Respiratory Deficiency Mediates the Regulation of CHO1-encoded Phosphatidylserine Synthase by mRNA Stability in Saccharomyces cerevisiae*  

Hyeon-Son Choi and George M. Carman#  

*From the Department of Food Science and Rutgers Center for Lipid Research, Rutgers University, New Brunswick, New Jersey 08901  

Running title: Respiratory Deficiency Mediates CHO1 mRNA Stability  

#To whom correspondence should be addressed. Dept of Food Science, Rutgers University, 65 Dudley Rd., New Brunswick, NJ 08901. Tel: 732-932-9611 (ext. 217); E-mail: carman@aesop.rutgers.edu  

The CHO1-encoded phosphatidylserine synthase (CDP-diacylglycerol:L-serine O-phosphatidyltransferase, EC 2.7.8.8) is one of the most highly regulated phospholipid biosynthetic enzymes in the yeast Saccharomyces cerevisiae. CHO1 expression is regulated by nutrient availability through a regulatory circuit involving a UASINO cis-acting element in the CHO1 promoter, the positive transcription factors Ino2p and Ino4p, and the transcriptional repressor Opi1p. In this work, we examined the posttranscriptional regulation of CHO1 by mRNA stability. CHO1 mRNA was stabilized in mutants defective in deadenylation (ccr4Δ), mRNA decapping (dcp1), and the 5'-3' exonuclease (xrn1) indicating that the CHO1 transcript is primarily degraded through the general 5'-3' mRNA decay pathway. In respiratory sufficient cells, the CHO1 transcript was moderately stable with a half-life of 12 min. However, the CHO1 transcript was stabilized to a half-life of greater than 45 min in respiratory deficient (rho- and rhoo) cells, the cox4Δ mutant defective in the cytochrome c oxidase, and wild type cells treated with KCN (a cytochrome c oxidase inhibitor). The increased CHO1 mRNA stability in response to respiratory deficiency caused increases in CHO1 mRNA abundance, phosphatidylserine synthase protein and activity, and the synthesis of phosphatidylserine in vivo. Respiratory deficiency also caused increases in the activities of CDP-diacylglycerol synthase, phosphatidylserine decarboxylase, and the phospholipid methyltransferases. Phosphatidylinositol synthase and choline kinase activities were not affected by respiratory deficiency. This work advances our understanding of phosphatidylserine synthase regulation and underscores the importance of mitochondrial respiration to the regulation of phospholipid synthesis in S. cerevisiae.
of the Kennedy pathway (Fig. 1). The PE synthesized by the Kennedy pathway may be methylated to PC via the CDP-DAG pathway (Fig. 1). Choline is used for PC synthesis via the CDP-choline branch of the Kennedy pathway (Fig. 1). In wild type cells, both the CDP-DAG and Kennedy pathways contribute to the synthesis of PC regardless of whether choline is supplemented to the growth medium (14-19). If choline is not present in the growth medium, the choline required for the Kennedy pathway is derived from the phospholipase D-mediated turnover of PC synthesized by way of the CDP-DAG pathway (19, 20).

PS synthase is regulated by biochemical and genetic mechanisms, both of which have an impact on the synthesis of PC via the CDP-DAG and Kennedy pathways (4-6, 21). The activity of PS synthase is modulated (i.e., inhibited or activated) by membrane phospholipids (e.g., PA, phosphatidylglycerol, cardiolipin,) (22-24), and is inhibited by inositol (25) and by the nucleotide CTP (17). In addition, the phosphorylation of the enzyme inhibits its activity, whereas dephosphorylation stimulates its activity (26, 27). In general, the inhibition of PS synthase activity favors PC synthesis via the Kennedy pathway (4, 5). The biochemical regulation of PS synthase activity also governs the partitioning of the substrate CDP-DAG between PS and PI; the inhibition of PS synthase activity favors PI synthesis (Fig. 1) (4).

The expression of the PS synthase (CHO1) gene is regulated by the supplementation of water-soluble phospholipid precursors (e.g., inositol) (28-31), zinc deprivation (32), and by growth phase (33, 34). CHO1 is maximally expressed in exponential phase cells when grown in the absence of inositol (28-31) and grown in the presence of zinc (32). CHO1 is repressed when inositol is supplemented to the growth medium (28-31) or when zinc is depleted from the growth medium (32). The zinc-mediated regulation of CHO1 occurs in the absence of inositol supplementation (32). Repression of CHO1 also occurs when cells enter the stationary phase of growth (33, 34). These forms of regulation are dependent on the UASNO cis-acting element in the promoter of the CHO1 gene (21). The derepression of CHO1 is mediated by a heterodimer complex of the positive transcription factors Ino2p and Ino4p that bind to a UASNO cis-acting element to drive transcription (5, 21, 35, 36). Repression of CHO1 is mediated by the repressor Opi1p, which interacts with Ino2p to attenuate transcription (5, 21, 35, 36). Opi1p repressor function is regulated by the cellular concentration of PA, which helps anchor the repressor to the nuclear/endoplasmic reticulum membrane apart from the Ino2p-Ino4p complex bound to the UASNO element (37). PA concentration and Opi1p repressor function is mediated in part by the PAH1-encoded Mg\(^{2+}\)-dependent PA phosphatase enzyme (38-40). Data (41) are consistent with a model (40) whereby activation of Mg\(^{2+}\)-dependent PA phosphatase activity results in a decrease in PA concentration followed by the translocation of Opi1p into the nucleus for interaction with Ino2p to repress CHO1 transcription. As discussed above for the biochemical regulation of PS synthase activity, the repression of CHO1 favors PI synthesis and the Kennedy pathway for PC synthesis (5).

Decay analysis of CHO1 mRNA in a cki1Δ eki1Δ mutant defective in the synthesis of phospholipids via the Kennedy pathway (Fig. 1) has revealed a novel mechanism by which CHO1 expression is regulated independent of the UASNO element in the CHO1 promoter (42). In wild type cells, CHO1 mRNA is moderately stable with a half-life of 12 min when compared with other S. cerevisiae mRNAs that have half-lives ranging from 1 to 60 min (43). However, CHO1 mRNA is greatly stabilized with a half-life > 45 min in the cki1Δ eki1Δ (KS106) mutant (42). This results in increased levels of the PS synthase protein and its associated activity (42). The objective of this work was to identify an intermediate or end product of the Kennedy that was responsible for regulation of CHO1 mRNA stability. During the course of this work, we discovered that the stabilization of CHO1 mRNA was not mediated by components of the Kennedy pathway, but instead it was mediated by a defect in mitochondrial respiration. This work underscores the importance of respiration to the regulation of phospholipid synthesis and
advances our understanding PS synthase regulation in yeast.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were reagent grade. Growth medium supplies were purchased from Difco Laboratories. The plasmid DNA purification and DNA gel extraction kits were from Qiagen, Inc. NEBlot kit, restriction endonucleases, recombinant Vent DNA polymerase, and modifying enzymes were purchased from New England Biolabs, Inc. RNA size markers were purchased from Promega. Yeast transformation kit was obtained from Clontech. Enhanced chemifluorescence Western blotting detection kit, polyvinylidene difluoride membranes, and ProbeQuant G-50 columns were purchased from GE Healthcare. Bio-Rad was the supplier of Zeta Probe blotting membranes, protein assay reagents, electrophoretic reagents, acrylamide solutions, immunochemical reagents, the DNA size ladder used for agarose gel electrophoresis, and protein molecular mass standards for SDS-PAGE. AdoMet, ampicillin, aprotinin, benzamidine, bovine serum albumin, choline, CTP, leupeptin, N-ethylmaleimide, pepstatin, phenylmethylsulfonyl fluoride, L-serine, and Triton X-100 were purchased from Sigma. Phospholipids were purchased from Avanti Polar Lipids. TLC plates were from EM science. Radiochemicals and scintillation counting supplies were from PerkinElmer Life Sciences and National Diagnostics, respectively. Thiolutin was a gift from Pfizer.

