Cloning of a Gene (PSD1) Encoding Phosphatidylserine Decarboxylase from Saccharomyces cerevisiae by Complementation of an Escherichia coli Mutant*

(Received for publication, May 18, 1993, and in revised form, July 16, 1993)

Constance J. Clancey, Shao-Chun Chang, and William Dowhan‡

From the Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, Texas 77225

A gene (PSD1) encoding a phosphatidylserine decarboxylase of Saccharomyces cerevisiae was cloned by complementation of a conditional lethal mutation in the homologous gene in Escherichia coli strain EH150. Expression of the cDNA clone in EH150 corrected growth, phospholipid, and phosphatidylserine decarboxylase activity defects. Expression of the genomic clone in wild type yeast resulted in 20-fold amplification of phosphatidylserine decarboxylase activity. A 1500-base pair open reading frame encodes a 56,558-Da protein with a potential mitochondrial targeting sequence. Upstream regulatory elements found in other enzymes of the phospholipid biosynthetic pathway are present in PSD1. The derived amino acid sequence shows 44 and 35% identity with the phosphatidylserine decarboxylase from CHO cells respectively. Near the carboxy terminus is an LGST sequence which, in E. coli, is the site of proteolytic cleavage of the proenzyme into the α and β subunits and formation of the pyruvate prosthetic group (Dowhan, W., and Li, Q.-X. (1992) Methods Enzymol. 209, 348–359). Disruption of the PSD1 gene in a haploid strain of yeast resulted in loss of detectable decarboxylase activity but little alteration of the growth properties or phospholipid composition. These results suggest that yeast has a second phosphatidylserine decarboxylation activity.

The conversion of phosphatidylserine to phosphatidylethanolamine is catalyzed by phosphatidylserine decarboxylase in both eukaryotic and prokaryotic organisms (Bishop and Bell, 1988; Carman and Henry, 1989; Raetz and Dowhan, 1990). In Escherichia coli the gene (psd) and gene product responsible for this essential step in phospholipid metabolism have been extensively studied (Dowhan et al., 1972; Hawrot and Kennedy, 1975; Satre and Kennedy, 1978). The psd gene encodes a proenzyme or α-subunit (Mr = 35,893) which is cleaved post-translationally to a β subunit (Mr = 28,579) and an α-subunit (Mr = 7,352) (Li and Dowhan, 1988). The α-subunit is derived from the carboxyl-terminal 69 amino acids of the α-subunit and is blocked at its amino terminus by an amide-linked pyruvate prosthetic group. Ser-254 of the α-subunit is essential for the formation of the two subunits and the pyruvyl group. The β-hydroxyl of Ser-254 functions as a nucleophile attacking the carbonyl of the adjacent peptide bond to form an ester intermediate. This is followed by an α,β-elimination reaction and hydrolysis resulting in the formation of the two subunits. The resulting pyruvyl-dependent decarboxylases thus far investigated (van Poelje and Snell, 1990). Since pyruvyl-dependent decarboxylases show extensive protein sequence homology within specific substrate groups and over several species (van Poelje and Snell, 1990), it is not completely surprising that the gene encoding phosphatidylserine decarboxylase from CHO cells was originally identified by the similarity of the derived protein sequence with that of the enzyme from E. coli (Kuge et al., 1991). The predicted α-subunit for the CHO cell enzyme is 32 amino acids in length, about half the size of the α-subunit of the E. coli enzyme.

Considerably less is known concerning the phosphatidylserine decarboxylase from Saccharomyces cerevisiae since neither the gene nor the gene product has been isolated. Analogous to the mammalian enzyme, yeast decarboxylase activity is localized to the inner mitochondrial membrane (Kuchler et al., 1988) suggesting the requirement for a mitochondrial targeting sequence. Expression of the partial cDNA clone from CHO cells in yeast increases the phosphatidylserine decarboxylase activity and suggests functional homology between these enzymes (Kuge et al., 1991), but the cDNA does not include sufficient information at the amino terminus to confirm the presence of an amino-terminal targeting region. Yeast phosphatidylserine decarboxylase activity is responsive to the presence in the growth medium of precursors utilized in phospholipid biosynthesis (Lamping et al., 1991; Overmeyer and Waechter, 1991). This has been demonstrated for other phospholipid biosynthetic enzymes as well (Nikoloff and Henry, 1991; Carman and Henry, 1989). Regulatory sequences present 5′ to the PSS (CHO1), INQ1, PEM1, PEM2, and PIS genes appear to be transcriptionally responsive to these precursors (Bailis et al., 1992; Kodaki et al., 1991a; Nikoloff and Henry, 1991; Carman and Henry, 1989), but the precise mechanism of transcriptional regulation is not known.

The sequence of events leading to formation of the pros-

*This work was supported in part by United States Public Health Service Grant GM 35143. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L20972.

‡ To whom reprint requests should be addressed. Tel.: 713-792-5600; Fax: 713-794-4150.

1 The abbreviations used are: CHO, Chinese hamster ovary; PCR, polymerase chain reaction; IPTG, isopropyl-β-D-thiogalactopyranoside; CL, cardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; HPTLC, high performance thin layer chromatography; kb, kilobase(s); bp, base pair(s).
thet group and inner mitochondrial membrane assembly of yeast phosphatidylserine decarboxylase is of interest, and the further study of this enzyme is important because of its potential role in all eukaryotic cells in the regulation of phospholipid biosynthesis and interorganellar trafficking of phospholipids. In this report we describe the construction of phosphatidylserine decarboxylase made in the endoplasmic reticulum, decarboxylated in the mitochondria, and translocated, as phosphatidylethanolamine, from the mitochondria to the other membrane systems of the cell (Voelker, 1991; Simbeni et al., 1991).

