Regulation of Phospholipid Synthesis in the Yeast cki1Δ eki1Δ Mutant Defective in the Kennedy Pathway

THE CHOI-ENCODED PHOSPHATIDYLSTERINE SYNTHASE IS REGULATED BY mRNA STABILITY

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In the yeast Saccharomyces cerevisiae, the most abundant phospholipid phosphatidylcholine is synthesized by the complementary CDP-diaclylglycerol and Kennedy pathways. Using a cki1Δ eki1Δ mutant defective in choline kinase and ethanolamine kinase, we examined the consequences of a block in the Kennedy pathway on the regulation of phosphatidylcholine synthesis by the CDP-diaclylglycerol pathway. The cki1Δ eki1Δ mutant exhibited increases in the synthesis of phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine via the CDP-diaclylglycerol pathway. The increase in phospholipid synthesis correlated with increased activity levels of the CDP-diaclylglycerol pathway enzymes phosphatidylinositol synthase, phosphatidylserine decarboxylase, phosphatidylethanolamine methyltransferase, and phospholipid methyltransferase. However, other enzyme activities, including phosphatidylinositol synthase and phosphatidate phosphatase, were not affected in the cki1Δ eki1Δ mutant. For phosphatidylserine synthase, the enzyme catalyzing the committed step in the pathway, activity was regulated by increases in the levels of mRNA and protein. Decay analysis of CHO1 mRNA indicated that a dramatic increase in transcript stability was a major component responsible for the elevated level of phosphatidylserine synthase. These results revealed a novel mechanism that controls phospholipid synthesis in yeast.

PC1 is the most abundant phospholipid in the membranes of eukaryotic cells (1–4). It serves as a structural component of the membrane and as a source of bioactive lipids (e.g. lyso-PC, PA, DAG) (1–5). The importance of PC is underscored by the fact that alterations in its metabolism are linked to apoptosis (6–9) and oncogenic transformation (10–12). In the model eukaryote Saccharomyces cerevisiae, PC is synthesized by complementary pathways that are common to those found in mammalian cells2 (Fig. 1) (1, 4, 13–17). In the CDP-DAG pathway, PC is synthesized from CDP-DAG via the reactions catalyzed by PS synthase (18–20), PS decarboxylase (21–23), PE methyltransferase (24, 25), and phospholipid methyltransferase (24, 26). In the CDP-choline branch of the Kennedy pathway, PC is synthesized from choline via the reactions catalyzed by choline kinase (27), phosphocholine cytidylyltransferase (28), and choline phosphotransferase (29, 30). Analyses of mutations in S. cerevisiae (4, 31, 32), as well as in mammalian cells (33, 34) indicate that the physiological role(s) of PC synthesized by the two pathways are different.

The contribution of the CDP-DAG and Kennedy pathways for PC synthesis in wild type S. cerevisiae is dependent on the exogenous supply of choline (35). When grown in the presence of choline, yeast primarily synthesizes PC via the Kennedy pathway (35). On the other hand, when cells are grown in the absence of choline, PC is primarily synthesized via the CDP-DAG pathway (35). Yet, even under this growth condition, the Kennedy pathway still contributes to the synthesis of PC (36–41). The choline required for the Kennedy pathway is derived from the phospholipase D-mediated turnover of PC synthesized via the CDP-DAG pathway (41, 42).

The Kennedy pathway is critical for PC synthesis when steps in the CDP-DAG pathway are blocked. The cho1 (43, 44), psd1 psd2 (23, 45), and pem1/cho2 pem2/opi3 (23–26, 46) mutants defective in PS synthase, PS decarboxylase, and the phospholipid methyltransferases, respectively, are choline auxotrophs. The cho1 (43, 44) and psd1 psd2 (23, 45) mutants can also synthesize PC if they are supplemented with ethanolamine instead of choline. The ethanolamine is used for PE synthesis via the CDP-ethanolamine branch of the Kennedy pathway through the reactions catalyzed by ethanolamine kinase (47), phosphoethanolamine cytidylyltransferase (48), and ethanolamine phosphotransferase (49, 50) (Fig. 1). The PE synthesized by this route is subsequently methylated to PC via the CDP-DAG pathway (Fig. 1).

