EUKARYOTIC CELL, June 2011, p. 776–781 Vol. 10, No. 6
1535-9778/11/$12.00 doi:10.1128/EC.05040-11

Involvement of the *Saccharomyces cerevisiae* Hydrolase Ldh1p in Lipid Homeostasis▽

Mykhaylo O. Debelyy,[1] Sven Thoms,[1]† Melanie Connerth,[2] Günther Daum,[2] and Ralf Erdmann[1]*

Abteilung für Systembiochemie, Institut für Physiologische Chemie, Medizinische Fakultät der Ruhr-Universität Bochum, D-44780 Bochum, Germany,[1] and Institute of Biochemistry, Graz University of Technology, A-8010 Graz, Austria[2]

Received 18 March 2011/Accepted 30 March 2011

Here, we report the functional characterization of the newly identified lipid droplet hydrolase Ldh1p. Recombinant Ldh1p exhibits esterase and triacylglycerol lipase activities. Mutation of the serine in the hydrolase/lipase motif GXSXG completely abolished esterase activity. Ldh1p is required for the maintenance of a steady-state level of the nonpolar and polar lipids of lipid droplets. A characteristic feature of the *Saccharomyces cerevisiae* Δ*ldh1* strain is the appearance of giant lipid droplets and an excessive accumulation of nonpolar lipids and phospholipids upon growth on medium containing oleic acid as a sole carbon source. Ldh1p is thought to play a role in maintaining the lipid homeostasis in yeast by regulating both phospholipid and nonpolar lipid levels.

Lipid droplets (LDs) are remarkable dynamic subcellular organelles of globular shape with a size range from 20 to 100 μm, depending on the cell type (9, 12, 15, 31). LDs are depots of neutral lipids with a complex biology that exist in virtually any kind of cell, ranging from bacteria to yeasts, plants, and higher mammals (3, 13, 15). In many cells, LDs occupy a considerable portion of the cell volume and weight (35). As the major intracellular storage organelles, LDs were first described in the works of R. Altmann and E. B. Wilson in the 19th century (1, 37). In contrast to the vesicular organelles, which have the aqueous content enclosed by a phospholipid bilayer membrane (12, 13), mature LDs have a unique physical structure: they have a neutral lipid core consisting of triacylglycerols (TG) and sterol esters (SE) surrounded by a phospholipid monolayer (3, 24, 38) that contains numerous peripheral or embedded proteins (26, 33). TG as well as SE play crucial roles for the cell: TG is the main energy store, and both TG and SE are depots of membrane lipid components (35). LDs can tightly regulate the level of intracellular free cholesterol by hydrolyzing sterol ester (26). The LD core also contains other endogenous neutral lipids, like monoacylglycerol, diacylglycerol, free cholesterol, and retinol ester, and xenobiotic hydrophobic compounds, such as polycyclic aromatic hydrocarbons (15, 17, 29, 32, 33). A number of proteins are specifically targeted to the LD surface (18), where they can regulate LD dynamics and the turnover of stored lipids (24). Lipid-metabolizing enzymes, including hydrolases and lipases, are the major class of LD enzymes (9). LDs play crucial roles in cellular energy homeostasis and lipid metabolism (35). LDs can provide a rapidly mobilized lipid source for many important biological processes. Neutral lipids may be mobilized for the generation of energy by β-oxidation or for the synthesis of membrane lipids and signaling molecules (9). It has been shown that nearly all cell types have the ability to generate LDs in response to elevated fatty acid levels and to subsequently metabolize and disperse these LDs when conditions are reversed (26), thereby providing an emergency energy pool for cell survival (3). Due to their unique architecture, LDs can protect cells from the effects of potentially toxic lipid species, such as unesterified lipids (23, 24) or toxic free fatty acids (3), by depositing them inside the LD's core. In addition to this lipid-scavenging function, LDs can transiently store certain proteins, which may be released or degraded at later time points (9, 13, 14, 36).

Here, we report the functional characterization of the newly identified LD hydrolase Ldh1p (34a). We demonstrate that recombinant Ldh1p exerts esterase and triacylglycerol lipase activities. The enzyme activity was abolished upon mutation of the conserved GXSXG-type lipase motif of the protein. The *Saccharomyces cerevisiae* Δ*ldh1* strain is characterized by the appearance of giant LDs and the accumulation of nonpolar lipids and phospholipids in LDs, indicative of a role of Ldh1p in maintaining lipid homeostasis.

