ACADEMIC PRESS

Biochemical and Biophysical Research Communications 293 (2002) 79–85

BBRC

www.academicpress.com

GDH1 expression is regulated by *GLN3*, *GCN4*, and *HAP4* under respiratory growth☆

Lina Riego, Amaranta Avendaño, Alexander DeLuna, Ekaterina Rodríguez, and Alicia González*

Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apartado Postal 70-242, 04510 Mexico City, México

Received 12 March 2002

Abstract

In the yeast *Saccharomyces cerevisiae*, two $NADP^+$-dependent glutamate dehydrogenase isoenzymes encoded by *GDH1* and *GDH3* catalyze the synthesis of glutamate from ammonium and α-ketoglutarate. In this work we analyzed *GDH1* transcriptional regulation, in order to deepen the studies in regard to its physiological role. Our results indicate that: (i) *GDH1* expression is strictly controlled in ethanol-grown cultures, constituting a fine-tuning mechanism that modulates the abundance of Gdh1p monomers under this condition, (ii) *GDH1* expression is controlled by transcriptional activators that have been considered as exclusive of either nitrogen (Gln3p and Gcn4p) or carbon metabolism (HAP complex), and (iii) chromatin remodeling complexes play a role in *GDH1* expression; *ADA2* and *ADA3* up-regulated *GDH1* expression on ethanol, while that on glucose was *ADA3*-dependent. *SPT3* and *SNF2* activated *GDH1* expression on either carbon source whereas *GCN5* played no role in any condition tested. The above described combinatorial control results in a refined mechanism that coordinates carbon and nitrogen utilizations.

Keywords: NADP-glutamate dehydrogenase; Glutamate biosynthesis; Carbon metabolism; Nitrogen metabolism

In most microorganisms, glutamate biosynthesis can be achieved through the action of two biosynthetic pathways. The first one is mediated by the NADP-glutamate dehydrogenase [16]. The second one depends on the concerted action of glutamine synthetase (GS) and glutamate synthase (GOGAT), respectively, encoded by *GLN1* and *GLT1* [29]. In *Saccharomyces cerevisiae*, there is a third route for glutamate biosynthesis constituted by the *GDH3*-encoded isoenzyme [1]. Thus, in this microorganism, mutations inactivating *GDH1*, *GLT1*, and *GDH3* are needed to attain full glutamate auxotrophy [1].

The purification and characterization of *GDH1*- and *GDH3*-encoded enzymes and heteromers of these two, showed that these enzymes have distinct metabolic roles, different allosteric properties, and rates of α-ketoglutarate utilization [10]. It was also shown that the expression of *GDH1*, *GDH3*, and *GDH2* (encoding the catabolic NAD-GDH) is coordinately regulated by the carbon source [6,10]. These findings indicate that glutamate biosynthesis and catabolism could provide a mechanism that modulates the utilization of α-ketoglutarate under fermentative and respiratory conditions without impairing the integrity of the connected energy-providing systems [10].

Since the GS/GOGAT pathway links α-ketoglutarate and glutamine metabolism, it has been suggested to play a regulatory link between carbon/energy and nitrogen metabolisms [12,30]. In *Escherichia coli* regulation of the GS/GOGAT pathway is crucial to control nitrogen metabolism and levels of α-ketoglutarate. Glutamine constitutes the signal that coordinately regulates carbon and nitrogen metabolisms [26]. Altogether, these observations indicate that a metabolic circuit that includes *GDH1*-, *GDH3*-, *GDH2*-, *GLN1*-, and *GLT1*-encoded enzymes plays a fundamental role in determining the

☆ *Abbreviations:* GDH, glutamate dehydrogenase; GS, glutamine synthetase; GOGAT, glutamate synthase; β-Gal, β-galactosidase; 3-AT, 3-aminotriazole.

* Corresponding author. Fax: +52-55-56225630.
E-mail address: amanjarr@ifisiol.unam.mx (A. González).

0006-291X/02/$ - see front matter
PII: S0006-291X(02)00174-2

intracellular levels of a variety of cofactors and energy yielding molecules [10,12,30]. Consequently, inquiries on the regulatory mechanisms determining the expression of this set of genes must consider this crucial role.

The *GDH1*-encoded NADP-GDH is the main catalyst for glutamate biosynthesis; its expression, mainly studied in glucose-grown cultures, is regulated by the HAP complex [8], *LEU3*- [17], and *GLN3*-encoded proteins [9]. Considering recent findings on the different roles that *GDH1*- and *GDH3*-encoded isoenzymes may play under fermentative and respiratory conditions [10], we undertook a comparative analysis of the transcriptional regulation of *GDH1* in either glucose- or ethanol-grown cells. The results herein described show that Gcn4p, Gln3p, and the HAP complex regulate *GDH1* expression in ethanol-grown cultures, and indicate that different chromatin remodeling and transcriptional complexes are involved in *GDH1* expression in glucose- and ethanol-grown cells.

Materials and methods

Strains. The following strains were transformed according to the method described by Ito et al. [18], with either pLIN1 (*GDH1-lacZ* 2μ *LEU2*) or pLIN10 (*GDH1-lacZ* 2μ *URA3*): CLA1, CLA-100, CLA-102 [31], CLA-302-0 [32], 27034b, 30078c [5], BWG1-7A, LWG1, JP40 [13], Y0000, Y04959 (Euroscarf Collection), PSY316, PSY316-ada2 [2], PSY316-gcn5, PSY316-ada3 [19], FY2, FY293, and FY1656 [28]. Construction of strain CLA-300 (*MAT*α *ura3 leu2*/p180 *GCN4-lacZ CEN4 ARS1 URA3*/pRS315 *CEN6 ARS4 LEU2*) has been previously reported [32].