The cki1Δ eki1Δ (47) and cpt1 ept1 (36, 38) mutants are defective in both the CDP-choline and CDP-ethanolamine branches of the Kennedy pathway, and they can only synthesize PC via the CDP-DAG pathway. However, unlike mutants defective in the CDP-DAG pathway (23–26, 43–46), these Kennedy pathway mutants do not exhibit any auxotrophic requirements (36, 47). Moreover, even in the absence of the Kennedy pathway, the cki1Δ eki1Δ (47) and cpt1 ept1 (36) mutants have an essentially normal complement of phospholipids including PC. In this work we showed that the activities of the CDP-DAG pathway enzymes were elevated in the cki1Δ eki1Δ mutant to compensate for the block in the Kennedy pathway. For PS synthase, the elevation in enzyme activity reactions for the conversion of PE to PC (PE methylation pathway) are catalyzed by a single enzyme (1).
was due to increased mRNA and protein levels. One component responsible for this regulation was a dramatic increase in mRNA stability. This identified a novel mechanism by which phospholipid synthesis is regulated in *S. cerevisiae.*

**EXPERIMENTAL PROCEDURES**

**Materials—**All the chemicals were reagent grade. Growth medium supplies were purchased from Difco. Restriction endonucleases, modifying enzymes, recombinant Vent DNA polymerase, and NEBlot kit were purchased from New England Biolabs, Inc. RNA size markers were purchased from Promega. ProbeQuant G-50 columns, polyvinylidene difluoride membranes, and an enhanced chemifluorescence Western blotting detection kit were purchased from Amersham Biosciences. Radiochemicals were from PerkinElmer Life Sciences. The DNA size ladder used for agarose gel electrophoresis, Zeta Probe membrane, and acrylamide solutions were purified from Bio-Rad.

**DNA Manipulations and Site-directed Mutagenesis—**Plasmid and genomic DNA preparation, restriction enzyme digestion, and DNA ligations were performed according to standard protocols (56). Transformations of yeast (57, 58) and *E. coli* (56) were performed as described previously. Plasmids were maintained and amplified in *E. coli* strain DH5α. Plasmid pAB709 (Ps*• lacZ*) contains 0.3 kilobases of the CHO1 promoter fused to the coding sequence of the *E. coli lacZ* gene (59). Plasmid pH2C is a derivative of pAB709, in which the core sequence of the *UASINO element* (13) in the CHO1 promoter was changed from 5’-CTTTCATACAT-3’ to 5’-CTTTCATAAAC-3’. Mutagenesis was performed with the Stratagene QuikChange™ site-directed mutagenesis kit using plasmid pAB709 as the template and the mutagenic primers 5’-CCCTAGCTTCTGATGCTCTAGGTTGATTTTTCGAGAGCTCAGGCT-GGAGG-3’.

**RNA Isolation and Northern Blot Analyses—**Total RNA was isolated from cells using the methods of Schmitt et al. (60) and Herrick et al. (61). The RNA was resolved overnight at 22 V on a 1% formaldehyde gel (62) and then transferred to Zeta Probe membrane by vacuum blotting. The CHO1 (39) and PGK1 (63) probes were labeled with [α-32P]dUTP using the NEBlot random primer labeling kit, and unincorporated nucleotides were removed using ProbeQuant G-50 columns. Pre-hybridization, hybridization with the probes, and washes to remove nonspecific binding were carried out according to the manufacturer’s instructions. Images of radiolabeled species were acquired by phosphorimaging analysis. Analysis of CHO1 mRNA decay was analyzed following the arrest of transcription as described by Gonzalez and Martin (64).

**Anti-PS Synthase Antibodies and Immunoblotting—**The peptide sequence MVESDEDFAPQEFPH (residues 1-15 at the N-terminal end of the deduced protein sequence of CHO1) was synthesized and used to raise antibodies in New Zealand White rabbits by standard procedures (64) at Bio-Synthesis, Inc. SDS-PAGE (65) using 12% slab gels and transfer of proteins to polyvinylidene difluoride membranes (66) were performed as described previously. The membrane was probed with a 1:500 dilution of the anti-PS synthase antibodies. 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. Immuno blot signals were in the linear range of detectability.

