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Mitochondrially-targeted bacterial phosphatidylethanolamine methyltransferase sustained phosphatidylcholine synthesis of a *Saccharomyces cerevisiae* Δ*pem1* Δ*pem2* double mutant without exogenous choline supply

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ABSTRACT

In eukaryotic cells, phospholipids are synthesized exclusively in the defined organelles specific for each phospholipid species. To explain the reason for this compartmental specificity in the case of phosphatidylcholine (PC) synthesis, we constructed and characterized a *Saccharomyces cerevisiae* strain that lacked endogenous phosphatidylethanolamine (PE) methyltransferases but had a recombinant PE methyltransferase from *Acetobacter aceti*, which was fused with a mitochondrial targeting signal from yeast Pet100p and a 3 × HA epitope tag. This fusion protein, which we named as mitopmt, was determined to be localized to the mitochondria by fluorescence microscopy and subcellular fractionation. The expression of mitopmt suppressed the choline auxotrophy of a double deletion mutant of *PEM1* and *PEM2* (*pem1*Δ*pem2*Δ) and enabled it to synthesize PC in the absence of choline. This growth suppression was observed even if the Kennedy pathway was inactivated by the repression of *PCT1* encoding CTP:phosphocholine cytidylyltransferase, suggesting that PC synthesized in the mitochondria is distributed to other organelles without going through the salvage pathway. The *pem1*Δ*pem2*Δ strain deleted for *PSD1* encoding the mitochondrial phosphatidylserine decarboxylase was able to grow because of the expression of mitopmt in the presence of ethanolamine, implying that PE from other organelles, probably from the ER, was converted to PC by mitopmt. These results suggest that PC could move out of the mitochondria, and raise the possibility that its movement is not under strict directional limitations.

1. Introduction

Organelle membranes in eukaryotic cells are composed of a variety of phospholipid species that are characterized by their hydrophilic head moieties. The membrane lipid compositions of organelles are not uniform throughout cells, and each organelle exhibits distinct phospholipid composition, which is believed to be important for the structure and function of the organelle. This uneven distribution of phospholipids is supposed to be generated and maintained by localized synthesis and metabolism of each phospholipid and by its directional transport between organelles.

In eukaryotic cells, major phospholipids, including phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and phosphatidylinositol (PI), are found in the membranes of all organelles, but they are biosynthesized exclusively in the defined organelles [1–4]. In yeast, PS is synthesized on the endoplasmic reticulum (ER) membrane by PS synthase Pss1p from CDP-diacylglycerol (CDP-DAG) and serine (Fig. 1) [5,6]. PS is then transported to the mitochondria and the endosome/Golgi/vacuole, and converted to PE by the PS decarboxylases Psd1p and Psd2p, respectively [7–10]. PE is then transported back to the ER and sequentially methylated to PC by PE methyltransferases Pem1p and Pem2p [11]. PC and PE are also synthesized through the Kennedy pathway, the last reactions of which are catalyzed by *sn*-1,2-diacylglycerol cholinephosphotransferase Cpt1p in yet ambiguous organelle(s), probably in the ER or Golgi, and *sn*-1,2-diacylglycerol ethanolaminephosphotransferase Ept1p in the ER, respectively [12–16]. PC is an important phospholipid for yeast, and mutants that are defective in PC synthesis through the methylation of PE require PC supply from the Kennedy pathway for growth, while it was reported that phosphatidylpropanolamine could substitute for PC in yeast [17].

Abbreviations: ER, endoplasmic reticulum; CDP-Cho, CDP-choline; CDP-DAG, CDP-diacylglycerol; CDP-Etn, CDP-ethanolamine; Cho, choline; Cho-P, choline phosphate; CL, cardiolipin; Etn, ethanolamine; Etn-P, ethanolamine phosphate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PDME, phosphatidyldimethylethanolamine; PMME, phosphatidylmonomethylethanolamine; PS, phosphatidylserine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; S1P, sphingosine-1-phosphate; 2D-TLC, two-dimensional thin layer chromatography

