The CDS1 Gene Encoding CDP-diacylglycerol Synthase In Saccharomyces cerevisiae Is Essential for Cell Growth*

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CDP-diacylglycerol is an important branch point intermediate in glycerophosphate-based phospholipid biosynthesis in both prokaryotic and eukaryotic organisms (1–4). In eukaryotic cells, phosphatidic acid is converted either to CDP-diacylglycerol by the CDP-diacylglycerol synthase or to diacylglycerol by a phosphatase. In mammalian cells, CDP-diacylglycerol is the precursor to phosphatidylserine (and its phosphorylated derivatives), phosphatidylethanolamine, and phosphatidylinositol. Diacylglycerol is the precursor to triacylglycerol, phosphatidylethanolamine, and phosphatidylcholine in all eukaryotic cells. Therefore, the partitioning of phosphatidic acid between CDP-diacylglycerol and diacylglycerol must be an important regulatory point in eukaryotic phospholipid metabolism. In all eukaryotic cells, CDP-diacylglycerol is required in the mitochondria for phosphatidylglycerol and cardiolipin synthesis and in the endoplasmic reticulum and possibly other organelles for the synthesis of phosphatidylinositol.

A cDNA (derived from the CDS gene) encoding a photoreceptor cell-specific isoform of CDP-diacylglycerol synthase has been isolated from Drosophila (5). The gene product shares sequence identity with CDP-diacylglycerol synthase from Escherichia coli (6, 7), suggesting that this enzyme has been highly conserved during evolution. This particular isoform is an important regulator of the reutilization of phosphatidylinositol 4,5-bisphosphate, which is the substrate for a phospholipase C-mediated signal cascade linked to a G-protein-initiated signal. Overexpression of the CDS gene increases the amplitude of the light response of photoreceptor cells, and CDS mutant cells undergo light-dependent retinal degeneration dependent on phospholipase C function. However, the mutant flies develop normally, indicating that the synthase isoform responsible for bulk phospholipid synthesis is unaffected by this mutation. These results are also consistent with there being multiple synthase activities in higher eukaryotic cells derived from either a single gene or multiple genes, which is in contrast to E. coli, which encodes a single synthase (6, 7).

The CDS1 gene lacks a convincing homology to known CDS genes in prokaryotes, but it shares a certain similarity with the CDS2 gene from Drosophila. The CDS1 gene is essential for cell growth and encodes a photoreceptor cell-specific isoform of the CDP-diacylglycerol synthase.

In order to gain more in-depth understanding of the cellular distribution, function, and regulation of CDP-diacylglycerol synthase in eukaryotic cells, we report in this paper the isolation of the CDS1 gene from S. cerevisiae. The gene product was verified by overexpression of CDP-diacylglycerol synthase in yeast transformants. By gene interruption, we demonstrate that CDS1 is an essential gene for cell growth and encodes the majority, if not all, of the synthase activity in yeast.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were of reagent grade or better. Radiochemicals, Hybaid N nylon membranes, and CTP were obtained from Amer sham Corp. Liquisint™ was purchased from National Diagnostics. Restriction endonucleases were from Promega Corp., New England
CDP-diacylglycerol Synthase of S. cerevisiae

**Table I**

| Strains or plasmids | Relevant characteristics | Source or reference |
|---------------------|--------------------------|---------------------|
| E. coli DH5α | α pho80lacZAM15 Δαα2ZYA-argF1616, endA1, recA1, hsdR17 (F-, m+) | (16) |
| Yeast YPH102 | α ura3-52, lys2-801, ade2-101, leu2-3, hisD-200 | (17) |
| Yeast YPH501 | α ura3-52, lys2-801, ade2-101, trp1-1, leu2-13, hisD-200 | (17) |
| Yeast YSD3 | α CDS1/ CDS1: TRP1, derivative of YPH501 | This work |
| Yeast YSD90A | α CDS1: TRP1, derivative of YSD3 | This work |
| Yeast YSD90B | α CDS1, derivative of YSD3 | This work |
| Yeast YSD90C | α CDS1: TRP1, derivative of YSD3 | This work |
| Yeast YSD90D | α CDS1, derivative of YSD3 | This work |
| Yeast MC13 | λ α101-13, lys2, can1 | (18) |