**Labeling and Analysis of Phospholipids—**Labeling of phospholipids with $^{32}$P and $[^{14}C]$serine were performed as described previously (43, 44). Phospholipid synthesis was followed by labeling cells for 30 min, whereas the steady state composition of phospholipids was determined by labeling cells for six generations. Phospholipids were extracted from labeled cells by the method of Bligh and Dyer (87) and analyzed by two-dimensional TLC. The solvent systems used in the first and second dimensions were chloroform/methanol/ammonium hydroxide/H$_2$O (90: 50:4:6) and chloroform/methanol/acetic acid/H$_2$O (64:8:10:2), respectively.

Identity of the labeled phospholipids on the chromatography plates was confirmed by comparison with standard phospholipids after exposure to iodine vapor. Radiolabeled phospholipids were visualized by phosphor imaging analysis. The relative quantities of $^{32}$P-labeled phospholipids were analyzed using ImageQuant software, whereas the amount of each $^{14}C$-labeled phospholipid was determined by liquid scintillation counting.

**Preparation of the Cell Extract and the Total Membrane Fraction—**The cell extract and total membrane fraction were prepared as described previously (68). Cells were disrupted at 4°C by homogenization with glass beads in 50 mM Tris-maleate buffer (pH 7.0) containing 1 mM EDTA, 0.3 mM 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 × g for 10 min. The total membrane fraction was obtained from the cell extract by centrifugation at 100,000 × g for 1 h. Membranes were resuspended in buffer containing 50 mM Tris-maleate (pH 7.0), 10 mM MgCl$_2$, 10 mM 2-mercaptoethanol, 3% glycerol (v/v), and 0.5 mM phenylmethylsulfonyl fluoride. Protein concentration was determined by the method of Bradford (69) using bovine serum albumin as the standard.

**Enzymes Assays—**All assays were conducted in triplicate at 30°C in a total volume of 0.1 ml. PS synthase activity was measured with 50 mM Tris-HCl buffer (pH 8.0), 0.6 mM MnCl$_2$, 3.2 mM Triton X-100, 0.2 mM CDP-DAG, and 0.5 mM [3$^{2}$H]serine (70). PS deacetylase activity was measured with 50 mM Tris-HCl buffer (pH 7.2), 10 mM 2-mercaptoethanol, 100 mM EDTA, 2 mM Triton X-100, and 0.5 mM [3$^{2}$H]PS (71, 72). PE methyltransferase activity was measured with 50 mM Tris-HCl buffer (pH 9.0), 0.2 mM PE, and 0.5 mM $^{3}S$-metlyl-2$^{3}$Hadenosylmethionine (73). Phospholipid methyltransferase activity was measured with 50 mM Tris-HCl buffer (pH 7.5), 10 mM MgCl$_2$, 0.2 mM phosphatidylin monomethylenolamine, and 0.5 mM $^{3}S$-metlyl-2$^{3}$Hadenosylmethionine (73). CDP-DAG synthase activity was measured with 50 mM Tris-maleate buffer (pH 6.5), 20 mM MgCl$_2$, 15 mM Triton X-100, 0.5 mM phosphatidate, and 1.0 mM [5$^{2}$H]CTP (74). PI synthase activity was measured with 50 mM Tris-HCl buffer (pH 8.0), 2 mM MnCl$_2$, 3.2 mM Triton X-100, 0.2 mM CDP-DAG, and 1 mM [2$^{3}$H]inositol (75). PA phosphatase activity was measured with 50 mM Tris-maleate buffer (pH 7.0), 10 mM 2-mercaptoethanol, 1 mM Triton X-100, and 0.1 mM [3$^{2}$P]IPA (76) in the presence and absence of 1 mM MgCl$_2$. β-Galactosidase activity was measured with 100 mM sodium phosphate buffer (pH 7.0), 3 mM O-nitrophenyl β-D-galactopyranoside, 1 mM MgCl$_2$, and 100 mM 2-mercaptoethanol (77). All assays were linear with time and protein concentration. The average S.D. 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/min. A unit of β-galactosidase activity was defined in μmol of product/min. Specific activity was defined as units/mg of protein.

**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**

**Effect of the cki1Δ eki1Δ Mutations on Cell Growth—**The effect of the cki1Δ eki1Δ mutations on cell growth was examined. Unless otherwise indicated, cells were grown in medium without inositol to preclude the regulatory effects that this compound has on phospholipid synthesis (4, 13, 14, 78–80). The cki1Δ eki1Δ mutant grew at a slower rate than the wild type control (Fig. 2). The doubling time for cki1Δ eki1Δ mutant was 3 h, compared with 2 h for the wild type, in complete synthetic medium. Plate count analysis showed that the cki1Δ eki1Δ mutations did not affect cell viability. In addition, microscopic examination did not reveal any gross morphological abnormalities in the cki1Δ eki1Δ mutant. The cell density at the stationary phase of growth showed little difference between the wild type and the cki1Δ eki1Δ mutant (Fig. 2).

