MOLECULAR AND CELLULAR BIOLOGY, Sept. 1991, p. 4455–4465
0270-7306/91/094455-11$02.00/0

Vol. 11, No. 9

Physiological and Genetic Analysis of the Carbon Regulation of the NAD-Dependent Glutamate Dehydrogenase of *Saccharomyces cerevisiae*

PETER W. COSCHIGANO, STEPHEN M. MILLER,† AND BORIS MAGASANIK*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received 1 April 1991/Accepted 12 June 1991

We found that cells of *Saccharomyces cerevisiae* have an elevated level of the NAD-dependent glutamate dehydrogenase (NAD-GDH; encoded by the *GDH2* gene) when grown with a nonfermentable carbon source or with limiting amounts of glucose, even in the presence of the repressing nitrogen source glutamine. This regulation was found to be transcriptional, and an upstream activation site (*GDH2* UAS_c) sufficient for activation of transcription during respiratory growth conditions was identified. This UAS was found to be separable from a neighboring element which is necessary for the nitrogen source regulation of the gene, and strains deficient for the *GLN3* gene product, required for expression of NAD-GDH during growth with the activating nitrogen source glutamate, were unaffected for the expression of NAD-GDH during growth with activating carbon sources. Two classes of mutations which prevented the normal activation of NAD-GDH in response to growth with nonfermentable carbon sources, but which did not affect the nitrogen-regulated expression of NAD-GDH, were found and characterized. Carbon regulation of *GDH2* was found to be normal in *hxk2*, *hap3*, and *hap4* strains and to be only slightly altered in a *ssn6* strain; thus, in comparison with the regulation of previously identified glucose-repressed genes, a new pathway appears to be involved in the regulation of *GDH2*.

The NAD-dependent glutamate dehydrogenase (NAD-GDH, product of the *GDH2* gene) of *Saccharomyces cerevisiae* catalyzes the oxidative deamination of glutamate to form α-ketoglutarate and ammonia, as follows:

$$\text{glutamate} + NAD^+ \rightarrow \alpha\text{-ketoglutarate} + NH_4^+ + NADH$$

It is thought that the chief role of the enzyme is in nitrogen metabolism (41), partly because regulation of NAD-GDH by the nitrogen source is well documented. Accordingly, in cells grown with 2% glucose as the carbon source, the level of this enzyme is high when glutamate is the source of nitrogen and low when glutamine or ammonia is the source of nitrogen (12, 16–18, 24, 32). The increase in the level of NAD-GDH by growth with glutamate has been shown to require the product of the *GLN3* gene, as cells carrying the *gln3* mutation have low levels of NAD-GDH regardless of the nitrogen source (46). However, we have now found that NAD-GDH levels are high in both *GLN3* and *gln3* strains grown with either glutamine or glutamate as the nitrogen source and with ethanol, acetate, or 0.1% glucose as the carbon source.

We report here studies of the regulation of NAD-GDH in response to the carbon source, including characterization of mutants found to be defective in this response. Our results indicate that *GDH2* is regulated by a carbon control pathway distinct from currently known regulatory circuits. This pathway was found to be primarily, although not completely, independent of the *SSN6* gene product, which has been demonstrated to be absolutely essential for the regulation of glucose-repressed genes in *S. cerevisiae*.

* Corresponding author.
† Present address: Department of Biology, Washington University, St. Louis, MO 63130.

MATERIALS AND METHODS

Strains. The strains used are listed in Table 1. All strains are derived from the PM38 background with the exception of 736-11D and the strains used to carry out the experiment in Table 3 (which are themselves isogenic).

Media. Media were prepared as described by Mitchell and Magasanik (45, 46) and Sherman et al. (57). All sources of carbon and energy were used at a final concentration of 2% with the exception of ethanol (3%) and, where indicated, glucose (0.1%).

Genetics. Standard yeast genetic methods were used (57).

Assays. Yeast cells were grown to an optical density of 80 to 120 Klett units as measured by a Klett-Summerson photoelectric colorimeter with a green filter (Klett Mfg. Co., New York, N.Y.) and then harvested as described by Mitchell and Magasanik (45). Cell extracts were made as described by Mitchell and Ludmerer (43) or Mitchell and Magasanik (44) with the modifications of Miller and Magasanik (41) and used immediately. Protein concentrations were determined by the method of Bradford (2), using reagents obtained from Bio-Rad Laboratories (Richmond, Calif.). Invertase assays were performed as described by Goldstein and Lampen (23). NAD-GDH assays were performed as described by Miller and Magasanik (41). Some variability is normally seen in assays performed on different days. To reduce this variability for each table, cultures were grown and assayed together. Assays for β-galactosidase were performed as described by Guarente (25) on 1-ml samples of cultures grown to an A_{600} of between 0.8 and 1.2.