**Notes:**
- The abbreviations used are: PCR, polymerase chain reaction; kb, kilobase pair(s); bp, base pair(s).
- Yeast strains were transformed using electrotransformation at 1.0 kV/mm and 186 μF.
- Amplification of DNA by Polymerase Chain Reaction—For both analytical and preparative purposes, PCR was performed after optimizing conditions as described by Innis and Gelfand (18). Amplification of the CDS1 gene from a YES yeast genomic DNA library (19), from a YEPD yeast genomic library (20), or from yeast chromosomal DNA employed the following primers: primer 1 (5' − CCATATCCTGGAGATGTGTGCAAC− CCGAG−3') and primer 2 (5' − CGCGTCTAGAAGAGTGTTTGT− CAA GTG−3'). They were designed according to the DNA sequence of open reading frame YBR029c (GenBank number Z35898); the underlined codons in primers 1 and 2 indicate the start and stop codons, respectively, for this open reading frame.

**DNA Labeling and Detection—** The Genus 1 kit was used according to the manufacturer's directions for preparation and detection of digoxigenin-labeled DNA probes. The kit utilizes random priming of template DNA and incorporation of digoxigenin-dUTP into the probe. Template DNA was produced by PCR and isolated by agarose gel electrophoresis. The amplified band was excised from the gel and extracted with the Geneclean II kit. An antibody against digoxigenin coupled to alkaline phosphatase, which in the presence of Lumi-Phos 530 produces a chemiluminescent signal, was used to permit detection of hybridized probe by x-ray film.

**Screening of a Genomic DNA Library—** E. coli colonies bearing a yeast genomic DNA library carried on the E. coli–yeast shuttle vector YEPI3 (20) were transferred to positively charged nylon membranes and screened for hybridization to the labeled PCR probe generated by using genomic DNA as template. Transfer of colonies to membranes, hybridization, and development of blots were carried out using the manufacturer's instructions for use of positively charged nylon membranes. Initial screenings were performed with the Genus 1 kit. SSC dilutions were prepared by combining 3 M NaCl, 0.3 M sodium citrate, pH 7.0. Hybridization was performed overnight at 68 °C in hybridization solution (5 × SSC, 0.5% Genus 1 kit blocking reagent, 0.1% N-lauroylsarcosine, 0.02% SDS) containing the labeled PCR probe (10 ng/ml). Following hybridization, membranes were washed twice for 5 min in 2 × SSC, 0.1% SDS at room temperature and twice for 15 min in 0.1% SSC, 0.1% SDS at 68 °C. Colonies corresponding to the positive signals on the blot were picked from the original plates for further screening, including a second round of hybridization screening and Southern blot analysis of their DNA.

**Southern Analysis of Genomic DNA—** DNA samples were digested with restriction enzymes and separated by agarose gel electrophoresis. DNA was transferred to positively charged nylon membranes by capillary transfer using 20 × SSC at room temperature. Membranes were rehydrated with 3 M NaCl, 0.3 M sodium citrate, pH 7.0. Hybridization was performed overnight at 68 °C in hybridization solution (5 × SSC, 0.5% Genus 1 kit blocking reagent, 0.1% N-lauroylsarcosine, 0.02% SDS) containing the labeled PCR probe (10 ng/ml). Following hybridization, membranes were washed twice for 5 min in 2 × SSC, 0.1% SDS at room temperature and twice for 15 min in 0.1% SSC, 0.1% SDS at 68 °C. Colonies corresponding to the positive signals on the blot were picked from the original plates for further screening, including a second round of hybridization screening and Southern blot analysis of their DNA.