**Effect of the cki1Δ eki1Δ Mutations on the Synthesis and Steady State Composition of Phospholipids—**Wild type cells synthesize phospholipids by both the CDP-DAG and Kennedy pathways (36–41), whereas the cki1Δ eki1Δ mutant can only synthesize phospholipids via the CDP-DAG pathway (47). The effects of the cki1Δ eki1Δ mutations on the synthesis and steady state composition of phospholipids were examined by labeling cells with $^{32}$P, and with $[^{14}C]$serine. In wild type cells, $^{32}$P is incorporated into phospholipids synthesized by both the CDP-DAG and Kennedy pathways, whereas the label from $[^{14}C]$serine is only incorporated into PS, PE, and PC synthesized by the CDP-DAG pathway (43). The cki1Δ eki1Δ mutations had a significant effect on phospholipid synthesis. The $^{32}$P labeling of the mutant showed increases in PS (54%), PE (21%), and PA (27%) but a decrease in PC (80%) (Fig. 3A). The $[^{14}C]$serine labeling of the mutant showed increases in the incorporation of label into PS (36%), PE (23%), and PC (28%) (Fig. 4A). The decrease in $^{32}$P incorporation into PC in the cki1Δ eki1Δ mutant reflected phospholipase D-mediated turnover of PC (41, 81) and the inability to reutilize choline for PC synthesis via the Kennedy pathway. The cki1Δ eki1Δ mutations did not have a major effect on the steady state composition of phospholipids labeled with either $^{32}$P (Fig. 3B) or with $[^{14}C]$serine (Fig. 4B). With the exception of a decrease in PS content (7.9 to 4.6%) for cells labeled with $^{32}$P, the phospholipid composition of the cki1Δ eki1Δ mutant was not significantly different from that of the wild type control. Thus, even in the absence of the Kennedy pathway, the cki1Δ eki1Δ mutant eventually contained an almost normal balance of phospholipids.

**Effect of the cki1Δ eki1Δ Mutations on the Levels of CDP-DAG Pathway Enzyme Activities—**The results of phospholipid labeling indicated that the cki1Δ eki1Δ mutant compensated for the defect in the Kennedy pathway by increasing the synthesis of phospholipids by the CDP-DAG pathway. Accordingly, we questioned whether the cki1Δ eki1Δ mutations affected the activities of the CDP-DAG pathway enzymes PS synthase, PS deacetylase, PE methyltransferase, and phospholipid methyltransferase. These enzymes, which are all associated with decarboxylase, PE methyltransferase, and phospholipid methyltransferase, were analyzed using the total membrane fraction isolated from cells grown to the exponential phase of growth. The cki1Δ eki1Δ mutant showed elevated activity levels of PS synthase (50%), PS deacetylase (33%), PE methyltransferase (36%), and phospholipid methyltransferase (44%) compared with the control (Fig. 5, A–D). CDP-DAG synthase (83), which is responsible for the formation of CDP-DAG, PE synthase (84, 85), which competes with PS synthase for the substrate CDP-DAG (Fig. 1), were also included in this analysis. The cki1Δ eki1Δ mutations caused a 27% increase in CDP-
were grown to the exponential (10^7 cells/ml) phase of growth. For pulse labeling of phospholipids (panel A), cells were incubated with [32P]Pi (10 μCi/ml) for 30 min. The steady state composition of phospholipids (Panel B) was determined by labeling cells for six generations with [32P]Pi (5 μCi/ml). Phospholipids were extracted and analyzed as described under "Experimental Procedures." The percentages shown for phospholipids were normalized to the total 32Pi-labeled chloroform-soluble fraction, which included sphingolipids and other unidentified phospholipids. Each data point represents the average of two independent experiments ± S.D.

