Ser3p (Yer081wp) and Ser33p (Yil074cp) Are Phosphoglycerate Dehydrogenases in *Saccharomyces cerevisiae*

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Two genes *YER081W* and *YIL074C*, renamed *SER3* and *SER33*, respectively, which encode phosphoglycerate dehydrogenases in *Saccharomyces cerevisiae* were identified. These dehydrogenases catalyze the first reaction of serine and glycine biosynthesis from the glycolytic metabolite 3-phosphoglycerate. Unlike either single mutant, the *ser3a ser33a* double mutant lacks detectable phosphoglycerate dehydrogenase activity and is auxotropic for serine or glycine for growth on glucose media. However, the requirement for the *SER*-dependent “phosphoglycerate pathway” is conditional since the “glyoxylate” route of serine/glycine biosynthesis is glucose-repressed. Thus, in cells grown on ethanol both expression and activity of all *SER*-encoded proteins are low, including the remaining enzymes of the phosphoglycerate pathway, Ser1p and Ser2p. Moreover, the available nitrogen source regulates the expression of *SER* genes. However, for only *SER33*, and not *SER3*, expression was regulated in relation to the available nitrogen source in a coordinated fashion with *SER1* and *SER2*. Based on these mRNA data together with data on enzyme activities, Ser33p is likely to be the main isoenzyme of the phosphoglycerate pathway during growth on glucose. Moreover, since phosphoglycerate dehydrogenase activity requires NAD⁺ as cofactor, deletion of *SER3* and *SER33* markedly affected redox metabolism as shown by substrate and product analysis.

Amino acid biosynthesis may proceed via different metabolic routes in *Saccharomyces cerevisiae*. For instance, glycine can potentially be formed from threonine via threonine aldolase, Gly1p (Fig. 1). This pathway, the “threonine pathway,” has been suggested to be the main glycine source on glucose media (1), but this function has not been verified. If glycine is formed, serine can subsequently be produced by interconversion of serine by tetrahydrofolate (THF)-dependent serine hydroxymethyltransferases, Shm1p and Shm2p (2–4). Alternatively, serine and glycine can be formed from glyoxylate, via the “glyoxylate pathway.” However, since both the formation of glyoxylate and the alanine glyoxylate aminotransferase (encoded by *YFL030W*) needed for this biosynthetic pathway is glucose-repressed, this alternative route for the production of serine and glycine can only be employed in the absence of glucose (5–8). However, a third alternative is to start biosynthesis of serine and glycine from the glycolytic intermediate 3-phosphoglycerate (Fig. 1). The first reaction in this pathway, the “phosphoglycerate pathway,” is catalyzed by phosphoglycerate dehydrogenase (EC 1.1.1.95), producing 3-phosphohydroxypyruvate plus NADH from 3-phosphoglycerate and NAD⁺. The enzymes of the subsequent two reactions are phosphoserine transaminase (Ser1p, EC 2.6.1.52) and phosphoserine phosphatase (Ser2p, EC 3.1.3.3). Deletion mutants of either *SER1* or *SER2* need an external source of serine during growth on glucose (5, 6, 9). However, genes encoding the first enzyme of the pathway, phosphoglycerate dehydrogenase, have as yet not been identified in *S. cerevisiae*.

Based on sequence similarity (47% identity) to the phosphoglycerate dehydrogenase gene in *Escherichia coli* we have previously suggested that yeast has two putative phosphoglycerate dehydrogenases (10). Those genes, *YER081W* and *YIL074C*, encode proteins that are 92% identical to each other. They are here denoted *SER3* and *SER33*, respectively. Sequence similarity analysis of available phosphoglycerate dehydrogenases revealed two groups of proteins; *S. cerevisiae* and *E. coli* enzymes representing one group and rat liver and *B. subtilis* enzymes representing the other (11). The *E. coli* and yeast enzymes also have in common their preference for the co-substrate NAD⁺ instead of NADP⁺ (5, 12).

Furthermore, Zhao and Winkler (12) have shown that the *E. coli* enzyme has hydroxylutarate dehydrogenase activity (EC 1.1.99.2) in addition to phosphoglycerate dehydrogenase activity, whereas this was not the case for the rat liver enzyme (11). Based on the above similarities we have recently suggested that the yeast enzyme may exert dual activities (10). In *S. cerevisiae*, we showed 2-hydroxyglutarate to be formed from glutamate, probably via the tricarboxylic acid cycle intermediate 2-oxoglutarate, when glutamate served as the sole nitrogen source (10). However, hydroxylutarate dehydrogenase activity has as yet not been reported in yeast.

Other proteins with homology to *Ser33p* may also be candidates for having hydroxylutarate dehydrogenase activity in *S. cerevisiae*: (i) Ypl113cp (27% identity), (ii) Ygl185cp (21–23% identity), with similarities to hydroxyacid dehydrogenases, (iii) Fdh1p (26–27% identity), a formate dehydrogenase, and (iv) Ynl274cp (25–26% identity), which is a putative hydroxysocaproate dehydrogenase (13).

In the present study, we have investigated physiological and expression properties of yeast strains with single and double deletions of *SER3* and *SER33*. Our data establish that *SER3* and *SER33* encode phosphoglycerate dehydrogenases and that they appear to be the only enzymes with such activity in...
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**S. cerevisiae.** Ser3p seems to be the main isoenzyme of the phosphoglycerate pathway of serine/glycine biosynthesis.