Growth conditions. Strains were routinely grown on minimal medium (MM), following the formula of yeast nitrogen base (Difco). Filter sterilized glucose (2%) or ethanol (2%) was used as the carbon source and 0.2% $(NH_4)_2SO_4$ or 0.1% glutamate, glutamine, or proline was used as the nitrogen source. Amino acids needed to satisfy auxotrophic requirements were added at 0.01%. Cells were incubated at 30 °C with shaking (250 rpm). For amino acid deprivation experiments, CLA1 and CLA-300 strains were treated as previously described [31]. Cultures were centrifuged and used for β-galactosidase (β-Gal) determinations.

Construction of lacZ fusions. Complementation of a *gdh1*Δ (MAR1) mutant [31] with a YCp50 genomic library led to select plasmid pCRI1. Sequencing of the 8 kb fragment present in pCRI1 confirmed that *GDH1* gene was carried by this plasmid. In order to construct a *lacZ* fusion with the *GDH1* promoter, we followed the procedure described by Dang et al. [8]. A 964 *Hin*dIII fragment including the *GDH1* promoter and 27 bp corresponding to the nine N-terminal codons of *GDH1* was obtained by digesting plasmid pCRI1 (Fig. 1). This fragment was fused in frame to the *E. coli lacZ* of YEp363 (2μ *LEU2*) or YEp353 (2μ *URA3*) [25] generating pLIN1 and pLIN10, respectively. To obtain a 5′ *GDH1* promoter deletion series, the pertinent forward deoxyoligonucleotides were designed based on the *GDH1* promoter sequence. Deoxyoligonucleotide R1 (5′-GCG CGA AGC TTG TTG AAA TTC TGG C-3′) was used as the reverse deoxyoligonucleotide in order to amplify the six individual deletions. Qiagen purified pLIN1 DNA was used as a template for PCR amplification, carried out in a Stratagene Robocycler 40 with the following program: one denaturing cycle for 3 min at 95 °C, followed by five cycles of 1 min denaturation at 95 °C, 1 min annealing at 52 °C and 2 min extension at 72 °C and 25 cycles of 1 min denaturation at 95 °C, 1 min annealing at 68 °C and 2 min extension at 72 °C, finally 5 min extension at 72 °C. The entire

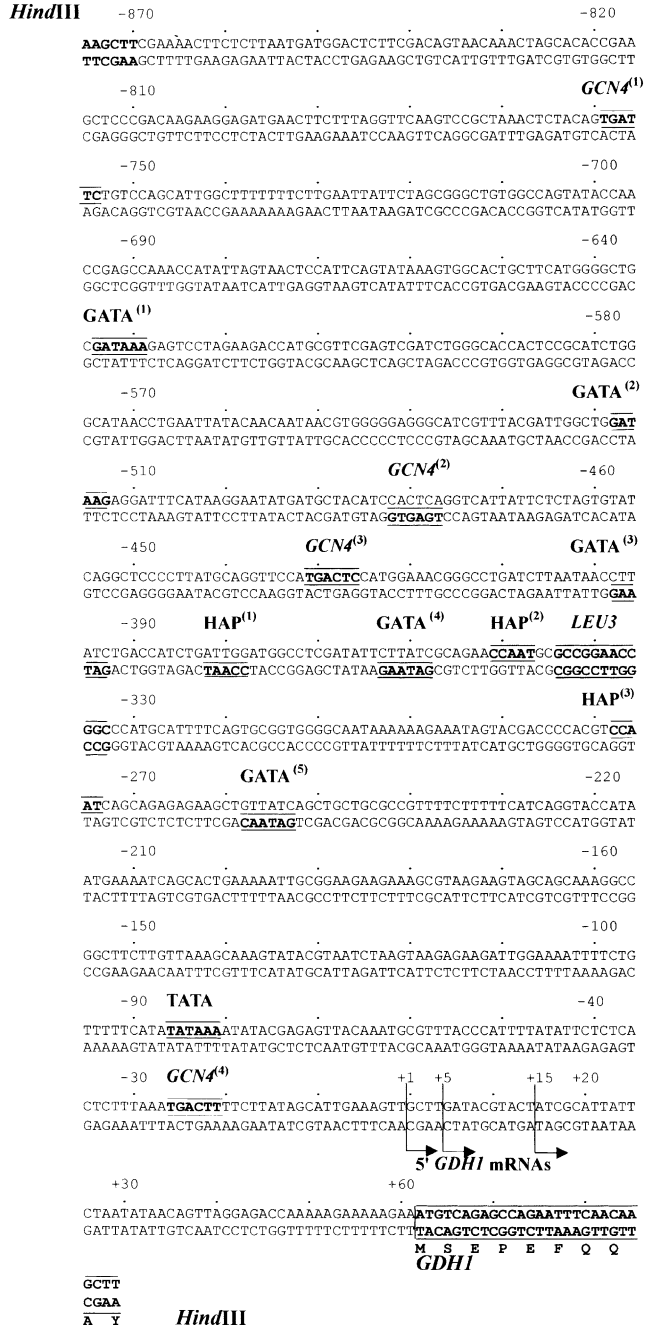


Fig. 1. *GDH1* promoter sequence. Putative binding sites for Gcn4p (*GCN4*), Gln3p, and Gat1p (GATA), HAP complex (HAP), and Leu3p (*LEU3*) are boxed and numbered starting from the most 5′. A putative TATA box as well as the three transcription initiation sites, at positions +1, +5, and +15 are indicated. *Hin*dIII sites were used to clone this fragment into YEp363 (2μ *LEU2 lacZ*) and YEp353 (2μ *URA3 lacZ*), generating plasmids pLIN1 and pLIN10, respectively.

family of PCR products was fused in-frame to the *E. coli lacZ* gene of YEp363 (2μ *LEU2*) [25], generating six fusion plasmids, pLIN2 through pLIN7. All fusion plasmids were sequenced with an ABI PRISM Genetic Analyzer, using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit from Applied Biosystems.