MATERIALS AND METHODS

Strains and plasmids. *S. cerevisiae* strains BY4742, BY4742 Δ*ybr204c*, BY4742 Δ*yor084w*, BY4742 Δ*ybr204c* Δ*yor084w*, BY4742 ERG6-RFP, and BY4742 ERG6-RFP Δ*ybr204c* are described in reference 34a. DNA plasmids pUG35-LDH1 (Ldh1p-GFP) and pUG36-LDH1 (GFP-Ldh1p) are described in reference 34a. Yeast media have been described previously (10, 11). pUG35-LDH1-M1 [Ldh1p-(S177A)-GFP] and pUG36-LDH1-M1 [GFP-Ldh1p-(S177A)] were cloned from pUG35-LDH1 and pUG36-LDH1 using a QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies) (primers RE2400 [5′-ATAGTGCTTGTAGGGCATGCTATGGGTTGTTTTCTGGCA-3′] and RE2401 [5′-TGCCAGAAAACAACCCATAGCATGCCCTACAAGCACTAT-3′]). pET21d-LDH1 was constructed by introducing PCR-amplified YBR204c (primers OST248 [5′-GCGAATTCCATATGAATATGGCAGAACGTGCAG-3′] and OST217 [5′-GCTGCGGCCGCCAATTTGGAATTATCAATCACC-3′]) into NdeI and NotI sites of pET21b (EMD Chemicals). pET21d-LDH1-M1 [Ldh1p-(S177A)-His_6] was cloned from pET21d-LDH1 using the QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies) (primers RE2400 [5′-ATAGTGCTTGTAGGGCATGCTATGGGTTGTTTTCTGGCA-3′] and RE2401 [5′-TGCCAGAAAA

* Corresponding author. Mailing address: Institut für Physiologische Chemie, Ruhr-Universität Bochum, Universitätsstraße 150, D-44780 Bochum, Germany. Phone: 49 234 322 4943. Fax: 49 234 321 4266. E-mail: Ralf.Erdmann@rub.de.

† Present address: Universitätsmedizin Göttingen, Abteilung für Pädiatrie und pädiatrische Neurologie, Georg-August-Universität Göttingen, D-37099 Göttingen, Germany.

▽ Published ahead of print on 8 April 2011.

CAACCCATAGCATGCCCTACAAGCACTAT-3′]). All constructs were confirmed by DNA sequencing.

Protein expression. Ldh1p was expressed from plasmid pET21b-LDH1 in *Escherichia coli* BL21(DE3). Cells were harvested by centrifugation and diluted in buffer A (1× phosphate-buffered saline [PBS], 300 mM sodium chloride, 1 mM dithiothreitol, 40 mM imidazole) containing a protease inhibitor mixture (8 μM antipain-dihydrochloride, 0.3 μM aprotinin, 1 μM bestatin, 10 μM chymostatin, 5 μM leupeptin, 1.5 μM pepstatin), together with 50 μg/ml lysozyme, 22.5 μg/ml DNase I, and 40 mM imidazole. The cells were sonicated using a 250D Branson (Danbury, CT) Digital Sonifier. After removal of cell debris by centrifugation, the supernatant was clarified by 0.22-μm filtration and loaded on His-Trap columns (GE Healthcare Life Sciences) equilibrated with buffer A. The column was washed in buffer A, and recombinant Ldh1p was eluted by a continuous 40 to 500 mM imidazole gradient. Peak fractions were identified by SDS-PAGE and pooled, and the isolated protein was concentrated with VivaSpin concentrators (30-kDa cutoff; Sartorius). The concentrated Ldh1p was subjected to size exclusion chromatography on an ÄKTA Purifier FPLC System with Superdex 200 (GE Healthcare Life Sciences). Peak fractions of Ldh1p were identified by SDS-PAGE and pooled, and the isolated protein was concentrated with VivaSpin (30-kDa cutoff; Sartorius).

Enzyme assays. Esterase activity was determined with *p*-nitrophenyl butyrate (PNB) (Sigma) in PBS (pH 7.4) in a total volume of 200 μl at 37°C. Free *p*-nitrophenol was determined at 410 nm in 96-well plates. Michaelis-Menten kinetics was analyzed using GraphPad Prism 5 (GraphPad Software). Triacylglycerol lipase (TGL) activity was determined using 1,2-dioleoyl-3-pyrenedecanol-rac-glycerol (DPG) (Marker Gene) in 0.1 M glycine, 19 mM sodium deoxycholate, pH 9.5, in a total volume of 200 μl at 37°C. Hydrolysis of DPG was followed in 96-well plates at 460 nm with 360-nm excitation in a Sirius HT fluorescence plate reader (MWG Biotech). The TGL activity of Ldh1p toward DPG was compared with the TGL activity of *Candida rugosa* triacylglycerol lipase (Lipase AT30 Amano; 1,440 units/mg; Sigma) as a control. We also adapted a specific and sensitive TGL assay originally developed for the measurement of bacterial TGLs (22). The TGL activity of Ldh1p on rhodamine B agar plates was determined by using agar plates containing trioleoylglycerol and rhodamine B. The agar (1% [wt/vol]) was dissolved in PBS, adjusted to pH 7.4, autoclaved, and cooled to 60°C. Then, trioleoylglycerol (2.5% [wt/vol]) and rhodamine B (0.001% [wt/vol]) were added to the agar medium with vigorous stirring for 1 min. The medium was kept for 10 min at 60°C to reduce foaming, and 20 ml of medium was poured into plastic petri dishes. To detect triacylglycerol lipase activity, holes with a diameter of 6 mm were punched into the agar and filled with 200 μl protein solution. Ldh1p and *C. rugosa* lipase (CRL) were diluted in PBS (pH 7.4). The plates were incubated for 48 h at 30°C. After 48 h, the plates began to show an orange fluorescence visible under UV light (350 nm).

