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Incapability of Utilizing Galactose by *pgs1* Mutation Occurred on the Galactose Incorporation Step in *Saccharomyces cerevisiae*

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Abstract A *Saccharomyces cerevisiae pgs1* null mutant, which is deficient with phosphatidyl glycerol (PG) and cardiolipin (CL) biosynthesis, grows well on most fermentable carbon sources, but fails to grow on non-fermentable carbon sources such as glycerol, ethanol, and lactate. This mutant also cannot grow on galactose medium as the sole carbon source. We found that the incorporation of [^{14}C]-galactose, which is the first step of the galactose metabolic pathway (Leloir pathway), into the *pgs1* null mutant cell was extremely repressed. Exogenously expressed *PGS1* (YCp*PGS1*) under indigenous promoter could completely restore the *pgs1* growth defect on non-fermentable carbon sources, and dramatically recovered [^{14}C]-galactose incorporation into the *pgs1* mutant cell. However, *PGS1* expression under the *GAL1* promoter (YEpP$_{GAL1}$-*PGS1-myc*) could not complement *pgs1* null mutation, and the *GAL2-lacZ* fusion gene (YEpP$_{GAL2}$-*lacZ*) also did not exhibit its β-galactosidase activity in the *pgs1* mutant. In wild-type yeast, antimycin A (1 μg/ml), which inhibits mitochondrial complex III, severely repressed not only the expression of the *GAL2-lacZ* fusion gene, but also uptake of [^{14}C]-galactose. However, exogenously expressed *PGS1* partially relieved these inhibitory effects of antimycin A in both the *pgs1* mutant and wild-type yeast, although it could not basically restore the growth defect on galactose by antimycin A. These results suggest that the *PGS1* gene product has an important role in utilization of galactose by *Gal* genes, and that intact mitochondrial function with *PGS1* should be required for galactose incorporation into the Leloir pathway. The *PGS1* gene might provide a clue to resolve the historic issue about the incapability of galactose with deteriorated mitochondrial function.

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In the eukaryotic cell, the minor anionic phospholipids, such as phosphatidylglycerol (PG) and cardiolipin (CL), are critical components of the mitochondrial inner membrane, which keeps a functional environment for the mitochondrial energy transducing system. Mitochondria have a crucial role for cell growth, apoptosis, cancer, regenerative disease, and reactive oxygen stress [1, 15, 21, 27], and it is known that the level of phosphatidylethanolamine (PE) in mitochondria is also important for cellular aerobic growth [6, 34]. Mitochondrial function is absolutely required in metabolic control of oxidative phosphorylation (OXPHOS), cell surface synthesis, and the expression of some nuclear genes [8, 31, 44]. Phosphatidylglycerol phosphatesynthase (*PGS1*), which catalyzes the synthesis of phosphatidylglycerol phosphate (PGP) from CDP-diacylglycerol (CDP-DG) and hydrolysis of *sn*-glycerol-3-phosphate to phosphatidylglycerol, the precursor of cardiolipin, is mostly located in mitochondria, carrying out their role in mitochondrial function [7, 10]. In *Saccharomyces cerevisiae*, the lack of PG/CL due to *PGS1* gene disruption is not lethal, but the *pgs1/cho1* double mutant, lacking phosphatidylglycerol, cardiolipin, and phosphatidylserine, is absolutely lethal [24]. In wild-type *E. coli*, PG and CL are strictly required for cell viability, but decreased amount of cardiolipin could be substituted by increased PG in *crd* (*cls1*) mutant [23, 46]. Whereas the growth of yeast *crd1* (*cls1*) mutant is not deterred but slow on non-fermentative carbon sources [17, 47], the *pgs1* null mutant, showing petite negative phenotype, fails to grow in non-fermentative carbon sources [7, 24]. In Chinese Hamster