**Fig. 3.** Effect of the cki1Δ eki1Δ mutations on the synthesis and steady state composition of phospholipids synthesized by the CDP-DAG and Kennedy pathways. Wild type (WT) and cki1Δ eki1Δ mutant cells were grown to the exponential (1 × 10^7 cells/ml) phase of growth. For pulse labeling of phospholipids (panel A), cells were incubated with [32P]Pi (10 μCi/ml) for 30 min. The steady state composition of phospholipids (Panel B) was determined by labeling cells for six generations with [32P]Pi (5 μCi/ml). Phospholipids were extracted and analyzed as described under "Experimental Procedures." The percentages shown for phospholipids were normalized to the total 32Pi-labeled chloroform-soluble fraction, which included sphingolipids and other unidentified phospholipids. Each data point represents the average of two independent experiments ± S.D.

**Fig. 4.** Effect of the cki1Δ eki1Δ mutations on the synthesis and steady state composition of phospholipids synthesized by the CDP-DAG pathway. Wild type (WT) and cki1Δ eki1Δ mutant cells were grown to the exponential (1 × 10^7 cells/ml) phase of growth. For pulse labeling of phospholipids (panel A), cells were incubated with [32P]Pi (10 μCi/ml) for 30 min. The steady state composition of phospholipids (Panel B) was determined by labeling cells for six generations with [32P]Pi (5 μCi/ml). Phospholipids were extracted and analyzed as described under "Experimental Procedures." Each data point represents the average of two independent experiments ± S.D.

**Fig. 5.** Effect of the cki1Δ eki1Δ mutations on the levels of CDP-DAG pathway enzyme activities. Wild type (WT) and cki1Δ eki1Δ mutant cells were grown to the exponential (1 × 10^7 cells/ml) phase of growth. The total membrane fraction was isolated and used for the assay of PS synthase (PSS, panel A), PS decarboxylase (PSD, panel B), PE methyltransferase (PEMT, panel C), phospholipid methyltransferase (PLMT, panel D), CDP-DAG synthase (CDS, panel E), and PI synthase (PIS, panel F) activities as described under "Experimental Procedures." Each data point represents the average of triplicate enzyme determinations from a minimum of two independent experiments ± S.D. U, units.

**DAG synthase activity (Fig. 5E),** whereas the level of PI synthase activity was not affected by the mutations (Fig. 5F). PA phosphatase is responsible for the formation of the DAG utilized for PE and PC synthesis via the Kennedy pathway (Fig. 1) (86). We questioned whether this enzyme would be regulated in cells blocked in the Kennedy pathway. The activities of the Mg^{2+}-dependent and Mg^{2+}-independent forms of PA phosphatase (86) were measured in both the membrane and cytosolic fractions of wild type and cki1Δ eki1Δ mutant cells. Neither of these activities was affected by the block in the Kennedy pathway (data not shown).

**Effect of the cki1Δ eki1Δ Mutations on the Expression of PS Synthase Protein and mRNA Levels—**To gain insight into the mechanism by which the CDP-DAG pathway enzyme activities were elevated in the cki1Δ eki1Δ mutant, we examined the expression of the PS synthase enzyme. PS synthase was chosen as a representative enzyme because it catalyzes the committed step in the CDP-DAG pathway (Fig. 1), and its gene expression is coordinately regulated with the other structural genes in the pathway (4, 13, 14, 78-80). The levels of the PS synthase protein (Cho1p) were examined by immunoblot analysis using antibodies generated against a peptide sequence found at the N-terminal end of the protein. PS synthase is a 30-kDa protein that is susceptible to proteolytic degradation (20, 87, 88). These antibodies recognized PS synthase (and its proteolysis product) in the total membrane fraction (Fig. 6A). Immunoblot analysis of membranes derived from a cho1Δ mutant served as a negative control for the antibodies (Fig. 6A). The level of PS synthase protein was elevated (~2-fold) in the membranes of the cki1Δ eki1Δ mutant when compared with the control (Fig. 6A). This indicated that the increase in PS synthase activity was a result of an increase in the level of enzyme protein. To determine whether the increase in enzyme content was due to an
increase in gene expression, we examined the level of CHO1 mRNA. Northern blot analysis of total RNA isolated from cells at the exponential phase of growth showed that the relative amount of CHO1 mRNA in the cki1Δ eki1Δ mutant was 2-fold greater than that present in the control wild type (Fig. 6B). These results indicated that a transcriptional mechanism was responsible for the regulation of PS synthase in the cki1Δ eki1Δ mutant.

