Sphingoid base 1-phosphate phosphatase: A key regulator of sphingolipid metabolism and stress response

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ABSTRACT   The sphingolipid metabolites ceramide and sphingosine-1-phosphate are second messengers with opposing roles in mammalian cell growth arrest and survival; their relative cellular level has been proposed to be a rheostat that determines the fate of cells. This report demonstrates that this rheostat is an evolutionarily conserved stress-regulatory mechanism that influences growth and survival of yeast. Although the role of sphingosine-1-phosphate in yeast was not previously examined, accumulation of ceramide has been shown to induce G_1 arrest and cell death. We now have identified a gene in *Saccharomyces cerevisiae*, *LBP1*, that regulates the levels of phosphorylated sphingoid bases and ceramide. *LBP1* was cloned from a yeast mutant that accumulated phosphorylated long-chain sphingoid bases and diverted sphingoid base intermediates from sphingolipid pathways to glycerophospholipid biosynthesis. *LBP1* and its homolog, *LBP2*, encode very hydrophobic proteins that contain a novel-conserved sequence motif for lipid phosphatases, and both have long-chain sphingoid base phosphate phosphatase activity. *In vitro* characterization of Lbp1p shows that this phosphatase is Mg^{2+}-independent with high specificity for phosphorylated long-chain bases, phytosphingosine and sphingosine. The deletion of *LBP1* results in the accumulation of phosphorylated long-chain sphingoid bases and reduced ceramide levels. Moreover, deletion of *LBP1* and *LBP2* results in dramatically enhanced survival upon severe heat shock. Thus, these phosphatases play a previously unappreciated role in regulating ceramide and phosphorylated sphingoid base levels in yeast, and they modulate stress responses through sphingolipid metabolites in a manner that is reminiscent of their effects on mammalian cells.

Branching pathways of sphingolipid metabolism may mediate growth arrest, stress, or proliferative responses depending on the cell type and the nature of the stimulus. Ceramide is emerging as an important regulatory component of stress responses and programmed cell death, known as apoptosis (1–5). In contrast, another sphingolipid metabolite, sphingosine-1-phosphate (SPP), has been implicated as a second messenger in cellular proliferation (6) and antagonizes ceramide-mediated apoptosis (7). Thus, it has been suggested that the relative intracellular levels of ceramide and SPP are a critical factor for cell survival. Although the ceramide/SPP rheostat may be an inherent characteristic of mammalian cells, external stimuli can reset this ratio (7–9). A variety of stress stimuli, including Fas ligand, TNF-α, IL-1, growth factor withdrawal, anticancer drugs, oxidative stress, heat shock, and ionizing radiation, increase ceramide levels (1, 2, 10, 11), whereas platelet-derived growth factor and other growth fac-

tors stimulate rapid, transient elevations in SPP levels (6). The mechanisms that regulate the levels of these sphingolipid second messengers are under intense investigation with most of the attention focused on degradative pathways: sphingomyelinase, which produces ceramide, and ceramidase, which generates sphingosine, the substrate for sphingosine kinase. SPP is rapidly metabolized via sphingosine lyase to produce phosphoethanolamine and *trans*-2-hexadecenal (12, 13) or is hydrolyzed back to sphingosine by a putative phosphatase (14). However, the relative contributions of the *de novo* biosynthesis and the metabolism of these sphingolipid metabolites have not yet been evaluated because most of the relevant enzymes have not been purified or cloned.

S. cerevisiae synthesizes sphingolipids that resemble mammalian sphingolipids, except that phytosphingosine and not sphingosine is the predominant sphingoid base, and phosphoinositol rather than phosphocholine is the polar head group attached to ceramide (15) (Fig. 1). Some of the downstream targets that link ceramide with signaling cascades in mammalian cells have been identified in yeast, and by analogy with its role in mammalian cells (1, 2), ceramide has been shown to induce G_1 arrest in yeast via a ceramide-activated protein phosphatase (16). Moreover, accumulation of ceramide in yeast mutants that lack inositol phosphorylceramide synthase results in cell death (17). These results further suggest the presence of a ceramide-activated death response in yeast. Although phosphorylated long-chain sphingoid bases (LCBPs) previously have not been detected in *Saccharomyces*, the degradative enzymes must be present because sphingolipid metabolites have been found to contribute to phosphatidylethanolamine synthesis (18) (Fig. 1).

To identify enzymes involved in sphingolipid metabolism, we took a yeast genetic approach and isolated mutants that diverted sphingolipid metabolites to glycerophospholipid synthesis. One mutant, *lbp1–1*, accumulated abnormally high levels of LCBP and was hypersensitive to australifungin, a ceramide synthase inhibitor (19). Here, we describe the cloning and characterization of *LBP1* and *LBP2*, two novel genes encoding sphingoid base–phosphate phosphatases that are important for the survival of yeast under stress conditions.

