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The *Saccharomyces cerevisiae* Leu3 Protein Activates Expression of *GDH1*, a Key Gene in Nitrogen Assimilation†

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The Leu3 protein of *Saccharomyces cerevisiae* has been shown to be a transcriptional regulator of genes encoding enzymes of the branched-chain amino acid biosynthetic pathways. Leu3 binds to upstream activating sequences (UAS_{LEU}) found in the promoters of *LEU1*, *LEU2*, *LEU4*, *ILV2*, and *ILV5*. In vivo and in vitro studies have shown that activation by Leu3 requires the presence of α-isopropylmalate. In at least one case (*LEU2*), Leu3 actually represses basal-level transcription when α-isopropylmalate is absent. Following identification of a UAS_{LEU}-homologous sequence in the promoter of *GDH1*, the gene encoding $NADP^+$-dependent glutamate dehydrogenase, we demonstrate that Leu3 specifically interacts with this UAS_{LEU} element. We then show that Leu3 is required for full activation of the *GDH1* gene. First, the expression of a *GDH1-lacZ* fusion gene is three- to sixfold lower in a strain lacking the *LEU3* gene than in an isogenic *LEU3*$^+$ strain. Expression is restored to near-normal levels when the *leu3* deletion cells are transformed with a *LEU3*-bearing plasmid. Second, a significant decrease in *GDH1-lacZ* expression is also seen when the UAS_{LEU} of the *GDH1-lacZ* construct is made nonfunctional by mutation. Third, the steady-state level of *GDH1* mRNA decreases about threefold in *leu3* null cells. The decrease in *GDH1* expression in *leu3* null cells is reflected in a diminished specific activity of $NADP^+$-dependent glutamate dehydrogenase. We also demonstrate that the level of *GDH1-lacZ* expression correlates with the cells' ability to generate α-isopropylmalate and is lowest in cells unable to produce α-isopropylmalate. We conclude that *GDH1*, which plays an important role in the assimilation of ammonia in yeast cells, is, in part, activated by a Leu3–α-isopropylmalate complex. This conclusion suggests that Leu3 participates in transcriptional regulation beyond the branched-chain amino acid biosynthetic pathways.

The role of the *LEU3* gene product as a transcriptional regulator in the biosynthesis of branched-chain amino acids is well established (7, 14, 33, 34, 36, 38). Leu3 is a DNA-binding protein of the $Zn(II)_2$-Cys_6 binuclear cluster type that interacts with an upstream activating sequence (UAS_{LEU}) in the promoter regions of *LEU1*, *LEU2*, *LEU4*, *ILV2*, and ILV5 (7, 14). Once bound to its target DNA, Leu3 can act either as a transcriptional activator or as a repressor. Leu3 mediates transcriptional activation only when α-isopropylmalate (α-IPM), the reaction product of the first committed step in leucine biosynthesis, is present (34). This dependence on α-IPM ties activation by Leu3 to the highly regulated *LEU4* gene product, α-IPM synthase (4, 27). In the absence of α-IPM, Leu3 reduces basal-level transcription four- to fivefold (34). α-IPM-dependent transcriptional activation and repression in the absence of α-IPM have been observed both in vivo (3, 7) and in a yeast-derived in vitro transcription system (33, 34). In the latter, the UAS_{LEU} of the *LEU2* promoter served as the Leu3 binding site.

The present investigation was prompted by the results of an earlier search for homologies to the UAS_{LEU} consensus sequence (5′-GCCGGNNCCGGC-3′) in the yeast nucleotide sequence database (36). The search produced one perfect match located upstream of the *GDH1* gene (25). The sequence, 5′-GCCGGAACCGGC-3′, is identical to that of the UAS_{LEU} element of *LEU2* (1). It is located between positions −405 and −394, with +1 signifying the beginning of the open reading frame of *GDH1*.