**Preparation of Cell Fractions and Measurement of CDP-diacylglycerol Synthase Activity—** All cell fractionation procedures were carried out at 4 °C. For the measurement of CDP-diacylglycerol synthase activity in the total membrane fraction, S. cerevisiae cells were grown to the exponential phase of growth, and the cells were collected by centrifugation in tared containers. Cells were washed in 50 ml Tris-maleate, pH 6.5, 0.25 M sucrose, 1 mM phenylmethylsulfonyl fluoride, centrifuged, and resuspended in the same solution. The cell suspension was washed with an equal volume of pre-chilled siliconized (0.25 mm) and disrupted in a Mini-Beadbeater (BioSpec Products) by six 15-s bursts with a 2-min pause between bursts. Silicon beads and unbroken cells were removed by centrifugation at 2,000 × g for 1 min. The total membrane fraction was separated from the cytoplasmic fraction by centrifugation at 100,000 × g for 1 h. The membrane pellet was suspended in 50 ml Tris-maleate, pH 6.5, containing 40 mM MgCl₂. CDP-diacylglycerol synthase activity was measured by the incorporation of [3H]CTP into chloroform-soluble material dependent on phosphatidic acid as described previously (9). For the measurement of CDP-
dicylglycerol synthase activity in the mitochondria and microsomes, yeast cells were grown to an optical density (A600) of 1.0. Isolation of yeast organelles was carried out by the method of Zinser and Daum (21). Mitochondria were isolated from the cell-free homogenate (3,000 × g supernatant) by centrifugation at 9,000 × g for 10 min, while microsomes were collected by centrifugation of the postmitochondrial supernatant (100,000 × g for 1 h).

Plasmid Shuffling and Overexpression of CDS1 by Galactose Induction—Plasmids (URA3) carrying the CDS1 gene under the regulation of the GAL1 promoter were introduced into the cds1 null mutant by “plasmid shuffling” as follows. Briefly, strain YSD90A/YEp30 was transformed with a pGAL-CDS1 plasmid. A transformant carrying both plasmids was grown in YEPG medium for 2 overnight cultures before plating for single colonies on CSMG-URA plates to select cells still containing the pGAL-CDS1 plasmid; resulting colonies were screened for lack of growth on CSMG-LEU and CSMG-URA plates to verify the absence of plasmid YEp30. Induction of the expression of CDS1 from the GAL1 promoter was carried out as follows. Cells transforming growth exponentially in either CSMR-URA or YEPR medium were diluted to an A600 of 0.1 unit with the same medium. Galactose (2%) was added to the cell culture when A600 reached 0.75 unit, and growth was continued before harvesting at various times; parallel growth after addition of glucose to 2% was carried out for those transformants that can grow in the presence of glucose. After preparation of membrane fractions, samples were assayed for CDP-diacylglycerol synthase activity; the samples were assayed for CDP-diacylglycerol synthase activity before they were assayed for CDP-diacylglycerol synthase activity.

Labeling and Analysis of Phospholipids—The CDS1 haploid strain YPH102 and the cds1:TRP1/GAL1-CDS1 transformant YSD90A/pSDG1 were grown in YEPR medium to the exponential phase of growth (A600 about 1.0–1.2). For steady state labeling, 5-ml aliquots of YEPRD and YEPRG medium were inoculated with the above strains to an A600 of 0.05. Following the addition of 50 μCi of [32P]orthophosphate, cells were grown for 16 h (six generations) to assure uniform labeling before harvesting by centrifugation at 1,500 × g. The cell pellets were resuspended in 1 ml of 80% ethanol and incubated at 80°C for 15 min. After centrifugation at 1,500 × g, the pellets were suspended in 0.67 ml of chlorform, methanol, 0.1 h HCl (1:2:0.8 (w/v)). Cells were lysed using glass beads, and the phospholipids were extracted as described previously (22). Isolated radiolabeled phospholipids were applied to boric acid-impregnated silica gel plates (8), which were developed in one dimension with chlorform/methanol/water/ammonium hydroxide (60:37.5:3:1) as the solvent system. Labeled phospholipids were detected and quantified directly from the thin layer plate using a Betascope (Betagen Corp.). For pulse labeling of phospholipids, 250 μCi of [32P]orthophosphate was added to each 5-ml cell culture (A600 = 1.0) in either YEPRD or YEPRG medium. Cells were grown for 30 min before harvesting. Phospholipid extraction and analysis were by the same procedure as described above.