**Effect of the UASNO Element on the Regulation of CHO1 Expression in the cki1Δ eki1Δ Mutant**—Maximum expression of CHO1 in wild type cells grown in the absence of inositol is dependent on the UASNO cis-acting element in its promoter (13, 59, 79). The UASNO element contains a consensus-binding site (5’-CANNTG-3’) for a heterodimer complex of the positive transcription factors Ino2p and Ino4p (13, 59, 79, 89). We questioned whether the UASNO element played a role in the regulation of PS synthase observed in the cki1Δ eki1Δ mutant. To address this question, the UASNO element was mutated to a nonconsensus sequence in the PCHO1-lacZ reporter gene where the expression of β-galactosidase activity is dependent on transcription driven by the CHO1 promoter (59). Cell extracts were prepared from exponential wild type and cki1Δ eki1Δ mutant cells bearing the wild type and mutant reporter genes and then assayed for β-galactosidase activity. As expected (59), the mutations in the UASNO element caused 56 and 51% decreases in β-galactosidase activity in wild type and cki1Δ eki1Δ mutant cells, respectively (Fig. 7). The β-galactosidase activity in the cki1Δ eki1Δ mutant bearing the wild type reporter gene was 13% higher than that of wild type cells with the wild type reporter gene (Fig. 7). In addition, the β-galactosidase activity in the cki1Δ eki1Δ mutant bearing the mutant reporter gene was 22% higher than that of wild type cells with the mutant reporter gene (Fig. 7). These results indicated that the mutations in the UASNO element did not affect the regulation of CHO1 expression mediated by the cki1Δ eki1Δ mutations. Interestingly, the increase in CHO1 expression in the cki1Δ eki1Δ mutant as monitored by β-galactosidase activity was not as great as that observed by Northern blot analysis (Fig. 6B). This raised the suggestion that an increase in transcription was not a major reason for the increase in CHO1 mRNA abundance in the cki1Δ eki1Δ mutant.

The expression of CHO1 in wild type S. cerevisiae is repressed by the addition of inositol to the growth medium (59, 90). To examine whether the cki1Δ eki1Δ mutations affected this regulation, β-galactosidase activity was measured in wild type and mutant cells grown in the absence and presence of 50 μM inositol. Inositol supplementation caused a reduction (35–
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50%) in β-galactosidase activity in both wild type and mutant cells, indicating that the cki1Δ eki1Δ mutations did not have a significant effect on the regulation of CHO1 expression by inositol.

Effect of the cki1Δ eki1Δ Mutations on the Stability of CHO1 mRNA—The abundance of mRNA in the cell reflects both its synthesis and decay. Because there was a small correlation between the levels of CHO1 mRNA and reporter gene expression, we questioned whether mRNA stability was responsible for the increased level of CHO1 transcript in the cki1Δ eki1Δ mutant. To address this hypothesis, transcription was arrested in wild type and cki1Δ eki1Δ mutant cells followed by a kinetic analysis of CHO1 mRNA decay. PGK1 mRNA was included in this analysis as a loading control because it is a highly stable transcript (63, 91, 92). In wild type cells, CHO1 mRNA decayed in a time-dependent manner with a half-life of 10 min (Fig. 8). When compared with other mRNAs in yeast, which have half-lives ranging from 1 to 60 min, CHO1 mRNA was a moderately stable transcript (61). In the cki1Δ eki1Δ mutant, however, the CHO1 mRNA was highly stable during the time course of the experiment, with a half-life greater than 25 min (Fig. 8). These results indicated that an increase in the stability of CHO1 mRNA had a major effect on the abundance of the CHO1 transcript in the cki1Δ eki1Δ mutant.

DISCUSSION

In Saccharomyces cerevisiae the most abundant phospholipid PC is synthesized by the complementary CDP-DAG and Kennedy pathways. In this work, we examined the consequences of a block in the Kennedy pathway on the regulation of phospholipid synthesis by the CDP-DAG pathway. The cki1Δ eki1Δ mutant compensated for the block in the Kennedy pathway by increasing the activity levels of the CDP-DAG pathway enzymes PS synthase, PE decarboxylase, PA methyltransferase, and phospholipid methyltransferase. The increase in these activities was reflected by an increase in the rate of phospholipid synthesis by the CDP-DAG pathway. CDP-DAG synthase, which supplies CDP-DAG for the pathway, was also elevated in cki1Δ eki1Δ mutant cells. However, other enzyme activities (i.e. PI synthase and PA phosphatase), which are not CDP-DAG pathway enzymes, were unaffected by the cki1Δ eki1Δ mutations. Although the steady state level of PC (and overall phospholipid composition) of cki1Δ eki1Δ mutant cells was not much different from that of wild type cells, the mutation caused a slower rate of growth.