**EXPERIMENTAL PROCEDURES**

*Strains, Media, and Growth Conditions—* We have used the *S. cerevisiae* strain, CEN.PK113–7D (MATa SUC2 GAL MAL2–8°) as the protoprophic wild type (14). Mutant strains of YER081W and YIL074C genes, YVL87 and YVL88, were obtained from the isogenic strains CEN.PK111–32D (MATa leu3–1,112 SUC2 GAL MAL2–8°) and CEN.PK111–10C (MATa his3Δ1 SUC2 GAL MAL2–8°), respectively. All the CEN.PK strains mentioned can be received from EUROSCARF, Frankfurt, Germany (www.uni-frankfurt.de/hbf/mikro/EUROSCARF/).

YPD, containing 10 g/liter of yeast extract, 20 g/liter of peptone, 20 g/liter of glucose, was used as complex medium. Agar (20 g/liter) plates were prepared from either YPD or, when defined conditions were needed, from YNB (1.7 g/liter of yeast nitrogen base without amino acids and ammonium sulfate, Difco), with appropriate additions of carbon and nitrogen sources as well as supplements. Sporulation medium contained 10 g/liter of potassium acetate and 20 g/liter of agar.

For liquid cultures, a synthetic defined medium was used, CBS (15), with glucose as carbon and energy source at a concentration of 20 g/liter and glutamate (3.5 g/liter) or ammonium sulfate (5 g/liter) as the nitrogen source. When needed, glycine (0.5 g/liter) or serine (0.7 g/liter) was added. The initial pH of the medium was adjusted to 6.0.

Inoculum cultures were grown on synthetic defined medium with glucose and were supplemented with serine. Cultures were grown in Erlenmeyer flasks (E-flasks) at 30 °C (the volume of flask was twice the volume of medium) on a rotary shaker resulting in semihomogeneous conditions and inoculated with 1.7% of the total culture volume.

**Gene Disruptions—** Each open reading frame (ORF) was substituted with a deletion cassette containing either the HIS3 gene (to replace ORF YIL074C) or the LEU2 gene (to replace ORF YER081C). Deletion cassettes were prepared with 2 rounds of PCR according to Wach (16), using primers L1 and L4, and restriction analysis of the amplified fragments at the end of both rounds. Deletion cassettes were then purified using the QIAquick Gel Extraction kit (Qiagen) and introduced in yeast cells according to Ito et al. (18).

For gene disruption, cells were routinely grown at 30 °C on YPD plates. Deletion mutants were selected on YNB-HIS or YNB-LEU plates (6.7 g/liter of yeast nitrogen base with ammonium sulfate but without amino acids, with addition of 0.77 g/liter CSM-HIS or 0.69 g/liter CSM-LEU, respectively). Correct integration of the deletion cassette was verified by PCR and enzymatic restriction. No defects in mutant mating were verified by PCR and enzymatic restriction. No defects in mutant mating were detected.

**Growth and Metabolite Analyses—** Biomass concentration was determined by comparison with a macroplaque of *S. cerevisiae* strain 10265 /H9251 MATa (21), using the following oligonucleotides (5′–3′, the sequence complementary to YDR338C): 5′-CTTGGATAAGCGGCTTTGATAC-3′ (YER081W), 5′-GCTTTGAGATCTCAG-3′ (YIL074C), 5′-TAATGCC; L2, GCTCAATCAATCACCGGATCCCCGGAATGCTTGTC-3′ (YER081W) and 5′-AGGCTTGCAGGAGCAATTGT-3′ (YIL074C). PCR reactions were performed as follows: (i) first round with Taq DNA polymerase (Fermentas), 2 min at 94 °C and 30 cycles of [30 s at 94 °C, 30 s at 50 °C, 1 min at 72 °C], and (ii) second round with the Expand Long Template PCR System (Roche Molecular Biochemicals), 1 min at 94 °C and 30 cycles of [15 s at 94 °C, 30 s at 45 °C, 1.5 min at 68 °C]. A final elongation step of 10 min was performed at the end of both rounds. Deletion cassettes were then purified using the QIAquick Gel Extraction kit (Qiagen) and introduced in yeast cells according to Ito et al. (18).

For labeling of proteins, duplicate cultures were grown in CBS medium supplemented with glucose as carbon source, and ammonium (5 g/liter), glycine (5 g/liter), serine (5 g/liter) or threonine (5 g/liter) as the nitrogen source. Cells were grown at 30 °C, and RNA was extracted from cells during respiro-fermentative exponential growth to the diauxic shift. Total RNA was isolated from yeast cells as described by Ausubel et al. (19) using a FastPrep apparatus (BIO101). RNA samples (10 μg/μl) were fractionated on 1% agarose gels containing formaldehyde, transferred by downward capillary blotting onto Hybond-N membranes (Amersham Biosciences) in 10× SSC buffer (22) and cross-linked using a GS GeneLinker (BioRad). Blots were then prehybridized at 65 °C for at least 2 h in 5× SSC, 50 mM NaHPO4 (from a 1× stock solution at pH 6.5), 5× Denhardt’s solution, 0.5% SDS, and 0.1 mg/ml denatured salmon sperm DNA, and then hybridized at 65 °C for 16 h with the RNA probe denaturing in 100% formamide.