Assessment of Inositol-Excretion Phenotype—The inositol excretion capacity of yeast strains was tested on CSMD-URA or CSMG-URA plates lacking inositol, choline, and ethanolamine. The inositol auxotrophic reporter strain YPH102; this construct carries 108 bp of DNA from the CDS1 gene to yield plasmid pSDG1, which was introduced into the wild-type yeast strain YPH102, yielding the strain YPH102/pSDG1 (not shown) grown on CSMD-URA medium for 1 h. The yeast strains (not shown) grown on CSMD-URA medium for 1 h were assayed for CDP-diacylglycerol synthase activity. Enzyme activity in strain YPH102 was increased about 10-fold when carrying either plasmid YEp2 or plasmid YEp30 as shown in Table II. Since both plasmids exhibited identical restriction patterns and brought about a similar overproduction of enzyme activity, they appeared to carry the same CDS1 structural gene. A 1.7-kb Ssp1 fragment from the genomic clone was subcloned into the plasmid pYES2 downstream of the GAL1 promoter (23). The construct carries 108 bp of CDS1 gene upstream sequence after the GAL1 promoter. Induction of the CDS1 gene in CSMG-URA medium brought about an 10-fold overexpression of CDS1 gene (Table II) when compared with either strain YPH102 alone or strain YPH102/pSDG1 (not shown) grown on CSMG-URA medium (uninduced).

CD51 Gene and CDP-diacylglycerol Synthase—Restriction digestion mapping and DNA sequencing from both ends (total of approximately 800 bp) of the gene (1371 bp) confirmed that the CDS1 gene is identical with open reading frame YBR029c (GenBank number Z35898). Inspection of the DNA sequence did not reveal any sequence motifs (27), suggesting the existence of introns in or near the CDS1 gene, which minimizes the possibility of multiple synthases being derived from alternate splicing of a common RNA transcript. In the 3’-region, two potential transcriptional termination sequences (28, 29) [1416-TAG-TAG-rich region—TAG-TATGT—AT-rich region—TTT-1502 and 1465-TAGNNATGTA-1475) and a polyadenylation site (30) (1548-AATAAA-1553) were found. A sequence (ATGG-AAA) homologous to the upstream activation sequence UA(S) (I, 31, 32) was found beginning 161 bp 5’ to the gene. No recognizable TATA promoter element was observed in this region. UAS(O) has been found 5’ to several genes (INO1, CH01, PEMS1, PEMS2, PIS, and PSE1) related to phospholipid metabolism (1, 33). This sequence appears to be related to the coordinate transcriptional depression of the expression of phospholipid biosynthetic enzymes via the products of the INO2 and INO4 genes (31) when cells are grown in the absence of inositol.
and either choline or ethanolamine. Its presence upstream of the CDS1 gene may explain the decreased level of CDP-diacylglycerol synthase when cells are grown in the presence of inositol, choline, and ethanolamine (34). Although multiple copies of this regulatory sequence exist in the promoter region of many of the genes reported above, only one conserved UA-SINO is located 5' to the CDS1 gene. This may explain the smaller response in synthase activity (about a 2-fold range) to these water-soluble precursors to phospholipids (34) when compared with the response of other enzymes of phospholipid metabolism (3-4 fold range).