Ovary (*CHO*) cell, *pgs1* mutation shows both dysfunctional mitochondria and only slow growth on galactose medium. However the overexpression of *CHO PGS* in *pgs CHO* mutant cell recovers both the growth defect on galactose and morphological and functional defects in mitochondria [26, 36, 37]. The crucial role of mitochondria in intracellular energy metabolism and programmed cell death is linked to the release of cytochrome *c*, resulting in caspase activation [9, 20, 38]. Cytochrome *c* is associated with cardiolipin, which is asymmetrically distributed across the lipid bilayer of the mitochondrial inner membrane, and the dissociation and release of cytochrome *c* from the mitochondrial inner membrane is necessary as the first step in apoptotic progress [20, 39]. Loss of mitochondrial PG/CL by *pgs1* mutation directly compromises the function of several essential enzymes in the mitochondrial process. The reduction of CL, release of cytochrome *c*, and consecutive decrease of membrane potential occur on respiratory reduction condition [36], and the decay of membrane potential was greatly accelerated when mitochondrial ATP hydrolysis was blocked by oligomycin [2, 36]. In non-oxidative condition or in dysfunctional mitochondrial condition, yeast can obtain only minimal energy, which is dependent on the fermentative glycolysis [17, 43].

Whereas wild-type yeast utilizes galactose for its growth, rho^0/rho^- mutants that lack a complete or have a deleted mitochondrial genome lose the capability for galactose utilization [13, 32]. The alteration of mitochondrial function in yeast delays the adaptation to grow on galactose, although most of *GAL* genes on the Leloir pathway are genetically normal [14, 33]. Although the relationship of mitochondria to the galactose metabolic pathway in *S. cerevisiae* has been known for a long time, little is known of the mechanism in yeast containing a mitochondrial defect. We hypothesized that the *PGS1* gene product of PG and the next step product of CL might be involved in galactose metabolism in relation to mitochondrial function, and the present study was undertaken to resolve the *pgs1*-mediated incapability of galactose. To the best of our knowledge, this is the first report dealing with the direct role of the *PGS1* gene in galactose metabolism related to mitochondrial respiratory function.

MATERIALS AND METHODS

Materials

All chemicals used were of reagent grade. Restriction enzymes and DNA modifying enzymes were purchased from Promega Corp., and New England Biolabs. The polymerase chain reaction (PCR) was performed using PCR SuperMIX (Life Technologies, Inc.) or Taq polymerase and the reagents from Promega Corp. Oligonucleotides were prepared commercially by Genosys Biotechnologies Inc. Radiochemicals were obtained from Amersham Pharmacia Biotech, and Perkins Elmer Life Science. The BCA kit for protein assay was a product of Pierce. Phospholipids were from Sigma. CDP-diacylglycerol (dioleolyl) stocked in this lab was used. Universal scintillation cocktail was purchased from ICN Biomedicals, Inc.

Strains and Media

Saccharomyces cerevisiae strains used in this study are DL1 (*MAT*a, *his311*, *his315*, *leu23*, *leu212*, *ura3251*, *ura3372*, *ura3328*) and YCD4 (*MAT*a, *his3-11*, *15*, *leu2-3*, *112*, *pgs1::HIS3*, *ura3-251*, *328*, *372*). All culture media and selection media for yeast and *E. coli* were purchased from BIO 101 Q-BIO gene, and Difco. *E. coli* strain DH5α was grown in LB medium (1% Bacto-tryptone, 0.5% Bacto-yeast extract, 1% NaCl, pH 7.4) at 37°C and supplemented with ampicillin (50 mg/ml) when needed for plasmid selection. YPD medium consisted of 1% Bacto-yeast extract, 2% Bacto-peptone, and 2% dextrose, and 2% or 4% galactose replaced dextrose as the carbon source in the YPG medium. For the selection of yeast transformant and mutant, yeast nitrogen base (YNB without amino acids, Difco) medium supplemented with required amino acids was used.