**Native Blot Analyses—** Gene expression was investigated in cells grown in CBS medium supplemented with glucose as carbon source, and serine (0.77 g/liter), glycine (0.69 g/liter) or ammonium sulfate (0.5 g/liter) as the nitrogen source. Cells were grown at 30 °C, and RNA was extracted from cells during respiro-fermentative exponential growth to the diauxic shift. Total RNA was isolated from yeast cells as described by Ausubel et al. (19) using a FastPrep apparatus (BIO101). RNA samples (10 μg/μl) were fractionated on 1% agarose gels containing formaldehyde, transferred by downward capillary blotting onto Hybond-N membranes (Amersham Biosciences) in 10× SSC buffer (22) and cross-linked using a GS GeneLinker (BioRad). Blots were then prehybridized at 65 °C for at least 2 h in 5× SSC, 50 mM NaHPO4 (from a 1× stock solution at pH 6.5), 5× Denhardt’s solution, 0.5% SDS, and 0.1 mg/ml denatured salmon sperm DNA, and then hybridized at 65 °C for 16 h with the RNA probe denaturing in 100% formamide. The RNA probe was labeled with [32P]dCTP (3000 Ci/ml) by use of a MegaPrime DNA labeling kit (Amersham Biosciences) and purified on a Nick column (Amersham Biosciences). SER1 and SER2 probes were prepared from the following PCR fragments (5′–3′). The resulting fragment length will be given in parenthesis. SER1 (1110 bp): TTGGGAAAGAGAGAAACCAACA and ATAGATGGAGGCTGACCAACC. SER2 (968 bp): GTTA-TCACCTGCTAAGTGGGAG and TTGACTGTTATCGTGGATTC. SER3 (1120 bp): TGTTGGCTCTATAAACCAGT and CCTGGCAAGAACATTATTTTCAAGCAGT. SER4 (900 bp): ATGTCGAAGGGAAAGGACAGGG and GCTCCT-ATCACTCCGCTTAAGTGAGCT. The resulting fragment length will be given in parenthesis. SER2 (968 bp): GGCTAGC and SER3 (880 bp): AGAGCAATTTGGGCGCAAGAA and AGGAAACCCTTTGTTAATAG.

These were labeled with [32P]ATP (3000 Ci/ml) by use of the T4 polynucleotide kinase (MBI) and purified on a microspin G25 column (Amersham Biosciences). The specificities of the oligonucleotides used for probing SER3 and SER3 probes were confirmed using the ser2A, ser3A and ser3B strains. Blots were washed 2× 5 min at room temperature and 2× 5 min at 65 °C in 0.5× SSC/0.1% SDS, then exposed 6–24 h to PhotonImager screens. Non-saturated signals were measured by a BioRad FX phospholmager and quantified by densitometry. Values were normalized by comparison with IPP1 signals.

**Expression Analysis—** For labeling of proteins, duplicate cultures were inoculated from overnight cultures giving a starting OD610 of 0.35–0.39 and grown to an OD610 of 0.35–0.39 in glucose/galactose medium supplemented with glycine. A volume of 1 ml was transferred to a separate flask (10 ml) and 8 μl (3.2 MBq) of [35S]methionine (specific activity >57 TBq/mmol, Amersham Biosciences) was added. The cultures were grown for another 30 min and then placed on ice. Cells were harvested (15,000 × g, 4 °C, 5 min), washed once with ice-cold milliQ water (Millipore), and the resulting cell pellet was stored at −80 °C until used. Proteins were extracted, the amount of incorporated [35S] was measured and proteins (in 2,000,000 dpm of extract) were separated by gel electrophoresis.

**Odor measurements at higher cell densities.** From these curves the maximal specific growth rate was determined.

Growth tests were performed on YNB plates, with a carbon source (20 g/liter of glucose or ethanol), a main nitrogen source (5 g/liter of ammonium, 3.5 g/liter of glutamate, or 10 g/liter glycine or threonine), and eventually an additional nitrogen source (120 mg/liter serine, glycine, or threonine). Inocula were grown in liquid YPD. Cells were washed in the corresponding YNB medium and diluted to an OD610 of 1. Aliquots of 10 μl of serial one-tenth dilutions (up to 10–7) were dropped on plates with appropriate medium and incubated for 2 days (ethanol plates for 6 days) at 30 °C.

Product formation and substrate consumption of the entire respiro-fermentative growth phase were determined from medium samples taken at inoculation and directly after glucose exhaustion. Glucose was checked using a Dihabur-Test (500 μl, Boehringer Mannheim).

Extracellular concentrations of metabolites (including glucose) were measured with HPLC (waters) using an ion-exchange column, HPX-87H, BioRad, as described previously (10). Glutamic acid concentration was determined using an enzymatic kit assay (Roche Molecular Biochemicals). Glycine was determined by measurements of the total free amino acid nitrogen concentration, by staining with ninhydrin (21), from which the glutamic acid contribution was subtracted. The standard solution contained both glutamate and glycine in the same concentration as present in the experiments.

The abbreviations used are: ORF, open reading frame; MALDI, matrix-assisted laser desorption; HPLC, high performance liquid chromatography.
two-dimensional PAGE (23). The protein pattern was detected by phos- 
horbimaging and protein quantification was performed by image ana-
lysis with the PDQuest software (BioRad). At least 2-fold and statisti-
tically significant changes (Student’s t test on log-transformed data) in 
protein levels were distinguished. Resolved proteins have previously 
been identified by microsequencing or MALDI-MS (23, 24). Protein 
identity data can be found at yeast-2DPAGE.gmm.gsu.edu.