The *GDH1* gene encodes NADP-dependent glutamate dehydrogenase (NADP-GDH) (17, 24, 25). In *Saccharomyces cerevisiae*, NADP-GDH occupies a key position in anabolic nitrogen metabolism: the combination of the NADP-GDH-catalyzed reaction (NH_3 + α-ketoglutarate + NADPH + H^+ → glutamate + $NADP^+$) with the glutamine synthetase reaction (NH_3 + glutamate + ATP → glutamine + ADP + P_i) constitutes the major pathway for the assimilation of ammonia (22). A second pathway, combining the glutamine synthetase reaction with the glutamate synthase-catalyzed reaction (glutamine + α-ketoglutarate + NADH + H^+ → 2 glutamate + NAD^+), is of minor importance, as shown by the fact that mutants lacking glutamate synthase grow as well as wild-type cells in media containing ammonium sulfate as the sole nitrogen source; in the same media, mutants lacking NADP-GDH grow at only about half the rate of wild-type cells (22). In spite of the key position of *GDH1*, our knowledge of what regulates the expression of this gene is scant. The specific activity of NADP-GDH was twofold higher in extracts from cells grown with ammonium sulfate as the sole nitrogen source when other nitrogen sources such as glutamate, aspartate, glutamine, or asparagine were used instead (29). The initial ammonium sulfate concentration in these experiments was 10 mM. Bogonez et al. (5) confirmed the induction by ammonium sulfate and added the observation that at much higher initial concentrations of ammonium sulfate (50 to 360 mM), the specific activity of the enzyme actually decreased about threefold. The authors showed that the decrease was not caused by accelerated degradation of the enzyme. They proposed the existence of some type of repression mechanism under these conditions. *GDH1*

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TABLE 1. Strains and plasmids used

	Description	Reference or source
Strains		
S. cerevisiae		
XK12-11B	*MATα leu4 leu5 ura3-52*	3
XK25-1B	*MATα ura3-52*	G. B. Kohlhaw's laboratory
XK83-4	*MATα LEU4*^{fbr} *ura3 can1*	8
XK157-3C	*MATα leu3-Δ2::HIS3 trp1-289 ura3-52 his-3Δ1*	37
XK157-R	*MATα LEU3 trp1-289 ura3-52 his3-Δ1*	This work
E. coli		
TG1	*K12 Δ(lac-pro) supE hsdDS/F' traD36 proA*$^+$ *B*$^+$ *lacI*^{q} *lacZM15*	
CJ236	*dut1 ung1 thi-1 relA1*/pCJ105 (Cm^r)	
Plasmids		
pCYG4	YEp13-derived plasmid carrying the *GDH1* gene	25
pSEYC102	*CEN4* plasmid containing a *'lacZ* gene preceded by a polylinker region	11
pYH100	See Fig. 1 and text	This work
pYH101	See Fig. 1 and text	This work
pKZ5	2μm plasmid carrying the *LEU3* gene under the control of the *PHO5* promoter	37

does not appear to be subject to the general control of amino acid biosynthesis (16, 35).

Here we show that *GDH1* gene expression is partially dependent upon Leu3. This conclusion is based on three lines of evidence. First, the expression of a *GDH1-lacZ* fusion construct is diminished in a *leu3* deletion mutant. The decrease is seen with all nitrogen sources tested and ranges from three- to sixfold. Second, a similar decrease in *lacZ* expression is seen when the UAS_{LEU} of the *GDH1* promoter connected to *lacZ* is destroyed by mutation. Third, the level of *GDH1* mRNA is diminished in a *leu3* deletion strain. These results strongly argue for positive regulation of *GDH1* by Leu3 and suggest that Leu3's effect reaches beyond the biosynthesis of the branched-chain amino acids, extending to the assimilatory step of nitrogen metabolism mediated by NADP-GDH. Correlated with this finding, we also show that the level of *GDH1-lacZ* expression is linked to intracellular production of α-IPM, suggesting that Leu3 activation of *GDH1* also requires α-IPM as a cofactor.