Gene Expression Plasmids

For YCp*PGS1* (*CEN*4, *ARS*1, *URA*3), and *PGS1* expression plasmid vector, the *PGS1* gene was amplified from wild-type yeast (DL1) chromosomal DNA by PCR, using the primers based on the reported DNA sequence (GenBank accession number Z48162). The amplicated 2.6 kb fragment with a designed SmaI site at 5', 3' primes was cloned into YCp50 single copy plasmid previously digested by SmaI. For $YEpP_{GAL2}$-*lacZ*, the amplified 910bp *GAL2* promoter region containing the *GAL2* start cordon from the pTV3 *GAL2* expression vector (generously given by Dr. J. Nikawa) was fused in frame with a *lacZ* reporter gene of the YEp363-*lacZ* expression vector. It was created by replacing the HindIII/BamHI fragment of YEp363 with amplified *GAL2* fragment. For a *c-myc* epitope-tagged *PGS1*, 1,557 bp of *PGS1* DNA fragments, which contained the BamH1 site at the N-terminal and Sal1 site at the C-terminal located at the end of the coding region, was generated by PCR. The PCR product was ligated into the pYES vector digested with the BamH1/Sal1 to be in frame with the human *c-myc* epitope, and finally produced $pYESP_{GAL1}$-*PGS1-myc*. All constructed plasmids were verified by sequencing for correct junction. The designed primers were as follows: *PGS1-myc*, forward, 5'-CGGATCCACTATGCATGGGA-GTGATTTCAC-3', reverse, 5'-GGTCGACTCATTAATATA-TATGTTATCCT-GA-3', PGS1; forward, 5'-GCCCGGGAG-CTATGCATGGGAGTGATTTCAC-3', reverse, 5'-GCCCGG-GTCAGCTCATTCTCTACCTTAATGGC-3', *GAL2* promoter; forward, 5'-CGGATCCGAGCATAACGGGCTGTACTAAT-

Table 1. The growth of yeast *pgs1* mutant on fermentable and non-fermentable carbon sources.

Strain	Genotype	Glucose	Maltose	Galactose	Raffinose	Glycerol
DL1	Wild-type	+	+	+	+	+
YCD4	*pgs1*	+	+	–	+	–
YCD4/YCp*PGS*	*pgs1/PGS1*	+	+	+	+	+

The growth was teasted on yeast complete agar medium; + growth; – no growth.

3', reverse, 5'-AGGCCAAGCTTGTTCAGACATGGGCT-TCTTGGG-3'. For each DNA amplification, a standard PCR reaction program (100 pmol each primer, 100 ng each DNA) was carried out.

Yeast Transformation

The transformation of yeast cells was performed as previously described [34]. Transformants were selected on YNB medium (Difco), which contained 2% glucose and required amino acids or bases. The transformation of *E. coli* was performed according to the calcium chloride method [40].

β-Galactosidase Assay

Yeast cells grown to mid-log phase in yeast nitrogen broth containing 2% glucose at 30°C were shifted to the fresh broth medium containing 2% galactose to induce *GAL2-lacZ* gene expression at 30°C. After 4 h of incubation, the cell suspension was disrupted with prechilled glass beads (diameter 0.3 mm) by voltexer for 5 min, with the cooling step repeated. The cell lysates were analyzed for β-galactosidase activity and expressed as Miller units [33].

[^{14}C]-Labeled Galactose Uptake

Yeast cells, grown in yeast nitrogen broth containing 2% glucose to mid-log phase at 30°C, were washed and resuspended in 2% galactose medium, and [^{14}C]-galactose (1 nmol) was added. At indicated time during the incubation, the [^{14}C]-galactose radioactivity incorporated was counted. Briefly, cells were rapidly collected on glass fiber filters (Whatman GF) in a vacuum manifolder and rinsed three times with 10 ml of ice-cold distilled water. The filtrates were dried and then transferred to 5 ml of scintillation universal cocktail (ICN Biomedicals, Inc.), and radioactivity was measured with a liquid scintillation counter (Amersham Pharmacia Biotech).

Protein Assay

The protein concentration in each cell extract was determined by the BCA protein assay kit (Pierce), using bovine serum albumin as a standard.

Other Methods

Yeast chromosomal DNA was prepared by the Sambrook method [40]. Plasmid DNA was extracted by the minipreparation method and purified with PEG solution (25% polyethylene glycol 6000, 2 M NaCl) for sequencing. DNA sequencing was carried out by the Molecular Genetics Core Facility (University of Texas Medical School, Houston, TX, U.S.A.).

