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Expression of a Novel 90-kDa Protein, Lsd90, Involved in the Metabolism of Very Long-chain Fatty Acid-containing Phospholipids in a Mitosis-defective Fission Yeast Mutant*

Kazuaki Yokoyama[1,2,†], Miyuki Nakagawa[1], Masayuki Satoh[1], Shigeaki Saitoh[3,‡], Naoshi Dohmae[4], Ayako Harada[1], Noriko Satoh[1], Ken Karasawa[1], Koji Takio[4,‡], Mitsuhiro Yanagida[2,3] and Keizo Inoue[1]

[1]Faculty of Pharmaceutical Sciences, Teikyo University, Suarashi, Sagamiko-machi, Sagamihara-shi, Kanagawa 229-0195; [2]CREST, Japan Science and Technology Agency, Sanbancho Building, 3, Sanbancho Chiyoda-ku, Tokyo 102-0075; [3]Graduate School of Biostudies, Kyoto University, Yoshida-Honmachi, Sakyo-ku, Kyoto 606-8501; and [4]Biomolecular Characterization Team, RIKEN, Wako-shi, Saitama 351-0198, Japan

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The fission yeast *lsd1/fas2* strain carries a temperature-sensitive mutation of the fatty-acid-synthase α-subunit, exhibiting an aberrant mitosis *lsd* phenotype, with accumulation of very-long-chain fatty-acid-containing phospholipid (VLCFA-PL). A novel 90-kDa protein, Lsd90 (SPBC16E9.16c), was found to be newly expressed in small particle-like structures in *lsd1/fas2* cells under restrictive conditions. Two mismatches leading to a double frame shift were found between the sequences of the *lsd90*$^+$ gene registered in the genomic database and the sequences determined experimentally at the amino acid, cDNA and genomic DNA levels. Unexpectedly, overexpression and disruption of the *lsd90*$^+$ gene in either *lsd1/fas2* or wild-type cells did not affect either cell growth or expression of the *lsd* phenotype. The amounts of VLCFA-PL that accumulated in *lsd90*-overexpressing *lsd1/fas2* cells were significantly lower than those in *lsd1/fas2* cells, suggesting the involvement of Lsd90 in the metabolism of VLCFA-PL.

Key words: fatty acid synthase mutant *lsd1/fas2*, Lsd90 (SPBC16E9.16c), proteome analysis, two-dimensional electrophoresis, very-long-chain fatty-acid-containing phospholipids.

Abbreviations: EMM, Edinburgh minimal medium; 2ME, 2-mercaptoethanol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PVDF, polyvinylidene difluoride; VLCFA-PLs, very-long-chain fatty-acid-containing phospholipids; YPD, yeast extract peptone dextrose.

Recently, various mutations of the genes responsible for fatty acid synthesis have been reported to bring about morphological defects in yeast cells (*1–5*). Mutations of either the *lsd1/fas2* gene encoding the fatty-acid-synthase α-subunit or acetyl-CoA carboxylase in a fission yeast, *Schizosaccharomyces pombe*, caused lethal morphological defects, *i.e.* expression of the *lsd* phenotype (a difference in daughter nuclear size and formation of a very thick septum, both of which were observed during mitosis), in a temperature-sensitive manner (*1*, *2*). It was also reported that mutations of fatty-acid desaturase or acetyl-CoA carboxylase in the budding yeast *Saccharomyces cerevisiae* brought about a defect in segregation of the mitochondria into the daughter cells and structural abnormality of the nuclear pores and vacuoles (*2–5*).

We have reported that three independent clones (*lsd1/fas2-H518*, *lsd1/fas2-H201* and *lsd1/fas2-H265*) expressing the *lsd* phenotype cultured at a restrictive temperature had a different point mutation in the same gene, *lsd1/fas2*, and that all *lsd1/fas2* strains showed temperature-dependent accumulation of VLCFA-PLs, which are derivatives of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Both of these contained triacontanate, a saturated fatty-acyl residue with 30 carbon atoms in a straight chain, at the *sn*−1 position of the glycerol backbone (C30:0-C18:1-PC and C30:0-C18:1-PE, respectively) (*6*).

The machinery responsible for the expression of this phenotype has not been elucidated. We therefore tried to identify the factor(s) responsible for induction of the *lsd* phenotype by monitoring proteins showing altered levels of expression or post-translational modification in response to induction of the phenotype.