Lipid extraction and TLC. The lipids were extracted by the method of Bligh and Dyer (4). The organic layer was washed three times with 1 M KCl, and the solvent was removed by evaporation in a vacuum. The lipids were dissolved in a small volume of chloroform and separated on thin-layer chromatography (TLC) plates (TLC Silica gel 60 F254; 20 by 20 cm; Merck) using chloroform-methanol-water (65:25:4 [vol/vol/vol]) as the developing solvent. Lipid classes were visualized with iodine vapor and identified according to TLC standard 18-5A (Nu-Chek Prep, Elysian, MN).

Electron microscopy. The ultrastructure of yeast cells was studied with oleate-induced cells that had been fixed with 1.5% $KMnO_4$ and processed as described previously (10).

Miscellaneous. Oil Red O staining, image acquisition, and the isolation of LDs are described in reference 34a. LD purification for lipid extraction was performed as described previously (8, 27). The weight of LDs was estimated gravimetrically in 1.5-ml reaction tubes (Eppendorf).

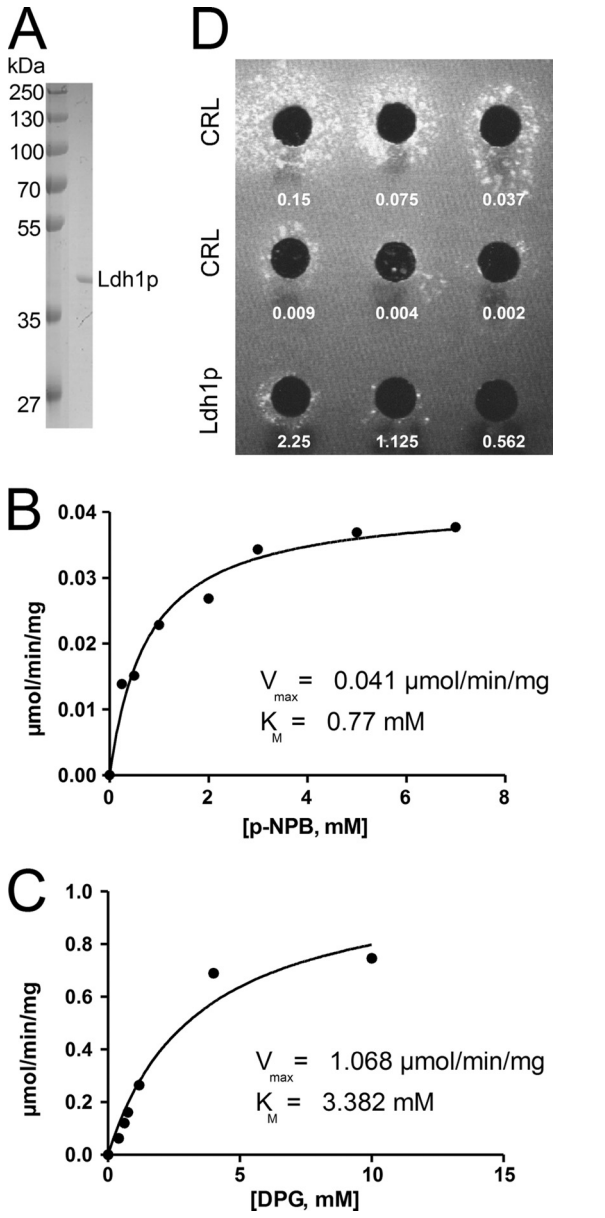

FIG. 1. Protein expression, purification, and enzymatic activity of Ldh1p. (A) Ldh1p was expressed as a fusion protein with a hexahistidine tag and purified by affinity chromatography. (B) Esterase activity of Ldh1p toward PNB. K_m and V_{max} values were calculated using Michaelis-Menten approximations. (C) TGL activity of Ldh1p toward DPG. (D) Purified Ldh1p and CRL were incubated on plates containing 2.5% trioleoylglycerol and 0.001% rhodamine B and, after 48 h, imaged at 350 nm. The numbers indicate the concentrations in mg/ml. In total, 200 μl was loaded per agar slot. Hydrolysis of trioleoylglycerol was identified by fluorescent halos.

RESULTS

Enzymatic activity of Ldh1p. Characteristic GXSXG motifs and similarities to α/β-hydrolases in the predicted protein sequences of Ldh1p suggest that the protein is an esterase or lipase (5, 28, 34). Indeed, Ldh1p was identified as a serine hydrolase by computational and chemical proteomics methods (2). We expressed Ldh1p as hexahistidine-tagged fusions in *E. coli* (Fig. 1A) and tested the isolated protein for esterase activity using PNB as a substrate. We found Ldh1p to be an active esterase hydrolyzing the model substrate PNB with a K_m of 0.77 mM and a V_{max} of 0.041 μmol/min/mg (Fig. 1B).