The predicted open reading frame encodes a protein of 457 amino acids with a molecular mass of 51,789 Da. A comparison of the deduced yeast CDP-diacylglycerol synthase amino acid sequence with the sequences of the CDP-diacylglycerol synthases from E. coli (6) and Drosophila (5) revealed a high degree of homology as shown in Fig. 1. The amino acid sequence from S. cerevisiae shares 37% identity and 60% similarity with the Drosophila enzyme, and 29% identity and 56% similarity with the E. coli enzyme. In regions that were highly conserved among the three enzymes, identities of approximately 90% were observed. Hydrophobicity analysis of the yeast enzyme by the method of Kyte and Doolittle (35) showed a highly hydrophobic protein containing several potential membrane spanning domains and a hydrophilic N terminus, which is absent in the E. coli CDP-diacylglycerol synthase. The profile of the yeast and Drosophila enzymes are remarkably similar. Analysis of the sequence by the PSORT program (36) showed no potential mitochondrial targeting sequence within the N-terminal hydrophilic region or any potential endoplasmic retention sequence (KKXX or HDEL) at the C terminus (37), although this enzyme activity has been localized primarily to the mitochondria and the endoplasmic reticulum (10). The lack in E. coli of the N-terminal and C-terminal extensions found in the eukaryotic enzymes may indicate that these sequences are required for organelle targeting. The PSORT program did predict possible plasma membrane and Golgi body localization for this sequence, which is consistent with finding the activity in secretory vesicles and the plasma membrane (11, 12).

The CDP-diacylglycerol synthase isolated from the total membranes of yeast (minus the nuclear fraction) was reported to have a molecular weight of 56,000 (9). The discrepancy with the predicted molecular weight could be explained by the inaccuracy of SDS gel electrophoresis methods with membrane-associated proteins. However, any additional posttranslational processing normally associated with organelle targeting of proteins would only make this discrepancy greater. Since the CDS1 gene encodes most if not all of the synthase activity in the cell (see below) and there are no sequence motifs consistent with alternate splicing of the primary transcript, how is this activity directed to multiple sites in the cell? Are there additional synthase isoforms that have not been yet identified?

There is precedence in yeast for the use of alternative AUG start sites on a common mRNA transcript for the synthesis of tRNA modification enzymes, which are localized to the mitochondria, cytoplasm, and nucleus (38-40). The protein products from a common transcript have different N termini, which appears to account for their different locations in the cell. In contrast to the CDP-diacylglycerol synthase, the N termini of the complete open reading frames of these modifying enzymes

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**Fig. 1. Comparison of predicted amino acid sequences of CDP-diacylglycerol synthases.** Identical amino acids are shaded in gray and boxed. The putative yeast (Sc) amino acid sequence and the Drosophila (Dm) (5) and E. coli (Ec) (6) amino acid sequences are numbered in the margins. These sequences are aligned using the Pileup program in GCG (56). A dash represents a gap placed by the computer program.

| Yeast CDS1 | Drosophila CDS1 | E. coli CDS1 |
|------------|-----------------|-------------|
| Sequence   | Sequence        | Sequence    |
| 1          | 2               | 3           |
| 4          | 5               | 6           |
| 7          | 8               | 9           |

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contain predicted mitochondrial targeting sequences. Phosphatidylycerine synthase of yeast is found associated with both the mitochondria (most likely the outer membrane) and the endoplasmic reticulum (41). Both forms have the same molecular weight of 30,804 and are derived from the \textit{CHO1} gene; whether this dual localization occurs in vivo and how it may happen has not been resolved. Removal of the first AUG of the open reading frame of the \textit{CHO1} gene results in localization of the majority of the protein (molecular weight of 22,400) to the cytoplasm in an inactive form but with sufficient membrane-associated active protein of reduced size to complement a \textit{cho1} mutant (42). This N terminus region cannot localize a soluble marker protein to either membranes or specific organelles, yet it appears to be important in localizing the \textit{CHO1} gene product.

Translation beginning at the second methionine of the CDP-diacylglycerol synthase would only reduce the size of the protein by 600 mass units, which would be indistinguishable from the complete open reading frame by SDS gel electrophoresis methods but may inactivate an unrecognized mitochondrial targeting sequence. The third AUG lies 72 codons from the start of the open reading frame, which would encode a protein much too small to be in agreement with the size of major protein thus far isolated; however, a smaller minor isof orm of the synthase may have been missed in the earlier work. Such a smaller product might still be active and membrane associated (with the plasma membrane for example) because it would still retain the regions homologous to the \textit{E. coli} enzyme.