Crude Cell Extract Preparation and Enzyme Activity Measure-
ments—Crude cell extracts were prepared from 50 ml of culture. Cells 
were harvested (2,000 × g, 4 °C, 5 min) and washed twice (20 and 1 ml) 
with washing buffer (10 mM KH₂PO₄, pH 7.5, containing 2 mM EDTA). 
The sedimented cell pellet was frozen in liquid nitrogen and stored at 
−20 °C until further treatments. The thawed cell pellet was resus-
pended in liquid dithiothreitol buffer (2 mM MgCl₂ and 1 mM dithiothreitol 
in 100 mM KH₂PO₄, pH 7.5) and supplemented with protease inhibitors 
(1 μl of 35 μl phenylmethanesulfonyl fluoride in ethanol and 0.6 μl of 
0.8 μl pepstatin and 1.2 μl leupeptin in ethanol). Cells were 
disrupted by vortexing with 1 g of glass beads (diameter 0.5 mm) for 
5 min at 4 °C. The suspension was then transferred to a 1.5-ml tube, and 
the beads were washed with 0.5 ml of extraction buffer (supplemented 
with appropriate amounts of protease inhibitors). After centrifugation 
at 15,000 × g, 4 °C, 15 min (if necessary twice), pooled supernatants 
were used immediately for measurement of enzyme activity and protein 
content. For preparation of crude extracts used in analysis of phospho-
serine phosphatase (Ser2p) activity (see below), Tris-HCl buffer at the 
same concentration and pH was used instead of the ordinary phosphate 
buffer.

Enzyme activities (except for Ser2p activity) and protein content 
were measured at 30 °C on a COBAS Fara Autoanalyzer (Roche Molec-
ular Biochemicals). Protein concentration was determined according to 
Lowry et al. (25), with bovine serum albumin as standard.

Phosphoglycerate dehydrogenase (EC 1.1.1.95) activity was mea-
sured by reduction of hydroxypyruvate phosphate, following the de-
crease of NADH at 340 nm. The reaction mixture contained 1 mM 
dithiothreitol, 0.25 mM NADH, 400 mM KCl, and 0.36 mM hydroxypyr-
ivate phosphate (prepared from the corresponding dimethylketal, 
Sigma) in 40 mM KH₂PO₄ buffer, pH 7.5. The activity of phosphoserine transaminase activity the assay of 
Hirsch-Kolb and Greenberg was essentially used (26), following the 
decrease of NADH. The reaction mixture contained 4 mM NaF, 0.25 mM 
NADH, 30 mM ammonium acetate, 20 mM pyridoxal phosphate, 10 
units/ml of glutamate dehydrogenase (NAD(P)-dependent, type III bo-
vine liver, Sigma), 8 mM Na-L-glutamate, and 0.36 mM hydroxypro-
pyruvate phosphate in 50 mM Tris-HCl buffer, pH 8.2.

Phosphoserine phosphatase was determined manually at 30 °C with 
crude extracts in a reagent solution containing 5 mM O-phospho-
t-serine and 5 mM MgCl₂ in 65 mM Tris-HCl buffer, pH 7.5. The reaction 
was stopped in aliquots of reaction mixture (320 μl) with 80 μl of 
trichloroacetic acid (250 g/liter) at appropriate time intervals after 
substrate addition (up to 10 min). Subsequent determination of released 
phosphate was performed according to Dryer et al. (27). There was no 
background release of phosphate in the reagent solution during the 
time of measurement, thus giving the enzymatic activity directly from 
the rate of phosphate release.

Hydroxysuccinylate dehydrogenase (EC 1.1.99.2) activity was mea-
sured by reduction of 2-oxosuccinate, following the decrease of NADH. 
The reaction mixture contained 1 mM dithiothreitol, 0.25 mM NADH, 
and 5 mM 2-oxoglutarate in 40 mM KH₂PO₄ buffer, pH 7.5. Formation of 
2-hydroxysuccinate during assay condition was checked by HPLC. 
Glutamate dehydrogenase activity was shown to negligibly contribute to 
the reduction of NADH in the hydroxysuccinate dehydrogenase assay, 
despite the fact that the crude extracts contained small amounts of 
ammonium.

Activity of hydroxysuccinylate dehydrogenase was measured in the same 
way as the hydroxysuccinate dehydrogenase, except that 6 mM of 
2-oxosuccinate was used as substrate.

Enzyme activity (units, μmol/min) was determined from the differ-
ence in absorbance (at 340 nm, ε = 6.3 mm⁻¹ cm⁻¹) after 
and before addition of substrate (3-phosphohydroxypropruvate, 3-phos-
phohydroxypropruvate plus glutamate, 2-oxoglutarate, or 2-oxoisocap-
route). When the slope prior to substrate addition was equal to or 
larger than the slope after substrate addition, then the detection limit 
was estimated as follows. The variances of the two slopes obtained in the 
linear regression of absorbance data were added and the 95% confi-
dence interval was calculated according to a t-distribution. The detec-
tion limit was around 0.2 units/g of protein for the phosphoserine dehydrogenase 
and between 0.1–0.6 units/g of protein for the hydroxy-
isocaproate dehydrogenase.

RESULTS

Ser3p and Ser33p Are Phosphoglycerate Dehydrogenases in 
S. cerevisiae—The full-length coding sequences of SER3 
(YER081W) and SER33 (YIL074C) were deleted and replaced with 
LEU2 and HIS3 markers, respectively, in the haploid strains 
CEN.PK113–32D and CEN.PK110–10C, respectively. 
This yielded isogenic strains without auxotrophic markers. A 
haploid double deletion mutant was obtained by mating and 
subsequent sporulation. The single and double deletion mu-
tants were viable and formed colonies of normal size and mor-
phology on complex media.