MATERIALS AND METHODS

Strains, Plasmids, and Reagents. *S. cerevisiae* W303–1A (*MAT*a, *ade2–1 can1–100 his3–11, 15 leu2–3, 112 trp1–1*
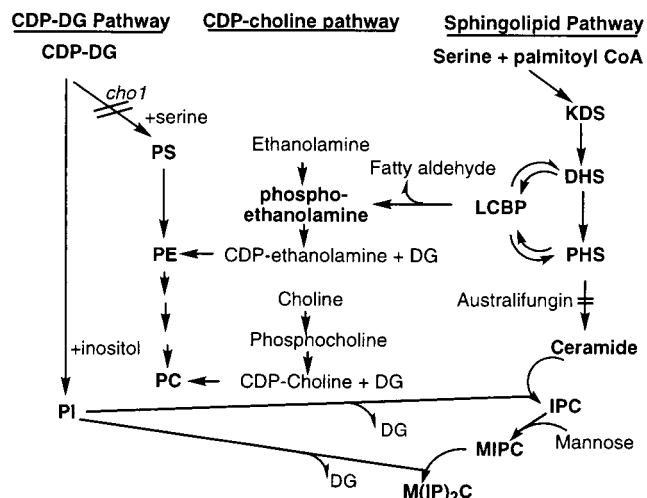

Lipid Synthesis in Yeast

FIG. 1. Pathways of sphingolipid and glycerophospholipid metabolism in *S. cerevisiae*. DG, diacylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; KDS, ketodihydrosphingosine; DHS, dihydrosphingosine; PHS, phytosphingosine; IPC, inositol phosphorylceramide; MIPC, mannose inositol phosphorylceramide; and $M(IP)_2C$, mannose diinositol diphosphorylceramide.

ura3–1) and its isogenic derivatives were provided by R. Rothstein (20), and BRS1187 (*MAT*a, *ade2–1 can1–100 his3–11, 15 leu2–3, 112 trp1–1 ura3–1 cho1*Δ::URA3) was the generous gift of S. Henry (21). A strain containing the *lbp1–1* mutation was isolated as a spontaneous suppressor of Δ*cho1* grown in yeast extract/peptone/dextrose (YPD) media in the absence of ethanolamine. *LBP1* was selected by screening a genomic DNA library (provided by S. Parent, Merck and constructed as described in ref. 22) for complementation of *lbp1–1* (strain 118–2A) on SC-URA plates that contained 0.1 μg/ml australifungin. *LBP2* was synthesized from W3031A DNA by using PCR with the following primers: 5′-GTGCGCACGTATTCTGCGGC and 5′-CGGTT-GAGTTTTTTACTTCTTCAC. *LBP1* was disrupted by the replacement of a 1.2-kb *Bam*HI/*Hpa*I fragment with the *LEU2* gene, and *LBP2* was disrupted by the replacement of a 1.3-kb *Mlu*I/*Eco*NI fragment with the *URA3* gene. After transformation into W303 strains, correctly targeted integrants were confirmed by using Southern analysis. Disruptants were mated with a *cho1*Δ::TRP1 strain, and the Δ*cho1*Δ*lbp1*, Δ*cho1*Δ*lbp2*, and Δ*cho1*Δ*lbp1*Δ*lbp2* strains were isolated. Australifungin was provided by G. Harris (Merck). A. Rosegay, Y. S. Tang, and A. Jones (Merck) synthesized 4, 5-[^{3}H]dihydrosphingosine by exposing acetylated sphingosine to tritium gas and palladium on carbon; the product was deacetylated and purified by using HPLC.