MATERIALS AND METHODS

Strains and media. *S. cerevisiae* and *Escherichia coli* strains and plasmids used in this work are shown in Table 1. To construct strain XK157-R, the *LEU3*-containing plasmid pTSC15 (6a) was digested with restriction enzyme *Eco*RI to remove the 2μm sequence. The resulting plasmid was used for integrative transformation of XK157-3C to Ura$^+$. Ura$^+$ transformants were then spread on minimal plates supplemented with 0.18 mM uracil, 0.1 mM tryptophan, 0.1 mM histidine, and 1 mg of 5-fluoroorotic acid per ml. Surviving colonies were purified and screened for the Ura$^-$ phenotype. Tests showed that several Ura$^-$ colonies were also His$^-$, indicating that they had lost the *HIS3* insert present in XK157-3C. The Ura$^-$ His$^-$ strains were shown to carry a *LEU3* gene at its original genomic position by PCR, using primers complementary to the *LEU3* flanking and coding region, respectively. One such strain was picked and labeled XK157-R (Table 1). The medium used for growing yeast cells contained 0.17% yeast nitrogen base without amino acids and ammonium sulfate (Difco Laboratories), 1% glucose, and 0.1% nitrogen sources as specified plus 0.15 mM leucine, 0.18 mM uracil, and 0.1 mM each of tryptophan and histidine when required. *E. coli* TG1 was routinely used for DNA manipulations. Strain CJ236 was used for the isolation of uridine-containing single-stranded DNA. *E. coli* cells were grown in L broth (9) containing ampicillin (100 μg/ml) alone or with chloramphenicol (50 μg/ml), as needed.

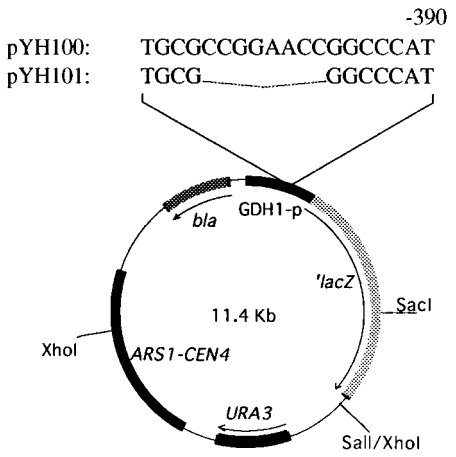

FIG. 1. Partial restriction maps and identification of important elements of plasmids pYH100 and pYH101. The position designation −390 refers to the *GDH1* promoter (GDH1-p), with +1 signifying the beginning of the open reading frame of *GDH1*. The UAS_{LEU} consensus sequence begins at −394. See Materials and Methods (construction of *GDH1-lacZ* fusions) for further details.

Construction of *GDH1-lacZ* fusions. Plasmid pCYG4, which contains the *GDH1* gene (25), was a gift from Benjamin Hall, University of Washington. Plasmid pSEYC102 was obtained from S. Emr, California Institute of Technology. To construct a *GDH1-lacZ* fusion, the termini of the 0.95-kb *Hin*dIII fragment from plasmid pCYG4 were filled in with the Klenow fragment of DNA polymerase. This fragment was then ligated to *Sma*I-digested plasmid pSEYC102. The resulting plasmid, pYH100 (Fig. 1), expresses a fusion protein containing nine amino acids from the *GDH1* gene, three amino acids from the linker region, and amino acids 10 to 1024 from the *lacZ* gene. To construct a *GDH1-lacZ* fusion lacking a functional UAS_{LEU} element, the 0.95-kb *Hin*dIII fragment from pCYG4 was cloned into plasmid pUC118. The subclone was transformed into strain CJ236, and uridine-containing single-stranded DNA was isolated. An oligonucleotide (5′-GAAAATGCATGGGCCCGCATTGGTTCT GCG-3′) was synthesized, and site-directed mutagenesis was carried out by the procedure of Kunkel et al. (20). The mutation was identified by the presence of a new *Apa*I site and confirmed by sequencing. The mutated *Hin*dIII fragment was recovered, the termini were filled in, and the fragment was ligated to *Sma*I-digested plasmid pSEYC102. The resulting plasmid was designated pYH101 (Fig. 1).