Proteome analysis using two-dimensional polyacrylamide gel electrophoresis and/or mass spectrometry of

*The nucleotide sequence of the *lsd90*$^+$ gene is available in the DDBJ/EMBL/GENBANK database with the accession number AB012755.

†To whom correspondence should be addressed. Tel: +042 685 3731, Fax: +042 685 3731,
E-mail: yokoyama@pharm.teikyo-u.ac.jp
‡Present addresses: Division of Cell Biology, Institute of Life Science, Kurume University, 1-1 Hyakunen-kohen, Kurume, Fukuoka 839-0864, Japan; Biometal Science Laboratory, RIKEN SPring-8 Center, Harima Institute, 1-1-1, Kouto, Sayo, Hyogo 679-5148 Japan

protease-digested peptide fragments of proteins from cells and tissues are widely accepted as effective tools for proteomic analysis, allowing the detection of not only changes in expression, but also any post-translational modifications, of the proteins (*7*). In the present study, we compared the proteome of both wild-type and *lsd1/fas2* mutant cells and found that at a restrictive temperature, expression of two novel proteins as well as catalase was induced in the mutant strains.

MATERIALS AND METHODS

Strains and Media—The haploid *S. pombe* strains including the wild-type (*HM123: h- leu1*), *lsd1/fas2-H518*, *lsd1/fas2-H201* and *lsd1/fas2-H265* (*h- leu1 lsd1*) were cultured at a permissive temperature of 26°C in rich yeast extract peptone dextrose (YPD) medium (*1*, *6*). All strains used in this study express a defect of the *leu1* gene, which can be compensated for by culturing in YPD medium or by transformation with a plasmid bearing the budding yeast *LEU2* gene. The plasmid-transformed cells were maintained in Edinburgh minimal medium 2 (EMM2), as described previously (*1*, *6*). YPD medium supplemented with 0.03% (w/v) palmitic acid also contained 1% (w/v) Tween-20 and 2% (w/v) agar. The *lsd* phenotype was induced by culturing at a restrictive temperature of 36°C for 4 h in YPD medium and for 6 h in EMM2 (*1*, *6*).

Proteome Analysis of lsd1/fas2 Strains in Response to Induction of the lsd Phenotype—Both wild-type and *lsd1/fas2* mutant cells were cultured at a restrictive temperature of 36°C for 4 h, harvested, washed and homogenized with glass beads. Debris from the cell walls was removed by centrifugation at 150*g* for 2 min. Two-dimensional polyacrylamide gel electrophoresis of total protein was performed under denaturing conditions according to a modification of the method described by O'Farrell (*8*). Briefly, the total cell homogenate, corresponding to 5×10^7 cells (~0.4 mg protein), was dissolved in 57% (w/v) urea, 2% (w/v) NP-40, 5% (v/v) 2-mercaptoethanol (2ME), 2% (final, w/v) ampholine (pH 3.5–9.5 preblend, Amersham Pharmacia Biotech, Uppsala, Sweden) and loaded onto an isoelectric focusing gel [3 mm i.d. × 12 cm, 48% (w/v) urea, 2% (w/v) NP-40, 1.6% (w/v, final) ampholine (pH 3.5–9.5 preblend) plus 0.4% (w/v, final) pharmalyte (pH 8.0–10.5, Amersham Pharmacia Biotech)] as a mixture of ampholyte and polyacrylamide (3.48% T, 5.4% C), and placed into a first-dimension electrophoresis system (model NA-1313, Nihon Eido, Tokyo, Japan). The gel was conditioned at 200 V for 15 min, at 300 V for 15 min and at 400 V for 30 min before loading. Isoelectric focusing was performed with 10 mM phosphoric acid in the upper chamber as an anode and 25 mM NaOH in the lower chamber as a cathode at 400 V for 16 h and 800 V for 1 h. The pH values at either end of the gel were 5.2 and 8.0, and the constructed pH gradient ranged from pH 5.2 to pH 7.3. The tube gel was then removed, equilibrated with a mixture comprising 62 mM Tris–HCl, 80 mM sodium dodecyl sulphate (SDS), 10% (v/v) glycerol and 5% 2ME (pH 6.8), and subjected to a second-dimension electrophoresis, which was performed with 12% polyacrylamide gel containing SDS, and the resulting gel was stained with Coomassie brilliant blue.