Strains, Plasmids, and Growth Conditions—The bacterial and yeast strains used in this work are listed in Table 1. Methods for the growth of bacteria and yeast were described previously (44, 45). Cells were grown at 30 °C in complete synthetic medium without inositol (46) using either 2% glucose or 2% glycerol as the carbon source. For selection of cells bearing plasmids, appropriate nutrients were omitted from synthetic complete medium. Glucose-grown cells were also cultured in the presence of 1 mM hydrogen peroxide to induce oxidative stress (47, 48). Cells in liquid media were grown to the exponential phase (1-2 x 10^7 cells/ml), and cell numbers were determined spectrophotometrically at an absorbance of 600 nm. Plasmids were maintained and amplified in Escherichia coli strain DH5α, which was grown in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl, pH 7.4) at 37 °C. Ampicillin (100 mg/ml) was added to cultures of DH5α carrying plasmids. For growth on plates, yeast and bacterial media were supplemented with 2% and 1.5% agar, respectively. Respiratory sufficiency was scored by growth on YPG (1% yeast extract, 2% peptone, 2% glycerol) and YPD (1% yeast extract, 2% peptone, 2% glucose) media plates (44, 45).

4′-6-Diamidino-2-phenylindole (DAPI)-staining of Mitochondria—Mitochondrial DNA of S. cerevisiae cells was examined by 4′-6-diamidino-2-phenylindole (DAPI) staining (49) using a Nikon Eclipse E800 fluorescence microscope equipped with a Hamamatsu Orca digital camera. Images were captured in monochrome and processed using Improvia Openlab software.

DNA Isolation and Manipulations—Plasmid and genomic DNA preparation, restriction enzyme digestion, and DNA ligations were performed according to standard protocols (45). Transformations of yeast (50) and E. coli (45) were performed as described previously. Plasmid maintenance and amplifications were performed in E. coli strain DH5α. Conditions for the amplification of DNA by PCR were optimized as described previously (51).

Construction of eki1Δ, ect1Δ, ept1Δ, and cki1Δ eki1Δ Mutants—A new eki1Δ mutant (HCY5) was constructed in the W303-1B background by the one-step gene replacement technique (52) with the eki1Δ::TRP1 disruption cassette as described by Kim et al. (53). The ect1Δ (HCY3) and ept1Δ (HCY4) mutants were similarly constructed in W303-1B using ect1Δ::TRP1 and ept1Δ::TRP1 disruption cassettes, respectively, that were prepared by PCR using appropriate primers and plasmid pRS414. Disruption of the chromosomal copies of the EKI1, ECT1, and EPT1 genes in the eki1Δ, ect1Δ, and ept1Δ, respectively, were
confirmed by PCR using the appropriate primers. Loss of the functions of the EKI1, ECT1, and EPT1 gene products in the mutants were confirmed by [1,2-14C]ethanolamine labeling and analysis of CDP-ethanolamine pathway intermediates (53-55). A new cki1Δ eki1Δ mutant (HCY7) was constructed by the one-step gene replacement technique (52) using the eki1Δ::TRP1 disruption cassette in the cki1Δ (KS105) mutant (53).

Analysis of CHO1 mRNA Decay—Total RNA was isolated from cells (43, 56), resolved overnight at 22V on a 1.1% formaldehyde gel (57), and then transferred to Zeta Probe membrane by vacuum blotting. The CHO1 (17) and PGK1 (58) probes were labeled with [α-32P]dTTP using the NEBlot random primer labeling kit. Unincorporated nucleotides were removed using ProbeQuant G-50 columns. Prehybridization, hybridization with the probes, and washes to remove non-specific binding were carried out according to manufacturer’s instructions. Images of radiolabeled mRNAs were acquired by phosphorimaging analysis. The half-life of CHO1 mRNA was determined after the arrest of transcription with thiolutin (15 µg/ml) as described by Gonzalez and Martin (58).

Immunoblotting—SDS-PAGE (59) using 12% slab gels and transfer of proteins to polyvinylidene difluoride membranes (60) were performed as described previously. The membrane was probed with a 1:500 dilution of anti-PS synthase antibodies (42). Goat anti-rabbit IgG alkaline phosphatase conjugate was used as a secondary antibody at a dilution of 1:5000. The PS synthase protein was detected using the enhanced chemifluorescence Western blotting detection kit, and the protein signals were acquired by Fluorimaging. The relative density of the protein was analyzed using ImageQuant software. Immunoblot signals were in the linear range of detectability.

Preparation of Yeast Cell Extract and the Total Membrane Fraction—The cell extract and total membrane fraction were prepared as described previously (61). Cell pellets were homogenized with glass beads at 4 °C in 50 mM Tris-maleate buffer (pH 7.0) containing 1 mM EDTA, 0.3 M sucrose, 10 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 5 µg/ml each of aprotinin, leupeptin, and pepstatin. The cell extract was obtained by centrifugation of the homogenate at 1,500 x g for 10 min. The cell extract was centrifuged at 100,000 x g for 1 h to obtain total membranes, which were then resuspended in 50 mM Tris-maleate (pH 7.0) buffer containing 10 mM MgCl2, 10 mM 2-mercaptoethanol, 20% glycerol (w/v), and 0.5 mM phenylmethylsulfonyl fluoride. Protein concentration was estimated by the method of Bradford (62) using bovine serum albumin as the standard.

Enzymes Assays—All assays were conducted in triplicate at 30 °C. CDP-DAG synthase activity was measured with 50 mM Tris-maleate buffer (pH 6.5), 20 mM MgCl2, 15 mM Triton X-100, 0.5 mM PA, and 1.0 mM [5-3H]CTP (63). PS synthase activity was measured with 50 mM Tris-HCl buffer (pH 8.0), 0.6 mM MnCl2, 3.2 mM Triton X-100, 0.2 mM CDP-DAG, and 0.5 mM [3-3H]serine (64). PS decarboxylase activity was measured with 50 mM Tris-HCl buffer (pH 7.2), 10 mM 2-mercaptoethanol, 5 mM EDTA, 2 mM Triton X-100, and 0.5 mM [3-3H]PS (65, 66). PE methyltransferase activity was measured with 50 mM Tris-HCl buffer (pH 7.5), 10 mM MgCl2, 0.2 mM phosphatidylinositol, and 0.5 mM [Me-3H]AdoMet (67). Phospholipid methyltransferase activity was measured with 50 mM Tris-HCl buffer (pH 7.5), 10 mM MgCl2, 0.2 mM phosphatidylmonomethylethanolamine, and 0.5 mM [Me-3H]AdoMet (67). PI synthase activity was measured with 50 mM Tris-HCl buffer (pH 8.0), 2 mM MnCl2, 3.2 mM Triton X-100, 0.2 mM CDP-DAG, and 1 mM [2-3H]inositol (68). Choline kinase activity was measured with 67 mM glycine-NaOH buffer (pH 9.5), 10 mM MgSO4, 1.3 mM dithiothreitol, 0.5 mM ATP, and 5 mM [methyl-3H]choline (69). All assays were linear with time and protein concentration. The average standard deviation of the assays was ±5%. A unit of phospholipid enzymatic activity was defined as the amount of enzyme that catalyzed the formation of 1 nmol of product per min.
Labeling and Analysis of Phospholipids—Labeling of phospholipids with $[14\text{C}]$serine was performed as described previously (12, 13). Phospholipid synthesis was followed by labeling exponential phase cells for 30 min. Phospholipids were extracted from labeled cells by the method of Bligh and Dyer (70). Two-dimension TLC with chloroform/methanol/ammonium hydroxide/H$_2$O (90:50:4:6) (dimension 1) and chloroform/methanol/acetic acid/H$_2$O (63:8:10:2) (dimension 2) was used to separate phospholipids (42). Radiolabeled phospholipids on the chromatography plates were visualized by phosphorimaging, and their identities were confirmed by comparison with standard phospholipids after exposure to iodine vapor. The amount of each $^{14}$C-labeled phospholipid was determined by liquid scintillation counting.

Analyses of Data—Statistical analyses were performed with SigmaPlot software. Statistical significance was determined by performing the Student’s $t$-test. $P$ values < 0.05 were taken as a significant difference.