Growth Assays. Growth inhibition was determined by using microtiter broth dilution assay in Synthetic Complete (SC) Yeast Nitrogen Base medium (Difco) containing 2% Glc and 0.078% complete supplement mixture (Bio101) or SC-URA for plasmid-containing strains. Cells were inoculated at OD_{600} = 0.001 (≈1 × 10^4 yeast cells/ml), and serial 2-fold dilutions of australifungin were made from 5 μg/ml. Growth after 24 hr at 30°C was measured by using absorbance readings with an SLT 340 ATTC (Tecan Instruments) after cell resuspension. To test suppression of the ethanolamine requirement of Δ*cho1*, the strains were inoculated into YPD and were grown overnight at 30°C to a density of 1 × 10^7 cells/ml. Serial dilutions of overnight cultures were spotted onto SC agar plates with or without 10 mM ethanolamine, and the plates were grown at 30°C for 2 days.
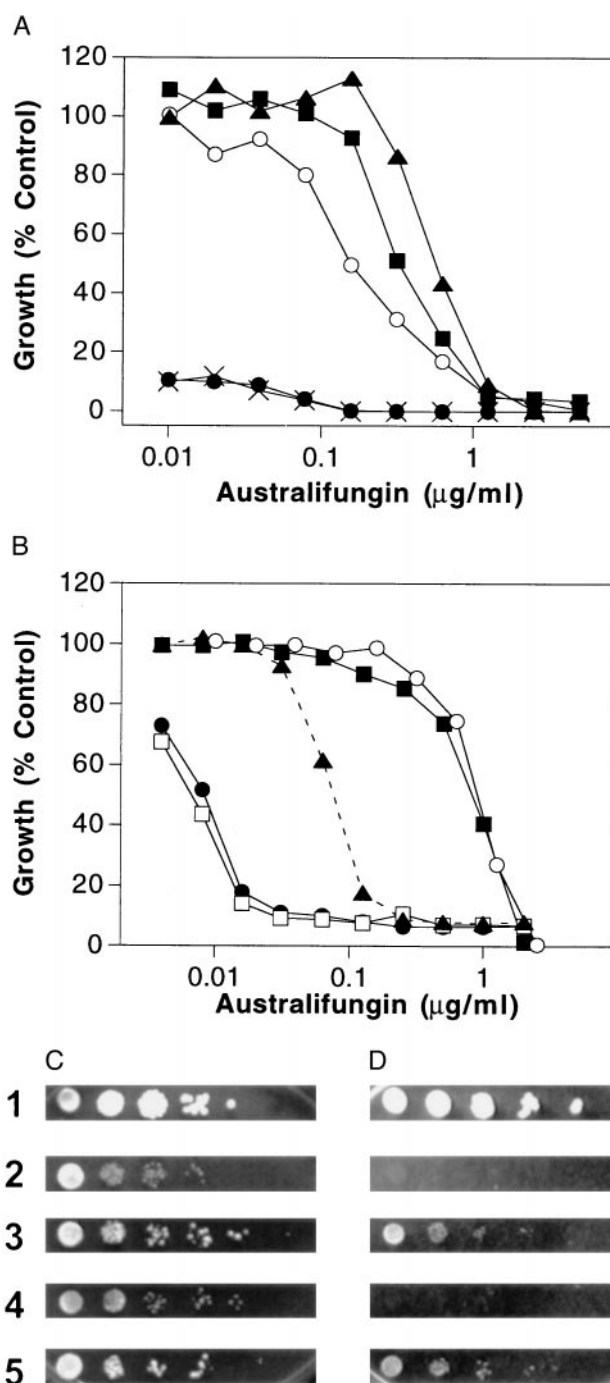

FIG. 2. (*A*) *LBP1* complements hypersensitivity of *lbp1–1* to australifungin. Wild-type strain W303-1A/YCp (○) or *lbp1–1* mutant transformed with control vector (●), *LBP1* on low copy YCp vector (■), *LBP1* on multicopy YEp vector (▲), or *LBP2* on multicopy YEp vector (X) were tested for their sensitivity to australifungin. The data are expressed as percent of growth of W303-1A. (*B*) Δ*lbp1* and *lbp1–1* are hypersensitive to australifungin. The sensitivity of *lbp1–1* (●), Δ*lbp1* (□), Δ*lbp2* (○) haploid mutants, and Δ*lbp1/LBP1* heterozygote (▲) to australifungin was compared with the sensitivity of wild-type diploid strain YSP047 (■). (*C* and *D*) Δ*lbp1*, but not Δ*lbp2*, suppresses the ethanolamine requirement of Δ*cho1*. Serial dilutions of overnight cultures were spotted onto SC agar plates with (*C*) or without (*D*) 10 mM ethanolamine. Strains: 1, W303–1A; 2, Δ*cho1*; 3, Δ*cho1*Δ*lbp1*; 4, Δ*cho1*Δ*lbp2*; and 5, Δ*cho1*Δ*lbp1*Δ*lbp2*.

Phosphatase Assays. Strains were grown to OD_{600} of 1.1 in SC (or SC-URA for plasmid-containing strains), and the membranes were prepared as described (19). For Fig. 5, cells were grown to OD_{600} of 11 in YPD media. To measure LCBP-

phosphatase activity, ^{32}P-labeled LCBPs were prepared by using phosphorylation of sphingosine or phytosphingosine with crude yeast sphingosine kinase, essentially as previously described (23). ^{32}P-LCBPs were extracted, purified by using TLC, and isolated as described (24). LCBP-phosphatase activity was measured by adding ^{32}P-labeled phytosphingosine-1-phosphate or ^{32}P-labeled SPP (1 nmol each, 25,000 cpm, 0.3% BSA complex) to yeast membrane preparations (2 µg) in 200 µl of 100 mM Hepes (pH 7.5) containing 10 mM EDTA and incubating for 10 min at 37°C (14). The remaining phosphorylated bases were extracted and analyzed by using TLC (25). In some experiments, the product, sphingosine, was measured after incubations with 20 µM unlabeled SPP (25).

