Short Communication

Isolation and characterization of a mutant defective in utilization of exogenous phosphatidylethanolamine in *Saccharomyces cerevisiae*

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Phospholipids in biological membranes contain diverse species of fatty acyl chains. Remodeling of fatty acyl chains is supposed to be vital for the maintenance of membrane structure and function. However, the mechanism and physiological roles of phospholipid remodeling are not fully clarified. To elucidate the mechanism of phospholipid remodeling, it is effective to establish a system in which exogenous phospholipids or phospholipid analogs are incorporated into the cells and to monitor their metabolism in the cells.

Phosphatidylethanolamine (PE) is one of the major and essential nonbilayer phospholipids and comprises ~20% of total phospholipids in eukaryotic cells (Daum et al., 1999; Storey et al., 2001). We have previously constructed a *Saccharomyces cerevisiae* strain, TKY12Ga, that is defective in phosphatidylserine (PS) decarboxylation by deletion of *PSD1* and *PSD2*, and its PE synthesis can be controlled by the carbon source in the media through replacement of the promoter of *ECT1*, encoding CTP: phosphoethanolamine cytidyltransferase, a key enzyme in PE synthesis through the Kennedy pathway, with the galactose-inducible and glucose-repressible *GAL1* promoter (Min-Seok et al., 1996; Deng, L., unpubl.). In galactose-con-

taining minimal medium (SG medium), TKY12Ga grew in the presence of ethanolamine. In contrast, its growth was inhibited in the glucose-containing minimal medium (SD medium), even in the presence of ethanolamine. However, when didecanoyl PE (diC10PE) was added to the SD medium, TKY12Ga grew despite the inability to synthesize PE. Since the length of acyl chains in PE in yeast is mainly C14 to C18, it was presumed that the PE with short acyl chains was remodeled to those containing acyl chains of normal length to be a component in the biological membrane. Here, to obtain information on the utilization of exogenous PE in yeast, we isolated mutants defective in utilization of exogenous PE to support growth when the synthesis of PE was suppressed, and analyzed one of them.

We mutagenized TKY12Ga with ethyl methanesulfonate (EMS) and isolated thirty-three mutants defective in growth on SD medium containing diC10PE from approximately 63,000 colonies. Among those, M25, M30, and M33 exhibited clear and stable phenotypes. These mutants did not grow in SD medium containing diC10PE, while they grew normally in SG medium containing ethanolamine at 30°C. In addition, M25 and M30 had the phenotype of temperature sensitivity in SG medium at 37°C (Fig. 2B–D, data not shown). However, the phenotypes of M30 and M33 were caused by multiple mutations, while that of M25 was by a single mutation (data not shown, see below). Therefore, M25 was chosen for further analysis.

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Fig. 1. Uptake of NBD-PE by M25.

TKY12Ga and M25 were pre-cultured in YPG medium for 24 h, then were shifted to YPD medium at a starting $OD_{600}=5 \times 10^{-3}$ and cultured for 48 h to consume ECT1p and/or PE. After that, both strains were transferred to new YPD medium at a starting $OD_{600}=0.3$ and cultured for 3 h, and then approximately 10^7 cells were harvested and incubated with NBD-PE for 30 min. The intensity of fluorescently labeled PE with the cells was analyzed with a FACScan cytometer.

Next, we examined the incorporation of exogenous PE into M25 cells using fluorescently labeled PE (1-myristoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]-hexanoyl}-sn-glycero-3-phosphoethanolamine, NBD-PE). M25 was incubated with NBD-PE for 30 min and washed, and then the intracellular fluorescence was determined with the FACScan cytometer as described previously (Kato et al., 2002). The intracellular fluorescence in M25 was similar to that in TKY12Ga (Fig. 1), suggesting that M25 has defects in the utilization of exogenous PE but not in its uptake.

After confirmation that the mutation in M25 is recessive and that a single mutation caused both the growth defect in PE-containing medium and temperature sensitivity of M25, the *S. cerevisiae* genomic DNA library, which was constructed in low-copy vector, YCp50, was introduced into M25 to isolate a gene that complements the defects of M25 in utilization of exogenous diC10PE. In approximately 6,300 transformants, 52 could grow on SD medium containing diC10PE. Plasmids were isolated from these 52 transformants and reintroduced into M25. Among those, only one transformant reproducibly showed positive growth on SD medium containing diC10PE. This transformant was named M25-66 and the plasmid derived from genomic library in M25-66 was denominated pM25-66.