RESULTS AND DISCUSSION

Exogenous *PGS1* Expression Recovers Impaired Growth of *pgs1* Mutant

The *pgs1* null mutant showed no growth defect on fermentative carbon sources such as glucose, maltose, and raffinose, but manifested complete growth defect on non-fermentative carbon source such as glycerol (Table 1). On galactose medium, the growth of *pgs1* was also severely inhibited. However, exogenously expressed *PGS1* gene in the *pgs1* null mutant by the YCp*PGS1* single copy plasmid under its indigenous promoter completely recovered the incapability of galactose (Table 1). For the growth curve of *pgs1* mutant, the cells previously cultured on YNB, yeast glucose minimal broth medium overnight were washed with sterile water, and 0.01 volume of the cell suspension was resuspended in yeast rich culture medium containing each of 2% glucose, 2% galactose, and 4% galactose as a sole carbon source, cultured at 30°C, and growth levels were determined at A_{600}. In 2% galactose medium as a sole carbon source, the *pgs1* mutant showed severe growth arrest during the culture, and it grew at a very low rate

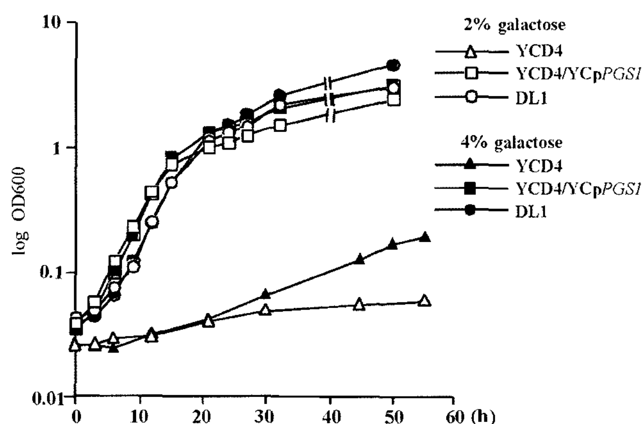

Fig. 1. Growth curve of *pgs1* mutant in galactose.
Yeast cells, such as DL1 (wild-type), YCD4 (*pgs1::HIS3*), and YCD4/YCp*PGS1*, were grown on YP (1% yeast extract, 2% peptone) medium containing either 2% galactose or 4% galactose. A_{600} value was measured at indicated times for log plots.

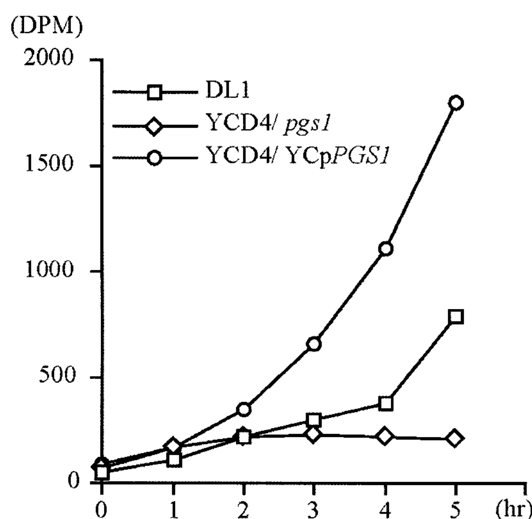

Fig. 2. Yeast *pgs1* mutant cells cannot take up [^{14}C]-galactose.
Yeast cells DL1 (wild-type), YCD4 (*pgs1*), and YCD4/YCp*PGS1* (*pgs1*/*PGS1*) were cultured overnight in yeast nitrogen broth containing 2% glucose at 30°C. The log phase cells were shifted to YPG medium (2% galactose) containing [^{14}C]-galactose (10,000 dpm/μl) and were incubated for 6 h. At indicated times, radioactivities were measured by a scintillation counter.