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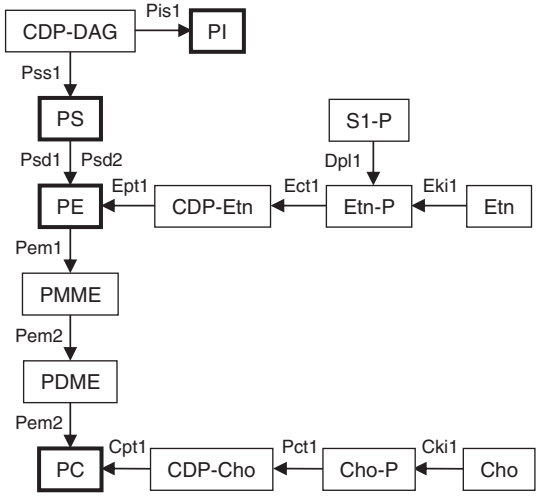

Fig. 1. Pathways for the synthesis of major phospholipids in yeast. See text for details. PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidyldimethylethanolamine; Etn, ethanolamine; Etn-P, ethanolamine phosphate; CDP-Etn, CDP-ethanolamine; Cho, choline; Cho-P, choline phosphate; CDP-Cho, CDP-choline; S1P, sphingosine-1-phosphate.

PI is synthesized from CDP-DAG and *myo*-inositol by PI synthase Pis1p in the ER or the mitochondrial outer membrane [12,18]. In contrast, the mitochondria-specific phospholipid cardiolipin (CL) is synthesized exclusively in the mitochondria [3].

The physiological significance of this compartmental specificity for localized phospholipid synthesis is poorly understood. PE and CL, phospholipids that are synthesized in the mitochondria, are important for the function and structure of this organelle. These phospholipids contribute to the formation or stability of the mitochondrial respiratory chain supercomplex, and Psd1p and enzymes involved in CL synthesis are required for respiratory growth [19–23]. PE and CL also play crucial roles in mitochondrial fusion [24,25]. In contrast, Psd2p is required for the maintenance of vacuolar PE level and heavy metal resistance [10].

PC is the bilayer-forming phospholipid that accounts for the largest fraction of membrane lipids in most organelles in yeast, but it is not clear whether PC synthesis through the methylation of PE by Pem1p and Pem2p needs to be carried out in the ER. To address this question, we constructed and characterized a yeast strain, in which PC is synthesized exclusively in the mitochondria by a bacterial PE methyltransferase localized to this organelle in a mutant deleted for *PEM1* and *PEM2*. Since this strain could synthesize PC and grow in the absence of the supplementation of choline even if PC synthesis through the Kennedy pathway is repressed, it is suggested that PC synthesized in the mitochondria is utilized in other organelles and supports the growth of yeast. The system we established in this study may be useful to evaluate PE import into the mitochondria from the organelle(s), in which Psd2p is localized to, and from the ER, in which PE is synthesized through the Kennedy pathway.

2. Materials and methods

2.1. Strains and media

Yeast strains used in this study are listed in Table 1. Primers used for the strain construction are listed in Table 2.

To delete *PEM2*, a DNA fragment was amplified by PCR using primers PEM2-delta-U and PEM2-delta-L with pAG32 [26] as a template, and was introduced into W3031A to obtain the strain *pem2*Δ. To delete *PEM1*, a DNA fragment was amplified using primers pem1-5 and pem1-3 with total DNA of the strain KEY02 [27] as a template, and this was introduced into *pem1*Δ to construct a strain *pem1*Δ*pem2*Δ (SKY010).

Table 1
Strains used in this study.

Name	Genotype	Source
W3031A	*MATa ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100*	ATCC
SKY010	W3031A *pem1*::*HIS3 pem2*::*hph*	This study
PCY12G	W3031A *pem1*::*HIS3 pem2*::*hph* P_{GAL1}*CCT1*::*nat1*	This study
SKY011	W3031A *pem1*::*HIS3 pem2*::*hph psd1*::*ADE2*	This study

To replace the *PCT1* promoter in the strain *pem1*Δ*pem2*Δ, a DNA fragment that contains 5′ non-coding region of *PCT1*, clonNAT resistant gene, *GAL1* promoter, *PCT1* ORF, and 3′ non-coding region of *PCT1* was amplified from total DNA of the strain KSY02 (*MATa his3 leu2 trp1 lys2 ura3 pem1*::*HIS3 pem2*::*LEU2* P_{GAL1}-*CCT1*::*NAT*^{r}) (H. Kishino, H. Eguchi, H. Horiuchi, R. Fukuda, and A. Ohta, unpublished) using primers ApaI-GPPCT1-F and ApaI-GPPCT1-R, and cloned into ApaI site of pBluescript II KS (+) to obtain pBS-pGAL-PCT. The nucleotide sequence of the ORF of *PCT1* was confirmed. The DNA fragment to replace the *PCT1* promoter was obtained by digestion of pBS-pGAL-PCT with ApaI and was introduced into SKY010, to obtain PCY12G.