Determination of β-Gal activities. Soluble extracts were prepared and β-Gal activities were determined as previously described [31]. One

unit of β-galactosidase corresponds to 1 μmol of *o*-nitrophenol produced per minute. Protein was measured by the method of Lowry et al. [23], with bovine serum albumin as the standard.

Results and discussion

Regulation of GDH1 expression by nitrogen and carbon sources

Determination of β-Gal activity in a wild type strain harboring plasmid pLIN1, containing the *GDH1* promoter fused to the complete β-Gal coding region, showed that in the presence of glucose as carbon source, *GDH1* transcriptional activity was similar in all the tested nitrogen sources (Fig. 2). However, when ethanol was used as carbon source, maximal transcriptional activity was found when ammonium was used as sole nitrogen source; on proline, glutamate, or glutamine, β-Gal activity decreased (Fig. 2). These results indicate that carbon repression is overimposed to that exerted by amino acids. The fact that *GDH1* transcriptional activity is repressed in the presence of amino acids is consistent with the role of *GDH1* as the main pathway involved in glutamate biosynthesis [1], which is the donor of the amino nitrogen utilized for the biosynthesis of most nitrogenous compounds.

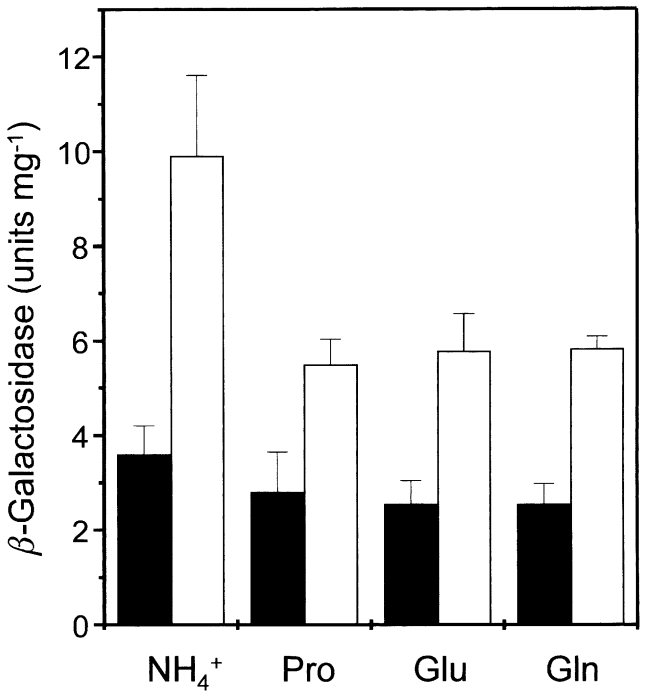


Fig. 2. Effect of different carbon and nitrogen sources on *GDH1* expression. β-Gal activity was determined in extracts obtained from the CLA1 wild type strain harvested during exponential growth on either glucose (*black bars*) or ethanol (*white bars*) as the carbon source, and ammonium sulfate (NH_4^+), proline (Pro), glutamate (Glu), or glutamine (Gln) as the nitrogen source. The reported β-Gal activities are averages of values obtained from three independent experiments ± SD.

In extracts of ethanol-grown cells, β-Gal activity fostered by *GDH1* (Fig. 2) was threefold higher, compared to that found on ammonium/glucose cultures. Northern analysis showed similar results, using the complete *GDH1* coding sequence as probe (data not shown). However, ethanol-increased expression did not correspond to an equivalent increment of NADP-GDH activity [8]. Extracts prepared from a *gdh3* null mutant grown on glucose or ethanol showed similar NADP-GDH activity (data not shown), indicating that the discrepancy between *GDH1* expression and NADP-GDH activity found in the wild type strain could not be attributed to a differential expression pattern of *GDH1* and *GDH3*. These results confirmed that ethanol-induced *GDH1* expression does not result in higher enzymatic activity and suggests the existence of a posttranscriptional regulatory mechanism, which remains to be identified. In this regard, it has been found that when *GDH1* or *GDH3* expression is fostered by the same promoter in glucose-grown cultures, Gdh1p activity is fivefold higher to that of Gdh3p, while in ethanol-grown cultures Gdh1p activity is only twofold higher compared to that of Gdh3p [10]. This supports the possibility that *GDH1* and *GDH3* transcripts could have a differential stability or translation rate in ethanol versus glucose. This would allow the organization of a NADP-GDH (Gdh1p/Gdh3p) isoenzyme with an appropriate rate of α-ketoglutarate utilization [10].

Effect of Gcn4p, Gln3p, and HAP complexes on GDH1 expression

Primer extension analysis performed with total RNA extracted from the CLA1 wild type strain harboring plasmid pLIN1 defined three transcription initiation sites (data not shown), which grossly corresponded to those previously determined [24] (Fig. 1).