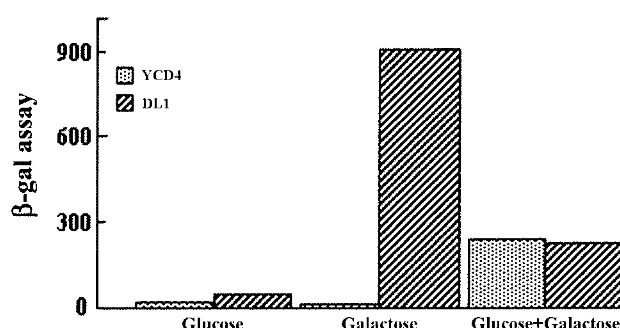

Fig. 3. Yeast *pgs1* mutant cannot induce *Gal2-lacZ* fusion gene expression in galactose medium.
Yeast cells (DL1, wild type; YCD4, *pgs1* mutant) were grown in YNB with 2% glucose to mid-log phase at 30°C. The cells were shifted to yeast-rich medium containing the following carbon sources; 2% glucose, 2% galactose, 0.1% glucose+2% galactose. *Gal2-lacZ* fusion gene expression was analyzed by β-galactosidase activity as Miller unit.

even in 4% galactose (Fig. 1). However, exogenous *PGS1* gene expression in the *pgs1* null mutant completely recovered the level of growth on galactose similar to that of wild-type yeast DL1 (Fig. 1).

[^{14}C]-Galactose Could Not Enter the *pgs1* Mutant Cell, but Exogenous *PGS1* Expression Accelerates the Entrance of [^{14}C]-Galactose

To investigate the incapability of growth of the *pgs1* null mutant on galactose, we examined the first galactose transport step in the Leloir pathway by counting the [^{14}C]-galactose incorporation rate in the *pgs1* null mutant and wild-type cells (Fig. 2). Thus, YCD4 *pgs1* mutant and its isogenic parental strain DL1 were cultured overnight in glucose medium, and the cells were shifted to 2% galactose medium ([^{14}C]-galactose 1 nmol) to determine the [^{14}C]-galactose incorporation rate. DL1 wild-type yeast cells showed highly increased [^{14}C]-galactose radioactivity, but YCD4 *pgs1* mutant could not increase [^{14}C]-galactose radioactivity during 5 h of culture. However, YCD4 cells harboring YCp*PGS1* fully recovered [^{14}C]-galactose incorporation to a level higher than that of wild-type yeast, indicating normal activity of indigenous *GAL2* gene in the galactose transport system by *PGS1* expression, and the role of *PGS1* in the galactose metabolic pathway.

P_{GAL2}-*lacZ* and P_{GAL1}-*PGS-myc* are Not Expressed in the *pgs1* Null Mutant Cell

In order to examine the level of *GAL2* gene expression in the *pgs1* mutant, we constructed a P_{GAL2}-*lacZ* fusion gene expression vector (YEpP_{GAL2}-*lacZ*), and determined β-galactosidase activity in *pgs1* mutant cells transformed with YEpP_{GAL2}-*lacZ*. As shown in Fig. 3, YEpP_{GAL2}-*lacZ* showed highly expressed β-galactosidase activity in 2% galactose medium in DL1 wild-type yeast, but not in glucose medium. In galactose (2%) and glucose (0.1%) media, very low β-galactosidase activity was found, showing normal carbon catabolitic repression in yeast. However, YEpP_{GAL2}-*lacZ* in the *pgs1* mutant did not express β-galactosidase activity in 2% galactose, but it induced a very low level of β-galactosidase activity in galactose (2%)-glucose (0.1%) medium (Fig. 3), showing that the *GAL2* gene could not be induced by galactose in the *pgs1* mutant cell. On the other hand, *PGS1* expression under the *GAL1* promoter (YESP$_{GAL1}$-*PGS1-myc*) surprisingly could not repress the growth defect of the *pgs1* null mutant in galactose medium (Fig. 4). The induction failure of *GAL1* promoter by galactose further

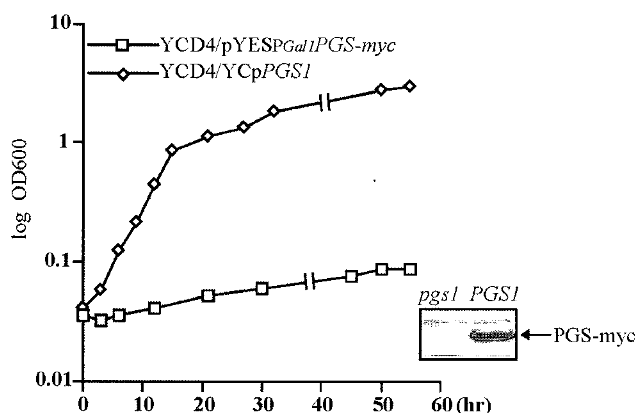

Fig. 4. Growth curve of *pgs1* mutant with YESP$_{GAL1}$-*PGS1-myc* in galactose medium.
Yeast cell density was measured at indicated times by spectrophotometery (A_{600}).