Yeast transformation, cell growth, and enzyme assays. Plasmids pYH100 and pYH101 were introduced into XK157-R and XK157-3C cells by a modified lithium acetate method (15). The transformants were purified once, and single colonies were inoculated into 10 ml of SD medium (13). After overnight growth at 30°C, yeast cultures were harvested by centrifugation and the cell pellets were washed once with sterile double-distilled water. The cells were inoculated into 50 ml of yeast nitrogen base-glucose medium containing 0.1% ammonium sulfate, L-asparagine, L-aspartate, L-glutamine, or L-glutamate as the sole nitrogen source. The cells were harvested at an optical density at 600 nm (OD_{600}) of between 0.9 and 1.0. They were washed once with double-distilled water and once with Z buffer (100 mM sodium phosphate buffer [pH 7.0], 10 mM KCl, 1 mM $MgSO_4$, 25 mM β-mercaptoethanol) and resuspended in Z buffer (final volume, 1 ml). Twenty microliters of 0.1% sodium dodecyl sulfate (SDS) and 50 μl of chloroform were added, and the mixture was vortexed for 30 s. After a 15-min preincubation at 30°C, 0.2 ml of *o*-nitrophenyl-β-D-galactopyranoside (4 mg/ml in Z buffer) was added, and the reaction mixture was further incubated until visible color developed. The reaction was stopped by adding 0.5 ml of 1 M Na_2CO_3. Cell debris was removed by centrifugation, and the OD_{420} of the samples was measured. Units of β-galactosidase activity were determined as specified by Miller (23). When NADP-GDH assays were to be performed, cells were grown as described above but resuspended in 0.1 M potassium phosphate buffer (pH 7.0). The cells were broken by two passages through a refrigerated French pressure cell (1,100 lb/in^2). The supernatant solution obtained after centrifugation at 27,000 × *g* for 20 min was used to assay NADP-GDH by the method of Doherty (10). The protein concentration was determined by the method of Bradford (6).

Electrophoretic mobility shift assays. A 510-bp fragment containing nucleo-

tides −485 to +25 of the *GDH1* gene (25) was excised from plasmid pCYG4 and purified. The termini were then filled in with the Klenow fragment of DNA polymerase in the presence of [α-^{32}P]dCTP. After passage through a Sephadex G-50 column, the labeled DNA was digested with *Kpn*I. Two labeled DNA fragments (309 and 205 bp) were recovered and used as probes. Synthetic oligonucleotides containing the UAS_{LEU} from the *GDH1* promoter (5′-[−409]ATGCGCCGGAACCGGCCCA[−391]-3′) and the *LEU2* promoter (7) were also used as probes. The electrophoretic mobility shift assays were performed as described previously (7, 37), using a partially purified Leu3 preparation (a gift from D. Wang of this laboratory).

Northern (RNA) blot analysis. Yeast strains XK157-3C and XK157-3C/pKZ5 were grown in medium containing either 0.1% L-asparagine or 0.1% ammonium sulfate and harvested at an OD_{600} of between 0.9 and 1.0. Total RNA was isolated by the hot phenol-glass bead method (19). Twenty micrograms of total RNA was fractionated on a 1.4% agarose gel containing 0.7 M formaldehyde. The RNA was transferred to a nylon membrane by using a downward alkaline transfer apparatus (Turboblotter; Schleicher & Schuell). The blot was first hybridized to a ^{32}P-labeled actin oligonucleotide probe (5′-CGAGGAGCGTCGTCACCGG-3′) in the rapid hybridization buffer supplied by Amersham. The membrane was washed twice for 15 min with 6× SSPE (1× SSPE contains 0.18 M NaCl, 10 mM sodium phosphate buffer [pH 7.7], and 1 mM EDTA)–0.1% SDS buffer at room temperature and then twice for 15 min with 2× SSPE–0.1% SDS buffer at 42°C. After exposure at −70°C, the actin probe was stripped off by incubating the membrane in 6× SSPE–50% formamide solution for 30 min at 42°C. The membrane was then hybridized to a 0.95-kb *Hin*dIII-*Stu*I fragment from the *GDH1* coding region. The blot was washed twice for 15 min with 6× SSPE–0.1% SDS buffer at room temperature, twice for 15 min with 2× SSPE–0.1% SDS buffer at 65°C, and once for 15 min in 0.8× SSPE–0.1% SDS buffer at 65°C. The blot was exposed at −70°C with an intensifying screen for several hours.