Examination of the *GDH1* promoter sequence revealed a canonical Gcn4p binding site, $GCN4^{(3)}$, and three noncanonical binding sites: $GCN4^{(1),(2),\text{and }(4)}$ (Fig. 1), suggesting that *GDH1* expression could be directly controlled by Gcn4p. The impairment of Gcn4p had no effect on *GDH1* basal expression on glucose, while it clearly affected *GDH1* ethanol-induced expression (Fig. 3A). To address if *GDH1* expression was regulated during amino acid deprivation by the Gcn4p-mediated general amino acid control [15], β-Gal activity was determined in extracts from cultures of the wild type strain carrying pLIN1, grown in the presence or absence of 3-AT, a competitive inhibitor of His3p. In the presence of this analog, cells become deprived of histidine. β-Gal activity was slightly lower in extracts obtained from glucose-grown cultures treated with 3-AT, as compared to that determined in cells grown in the absence of this

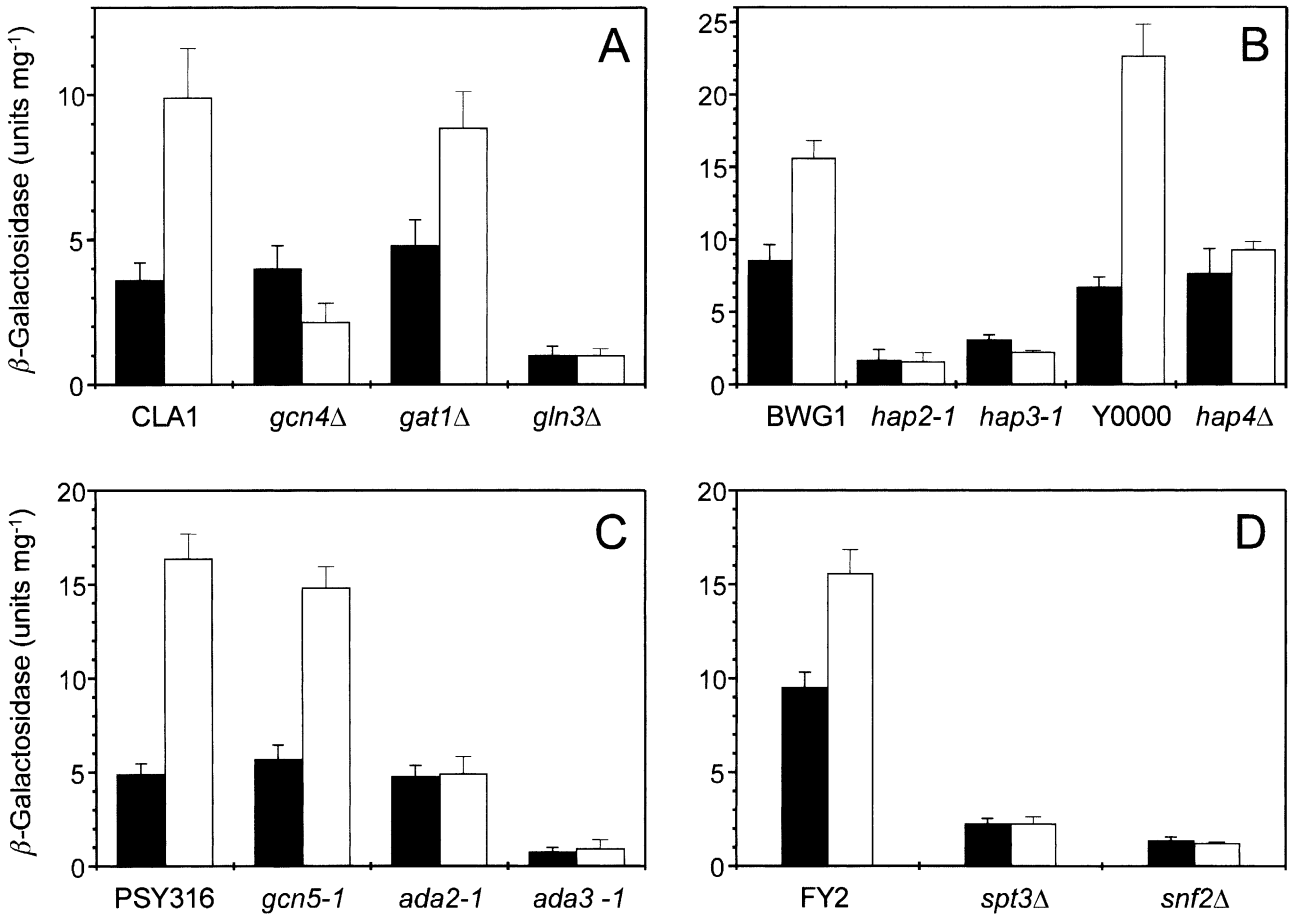


Fig. 3. *GDH1* expression in mutants altered in various transcriptional activators and chromatin remodeling factors. β-Gal activity was determined in extracts obtained from yeast cells harvested during exponential growth on either glucose (*black bars*) or ethanol (*white bars*) as carbon sources, and ammonium sulfate as the nitrogen source. (A) Parental strain CLA1 (*MATα ura3 leu2*) and its isogenic *gcn4Δ*, *gat1Δ*, and *gln3Δ* derivatives transformed with pLIN1 (*GDH1-lacZ* 2μ *LEU2*). (B) Parental strain BWG1-7A (*MATα ade1 leu2 ura3 his4*) and its isogenic *hap2-1* and *hap3-1* derivatives transformed with pLIN1, together with the parental strain Y0000 (*MATα his3Δ leu2Δ met15Δ ura3Δ*) and its isogenic *hap4Δ* derivative transformed with pLIN1. (C) Parental strain PSY316 (*MATα ura3 lys2 leu2 ade2 his3Δ*) and its isogenic *gcn5-1*, *ada2-1*, and *ada3-1* derivatives transformed with pLIN1. (D) Parental FY2 (*MATα ura3*) and its isogenic *spt3Δ* and *snf2Δ* derivatives transformed with pLIN10 (*GDH1-lacZ* 2μ *URA3*). The reported β-Gal activities are averages of values obtained from three independent experiments ± SD.