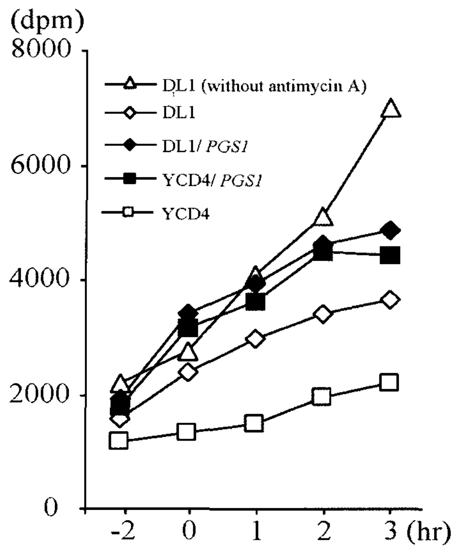

Fig. 5. Antimycin A inhibits [^{14}C]-galactose uptake in yeast.
To measure the rate of [^{14}C]-galactose incorporation into yeast cell under condition of mitochondrial respiration deficiency, yeast cells were treated with antimycin A (1 μg/ml). Before and after the addition of antimycin A, radioactivity was determined, as shown in Fig. 2.

supports the unexpression of the *GAL2-lacZ* fusion gene in the *pgs1* null mutant, thus indicating that the *GAL1* promoter and *GAL2* promoter do not respond to galactose by *pgs1* mutation.

Mitochondrial Electron Transport Chains Affect Galactose Dynamics

It is known that wild-type yeast cells show minimal growth in respiration deficient condition, when ethidium bromide or antimycin A is added, which induces mitochondrial DNA damage or mitochondrial respiratory defect, respectively.

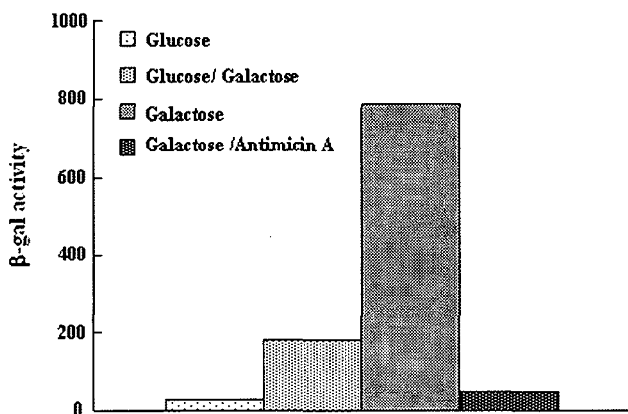

Fig. 6. Antimycin A inhibits *Gal2-lacZ* fusion gene expression in yeast.
DL1/YEpP$_{GAL2}$-*lacZ* cells in log phase were cultured on yeast minimal growth medium containing different kinds of carbon source. Antimycin A (1 μg/ml) as mitochondrial respiration inhibitor was added and β-galactosidase activity was determined.

However, *pgs1* mutant cell YCD4 could not grow on these restricted conditions. Both [^{14}C]-galactose incorporation and P$_{GAL2}$-*lacZ* expression by β-galactosidase activity were examined to determine whether the mitochondrial respiration deficient state by antimycin A affects galactose utilization in DL1 wild-type and YCD4 *pgs1* mutant cells (Figs. 5 and 6). The addition of antimycin A (1 μg/ml), which inhibits mitochondrial complex III, reduced [^{14}C]-galactose uptake in both DL1 and YCD4. Exogenously expressed *PGS1* by YCp*PGS1* increased [^{14}C]-galactose incorporation in the *pgs1* mutant and wild-type DL1. However, it could not overcome the substantial mitochondrial respiratory damage induced by antimycin A (Fig. 5). YEp P$_{GAL2}$-*lacZ* expression in DL1 cells by galactose was also significantly depressed by the addition of antimycin A, suggesting *GAL2* gene repression by mitochondrial respiratory inhibition.