Phospholipase A, C, and D activities were not detected (data not shown). For the analysis of phospholipase A activity, we used the fluorogenic phospholipase A substrate bis-BODIPY FL C_{11}-PC (B7701; Invitrogen) [1,2-bis-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-undecanoyl)-sn-glycero-3-phosphocholine]. For analysis of phospholipase C

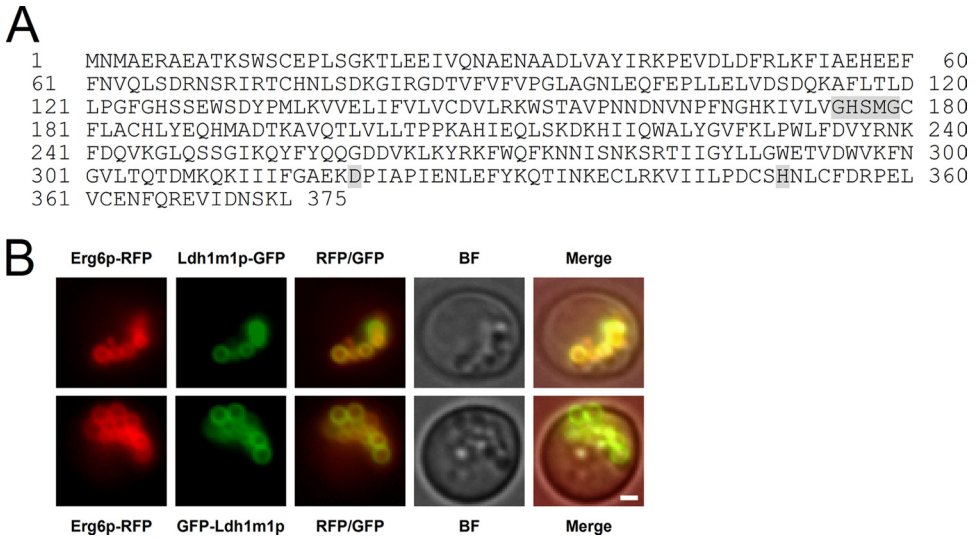

FIG. 2. Hydrolase activity is not required for Ldh1p targeting to LDs. (A) Ldh1p is a hydrolytically active serine hydrolase with a classical catalytic triad containing a conserved serine (GXSXG motif), histidine, and aspartate (grey shading). (B) GFP-Ldh1m1p and Ldh1m1p-GFP were coexpressed in a yeast strain with genomically tagged Erg6p-RFP. Ldh1m1p colocalizes with the LD marker protein Erg6p [δ(24)-sterol methyl transferase]. Ldh1p containing a mutation of the active site (S177A) still localizes to LDs, indicating that the lipid targeting is independent of its catalytic activity. Bar, 1 μm. BF, bright field.

activity, we used the Amplex Red Phosphatidylcholine-Specific Phospholipase C Assay Kit (A12218; Invitrogen). Phospholipase D activity was assayed with the Amplex Red Phospholipase D Assay Kit (A12219; Invitrogen).

Next, we assayed TGL activity using DPG as a substrate (Fig. 1C). One of the acyl residues of DPG contains the eximer-forming pyrene decanoic acid. Upon hydrolytic cleavage, the released pyrene decanoic acid leads to a decrease in eximer fluorescence. We found Ldh1p to be an active triacylglycerol lipase hydrolyzing the model substrate DPG with a K_m of 3.3 mM and a V_{max} of 1 μmol/min/mg (Fig. 1C). TGL activity was also confirmed by an assay with fluorescein dilaurate as the substrate (not shown). We also adapted a specific and sensitive TGL assay originally developed for the measurement of bacterial TGLs (22). This assay is based on the hydrolysis of trioleoylglycerol and the formation of orange fluorescent rhodamine B halos. The results shown in Fig. 1D revealed that Ldh1p exerts a weak TGL activity. In summary, the purified Ldh1p exerts esterase and TG lipase activities.

Mutational analysis of the GXSXG-type lipase motif. The characteristic GXSXG motif of α/β-hydrolases is present in Ldh1p and is thought to contribute to the active site of the enzyme (Fig. 2A). To test this experimentally, we introduced a point mutation into the putative active site of Ldh1p (S177A) and analyzed the mutated protein for esterase activity. Replacement of serine with alanine in the hydrolase/lipase motif of Ldh1p completely abolished hydrolase activity. The mutated protein (Ldh1m1p) still localized to LDs, suggesting that the catalytic activity is not required for its topogenesis (Fig. 2B).

The Δ*ldh1* mutant is characterized by the accumulation of lipids. Ldh1p has been shown to be predominantly localized to LDs (34a). To characterize the function of Ldh1p in more detail, we investigated whether the enzyme is involved in the biogenesis of LDs. To this end, LDs were isolated from oleic acid-induced wild-type and Δ*ldh1* mutant cells and appeared as a thick layer on top of a gradient of the mutant (Fig. 3A). The total weight of LDs was drastically increased in the Δ*ldh1* yeast strain in comparison to the wild type (Fig. 3B). These data were corroborated by TLC separation of extracted lipids from