The nucleotide sequences of approximately 500 bp regions at both ends of the insert in pM25-66 were analyzed and the corresponding sequence was searched in *Saccharomyces* Genome Database ([\[www.stanford.edu/Saccharomyces/\]\(http://www.stanford.edu/Saccharomyces/\)\). The insert of pM25-66 was a 12 kb fragment on chromosome XV from 35975 to 48125, containing six ORFs of *YOR153C*, *FRE7*, *GRE2*, *YOL150C*, *DCP1* and *SPT20*, besides a part of *PEX11* \(Fig. 2A\).](http://genome-</p>
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To identify which ORF in the insert of pM25-66 was responsible for the complementation of growth deficit of M25 in SD medium containing diC10PE, we digested the insert of pM25-66 with restriction endonucleases, cloned the fragments into the low copy vector YCplac33, and obtained plasmids YCpYOL153C, YCpFRE7, YCpGRE2, YCpYOL150, YCpDCP1, and YCpSPT20 (Fig. 2A). The constructed plasmids were introduced into M25 and growth of transformants on SD medium containing diC10PE and on SG medium at 37°C were examined. As shown in Fig. 2B–D, only a transformant containing YCpDCP1 showed positive growth both on SD medium containing diC10PE and on SG medium containing ethanolamine at 37°C.

DCP1 encodes an mRNA decapping enzyme in *S. cerevisiae*, and *dcp1* mutant is defective in 5' to 3' mRNA degradation and has impaired growth (Beelman et al., 1996; Hajji et al., 1999). *DCP1* is essential for growth in certain genetic backgrounds. We next analyzed the nucleotide sequence of *DCP1* region of M25 to identify the mutation site. Total DNA of M25 was prepared and used as a template to amplify the *dcp1* ORF, 260 bp of 5'-non-coding region, and 28 bp of 3'-non-coding region in three independent PCR reactions, and the nucleotide sequences were determined. We found that the *dcp1* gene of M25 had a point mutation from guanine to adenine at nucleotide residue 421 in the *DCP1* ORF. This mutation leads to a substitution of Gly to Arg at amino acid residue 141 within the highly conserved region (Fig. 2E) (She et al., 2004). This result suggests that M25 likely has an aberrant Dcp1p and this aberrant Dcp1p makes the yeast cells defective in utilizing exogenous PE when the synthesis of PE is suppressed.

It is known that mRNA turnover contributes to the control and regulation of gene expression. In *S. cerevisiae*, the major pathway of mRNA decay is the deadenylation-dependent decapping pathway (Kushner, 2004; Meyer et al., 2004). Decapping of mRNA by the combined action of Dcp1p and Dcp2p is a key step in this pathway because it induces degradation of the mRNA, and is also critical in an aspect of mRNA surveillance in which aberrant mRNAs containing non-sense mutant codons are decapped without a require-

Fig. 2. *DCP1* complements the growth defect of M25 in SD medium containing diC10PE.

(A) Inserts of the constructed plasmids, which were obtained by digestion of pM25-66. (B–D) M25 cells containing indicated plasmids were plated on SD medium, and incubated for 2–3 days to consume ECT1p and/or PE in TKY12Ga. Cells were then plated on SG medium (B, D) or SD medium containing 20 mM diC10PE (C). Incubation temperature was 30°C (B, C) or 37°C (D). (E) The mutation site of the *dcp1* gene in M25 strain. Changes in nucleotide and amino acid sequences are shown.

ment for deadenylation and are degraded in a 5' to 3' direction (Cao and Parker, 2003; Hagan et al., 1995; Muhlrud and Parker, 1994). Containing such an important function, the Dcp1p is highly conserved in eukaryotes (She et al., 2004). It was reported that a *DCP1* null mutation caused a defect in 5' to 3' mRNA degradation and stabilized unstable transcripts that were degraded via the deadenylation-dependent decapping pathway (Beelman et al., 1996).

For the engagement of Dcp1 in the metabolism of PE, two possibilities exist. One is that Dcp1 is involved in the degradation process of the transcript(s) which is/are essential for utilization of exogenous PE and

that abnormal Dcp1p in M25 is hyper-activated or constitutively activated because of the mutation and therefore the amount of the essential transcript(s) for the utilization of exogenous PE is decreased in M25. The other is that Dcp1p participates in the turnover of transcripts(s) whose translation product(s) negatively regulates the utilization of exogenous PE and that in M25 the amount of those transcript(s) is increased and therefore the utilization of exogenous PE is suppressed. Analysis of transcript profile in M25, in addition to the effect of the mutation on the Dcp1p activity in M25, may provide valuable information on the metabolism of PE in eukaryotic cells.

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