Relationship Between *PGS1*, Galactose, and Mitochondria

In the *pgs1* null mutant, exogenous *PGS1* expression under indigenous promoter or under *ADH* promoter (YEpP$_{ADH}$-*PGS1*) can completely recover the *pgs1* growth defect on non-fermentable carbon sources and galactose. In the present study, the inability of galactose in the *pgs1* null mutant cell was found to be due to inactive galactose incorporation, because the *pgs1* mutant incorporated only very low level of [^{14}C]-galactose in the cell, compared with wild-type cells. Low intracellular [^{14}C]-galactose in the *pgs1* mutant could definitely explain how *GAL1-PGS1* gene expression was repressed, and why *GAL2-lacZ* did not exhibit β-galactosidase activity in the *pgs1* mutant.

Usually, wild-type yeast can take up galactose by the high affinity transport system, and the level of cytoplasmic galactose depends mainly on galactose transporter activity by the *GAL2* gene [14]. *GAL* genes are highly induced in galactose, and the rapid response to galactose is mediated by a serial interaction of *GAL3*, *GAL80*, and *GAL4* regulatory genes in the Leloir pathway [35, 42]. Although most *gal* mutants, including *gal1*, *gal2*, *gal3*, or *gal4*, need long-term adaptation to grow on galactose medium in respiratory sufficient condition, the *pgs1* mutant cell never shows this adaptable growth in galactose. Structural genes of *GAL1* (galactokinase), *GAL7* (galactose transferase), and *GAL10* (galactose epimerase), and regulatory genes of *GAL4*, *GAL80*, and *GAL3*, are involved in the galactose metabolic pathway [3, 4, 14]. The *gal2* mutant can grow only on high galactose (5%) medium, and the *gal3* mutant can grow slowly on galactose (2%) after the adaptation of 3–5 days in respiratory sufficient condition; however, the *gal1*, *gal4*, and *gal80* mutants grow well on galactose [4, 5, 28, 42]. Defect of *GAL3* causes a long delay for *GAL* gene induction, but the overexpression of *GAL3* causes constitutive expression of *GAL* genes [42]. In these defective growth phenotypes of *GAL* gene mutants *gal2*, *gal3* mutant is similar to the *pgs1* yeast mutant and *pgs1* *CHO* mutant

[7, 26], but Yeast *pgs1* mutation shows more severe growth defect than *gal* mutation. In the present study, we showed that *PGS1* expression is required for normal induction of the *GAL* gene in galactose catabolism, because *pgs1* mutation alters the *GAL1* and *GAL2* gene expression pathway at the galactose transport step into the cell, and *GAL* promoters on YESP$_{GAL1}$-*PGS1-myc*, and the YEpP$_{GAL2}$-*lacZ* fusion gene, fail to express in the *pgs1* mutant cell. However, the exact interaction between the *PGS1* gene and these *GAL* genes remains unclear.

One possibility is that the *pgs1* gene could affect the galactose metabolic pathway via concomitant mitochondrial dysfunction due to PG/CL deficiency. Substantial *pgs1* null mutation means no PG and no CL in the mitochondrial membrane, but the *crd1* null mutant lacks only CL; therefore, *crd1* mutation exhibits less cardiolipin-dependant obstacles than *pgs1* mutation in yeast cell growth [7, 47]. The growth defect of the *pgs1* mutant on glycerol, lactate, or galactose in respiration sufficient condition could most likely be due to severely accumulated damages in mitochondrial function, probably including mitochondrial membrane potential, electron transport chain, and mitochondrial morphology by lack of PG/CL.

In the eukaryotic cell, the loss of PG/CL might cause mitochondrial disorder such as MPT, release cytochrome *c* that activates the apoptotic cascade pathway [15, 38,], and compromise directly the function of several essential enzymes in mitochondria. The release of cytochrome *c* and the decrease of membrane potential often occur under respiratory reduction condition [36, 39]. Human cancer cell line *SNU668* also severely induces growth defect on galactose medium in mitochondrial respiration deficient condition induced by antimycin A (1 μg/ml), oligomycin (1 μg/ml), and EtBr (1 μg/ml) (data not shown).