During growth on glucose, deletion mutants of all functional phosphoglycerate dehydrogenases should result in a block in the 
phosphoglycerate pathway of serine/glycine biosynthesis. 
Such mutants would require externally available serine or 
glycine, provided that no alternative pathways are active, such 
as the glyoxylate or threonine pathways (Fig. 1). As shown in 
Fig. 2A, the SER3 and SER33 genes encode the only functional 
phosphoglycerate dehydrogenases since the double mutant 
requires serine for growth, while the single ser3Δ and 
ser33Δ mutants could grow in the absence of serine. 
Furthermore, the glyoxylate pathway provides no alternative 
during growth on glucose since it is glucose-repressed (5–8). In contrast, as 
expected the ser3Δ ser33Δ mutant grew well on ethanol as 
the only carbon source with ammonium as the only nitrogen source 
(Fig. 2B).

In addition, the double deletion mutant (ser3Δ ser33Δ) provides 
an opportunity to investigate the function of the second 
alternative pathway, the threonine pathway, which has been 
suggested to be important during growth on glucose (1). 
However, addition of threonine did not allow growth of the double 
mutable, either when added to ammonium (data not shown) or 
glutamate (Fig. 2C) as the main nitrogen sources or when used 
as the sole nitrogen source (data not shown). Thus, the thr3-
mine pathway seems to be non-functional during all conditions 
tested.

Surprisingly, the addition of glycine to ammonium-containing 
medium did not support growth of the double mutant (Fig. 2A). 
However, ammonium mediates strong nitrogen catabolite 
repression on for example uptake systems (28). We therefore 
tested other nitrogen sources (Fig. 2C). The double mutant 
could grow on both glycine (or serine; data not shown)—supple-
mented glutamate medium or on glycine as the sole nitrogen 
source (data not shown). Both single deletion mutants 
could also grow on glutamate as sole nitrogen source (Fig. 2C).

We have measured enzymatic activities in crude extracts to 
confirm the function of Ser3p and Ser33p as phosphoglycerate dehydrogenases (Table I). The activity of the phosphoglycerate 
dehydrogenase in Escherichia coli is inhibited by serine and to 
a lesser extent by glycine (12, 29, 30). Since inhibition by serine 
has been reported also for yeast (5), glycine was used as a 
supplementing nitrogen source to glutamate in the following 
experiments in order to minimize inhibition of phosphoglycerate 
dehydrogenase while still allowing growth on glucose of the 
ser3Δ ser33Δ double mutant. The ser3Δ ser33Δ mutant showed 
no phosphoglycerate dehydrogenase activity (Table I), indicat-
ing that only these two genes encode such an activity. The 
specific activity determined in the wild-type strain was equi-
valent to the sum of the activities found in the single mutants. 
The reduction of phosphoglycerate dehydrogenase activity was 
more pronounced in the ser3Δ mutant, indicating that Ser3p is 
the major isoenzyme. As may be expected, since externally 
accessible glycine reduces the need for its biosynthesis, addi-
tion of glycine reduced the activity in all strains tested, most 
prominently in the ser3Δ mutant. Externally available glycine 
may affect the expression of the SER3 and SER33 genes or
modulate the activity of Ser3p and Ser33p directly. To clarify the situation, the mRNA expression was studied during growth with different nitrogen sources, see below. Also when glycine was added to the medium, the activities measured in the single mutants added up to that of the wild-type strain.

Expression of the SER Genes and Enzyme Activities during Respiro-fermentative Growth—The expression of genes encoding the enzymes of the serine/glycine pathway was analyzed by Northern blot analysis during respiro-fermentative growth and at the transition to respiratory growth. Small but significant differences in gene expression between the wild-type strain and the single mutants were observed (Fig. 3, A and B). The SER1, 2, and 33 genes showed a declining expression during late exponential growth. After glucose exhaustion, the mRNA level of SER1 seemingly increased. However, this apparent increase of SER1 mRNA probably results from the normalization of mRNA levels with IPP1 expression. IPP1 seemed to be down-regulated at the diauxic shift, since despite that the same amount (concentration was measured) of total mRNA was loaded on the gels the amount of IPP1 mRNA at this growth phase was low for all strains and irrespective of type of nitrogen source (Fig. 3B; data not shown). The low enzymatic activity of Ser1p determined at diauxic shift also indicates a true low expression of SER1. However, down-regulation of IPP1 has

Fig. 1. Serine and glycine synthesis in S. cerevisiae, adapted from Refs. 1-3, 5, 8, 48, and 49. Enzymes: Ser3p, Ser33p: phosphoglycerate dehydrogenases; Ser1p: phosphoserine transaminase; Ser2p: phosphoserine phosphatase; Shm1p, Shm2p: serine hydroxymethyltransferases; Gcv1p + Gcv2p + Gcv3p + Lpd1p: a protein complex of these proteins in the glycine cleavage system, i.e. glycine decarboxylase; Gly1p: threonine aldolase; Yf030wp: alanine glyoxylate aminotransferase; and Icl1p, Icl2p: isocitrate lyases. THF, tetrahydrofolate.