analog (2.64 versus 3.60 units mg^{-1}). These results indicate that *GDH1* is not regulated by the general amino acid control during amino acid deprivation on glucose. *GDH1* expression of ethanol-grown cells in the presence of 3-AT was similar to that found on ethanol alone (10.0 versus 9.90 units mg^{-1}), showing that *GDH1* transcription did not respond to histidine deprivation on ethanol. Accordingly, β-Gal activity fostered by the translational *GCN4-lacZ* gene fusion [14] in glucose-grown cultures (0.039 units mg^{-1}) increased 10-fold in ethanol-grown cultures (0.370 units mg^{-1}), or after 3-AT treatment (0.460 units mg^{-1}), indicating that translation of *GCN4* mRNA is equally increased in the presence of either 3-AT or ethanol. These results demonstrate that Gcn4p plays a crucial role in *GDH1* regulation on ethanol-grown cultures, and are in agreement with previous observations showing that glucose starvation stimulates *GCN4* translational expression through a different mechanism to that elicited by amino acid limitation [33]. The fact that *GCN4* translation is similarly increased in ethanol and 3-AT (0.37 versus 0.46 units mg^{-1}), but that *GDH1* expression is only induced by ethanol, possesses an interesting question in regard to the mechanisms determining the selective induction of *GDH1* by Gcn4p, which remains unsolved.

The *GDH1* promoter contains five GATAA sequences, four of which have been previously described [9] (Fig. 1). These sequences can constitute the *cis*-acting elements, UAS_{NTR}, acting as binding site(s) for two transcriptional activators, Gln3p and Gat1p/Nil1p, known to regulate the expression of nitrogen-modulated genes [4]. These sites could also have a role in down regulation of nitrogen-controlled gene expression exerted by the GATA family member Dal80p/Uga43p [5,7]. The lack of Dal80p/Uga43p (data not shown) or Gat1p/Nil1p (Fig. 3A), had no effect on *GDH1* expression in either

glucose- or ethanol-grown cells. The impairment of Gln3p diminished β-Gal activity in extracts obtained from glucose-grown cells. However, it had a more severe effect on *GDH1* expression in ethanol-grown cells, in which a 10-fold lower β-Gal activity was obtained compared to that found in the wild type strain (Fig. 3A). Recent findings obtained from the study of whole-genome transcription profiling, led to the proposition that proteins of the TOR signaling pathway communicate with transcription factors as part of a nutrient-response network [20]. It was considered that Gln3p was mainly involved in the nitrogen discrimination pathway, while Gat1p played this role in the carbon discrimination pathway. Our results show that Gln3p and Gat1p could have overlapping functions, since *GDH1* carbon-induced expression is Gln3p-dependent, while Gat1p plays no role in this regulation. To our knowledge, *GDH1* is the first example of a gene whose carbon regulation is *GLN3*-dependent. These findings strengthen the notion that ammonium assimilation constitutes the link between carbon and nitrogen regulations.

It has been previously shown that *GDH1* expression is glucose-repressed and highly induced in the presence of nonfermentable carbon sources [8]. Our results confirmed that impairment of either *HAP2* or *HAP3*, resulted in a decreased β-Gal activity fostered by *GDH1-lacZ* gene fusion in extracts obtained from glucose-grown cells, while the *hap4* null mutant showed no effect on β-Gal activity. However, impairment of either *HAP2*, *HAP3*, or *HAP4* diminished *GDH1*-dependent β-Gal activity in ethanol-grown cells (Fig. 3B). These results show that Hap4p is not involved in glucose-dependent regulation, but that it does play a role in *GDH1* expression in ethanol-grown cultures. Thus, in glucose-grown cells, the HAP complex operates in a Hap4p independent manner, while in ethanol the three subunits are involved in *GDH1* expression; supporting previous observations indicating that under respiratory metabolism, the α-ketoglutarate yielding enzymes are HAP-controlled [22]. The *GDH1*-encoded enzyme, being regulated by the HAP complex, could form part of a HAP-dependent network determining synthesis and utilization of α-ketoglutarate.

The above results show that *GDH1* transcription of yeast cells grown on glucose is regulated by Gln3p, Hap2p, and Hap3p; however, the lack of these effectors has a more dramatic effect on ethanol-grown cells. Furthermore, results show that Gcn4p- and Hap4p-mediated regulation is only observed in ethanol-grown yeast cells.