We report here the isolation of the PSD1 gene of S. cerevisiae by complementation of a conditional lethal mutation in the E. coli psd gene. The yeast gene has been sequenced and compared to the related genes from other organisms. A potential mitochondrial targeting sequence and the predicted site of the proteolytic cleavage and formation of a pyruvate prosthetic group were found. We have also constructed a null allele in the yeast PSD1 gene and characterized the phenotype of this mutant. The disruption of the gene resulted in loss of detectable phosphatidylserine decarboxylase activity but did not result in loss of viability or any major alterations in phospholipid composition.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals were reagent grade or better. Radiochemicals and Hyclone liquid media were purchased from Amersham Corp. Lipontin™ was purchased from National Diagnostics. Restriction endonucleases, DNA modifying enzymes, and the phage M13mp18 were obtained from New England Biolabs, Bethesda Research Laboratories, Boehringer Mannheim, Stratagene, and Promega Corp. Phospholipids were from Sigma. Agarose was from BBI and Sea Plaque, and agarose agarose gel from FMC. The PCR Reagent Kit was from Perkin-Elmer Cetus, and the Sequenase™ Version 2.0 Sequencing Kit was from U. S. Biochemical Corp. Long Ranger™ polycrylamide solution was from J. T. Baker Chemical Co. The Genus™ 1 Kit (DNA Labeling and Detection Kit, Nonradioactive), digoxigenin-labeled DNA molecular weight markers, positively charged nylon membranes, and Lumi-Phos™ 530 were purchased from Boehringer Mannheim. Oligonucleotides were prepared commercially by Genosys Biotechnologies, Inc. or synthesized by the Molecular Genetics Core Facility, University of Texas Medical School at Houston using an Applied Biosystems 394 oligonucleotide synthesizer. The RCAB kit was from Pierce. Yeast lytic enzyme (A. throbacter luteus, 100,000 units/g) was from ICN Biochemicals. Promase (Streptococcus gravisus) was from Calbiochem. Components of bacterial and yeast growth media were purchased from Difco. Ultrafree-MC 0.45-μm filter units were from Millipore. Thin layer plates were from J. T. Baker Chemical Co., and HPTLC plates were from E. Merck. K-Prep phosphatidyl-[H-1]cholesterol was prepared enzymatically as previously described (Dowhan and Li, 1992) from phosphatidic acid derived from egg lecithin.

**Growth Conditions, Strains, and Plasmids**—Methods of yeast growth, sporulation, and tetrad analysis were as described by Sherman et al. (1986). YPD medium consisted of 1% Bacto-yeast extract, 2% Bacto-peptone, and 2% dextrose. In YPG, YPL or YPGm medium, 2% glycerol, lactate or galactose, respectively, replaced dextrose as the carbon source. YPGE medium contained 2% ethanol in addition to the glycerol in YPG medium. Minimal defined media were prepared from Yeast Nitrogen Base without amino acids and supplemented as described by Hirsch and Henry (1986). Inositol-free media were prepared according to Hirsch and Henry (1986), except that Vitamin-free Yeast Nitrogen Base was not available from suppliers, and components were supplied individually. Selection media omitted one component as required. Growth medium was supplemented with 1 mg/ml ampicillin, 100 U/ml penicillin, 25 μg/ml kanamycin, 50 mg/ml geneticin, 1 mg/ml XhoI or EcoRI endonuclease, are positioned between the lacOOP and tetracycline resistance gene of the plasmid pBR322 contained in a suitable host strain. The resulting plasmids contain cDNAs in a vector (Y-pSE336) which permits replication, expression, and selection in E. coli or S. cerevisiae. cDNA inserts, which can be excised by either XhoI or EcoRI endonuclease, are positioned between the lacOOP promoter of E. coli and the GAL10 promoter of yeast directed in opposite orientations into the insert (Ellode et al., 1991). The yeast genomic DNA library (Nasmyth and Tatchell, 1980) from which the PSD1 gene and characterized the phenotype of this mutant. The disruption of the gene resulted in loss of detectable phosphatidylserine decarboxylase activity but did not result in loss of viability or any major alterations in phospholipid composition.

**DNA Sequencing**—Single-stranded DNA sequencing of the yeast PSD1 gene was performed on sequencing fragments of a HindIII digest of plasmid pN9 in phase M13mp18 by standard protocols (Sambrook et al., 1989). DNA sequencing using double-stranded DNA as a template was performed on plasmid pCC8. The dideoxy chain termination reaction using custom-tailored primers was performed as previously described (Sanger et al., 1977) following protocols outlined in the Sequenase™ Version 2.0 DNA Sequencing Kit. The reaction products were run on Long Ranger™ polycrylamide electrophoresis gels as in the protocol outlined by J. T. Baker Chemical Co. DNA sequence and putative amino acid sequence were analyzed by Genetics Computer Group Sequence Analysis Software Package, Version 7.2 (available from the University of Wisconsin Computer Graphics Laboratory). DNA sequence data are deposited in GenBank.

**Screening of the Genomic DNA Library**—E. coli colonies bearing a yeast genomic DNA library carried on the E. coli yeast shuttle vector YEP13 (Nasmyth and Tatchell, 1980) were transferred to positively charged nylon membranes and screened for hybridization to the yeast insert DNA. Positive clones were isolated by hybridization to membranes. Hybridization, and development of blots were carried out using the manufacturer’s instructions for use of positively charged

---

**Phosphatidylserine Decarboxylase in S. cerevisiae**

42581

---

**DNA Labeling and Detection**—The Genus™ 1 Kit was used according to the manufacturer’s instructions for preparation and detection of nonradioactive DNA probes. The kit utilized random priming of template DNA and incorporation of a digoxigenin-dUTP into the probe. Template DNA was used by PCR and isolated by agarose (low melt) gel electrophoresis, excision of the desired band, and centrifugal filtration. (0.45-μm filter unit). An antibody to 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 autoradiography.

**Screening of the Genomic DNA Library**—E. coli colonies bearing a yeast genomic DNA library carried on the E. coli yeast shuttle vector YEP13 (Nasmyth and Tatchell, 1980) were transferred to positively charged nylon membranes and screened for hybridization to the yeast insert DNA. Positive clones were isolated by hybridization to membranes. Hybridization, and development of blots were carried out using the manufacturer’s instructions for use of positively charged
nylon membrane and the Genius Kit. SSC dilutions were prepared from 20 × SSC (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% Genomic DNA—Genomic DNA was digested with restriction enzymes and separated by agarose gel electrophoresis. DNA was then transferred to positively charged nylon membrane by capillary transfer using 20 × SSC, and the membranes were baked 2 h at 80 °C under vacuum. The labeled cDNA probe used for screening the genomic library was also used for hybridization to Southern blots. Methods for hybridization and development of blots were the same as those described for library screening.