In glucose medium, the *pgs1* mutant also shows growth defect in conditional unfunctional mitochondria after the addition of EtBr (1 μg/ml) or antimycin A (1 μg/ml). However the *crd1* null mutant shows a growth phase similar to its parental wild-type cell in respiration deficient condition [7, 17]. The *CHO* cell *pgs1* mutant (*PGS-S*) also could not survive in galactose medium, and displays mitochondrial disorder [26]. Although wild-type yeast can grow very slowly in galactose in respiratory deficient condition, the growth of *rho* mutants are completely inhibited on galactose medium by the addition of antimycin A or ethidium bromide [17, 32]. Usually, *rho*^{0} and *rho*$^-$ mutants have less *PGS1* activity than the *rho*$^+$ strain [16], and the repression of [^{14}C]-galactose incorporation in wild-type yeast cell by antimycin A and the dramatic dynamics to recover the [^{14}C]-galactose incorporation in the *pgs1* mutant by exogenous *PGS1* represent the critical role of *PGS1* in the galactose metabolic system. Oxidative phosphorylation depends on the assembly of both respiratory chain generating electrochemical potential and the F_0F_1-ATPase complex [2, 29]. However, loss of the *PGS1* gene alters the phospholipid composition in the mitochondrial membrane, resulting in the loss of mitochondrial membrane potential and lack of respiratory enzymatic activities [36, 37].

In the *pgs1* mutant cell, the galactose metabolic pathway should not be constitutively damaged, because only exogenously expressed *PGS1* restored [^{14}C]-galactose incorporation and the *pgs1* mutant responded to (2%) galactose-(0.1%) glucose, but not 2% galactose, thus showing low β-galactosidase activity because of carbon catabolite repression by the transformed *Gal2-lacZ* fusion gene. Inactivation of *GAL1* and the *GAL2* gene promoter in the *pgs1* mutant showed that this mutant could not incorporate enough galactose to induce *GAL1* and *GAL2* gene expression. The threshold of galactose concentration and the expressional level of exogenous *PGS1* gene needed for *GAL* gene expression could be determined in the *pgs1* mutant with the *tet*-regulated *PGS1* expression vector system that we constructed [37].

On the other hand, these specific incapabilities of galactose in the *pgs1* mutant might occur on cellular membrane barriers and cellular surface synthesis, which might directly be related with inactivation of the *GAL2* transporter in the extracellular nutritional environment. Some respiratory deficient petite mutants show abnormal cell surface characteristics [13, 32].

Some nuclear genes are also differentially expressed depending on mitochondrial respiratory conditions [22, 32]. A large number of nuclear genes and their products are also required for the assembly of functional mitochondria, and nuclear petite mutants are dependent on proper function in the mitochondrial genetic and protein synthetic system [22]. In *Saccharomyces cerevisiae*, the *RTG1* and *RTG2* genes play a role in controlling interorganella communication such as mitochondria, peroxisome, and nucleus [8, 18]. Nuclear genes, *imp1*, *imp2*, and *Fzo1* mutants cannot grow on non-fermentable carbon sources in respiratory deficient condition. *IMP1* is allelic to the *GAL2* gene, because exogenous *GAL2* gene expression allows *imp1*/*rho*^{0} strains to grow on galactose [11, 44]. The mutation of *IMP2*, which is a positive regulator of maltose permease and galactose permease, also cannot grow on galactose medium in the presence of a mitochondrial respiratory inhibitor such as EtBr and erythromycin [12, 25, 30]. Probably, *pgs1* might be involved in the nucleo-mitochondrial control system for *GAL* gene expression.

However, it is not conclusive that galactose incapability by *pgs1* mutation includes alteration of signals for *GAL2* gene induction and low ATP production of *pgs1* mutant, which depends only on glycolysis without a mitochondrial high-efficient ATP production system. Deficient energy might make the galactose transporting system inactive, because cellular biochemical reactions depend on a tightly regulated ATP/ADP ratio [19, 39, 41].

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