PSD1 was deleted in the strain *pem1*Δ*pem2*Δ using *ADE2* as a marker. The deletion cassette was constructed by fusion PCR [28] with the primers, ADE2-u, ADE2-l-2, PSD1-K1, delta-psd1-A-r, PSD1-K2, delta-psd1-B-f, delta-psd1-nest-f, and delta-psd1-nest-r. This fragment was introduced into SKY010, to obtain *pem1*Δ*pem2*Δ*psd1*Δ (SKY011).

Acetobacter aceti strain (NBRC 3283) was obtained from NITE Biological Resource Center.

The minimal medium contained 0.17% yeast nitrogen base (w/o amino acid and ammonium sulfate, Difco), 0.5% ammonium sulfate,

Table 2
Primers used in this study.

Name	Sequence
PEM2-delta-U	5′-CCATAAAACAGCAATTGAAGACAACAAGAATAGCGGCAAGTCAAGCGAGCTTGCCTTGTCCCCGCCG-3′
PEM2-delta-L	5′-GGTTAGCGTAGATCATGGCAGTAAAAGGTTCTTCCCAACGAAGAGTCGACACTGGATGGCGGCGT-3′
pem1-5	5′-GTAAACCTATCTCGCTACCCC-3′
pem1-3	5′-GCGGCACTAAAACTTCCAAC-3′
ApaI-GPPCT1-F	5′-TTGGGCCCACGTTTGTTATTGAGTACGAGTCCATAT-3′
ApaI-GPPCT1-R	5′-TTGGGCCCTCGAGTTCGCTTCGCACTGCT-3′
ADE2-u	5′- GCCGTATCGTGATTAACG-3′
ADE2-l-2	5′-TTAATCGCAGACTTAAGC-3′
PSD1-K1	5′-GCTCTAGCATTGGATCTTAC-3′
delta-psd1-A-r	5′-TACGTTAATCACGATACGGCGCTGGCTTTGCTTTTCCTC-3′
PSD1-K2	5′-TACCACCTCCTTCGCAACTGG-3′
delta-psd1-B-f	5′-CTGCTTAAGTCTGCGATTAAAAGCAATCATATGTAAAGTT-3′
delta-psd1-nest-f	5′-AGCAAGAAATGTCCAAGATG-3′
delta-psd1-nest-r	5′-TTGAAAGTTTGGGTATTCAA-3′
EcoRI-pMET3	5′-GGAATTCCGATATGTACGTAGTGGTATA-3′
pMET3-SmaI	5′-TCCCCCGGGGGAGTTAATTATACTTTATTCTTG-3′
SmaI-PET100-U	5′-TCCCCCGGGATGGGTCTATTTAATAACTT-3′
BamHI-PET100-L	5′-CGGGATCCACCAATATAGTACATTACAG-3′
pmt-BamHI-f	5′-CGCGGATCCATGTCCACCAGCAGGCAGAG-3′
pmt-SacI-r	5′-CGAGCTCTACAGGCAGATTTTCCAGTG-3′
3HA-SacI-f	5′-CGAGCTCTACCCATACGATGTTCCTGA-3′
3HA-SalI-r	5′-ACGCGTCGACTCAGCACTGAGCAGCGTAATCT-3′
pPGK1-EcoRI-fw	5′-CCCGGAATTCTGTTTGCAAAAAGAACAAAAC-3′
pPGK1-SmaI-rv	5′-TCCCCCGGGTGTTTTATATTTGTTGTAAAAAGTAG-3′
EGFP-EcoRI-f	5′-CCGGAATTCGTGAGCAAGGGCGAGGAGCT-3′
EGFP-SalI-r	5′-ACGCGTCGACTTACTTGTACAGCTCGTCCA-3′
TOM70-BamHI-f	5′-CGCGGATCCCTTGATAGAATTATATTTTT-3′
TOM70-EcoRI-r	5′-CCGGAATTCCATTAAACCCTGTTCGCGTA-3′