Amino Acid Sequence—The array of 90-kDa proteins separated in a two-dimensional gel was transferred onto a polyvinylidene difluoride (PVDF, PE Applied Biosystems, Foster City, CA, USA) membrane in a buffer containing 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid-NaOH (pH 11.0), 10% methanol (by volume) and 0.005% SDS (w/v) (*9*), and the individual spot was subjected to N-terminal amino acid sequence analysis using a protein sequencer Model 477A (PE Applied Biosystems). To determine the internal amino acid sequence, the protein spots of 90, 58 and 43 kDa were excised separately from Coomassie-stained gels and subjected to in-gel digestion with *Achromobacter* protease I (a gift from Dr Masaki of Ibaraki University) in 100 mM Tris–HCl (pH 9.0), 2 mM ethylenediaminetetraacetate (EDTA) and 0.1% (w/v) SDS. The resulting peptide fragments were extracted and separated by tandem columns of DEAE-5PW (2 mm i.d. × 20 mm, Tosoh, Japan) and Mightysil RP-18 (2 mm i.d. × 50 mm, Kanto Chemical, Japan). Collected fractions were subjected to matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry on a REFLEX mass spectrometer (Bruker Daltonics) with α-cyano-4-hydroxy cinnamic acid (Aldrich) as a matrix and automated Edman degradation (*10*).

Determination of Nucleotide Sequence, Real-time PCR and Overexpression and Disruption of lsd90$^+$—Total RNA was extracted from wild-type cells that had been disrupted by glass beads in a lysis buffer, using an RNeasy Kit (Qiagen, Hilden, Germany). The cDNA of *lsd90*$^+$ was amplified as two overlapping fragments from 5,903 to 7,930 and from 6,898 to the poly(A) tail by reverse transcriptase–polymerase chain reaction (PCR). The base numbers are represented as those of the reverse-complement sequence of Acc. No. Z99759, DDBJ release 33. The PCR products were directly sequenced for both directions using an automated DNA sequencer model 373S with a dye terminator cycle sequencing kit (PE Applied Biosystems).

The genomic DNA of the wild-type cells was extracted with a QIAamp Tissue Kit (Qiagen) and employed as a template for PCR using Ex-*Taq* DNA polymerase (Takara, Shiga, Japan). The entire coding region of *lsd90*$^+$, from 6,064 to 8,334, was amplified and ligated into pREP81, an expression vector for fission yeast derived from pREP1 bearing autonomously replicating sequence 1, budding yeast *LEU2* and the *nmt1* promoter, which is inducible by removal of thiamine (*11*) with a modification in the TATA box of the *nmt1* promotor. The resulting plasmid pLSD90 was transformed into wild-type cells and *lsd1/fas2* mutant cells by the lithium method (*12*).

The expression level of *lsd90*$^+$ mRNA was quantified by the real-time PCR method in comparison with the expression level of *actin* (Acc. No. NM_001021513) as a control gene (*13*) using a real-time PCR system model 7,500 (Applied Biosystems). The cDNA synthesized from total RNA extracted as described above using PrimeScript RTase (Takara) was used as the template.

Disruption of *lsd90*$^+$ was performed using the following strategy. Two genomic DNA fragments containing the