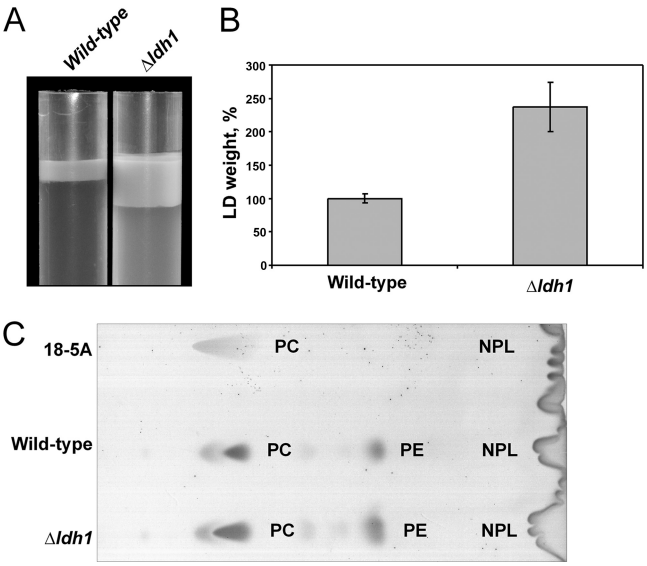

FIG. 3. The Δ*ldh1* yeast strain exhibits excessive accumulation of nonpolar and polar lipids in LDs during growth on medium containing oleic acid as a sole carbon source. (A) LDs were isolated from wild-type and Δ*ldh1* mutant cells and appeared as a thick layer on top of a gradient of the mutant. (B) The total weight of LDs was strongly increased in the Δ*ldh1* yeast strain in comparison to the wild type. The error bars indicate standard deviations. (C) TLC separation of extracted nonpolar and polar lipids from purified LDs, which showed the increase in nonpolar lipids and phospholipids in the Δ*ldh1* yeast strain. PC, phosphatidylcholine; PE, phosphatidylethanolamine; NPL, nonpolar lipids.

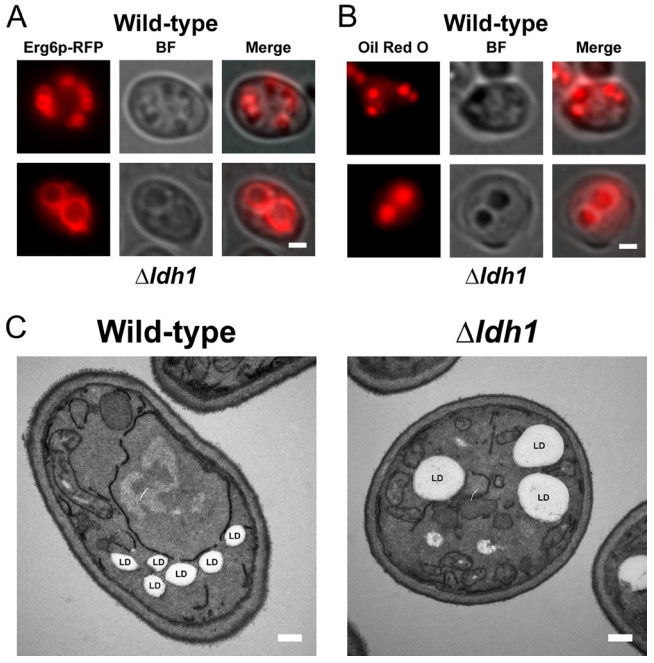

FIG. 4. Giant LDs in the Δ*ldh1* mutant. (A) Comparison of LD morphologies of the wild type (BY4742 Erg6p-RFP) and a deletion strain (BY4742 Δ*ldh1* Erg6p-RFP) by fluorescence microscopy. Bar, 1 μm. (B) Localizations and morphologies of Oil Red O-stained wild-type (BY4742) and deletion strain (BY4742 Δ*ldh1*) LDs. Bar, 1 μm. (C) Absence of *LDH1* leads to the formation of giant LDs, as well to the reduction of the total LD number in a cell. Shown are electron microscopic images of cells: the wild type (BY4742) and a deletion strain (BY4742 Δ*ldh1*). Bars, 1 μm.

purified LDs, which showed the increase in nonpolar lipids and phospholipids in the Δ*ldh1* yeast strain (Fig. 3C).

Giant lipid droplets in Δ*ldh1* mutant cells. To analyze whether the accumulation of lipids in mutant cells lacking the LD protein Ldh1p is accompanied by changes in LD morphology, the LDs of oleic acid-induced wild-type and Δ*ldh1* knockout cells expressing genomically encoded Erg6p-red fluorescent protein (RFP) were visualized by fluorescence microscopy (Fig. 4A), and the LDs of oleic acid-induced wild-type and Δ*ldh1* knockout cells were stained with Oil Red O and inspected by fluorescence microscopy (Fig. 4B). The data demonstrate that LDs can still be formed in the absence of Ldh1p, indicating that Ldh1p *per se* is not required for the formation of LDs. However, the morphological appearance of LDs in Δ*ldh1* mutant cells differed significantly from that in wild-type cells. The LDs of the mutant exhibited brighter fluorescence, indicating the existence of bigger LDs. These data were corroborated by electron microscopic inspection of wild-type and mutant cells, which revealed the presence of giant LDs in the Δ*ldh1* mutant (Fig. 4C).