RESULTS

Leu3 interacts with a UAS_{LEU} element present in the *GDH1* promoter. To ascertain whether the UAS_{LEU} element located between positions −405 and −394 of the *GDH1* promoter interacted with Leu3, we conducted a series of electrophoretic mobility shift assays (Fig. 2). First, dividing the promoter region into a distal and a proximal portion, we found that only the distal portion, extending from positions −283 to −487, was capable of forming a specific complex with Leu3 (Fig. 2A). The more slowly moving complex in lanes 1 and 3 of Fig. 2A represented specific binding since its formation was competed against by a DNA fragment containing the UAS_{LEU} element of the *LEU2* promoter (Fig. 2A, lane 4). That fragment had been shown previously to interact specifically with Leu3 (7). We also observed specific interaction between a synthetic 19-bp fragment consisting of positions −409 to −391 of the *GDH1* promoter and Leu3 (Fig. 2B). Competition for binding was seen both with unlabeled *GDH1*-UAS_{LEU} DNA and with unlabeled *LEU2*-UAS_{LEU} DNA (Fig. 2B, lanes 2 and 3) but not with unlabeled *LEU2* DNA whose UAS_{LEU} had been destroyed by the deletion of six core base pairs (Fig. 2B, lane 4). These results are consistent with the idea that the UAS_{LEU}-related sequence identified by the database search is the only segment within the *GDH1* promoter that Leu3 will interact with.

Both the *LEU3* gene product and the UAS_{LEU} element are required for enhanced transcription from the *GDH1* promoter. To determine whether *LEU3* affected the expression of *GDH1* and to evaluate any effects that *LEU3* might have, we constructed plasmid pYH100 (Fig. 1), in which the promoter region of *GDH1* (−935 to +27) was fused to the bacterial *lacZ* gene. The presumptive UAS_{LEU} core sequence is located between positions −405 and −394, and a TATA sequence (TATATAAA) is located between positions −149 and −142. Transcription start sites are present at positions −69, −65, and −56 (24, 25). The *GDH1-lacZ* fusion plasmid was used to transform a wild-type (with respect to *LEU3*) (strain XK157-R), an isogenic *leu3* deletion strain (XK157-3C), and the same *leu3* deletion strain that had been made *LEU3*$^+$ by transformation with plasmid pKZ5. The transformed strains were grown with different nitrogen sources, and β-galactosidase activities were determined (Table 2). Irrespective of the nitrogen source, the activities obtained with the *leu3* deletion strain were significantly below those obtained with the *LEU3*$^+$ strains, the difference ranging from three- to sixfold. Upon transformation of the *leu3* deletion strain with a *LEU3*-carrying plasmid, the β-galactosidase activity returned to levels similar to those seen with the wild-type strain. These results suggest that the differences in specific activities for a given nitrogen source are related to the availability to the Leu3 protein and that Leu3 is partially required for the expression of *GDH1*.