Lipid Synthesis and TLC Analysis. One milliliter cultures were grown to 0.25 OD_{600} in SC medium at 30°C and labeled with sphingolipid precursors. Lipids were extracted, processed, and resolved by TLC in $CHCl_3$:methanol:4.2 N NH_4OH (9:7:2) as described (26). Labeling and extraction conditions used were: (*i*) 2-hr labeling with 5 µCi/ml [3H]palmitate, after which lipids were deacylated (26) and acidified, and free fatty acids were removed by extraction with petroleum ether; (*ii*) 6-hr labeling with 20 µCi/ml [3H]serine, after which lipids were deacylated; and (*iii*) 30-min labeling with 2 µCi/ml [3H]dihydrosphingosine, after which total lipid extracts were analyzed. Radiolabeled lipids were quantified on a Molecular Dynamics PhosphorImager by using a tritium-sensitive screen and were visualized with XAR-5 film (Kodak) after treatment with EN[3]HANCE (DuPont/NEN).

Stress Response. Yeast strains were grown in SC, SC-URA, or YPD medium overnight, and stationary phase cultures were heat-shocked at 50°C for the indicated times. Cell growth after 24 hr was determined using the *A* at 660 nm. Viability was determined by plating diluted cultures before and after heat shock and by counting colony-forming units. Results are expressed relative to the same strains that were not subject to heat shock.

RESULTS AND DISCUSSION

Cloning and Disruption of *LBP1*. We used a genetic approach to identify enzymes involved in sphingolipid metabolism in yeast, taking advantage of the diversion of sphingoid base intermediates from sphingolipid metabolism to phospholipid synthesis (Fig. 1). Our strategy was to isolate suppressors of Δ*cho1*, a strain that lacks phosphatidylserine synthase and requires exogenously supplied choline or ethanolamine to synthesize methylated phospholipids that are essential for growth (21). It was expected that mutations in sphingolipid pathways that increase metabolism of LCB to phosphoethanolamine would support the growth of Δ*cho1* in the absence of an exogenous supply of ethanolamine (27). Of several suppressors with altered sphingolipid metabolism that were isolated, one mutant, *lbp1–1*, showed dramatic accumulation of LCBP and was hypersensitive to australifungin, a ceramide synthase inhibitor (19).

The drug hypersensitivity phenotype of *lbp1–1* was used to clone the complementing gene from *Saccharomyces*. Genomic library screening resulted in the identification of ORF YJL134 as the only gene that restored normal drug sensitivity to *lbp1–1* on either low copy or multicopy vectors but did not affect the

sensitivity of wild-type cells to australifungin (Fig. 2*A*). *LBP1* has a close homolog in the yeast database, YKR053/*LBP2*, that shares 56% identity at the amino acid level; both genes encode highly hydrophobic proteins with at least five predicted transmembrane domains. To determine whether the *lbp1–1* phenotypes resulted from the function of *LBP1* and/or *LBP2* gene products, strains containing disruptions of each gene were constructed. Analysis of the disrupted strains revealed that Δ*lbp1* had all of the phenotypes associated with *lbp1–1*; both mutations conferred the same level of hypersensitivity to australifungin (Fig. 2*B*), suppressed the ethanolamine requirement of the Δ*cho1* mutant (Fig. 2*D*), and caused the accumulation of phosphorylated sphingoid bases, as described below. In contrast, the disruption of *LBP2* did not confer sensitivity to australifungin (Fig. 2*B*), suppress Δ*cho1* (Fig. 2*D*), or alter sphingolipid synthesis. Furthermore, strains containing null alleles of both *LBP1* and *LBP2* had all of the phenotypes of Δ*lbp1*, without additional effects. Thus, the *lbp1–1* mutation is due to loss of function of *LBP1*, and *LBP2* cannot substitute for *LPB1*. Interestingly, heterozygous diploids made with either *lbp1–1* or Δ*lbp1* had an intermediate level of sensitivity to australifungin (Fig. 2*B*). This gene dosage effect suggests that cellular levels of Lbp1p may be limiting.