Esterase activity of Ldh1p is required for lipid homeostasis. The Δ*ldh1* yeast strain exhibits an excessive accumulation of lipids in LDs during growth on medium containing oleic acid as a sole carbon source. To test whether the loss of hydrolase activity of Ldh1p is responsible for the observed phenotype, we tested complementation of the mutant with functional and catalytic dead Ldh1p harboring a substitution of the active-site serine (Ldh1m1p). LDs were isolated from oleic acid-induced wild-type cells, Δ*ldh1* mutant cells, and mutant cells expressing plasmids encoding either wild-type Ldh1p or the mutant Ldh1m1p. LDs appeared as a thick layer on top of the gradient, and comparison of the gradients revealed a thin lipid layer on top of the gradient for the wild type and the Δ*ldh1* mutant complemented with wild-type Ldh1p. A thicker layer, which is typical of the Δ*ldh1* mutant, was monitored for mutant cells that contained the catalytic dead Ldh1p (Fig. 5A). These data were corroborated by determination of the total weight of LDs, which was increased in the Δ*ldh1* strain and remained increased upon expression of the mutant protein (not shown). Accordingly, staining with Oil Red O and inspection of the cells by fluorescence microscopy (Fig. 5B), as well as by electron microscopy (Fig. 5C), revealed that the giant-LD phenotype of the Δ*ldh1* strain could be complemented with wild-type Ldh1p, but not with the catalytic dead mutant Ldh1p. These data demonstrate that functional complementation of the Δ*ldh1* mutant phenotype requires expression of enzymatically active Ldh1p, indicating that the hydrolase activity of the enzyme is required for its function in lipid homeostasis.

DISCUSSION

Ldh1p is a hydrolytically active serine hydrolase with a classical catalytic triad containing a serine (GXSXG motif). A conserved histidine was revealed by profile hidden Markov models (9a), and the aspartate of the probable triad was derived from an alignment with canine gastric triacylglycerol lipase (Fig. 2A). The putative active-site serine of Ldh1p is located next to the regions of highest hydrophobicity, suggesting that Ldh1p is a membrane-active hydrolase. We demonstrated that the hydrolase activity of Ldh1p could be completely abolished by the replacement of the active-site serine by alanine. Fluorescence microscopy analysis indicated that Ldh1p targets to the boundary of the LD monolayer membrane, supporting the idea that Ldh1p is involved in metabolic processes. Taken together, these features characterize Ldh1p as an active LD hydrolase. Mutation of the active site of Ldh1p does not lead to protein mislocalization, indicating that the lipase active site of Ldh1p is not involved in LD targeting.

Cells deficient in Ldh1p are characterized by giant LDs accompanied by the accumulation of nonpolar lipids and phospholipids. Thus, Ldh1p seems to be required for the mobilization of LD-stored lipids, which would also explain the dependency of the Ldh1p function on its hydrolase activity. We speculate that Ldh1p plays a role in maintaining lipid homeostasis by regulating both phospholipid and nonpolar lipid levels. Interestingly, the Δ*ldh1* (Δ*ybr204c*) strain has been reported to exhibit resistance to the lipophilic drug camptothecin (16, 19, 20). Camptothecin is a cytotoxic quinoline alkaloid that inhibits the DNA enzyme topoisomerase I. The resistance to camptothecin might be explained by increased detoxification properties of LDs with an excessive amount of nonpolar lipids, which may serve as a reservoir for hydrophobic toxic molecules (3, 7, 21, 35). Global genomic screening research recently disclosed the transient induction of *LDH1* by growth on oleate medium (30). It was shown that the level of Ldh1p increased within the first 3 h of induction, followed by a decrease within the subsequent 6 h and complete reduction to basal levels within the next 17 h. Such an expression profile might hint at a