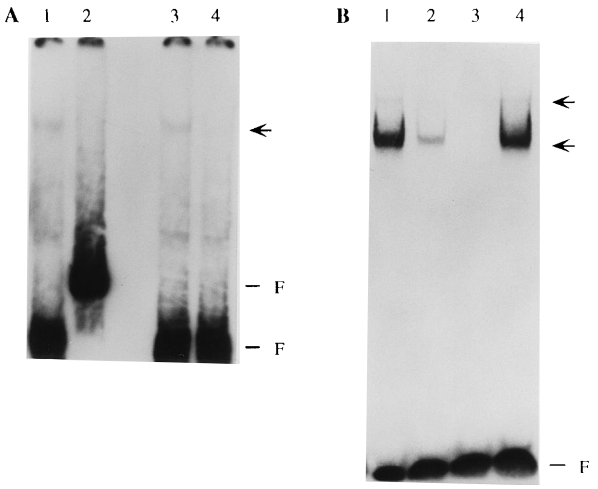

FIG. 2. Electrophoretic mobility shift assays showing specific interaction between Leu3 and *GDH1* promoter sequences. A partially purified Leu3 preparation from Leu3-overproducing cells (purified through the ammonium sulfate step [32]) was used throughout. The total protein concentration was 20 μg/40-μl assay volume. (A) Band shifts with *GDH1* promoter fragments (see Materials and Methods for preparation of fragments). Lanes: 1 and 3, distal promoter fragment covering positions −487 to −283, ca. 50 ng per assay; 2, proximal promoter fragment covering positions −282 to +27, ca. 5 ng per assay; 4, same as lanes 1 and 3 except that a competing oligonucleotide (20 ng of unlabeled 30-mer containing the UAS_{LEU} of the *LEU2* promoter [7]) was also present. (B) Band shifts with a synthetic oligonucleotide containing the presumed UAS_{LEU} element of the *GDH1* promoter (*GDH1* 19-mer; see Materials and Methods). The concentration of the labeled 19-mer was 0.2 ng per assay throughout. Lanes: 1, labeled *GDH1* 19-mer, no additions; 2, labeled *GDH1* 19-mer in the presence of a 100-fold molar excess of unlabeled *GDH1* 19-mer; 3, labeled *GDH1* 19-mer in the presence of a 100-fold molar excess of unlabeled 30-mer of the *LEU2* UAS_{LEU} (7); 4, labeled *GDH1* 19-mer in the presence of a 100-fold molar excess of unlabeled, noncompeting 24-mer of the *LEU2* UAS_{LEU} (7). The reaction mixtures contained 10 μg (A) or 1 μg (B) of poly(dI-dC) to reduce nonspecific binding. The arrows point to the positions of specific shifted complexes. F indicates the position of free DNA. The low intensity of the shifted bands in panel A was caused by low labeling efficiencies of the promoter fragments (i.e., the presence of large amounts of unlabeled DNA) and the relatively large amount of poly(dI-dC) that had to be used to reduce nonspecific binding.

Previous determinations of NADP-GDH activity in a wild-type strain had shown it to be highest when cells were grown on ammonium sulfate as the sole nitrogen source. With asparagine, aspartate, glutamine, or glutamate as the sole nitrogen source, the specific activity dropped to about half the value obtained with ammonium sulfate (29). The expression of *GDH1-lacZ* as a function of the nitrogen source yielded a similar pattern except that cells grown on asparagine as the sole nitrogen source showed a fourfold decrease in β-galactosidase activity compared with ammonium sulfate-grown cells (Table 2).

Further evidence for the involvement of Leu3 in the regulation of *GDH1* came from studying the effect caused by eliminating the UAS_{LEU} site from the *GDH1* promoter. The UAS_{LEU} site of the *GDH1* promoter was mutated by deleting 8 bp of the 12-bp core sequence (Fig. 1). The resulting con-

TABLE 2. Expression of a *GDH1-lacZ* fusion in *LEU3*$^+$ and *leu3* deletion strains grown on different nitrogen sources and effect of deleting the UAS_{LEU} element from the *GDH1* promoter[a]

Nitrogen source[b]	Mean β-galactosidase sp act (Miller units) ± SD				
	XK157-R/pYH100 (WT[c]/WT UAS_{LEU})	XK157-3C/pYH100 (*leu3*Δ/WT UAS_{LEU})	XK157-3C/pYH100,pKZ5 (*leu3*Δ/WT UAS_{LEU} *LEU3*)	XK157-R/pYH101 (WT/mutant UAS_{LEU})	XK157-eC/pYH101 (*leu3*Δ/mutant UAS_{LEU})
Ammonium sulfate	581 ± 32	97 ± 10	455 ± 9	206 ± 39	71 ± 13
Asparagine	146 ± 37	44 ± 6	133 ± 15	32 ± 4	26 ± 4
Aspartate	252 ± 17	61 ± 12	155 ± 64	65 ± 1	ND[d]
Glutamine	226 ± 20	86 ± 8	348 ± 138	47 ± 5	ND
Glutamate	333 ± 38	61 ± 13	240 ± 50	77 ± 10	ND