LBP1 and LBP2 Are Phosphatases. The sequence analysis of *LBP1* and *LPB2* revealed the presence of a novel motif recently identified in several lipid phosphate phosphatases (refs. 28, 29; Table 1). When phytosphingosine-1-^{32}P and sphingosine-1-^{32}P were used as substrates for the phosphatase activity present in membrane preparations from log phase cells grown in SC media, the Δ*lbp1* strain had low phosphatase activity, whereas a strain containing multiple copies of *LBP1* (Yep*LBP1*) had markedly higher activity than did the wild-type strain (Fig. 3*A*). In contrast, the LCBP-phosphatase activity of Δ*lbp2* was similar to that of the wild type, and the strain containing multiple copies of *LBP2* had slightly enhanced activity. LCBP- phosphatase appears to be unique because, unlike the previously described yeast phospholipid phosphatases, phosphatidate phosphohydrolase and DGPP phosphatase (29, 30), it was inhibited by a low concentration of Triton X-100 (0.05%) and was not inhibited by divalent cations or aliphatic cations, such as propanolol. Moreover, the phosphatase was not inhibited by EDTA, and its activity was even increased >40% in the presence of EDTA. As with these other yeast phosphohydrolases, LCBP-phosphatase activity was relatively insensitive to thioreactive agents (Fig. 3*B*) and was inhibited by NaF, but only in the absence of EDTA (unpublished data). Moreover, there was a striking similarity between this phosphatase activity and that of SPP phosphatase isolated from plasma membranes of rat liver (14). In competition studies, Lbp1p appeared to be highly specific for LCBP because a wide variety of other phospholipids, including phosphatidate and lysophosphatidate, were not competitive substrates (Fig. 3*C*), and, in addition to unlabeled SPP, only sphingosine, dihydrosphingosine, and phytosphingosine markedly inhibited this phosphatase (Fig. 3*C*).

Loss of LCBP-phosphatase activity in *lbp1–1* resulted in abnormally high levels of LCBP, as shown by labeling the strains with [3H]palmitate and resolving the labeled lipids by using TLC (Fig. 4, lanes 1 and 2). [3H]Serine labeling revealed that in the disrupted strains, the Δ*lbp1* mutant, but not the

Table 1.   *LBP1* and *LBP2* phosphatase domains

Protein	Domain 1	Domain 2	Domain 3
Lbp1p	127-KDYWCLPRP-20-PSSH-42-GRIYCGMHGILD-409		
Lbp2p	128-KDYWCLPRP-20-PSSH-42-GRVYCGMHGMLD-404		
Consensus	K RP PSGH SR H D		

Domains important for phosphatase activity and consensus sequence taken from ref. 28. The entire sequence for *LBP1* can be found in GeneBank database accession no. Z49410 and for *LBP2* in GeneBank database accession no. Z28278.
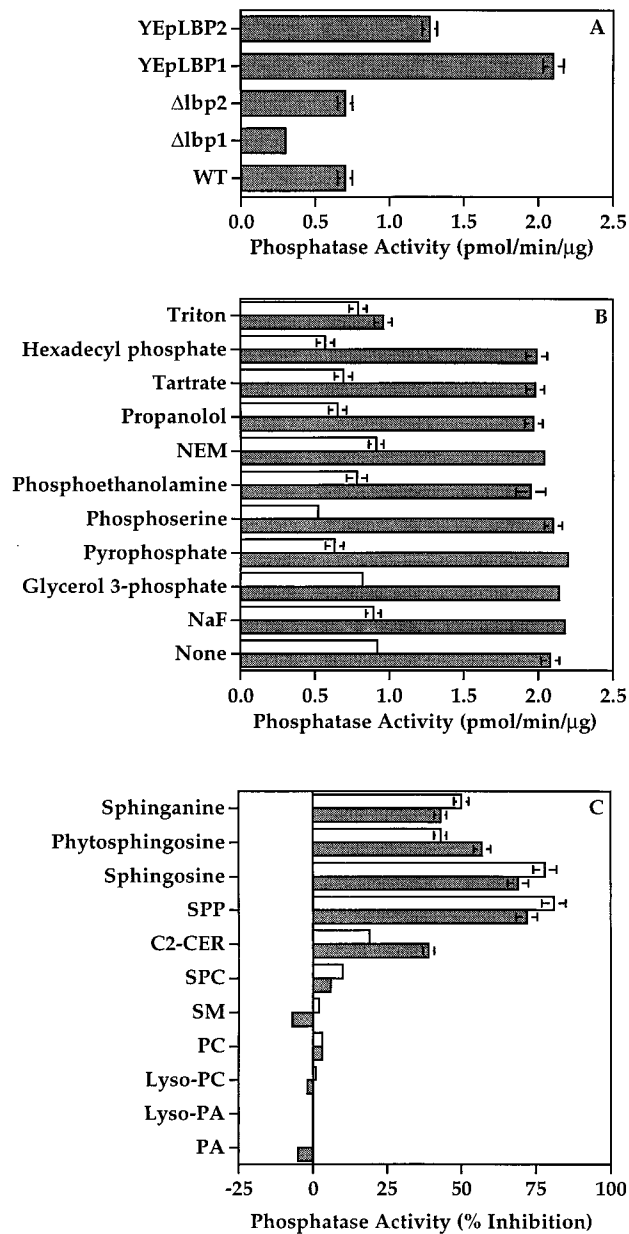
FIG. 3. The product of the *LBP1* gene is an LCBP phosphatase. (*A*) LCBP-phosphatase activity was measured in wild-type (WT) W303-1A, Δ*lbp1*, Δ*lbp2*, and W303 cells carrying *LBP1* or *LBP2* on multicopy YEp plasmids by using phytosphingosine-1-^{32}P as substrate. (*B*) Effects of lipids and phosphatase inhibitors on LCBP-phosphatase activity of wild-type W303 cells (open bars) and the *LBP1* overexpressor strain (hatched bars). The following agents were used: Triton X-100, 0.05%; hexadecylphosphate (100 μM); tartrate (10 mM); propanolol (4 mM); *N*-ethylmaleimide (NEM, 5 mM); phosphoethanolamine (10 mM); phosphoserine (10 mM); pyrophosphate (10 mM); glycerol 3-phosphate (6 mM); and NaF (10 mM). (*C*) Inhibition of LCBP-phosphatase activity by 100 μM concentrations of the indicated lipids in membrane fractions from the *LBP1* overexpressor strain using phytosphingosine-1-^{32}P (open bars) or sphingosine-1-^{32}P (hatched bars) as substrates.