Disruption of the \textit{CDS1} Gene—The \textit{CDS1} gene was disrupted in vitro and introduced into the genome by homologous recombination as described below. A 3-kb \textit{EcoRI-BamHI} fragment from plasmid YE30 containing the \textit{CDS1} gene was subcloned into pUC19. The NruI-MscI region (853 bp in length) internal to the \textit{CDS1} gene was replaced by a 2 kb \textit{PstI-BspHI} fragment carrying the \textit{TRP1} gene from plasmid pRS304 (43). The disrupted \textit{CDS1} gene (\textit{cds1::TRP1}) was excised by \textit{EcoRI-BamHI} digestion and used to transform the trp1 homozygous diploid \textit{YPH501} (43). Tryptophan prototrophy (growth on CSM-trp plates) was used to select for replacement of one of the \textit{CDS1} genes by homologous recombination with the \textit{cds1::TRP1} fragment. PCR reactions using genomic DNA of the interrupted diploid as template confirmed that the disrupted \textit{CDS1} gene had integrated at the \textit{CDS1} locus of one of the two chromosomes (Fig. 2).

The \textit{CDS1/ cds1::TRP1} heterozygous diploid strain \textit{YSD3} was sporulated and subjected to tetrad analysis. Each of the 10 tetrads dissected gave rise to only two viable spores, all of which were tryptophan auxotrophs; no spores with a \textit{TRP1} phenotype survived. Inspection of the nonviable spores by microscopy showed that none of them had undergone germination, single cell division, or budding, indicating that the residual amount of CDP-diacylglycerol synthase in the spore was not sufficient for germination. The 2:2 ratio of viable to nonviable spores, together with the segregation of tryptophan auxotrophy and the \textit{CDS1} gene with the viable spores, indicates that the \textit{CDS1} gene is essential for cell growth.

In order to rescue the nonviable spores, plasmid YE30 (\textit{CDS1}), which also carries a \textit{LEU2} marker, was transformed into the heterozygous diploid \textit{YSD3} (\textit{CDS1/ cds1::TRP1}) prior to sporulation. Each of the four tetrads dissected gave rise to four viable spores. These spores were tested for growth in the absence of leucine and tryptophan. Among the four spores within each tetrad, two were tryptophan auxotrophs (\textit{YSD90B, YSD90D}) and the other two were prototrophic for tryptophan (\textit{YSD90A, YSD90C}). All spores were leucine prototrophs, indicating that plasmid YE30 segregated efficiently during meiosis and that a plasmid borne copy of the \textit{CDS1} gene had rescued the nonviable spores. These spores were grown in YEPD medium for one or two overnights, and the cell cultures were sampled. After 24 h of growth, 90% of the tryptophan auxotrophs (\textit{CDS1} wild type) were leucine auxotrophs, indicating that the wild-type cells lost the YE30 plasmid rapidly. The tryptophan prototrophs (\textit{cds1::TRP1}) remained prototrophic for leucine even after 48 h of growth. Supplementation of the liquid growth medium and the selection plates with choline and ethanomine did not result in the loss of the covering plasmid YE30 from the null mutants. Therefore, lack of dependence on CDP-diacylglycerol for phosphatidylethanolamine and phosphatidylcholine biosynthesis by utilization of the diacylglycerol-dependent pathway does not suppress the need for the \textit{CDS1} gene.