[a] Three independent colonies were used for each condition. Assays were performed in quadruplicate. See Table 1 for genotypes of strains used.
[b] Each was present at a concentration of 0.1%. All media were supplemented with 0.15 mM leucine. The concentration of leucine is insufficient to affect regulation (27).
[c] WT, wild type.
[d] ND, not determined.

struct (plasmid pYH101) was introduced into the wild-type and *leu3* deletion strains, and β-galactosidase levels were measured in cells grown on different nitrogen sources (Table 2). With respect to the XK157-R/pYH101 cells, it is obvious that under all conditions, expression of the *GDH1-lacZ* fusion was considerably less than in the XK157-R/pYH100 cells, with the decrease ranging from three- to fivefold (Table 2). The β-galactosidase levels in the strain containing an intact *GDH1* promoter but a *leu3* deletion were similar to those in the strain containing a UAS_{LEU}-less *GDH1* promoter but an intact *LEU3* gene (with differences ranging from essentially none to twofold) (Table 2). β-Galactosidase levels in strain XK157-3C/pYH101 (*leu3* deletion, mutated *GDH1* UAS_{LEU}) were 30 to 40% below the levels in strain XK157-3C/pYH100 (*leu3* deletion, intact *GDH1* UAS_{LEU}). These results raise the question of whether a protein other than Leu3 might interact with UAS_{LEU}. So far, no evidence for such a protein has been obtained.

To further test the idea that regulation of *GDH1* by Leu3 occurs at the transcriptional level, we determined the steady-state concentration of *GDH1* mRNA under selected conditions. The results (Fig. 3) support the conclusions drawn on the basis of the *GDH1-lacZ* fusion data. The *GDH1* mRNA level in the *leu3* deletion strain was three- to fourfold lower than the mRNA level in the isogenic (transformed) *LEU3*$^+$ strain when cells were grown on asparagine as the sole nitrogen source (Fig. 3, lanes 1 and 3); the difference was about threefold with ammonium sulfate-grown cells (Fig. 3, lanes 2 and 4). The corresponding differences in terms of β-galactosidase activities were three- to fivefold (Table 2). The mRNA levels were 3-fold higher in the *leu3* deletion strain when ammonium sulfate was used as nitrogen source (compared with asparagine) and 2.4-fold higher in a corresponding wild-type strain grown under the same conditions. A similar effect had been seen with the *GDH1-lacZ* fusion (Table 2).

The decreased expression of the *GDH1* gene in a *leu3* deletion strain was reflected in the specific activity of NADP-GDH. In crude extracts prepared from cells grown with ammonium sulfate (0.1%) as the sole nitrogen source, the specific activities were 0.73 and 0.22 μmol of NADPH per min per mg of protein, respectively, for a wild-type (XK25-1B) and a *leu3* deletion strain (XK157-3C).

Expression of a *GDH1-lacZ* fusion is also affected by the intracellular concentration of α-IPM. Gene activation by Leu3 requires the presence of α-IPM (3, 7, 27, 34). To determine whether this requirement also holds for the activation of *GDH1*, we measured the level of expression of our *GDH1-lacZ* fusion in strains with different capacities to synthesize α-IPM (Table 3). All strains were grown on 0.1% ammonium sulfate as the sole nitrogen source. In a wild-type strain and in a strain producing feedback-resistant α-IPM synthase (*LEU4*^{fbr}), expression of the *GDH1-lacZ* fusion gene was high. When the two strains were grown in the presence of leucine, a condition expected to lower α-IPM production in the wild-type but not the *LEU4*^{fbr} strain, the level of *GDH1-lacZ* expression dropped by more than twofold in the wild-type strain and by less than 20% in the *LEU4*^{fbr} strain. Furthermore, in a strain devoid of α-IPM synthase activity and hence α-IPM (3), the expression of *GDH1-lacZ* was between 7 and 10% of what was seen with the other two strains. Taken together, these results are consistent with the idea that α-IPM is a coactivator of *GDH1* gene expression.