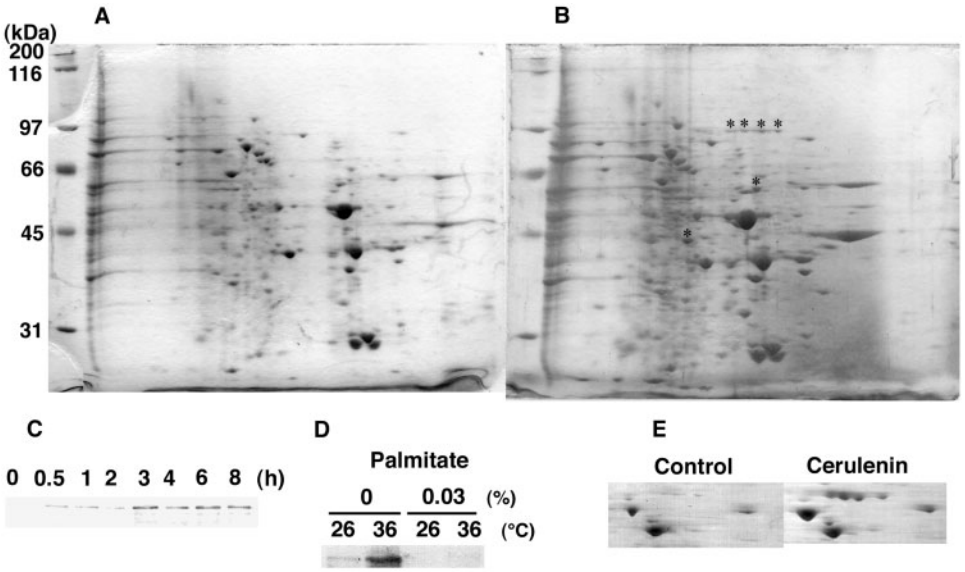

Fig. 1. **Proteome analysis of *lsd1/fas2* mutants.** Cells were lysed using glass beads and then subjected to two-dimensional polyacrylamide gel electrophoresis under first-dimension denaturing conditions with a tube gel containing 48% (w/v) urea, 2% (w/v) NP-40, 1.6% (final) ampholine 3.5–9.5 (preblend) plus 0.4% pharmalyte 8.0–10.5 as a mixture of ampholyte and polyacrylamide (3.48% T, 5.4% C). The tube gel was then applied to a SDS–PAGE gel containing 12% polyacrylamide; (A) wild-type cells cultured at 36°C for 4 h, (B) *lsd1/fas2-H518* cells cultured at a restrictive temperature of 36°C for 4 h. The gels were stained with Coomassie brilliant blue, and asterisks indicate the spots that represent the level of expression. (C) Lysate of *lsd1/fas2-H518* cells cultured for the indicated period at 36°C, and (D) *lsd1/fas2-H518* cells rescued in the presence of 0.03% palmitic acid plus 1% Triton X-100, cultured at 36°C overnight, were subjected to SDS–PAGE and blotted with affinity-purified anti-Lsd90. (E) wild-type cells cultured at 36°C for 4h in the presence of 10 μg/ml cerulenin.

5′ and 3′ flanking regions with ~100 bp of the coding region of the *lsd90*$^+$ gene, from 5,009 to 6,177 and from 8,196 to 9,339, were amplified by PCR, and the *ura4*+ gene was ligated in the middle of both fragments. The construct was cut out with XhoI and EcoRI, located at 5,098–5,103 and 9,316–9,321, and transformed into diploid cells to replace the *lsd90*$^+$ gene. The resulting heterozygotes were selected by genomic PCR and allowed to sporulate. The spores were then dissected and germinated.

Immunostaining and Immunoblotting—Full-length genomic DNA of *lsd90*$^+$ was amplified and ligated to pPROEX1 (Gibco BRL) to fuse with the 6× His-tag at the N-terminal. The gene product expressed in *Escherichia coli* was purified using Ni–NTA agarose (Qiagen), and the resulting product was used to immunize rabbits. The antibody for Lsd90 was affinity purified from the rabbit serum and used for immunostaining as described previously (*14*).

Phospholipid Analysis—Both wild-type and *lsd1/fas2* mutant cells were cultured at a restrictive temperature of 36°C or at a permissive temperature of 26°C for 4 h in the case of YPD medium and for 6 h in the case of EMM2 medium, and then incubated with 120 nCi/ml [1-^{14}C]acetic acid sodium salt (Amersham Pharmacia Biotech, Uppsala, Sweden) for 1 h before harvesting. The cells were then harvested, washed and homogenized with glass beads. Total lipids were extracted according to Bligh and Dyer (*15*), applied to a corner of a high-performance thin-layer chromatography plate (Merck #5641, 10 × 10 cm) and developed by two-dimensional chromatography with chloroform/methanol/formic acid/water (65/25/8.9/1.1, by volume) and chloroform/methanol/aqueous ammonia/water (50/40/3/7, by volume), as described previously (*6*, *16–18*). The radioactivity of each individual spot was measured by an imaging analyzer BAS1500Mac (Fuji Photo Film).

