alternative explanation for our data would argue that *LCB1* is a regulatory gene whose product controls the synthesis or activity of serine palmitoyltransferase. While this possibility seems unlikely, further data will be needed to prove that *LCB1* codes for serine palmitoyltransferase or a subunit of the enzyme. Isolation of *LCB1* has already proven to be useful by allowing the isolation of mutant yeast strains that lack detectable sphingolipids (9). The gene should be useful for a variety of other studies.

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