Preparation of Cell Fractions and Measurement of Phosphatidylserine Decarboxylase Activity—All cell fractionation procedures were carried out at 4 °C. S. cerevisiae were grown to mid-log phase and the cells collected by centrifugation in tared containers. Wet weight of the positive regions of the blot were the same as those described for library screening.

Southern Hybridization Analysis of Genomic cDNA—Genomic DNA was digested with restriction enzymes and separated by agarose gel electrophoresis. DNA was then transferred to positively charged nylon membranes by capillary transfer using 20 × SSC, and the membranes were baked 2 h at 80 °C under vacuum. The labeled cDNA probe used for screening the genomic library was also used for hybridization to Southern blots. Methods for hybridization and development of blots were the same as those described for library screening.

Preparation of Cell Fractions and Measurement of Phosphatidylserine Decarboxylase Activity—All cell fractionation procedures were carried out at 4 °C. S. cerevisiae were grown to mid-log phase and the cells collected by centrifugation in tared containers. Wet weight of the positive regions of the blot were the same as those described for library screening.

Southern Hybridization Analysis of Genomic cDNA—Genomic DNA was digested with restriction enzymes and separated by agarose gel electrophoresis. DNA was then transferred to positively charged nylon membranes by capillary transfer using 20 × SSC, and the membranes were baked 2 h at 80 °C under vacuum. The labeled cDNA probe used for screening the genomic library was also used for hybridization to Southern blots. Methods for hybridization and development of blots were the same as those described for library screening.

Preparation of Cell Fractions and Measurement of Phosphatidylserine Decarboxylase Activity—All cell fractionation procedures were carried out at 4 °C. S. cerevisiae were grown to mid-log phase and the cells collected by centrifugation in tared containers. Wet weight of the positive regions of the blot were the same as those described for library screening.

Southern Hybridization Analysis of Genomic cDNA—Genomic DNA was digested with restriction enzymes and separated by agarose gel electrophoresis. DNA was then transferred to positively charged nylon membranes by capillary transfer using 20 × SSC, and the membranes were baked 2 h at 80 °C under vacuum. The labeled cDNA probe used for screening the genomic library was also used for hybridization to Southern blots. Methods for hybridization and development of blots were the same as those described for library screening.

Preparation of Cell Fractions and Measurement of Phosphatidylserine Decarboxylase Activity—All cell fractionation procedures were carried out at 4 °C. S. cerevisiae were grown to mid-log phase and the cells collected by centrifugation in tared containers. Wet weight of the positive regions of the blot were the same as those described for library screening.

Southern Hybridization Analysis of Genomic cDNA—Genomic DNA was digested with restriction enzymes and separated by agarose gel electrophoresis. DNA was then transferred to positively charged nylon membranes by capillary transfer using 20 × SSC, and the membranes were baked 2 h at 80 °C under vacuum. The labeled cDNA probe used for screening the genomic library was also used for hybridization to Southern blots. Methods for hybridization and development of blots were the same as those described for library screening.

Preparation of Cell Fractions and Measurement of Phosphatidylserine Decarboxylase Activity—All cell fractionation procedures were carried out at 4 °C. S. cerevisiae were grown to mid-log phase and the cells collected by centrifugation in tared containers. Wet weight of the positive regions of the blot were the same as those described for library screening.

Southern Hybridization Analysis of Genomic cDNA—Genomic DNA was digested with restriction enzymes and separated by agarose gel electrophoresis. DNA was then transferred to positively charged nylon membranes by capillary transfer using 20 × SSC, and the membranes were baked 2 h at 80 °C under vacuum. The labeled cDNA probe used for screening the genomic library was also used for hybridization to Southern blots. Methods for hybridization and development of blots were the same as those described for library screening.

Preparation of Cell Fractions and Measurement of Phosphatidylserine Decarboxylase Activity—All cell fractionation procedures were carried out at 4 °C. S. cerevisiae were grown to mid-log phase and the cells collected by centrifugation in tared containers. Wet weight of the positive regions of the blot were the same as those described for library screening.

Southern Hybridization Analysis of Genomic cDNA—Genomic DNA was digested with restriction enzymes and separated by agarose gel electrophoresis. DNA was then transferred to positively charged nylon membranes by capillary transfer using 20 × SSC, and the membranes were baked 2 h at 80 °C under vacuum. The labeled cDNA probe used for screening the genomic library was also used for hybridization to Southern blots. Methods for hybridization and development of blots were the same as those described for library screening.
ing the addition of 50 μCi of [32P]orthophosphate, cells were grown 
for at least six generations to assure uniform labeling before harvest-
ing by centrifugation at 1500 × g. The cell pellets were suspended 
in 1 ml of 80% ethanol at 80 °C and incubated at 80 °C for 15 min to 
inactivate degradative enzymes (Henschke and Rose, 1991). After 
 centrifugation at 1500 × g, the ethanol supernatants were discarded 
and the pellets resuspended in 1 ml of chloroform/methanol/0.1 N 
HCl (1:2:0.8, v/v) containing 100 μg of a mixture of unlabeled carrier 
phospholipid (PC/PE/PS/PI, in the ratio of 15:10:2:2). Cells were 
lysed using glass beads, and the phospholipids were extracted as 
described by Homann et al. (1987). Isolated radiolabeled phospho-
lipids were applied to boric acid-impregnated silica gel plates (Fine 
and Sprecher, 1982) which were developed in one dimension with 
chloroform/methanol/water/ammonium hydroxide (120:75:6:2) as 
the solvent system. Labeled phospholipids were detected and quan-
tified from the thin layer plate using a Betascope (Betagen Corp.). 

The areas corresponding to phosphatidylinositol, phosphatidyl-
seryine, and phosphatidylethanolamine were scraped from the 
plate, eluted, and concentrated as described by Fine and Sprecher 
(1982) and applied to silica gel HPTLC plates. Plates were developed 
in the solvent system chloroform/methanol/acetic acid (65:25:10) or 
chloroform/methanol/formic acid (65:25:10).