RESULTS AND DISCUSSION

Induction of Expression of 90, 58 and 43-kDa Proteins Upon Induction of the lsd Phenotype in lsd1/fas2 Strains—The protein expression pattern of both wild-type and *lsd1/fas2* mutant cells cultured at 26°C (a permissive temperature for the mutant cells) and at 36°C (a restrictive temperature for the mutant cells) was analysed using two-dimensional electrophoresis. The protein patterns of wild-type and mutant cells (*lsd1/fas2-H518*) cultured at 26°C were the same as those observed for wild-type cells cultured at 36°C (Fig. 1A). In contrast, an array of 90-kDa proteins with pI 6.4, 6.5, 6.6 and 6.8 was newly expressed in *lsd1/fas2-H518* at 36°C (Fig. 1B). The other mutants (*lsd1/fas2-H201* and *lsd1/fas2-H265*) exhibited the same change in protein expression pattern when the temperature was increased (data not shown).

The four spots of molecular mass 90 kDa were found to be charge isomers of the same protein as described below; this protein will be referred to as Lsd90 throughout this article. The time course of induction of Lsd90 after the temperature shift is shown in Fig. 1C. Expression of Lsd90 paralleled induction of the *lsd* phenotype (*1*). It has been reported that the lethality and *lsd* phenotype of the *lsd1/fas2* strain at 36°C may be compensated for by supplementation of the

culture medium with palmitic acid (*1*, *6*). Palmitic acid supplementation almost completely inhibited the induction of Lsd90 (Fig. 1D). Cerulenin, an inhibitor of fatty acid synthase that has been shown to induce the *lsd* phenotype in wild-type cells (*1*), induced the expression of Lsd90 (Fig. 1E). Induction of Lsd90 was also observed in *cut6*, a temperature-sensitive mutant of acetyl-CoA carboxylase, at 36°C (data not shown). This mutant also exhibited the *lsd* phenotype at the restrictive temperature of 36°C (*1*). These findings show that expression of Lsd90 is highly related to expression of the *lsd* phenotype.

In addition to Lsd90, the level of expression of two proteins, 58 kDa pI 6.6 and 43 kDa pI 6.0, was found to increase in *lsd1*/*fas2-H518*, *-H265* and *-H201* at 36°C (Fig. 1B, and data not shown).

Determination of the Sequence of Lsd90 and the 58 and 43-kDa Proteins—N-terminal amino acid sequencing of four individual 90-kDa spots was performed, and an identical sequence, VGTINESMQNMKIGAKETAQ, was obtained from all of them, suggesting that they are all derived from the same protein. A BLAST search (*19*) of the DDBJ/GenBank/EMBL DNA database (based on DDBJ release 33) revealed that this sequence is identical to that of a fission yeast hypothetical protein SPBC16E9.16c (Acc. No. Z99759). SPBC16E9.16c was predicted to encode a 677-amino-acid protein with two introns ranging from 34,002 to 31,732 (predicted coding regions; 34,002–34,000, 33,805–32,663 and 32,619–31,732) of fission yeast genome cosmid clone SPBC16E9 containing 40,065 bp in the reverse-complement direction. The automated splicing prediction was updated on 9 November 2005, and the open reading frame is now predicted to be 34,002–34,000, 33,805–32,965 and 32,816–31,732 with an intron and a frame-shift encoding a 642-amino-acid protein. For convenience, in this article, the base numbers are represented as those of the reverse-complement sequence of clone SPBC16E9, and the coding region ranged from 6,064 to 8,334 (6,064–6,066, 6,261–7,101, 7,250–8,334). The automated splicing prediction was found to be incorrect, because the determined amino acid sequence corresponded to 6,067–6,126 in terms of base numbers that were actually translated into protein. This reading frame contained several stop codons beginning from 6,505–6,507 which gave only 147 aa and 15.3 kDa, while the protein is 90 kDa, suggesting the existence of intron(s) or frame shift(s). In order to clarify the assignment of this protein as the product of the SPBC16E9.16c gene and to identify the reading frame(s), internal peptide fragments were sequenced and LYTTEPVHPHAVTTNEPTDVSTK (607–629), DTTSTY EGAQxL (112–123), NEAAWK (284–289), xxQDAEL AxExAxxxQxxxE (215–234), AELASYQK (404–411) and NPSGNADIGATSSERTAYAAPQRAAVDTxNVSPPSTQ TG (68–106) were obtained. The numbers in parenthesis indicate the positions of the amino acid residues determined as described below. The first sequence was in the same reading frame as that of the N-terminal 'first frame' but presumably located near to the C-terminus. In contrast, the following four sequences were not in the same reading frame of the N-terminus but in a different reading frame, the 'third frame', and located between the first sequence and the N-terminus. In addition, while most