RESULTS

CHO1 mRNA Decay Involves Deadenylation, Decapping, and 5’-3’ Exonuclease Cleavage—Many mRNAs in S. cerevisiae are degraded by a general deadenylation-dependent pathway involving the deadenylation of the poly(A) tail by Ccr4p, the removal of the 5’ cap by the Dep1p-Dep2p complex, and the 5’-3’ exonuclease cleavage by Xrn1p (71). To examine whether CHO1 mRNA is degraded by this general pathway, a decay analysis was performed in mutants defective in each of the three steps. The rate of CHO1 mRNA decay was reduced in the ccr4Δ mutant defective in deadenylation, in the dep1Δ mutant defective in decapping, and in the xrn1Δ mutant defective in 5’-3’ exonuclease cleavage (Fig. 2, Table 3). That CHO1 mRNA was stabilized in these mutants indicated that the CHO1 transcript is degraded by the general decay pathway (71).

Effects of the ckiΔ, ekiΔ, and ckiΔ ekiΔ Mutations on CHO1 mRNA Decay—Previous studies have shown that the ckiΔ ekiΔ mutations have a stabilizing effect on the abundance of CHO1 mRNA (42). It is unclear, however, whether the signal responsible for increased CHO1 mRNA stability in the ckiΔ ekiΔ (KS106) mutant results from a defect in the CDP-choline branch, the CDP-ethanolamine branch, or from both branches of the Kennedy pathway. To address this question, CHO1 mRNA decay was examined in the ckiΔ (KS105) mutant (53) defective in the first step of the CDP-choline pathway and in the ekiΔ (KS101) mutant (53) defective in the first step of the CDP-ethanolamine pathway (Fig. 1). CHO1 mRNA decay was also reexamined in the ckiΔ ekiΔ (KS106) mutant. The half-life of CHO1 mRNA decay in the ckiΔ mutant was similar to that observed in the wild type control (Fig. 3, Table 3). In contrast, CHO1 mRNA was stabilized in the ekiΔ (KS101) mutant (Fig. 3, Table 3). Thus, the increase in CHO1 mRNA stability in the ckiΔ ekiΔ (KS106) mutant was due to the ekiΔ mutation (i.e., defect in the CDP-ethanolamine branch).

Effects of CDP-ethanolamine Pathway Mutations on CHO1 mRNA Decay—Mutations in the second (ect1Δ) and third (ept1Δ) steps of the CDP-ethanolamine pathway (i.e., phosphoethanolamine cytidylyltransferase and phosphocholine cytidylyltransferase, respectively) were constructed and examined for their effects on CHO1 mRNA decay. Of the three mutants, ekiΔ (KS101) was the only mutant in which CHO1 mRNA was stabilized (Fig. 4, Table 3). The effects of the three CDP-ethanolamine pathway mutations on the abundance of CHO1 mRNA and PS synthase protein (Cho1p) were also examined (Fig. 5). The levels of CHO1 mRNA and PS synthase protein were elevated by about 2-fold in the ekiΔ (KS101) mutant when compared with the wild type control (Fig. 5). As described previously (42), CHO1 mRNA and Cho1p were elevated in the ckiΔ ekiΔ (KS106) mutant. These results correlated with the increase in CHO1 mRNA stability observed in the ekiΔ and ckiΔ ekiΔ mutants. On the other hand, the
CHO1 gene products were not affected in the ect1Δ and ept1Δ mutants (Fig. 5).

Loss of the EKI1 Gene Is Not Responsible for Increased CHO1 mRNA Stability in the eki1Δ (KS101) Mutant—To confirm that the deletion of the EKI1 gene was responsible for the increased stability of CHO1 mRNA, the eki1Δ (KS101) mutant was transformed with a single copy plasmid bearing the EKI1 gene. Unexpectedly, the introduction of the EKI1 gene into the eki1Δ (KS101) mutant did not reverse the stable CHO1 mRNA phenotype of the mutant (Fig. 6, Table 3). This result indicated that a second mutation in the eki1Δ mutant was responsible for increasing CHO1 mRNA stability. Accordingly, a new eki1Δ (HCY5) mutant was constructed by the same method (53) used to construct the original eki1Δ (KS101) mutant. The analysis of CHO1 mRNA decay in the new eki1Δ mutant confirmed that the loss of the EKI1 gene was not responsible for the increased stability of CHO1 mRNA. Accordingly, a new eki1Δ (HCY5) mutant was used to construct the original eki1Δ (KS101) mutant. The analysis of CHO1 mRNA decay in the new eki1Δ mutant confirmed that the loss of the EKI1 gene was not responsible for the increased stability of CHO1 mRNA (Fig. 6, Table 3). Moreover, a new cki1Δ eki1Δ HCY7 mutant was constructed, and it exhibited the wild type rate of CHO1 mRNA decay.

The eki1Δ (KS101) and cki1Δ eki1Δ (KS106) Mutants Are Respiratory Deficient—Unlike wild type (W303-1B) cells that formed pink colonies on agar plates, the eki1Δ (KS101) and cki1Δ eki1Δ (KS106) mutant colonies were white and relatively small (i.e., petite) in appearance. These characteristics were first thought to be due to the eki1Δ mutation. However, the colonies produced by the new eki1Δ (HCY5) and cki1Δ eki1Δ (HCY7) mutants were similar in appearance (i.e., normal size and pink) to their wild type parent W303-1B. Petite white colonies are characteristics of respiratory-deficient mutants (44). Respiratory deficient mutants are defective in mitochondrial function and cannot grow on non-fermentable carbon sources (44, 72). With this in mind, the respiratory sufficiency of the original eki1Δ (KS101) and cki1Δ eki1Δ (KS106) mutants were examined by growth on agar plates containing glucose (fermentable) or glycerol (non-fermentable) as the carbon source. Like known respiratory mutants (MGY100 and W303 [rho0]), the original eki1Δ (KS101) and cki1Δ eki1Δ (KS106) mutants only grew on glucose agar plates, whereas the new eki1Δ (HCY5) and cki1Δ eki1Δ (HCY7) mutants grew on both glucose and glycerol agar plates (Table 4). These data indicated that the original mutants were respiratory deficient. The white colony phenotype can be attributed to respiratory deficiency because the development of a pink color in the ade2 genetic background found in the eki1Δ (KS101) and cki1Δ eki1Δ (KS106) mutants and the parent W303-1B strain would be dependent on oxidative phosphorylation (73). Another characteristic common to respiratory-deficient mutants is the inability to grow at elevated temperatures (74). Indeed, the original eki1Δ (KS101) and cki1Δ eki1Δ (KS106) mutants were temperature sensitive for growth at 37°C, whereas the new mutants were not temperature sensitive for growth (Table 4).

Interestingly, the new eki1Δ (HCY5) mutant had a tendency to give rise to spontaneous petite colonies that were white in color. Likewise, the wild type parent W303-1B also gave rise to petite white colonies, but at a lower frequency. One petite colony (strain HCY6) that was derived from the new eki1Δ (HCY5) mutant and one petite colony (strain HCY8) that was derived from the parent strain W303-1B were examined for their growth on glucose and glycerol, and for their growth on glucose at 37°C. Strains HCY6 and HCY8 exhibited the phenotypes of respiratory deficiency (Table 4).

Respiratory deficient mutants are differentiated by the extent of mitochondrial DNA that they lack. Cells that have a reduced amount of mitochondrial DNA are designated rho−, whereas cells that lack mitochondrial DNA are designated rho0 (44, 72). Florescence microscopy of cells stained with 4',6-diamidino-2-phenylindole (DAPI) (44) indicated that KS101, KS106, and HCY6 were rho0, whereas HCY8 was rho−.

Respiratory Deficiency Is Responsible for Increased CHO1 mRNA Stability—CHO1 mRNA decay was examined in two respiratory deficient mutants. These mutants included a
known rho\(^{-}\) mutant (MGY100) and a known rho\(^{o}\) mutant (W303-1A [rho\(^{o}\)]). The two respiratory mutants exhibited an increase in the half-life of CHO1 mRNA when compared with that of the respiratory sufficient control (Fig. 7, Table 3). Thus, respiratory deficiency, whether due to a partial or total lack of the mitochondrial genome, gave rise to increased CHO1 mRNA stability. These data also confirmed that the basis for the increased stability of CHO1 mRNA in the original eki1\(^{\Delta}\) (KS101) mutant was respiratory deficiency.