Regulated Expression of the \textit{CDS1} Gene—To study the cellular response to different CDP-diacylglycerol synthase levels, plasmid pSDG1 (\textit{P}_{GAL1}-\textit{CDS1}, multicopy) was introduced into the null mutant \textit{YSD90A (cds1::TRP1)} by “plasmid shuffling” as described under “Experimental Procedures.” This transformant also showed an 10-fold increase of the CDP-diacylglycerol synthase activity above the wild-type yeast background, which was dependent on growth in CSMG-URA induction medium (Table II). The increase in CDP-diacylglycerol synthase specific activity relative to a wild-type control was the same (9-fold) in the mitochondrial- and the endoplasmic reticulum-enriched fractions dependent on galactose induction of the only \textit{CDS1} gene in a haploid \textit{cds1} null background. One-third of the total enzyme activity was in the mitochondria, and the remainder was in the microsomal fraction as was also observed in wild-type cells lacking any plasmids. Although the reported distribution of activity between these two organelles varies (8, 10, 44), both fractions were proportionately enriched in synthase activity when the \textit{CDS1} gene was overexpressed, indicating that the \textit{CDS1} gene product is associated with both subcellular fractions. When strain \textit{YSD90A/pSDG1} was grown in either CSMD-URA or CSMR-URA medium (noninducing conditions), only 10% of the wild-type CDP-diacylglycerol synthase activity was detected (Table II), which was sufficient to support robust cell growth on agar plates and in liquid medium. Introduction of the low copy number plasmid pSDG2 (\textit{P}_{GAL1}-\textit{CDS1}), which carries a \textit{LEU2} marker into strain \textit{YSD90A}, also supported growth and overproduction of synthase activity (10-fold) in CSMG-LEU medium; this plasmid contains no DNA derived from the 5’ upstream region of the \textit{CDS1} gene. However, unlike the high copy number plasmid pSDG1, the latter plasmid could not complement the null allele when grown on CSMD-LEU agar plates. Plating cultures for single colonies resulted in the appearance after 4 days incubation of very small colonies,
which when restreaked to CSMD-LEU plates did not form single colonies. Therefore, the original colonies appear to have resulted from utilization of residual synthase activity produced under induction conditions followed by cell arrest on glucose-containing media once insufficient synthase activity was present to sustain growth. Similarly, liquid cultures of this transformant growing in CSMG-LEU arrested several generations after switching to CSMD-LEU media. These results are consistent with the earlier conclusion that the CDS1 gene is essential and encodes the majority of the synthase activity. The ability of plasmid pSDG1 and not plasmid pSDG2 to complement the null allele under repressed conditions is most likely due to leak through transcription, which would result in higher levels of transcript from the multicopy plasmid (about 10–20 copies/cell) than the low copy number plasmid (about 1–2 copies/cell) (45).

**Inositol Excretion Phenotype**—The cdg1 mutant of yeast (46) exhibits about a 75% reduction in the derepressed level of CDP-diacylglycerol synthase activity. The synthase activity in this mutant also no longer responds to regulation by inositol and choline, and the mutant excretes inositol into the growth medium. The product of the CDG1 gene has not been established. In order to determine whether inositol excretion could be caused by simply reducing the steady state level of synthase activity, strain YSD90A/pSDG1 was streaked as patches to YEPD media as described under “Experimental Procedures” (Fig. 3A) versus the wild-type strain YPH102 grown under similar conditions; the results for the latter strain were independent of the carbon source. Changes in the relative percent incorporation of label into the various phospholipid classes during a pulse labeling experiment should be related to changes in the initial rate of synthesis of each phospholipid. The most significant difference brought about by overproduction of the synthase (induced) is a marked increase in the rate of synthesis of phosphatidylinositol and a decrease in the rate of synthesis of phosphatidylinositol and a decrease in phosphatidylinositol labeling. The increase in phosphatidyl serine from 0.2% for wild-type and induced cells to 0.8% for overproduction of the synthase (induced) is a marked increase in the rate of synthesis of phosphatidylserine and its downstream metabolic products. A 90% reduction in the level of the synthase over wild-type levels (uninduced) resulted in a significant increase in phosphatidylinositol labeling with a reduction in phosphatidylserine labeling. The increase in phosphatidic acid from 0.2% for wild-type and induced cells to 0.8% for uninduced synthase was significant and reproducible consistent with this synthase being involved in the major phospholipid biosynthetic pathways of the cell. To analyze phospholipid composition, both strain YSD90A/pSDG1 and strain YPH102 were labeled to steady state with [32P]orthophosphate in the above media as described under “Experimental Procedures”(Fig. 3B). Strain YPH102 grown in YEPG medium (data not shown) gave the same results as cells grown in YEPD medium. Except possibly for phosphatidylethanolamine, the differences in labeling patterns among the strains reflected the pulse labeling results. Relative to wild-type cells, overproduction of the synthase increased the proportion of phosphatidylinositol while underexpression of the synthase reduced the proportion of phosphatidylserine. Although the levels were low, phosphatidic acid appears to be elevated under uninduced conditions and cardiolipin levels were highest under induced conditions. These results are consistent with variations in either the steady state level of CDP-diacylglycerol or its rate of synthesis affecting the relative rate of synthesis of phospholipid at this branch point in metabolism. Accumulation of phosphatidic acid and increases in cardiolipin are also consistent with low and high levels, respectively, of synthase activity.