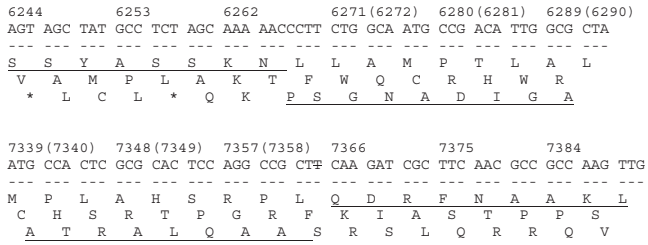

Fig. 2. **Nucleic acid sequence and deduced amino acid sequence of *lsd90*$^+$.** Two 'frame shifts' in the nucleotide sequence of the *lsd90*$^+$ gene in the DNA database are indicated with the corresponding amino acids. The underlining indicates the 'correct' sequence.

of the last sequence, NPSGNADIGATSSERTAYAAPQR AAVDTxNVSPPSTQTG, was encoded by the third frame, the first N residue was encoded by the first frame. Therefore, it was concluded that a cytosine was missing at 6,267–6,268, and in addition, another frame shift was present between 7,297 and 7,882. In order to locate the frame shift, the remaining peptide fragments were screened by mass-spectrometry, and a fragment EAxDRE LDDATxALQAAQDxFNAAK (418–442) was identified by Edman degradation. This was different from the sequence EACDRELDDATRALQAASRSLQRRQVESSEPVstop predicted from the database. In parallel, two overlapping cDNA fragments of this gene were amplified and sequenced. As a result, two mismatches against the database sequence were found; CC in 6,267–6,268 and TT in 7,364–7,365 were CCC and T, respectively, indicating that the *lsd90*$^+$ gene possesses no intron and encodes 756 amino acids. In addition, the genomic DNA sequence of this gene was determined, and again it was identical to the cDNA sequence. Taken together, it was clearly proven that there is no intron in this gene. The correct sequence of *lsd90*$^+$ (Fig. 2) was submitted to the DDBJ/GenBank/EMBL database under Acc. No. AB012755. A search of this database revealed that *lsd90*$^+$ has an ATP/GTP-binding site motif A near the C-terminus and that there is no homologous protein exhibiting more than 60% similarity or 40% identity. SPBC16E9.16c was originally categorized as a pseudogene and described as a protein expressed under stress conditions in the latest version of the database listing (revised 9 November 2005). Although mistakes are unavoidable, submission of any data to the database should be done with extreme caution. Once listed, the information tends to be considered established; for example, proteomic analysis relies heavily on the database and such mistakes may create considerable confusion.

The amino acid sequences of internal peptide fragments of the 58 and 43-kDa proteins were also determined. WHFITNQGTK was obtained from the 58-kDa protein, suggesting that this protein is catalase (Acc. No. D55675 and SPCC757.07c). The 43-kDa protein gave a sequence of TTQGGDYGEDYSQSYPTDTYGS xQK, which is identical to the product of an open reading frame of fission yeast Acc. No. D89241, as well as SPAC23H3.15c of Acc. No. Z99163 and SPAC25H1.01c of AL031825.

mRNA Expression Level, Overexpression and Disruption of Lsd90—Then, Lsd90 was overexpressed