One liter of E. coli was inoculated to A0 = 0.01, grown at 
30 °C in the presence of 5 μCi of [32P]orthophosphate for 1 h, then 
shifted to 42 °C for 2.5 h. Lipids were extracted using the method 
described by DeChavigny et al. (1991). Isolated radiolabeled phospho-
lipids were applied to silica gel plates which were developed in one 
dimension with chloroform/methanol/water/ammonium hydroxide 
(65:25:4) as the solvent system. Labeled phospholipids were detected and quantified directly from the thin layer plate using a Betascope.

RESULTS

Complementation of Growth Phenotype of Strain EH150—
Incubation of 5 × 10^9 EH150/XKC cells with 4 × 10^9 phage 
from the XYES yeast cDNA library resulted in 2.5 × 10^9 total 
transfectants, six of which grew at the nonpermissive temper-

ature. Plasmids isolated from the six colonies were used to 
retransform strain EH150. All plasmids corrected the condi-
tional lethal growth phenotype of strain EH150 indicating 
that in each case the correction was due to the presence of 
plasmid and not to a reversion event. The cDNA inserts 
ranged in size from 0.6 to approximately 5 kb. By restriction 
mapping, three of the inserts appeared to be the same and 
represented by plasmid pCC8, resulting in a panel of four 
unique positive plasmids (pCC8, pCC9, pCC15, and pCC21).

As shown in Fig. 1A, these four positive plasmids, when 
carried in strain EH150, elevated the level of phosphatidyl-
seryine decarboxylase activity (E. coli assay conditions) in 
cell extracts when compared to strain EH150 carrying no plasmid 
or carrying the control plasmid (V-pSE936). This result 
was independent of growth temperature of cells or preincubation 
temperature of the cell extracts. As expected, all cells grown 
at 30 °C and cell extracts preincubated at 30 °C showed 
phosphatidylserine decarboxylase activity; however, cells with 
the four positive plasmids showed overexpression of the decar-
boxylase activity. When the cells were grown at 30 °C and 
the cell extracts preincubated at 42 °C, all extracts showed some 
derate in phosphatidylserine decarboxylase activity consist-
ent with the loss of temperature-sensitive E. coli activity 
expressed at 30 °C. When cells were grown at 42 °C and 
preincubated at 30 °C, lysates from control cells displayed 
little phosphatidylserine decarboxylase activity, while lysates 
from cells carrying the positive plasmid showed significant 
phosphatidylserine decarboxylase activity. When cells were 
grown at 42 °C, preincubation of lysates at 42 °C completely 
abolished phosphatidylserine decarboxylase activity in the 
control lysates while the lysates from cells carrying the posi-
tive plasmids showed an increase in phosphatidylserine de-
carboxylase activity. These results are consistent with the 
presence of phosphatidylserine decarboxylase activities from 
the host strain as well as from the plasmid. Similar results 
were obtained when activity measurements were made under 
yeast assay conditions. Labeling of phospholipids with 32PO4, 
also showed that the accumulation of phosphatidylserine and the 
decrease in phosphatidylethanolamine which occurs in 
control cells after a shift of growth temperature to 42 °C does 
not occur in cells with the positive plasmids (Fig. 1B). There-
fore, all four plasmids corrected the temperature-dependent 
loss of viability, loss of decarboxylase activity, and increase 
in phosphatidylserine levels.

Screening of the Genomic DNA Library—The insert derived 
from plasmid pCC8 was chosen as the probe used to screen a 
SSS. Phosphatidylserine Decarboxylase in S. cerevisiae

![Fig. 1. Effects of cDNA clones on E. coli strain EH150. A.](image-url)
of the growth phenotype of strain EH150 contained this cDNA. The probe showed hybridization to a dot-blot of yeast genomic DNA (strain DL1) but not E. coli genomic DNA (strain EH150) confirming its yeast origin (data not shown). Southern hybridization analysis (using the same probe) of strain DL1 genomic DNA revealed two hybridization-positive DNA fragments after digestion with NcoI, EcoRI, or HpaI, but only one hybridization-positive DNA fragment after digestion with either XhoI or BglII (data not shown). These results were consistent with the restriction map of plasmid pCC8 shown in Fig. 2A and indicated a sequence in yeast genomic DNA that was structurally related to plasmid pCC8. Screening of the yeast genomic DNA library carried in E. coli was performed as described under "Experimental Procedures." From 20,000 colonies screened, two colonies (containing plasmids pN9 and pN11) were identified as potentially carrying a genomic copy of the yeast PSDI gene. Restriction mapping of the two clones suggested that they were identical. As shown in Fig. 2B, the restriction endonuclease map of plasmid pN9 reveals a HindIII fragment that contains the entire cDNA insert in plasmid pCC8.

Phosphatidylserine Decarboxylase Activity of Genomic Clones pN9 and pN11—Total membrane fractions from mid-log cultures of yeast strain DL1 transformed with plasmids pN9 and pN11 were examined for phosphatidylserine decarboxylase activity under yeast assay conditions. Phosphatidylserine decarboxylase activity in strain DL1 (0.45 nmol/mg/min) was increased 25- or 18-fold when carrying plasmid pN9 (11 nmol/min/mg) or plasmid pN11 (8.2 nmol/min/mg), respectively. Activity was linear with respect to time for at least 60 min in assays with 60-100 pg of membrane protein (data not shown). Since both plasmids exhibited identical restriction patterns (data not shown) and brought about a similar overproduction of enzymatic activity, they appeared to carry the same PSDI structural gene.

DNA Sequence of the PSDI Gene—Single-stranded sequencing of the genomic clone pN9 was performed as described under "Experimental Procedures." Both DNA strands were sequenced using overlapping primers as summarized in Fig. 2B. An open reading frame of 1500 bp corresponding to a predicted 500-amino acid protein (56,558 Da) was observed (Fig. 3). The region 5' of the open reading frame contained three sequences homologous to the consensus sequence 5'-ATGTGAAT-3', which is postulated to confer inositol and choline regulation on several yeast phospholipid biosynthetic genes (Ballal et al., 1992; Kodaki et al., 1991b; Carman and Henry, 1989). Downstream of these sequences were two potential TATA promoter elements (TATAAA consensus sequence). Within the expected range of 40 to 120 bp downstream of the TATA promoter elements (Struhl, 1989), two sequences conformed to either consensus sequence RYRR (where R = purines and Y = pyrimidines) or TC(A/G)A, which account for more than half of the known yeast transcription initiation sites (Guerente, 1987). Using either of these potential initiation sites, the predicted length of the 5'-untranslated region would be consistent with the average of 52 nucleotides reported for many yeast genes (Cigan and Donahue, 1987). The common preference for adenine nucleotides was observed 5' to the ATG start codon, and the region surrounding the start codon loosely matched the yeast translation initiation site consensus sequence, (A/Y)(A/A)/(T)ATG(TCT (Cigan and Donahue, 1987). In the 3'-untranslated region, sequences similar to consensus sequence, (TAA)/(TAG)/(TGA)...1-140...(T-rich)...TAG... (TAGT) ... (A-T-rich)...TTT (Zaret and Sherman, 1982), and motifs, TAG...TATA and TAG...TATGTA (Russo et al., 1991), postulated to be involved in transcription termination were found. Finally a polyadenylation site (Wickens and Stephenson, 1984) appeared near the end of the determined sequence.