Data indicate that the PC synthesized by the CDP-DAG and Kennedy pathways is not functionally equivalent (4, 31, 32). Boumann et al. (32) have recently shown that the two pathways leading to PC in S. cerevisiae produce different sets of molecular species. For example, the PC synthesized via the Kennedy pathway is enriched in the monounsaturated species 32:1 and 34:1 when compared with the PC synthesized via the CDP-DAG pathway (32). Thus, the two pathways may yield structurally different PC species for different membrane functions (32). There is evidence that the fatty acyl composition of PC may be remodeled after its synthesis (32). Therefore, the PC synthesized via the CDP-DAG pathway in the cki1Δ eki1Δ mutant may be remodeled to compensate for the PC that was not synthesized via the Kennedy pathway.

The CHO1-encoded PS synthase is one of the most highly regulated enzymes in the CDP-DAG pathway (4, 78, 88). PS synthase is regulated by genetic and biochemical mechanisms, which have an impact on the synthesis of PC via the CDP-DAG and Kennedy pathways (4, 78, 88). CHO1 expression is regulated by water-soluble phospholipid precursors (e.g. inositol supplementation) (59, 90, 94, 95) and by growth phase (96, 97). The activity of PS synthase is modulated by membrane phospholipids (98–100) and is inhibited by inositol (101) and by CTP (39). The enzyme is also phosphorylated and inactivated by protein kinase A (102, 103). In this study, we showed that the cki1Δ eki1Δ mutations caused an increase in CHO1 mRNA abundance, and the corresponding increase in the levels of PS synthase protein and activity played a role in the activation of the CDP-DAG pathway to compensate for the block in the Kennedy pathway. However, this regulation was not mediated by the UASINO element in the CHO1 promoter that is required for maximum gene expression (59). Moreover, the cki1Δ eki1Δ mutations did not affect the inositol-mediated repression of the CHO1 gene.

A dramatic increase in CHO1 mRNA stability, as opposed to an increase in CHO1 transcription, contributed to the elevated levels of CHO1 transcript in the cki1Δ eki1Δ mutant. The process of mRNA decay/stability is a major control point in gene expression (104). Transcript stability is influenced by several developmental and environmental factors (104, 105). For example, studies with E. coli (106) and mammalian cells (104, 105, 107) show that the stability of mRNAs increases under conditions of stress. The inability to synthesize PC by the Kennedy pathway was a stressful condition in the cki1Δ eki1Δ mutant, and this may have contributed to the stability of the CHO1 transcript.

PS synthase is not the first enzyme in lipid metabolism to be regulated by mRNA stability. For example, the S. cerevisiae OLE1-encoded Δ9 fatty acid desaturase (63, 108) and the mammalian FAS-encoded fatty acid synthase (109) are also regulated by this mechanism. The stability of these mRNAs is regulated by nutrient availability (63, 108, 109). The OLE1 transcript is rapidly degraded upon fatty acid supplementation (63, 108), whereas the stability of the FAS transcript increases upon glucose supplementation (109).

CHO1 expression is coordinately regulated with the expression of the other genes in the CDP-DAG pathway in response to inositol supplementation and growth phase (4, 14, 79). The increase in the other CDP-DAG pathway enzyme activities in the cki1Δ eki1Δ mutant also reflected the coordinate regulation of the pathway. Whether or not the increased activity levels of the CDP-DAG pathway enzymes in cki1Δ eki1Δ mutant cells would have increased stability of their transcripts will be addressed in future studies.

In summary, we showed that S. cerevisiae compensated for a block in PC synthesis via the Kennedy pathway by increasing the levels of enzyme activities responsible for synthesis of PC via the CDP-DAG pathway. For the PS synthase enzyme, the increased level of activity was due to increased CHO1 mRNA and protein levels. A dramatic increase in CHO1 mRNA stability was a major component of this regulation. To our knowledge, this is the first report describing mRNA stability as a mechanism to control phospholipid synthesis in S. cerevisiae.

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