Respiration is a major function of the mitochondrion, which takes place at the inner mitochondrial by five-enzymatic complexes (75). KCN, a specific inhibitor of cytochrome c oxidase (complex IV), is commonly used to halt the electron transport chain and mitochondrial respiration (75, 76). To determine the effects of inhibiting respiration on CHO1 mRNA stability, respiratory-sufficient cells (W303-1B) were grown in the absence and presence of KCN. The addition of KCN to the growth medium resulted in a dose-dependent increase in the half-life of CHO1 mRNA (Fig. 8, Table 3). To further confirm that a block in respiration was responsible for the increase in CHO1 mRNA stability, the decay of the CHO1 transcript was analyzed in the cox4\(^{\Delta}\) mutant. COX4 is a nuclear gene that encodes an indispensable subunit of cytochrome c oxidase (77). The decay rate of CHO1 mRNA was much longer in the cox4\(^{\Delta}\) mutant when compared with the wild type control (Fig. 9, Table 3). Taken together, these data provided strong evidence that respiratory deficiency mediates CHO1 mRNA stability.

**Effects of Respiratory Deficiency on the Abundance of CHO1 mRNA, PS Synthase Protein, PS Synthase Activity, and on the Synthesis of PS In Vivo**—Northern blot and Western blot analyses for the CHO1 gene products were performed with a respiratory deficient mutant and the results are presented in Figs. 10A and B, respectively. The levels of both CHO1 mRNA and PS synthase protein (Cho1p) were about 2-fold higher in the respiratory deficient mutant when compared with the levels in the wild type control. The increased levels of CHO1 mRNA and PS synthase protein in the respiratory mutant correlated with an increased level of PS synthase activity (Fig. 10C). Elevated PS synthase activity was also characteristic of respiratory deficient eki1\(^{\Delta}\) (KS101) and eki1\(^{\Delta}\) eki1\(^{\Delta}\) (KS106) mutants, but not respiratory sufficient eki1\(^{\Delta}\) (HCY5) and eki1\(^{\Delta}\) eki1\(^{\Delta}\) (HCY7) mutants. To examine the effect of respiratory deficiency on the synthesis of PS in vivo, exponential phase cells were labeled with \(^{14}C\)serine for 30 min followed by the extraction and analysis of phospholipids. \(^{14}C\)Serine is directly incorporated into PS via the PS synthase enzyme, followed by the incorporation of the label into PE and PC via the reactions catalyzed by the PS decarboxylase and phospholipid methyltransferase enzymes (i.e., CDP-DAG pathway) (5, 12). The respiratory deficient mutant showed a 1.9-fold increase in the incorporation of \(^{14}C\)serine into PS after the 30 min labeling period (Fig. 10D). The effects of respiratory deficiency on the incorporation of the label into PE and PC were less dramatic (Fig. 10D). Respiratory deficiency did not have a significant effect on the steady state composition of phospholipids labeled \(^{14}C\)serine.

*Effects of Respiratory Deficiency on the Levels of Phospholipid Synthesis Enzyme Activities*—In addition to PS synthase, the activities of other CDP-DAG pathway enzymes were elevated in the original cki1\(^{\Delta}\) eki1\(^{\Delta}\) (KS106) mutant (42). To address whether this regulation was due to the Kennedy pathway mutations or due to respiratory deficiency, the enzyme activity analyses were performed with the rho\(^{\circ}\) (HCY8) mutant. The levels of CDP-DAG synthase (29%), PS decarboxylase (26%), PE methyltransferase (60%), and phospholipid methyltransferase (34%) activities were elevated in respiratory deficient cells when compared with the control (Fig. 11). On the other hand, the activities of PI synthase and choline kinase were not affected by respiratory deficiency (Fig. 11). The analyses of these activities in the new cki1\(^{\Delta}\) eki1\(^{\Delta}\) mutant (HCY7) showed that the Kennedy pathway mutations did not affect the
activity levels of the CDP-DAG pathway enzymes.

Effects of Oxidative Stress and Respiratory Growth on CHO1 mRNA Decay/Stability—We questioned whether oxidative stress affected the rate of CHO1 mRNA decay. For this experiment, glucose-grown wild type cells were incubated with hydrogen peroxide, a treatment commonly used to induce oxidative stress (47, 48). This treatment did not affect the rate of CHO1 mRNA decay. We also questioned whether respiratory growth affected the decay of the CHO1 transcript. The rate of CHO1 mRNA decay of wild type cells grown with glycerol was not significantly different from cells grown with glucose.

DISCUSSION

In this work, we showed that the increased stability of CHO1 mRNA in the originalcki1Δeki1Δ (KS106) mutant was caused by respiratory deficiency associated with the loss of ethanolamine kinase (i.e., eki1Δ mutation) and not from the Kennedy pathway mutations per se. Decay analysis of CHO1 mRNA using respiration-deficient mutants (rho' and rho"), the cox4Δ mutant defective in the cytochrome c oxidase, and wild type cells treated with KCN (a cytochromec oxidase inhibitor) confirmed that respiratory deficiency was responsible for the increase in the half-life of the CHO1 transcript. Moreover, the increased CHO1 mRNA stability in response to respiratory deficiency was responsible for increased PS synthase protein and activity, and the synthesis of PS in vivo.

It was not too surprising that the eki1Δ mutation gave rise to respiratory deficient cells. Phospholipid synthesis mutants (e.g., cho1, psd1Δ, opi3) commonly give rise to respiratory deficient petite colonies (13, 78, 79). Although it is not yet clear how the eki1Δ mutation caused respiratory deficiency, the reason for this general phenomenon may be attributed to the important roles that phospholipids (e.g., cardiolipin and PE) play in both the composition and function of mitochondrial membranes (78, 80-88).

Genome-wide analyses of gene expression in S. cerevisiae have shown that the levels of CHO1 mRNA decrease when cells transit from glucose-based fermentative to glycerol-based respiratory growth (89), and that CHO1 mRNA levels increase in response to oxygen deprivation (90). These findings indicate that a lack of mitochondrial respiration is a physiological condition that requires an increase in the abundance of the CHO1 transcript, supporting the role of mitochondrial respiration in the control of CHO1 expression. We showed in this study that the rate of CHO1 mRNA decay was not affected in glycerol-grown cells. Thus, the regulation that occurs when cells transit from fermentative to non-fermentative growth occurs at the level of transcription. Because respiratory deficiency may lead to oxidative stress, we questioned whether incubation with hydrogen peroxide affected the rate CHO1 mRNA stability. This stress condition did not affect the decay rate of CHO1 mRNA.

CHO1 mRNA was stabilized in mutants defective in deadenylation (ccr4Δ), mRNA decapping (dcp1), and the 5'-3' exonuclease (xrn1). These results indicated that the CHO1 transcript is primarily degraded through the general 5'-3' mRNA decay pathway (71). The specific mechanisms and regulators that control the stabilization of CHO1 mRNA in response to respiratory deficiency are unknown. Given that CHO1 mRNA decays by the primary 5'-3' decay pathway when cells are respiratory sufficient, it is reasonable to predict that the rate of deadenylation and/or decapping may be reduced when respiration is blocked. Extensive studies will be required to identify cis-acting elements in the transcript as well as components of the signal transduction system that lead from the respiratory defect to the mRNA stabilization response.

The activities of other CDP-DAG pathway enzymes (e.g., CDP-DAG synthase, PS decarboxylase, and the phospholipid methyltransferase enzymes) were also elevated in respiratory deficient cells. These results were similar to that found in the respiratory deficientcki1Δeki1Δ (KS106) mutant (42). A reanalysis of these enzyme activities in the respiratory
sufficient ck1Δ eklΔ (HCY7) mutant confirmed that the regulation was solely due to the rho° mutation. The regulation of phospholipid synthesis enzymes in response to respiratory deficiency was not a universal response as the PI synthase and choline kinase activities were not affected by respiratory deficiency.

OLE1, which encodes the Δ-9 fatty acid desaturase, is another yeast gene of lipid metabolism whose expression is independently regulated at the levels of transcription and by mRNA stability (58, 91, 92). The OLE1 transcript is destabilized when cells are supplemented with unsaturated fatty acids (58, 91, 92). This fatty acid-regulated decay of OLE1 mRNA occurs through both the 5'-3' general degradation pathway and via exosomal 3'-5' degradation activities (92). Interestingly, like CHO1 mRNA, OLE1 transcript levels are more abundant under oxygen-deprived conditions (92). It is not clear, however, whether this regulation is due to an increased rate of transcription, a decreased rate of mRNA decay or contributions from both regulatory systems.