Δ*lbp2* mutant, accumulated LCBP and also had reduced levels of ceramide (Fig. 4, lanes 3–5). Quantitative analysis of [^{3}H]serine-labeled phospholipids revealed that the LCBPs were elevated 2.5-fold in Δ*lbp1*, and ceramide levels were consistently reduced 35–50% compared with wild-type or Δ*lbp2* cells. These results suggest that sphingoid bases are normally phosphorylated to a greater extent than was previously appreciated but do not accumulate because of LCBP-
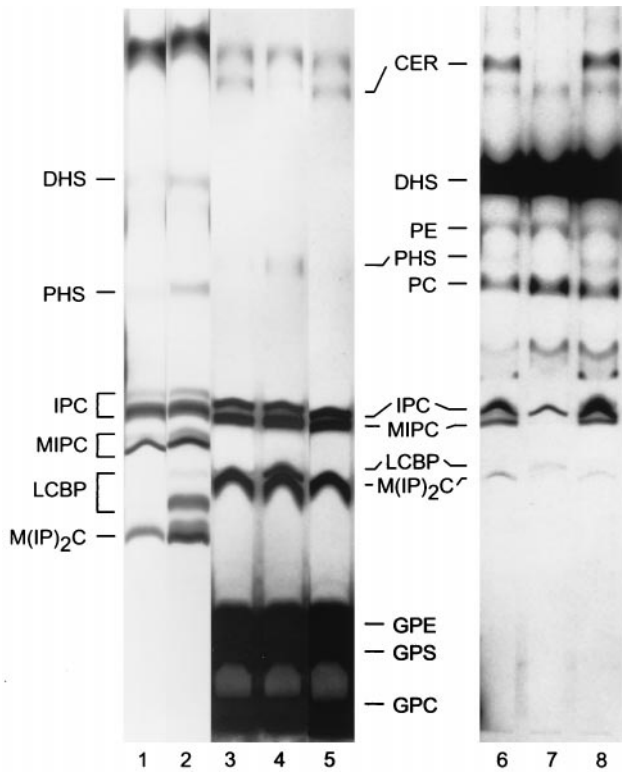

FIG. 4. Δ*lbp1* accumulates LCBP, has reduced levels of ceramide, and fails to incorporate exogenous dihydrosphingosine into sphingolipids. Strains W3031A (lanes 1, 3, and 6), *lbp1–1* (lane 2), Δlbp 1 (lanes 4 and 7), or Δ*lbp2* (lanes 5 and 8) were labeled with the sphingolipid precursors: [^{3}H]palmitate (lanes 1 and 2), [^{3}H]serine (lanes 3–5), or [^{3}H]dihydrosphingosine (lanes 6–8), and the lipids were extracted and processed as described in *Materials and Methods* and separated by using TLC. Migration of standards is indicated: CER, ceramide; GPE, glycerophosphate ethanolamine; GPS, glycerophosphate serine; GPC, glycerophosphate choline; all other standards as described in Fig. 1.

phosphatase activity. Thus, Lbp1p is an important regulator of the flux of sphingoid base intermediates from ceramide/sphingolipid pathways to glycerophospholipid synthesis.

Although total sphingolipid synthesis was only slightly reduced in *lbp1* mutants as measured by [^{3}H]serine, [^{3}H]palmitate, [^{3}H]inositol labeling, or by mass measurements of long-chain bases (unpublished data), exogenous [^{3}H]dihydrosphingosine was not incorporated into sphingolipids in Δ*lbp1* (Fig. 4, lanes 6–8). In a recent study using a sphingolipid compensatory mutant that failed to incorporate exogenous LCB into sphingolipids, *LBP1* was isolated as the complementing gene and named *LCB3*. *LBP1/LCB3* restored normal sphingolipid synthesis to the mutant, and Lcb3p was suggested to be acting as a plasma membrane transport protein for LCB (31). In contrast, we have found that Lbp1p is localized to the endoplasmic reticulum (unpublished data). Moreover, our results argue that Lbp1p/Lcb3p is a lipid phosphatase and is not involved in LCB uptake, because incorporation of exogenous [^{3}H]dihydrosphingosine into phospholipids was significantly enhanced in *lpb1* mutants, and only sphingolipid synthesis was impaired (Fig. 4). This defect in sphingolipid synthesis raises the intriguing possibility that externally supplied LCBs are phosphorylated after their entry and require dephosphorylation by Lbp1p before they can be used for ceramide synthesis.