Fig. 2. Growth tests of wild type (wt) and ser3Δ or/and ser33Δ deletion mutants using serial one-tenth dilutions on YNB plates. A, serine or glycine supplementation. Glucose was used as carbon and energy source and the ammonium ion as the main nitrogen source. B, ethanol was used as carbon and energy source with the ammonium ion as the sole nitrogen source. C, glycine or threonine supplementation. Glucose was used as carbon and energy source and glutamate as the main nitrogen source.

| Type of strain | Glutamate medium (units/g protein) | Glutamate/glycine medium (units/g protein) |
|---------------|----------------------------------|------------------------------------------|
| wt            | 3.2                              | 2.2                                      |
| ser3Δ         | 2.9                              | 1.7                                      |
| ser33Δ        | 1.3                              | 0.5×                                     |
| ser3Δser33Δ   | <0.3×                            | 0.2×                                     |

* The preculture was grown on glutamate medium supplemented with serine. At OD₆10 = 4.9 (after glucose exhaustion), cells were transferred to a medium containing only glutamate as nitrogen source. Samples were taken before glucose exhaustion after 1 day of incubation. After two more days of incubation, no activity was detectable. Two independent determinations were done.

* One sample was below the detection limit.

* The activity in five samples was below the detection limit, while the activity in three other samples was close to the detection limit.
whereas SER3 expression was not affected by the deletion of SER33, whereas SER33 expression was slightly decreased in the ser3Δ mutant.

The enzyme activities of the three reactions of the pathway (Ser1p, Ser2p, Ser33p) were also analyzed to reveal correlations between gene expression and protein activity (Fig. 3, A versus C). The phosphoserine transaminase activity stayed constant during the respiro-fermentative growth phase but strongly decreased after glucose depletion. The phosphoserine phosphatase activity behaved similarly, although decreased already before glucose exhaustion. The specific activity of phosphoglycerate dehydrogenase first increased monotonically until glucose depletion and then decreased drastically at the diauxic shift. The SER enzyme activities followed the mRNA expression in that both decreased during the shift from respiro-fermentative to respiratory growth. Hence, the activity of the phosphoglycerate pathway seemed to some extent be regulated at the level of gene expression.

Expression of SER Genes during Growth on Different Nitrogen Sources—Since glycine addition was found to have an impact on the phosphoglycerate dehydrogenase activity, the effect of different nitrogen sources on the expression of the SER genes was then investigated. The expression of all SER genes, except for SER3, was affected by the nitrogen source in a similar way (Fig. 4). The SER3 mRNA level was very sensitive to additions of serine, glycine, and threonine, which largely reduced the expression, while the expression of the other SER genes was only sensitive to threonine addition (Fig. 4). Hence, the reduced activities found in glycine-supplemented medium (Table I) may partly be explained by reduced expression of SER3.

Although there was a tendency of decreased expression of the SER1, SER2, and SER33 genes in the mid-exponential phase during growth on ammonium as compared with glutamate, this difference was not statistically significant. However, a consistently lower enzymatic activity of phosphoglycerate dehydrogenase (0.99 units/g of protein) was measured in the wild-type strain when ammonium was used as the sole nitrogen source (data not shown). This observed activity is similar to that reported previously (1.1–1.5 units/g of protein) for growth on ammonium.

Consequences of Growth and Metabolism When Deleting the SER3/33 Genes—In order to determine consequences of SER3 and SER33 deletions on growth and metabolism, we first determined the maximal specific growth rates (Table II). Cells were grown on microtiter plates in liquid medium, with and without additional glycine, in the presence of glucose as carbon source and glutamate as main nitrogen source. The wild-type strain grew only slightly faster than both single mutants. Addition of glycine reduced the growth rate of both the wild-type strain and the single mutants about 15%. The double mutant, which was able to grow when glycine was supplemented, showed the slowest growth rate of all strains (85% of the wild-type level). The growth rate of the ser3Δ ser33Δ mutant was not reduced because the interconversion of glycine to ser-
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FIG. 4. mRNA levels of SER1, SER2, SER33, and SER3 relative to IPP1 in the midexponential growth phase (OD610 2.1–2.6) in the wild-type strain. Cells were grown using glucose as carbon source and ammonium sulfate (NH4+) or glutamate as the main nitrogen sources. Different nitrogen supplements were added to the glutamate medium: No supplement (No), serine (+S), threonine (+T), or glycine (+G). S.D. of the analytical procedures was 15% for SER1 and SER2 and 20% for SER3 and SER33. The images of Northern blots are shown.

Table II

Maximal specific growth rates

| Supplement | Type of strain | \( \mu_{\text{max}} \ \text{h}^{-1} \) |
|------------|----------------|------------|
|            | wt             | ser3Δ      | ser33Δ      | ser3Δser33Δ |
| None       | 0.330          | 0.300      | 0.292       | No growth*  |
| Glycine    | 0.269          | 0.263      | 0.246       | 0.234       |

* A slight increase in OD was recorded. Growth of the double mutant ceased in extended experiments with nutrients in excess, except for serine or glycine, which were absent.

The metabolite product pattern during the entire period of respiro-fermentative growth was also monitored. The strains were grown on medium with glutamate as nitrogen source, without and with glycine; to fulfill the auxotrophic requirement of the double deletion mutant (Table III). With glutamate as the sole nitrogen source, deletion of either SER3 or SER33 only slightly affected the production of biomass and of major end products of growth (ethanol and glycerol) compared with that of the wild type (Table III). On the other hand, acetic acid production decreased in the mutants, which was balanced with a substantial increase in pyruvic acid production. Despite the fact that glutamate was consumed in similar amounts, two products of its metabolism, succinic and 2-hydroxyglutaric acids, were formed in lower amounts in both single mutants, while this reduction was to some extent balanced with a slightly increased production of its first conversion product, 2-oxoglutarate and of fumaric acid. The recorded changes in metabolite production in both single mutants, indicate an altered flux distribution to and within the tricarboxylic acid cycle in ser3Δ and ser33Δ mutants compared with the wild type during growth on glutamate as sole nitrogen source. In total, there were small differences between the ser3Δ and ser33Δ mutants grown on glutamate as the sole nitrogen source.