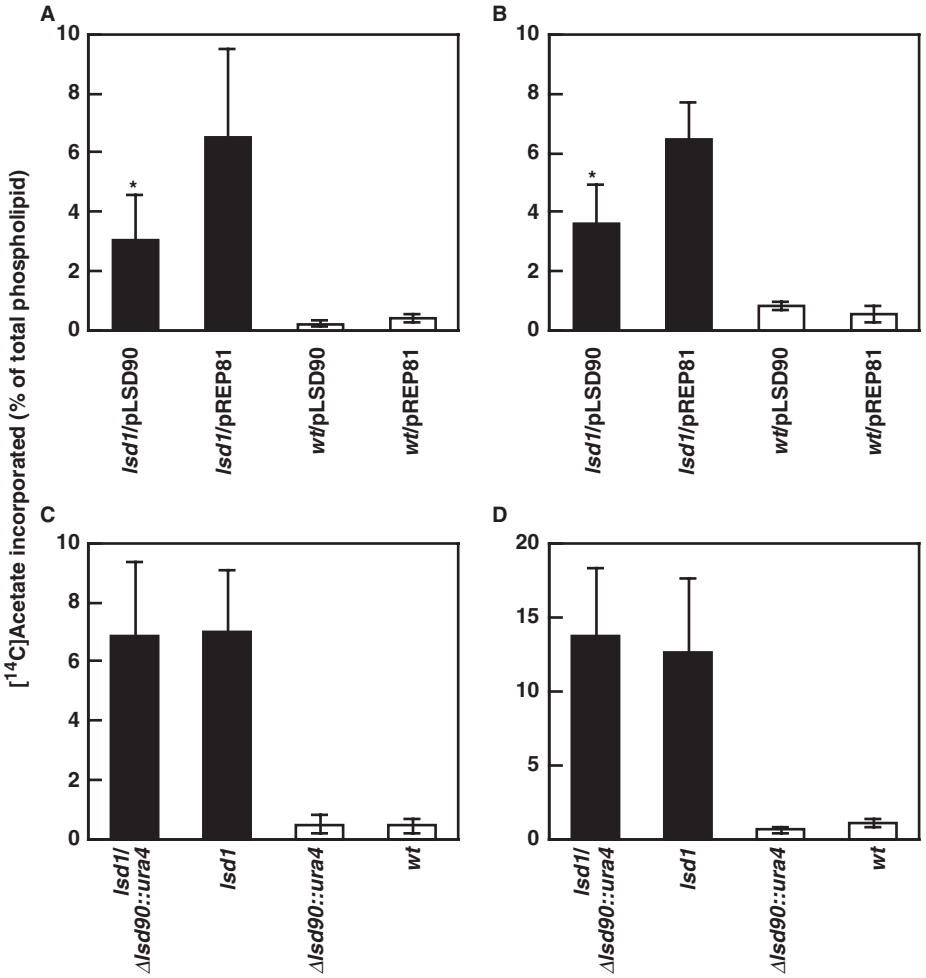

Fig. 3. **Effect of overexpression and disruption of the *lsd90*$^+$ gene on the metabolism of VLFCA-containing phospholipids.** Wild-type and *lsd1/fas2-H518* cells were transformed with pLSD90 or pREP81, cultured in EMM2 without thiamine for 12 h at 26°C, then treated at 36°C for 6 h and labelled with 120 nCi/ml [1-^{14}C]acetate for 1 h before harvesting. Phospholipids were extracted and separated on a thin-layer chromatography plate, and radioactivities were measured by a BAS1500Mac imaging analyzer. C30:0-C18:1-PC (A) and C30:0-C18:1-PE (B). Wild-type, *lsd1/fas2-H518* and double mutant *lsd1/fas2-H518*, *Δlsd90::ura4*$^+$ cells were cultured in YPD, treated at 36°C for 4 h and labelled with 120 nCi/ml [1-^{14}C]acetate for 1 h before harvesting. Phospholipids were analysed as described above. C30:0-C18:1-PC (C) and C30:0-C18:1-PE (D).

in both the wild-type cells and the *lsd1/fas2-H518* strain. The cells that were transformed with pLSD90 were maintained in the presence of 2 μM thiamine. We used pREP81 as the expression vector, as it has a weak mnt1 promoter derived from pREP1 with a normal nmt1 promoter. The normal nmt1 promoter was too strong for use in our study, because the expression level of Lsd90 protein was much higher than the level in the *lsd1/fas2-H518* strain under restrictive conditions with many unnaturally modified spots evident on two-dimensional electrophoresis and the overexpressed protein was mislocalized to vacuole-like structures. Induction of gene expression was performed by removal of thiamine, and confirmed by western blotting (data not shown). Unexpectedly, overexpression of Lsd90 did not affect cell growth appreciably in either the wild-type cells or the *lsd1/fas2* mutants. In addition, transformation of the *lsd90*$^+$ gene had no effect on expression of the *lsd* phenotype in *lsd1/fas2* mutants (data not shown).