Heat Stress Response. The changes in levels of LCBP and ceramide that we detected in *lbp1* mutants prompted us to investigate the function of the LCBP phosphatase in yeast growth and stress responses. Growth of strains containing null alleles of either or both of the *LBP* genes did not differ significantly from growth of control cells grown on different
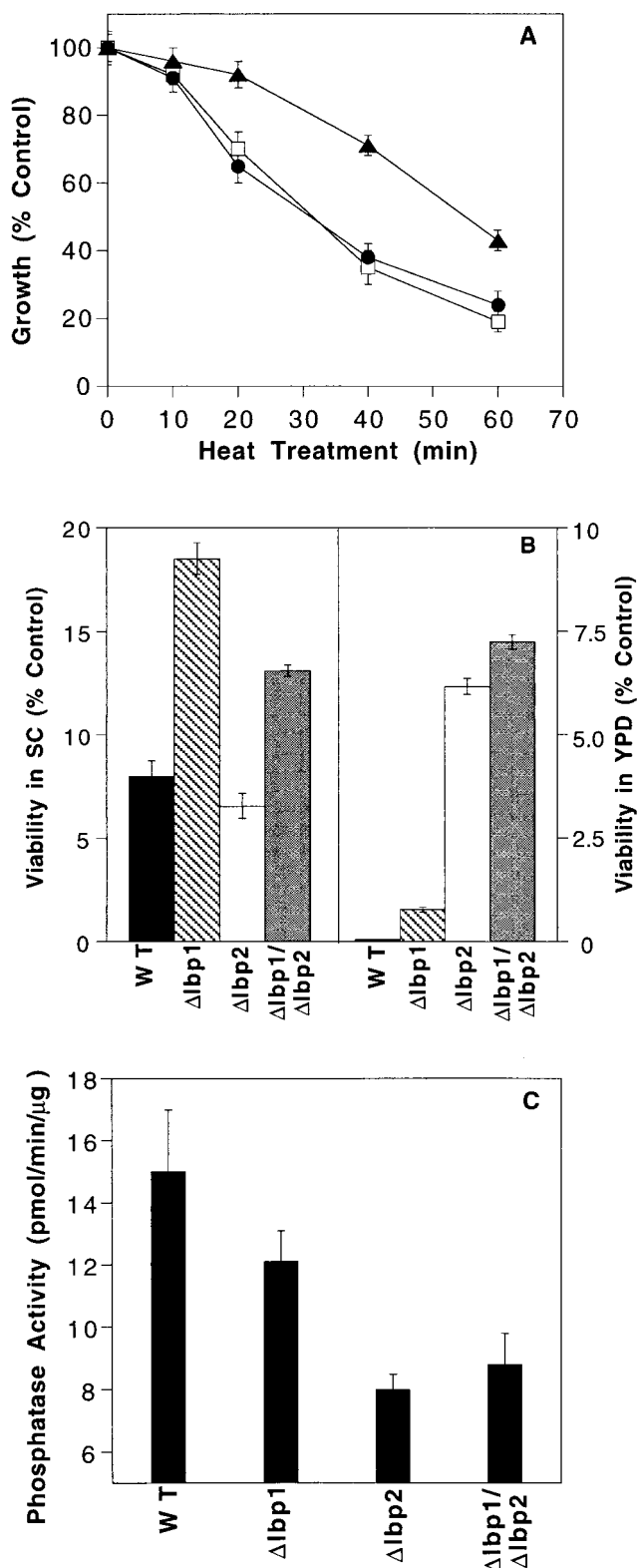
FIG. 5. (*A, B*) Δlbp mutants are protected from loss of viability upon heat shock. (*A*) Yeast strains were grown in SC medium and heat-shocked at 50°C for the indicated times, and then cell growth after 24 h was determined; W303 (□), Δ*lbp1* (▲), and W303 cells carrying *LBP1* on multicopy plasmids (●). (*B*) Strains were grown overnight in SC (Left) or YPD (Right), and heat-shocked for 45 min, and viability was determined. (*C*) Δ*lbp2* has reduced LCBP phosphatase activity in YPD media. W3031A (WT), Δ*lbp1*, Δ*lbp2*, and Δ*lbp1*Δ*lbp2* strains were grown in YPD media to OD$_{600}$ of 11, and LCBP phosphatase activity was measured, as described in *Materials and Methods*, using sphingosine-1-^{32}P as substrate.