DISCUSSION

In this report, we have provided evidence that expression of the *GDH1* gene of *S. cerevisiae* possesses a partial requirement for Leu3, a regulator previously reported to be involved only in the control of branched-chain amino acid biosynthesis. Eliminating either Leu3 itself or the *cis* element to which Leu3 binds reduces the expression of the *GDH1-lacZ* fusion and the mRNA level by three- to sixfold. We conclude that Leu3 participates in transcriptional activation of *GDH1*. Leu3 activation of *GDH1* requires α-IPM, correlating with the results obtained in other studies of this activator protein.

Is a regulation of *GDH1* expression by Leu3–α-IPM physiologically significant? We believe that it is since it would establish a link between the cellular pool of a relatively abundant reporter amino acid, i.e., leucine, and a key reaction in the early stages of assimilatory nitrogen metabolism. Leucine

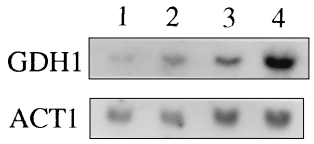

FIG. 3. Northern (RNA) blotting analysis of *GDH1* mRNA steady-state levels in cells grown on ammonium sulfate or asparagine as the sole nitrogen source. See Materials and Methods for experimental details. Lanes: 1, RNA from XK157-3C cells (pertinent genotype, *leu3*-Δ*2*) grown on asparagine as the sole nitrogen source; 2, same as lane 1 except that cells were grown on ammonium sulfate as the sole nitrogen source; 3, RNA from XK157-3C/pKZ5 cells (pertinent genotype, *LEU3*$^+$) grown on asparagine as the sole nitrogen source; 4, same as lane 3 except that cells were grown on ammonium sulfate as the sole nitrogen source. GDH1, blotting with a *GDH1* probe; ACT1, blotting with an actin gene probe. The blots were quantitated by densitometry. After normalization against actin mRNA, the *GDH1* blots had the following relative values: lane 1, 0.32; lane 2, 1.00; lane 3, 1.15; lane 4, 2.76.

TABLE 3. Expression of a *GDH1-lacZ* fusion in cells with different α-IPM-synthesizing capabilities[a]

Addition to standard growth medium[b]	Mean β-galactosidase sp act (Miller units) ± SD		
	XK157-R/pYH100 (WT[c]/*WT* UAS_{LEU})	XK83-4/pYH100 (*LEU4*^{fbr}/WT UAS_{LEU})	XK12-11B/pYH100 (*leu4 leu5*/WT UAS_{LEU})
No addition	581 ± 32	428 ± 12	
Leucine (2 mM) + isoleucine (1 mM)	253 ± 15	367 ± 17	43 ± 11

[a] The β-galactosidase assays were performed as described in Table 2, footnote *a*, and Materials and Methods. See Table 1 for genotypes of strains used.
[b] All media contained 0.1% ammonium sulfate as the primary nitrogen source. Strain XK12-11B requires leucine for growth. Since higher concentrations of leucine alone cause growth retardation, isoleucine was added to alleviate that effect.
[c] WT, wild type.

would act by controlling the production of α-IPM, mainly through feedback inhibition of α-IPM synthase (8). In addition, the Leu3–α-IPM connection would subject *GDH1*, and hence the rate by which ammonia can be assimilated, to a subtle, indirect control by the general amino acid control system since the expression of both *LEU4* (the gene encoding α-IPM synthase) and *LEU3* is regulated by Gcn4, the transcriptional activator that mediates the general amino acid control (17a, 27, 38). *GDH1* is apparently not a direct target for Gcn4 (16, 35). Regarding the role of Leu3 as a more general regulator, it is important to recall that the *Neurospora crassa leu-3* gene also pleiotropically regulates expression of genes beyond those of the branched-chain amino acid pathway (26, 28). For example, the *N. crassa* gene has been shown to regulate *his-1*, the gene encoding imidazoleglycerol-phosphate dehydratase (18), and α-IPM has been reported to mediate transient repression of overall protein biosynthesis (2). Additional work will be required to identify the other *cis*-acting elements and *trans*-acting factors that may participate along with Leu3 in controlling the expression of *GDH1*. It will then be possible to more completely understand the precise physiological role of Leu3 in *GDH1* expression in particular and ammonia assimilation and utilization in general.

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