The fact that only 10% of the wild type level of the synthase...
Serine decarboxylase (gene exists, as is the case with the expression of phosphatidylserine synthase, even though the affinity for CDP-diacylglycerol synthase activity associated with both the yeast endoplasmic reticulum and mitochondrial fractions. Reverse transcription of CDS1 and CDS2 demonstrate that the expression of the latter enzyme for the CDP-diacylglyc-erol synthase of S. cerevisiae is consistent with the level of CDPA-diacylglycerol being limiting for phosphatidylinositol synthesis. The phosphatidylinositol synthase would appear to be more sensitive to changes in the in vivo concentration of CDPA-diacylglycerol than the phosphatidylserine synthase, even though the affinity for CDPA-diacylglycerol by these two enzymes measured in vitro at saturation for their second substrates is the same (48–50). With two substrate enzymes, the apparent Km of one substrate is in-versed related to the concentration of the second substrate when the latter is below its saturation concentration. At physi-ological concentrations of serine, the phosphatidylinerine synthase should be saturated for serine and operating at its min-imum apparent Km for CDP-diacylglycerol (50). However, the physiological concentration of inositol is 9-fold below its Km in the case of the phosphatidylinositol synthase (50). Therefore, the apparent Km of the latter enzyme for the CDPA-diacylglycerol should be much higher than the former enzyme under physiological conditions, which is reflected in the effects brought about by overproduction and repression of the CDPA-diacylglycerol synthase activity under the control of Pgal1.

Conclusions—Clearly the CDS1 gene encodes an essential CDPA-diacylglycerol synthase activity associated with both the yeast endoplasmic reticulum and mitochondrial fractions. Results with complementation of the cds1 null mutant with plas-mids pSDG1 and pSDG2 demonstrate that the CDS1 gene encodes more than 90% of the synthase activity in the cell and does not encode an activity that is targeted solely to either the mitochondria or the endoplasmic reticulum. If a second CDS gene exists, as is the case with the expression of phosphatidylinerine decarboxylase (PDS1 and PDS2) activity (33, 51, 52), it would account for significantly less than 10% of the total activity. Unlike the PDS genes (52, 53), which can complement each other, a possible second CDS gene does not support growth in the absence of the CDS1 gene.

There is a clear difference in the germination phenotype of null mutants of the CDS1 and PIS (encoding phosphatidylinositol synthase) genes. Although both genes are essential for vegetative growth, null spouses derived from heterozygous null/wild-type diploids of the PIS gene undergo sporulation and at least one cell division before arresting with buds (54), while spouses containing the null cds1 gene do not germinate. Therefore, supplying CDPA-diacylglycerol for functions other than bulk phosphatidylinositol biosynthesis may be crucial to cell viability. One such function might be in supplying substrate for the plasma membrane-associated signal transduction pathway responsible for phosphoinositide formation, which has been linked to regulation of cell growth in yeast (13, 55).

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