The genomic library containing the plasmid pN9 was generated from a partial Sau3AI digest of yeast genomic DNA (Nasmyth and Tatchell, 1980). Since there were no Sau3AI sites in the sequence left of the left-most HindIII site (Fig. 2B), the possibility that this region is comprised of randomly ligated yeast genomic DNA fragments from the partial Sau3AI digest is unlikely. The sequence of the 2.1-kb region between the two HindIII sites contained five Sau3AI sites, but also contained NcoI, EcoRI, and HpaI sites in complete agreement with the plasmid pCC8 restriction map. Further-
**Phosphatidylserine Decarboxylase in S. cerevisiae**

The predicted amino acid sequence and its homology with the phosphatidylserine decarboxylase from CHO cells (Kuge et al., 1989; Li and Dowhan, 1988) are described in Fig. 4. From computer analysis of the protein sequence, the amino acid sequence from S. cerevisiae shared 44% identity and 60% similarity with the CHO cell enzyme and 35% identity and 55% similarity with E. coli enzyme, while the enzyme from CHO cells and E. coli were 33% identical and 53% similar. There were two distinct areas of high homology among the three enzymes. Both the amino- and carboxy-terminal ends of the proteins showed significant homology, with little homology among the central domains of the enzymes. Hydrophobic analysis by the method of Kyte and Doolittle (1982) showed a very similar homology profile for all three proteins (Fig. 5). The significance of the homology at the amino-terminal end was not clear but homology at the carboxy-terminal end included the highly conserved LG$T(V/I)(V/I)$ sequence corresponding to the post-translational processing site in E. coli which results in Ser-254 being converted to the pyruvoyl prosthetic group at the amino terminus of the $\alpha$ subunit of 7,332 Da (Li and Dowhan, 1988). Similar post-translational activation of the yeast enzyme would result in a pyruvoyl-containing $\alpha$ subunit of 4,192 Da and a $\beta$-subunit of 52,367 Da; the predicted size for the $\alpha$-subunit of the CHO cell enzyme is 3,547 Da (Kuge et al., 1991). The pyruvoyl prosthetic group appears to lie in a hydrophobic environment in all three enzymes.

The amino-terminal sequence (57 amino acids) was examined for potential as a mitochondrial targeting sequence. Computer analysis showed a weak $\alpha$-helical but a large $\beta$-sheet hydrophobic moment (0.74) by the method of Eisenberg et al. (1984). Previous work has suggested that an $\alpha$-helical hydrophobic moment is important for a targeting structure, but a $\beta$-sheet hydrophobic moment also appears to be functional (Lemire et al., 1989; Schatz, 1987). In addition, this region exhibited an abundance of positively charged residues and an absence of either acidic amino acids, two adjacent "helical breakers," or extended hydrophobic regions (Lemire et al., 1988; Hartl et al., 1989). Hence, the overall characteristics of this region are suggestive of a mitochondrial targeting sequence.

---

**Fig. 3. DNA sequence of the PSD1 gene from S. cerevisiae.** The 1500-bp open reading frame is shown between the start codon (+1) and stop codon (+1051) which are boxed. The left margin shows complete bp numbering, and the right margin shows the bp number relative to the first base of the start codon. In the 3'-region, potential transcription initiation sites are single underlined. In the 5'-region, two potential transcription termination sequences are shown either in bold or single underlining, for each respective motif, and the putative polyadenylation site is double underlined.

| Coord. | Sequence |
|--------|----------|
| 1051   | GGCTCTTTTTCTGCTATTCTACTTTACCGCTGAATGCGATGTCTCGATTA~CAAGTAAATTCTCT 440 |
| 1331   | TATAATCAACTCTGAAACTGAAATCGAACAAGTCAAGGGAATGACATATTCCATCAAAGAATTCCTT 720 |
| 1401   | GGCACTCACTCCCACCCCATGTCTAAGAG~ATCTAGTCTAGATTTGACTTCTGATGAGGAAAAGC 790 |
| 1471   | ATAGAGAATTCGCCAGGGTAAATAGAATACAATTAGCGGGTTCCGAAGACACTGAACAGCCTCTTCTTAA 860 |
| 1490   | TTTGATTT-GCAATCATATGTAAAGTTUCATTTATTTTGCTG~TATTATCTGCACAC 1560 |
| ...    | ...      |