Regulation of GDH1 expression by chromatin remodeling factors

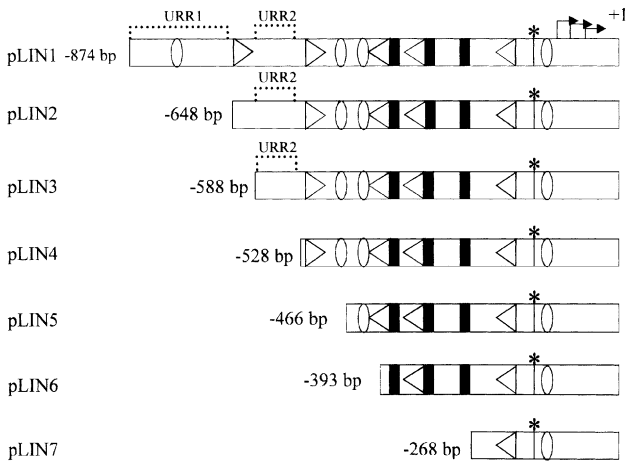
Two distinct Gcn5p-dependent histone acetyltransferase complexes [11]: the SAGA (Spt–ADA–Gcn5–acetyltransferase) and ADA (ADA–Gcn5–acetyltransferase), and the Swi–Snf complex [27] have been shown to remodel chromatin in vivo and to stimulate the binding of activators and basal factors to nucleosomal DNA.

To learn if the SAGA, ADA, or the Swi–Snf complexes were involved in *GDH1* expression, β-Gal activity was determined in a wild type strain and in *gcn5*, *ada2*, *ada3*, *spt3*, and *snf2* mutants transformed with either plasmid pLIN1 or pLIN10. The lack of Spt3p or Ada3p affected *GDH1* expression in extracts obtained from either glucose or ethanol cultures (Figs. 3C and D), showing that the SAGA complex participates in *GDH1* transcriptional regulation. Lack of Ada2p affected ethanol-induced *GDH1* expression, indicating that depending on the carbon source, different members of the SAGA remodeling complex regulate *GDH1* expression. A *gcn5* null mutation had no effect on *GDH1* expression on either glucose or ethanol. Even though the SAGA complex includes Gcn5p, apparently not all SAGA-dependent promoters are affected by Gcn5p-dependent acetylation [21]. In this regard, it has been recently shown that in strains lacking *GCN5*, *GAL1* transcription ocurred at wild type levels [3]. These data indicate that individual SAGA components are differentially required for the transcription of a given gene. Our results also suggest that either *GDH1* expression is not regulated by acetylation or that the $TAF_{II}145$ histone acetylase present in the TFIID transcription complex could play this role [21].

β-Gal activity was several-fold lower in the *snf2* mutant compared to that found in the wild type strain in extracts from both glucose- and ethanol-grown cells (Fig. 3D). These results show that the Swi–Snf complex is readily involved in *GDH1* transcriptional regulation.

Deletion analysis of GDH1 promoter

A collection of 5′ deletions of increasing size affecting the *GDH1* promoter was constructed as described in Materials and methods. When a wild type strain harboring pLIN2, which lacks the most 5′ 226 bp of the *GDH1* promoter, was grown on ammonium–glucose β-Gal activity was higher than that obtained with the wild type strain carrying plasmid pLIN1 (Fig. 4). This increment was more evident in yeasts grown on ammonium with ethanol as carbon source, a condition in which β-Gal activity was 2.2-fold higher than that found in the wild type strain carrying pLIN1. These results suggest that a target for negative regulation (upstream repressing region 1 [URR1]) was missing in pLIN2. Loss of the neighboring 60 bp (pLIN3) resulted in a twofold decrease in β-Gal activity on both glucose and ethanol compared to that fostered by pLIN2, suggesting that the $GATA^{(1)}$ box lost in pLIN3 may play a role in *GDH1* expression. The loss of a second URR localized between −588 and −528 (pLIN4), doubled β-Gal activity (Fig. 4). Enzymatic activity fostered by pLIN5 was decreased



	β-Galactosidase (units mg^{-1})							
	CLA 1 *GCN4 GLN3*				CLA 100 *gcn4Δ GLN3*		CLA 302-0 *GCN4 gln3Δ*	
	glucose glutamate	glucose NH_4^+	ethanol glutamate	ethanol NH_4^+	glucose NH_4^+	ethanol NH_4^+	glucose NH_4^+	ethanol NH_4^+
pLIN1	2.55	3.60	5.78	9.90	4.00	2.16	1.00	1.00
pLIN2	2.80	5.17	7.20	22.34	5.61	7.64	0.59	1.00
pLIN3	2.13	3.66	4.56	12.50	4.50	5.00	0.50	0.65
pLIN4	4.10	6.23	9.43	20.46	5.50	3.26	0.75	1.77
pLIN5	3.00	3.56	10.50	13.77	3.50	3.00	0.65	1.45
pLIN6	3.50	4.00	10.00	11.72	5.17	4.00	0.70	1.50
pLIN7	ND	1.44	ND	5.88	2.40	2.92	1.12	1.41

Fig. 4. β-Gal specific activities of 5′ deletions of the *GDH1* promoter. The *GDH1* full promoter and 5′ deletions were cloned into the 2μ *LEU2 lacZ* vector YEp363, generating plasmids pLIN1 through pLIN7. These plasmids were transformed into the wild type strain CLA1 and its isogenic derivatives CLA100 (*gcn4Δ*) and CLA101 (*gln3Δ*). The 5′ region carried in each plasmid is indicated in rows pLIN1 to pLIN7. Activity was determined in extracts obtained from cells grown on either ammonium sulfate or glutamate as nitrogen sources, with glucose or ethanol as carbon sources. The reported β-Gal activities are averages of values obtained from three independent experiments; variation was always $\leqslant$15%. ND, not determined. Diagrams depict putative binding sites for Gcn4p ((·)), Gln3p (▷, ◁), and HAP complex (■), putative TATA box (∗), transcription initiation sites (→), and putative URRs (·····).