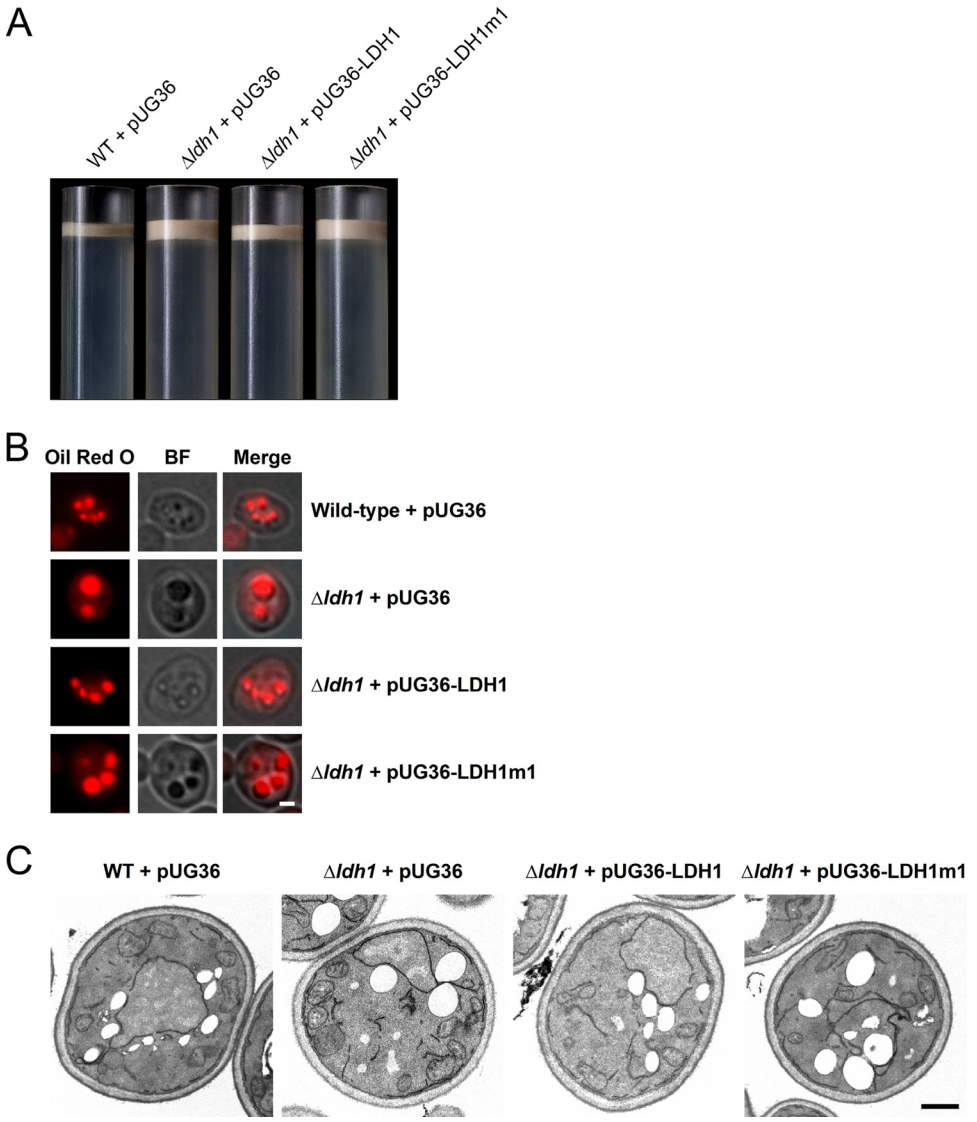

FIG. 5. The esterase activity of Ldh1p is required for lipid homeostasis. The Δ*ldh1* yeast strain exhibits excessive accumulation of lipids in LDs during growth on medium containing oleic acid as a sole carbon source. LDs were isolated from oleic acid-induced wild-type cells and Δ*ldh1* mutant cells expressing plasmids encoding either wild-type Ldh1p or the mutant Ldh1m1p. (A) LDs appeared as a thick layer on top of the gradient, and comparison of the gradients revealed a thin lipid layer on top of the gradient for the wild type (WT) and the Δ*ldh1* mutant complemented with wild-type Ldh1p. A thicker layer, typical of the Δ*ldh1* mutant, was monitored for mutant cells that contained the catalytic dead Ldh1p. (B) Staining with Oil Red O and inspection by fluorescence microscopy revealed that the giant-LD phenotype of the Δ*ldh1* strain could be complemented with wild-type Ldh1p, but not with the catalytic dead mutant Ldh1p. Bar, 1 μm. (C) Electron microscopy revealed that the giant-LD phenotype of the Δ*ldh1* strain could be complemented with wild-type Ldh1p, but not with the catalytic dead mutant Ldh1p. Bar, 1 μm.

regulatory or signaling function instead of direct involvement of the enzyme in lipid metabolism. Interestingly, *LDH1* expression is also induced upon sporulation (6), which is mildly affected in cells deficient in Ldh1p (25). Our data clearly show that Ldh1p *per se* is not required for the biogenesis of LDs, but the severe accumulation of lipids and the corresponding appearance of the giant LDs in Δ*ldh1* mutant cells strongly suggest a role for the enzyme in LD lipid homeostasis.

ACKNOWLEDGMENTS

We thank Elisabeth Becker, Monika Bürger, and Uta Ricken for technical assistance; Robert Rucktäschel for scientific input; and Wolfgang Girzalsky for reading the manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB642 and ER178/4-1).

REFERENCES

1. **Altmann, R.** 1890. Die Elementarorganisem und ihre Beziehungen zu den Zellen. Veit, Leipzig, Germany.
2. **Baxter, S. M., et al.** 2004. Synergistic computational and experimental proteomics approaches for more accurate detection of active serine hydrolases in yeast. Mol. Cell Proteomics **3:**209–225.
3. **Beller, M., K. Thiel, P. J. Thul, and H. Jackle.** 2010. Lipid droplets: a dynamic organelle moves into focus. FEBS Lett. **584:**2176–2182.
4. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37:**911–917.
5. **Brenner, S.** 1988. The molecular evolution of genes and proteins: a tale of two serines. Nature **334:**528–530.
6. **Chu, S., et al.** 1998. The transcriptional program of sporulation in budding yeast. Science **282:**699–705.

7. **Czabany, T., K. Athenstaedt, and G. Daum.** 2007. Synthesis, storage and degradation of neutral lipids in yeast. Biochim. Biophys. Acta **1771:**299–309.
8. **Daum, G., P. C. Bohni, and G. Schatz.** 1982. Import of proteins into mitochondria. Cytochrome b2 and cytochrome c peroxidase are located in the intermembrane space of yeast mitochondria. J. Biol. Chem. **257:**13028–13033.
9. **Digel, M., R. Ehehalt, and J. Fullekrug.** 2010. Lipid droplets lighting up: insights from live microscopy. FEBS Lett. **584:**2168–2175.

9a. **Eddy, S. R.** 1998. Profile hidden Markov models. Bioinformatics **14:**755–763.