Glycine supplementation influenced largely the pattern of formed metabolites in all strains (Table III). The addition of glycine to the medium resulted in a diminished consumption of glutamate, while the total amount of nitrogen taken up was similar for all strains with and without glycine supplementation (0.80–0.83 mmol of nitrogen/g of glucose).

There were many indications of an altered metabolism during growth on glutamate plus glycine when both SER3 and SER33 was deleted as compared with the wild type (Table III). Thus, in the double mutant the amounts of ethanol, glycerol, 2-oxoglutaric acid, 2-hydroxyglutaric acid, and fumaric acid formed decreased, while the amounts of acetic acid and succinic acid increased. Formation of 2-hydroxyglutaric acid was not even detectable in the double mutant (Table III). All these altered levels indicate a changed redox metabolism in the double deletion mutant as compared with the wild-type strain.

In order to determine if effects of SER3 and SER33 deletions on metabolism could be explained by altered expression levels of cellular proteins, two-dimensional PAGE analysis was performed with protein extracts of the wild type and the double mutant from samples taken in mid-exponential growth phase in glutamate plus glycine medium. However, very few and only minor changes were observed when about 500 proteins were analyzed (data not shown). All recorded expression changes corresponded to unidentified proteins except for Tdh1p. This minor isoform of glyceraldehyde-3-phosphate dehydrogenase was up-regulated 3-fold in the double mutant (from 325 ± 75 ppm in the wild-type strain to 974 ± 75 ppm in the double mutant, with the maximal deviation given). Interestingly, Shm2p, one of the serine/glycine-interconverting serine hydroxymethyltransferases, was present in similar amounts in both strains. Hence, the difference in metabolite levels were not due to an altered amount of proteins. Possible spots in the two-dimensional PAGE gels for Ser3p and Ser33p, which were absent for the double mutant, were found at a molecular mass
of 50 kDa and pI of 5.6 and 6.2, respectively, close to their theoretical values (5.39 and 6.18). However, further identification is required.

Other Functions of the SER3/33 Genes—In bacteria, the formation of 2-hydroxyglutarate from 2-oxoglutarate is dependent on the presence of a hydroxyglutarate dehydrogenase activity (12, 32). Indeed, hydroxyglutarate dehydrogenase activity was also present in crude extracts of S. cerevisiae (Table IV). The activity was highly dependent on NADH as cofactor. Use of NADPH as cofactor in the activity assay yielded only 20% of the activity as measured with NADH (data not shown). The specific hydroxyglutarate dehydrogenase activity increased about 4-fold during respiro-fermentative growth for both the wild-type strain (Fig. 5) and all mutants (data not shown).

Since the E. coli phosphoglycerate dehydrogenase has a hydroxyglutarate dehydrogenase activity, candidates for yeast enzymes with such activity were Ser3p and Ser33p, as well as their homologues, Ynl274cp, Ygl185cp, Ypl113cp, and Fdh1p. However, absence of Ser33p, or of both Ser3p and Ser33p, rather resulted in a higher hydroxyglutarate dehydrogenase activity (Table IV). On the other hand, the ser3Δ ser33Δ mutant did not form any 2-hydroxyglutarate in glutamate/glycine medium (Table III) but traces of this metabolite were found during anaerobic growth in glutamate/serine medium (data not shown).

One of the candidates for hydroxyglutarate dehydrogenase activity, Ynl274cp, has been assigned a hydroxyisocaproate dehydrogenase activity (13). The activity of hydroxyisocaproate dehydrogenase increased during respiro-fermentative growth and reached a value of 1-5 units/g of protein at the diauxic shift in both wild-type and mutants strains (Fig. 5B and data not shown), which was in accordance with the range reported previously (13) for a wild-type strain growing in ethanol medium (2.3–5.9 units/g of protein). The Northern blot analysis (Fig. 5B) confirmed that expression of YNL274C is glucose-repressed (33). Also expression of both YGL185C and YPL113C was strongly diminished in glucose medium (Fig. 5C), and the mRNA of the FDL1C could not be detected. No clear difference in expression of the homologues was seen comparing the ser3/33 mutants with the wild type (data not shown).

### DISCUSSION

**Phosphoglycerate Dehydrogenase Activity**—We have in this work demonstrated that the gene products of YER081W and YIL074C (in this study renamed SER3 and SER33, respectively) both have phosphoglycerate dehydrogenase activity. The double deletion mutant, ser3Δ ser33Δ, is auxotrophic for serine and glycine during growth on glucose. Furthermore, phosphoglycerate dehydrogenase activity was absent in crude extracts of the double mutant and lowered in single deletion mutants. Consequently, Ser3p and Ser33p appear to be the only enzymes with phosphoglycerate dehydrogenase activity present in S. cerevisiae. In addition, the previously suggested pathway for formation of glycine from threonine (1), the threonine pathway, could not be confirmed. Even if threonine addition reduced the expression of all SER genes, the ser3Δ ser33Δ mutant could neither grow on threonine as sole nitrogen source nor as supplement to glutamate or ammonium. Hence, the main pathway during growth on glucose for both serine and glycine biosynthesis seems to be via Ser3p and Ser33p, i.e. by the phosphoglycerate pathway.
a strong repression on amino acid transport proteins. The main protein involved in uptake of glycine is Gap1p (36), and both protein function and production are repressed in the presence of ammonium ions (37). Also glutamate decreases the activity of Gap1p (38). The level of GAPI expression remains one fifth of that under derepressed conditions (37). Also other proteins involved in glycine uptake, such as Agp1p, Tat2p, Dip5, and Put4p, are all under nitrogen catabolite repression (39). Thus, the effects of nitrogen repression on the uptake of glycine provide a plausible explanation for the observed inhibited growth when ammonium was accessible as the main nitrogen source. On the other hand, the level of uptake mediated by Gap1p in presence of glutamate was sufficient for growth of the double mutant when glutamate was the main nitrogen source (Fig. 2). In addition to effects on uptake, we cannot exclude the possibility that ammonium ions repress components involved in the conversion from glycine to serine, for example the serine hydroxymethyltransferases (Fig. 1). Assimilation of glycine into serine is catalyzed by the minor isoenzyme, the mitochondrial Shm1p, together with the glycine cleavage system (3, 4).