In summary, we showed that the CHO1 transcript is degraded by the general mRNA decay pathway, and clarified that a block in the Kennedy pathway for phospholipid synthesis was not responsible for the regulation of PS synthase by mRNA stability. The rate of mRNA decay plays an important role in the control of gene expression (93). The half-life of an mRNA governs the number of times a transcript can be translated, which in turn governs the amount of protein that can be produced at a given rate of transcription (93). We hypothesize that S. cerevisiae cells compensated for the stress of respiratory deficiency by conserving the CHO1 transcript for translation to the PS synthase enzyme for the synthesis of PS. This work advances our understanding of PS synthase regulation and underscores the importance of mitochondrial respiration to the regulation of phospholipid synthesis in S. cerevisiae.

Acknowledgments—We thank Roy Parker, Miriam Greenberg, and William Dowhan for mutants defective in the mRNA decay pathway, respiratory deficient strains, and the cox4Δ mutant, respectively. Dawn Brasaemle is acknowledged for the use of the Nikon Eclipse E800 microscope, and we thank Jeanelle Morgan for assistance with fluorescence microscopy. Gil-Soo Han, Charles Martin, and Robert Ramirez are acknowledged for helpful discussions during the preparation of this manuscript.
REFERENCES

1. Letts, V. A., Klig, L. S., Bae-Lee, M., Carman, G. M., and Henry, S. A. (1983) *Proc.Natl.Acad.Sci.USA* **80**, 7279-7283

2. Nikawa, J., Tsukagoshi, Y., Kodaki, T., and Yamashita, S. (1987) *Eur.J.Biochem.* **167**, 7-12

3. Kiyono, K., Miura, K., Kushima, Y., Hikiji, T., Fukushima, M., Shibuya, I., and Ohta, A. (1987) *J.Biochem.* **102**, 1089-1100

4. Carman, G. M. and Zeimetz, G. M. (1996) *J.Biol.Chem.* **271**, 13293-13296

5. Carman, G. M. and Henry, S. A. (1999) *Prog.Lipid Res.* **38**, 361-399

6. Yamashita, S. and Nikawa, J. (1997) *Biochim.Biophys.Acta* **1348**, 228-235

7. Natter, K., Leitner, P., Faschinger, A., Wolinski, H., McCraith, S., Fields, S., and Kohlwein, S. D. (2005) *Mol.Cell Proteomics.* **4**, 662-672

8. Bae-Lee, M. and Carman, G. M. (1984) *J.Biol.Chem.* **259**, 10857-10862

9. Rattray, J. B., Schibeci, A., and Kidby, D. K. (1975) *Bacteriol.Reviews* **39**, 197-231

10. Henry, S. A. (1982) in *The molecular biology of the yeast Saccharomyces. Metabolism and gene expression* (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) pp. 101-158, Cold Spring Harbor Laboratory, Cold Spring Harbor

11. Paltauf, F., Kohlwein, S. D., and Henry, S. A. (1992) in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression* (Jones, E. W., Pringle, J. R., and Broach, J. R., eds) pp. 415-500, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

12. Atkinson, K., Fogel, S., and Henry, S. A. (1980) *J.Biol.Chem.* **255**, 6653-6661

13. Atkinson, K. D., Jensen, B., Kolat, A. I., Storm, E. M., Henry, S. A., and Fogel, S. (1980) *J.Bacteriol.* **141**, 558-564

14. Morash, S. C., McMaster, C. R., Hjelmstad, R. H., and Bell, R. M. (1994) *J.Biol.Chem.* **269**, 28769-28776

15. McGee, T. P., Skinner, H. B., Whitters, E. A., Henry, S. A., and Bankaitis, V. A. (1994) *J.Cell Biol.* **124**, 273-287

16. McMaster, C. R. and Bell, R. M. (1994) *J.Biol.Chem.* **269**, 28010-28016

17. McDonough, V. M., Buxeda, R. J., Bruno, M. E. C., Ozier-Kalogeropoulos, O., Adeline, M.-T., McMaster, C. R., Bell, R. M., and Carman, G. M. (1995) *J.Biol.Chem.* **270**, 18774-18780

18. Ostrander, D. B., O'Brien, D. J., Gorman, J. A., and Carman, G. M. (1998) *J.Biol.Chem.* **273**, 18992-19001
19. Patton-Vogt, J. L., Griac, P., Sreenivas, A., Bruno, V., Dowd, S., Swede, M. J., and Henry, S. A. (1997) J. Biol. Chem. **272**, 20873-20883

20. Xie, Z. G., Fang, M., Rivas, M. P., Faulkner, A. J., Sternweis, P. C., Engebrecht, J., and Bankaitis, V. A. (1998) Proc. Natl. Acad. Sci. USA **95**, 12346-12351

21. Chen, M., Hancock, L. C., and Lopes, J. M. (2007) Biochim. Biophys. Acta **1771**, 310-321

22. Hromy, J. M. and Carman, G. M. (1986) J. Biol. Chem. **261**, 15572-15576

23. Bae-Lee, M. and Carman, G. M. (1990) J. Biol. Chem. **265**, 7221-7226

24. Oshiro, J., Rangaswamy, S., Chen, X., Han, G.-S., Quinn, J. E., and Carman, G. M. (2000) J. Biol. Chem. **275**, 40887-40896

25. Kelley, M. J., Bailis, A. M., Henry, S. A., and Carman, G. M. (1988) J. Biol. Chem. **263**, 18078-18085

26. Kinney, A. J. and Carman, G. M. (1988) Proc. Nat. Acad. Sci. USA **85**, 7962-7966

27. Kinney, A. J., Bae-Lee, M., Singh Panghaal, S., Kelley, M. J., Gaynor, P. M., and Carman, G. M. (1990) J. Bacteriol. **172**, 1133-1136

28. Klig, L. S., Homann, M. J., Carman, G. M., and Henry, S. A. (1985) J. Bacteriol. **162**, 1135-1141

29. Poole, M. A., Homann, M. J., Bae-Lee, M., and Carman, G. M. (1986) J. Bacteriol. **168**, 668-672

30. Bailis, A. M., Poole, M. A., Carman, G. M., and Henry, S. A. (1987) Mol. Cell. Biol. **7**, 167-176

31. Bailis, A. M., Lopes, J. M., Kohlwein, S. D., and Henry, S. A. (1992) Nucleic Acids Res. **20**, 1411-1418

32. Iwanyshyn, W. M., Han, G. S., and Carman, G. M. (2004) J. Biol. Chem. **279**, 21976-21983

33. Homann, M. J., Poole, M. A., Gaynor, P. M., Ho, C.-T., and Carman, G. M. (1987) J. Bacteriol. **169**, 533-539

34. Lamping, E., Luckl, J., Paltuf, F., Henry, S. A., and Kohlwein, S. D. (1995) Genetics **137**, 55-65

35. Greenberg, M. L. and Lopes, J. M. (1996) Microbiol. Rev. **60**, 1-20

36. Henry, S. A. and Patton-Vogt, J. L. (1998) Prog. Nucleic Acid Res. **61**, 133-179

37. Loewen, C. J. R., Gaspar, M. L., Jesch, S. A., Delon, C., Ktistakis, N. T., Henry, S. A., and Levine, T. P. (2004) Science **304**, 1644-1647

38. Santos-Rosa, H., Leung, J., Grimsey, N., Peak-Chew, S., and Siniossoglou, S. (2005) EMBO J **24**, 1931-1941

39. Han, G.-S., Wu, W.-I., and Carman, G. M. (2006) J Biol. Chem. **281**, 9210-9218

40. Carman, G. M. and Han, G. S. (2006) Trends Biochem Sci **31**, 694-699
41. O'Hara, L., Han, G. S., Peak-Chew, S., Grimsey, N., Carman, G. M., and Siniosoglou, S. (2006) *J Biol.Chem.* **281**, 34537-34548

42. Choi, H. S., Sreenivas, A., Han, G.-S., and Carman, G. M. (2004) *J.Biol.Chem.* **279**, 12081-12087

43. Herrick, D., Parker, R., and Jacobson, A. (1990) *Mol Cell Biol* **10**, 2269-2284

44. Rose, M. D., Winston, F., and Heiter, P. (1990) *Methods in Yeast Genetics: A Laboratory Course Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

45. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning, A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

46. Culbertson, M. R. and Henry, S. A. (1975) *Genetics* **80**, 23-40

47. Jamieson, D. J. (1992) *J.Bacteriol.* **174**, 6678-6681

48. Jamieson, D. J., Rivers, S. L., and Stephen, D. W. (1994) *Microbiology* **140**, 3277-3283

49. Kaiser, C., Michaelis, S., and Mitchell, A. (1994) *Methods in Yeast Genetics: A Laboratory Course Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

50. Ito, H., Yasuki, F., Murata, K., and Kimura, A. (1983) *J.Bacteriol.* **153**, 163-168

51. Innis, M. A. and Gelfand, D. H. (1990) in *PCR Protocols. A Guide to Methods and Applications* (Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J., eds) pp. 3-12, Academic Press, Inc., San Diego

52. Rothstein, R. (1991) *Methods Enzymol.* **194**, 281-301

53. Kim, K., Kim, K.-H., Storey, M. K., Voelker, D. R., and Carman, G. M. (1999) *J.Biol.Chem.* **274**, 14857-14866

54. Elabbadi, N., Ancelin, M. L., and Vial, H. J. (1997) *Biochem J* **324**, 435-445

55. Kersting, M. C., Choi, H. S., and Carman, G. M. (2004) *J Biol Chem.* **279**, 35353-35359

56. Schmitt, M. E., Brown, T. A., and Trumpower, B. L. (1990) *Nucleic Acids Res* **18**, 3091-3092

57. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1993) *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York

58. Gonzalez, C. I. and Martin, C. E. (1996) *J.Biol.Chem.* **271**, 25801-25809

59. Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685

60. Haid, A. and Suissa, M. (1983) *Methods Enzymol.* **96**, 192-205

61. Oshiro, J., Han, G.-S., Iwanyshyn, W. M., Conover, K., and Carman, G. M. (2003) *J.Biol.Chem.* **278**, 31495-31503

62. Bradford, M. M. (1976) *Anal.Biochem.* **72**, 248-254
63. Carman, G. M. and Kelley, M. J. (1992) *Methods Enzymol.* **209**, 242-247
64. Carman, G. M. and Bae-Lee, M. (1992) *Methods Enzymol.* **209**, 298-305
65. Carson, M. A., Emala, M., Hogsten, P., and Waechter, C. J. (1984) *J.Biol.Chem.* **259**, 6267-6273
66. Lamping, E., Kohlwein, S. D., Henry, S. A., and Paltauf, F. (1991) *J.Bacteriol.* **173**, 6432-6437
67. Gaynor, P. M. and Carman, G. M. (1990) *Biochim.Biophys.Acta* **1045**, 156-163
68. Carman, G. M. and Fischl, A. S. (1992) *Methods Enzymol.* **209**, 305-312
69. Porter, T. J. and Kent, C. (1992) *Methods Enzymol.* **209**, 134-146
70. Bligh, E. G. and Dyer, W. J. (1959) *Can.J.Biochem.Physiol.* **37**, 911-917
71. Parker, R. and Song, H. (2004) *Nat.Struct.Mol.Biol.* **11**, 121-127
72. Traven, A., Wong, J. M., Xu, D., Sopta, M., and Ingles, C. J. (2001) *J.Biol.Chem.* **276**, 4020-4027
73. Meskauskas, A., Ksenzenko, V., Shlyapnikov, M., Kryukov, V., and Citavicius, D. (1985) *FEBS Lett.* **182**, 413-414
74. Cause, G. F. and Kusovkova, L. I. (1970) *Experientia* **26**, 209-210
75. Garett, R. H and Grisham, C. M. Biochemistry. SECOND. 1999. Saunders College Publishing.
76. Mootha, V. K., Wei, M. C., Buttle, K. F., Scorrano, L., Panoutsakopoulou, V., Mannella, C. A., and Korsmeyer, S. J. (2001) *EMBO J.* **20**, 661-671
77. Dowhan, W., Bibus, C. R., and Schatz, G. (1985) *EMBO J.* **4**, 179-184
78. Birmer, R., Burgermeister, M., Schneiter, R., and Daum, G. (2001) *Mol.Biol.Cell* **12**, 997-1007
79. Greenberg, M. L., Hubbell, S., and Lam, C. (1988) *Mol.Cell.Biol.* **8**, 4773-4779
80. Li, G., Chen, S., Thompson, M. N., and Greenberg, M. L. (2007) *Biochim.Biophys.Acta* **1771**, 432-441
81. Gohil, V. M., Hayes, P., Matsuyama, S., Schagger, H., Schlame, M., and Greenberg, M. L. (2004) *J Biol.Chem.* **279**, 42612-42618
82. Zhong, Q., Gohil, V. M., Ma, L., and Greenberg, M. L. (2004) *J Biol.Chem.* **279**, 32294-32300
83. Schlame, M., Rua, D., and Greenberg, M. L. (2000) *Prog.Lipid Res.* **39**, 257-288
84. Su, X. and Dowhan, W. (2006) *Mol.Cell Biol.* **26**, 743-753
85. Zhang, M., Mileykovskaya, E., and Dowhan, W. (2005) *J Biol.Chem.* **280**, 29403-29408
86. McMillin, J. B. and Dowhan, W. (2002) *Biochim.Biophys.Acta* **1585**, 97-107
87. Ostrander, D. B., Zhang, M., Mileykovskaya, E., Rho, M., and Dowhan, W. (2001) *J Biol.Chem.* **276**, 25262-25272

88. Gohil, V. M., Thompson, M. N., and Greenberg, M. L. (2005) *J Biol.Chem.* **280**, 35410-35416

89. Roberts, G. G. and Hudson, A. P. (2006) *Mol.Genet.Genomics* **276**, 170-186

90. Lai, L. C., Kosorukoff, A. L., Burke, P. V., and Kwast, K. E. (2006) *Eukaryot.Cell* **5**, 1468-1489

91. Vemula, M., Kandasamy, P., Oh, C. S., Chellappa, R., Gonzalez, C. I., and Martin, C. E. (2003) *J.Biol.Chem.* **278**, 45269-45279

92. Martin, C. E., Oh, C. S., and Jiang, Y. (2007) *Biochim.Biophys.Acta* **1771**, 271-285

93. Guhaniyogi, J. and Brewer, G. (2001) *Gene* **265**, 11-23

94. Vance, J. E. (1998) *Trends Biochem Sci* **23**, 423-428

95. Hatfield, L., Beelman, C. A., Stevens, A., and Parker, R. (1996) *Mol.Cell Biol.* **16**, 5830-5838

96. Tucker, M., Valencia-Sanchez, M. A., Staples, R. R., Chen, J., Denis, C. L., and Parker, R. (2001) *Cell* **104**, 377-386

97. Thomas, B. and Rothstein, R. (1989) *Cell* **56**, 619-630

98. van Loon, A. P., Van Eijk, E., and Grivell, L. A. (1983) *EMBO J* **2**, 1765-1770

99. Sikorski, R. S. and Hieter, P. (1989) *Genetics* **122**, 19-27

100. Hill, J. E., Myers, A. M., Koerner, T. J., and Tzagoloff, A. (1986) *Yeast* **2**, 163-167

101. He, F., Peltz, S. W., Donahue, J. L., Rosbash, M., and Jacobson, A. (1993) *Proc.Natl.Acad.Sci.U.S.A* **90**, 7034-7038
FOOTNOTES

*This work was supported in part by United States Public Health Service, National Institutes of Health Grant GM-50679.

1The abbreviations used are: PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PA, phosphatidate; CDP-DAG, CDP-diacylglycerol.