More, results of Southern and PCR analyses of yeast genomic DNA (see below) are consistent with the size and sequence reported here. Therefore, the entire sequenced region should correspond to a contiguous yeast genomic sequence. The predicted amino acid sequence and its homology with the phosphatidylserine decarboxylase from CHO cells (Kuge et al., 1991) and E. coli (Li and Dowhan, 1988) are depicted in Fig. 4. From computer analysis of the protein sequence, the amino acid sequence from S. cerevisiae shared 44% identity and 60% similarity with the CHO cell enzyme and 35% identity and 55% similarity with E. coli enzyme, while the enzyme from CHO cells and E. coli were 33% identical and 53% similar. There were two distinct areas of high homology among the three enzymes. Both the amino- and carboxy-terminal ends of the proteins showed significant homology, with little homology among the central domains of the enzymes. Hydrophobic analysis by the method of Kyte and Doolittle (1982) showed a very similar homology profile for all three proteins (Fig. 5). The significance of the homology at the amino-terminal end was not clear but homology at the carboxy-terminal end included the highly conserved LG$T(V/I)(V/I)$ sequence corresponding to the post-translational processing site in E. coli which results in Ser-254 being converted to the pyruvoyl prosthetic group at the amino terminus of the $\alpha$ subunit of 7,332 Da (Li and Dowhan, 1988). Similar post-translational activation of the yeast enzyme would result in a pyruvoyl-containing $\alpha$ subunit of 4,192 Da and a $\beta$-subunit of 52,367 Da; the predicted size for the $\alpha$-subunit of the CHO cell enzyme is 3,547 Da (Kuge et al., 1991). The pyruvoyl prosthetic group appears to lie in a hydrophobic environment in all three enzymes. The amino-terminal sequence (57 amino acids) was examined for potential as a mitochondrial targeting sequence. Computer analysis showed a weak $\alpha$-helical but a large $\beta$-sheet hydrophobic moment (0.74) by the method of Eisenberg et al. (1984). Previous work has suggested that an $\alpha$-helical hydrophobic moment is important for a targeting structure, but a $\beta$-sheet hydrophobic moment also appears to be functional (Lemire et al., 1989; Schatz, 1987). In addition, this region exhibited an abundance of positively charged residues and an absence of either acidic amino acids, two adjacent "helical breakers," or extended hydrophobic regions (Lemire et al., 1988; Hartl et al., 1989). Hence, the overall characteristics of this region are suggestive of a mitochondrial targeting sequence.
Interruption of the Genomic Copy of the PSD1 Gene—The diploid yeast strain YPH501 (leu2/leu2) was transformed with a linear DNA construct carrying the PSD1 gene interrupted by insertion of the LEU2 gene as described under “Experimental Procedures.” The resulting diploid strain YCD1 was sporulated, and 20 sets of tetrads were isolated on dissection plates made of YPD medium. Four spores from each tetrad produced colonies. These colonies were streaked to complete inositol-free medium and to inositol-free medium without leucine. In addition, the colonies were streaked to analogous plates supplemented with ethanolamine and choline, to YPD medium or to YPGE medium. None of the 20 sets of tetrads demonstrated auxotrophy for ethanolamine or choline. Eighteen of the tetrads demonstrated 2:2 segregation of the leu2LEU2 markers as shown by growth of haploids on inositol-free media with and without leucine. Two of the tetrads showed 3:1 (LEU2:leu2) segregation and were not considered further. Those colonies prototrophic for leucine should represent cells derived from the spores carrying the interrupted psd1::LEU2 gene. All colonies prototrophic for leucine grew slowly on YPGE plates relative to the colonies auxotrophic for leucine. There was only a slight difference in growth on YPD plates between any of the colonies. Colonies from the four spores of one tetrad were also streaked to the following media: YPGal, YPL, YPG, and YPGE. In all cases, colonies from spores containing the psd1::LEU2 grew more slowly than colonies containing wild type PSD1 gene.

Genomic DNA was prepared from four haploid cultures derived from one tetrad. Fig. 6A shows the results obtained when PCR was performed using genomic DNA from these haploid strains as template and the oligonucleotide primers 1 and 2. Primers 1 and 2 should produce a fragment extending from nucleotide 693 to 1164 of the sequence shown in Fig. 3. The resulting fragment should be 471 bp in length, the length of the PCR fragment observed when genomic DNA from strain YCD1B or YCD1C (PSD1, leu2) was used as template. Since primers 1 and 2 flank the NcoI site (nucleotides 1143-1148) which was used for the insertion of LEU2 gene into the PSD1 sequence, the interruption of PSD1 by LEU2 should result in a 3.5-kb PCR fragment, the length of the fragment...
observed when genomic DNA from strain YCD1A or YCD1D (psd1::LEU2, leu2) was used as template. These results confirmed that the genomic copy of the PSD1 gene has been interrupted by the LEU2 gene in strains YCD1A and YCD1D and that no other sequence existed in these strains which could be detected by PCR using primers 1 and 2. In a second experiment, genomic DNA was digested with HindIII and subjected to Southern hybridization analysis using the labeled insert of plasmid pCC8 as a probe. As shown in Fig. 6B, DNA from the strains YCD1B and YCD1C contained a 2.1-kb HindIII fragment which hybridized to the probe. In contrast, DNA from strains YCD1A and YCD1D contained a 5.1-kb HindIII fragment which hybridized to the probe. The 5.1-kb fragment corresponds to the wild type 2.1-kb fragment into which a 3-kb LEU2 fragment has been inserted.

Phosphatidylserine Decarboxylase Activity of Haploid Strains Derived from the Diploid Strain YCD1—Total membrane preparations were made and assayed for phosphatidylserine decarboxylase activity under yeast conditions as described under “Experimental Procedures.” The phosphatidylserine decarboxylase activities for the two haploid strains were linear with time up to 100 min and had an average specific activity over this time scale of 0.82 and 1.24 nmol/min/mg (Fig. 7A). The two haploid strains with the interrupted psd1::LEU2 gene showed no reproducible or convincing activity over the background level.

To more fully investigate the presence of residual activity and to rule out an inhibitor in the extract from mutant cells, membrane preparations from one of the psd1::LEU2 haploid strains was supplemented on a percent protein basis (w/w) with a membrane preparation from a PSD1 haploid strain. This mixture of membrane preparations was assayed for 60 and 120 min (Fig. 7B). Membrane preparations from the PSD1 haploid strains exhibited a specific activity of 0.55 nmol/min/mg, which was comparable to wild type haploid strain YPH499 (0.24 nmol/min/mg). The membranes from the psd1::LEU2 haploid strains again showed no significant activity. The supplementation of the psd1::LEU2-derived membrane preparation with the PSD1-derived membrane preparation showed activity above background consistent with the amount of supplementation by wild type membrane preparations. Based on the above supplementation experiment, a residual activity of less than 5% of wild type would not have been convincingly detected. This experiment also ruled out an inhibitor of the phosphatidylserine decarboxylase activity in the mutant membrane preparations. No significant activity was observed in the cytosolic fraction of any of the four haploids (data not shown), ruling out the presence of a stable non-membrane associated form of the activity.