twofold compared to that sustained by pLIN4, suggesting that the GATA[2] and the GCN4[2] *cis*-acting sites could also play a role in *GDH1* transcriptional activation. A further 5′ extended deletion construct (pLIN7) fostered β-Gal activity which was half-fold lower to that found with the full promoter (pLIN1), suggesting that the GATA[5] and GCN4[4] sites could be determining *GDH1* expression from this plasmid. Strains harboring constructs from pLIN1 through pLIN4 showed lowest β-Gal activity in the presence of glucose and glutamate, increasing gradually as nitrogen and carbon repressions were relieved, the highest expression being attained on ethanol plus ammonium. These results show that carbon repression is stronger than that exerted by the nitrogen source. Most interesting is the fact that strains harboring plasmids pLIN5 and pLIN6 failed to show repression by glutamate (Fig. 4). However, sequences that could participate in glutamate repression were not identified and further analysis will be needed to determine these *cis*-acting elements. The net increase in β-Gal activity in ethanol-grown cultures was evident in all deletion constructs, suggesting that this effect could be dependent on the GATA[5] and GCN4[4] *cis* elements or in a so far unidentified element which was retained in pLIN7 construct.

Null *gcn4* and *gln3* mutants were transformed with pLIN1 through pLIN7, and β-Gal activity was determined. As Fig. 4 shows, results obtained for β-Gal expression confirmed that Gcn4p has a role in *GDH1* expression in ethanol-grown cultures, and that although Gln3p affects *GDH1* expression on either glucose or ethanol-grown cultures, its effect is more pronounced in ethanol.

Concluding remarks

Results presented throughout this paper indicate that *GDH1* expression in ethanol is highly regulated, and are in agreement with the role that has been previously assigned to the NADP-glutamate dehydrogenase isoenzymes [10]. Gdh1p, Gdh3p, and the heteromeric enzyme present in ethanol-grown cultures show different allosteric properties and rates of α-ketoglutarate utilization. This constitutes a pacemaker mechanism in which the relative abundances of Gdh1p and Gdh3p monomers result in a balanced utilization of α-ketoglutarate under respiratory conditions [10]. It is tempting to speculate that the stringent regulation of *GDH1* expression in ethanol-grown cultures determines the amount of Gdh1p present in this condition, and consequently the overall Gdh1p/Gdh3p ratio that governs the rate of α-ketoglutarate utilization for glutamate biosynthesis.

Acknowledgments

We acknowledge Cristina Aranda for skillful technical assistance, Lourdes Valenzuela for helpful discussions, to the Molecular Biology Unit of the Instituto de Fisiología Celular for the synthesis of deoxyoligonucleotides, and Fernando Bastarrachea for his critical review of the manuscript. We also thank Allan Hinnebusch for kindly providing plasmid p180, and Bruno Andre, Mark Jhonston and Leonard Guarente, for providing yeast strains. The work was supported in part by the DGAPA, Universidad Nacional Autónoma de México (IN212898), and by the Consejo Nacional de Ciencia y Tecnología (31774-N). L.R. received a fellowship and two grants (PAEP-201371 and 102358) from the Dirección General de Estudios de Posgrado, UNAM.

References

[1] A. Avendaño, A. DeLuna, H. Olivera, L. Valenzuela, A. González, *GDH3* encodes a glutamate dehydrogenase isozyme a previously unrecognized route for glutamate biosynthesis in *Saccharomyces cerevisiae*, J. Bacteriol. 179 (1997) 5594–5597.

[2] S.L. Berger, B. Piña, N. Silverman, G.A. Marcus, J. Agapite, J.L. Regier, S.J. Triezenberg, L. Guarente, Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains, Cell 70 (1992) 251–265.

[3] S.R. Bhaumik, M.R. Green, SAGA is an essential in vivo target of the yeast acidic activator Gal4p, Genes Dev. 15 (2001) 1935–1945.

[4] D. Blinder, B. Magasanik, Recognition of nitrogen-responsive upstream activation sequences of *Saccharomyces cerevisiae* by the product of the *GLN3* gene, J. Bacteriol. 177 (1995) 4190–4193.

[5] D. Coornaert, S. Vissers, B. Andre, M. Grenson, The *UGA43* negative regulatory gene of *Saccharomyces cerevisiae* contains both a GATA-1 type zinc finger and a putative leucine zipper, Curr. Genet. 21 (1992) 301–307.

[6] P.W. Coschigano, S.M. Miller, B. Magasanik, Physiological and genetic analysis of the carbon regulation of the NAD-dependent glutamate dehydrogenase of *Saccharomyces cerevisiae*, Mol. Cell. Biol. 11 (1991) 4455–4465.

[7] T.S. Cunningham, T.G. Cooper, The *Saccharomyces cerevisiae DAL80* repressor protein binds to multiple copies of GATAA-containing sequences (URS_{GATA}), J. Bacteriol. 175 (1993) 5851–5861.

[8] V.D. Dang, C. Bohn, M. Bolotin-Fukuhara, B. Daigan-Fornier, The CCAAT box-binding factor stimulates ammonium assimilation in *Saccharomyces cerevisiae*, defining a new cross-pathway regulation between nitrogen and carbon metabolisms, J. Bacteriol. 178 (1996) 1842–1849.

[9] J.R. Daugherty, R. Rai, H.M.E. Berry, T.G. Cooper, Regulatory circuit for responses of nitrogen catabolic gene expression to the *GLN3* and *DAL80* proteins and nitrogen catabolite repression in *Saccharomyces cerevisiae*, J. Bacteriol. 175 (1993) 64–73.