Next, the *lsd90*$^+$ gene was disrupted by homologous recombination. All four spores for each tetrad were able to germinate. The disruptant showed no abnormality of either cell growth or cell morphology (data not shown). The disruptant was crossed with *lsd1/fas2-H518*, and a double mutant, *lsd1/fas2-H518*, *Δlsd90::ura4*$^+$ was isolated. Again, the double mutant showed no change in either cell growth or expression of the *lsd* phenotype (data not shown).

Involvement of Lsd90 in the Metabolism of VLCFA-PLs—We have reported that VLCFA-PLs accumulates in *lsd1/fas2* mutants under restrictive conditions (*6*). Because peroxisomes are known to be sites of VLCFA degradation (*20*, *21*), and catalase, a peroxisomal enzyme, was also induced under the same conditions (Fig. 1B), we examined the effect of overexpression of the *lsd90*$^+$ gene on the metabolism of VLCFA-PLs. VLCFA-PLs, C30:0-C18:1-PC and C30:0-C18:1-PE, accumulated in the pLSD90 transformant to a lesser extent than in

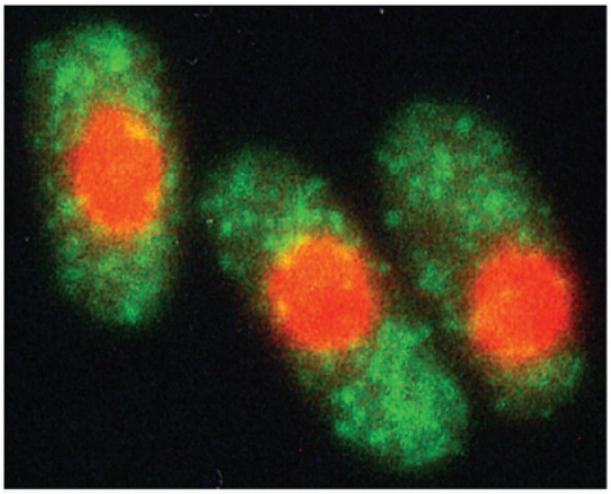

Fig. 4. **Subcellular localization of Lsd90.** *lsd1/fas2-H518* cells were cultured at 36°C for 4 h and stained with affinity-purified anti-Lsd90 (green) and DNA was stained with propidium iodide (red).

lsd1/fas2-H518 transformed with the vehicle vector at a restrictive temperature (Fig. 3A and B). This result suggests that Lsd90 plays an inhibitory role in the accumulation of VLCFA-containing phospholipids. VLCFA metabolism in the Lsd90 disruptant was then examined. Unexpectedly, the amounts of both C30:0-C18:1-PC and C30:0-C18:1-PE that accumulated in the double mutant, *lsd1/fas2-H518*, *Δlsd90::ura4*$^+$, were almost the same as those in *lsd1/fas2-H518* (Fig. 3C and D). The possible mechanisms underlying this action could include inhibition of the synthesis of VLCFA itself, introduction of VLCFA into phospholipids, or stimulation of either the degradation of VLCFA itself or VLCFA-PLs or transport of VLCFA/VLCFA-PL to the site of degradation. The lack of increased accumulation of VLCFA-PL in the double mutant cells could have resulted from compensation of *lsd90*$^+$ gene disruption by other protein(s), and this possibility needs to be investigated further.

Subcellular Localization of Lsd90—In order to analyse the subcellular localization of Lsd90, the cells were stained with an affinity-purified antibody against Lsd90. The Lsd90 induced in *lsdl/fas2* cells at a restrictive temperature was localized in a large number of small particle-like structures, but was not found in the nucleus or the abnormal septum of the cells, indicating that this protein is not a constituent of the *lsd* phenotype structures (Fig. 4). The Lsd90-containing structures appeared to differ from either the mitochondria located along microtubles or the endoplasmic reticulum near the nucleus, but looked similar to peroxisomes found in other organisms, such as various yeasts (*22*) and mammals (*23*). Recently, a systematic ORFome analysis was reported at the URL http://cgl.riken.go.jp/ (*24*). Based on the results of this project, a peroxisomal enzyme catalase fused with yellow fluorescent protein was found to be located in a relatively small number of larger particle-like structures whose locations differed from those of Lsd90, indicating that Lsd90 is not located in peroxisomes. The site of localization and the precise function of Lsd90 remain to be elucidated.

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