10. **Erdmann, R., M. Veenhuis, D. Mertens, and W. H. Kunau.** 1989. Isolation of peroxisome-deficient mutants of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. U. S. A. **86:**5419–5423.
11. **Erdmann, R., et al.** 1991. PAS1, a yeast gene required for peroxisome biogenesis, encodes a member of a novel family of putative ATPases. Cell **64:**499–510.
12. **Farese, R. V., Jr., and T. C. Walther.** 2009. Lipid droplets finally get a little R-E-S-P-E-C-T. Cell **139:**855–860.
13. **Fujimoto, T., Y. Ohsaki, J. Cheng, M. Suzuki, and Y. Shinohara.** 2008. Lipid droplets: a classic organelle with new outfits. Histochem. Cell Biol. **130:**263–279.
14. **Goodman, J. M.** 2008. The gregarious lipid droplet. J. Biol. Chem. **283:**28005–28009.
15. **Guo, Y., K. R. Cordes, R. V. Farese, Jr., and T. C. Walther.** 2009. Lipid droplets at a glance. J. Cell Sci. **122:**749–752.
16. **Hsiang, Y. H., R. Hertzberg, S. Hecht, and L. F. Liu.** 1985. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J. Biol. Chem. **260:**14873–14878.
17. **Imanishi, Y., V. Gerke, and K. Palczewski.** 2004. Retinosomes: new insights into intracellular managing of hydrophobic substances in lipid bodies. J. Cell Biol. **166:**447–453.
18. **Ingelmo-Torres, M., et al.** 2009. Hydrophobic and basic domains target proteins to lipid droplets. Traffic **10:**1785–1801.
19. **Knab, A. M., J. Fertala, and M. A. Bjornsti.** 1993. Mechanisms of camptothecin resistance in yeast DNA topoisomerase I mutants. J. Biol. Chem. **268:**22322–22330.
20. **Knab, A. M., J. Fertala, and M. A. Bjornsti.** 1995. A camptothecin-resistant DNA topoisomerase I mutant exhibits altered sensitivities to other DNA topoisomerase poisons. J. Biol. Chem. **270:**6141–6148.
21. **Kohlwein, S. D.** 2010. Triacylglycerol homeostasis: insights from yeast. J. Biol. Chem. **285:**15663–15667.
22. **Kouker, G., and K. E. Jaeger.** 1987. Specific and sensitive plate assay for bacterial lipases. Appl. Environ. Microbiol. **53:**211–213.
23. **Krahmer, N., Y. Guo, R. V. Farese, Jr., and T. C. Walther.** 2009. SnapShot: lipid droplets. Cell **139:**1024–1024.e1021.
24. **Listenberger, L. L., and D. A. Brown.** 2008. Lipid droplets. Curr. Biol. **18:**R237–R238.
25. **Marston, A. L., W. H. Tham, H. Shah, and A. Amon.** 2004. A genome-wide screen identifies genes required for centromeric cohesion. Science **303:**1367–1370.
26. **Martin, S., and R. G. Parton.** 2006. Lipid droplets: a unified view of a dynamic organelle. Nat. Rev. Mol. Cell Biol. **7:**373–378.
27. **Mlíčková, K., et al.** 2004. Lipid accumulation, lipid body formation, and acyl coenzyme A oxidases of the yeast Yarrowia lipolytica. Appl. Environ. Microbiol. **70:**3918–3924.
28. **Schrag, J. D., and M. Cygler.** 1997. Lipases and alpha/beta hydrolase fold. Methods Enzymol. **284:**85–107.
29. **Skinner, J. R., et al.** 2009. Diacylglycerol enrichment of endoplasmic reticulum or lipid droplets recruits perilipin 3/TIP47 during lipid storage and mobilization. J. Biol. Chem. **284:**30941–30948.
30. **Smith, J. J., et al.** 2002. Transcriptome profiling to identify genes involved in peroxisome assembly and function. J. Cell Biol. **158:**259–271.
31. **Stobart, A. K., S. Stymne, and S. Höglund.** 1986. Safflower microsomes catalyse oil accumulation in vitro: a model system. Planta **169:**33–37.
32. **Tauchi-Sato, K., S. Ozeki, T. Houjou, R. Taguchi, and T. Fujimoto.** 2002. The surface of lipid droplets is a phospholipid monolayer with a unique fatty acid composition. J. Biol. Chem. **277:**44507–44512.
33. **Thiele, C., and J. Spandl.** 2008. Cell biology of lipid droplets. Curr. Opin. Cell Biol. **20:**378–385.
34. **Thoms, S., M. O. Debelyy, K. Nau, H. E. Meyer, and R. Erdmann.** 2008. Lpx1p is a peroxisomal lipase required for normal peroxisome morphology. FEBS J. **275:**504–514.

34a. **Thoms, S., M. O. Debelyy, M. Connerth, G. Daum, and R. Erdmann.** 2011. The putative *Saccharomyces cerevisiae* hydrolase Ldh1p is localized to lipid droplets. Eukaryot. Cell **10:**●.

35. **Walther, T. C., and R. V. Farese, Jr.** 2009. The life of lipid droplets. Biochim. Biophys. Acta **1791:**459–466.
36. **Welte, M. A.** 2007. Proteins under new management: lipid droplets deliver. Trends Cell Biol. **17:**363–369.
37. **Wilson, E.** 1896. The cell in development and inheritance. Macmillan, New York, NY.
38. **Zweytick, D., K. Athenstaedt, and G. Daum.** 2000. Intracellular lipid particles of eukaryotic cells. Biochim. Biophys. Acta **1469:**101–120.