2The S. cerevisiae PS synthase enzyme should not be confused with the PS synthase enzyme from mammalian cells that catalyzes an exchange reaction between PE or PC with serine (94).
### TABLE 1

**Strains used in this work**

| Strain          | Relevant characteristics                                                                 | Source or Ref. |
|-----------------|------------------------------------------------------------------------------------------|----------------|
| E. coli         |                                                                                         | (45)           |
| DH5α            | F' φ80d/lacZΔM15 Δ(lacZYA-argF)U169 deoR recA1 endA1 hsdR17(rk-mk+) phoA supE44 λ thi-1 gyrA96 relA1 |                |
| S. cerevisiae   |                                                                                         |                |
| yRP840          | MATα cup1::LEU2 (PM) his4-539 leu2-3,112 trp1 ura3-52                                   | (95)           |
| yRP841          | MATα cup1::LEU2 (PM) leu2-3,112 lys2-201 trp1 ura3-52                                   | (95)           |
| yRP1616         | ccr4Δ::NEO derivative of yRP840                                                          | (96)           |
| yRP1069         | dep1::URA3 derivative of yRP841                                                          | (95)           |
| yRP884          | xrn1::URA3 derivative of yRP840                                                          | (95)           |
| W303-1B         | MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1                                | (97)           |
| KS105           | cki1Δ::HIS3 derivative of W303-1B                                                         | (53)           |
| KS101           | eki1Δ::TRP1 derivative of W303-1B [rho<sup>o</sup>]                                    | (53)           |
| KS106           | cki1Δ::HIS3 eki1Δ::TRP1 derivative of W303-1B [rho<sup>o</sup>]                         | (53)           |
| HCY3            | ekt1Δ::TRP1 derivative of W303-1B                                                         | This study     |
| HCY4            | ekt1Δ::TRP1 derivative of W303-1B                                                         |                |
| HCY5            | eki1Δ::TRP1 derivative of strain W303-1B                                                  | This study     |
| HCY6            | rho<sup>o</sup> derivative of HCY5                                                        | This study     |
| HCY7            | cki1Δ::HIS3 eki1Δ::TRP1 derivative of W303-1B                                             | This study     |
| HCY8            | rho<sup>+</sup> derivative of W303-1B                                                     | This study     |
| W303 [rho<sup>o</sup>] | MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 [rho<sup>o</sup>] | M. Greenberg  |
| MGY100          | MATα ade1 oxi2 [rho<sup>+</sup>]                                                         | M. Greenberg  |
| DL1             | MATα his3-11 15 leu2-3 12 ura3-251 328,372                                               | (98)           |
| WD1             | cex4Δ::LEU2 derivative of DL1                                                           | (77)           |
### TABLE 2

Plasmids used in this work

| Plasmid     | Relevant characteristics                                                                 | Source or Ref. |
|-------------|------------------------------------------------------------------------------------------|---------------|
| pRS414      | Single copy vector containing *TRP1*                                                      | (99)          |
| pRS416      | Single copy vector containing *URA3*                                                      | (99)          |
| YEp352      | Multicopy *E. coli*/*yeast shuttle vector containing *URA3*                               | (100)         |
| pAS103      | Plasmid containing a 1.2 kb fragment of the *CHO1* gene                                   | (17)          |
| pRIP1PGK    | Plasmid containing a 1.0 kb fragment of the *PGK1* gene                                   | (101)         |
| pKSK1       | *EKII* gene derived from PCR ligated into the SrfI site of pCRScript\textsuperscript{TM}   | (53)          |
|             | AMP SK(+)                                                                                 |               |
| pKSK2       | *TRP1* disruption cassette from pJA52 ligated into the BglIII/BsaBI sites of plasmid pKSK1 | (53)          |
| pHS9        | *EKII* gene from pKSK1 ligated into the PstI/SacI sites of YEp352                         | This study    |
| pHS12       | *EKII* gene from pKSK1 ligated into the BamHI/SacI site of pRS416                         | This study    |
### TABLE 3

**CHO1 mRNA half-lives determined in this work**

| Strain                                    | Half-life (min)¹ |
|-------------------------------------------|-----------------|
| Wild type (yRP840)                        | 15.4 ± 4.4      |
| ccr4Δ (yRP1616)                           | > 45            |
| xrn1Δ (yRP884)                            | > 45            |
| Wild type (yRP841)                        | 11.6 ± 2.4      |
| dcp1Δ (yRP1069)                           | > 45            |
| Wild type (W303-1B)                       | 12.3 ± 1.3      |
| cki1Δ (KS105)                             | 14.5 ± 2.2      |
| eki1Δ [rho0] (KS101)                      | > 45            |
| eki1Δ cki1Δ [rho0] (KS106)                | > 45            |
| ect1Δ (HCY3)                              | 12.9 ± 1.8      |
| ept1Δ (HCY4)                              | 14.6 ± 2.1      |
| eki1Δ [rho0]/EKII (KS101 containing pH9)  | > 45            |
| eki1Δ (HCY5)                              | 17 ± 1.25       |
| eki1Δ [rho0] (HCY6)                       | > 45            |
| rho⁻ (MGY100)                             | > 45            |
| rho⁻ (W303 [rho⁻])                        | > 45            |
| rho⁻ (HCY8)                               | > 45            |
| Wild type treated with 1 mM KCN           | 22.3 ± 1.3      |
| Wild type treated with 2 mM KCN           | 32.0 ± 1.4      |
| Wild type treated with 4 mM KCN           | > 45            |
| Wild type (DL1)                           | 18.5 ± 4.5      |
| cox4Δ (WD1)                               | > 45            |

¹The half-life values are the average of triplicate determinations ± S.D.
### TABLE 4

Growth of mutants using glycerol and glucose as the carbon source

| Relevant genotype (strain) | Glycerol, 30 °C | Glucose, 30 °C | Glucose, 37 °C |
|----------------------------|-----------------|----------------|----------------|
| Wild type (W303-1B)        | +               | +              | +              |
| eki1Δ [rho°] (KS101)       | -               | +              | -              |
| eki1Δ (HCY5)               | +               | +              | +              |
| eki1Δ (HCY6)               | -               | +              | -              |
| cki1Δ eki1Δ [rho°] (KS106) | -               | +              | -              |
| cki1Δ eki1Δ (HCY7)         | +               | +              | +              |
| rho° (HCY8)                | -               | +              | -              |
| rho° (MGY100)              | -               | +              | -              |
| rho° (W303-1B [rho°])      | -               | +              | -              |

1The indicated cells were grown in YPD medium to stationary phase at 30 °C. Cells were diluted and spotted onto YPG and YPD media plates. The plates were incubated at the indicated temperatures, and colony growth was scored after 5 days. +, growth; -, no growth.
FIGURE LEGENDS

FIGURE 1. Phospholipid synthetic pathways in *S. cerevisiae*. The pathways shown for the synthesis of phospholipids include the relevant steps discussed throughout the paper. The genes encoding enzymes responsible for the reactions in the CDP-DAG and Kennedy pathways are indicated in the figure. PA, phosphatidate; CDP-DAG, CDP-diacylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; DAG, diacylglycerol; P-choline, phosphocholine; P-ethanolamine, phosphoethanolamine.

FIGURE 2. Effects of the ccr4Δ, dcp1Δ, and xrn1Δ mutations on CHO1 mRNA decay. Wild type (*WT*, yRP840 for ccr4Δ and xrn1Δ, and yRP841 for dcp1Δ), ccr4Δ (yRP1616), dcp1Δ (yRP1069), and xrn1Δ (yRP884) mutant cells were grown to the exponential phase (1x10⁷ cells/ml) of growth. Following the arrest of transcription, 5-ml samples were taken every 5 min, total RNA was extracted, and the levels of CHO1 mRNA and PGK1 mRNA were determined by Northern blot analysis. The relative amounts of CHO1 and PGK1 mRNAs were determined by ImageQuant analysis. The figure shows a plot of the log of the relative amount of CHO1 to PGK1 mRNAs *versus* time. The lines drawn were the result of a least-squares analysis of the data. The data shown in the figure are representative of three independent experiments. Half-life values are presented in Table 3.

FIGURE 3. Effects of the cki1Δ, eki1Δ, and cki1Δ eki1Δ mutations on CHO1 mRNA decay. Wild type (*WT*, W303-1B), cki1Δ (KS105), eki1Δ (KS101), and cki1Δ eki1Δ (KS106) mutant cells were grown to the exponential phase (1x10⁷ cells/ml) of growth. Following the arrest of transcription, CHO1 mRNA decay was quantified as described in the legend to Fig. 2. The figure shows a plot of the log of the relative amount of CHO1 to PGK1 mRNAs *versus* time. The lines drawn were the result of a least-squares analysis of the data. The data shown in the figure are representative of three independent experiments. Half-life values are presented in Table 3.