carbon or nitrogen sources, at different pH (4.0 to 7.0), with 10 mM Ca^{2+} or Mg^{2+}, or under high NaCl or sorbitol osmotic stress, and all strains were capable of mating and sporulating. Dramatic differences were seen, however, with heat stress, which has been shown to elevate ceramide levels (32). In SC medium, the Δ*lbp1* mutant, but not Δ*lbp2*, was markedly protected from the loss of viability after severe heat shock as measured by recovery of cell growth (Fig. 5*A*) and viability counts (Fig. 5*B*). Similar results were obtained with Δ*lbp1* in rich YPD media, but in this case, the Δ*lbp2* mutant and, to an even greater extent, the Δ*lbp1*Δ*lbp2* strain showed surprising and dramatic enhancement of survival (Fig. 5*B*). Because of the unexpected heat shock response of the Δ*lbp2* mutants in YPD media, we reexamined the phosphatase activity in strains grown to stationary phase in YPD. In this case, sphingosine-1-^{32}P-phosphatase activity was almost 2-fold lower in the Δ*lbp2* mutant and only slightly reduced in the Δ*lbp1* mutant (Fig. 5*C*). Moreover, Northern blot analysis showed that the *LBP1* message predominated in log phase cells, and the *LBP2* message, which was almost undetectable except when expressed on multicopy vectors, increased upon heat stress (unpublished data). These data suggest that there are two alternatively regulated LCBP phosphatases: *LBP1*, which can regulate the flux of metabolites between ceramide/sphingolipids and LCBP/phospholipids in actively growing cells, and *LBP2*, which plays a major role under stress conditions.

LCBP phosphatase emerges from this study as a key regulator of sphingolipid signaling molecules, a role that has not been previously appreciated. With the exception of one study describing SPP-phosphatase activity in rat liver extracts (14), all prior attention has focused on SPP-lyase as the activity responsible for attenuating the SPP signal. Here, we show that yeast mutants that lack LCBP phosphatase not only have elevated levels of phosphorylated sphingoid bases but also have reduced amounts of ceramide. These results suggest that significant amounts of sphingoid base intermediates cycle through phosphorylation/dephosphorylation reactions, and the activity of the phosphatase is an important regulator of the ultimate fate of the sphingoid bases. Reduced phosphatase activity in Δ*lbp1* results in an increased flux of LCB through phosphorylated forms at the expense of ceramide synthesis; LCBP can then be degraded in a lyase-catalyzed reaction, supplying the phosphoethanolamine required by the Δ*cho1* mutant for phosphatidylethanolamine and phosphatidylcholine synthesis. Disruption of *LBP1* and *LBP2* significantly decreased but did not abolish LCBP-phosphatase activity *in vitro*. Rat liver extracts were found to have multiple SPP-phosphatase activities (14), and purified *Saccharomyces* DGPP phosphatase preferred DGPP as substrate but was inhibited by SPP and ceramide 1-phosphate, which suggest that these lipids might also serve as substrates for DGPP phosphatase (30). Thus, other lipid phosphatases may also play a role in regulating cellular LCBP levels. Likewise, the LCBP phosphatases may have activity against other lipid substrates, although our *in vitro* competition studies suggest a high level of specificity for phosphorylated sphingoid bases.

The occurrence of elevated levels of LCBP and reduced levels of ceramide in *lbp1* mutants did not appear to have dramatic consequences to yeast cells growing under normal conditions. However, we expect that many metabolic processes are probably altered in these cells, based on observations obtained with fumonisin B$_1$, a ceramide synthase inhibitor (33). In addition to lowering ceramide levels, fumonisin B$_1$ causes accumulation of sphingoid bases and their phosphorylated derivatives (34), giving rise to profound and diverse effects in mammalian cells (5). In *Saccharomyces*, fumonisin B$_1$ decreased the synthesis of many phospholipids, an effect that was attributed to the regulatory activity on key lipid biosyn-

thetic enzymes by the accumulating sphingoid bases, and may also have been due, in part, to LCBP accumulation (35).

In contrast to the minimal effects on growth rates under normal conditions, mutants lacking LCBP phosphatase had dramatically enhanced survival with severe heat shock. Moreover, we detected *LBP2* activity only under stress conditions, suggesting a critical role for sphingolipid intermediates in stress physiology. These effects in yeast are reminiscent of mammalian cells, in which ceramide levels increase in response to stress, and ceramide and SPP are important regulatory components of stress responses (1–5, 7, 32). Thus, the balance between the levels of ceramide that favor death to levels of LCBP that inhibit death also is critical for the survival of yeast. We propose that the ceramide/LCBP rheostat is an evolutionarily conserved regulatory mechanism.

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