Phospholipid Composition of Haploid Strains Derived from the Diploid Strain YCD1—Labeling of phospholipids with $^{32}$P$_4$ showed that even in the absence of detectable phosphatidylserine decarboxylase activity, the percentage of phospholipids represented by PS and PE did not change when the PSD1 gene was interrupted by LEU2 (Table II). PI and PC levels appeared to be slightly higher in cells bearing the interrupted PSD1 gene. Rechromatography of recovered PI, PE, and PS on HPTLC plates in solvent systems which would
Phosphatidylserine Decarboxylase in S. cerevisiae

The evidence presented supports the cloning and disruption of the gene encoding the major phosphatidylserine decarboxylase activity thus far identified in yeast. The complementation of the E. coli mutant, the sequence homology with the E. coli enzyme, and the overproduction in yeast of phosphatidylserine decarboxylase activity by multiple copies of the genomic clone establish that a gene encoding yeast phosphatidylserine decarboxylase activity was isolated rather than a gene responsible for regulation of PSD1 gene expression. The genetic analysis of haploid strains following sporulation of the PSD1::LEU2 strain, the Southern hybridization and PCR analysis of the genomic DNA from the haploid strains, and the lack of phosphatidylserine decarboxylase activity in the two haploids prototrophic for leucine support the disruption of the gene encoding the major activity.

Our assay conditions could have detected at least 5% of normal phosphatidylserine decarboxylase activity if it were present in the extracts from the disrupted haploid strains. There was no evidence of any activity in either the total membrane or cytoplasmic fractions which eliminates a mis-targeted fragment of the enzyme or the presence of a non-membrane-associated form of the enzyme; given what is known about the enzymological properties of the closely related enzyme from E. coli (Warner and Dennis, 1975), it is unlikely that the enzyme could catalyze decarboxylation if it did not have affinity for membranes or detergent micelles. No indication of an inhibitor of decarboxylase activity was found in the mutant extracts. Therefore, the fact that disruption of this gene in haploid strains did not result in ethanolamine auxotrophy, elimination of phosphatidylethanolamine, or the accumulation of phosphatidylserine was unexpected. The possibility exists that the disrupted gene could still express a truncated enzyme from a new promoter originating within the LEU2 insert. However, partial expression of the PSD1 gene is an unlikely explanation for the phenotype of our mutant since a deletion of the PSD1 gene including the region encoding the putative catalytic subunit (Trotter et al., 1993) does not result in either ethanolamine or choline auxotrophy. Since phosphatidylserine levels did not dramatically increase and phosphatidylethanolamine levels were normal in cells with no exogenous supply of ethanolamine, the most likely explanation for these results is a second phosphatidylserine decarboxylase activity which is below the level of detection of our in vitro assay.

The possibility of two genes encoding phosphatidylserine decarboxylase activity is supported by our isolation of several other cDNAs which complement the E. coli mutant, but which show little homology with the insert in plasmid pC8. There is precedence in yeast for a minor isozyme of a subunit of the inner mitochondrial membrane-associated cytochrome oxidase which can provide sufficient activity for mitochondrial

![Image of graph](image-url)
function (Cumsky et al., 1985). CDP-diacylglycerol synthase activity, which is required for synthesis of phospholipids both in the endoplasmic reticulum and the mitochondria, is also found associated with both of these organelles (Zinser et al., 1991) although it is not known if these activities are encoded by the same or different genes. Therefore, the question of whether a phosphatidylserine decarboxylase activity is essential in yeast still remains unresolved, but our results explain why it has been difficult to isolate a mutant in this activity based on screening for a conditional lethal phenotype or activity, which is required for synthesis of phospholipids both by the same or different genes. Therefore, the question of whether this event occurs before or after membrane assembly, but in E. coli a mutant phosphatidylserine decarboxylase which cannot undergo activation (Ser-254 replaced by alanine) or mutants in which activation is very slow (Ser-254 replaced by cysteine or threonine) accumulate unprocessed enzyme in the membrane and not in the supernatant (Li and Dowhan, 1990). This would suggest that membrane assembly does not require enzyme activation, and membrane assembly may even be required for activation. In the histidine decarboxylases, the activation event requires a folded proenzyme form (van Poelje and Snell, 1990). Since translocation of proteins targeted to the inner mitochondrial membrane occurs while the precursor is in a largely an unfolded state (Glick and Schatz, 1991) and activation of the decarboxylases results in a heterologous dimer as the minimum catalytic unit, the activation step probably occurs after translocation and assembly.

Now that a psd1 mutant and a PSD1 gene of yeast are available, several future experiments are possible. First, the issue of the mechanism by which phosphatidylethanolamine is synthesized in the absence of the major phosphatidylserine decarboxylase and ethanolamine supplementation must be resolved. Further characterization of the remaining cDNAs which correct the E. coli mutant may aid in answering this problem. Interesting questions concerning the mechanism of targeting and assembly of this inner mitochondrial membrane enzyme can be addressed. Further resolution of the genetics and biochemistry of this step in phospholipid metabolism will shed light on the synthesis and regulation of phospholipids in both the mitochondria and throughout the cell, particularly if two decarboxylases exist. Finally, a more direct approach to the complex problem of phosphatidylserine/phosphatidylethanolamine trafficking between the endoplasmic reticulum and the mitochondria can be formulated.

Acknowledgments—We thank Dr. Steve Elledge of Baylor College of Medicine for providing us with the YES library and the phage M13 without which this work would not have been possible. We also thank Dr. Mike Gustin of Rice University for his assistance in the tetrad analysis and Dr. Dennis R. Voelker and colleagues for sharing some of their observations prior to publication.