FIGURE 4. Effects of the eki1Δ, ect1Δ, and ept1Δ mutations on CHO1 mRNA decay. Wild type (*WT*, W303-1B), eki1Δ (KS101), ect1Δ (HCY3), and ept1Δ (HCY4) mutant cells were grown to the exponential phase (1x10⁷ cells/ml) of growth. Following the arrest of transcription, CHO1 mRNA decay was quantified as described in the legend to Fig. 2. The figure shows a plot of the log of the relative amount of CHO1 to PGK1 mRNAs *versus* time. The lines drawn were the result of a least-squares analysis of the data. The data shown in the figure are representative of three independent experiments. Half-life values are presented in Table 3.

FIGURE 5. Effects of Kennedy pathway mutations on the levels of CHO1 mRNA and PS synthase protein. Wild type (*WT*, W303-1B), cki1Δ (KS105), eki1Δ (KS101), cki1Δ eki1Δ (KS106), ect1Δ (HCY3), and ept1Δ (HCY4) mutant cells were grown to the exponential phase (1x10⁷ cells/ml) of growth. A, the abundance of CHO1 mRNA was determined with 10 µg of total RNA by Northern blot analysis. The relative amounts of CHO1 and PGK1 mRNAs from wild type and mutant cells were determined by ImageQuant analysis of the data. The relative amount of CHO1 to PGK1 mRNA in wild type cells was arbitrarily set at 1. B, the total membrane fraction (12.5 µg of protein) was subjected to immunoblot analysis using a 1:500 dilution of anti-PS synthase antibodies. The relative amounts of the PS synthase protein from wild type and mutant cells were determined by ImageQuant analysis of the data. The amount of PS synthase protein found in wild type cells was arbitrarily set at 1. The data shown in panels A and B are the average of three experiments ± S.D.
FIGURE 6. **Effect of the EKI1 gene on CHO1 mRNA decay.** Wild type (WT, W303-1B), eki1Δ (KS101 containing plasmid YEp352), eki1Δ/eki1 (KS101 containing plasmid pH9), and eki1Δ (HCY5) mutant cells were grown to the exponential phase (1x10^7 cells/ml) of growth. Following the arrest of transcription, CHO1 mRNA decay was quantified as described in the legend to Fig. 2. The figure shows a plot of the log of the relative amount of CHO1 to PGK1 mRNAs versus time. The lines drawn were the result of a least-squares analysis of the data. The data shown in the figure are representative of three independent experiments. Half-life values are presented in Table 3.

FIGURE 7. **Effect of respiratory deficiency on CHO1 mRNA decay.** Wild type (WT, W303-1B), rho- (MGY100), and rhoo (W303 [rho^o]) mutant cells were grown to the exponential phase (1x10^7 cells/ml) of growth. Following the arrest of transcription, CHO1 mRNA decay was quantified as described in the legend to Fig. 2. The figure shows a plot of the log of the relative amount of CHO1 to PGK1 mRNAs versus time. The lines drawn were the result of a least-squares analysis of the data. The data shown in the figure are representative of three independent experiments. Half-life values are presented in Table 3.

FIGURE 8. **Effects of KCN on CHO1 mRNA decay.** Wild type (W303-1B) cells were grown to the exponential phase (1x10^7 cells/ml) of growth in the absence and presence of the indicated concentrations of KCN. Following the arrest of transcription, CHO1 mRNA decay was quantified as described in the legend to Fig. 2. The figure shows a plot of the log of the relative amount of CHO1 to PGK1 mRNAs versus time. The lines drawn were the result of a least-squares analysis of the data. The data shown in the figure are representative of three independent experiments. Half-life values are presented in Table 3.

FIGURE 9. **Effect of the cox4Δ mutation on CHO1 mRNA decay.** Wild type (WT, W303-1B) and cox4Δ (WD1) mutant cells were grown to the exponential phase (1x10^7 cells/ml) of growth. Following the arrest of transcription, CHO1 mRNA decay was quantified as described in the legend to Fig. 2. The figure shows a plot of the log of the relative amount of CHO1 to PGK1 mRNAs versus time. The lines drawn were the result of a least-squares analysis of the data. The data shown in the figure are representative of three independent experiments. Half-life values are presented in Table 3.

FIGURE 10. **Effects of respiratory deficiency on the levels of CHO1 mRNA, PS synthase protein, PS synthase activity, and the synthesis of PS in vivo.** Wild type (WT, W303-1B) and rho- (HCY8) mutant cells were grown to the exponential phase (1x10^7 cells/ml) of growth. A, the abundance of CHO1 mRNA was determined with 10 µg of total RNA by Northern blot analysis. The relative amounts of CHO1 and PGK1 mRNAs from wild type and mutant cells were determined by ImageQuant analysis of the data. The relative amount of CHO1 to PGK1 mRNA in wild type cells was arbitrarily set at 1. B, the total membrane fraction (12.5 µg of protein) was subjected to immunoblot analysis using a 1:500 dilution of anti-PS synthase antibodies. The relative amounts of the PS synthase protein from wild type and mutant cells were determined by ImageQuant analysis of the data. The amount of PS synthase protein found in wild type cells was arbitrarily set at 1. C, the total membrane fraction was isolated and used for the assay of PS synthase activity. D, cells were incubated with [14C]serine for 30 min. Phospholipids were extracted and analyzed by two-dimensional TLC. The data shown in panels A-D are the average of three experiments ± S.D.
FIGURE 11. Effects of the rho− mutation on the levels of phospholipid synthesis enzyme activities. Wild type (WT, W303-1B) and rho− (HCY8) mutant cells were grown to the exponential phase (1x10^7 cells/ml) of growth. The total membrane fraction was isolated and used for the assay of CDP-DAG synthase (CDS), PS synthase (PSS), PS decarboxylase (PSD), PE methyltransferase (PEMT), phospholipid methyltransferase (PLMT), and PI synthase (PIS). The cell extract was used for the assay of choline kinase (CK) activity. The specific activities (nmol/min/mg) of these enzymes from wild type cells were 0.92 ± 0.04, 2.2 ± 0.03, 0.41 ± 0.02, 0.4 ± 0.05, 0.64 ± 0.01, 2.5 ± 0.14, and 4.5 ± 0.14. Each data point represents the average of triplicate enzyme determinations from two independent experiments ± S.D.
Fig. 1

CDP-DAG Pathway

PA → CDP-DAG

CDP-choline

P-choline

Ethanolamine

Choline

Kennedy Pathway
Fig. 2

- **Log Relative CHO1 mRNA**
  - **WT**
  - **ccr4Δ**
  - **dcp1Δ**
  - **xrn1Δ**

Time, min

Log Relative CHO1 mRNA
Fig. 3

Log Relative CHO1 mRNA

0 1 2 3 4 5 6

Time, min

0 10 20 30 40

WT

cki1Δ

eki1Δ [rho^0]

cki1Δ eki1Δ [rho^0]
Fig. 4

Log Relative CHO1 mRNA

Time, min

WT
eki1Δ [rho^0]
ect1Δ
ept1Δ
A

Fig. 5

B

CHO1/PGK1

CHO1p, Relative Amount

WT cki1Δ eki1Δ [rho9] cki1Δ eki1Δ [rho9] ect1Δ ept1Δ

0 1 2 3

0 1 2 3

WT cki1Δ eki1Δ [rho9] cki1Δ eki1Δ [rho9] ect1Δ ept1Δ
Fig. 6

Log Relative CHO1 mRNA

Time, min

WT
eki1Δ [rho^0]
eki1Δ [rho^0]/EKI1
eki1Δ
Fig. 7

Log Relative CHO1 mRNA

WT

rho−

rho0

Time, min

0 10 20 30 40

0 1.0 1.5 2.0
Fig. 9

![Graph showing time in minutes on the x-axis and log relative CHO1 mRNA on the y-axis. The graph compares WT and cox4Δ samples.](image-url)
Fig. 10

A

CHO1/PGK1

WT  rho-

B

Cho1p, Relative Amount

WT  rho-

C

PS Synthase, Units/mg

WT  rho-

D

Phospholipid, cpm/10^6 cells

WT  rho-

PS  PE  PC
Respiratory deficiency mediates the regulation of CHO1-encoded phosphatidylserine synthase by mRNA stability in Saccharomyces cerevisiae
Hyeon-Son Choi and George M. Carman

J. Biol. Chem. published online August 30, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M705098200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts