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Loss of phosphatidylserine synthesis results in aberrant solute sequestration and vacuolar morphology in *Saccharomyces cerevisiae*

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Abstract

Null *cho1* mutants of *Saccharomyces cerevisiae* are incapable of phosphatidyl-serine synthesis. They were more susceptible than wild-type strains to 100 mM $CaCl_2$, 3 mM $ZnCl_2$ or 1 mM $MnCl_2$, but not to $MgCl_2$ nor KCl. They were also susceptible to high concentrations of basic amino acids, L-lysine and L-arginine, and to an L-lysine analog, *S*-2-aminoethyl-L-cysteine. Their vacuolar pools of amino acids, especially those of basic ones, were decreased. Pigmentation of *cho1 ade2* double mutants was obscured and vacuoles of *cho1* mutants were considerably fragmented. These indicate that phosphatidylserine plays vital roles in normal vacuolar function and morphogenesis.

Key words: *Saccharomyces cerevisiae*; Phosphatidylserine; Vacuole; *cho1* mutant

1. Introduction

Phosphatidylserine (PtdSer) is one of the major glycerophospholipids in eukaryotic membranes and is distributed among wide variety of eukaryotic organisms. It is not only an immediate precursor for another major glycerophospholipid, phosphatidylethanolamine, but is also supposed to have vital roles in many biological processes as reviewed recently [1,2]. In a budding yeast *Saccharomyces cerevisiae*, PtdSer is synthesized from CDP-diacylglycerol and L-serine by a microsomal enzyme, PtdSer synthase (CDP-diacylglycerol:L-serine *O*-phosphatidyltransferase; EC 2.7.8.8), encoded by *CHO1* [3,4]. Disruption of the *CHO1* gene made cellular PtdSer undetectable, but the resultant *cho1* mutants grew at nearly normal rates when supplemented with ethanolamine or choline to their culture media, and hence the physiological roles of PtdSer in yeast have been obscured [5,6,7].

In this report, we describe several abnormal features of the *S. cerevisiae cho1* disruptants, which suggest that PtdSer synthesis is necessary for maintaining normal intracellular compartments for divalent ions and basic amino acids and vacuolar morphology in yeast.

2. Materials and methods

2.1. Yeast and plasmids

S. cerevisiae strains SRD3–37 (*MAT*α *leu2–3,112 arg6*), SRD3–38 (*MAT*a *leu2–3,112 ura2 cho1::LEU2*), SRD3–39 (*MAT*α *leu2–3,112 arg6*) and SRD3–40 (*MAT*a *leu2–3,112 ura2 cho1::LEU2*) are tetrads from a single ascus and expected to have close genetic backgrounds [6]. Strains Lu7a (*MAT*a *leu2–3,112 ura2*) and La8α (*MAT*α *leu2–3,112 arg6*) are their parental haploids [6]. Strain YB1803 (*MAT*a *trp1 cho1::LEU2*) was free of flocculation, derived from the strain SRD3–38 by two successive crosses with strains C5558–2B (*MAT*α *trp1 ura3*) and SEY2101 (*MAT*a *ura3–52 leu2–3,112 suc2-d9 ade2–1*)[8]. Plasmid pPSY18 was a multicopy plasmid with the intact *CHO1* gene [4]. Plasmid YCpGPSS was constructed as follows; a 990-bp *CHO1* DNA fragment that lacked promoter region was excised from plasmid pSKY3224 [4] and combined with a *Bam*HI-*Bgl*II fragment that contained promoter region of the *GAL7* gene from plasmid pMT34 + 3 [9]. The resultant *GAL7-CHO1* fusion gene was inserted between the *Bam*HI and *Sal*I sites of a centromere plasmid, YCpG11 [10].

Yeast strains were grown at 30°C in a nutrient medium, YPD, or in a synthetic minimal medium, SD, supplemented with 1 mM ethanolamine or choline [6].

2.2. Chemical analyses

Yeast phospholipids that were labeled with [^{32}P]P_i for at least five generations were extracted, separated and quantified as described previously [6].

Cytosolic and vacuolar pools of amino acids were extracted differentially by $CuCl_2$ treatment and measured according to the method of Ohsumi et al. [11]. Strain YB1803 with plasmid YRp7 or pPSY18 was grown to early exponential phase (OD_{600} = 0.1) in SD medium supplemented with 1 mM ethanolamine. Each culture was divided into two parts and 10 mM L-lysine was added to the one part. After 4.5 h incubation, cells in 6 ml culture were collected by brief microfuge-centrifugation, washed twice with water and suspended in 1.5 ml of 2.5 mM potassium phosphate buffer (pH 6.0) containing 0.6 M D-sorbitol, 0.2 mM $CuCl_2$ and 10 mM D-glucose. After 10 min at 30°C, the suspensions were filtered through a glass fiber filter (Whatman GF/C) and washed 4 times with the same buffer. Filtrates were combined and used to measure cytosolic amino acid pools. Cells remaining on the filters were boiled in 3 ml water for 15 min. Solid materials were removed by centrifugation and the supernatents were used to measure vacuolar amino acid pools. Fluids containing extracted amino acids were acidified by 5% trichloroacetic acid to remove proteins and washed several times with diethylether. Amino acids were concentrated by lyophilization and analyzed with Hitachi L8500 amino acids analyzer. The data in Table 1 are the average of two culture samples and their differences were within 10%.

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3. Results

3.1. *Reduced* ade2*-pigmentation in* cho1 *mutants*

While growing a *cho1*::*LEU2* mutant with *ade2* mutation, we were aware that its colony color was pale pink, not reddish as typically seen with ordinary *ade2* mutants. To test whether such absence of coloration was related to *cho1* mutation, a *cho1*-disrupted derivative of strain SRD3–38 was crossed with an *ade2* mutant, SEY2101, and colony color of resultant 6 sets of tetrads were examined. All *cho1 ade2* colonies were pink and *CHO1 ade2* colonies were red. Furthermore, among randomly obtained 204 spores with *ade2* and *cho1*::*LEU2* mutations simultaneously, 193 gave pink or white colonies and 11 gave red colonies, although not as reddish as most *ade2 CHO1* spores. Introduction of plasmid pPSY18 that carried the wild-type *CHO1* gene into one of the colorless *ade2 cho1*::*LEU2* clones, CTA2–6 (*MATα trp1 ade2 leu2–3,112 cho1*::*LEU2*), gave red-colored transformants. These results indicate that *ade2*-pigmentation and PtdSer synthesis are highly correlated. Since *ade2*-pigments are known to accumulate in vacuoles [12], we suspected that vacuoles of the *cho1* mutants might not function properly.

3.2. *Effect of divalent cations*

Some yeast mutants defective in vacuolar acidification and morphogenesis were sensitive to high concentrations of extracellular Ca^{2+} and other divalent cations, such as Zn^{2+} and Mn^{2+} [13–17]. Therefore, we tested the effect of these ions on the growth of *cho1*::*LEU2* mutants. As shown in Table 1, strains SRD3–38 and SRD3–40 failed to grow at over 100 mM $CaCl_2$, whereas *CHO1* strains grew normally even at 300 mM. Another *cho1*::*LEU2* mutant, YB1803, exhibited $CaCl_2$-sensitivity but the introduction of plasmid pPSY18 turned it $CaCl_2$-resistant (data not shown). $ZnCl_2$ and $MnCl_2$, were also inhibitory to the *cho1* mutants at low concentrations (3 mM and 1 mM, respectively). Magnesium chloride and KCl at over 300 mM did not affect the growth of the *cho1* mutants at all.

Table 1
Sensitivity of *cho 1* mutants to divalent metal ions

Strain	*CHO1*	Salt added[a] None	$CaCl_2$ (100)	$ZnCl_2$ (3)	$MnCl_2$ (1)	$MgCl_2$ (300)	KCl (300)
SRD3–37	wild	+++	+++	++	++	+++	+++
SRD3–38	*cho1*::*LEU2*	+	–	–	–	+	+
SRD3–39	wild	+++	+++	++	++	+++	+++
SRD3–40	*cho1*::*LEU2*	+	–	–	–	+	+
Lu7a	wild	++	++	+	+	++	++
La8α	wild	+++	+++	++	++	+++	+++

[a] In parentheses; concentrations (mM) of salts. Colony growth on YPD agar medium after 3 days is indicated by +++, ++, + or – (no growth).

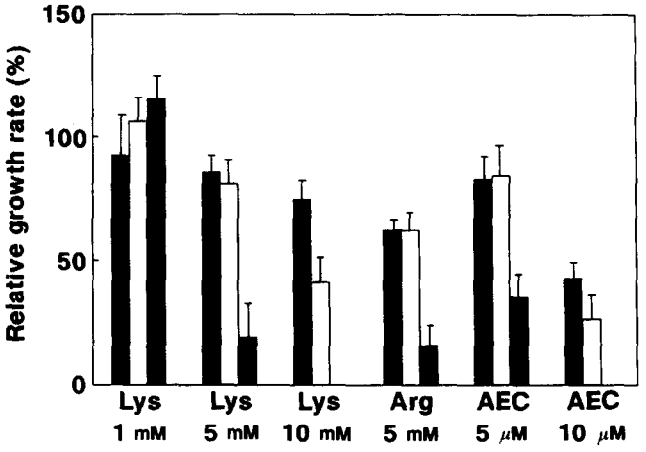

Fig. 1. Impaired growth of *cho1* mutants by basic amino acids and AEC. Strain YB1803 with YCpG11 or YCpGPSS was grown in SD medium supplemented with 1 mM ethanolamine or in the same medium of which carbon source was replaced with 1% D-glucose plus 2% D-galactose. Growth rates in the exponential phase are indicated by bars the heights of which indicate relative rates to those without amino acid supplementation. Values are average of three cultures. Hatched bar = YB1803/YCpGPSS grown on D-glucose + D-galactose; open bar = YB1803/YCpGPSS; closed bar = YB1803/YCpG11. The strain YB1803/YCpG11 did grow in the medium with 10 mM L-lysine or 10 μM AEC.

3.3. *Sensitivity to high concentration of basic amino acids*

Another feature of some types of vacuolar mutants is their sensitivity to high concentrations of basic amino acids and their analogs [17]. We found that the growth rate of strain YB1803 without the *CHO1* gene was significantly low in the presence of 10 mM L-lysine or 5 mM L-arginine, in comparison with those harboring the plasmid YCpGPSS when *CHO1* gene was induced by D-galactose (Fig. 1). The PtdSer content under this condition (1% D-glucose plus 2% D-galactose) was 5.6% of the total phospholipids, nearly the wild-type level, and the strain YB1803 with YCpGPSS under this condition was not auxotrophic for ethanolamine or choline. The growth inhibition by these basic amino acids seems to be antagonized by a relatively low level of PtdSer synthesis, because the strain YB1803/YCpGPSS under the repressed condition contained a small amount of PtdSer (0.8% of the total phospholipids) and was less sensitive to L-lysine than the strain YB1803 with vector plasmid YCpG11. The *cho1* mutant was also sensitive to low concentration of an L-lysine analog *S*-2-aminoethyl-L-cysteine (AEC) (Fig. 1), but not to high concentration of L-glutamic acid, L-leucine or L-serine (data not shown).

3.4. *Intracellular pools of amino acids*

Since the sensitivity to basic amino acids and AEC has been supposed to be related to the increase in their cytosolic concentrations [17], we examined the effect of *cho1* mutation on intracellular pools of amino acids (Table 2). Vacuolar pool of L-lysine of a *cho1* mutant

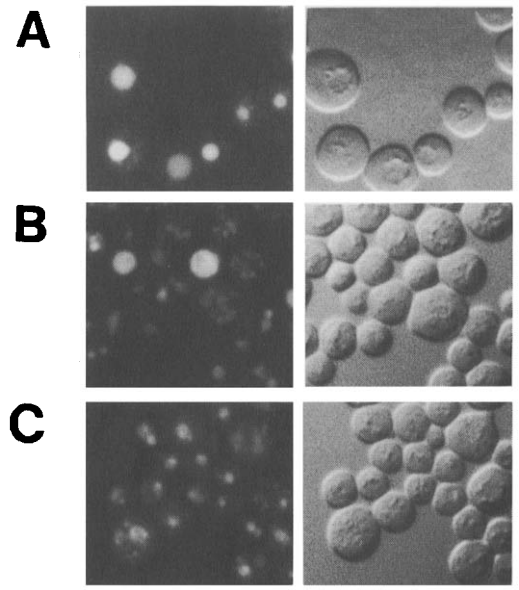

Fig. 2. Effect of *cho1* mutation on vacuolar profile. A fresh culture of strain YB1803/YCpGPSS in SD medium containing 1% D-glucose plus 2% D-galactose as carbon sources was transferred (2% inoculation) into the same medium (A) and the medium with 5% D-glucose plus 1 mM ethanolamine (B) or 5% D-glucose plus 1 mM choline (C). After 23 h at 30°C, cells were stained by 0.2 mM quinacrine for 5 min according to the method of Weisman et al. [12] and observed by fluorescence (left panels) and Nomarski optics (right panels). Until this time, PtdSer synthase activity was undetectable and PtdSer content was at its lowest. Those with bright fluorescence in panel B are of dead cells.

(YB1803 with YRp7) was 3.8 nmol/10^8 cells and much smaller than the cytosolic pool (12.4 nmol/10^8 cells), whereas that of the *CHO1* strain (YB1803 with pPSY18) was 18.0 nmol/10^8 cells and larger than its cytosolic pool (9.7 nmol/10^8 cells). The vacuolar pools of L-histidine and L-arginine in the mutant were also smaller than their cytosolic pools, whereas those in the *CHO1* strain were not. Intracellular contents of other amino acids were somewhat smaller in the *cho1* mutant. The presence of 10 mM L-lysine in the medium resulted in an increase in the overall L-lysine pool in both the *CHO1* and *cho1* strains to similar levels, although the vacuolar pool of the *cho1* strain was small (16.7% of total) and its cytosolic pool (228.1 nmol/10^8 cells) was larger than that (118.5 nmol/10^8 cells) of the *CHO1* strain.

3.5. Vacuolar morphology

Vacuoles under the condition where PtdSer synthesis was repressed could be stained by quinacrine or chloroquine, although they appeared fragmented and somewhat faint (Fig. 2). This was most evident in the early stationary phase. Vacuoles in the *CHO1* mutant were not clearly observed by Nomarski optics. Similar results were obtained with other *CHO1*-disrupted mutants.

4. Discussion

The diminished *ade2*-pigmentation and the Ca^{2+}-sensitive growth phenotype are known in both types of mutants defective in vacuolar H^+-ATPase [13,14,18] and defective in vacuolar formation [15–17]. In the former type mutants, vacuolar Ca^{2+}-uptake does not occur and hence cytosolic Ca^{2+} concentration is elevated [14], which is thought to be a reason for their sensitivity to high concentration of extracellular Ca^{2+}. They are also sensitive to Zn^{2+} and Mn^{2+} as observed in the *cho1* mutants

Table 2
Vacuolar and cytosolic amino acid pools of the *cho1* mutant[a]

Amino acid	YB1803/YRp7				YB1803/pPSY18			
	− Lysine		+ Lysine		− Lysine		+ Lysine	
	C	V	C	V	C	V	C	V
L-Lysine	12.4	3.8	228.1	45.7	9.7	18.0	118.5	108.6
L-Arginine	39.8	14.7	34.7	8.8	26.5	47.9	22.4	34.9
L-Histidine	3.9	3.6	2.3	1.7	2.6	5.8	1.4	3.6
L-Serine	6.5	2.6	5.8	1.3	4.0	1.8	3.5	0.9
L-Threonine	10.0	1.7	10.9	0.9	9.2	3.6	6.8	2.1
L-Alanine	15.3	4.3	18.2	2.7	10.9	5.0	8.4	2.3
Glycine	4.2	3.1	4.1	2.4	2.4	2.8	1.9	2.0
L-Leucine	3.0	1.6	3.0	0.9	1.3	0.8	1.1	0.4
L-Isoleucine	1.2	1.3	1.1	1.4	0.7	1.0	0.6	0.5
L-Valine	17.7	4.9	9.9	5.0	5.3	4.1	5.7	2.9
L-Aspartic acid	14.2	2.0	20.7	1.1	11.4	2.6	10.7	1.7
L-Glutamic acid	46.9	11.1	56.5	5.2	35.3	18.5	32.7	8.7
L-Asparagine	40.4	16.8	55.1	9.8	33.3	16.5	32.8	11.3
L-Glutamine	35.5	7.5	39.6	3.3	22.1	13.1	12.3	6.1
Total	251.0	79.0	490.0	90.2	174.7	141.5	258.8	186.0

[a] Values are amounts of amino acids (nmol/10^8 cells). Yeast cells were grown in the presence (+Lysine) or absence (−Lysine) of 10 mM L-lysine. C = cytosolic pool; V = vacuolar pool.

[14]. Cytosolic Ca^{2+}-concentration has not been measured for the mutants defective in vacuolar formation, but it is quite conceivable that the disappearance or shrinkage of vacuole, a cellular Ca^{2+} reservoir [19], will result in a similar change. The sensitivity to basic amino acids or AEC was also reported with the mutant defective in vacuolar formation [17]. The vacuolar pools for basic amino acids in the *slp1/vps33* mutants were decreased, whereas their cytosolic pools were increased significantly, which likely caused their sensitivity to basic amino acids [17]. Similar phenomenon has not yet been reported for the mutants defective in vacuolar H^+-ATPase, but it should happen to them because of the lack of H^+-gradient which is necessary to excrete excess amino acids across the vacuolar membrane [20]. In the case of the *cho1* mutant, cytosolic pools of basic amino acids were larger than their vacuolar pools and the cytosolic L-lysine pool expanded further by the addition of L-lysine to the medium, which should be the reason why the *cho1* mutant is sensitive to high concentration of L-lysine. Thus, the growth phenotypes similar to the two types of vacuolar mutants and the actual reduction of vacuolar pool size for basic amino acids lead us to the notion that, without PtdSer synthesis, the vacuolar pools are limited for various molecules, such as the *ade2*-pigments, divalent cations like Ca^{2+}, and basic amino acids.

How such limitation of vacuolar pools occurred in the *cho1* mutant is presently not certain. Although the vacuoles of the *cho1* mutant were stained by quinacrine, which accumulates only in acidified vacuoles [12], it is still possible that the vacuolar pH is not maintained within the most suitable range, since the observed fluorescence was not so intense as seen in the wild-type strain. The mutants defective in vacuolar H^+-ATPase show *pet*$^-$ phenotype [14] and the *cho1* mutant also grew very slowly in the media that contained non-fermentable carbon sources (data not shown). PtdSer may be necessary for the full activity of H^+-ATPase or for the intactness of permeability barrier of vacuolar membrane, even though the content of PtdSer in vacuolar membrane is small (4.4% of the total vacuolar phospholipids [21]).

The observed fragmentation of vacuoles provides another clue about the cause of the likely limitation of vacuolar pools in the *cho1* mutant. Fragmentation of vacuoles should limit their total volume and may partially, at least, result in the observed phenotypes of the *cho1* mutants as supposed in the mutants defective in vacuolar formation [15]. It is likely that PtdSer is required for the efficient fusion of vacuolar precursor vesicles to form large central vacuoles, since PtdSer stimulates fusion of artificial membranes in the presence of Ca^{2+} [22].

The results so far presented here relieve the involvement of PtdSer in yeast vacuolar function and morphology. Further analyses on the properties of the vacuolar membrane of the *cho1* mutant are now in progress.

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