[10] A. DeLuna, A. Avendaño, L. Riego, A. González, NADP-glutamate dehydrogenase isoenzymes of *Saccharomyces cerevisiae*. Purification, kinetic properties and physiological roles, J. Biol. Chem. 276 (2001) 43775–43783.

[11] A. Eberharter, D.E. Sterner, D. Schieltz, A. Hassan, J.R. Yates 3rd, S.L. Berger, J.L. Workman, The ADA complex is a distinct histone acetyltransferase complex in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 19 (1999) 6621–6631.

[12] B. Flores-Samaniego, H. Olivera, A. González, Glutamine synthesis is a regulatory signal controlling glucose catabolism in *Saccharomyces cerevisiae*, J. Bacteriol. 175 (1993) 7705–7706.

[13] S.L. Forsburg, L. Guarente, Identification and characterization of *HAP4*: a third component of the CCAAT-bound HAP2/HAP3 heteromer, Genes Dev. 8 (1989) 1166–1178.

[14] A.G. Hinnebusch, A hierarchy of trans-acting factors modulates translation of an activator of amino acid biosynthetic genes in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 5 (1985) 2349–2360.

[15] A.G. Hinnebusch, Mechanism of gene regulation in the general control of amino acid biosynthesis in *Saccharomyces cerevisiae*, Microbiol. Rev. 52 (1988) 248–273.

[16] H. Holzer, S. Schneider, Anreicherung und Trennung einer DPN-spezifischen und einer TPN-spezifischen Glutaminasaure Dehydrogenase aus Hefe, Biochem. Z. 329 (1957) 361–367.

[17] Y. Hu, T.G. Cooper, G.B. Kohlhaw, The *Saccharomyces cerevisiae* Leu3 protein activates expression of *GDH1*, a key gene in nitrogen assimilation, Mol. Cell. Biol. 15 (1995) 52–57.

[18] H. Ito, Y. Fukuda, K. Murata, A. Kimura, Transformation of intact yeast cells treated with alkali cations, J. Bacteriol. 153 (1983) 163–168.

[19] A.J. Koleske, S. Buratowski, M. Nonet, R.A. Young, A novel transcription factor reveals a functional link between the RNA polymerase II, CTD and TFIID, Cell 69 (1992) 883–894.

[20] F.G. Kuruvilla, A.F. Shamji, S.L. Schreiber, Carbon- and nitrogen-quality signaling to translation are mediated by distinct GATA type transcription factors, Proc. Natl. Acad. Sci. USA 98 (2001) 7283–7288.

[21] T.H. Lee, H.C. Causton, F.C.P. Holstege, W. Shen, N. Hannett, E.G. Jemmings, F. Winston, M.R. Green, R.A. Young, Redundant roles for the TFIID and SAGA complexes in global transcription, Nature 405 (2000) 701–704.

[22] Z. Liu, R. Butow, A transcriptional switch in the expression of yeast tricarboxilic acid cycle genes in response to a reduction or loss of respiratory function, Mol. Cell. Biol. 19 (1999) 6720–6728.

[23] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[24] W.S. Moye, N. Amuro, J.K.M. Rao, H. Zalkin, Nucleotide sequence of yeast *GDH1* encoding nicotinamide adenine dinucleotide phosphate-dependent glutamate dehydrogenase, J. Biol. Chem. 260 (1985) 8502–8508.

[25] A.M. Myers, A. Tzagaloff, D.M. Kinney, C.J. Lusty, Yeast shuttle integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions, Gene 45 (1986) 299–310.

[26] A.J. Ninfa, P. Jiang, M.R. Atkinson, J.A. Peliska, Integration of antagonistic signals in the regulation of nitrogen assimilation in *Escherichia coli*, Curr. Top. Cell Regul. 36 (2000) 31–75.

[27] C.L. Peterson, J.W. Tamkun, The SWI–SNF complex: a chromatin remodeling machine?, TIBS 20 (1995) 143–146.

[28] D.E. Sterner, P.A. Grant, S.M. Roberts, L.J. Duggan, R. Belotserkovskaya, L.A. Pacella, F. Winston, J.L. Workman, S.L. Berger, Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation and TATA–binding protein interaction, Mol. Cell. Biol. 19 (1999) 86–98.

[29] D.W. Tempest, J.L. Meers, C.M. Brown, Synthesis of glutamate in *Aerobacter aerogenes* by a hitherto unknown route, Biochem. J. 117 (1970) 405–407.

[30] N.A.W. Van Riel, M.L.F. Giuseppin, E.G. TerSchure, C.T. Verrips, A structured minimal parameter model of the central nitrogen metabolism in *Saccharomyces cerevisiae*: the prediction of the behaviour of mutants, J. Theor. Biol. 191 (1998) 397–414.

[31] L. Valenzuela, P. Ballario, C. Aranda, P. Filetici, A. González, Regulation of expression of *GLT1*, the gene encoding glutamate synthase in *Saccharomyces cerevisiae*, J. Bacteriol. 180 (1998) 3533–3540.

[32] L. Valenzuela, C. Aranda, A. González, TOR modulates *GCN4*-dependent expression of genes turned on by nitrogen limitation, J. Bacteriol. 183 (2001) 2331–2334.

[33] R. Yang, S.A. Wek, R.C. Wek, Glucose limitation induces *GCN4* translation by activation of Gcn2 protein kinase, Mol. Cell. Biol. 20 (2000) 2706–2717.