Serine and glycine fulfill also a role as sources of one-carbon units (4). The amounts of serine and glycine, as measured to be present in yeast cells (15), corresponds to only 32% of the glycine taken up by the cells (Table III). Thus, the residual glycine taken up must be converted and is most probably used as one-carbon units. It also seems, as the opposite is true, i.e. that one-carbon units can serve as a source for serine and glycine production since addition of exogenous one-carbon units (as formate, 10 mM) allowed slow growth of the double mutant on ammonium salt as the nitrogen source (data not shown). A similar observation has been reported for the ser\(\Delta\) mutant (3).

Addition of amino acids largely reduced the expression of SER3. Repression of SER3 mRNA by amino acids is consistent with that found in rich medium as compared with minimal medium (34). The promoter of the SER3 gene contains as many as 8 potential Gcn4p binding sites. Gcn4p is required for stimulated expression of genes encoding amino acid biosynthetic enzymes in response to amino acid starvation (35). Hence, amino acid depletion may stimulate expression of SER3 via the Gcn4p pathway.

Effects on Redox Metabolism—Glycerol production is crucial during anaerobic conditions in order to reoxidize cytosolic NADH. The largest source of cytosolic net NADH production is amino acid synthesis for protein production (15, 40, 41). When glycine is added as an additional nitrogen source the need for synthesis of glycine and serine results in less NADH formation via the redox reaction catalyzed by Ser3/33p. The amount of serine and glycine in biomass (15) corresponds to about 15 mg of glycerol per g of glucose consumed in terms of redox equivalents (NADH). This is comparable to the decreased level of glycerol formation observed in the wild-type strain when glycine was added to the medium (Table III).

When glutamate is used as nitrogen source, its carbon skeleton is converted to 2-oxoglutarate, succinate and 2-hydroxyglutarate, which appear as extracellular products (10). This degradation pathway is in accordance with our data. The ser\(\Delta\) double mutant consumed less glutamate than the wild-type strain. The altered yield of degradation products calculated in the double mutant (\(-0.068 \text{ mmol/g of glucose}\)) fits with the observed reduced glutamate consumption (\(-0.061 \text{ mmol/g of glucose}\)), which has consequences on the redox metabolism. The 2-oxoglutarate formed is a product of glutamate in transamination reactions (10, 42, 43), but no NADH formation is expected from these reactions. In contrast, both the de-
increased 2-hydroxylutarate and increased succinate formation in the double mutant contribute to an increased production of NADH (Fig. 1), which needs to be reoxidized. In addition, acetate formation increased in the double mutant. Formation of acetate from glucose is associated with formation of 1 or 2 NADH/acetate, depending on the use of NADP⁺ or NAD⁺-dependent isoenzymes of aldehyde dehydrogenase (15, 44). In addition, less glycerol was produced and consequently almost all observed changes in metabolite formation in the double mutant compared with the wild type resulted in increased cytosolic NADH production. The amount of the minor isoenzyme of the glyceraldehyde phosphate dehydrogenases in S. cerevisiae, Tdh1p seems to be redox-regulated. Accordingly, the amount of Tdh1p was increased in the serΔ3Δ ser33Δ double mutant indicating an increased cytosolic NADH/NAD⁺ ratio. However, since the cells were cultured under aerobic conditions, the surplus of NADH formed, both cytosolically and/or in the mitochondria (45, 46), should not create a redox problem since it can still be reoxidized in the respiratory chain. This is also reflected by a relatively non-affected growth rate of the double mutant. In fact, the slightly increased biomass formation, and the simultaneously decreased ethanol and glycerol production in the double mutant contribute to an increased production of 2-hydroxylutarate and increased succinate formation in the double mutant as compared with the wild type, indicates an increased oxidative metabolism in the former.

Hydroxylutarate Dehydrogenase Activity—Enzyme activity measurements did not indicate that Ser3p or Ser33p might have the sought hydroxylutarate dehydrogenase activity, but still the ser3Δ ser33Δ double mutant formed only traces of 2-hydroxylutarate. However, the amount of 2-hydroxylutarate found extracellularly reflects the balance between formation and consumption. Hence, the ser3Δ and ser33Δ mutations may indirectly enhance the degradation of 2-hydroxylutarate. We have previously found that yeast cells are able to consume 2-hydroxylutarate added to the medium (47), but no degradation pathways have yet been suggested in the literature. It appears that yet unidentified enzyme(s) are responsible for 2-hydroxylutarate formation during glucose growth, since the alternative enzymes investigated in this study (Ynl274p, Ypl113p, and Ygl185p) are all glucose-repressed.

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Ser3p (Yer081wp) and Ser33p (Yil074cp) Are Phosphoglycerate Dehydrogenases in *Saccharomyces cerevisiae*
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