REFERENCES

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

Blumenthal, W. R., and Bell, R. M. (1988) J. Bio. Chem. 263, 570-610

Broch, J., Strathern, J. N., and Hicks, J. B. (1979) Gene (Amst.) 1, 121-133

Carmen, G. M., and Henry, S. A. (1988) Annu. Rev. Biochem. 57, 635-669

Cigan, M. A., and Donahue, T. F. (1987) Gene (Amst.) 58, 1-18

Cumisky, M. G., Ko, C., Trefilm, C. E., and Foyton, R. O. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 2255-2259

DeChavigny, A., Heacock, P. N., and Dowhan, W. (1991) J. Biol. Chem. 266, 3253-3262

Dowhan, W. (1992) Methods Enzymol. 209, 7-20

Dowhan, W., and Li, Q.-X (1992) Methods Enzymol. 209, 348-359

Dowhan, W., Wickner, W. T., and Kennedy, E. F. (1972) J. Biol. Chem. 249, 3079-3084

Eisenberg, D., Weiss, R. M., and Terwilliger, T. C. (1984) Biophysics 84, 140-144

Elledge, S. J., Mulligan, J. T., Ramer, S. W., Spotwood, M., and Davis, R. W. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 1731-1735

Ferber, G., Villarel, A., and Mortale, J. (1983) Biotechniques 3, 442-452

Fine, J. B., and Sprecher, H. (1980) J. Biol. Chem. 255, 660-666

Genetic Computer Group (1989) Program Manual for the GCG Package, Version 7, Madison, WI 53711

Glick, B., and Schatz, G. (1991) Annu. Rev. Genet. 25, 21-44

Guarnere, L. (1987) Annu. Rev. Genet. 21, 425-452

Hartl, F.-U., Pflanzer, N., Nicholson, D. W., and Neupert, W. (1989) Biochem. Biophys. Acta 988, 1-45

Hewitt, E., and Kennedy, E. P. (1976) Proc. Natl. Acad. Sci. U.S.A. 72, 1112-1116

Hirschhorn, P. A., and Rose, A. H. (1991) The Yeasts: A Taxonomic Study of the Yeasts. A Monograph (Kluwer Academic Publishers, The Netherlands, 1991) 4th eda Vol. 4, p. 313, Academic Press Inc., San Diego, CA

Hirsch, P. J., and Henry, S. A. (1986) Mol. Cell. Biol. 6, 3230-3239

Homann, M., J. Poole, M. A. Gaynor, P. M., Ho, C.-T., and Carman, G. M. (1987) J. Bacteriol. 169, 553-559

Hosaka, K., Kodaki, T., and Yamanishi, S. (1986) J. Biol. Chem. 261, 2535-2553

Innis, M. A., and Gelfand, D. H. (1990) in PCR Protocols: A Guide to Methods

Phosphatidylserine Decarboxylase in E. cavaeias
Phosphatidylserine Decarboxylase in S. cerevisiae and Applications (Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J., eds) pp. 3–12, Academic Press, Inc., San Diego, CA

Jennings, S. M., Tsay, Y. H., Flach, T. M., and Robinson, G. W. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6039–6042

Kodaki, T., Nikawa, J., Hosaka, K., and Yamashita, S. (1991a) J. Bacteriol. 173, 7092–7095

Kodaki, T., Hosaka, K., Nikawa, J., and Yamashita, S. (1991b) J. Biochem. (Tokyo) 100, 276–287

Kuchler, K., Daum, G., and Paltauf, F. (1989) J. Bacteriol. 165, 901–910

Kuge, O., Nashijima, M., and Akamatsu, Y. (1991) J. Biol. Chem. 266, 6370–6376

Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132

Lamping, E., Kohlwein, S. D., Henry, S. A., and Paltauf, F. (1991) J. Bacteriol. 173, 6420–6437

Lemire, B. D., Fankhauser, C., Baker, A., and Schatz, G. (1989) J. Biol. Chem. 264, 20206–20215

Li, Q.-X., and Dowhan, W. (1988) J. Biol. Chem. 263, 11516–11522

Li, Q.-X., and Dowhan, W. (1990) J. Biol. Chem. 265, 4111–4115

Maniatis, T., Fritsch, E. F., and Sambrook, F. (1982) Molecular Cloning, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY

Marck, C. (1988) Nucleic Acid Res. 16, 1829–1836

Nasymth, K. A., and Tatchell, K. (1980) Cell 19, 753–764

Nikawa, J. I., Kodaki, T., and Yamashita, S. (1988) J. Bacteriol. 170, 4727–4731

Nikoloff, D. M., and Henry, S. A. (1991) Annu. Rev. Genet. 25, 559–583

Overmeyer, J. H., and Warachter, C. J. (1991) Arch. Bioch. Biophys. 290, 511–516

Rao, C. R. H., and Dowhan, W. (1990) J. Biol. Chem. 265, 1235–1238

Russo, P., Li, W.-Z., Hampsey, D. M., Zaret, K. S. and Sherman, F. (1991) EMBO J. 10, 563–571

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning, 2nd Ed, Cold Spring Harbor Laboratory, New York

Sanger, F., Miklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467

S鲍, M., and Kennedy, E. P. (1978) J. Biol. Chem. 253, 479–483

Schacht, G. (1987) Eur. J. Biochem. 165, 1–6

Schiestl, R. H., and Gietz, R. D. (1989) Curr. Genet. 16, 339–346

Sherman, F., Fink, G. R., and Hicks, J. B. (1986) Laboratory Course Manual for Methods in Yeast Genetics, pp. 117–168, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Simchen, R., Gervois, Y., and Kaper, J. B. (1979) Res. Microbiol. 130, 417–427

Simchen, R., Gervois, Y., and Kaper, J. B. (1982) J. Gen. Microbiol. 128, 2575–2586

Struhl, K. (1989) Annu. Rev. Biochem. 58, 1051–1077

Trotter, P. J., Pezet, J., and Voelker, D. R. (1983) J. Biol. Chem. 268, 21416–21424

Tsubokawa, Y., Nikawa, J.-I., Hosaka, K., and Yamashita, S. (1991) J. Bacteriol. 173, 2134–2136

Van Loon, A. P. G. M., Van Bijk, E., and Grivell, L. A. (1983) EMBO J. 2, 1753–1759

Van Poelje, P. D., and Smell, E. E. (1990) Annu. Rev. Biochem. 59, 29–59

Voelker, D. R. (1991) Mol. Microbiol. 5, 453–460

Warner, T. G., and Dennis, E. A. (1975) J. Biol. Chem. 250, 8004–8009

Wickens, M., and Stephenson, P. (1984) Science 226, 1045–1051

Zagursky, R. J., and Berman, M. L. (1984) Gene (Amst.) 27, 183

Zaret, K. S. and Sherman, F. (1982) Cell 28, 563–573

Zinser, E., Sperka-Gottlieb, C. D. M., Pass, E.-V., Kohlwein, S. D., Paltauf, F., and Daum, G. (1991) J. Bacteriol. 173, 2026–2034