Plasmid preparations. Bacterial plasmids were prepared by the boiling method of Holmes and Quigley (33). Plasmid DNA was prepared for sequencing with a mini kit from Qiagen (Studio City, Calif.). The alkaline lysis method of Maniatis et al. (40) was used for large-scale CsCl bacterial plasmid preparations.

Plasmid constructions. Plasmid pSM15 was constructed by

TABLE 1. Strains and plasmids

Strain or plasmid	Genotype[a]	Source or reference
S. cerevisiae		
PM38	*MATα leu2-3,112 ura3-52*	P. Minehart
PM71	*MATα leu2-3,112 ura3-52 gln3Δ5::LEU2*	P. Minehart
PS100	*MATα leu2-3,112 ura3-52* (pL15-6)	This study
PS100-113	*MATα leu2-3,112 ura3-52 rgc1-113* (pL15-6)	This study
PS100-115	*MATα leu2-3, 112 ura3-52 rgc2-115* (pL15-6)	This study
PS101	*MATα leu2-3,112 ura3-52 rgc1-113*	This study
PS102	*MATα leu2-3,112 ura3-52 rgc2-115*	This study
PH6	*MATα leu2-3,112 ura3-52 hxk2Δ::URA3*	This study
PH9	*MATα leu2-3,112 ura3-52 ssn6Δ6::URA3*	This study
736-11D	*MATa ade2-102 ura3-52*	1
BWG1-7A	*MATa leu2-3,112 ura3-52 ade1-100 his4-519*	28
SHY40	*MATa leu2-3,112 ura3-52 ade1-100 his4-519 hap3Δ::HIS4*	30
SLF401	*MATa leu2-3,112 ura3-52 ade1-100 his4-519 hap4Δ::LEU2*	21
E. coli XL1-Blue	*recA1 lac endA1 gyrA96 thi hsdR17 supE44 relA1*/F' *proAB lacI*^{q} *lacZΔM15* Tn*10*(Tet^r)	Stratagene
Plasmids		
Bluescript KS$^-$		Stratagene
pSLFΔ178K	*CYC1-lacZ* fusion without any UAS	20
pLGΔ-265	*CYC1-lacZ* fusion with UAS2 of *CYC1*	28
pSM2		41
pSM15	1.0-kb *Dra*I-*Dra*I fragment from pSM2 ligated into *Sma*I site of Bluescript KS$^-$	This study
pSM16	1.1-kb *Eco*RI-*Xho*I fragment from pSM2 ligated into *Eco*RI-*Xho*I-digested Bluescript KS$^-$	This study
pL15-1	0.1-kb *Ase*I-*Bst*EII fragment from pSM16 ligated in opposite orientation into *Sma*I site of pSLFΔ178K	This study
pL15-6	Two copies of 0.1-kb *Ase*I-*Bst*EII fragment from pSM16, both in opposite orientation, ligated into *Sma*I site of pSLFΔ178K	This study
pL15-7	0.1-kb *Ase*I-*Bst*EII fragment from pSM16 ligated in native orientation into *Sma*I site of pSLFΔ178K	This study

[a] Plasmids carried by yeast strains are given in parentheses.

cutting the *GDH2*-bearing plasmid pSM2 (41) with restriction endonuclease *Dra*I and ligating the 1.0-kb fragment from the digest into *Sma*I-digested Bluescript KS$^-$ vector (Stratagene, La Jolla, Calif.). Plasmid pSM16 was made by cutting plasmid pSM2 with restriction enzymes *Eco*RI and *Xho*I and ligating the resulting 1.1-kb fragment into Bluescript KS$^-$ plasmid linearized with *Eco*RI and *Xho*I. Plasmid pSM17 was made by cutting plasmid pSM2 with the enzymes *Bal*I and *Sal*I and ligating the 0.3-kb fragment from the digest into Bluescript KS$^-$ plasmid cut with *Eco*RV and *Sal*I. *GDH2-CYC1-lacZ* fusion constructs pL15-1, pL15-6, and pL15-7 were derived from a single ligation of Klenow-blunted *Ase*I-*Bst*EII fragment from pSM16 into pSLFΔ178K (20) linearized with restriction enzyme *Asp* 718 and blunted with Klenow enzyme. Sequence analysis of these plasmids indicated that pL15-1 has one insert in the reverse orientation, pL15-7 has one insert in the native orientation, and pL15-6 has two inserts, both in the reverse orientation, with respect to the orientation of the fragment in the *GDH2* gene. Vector DNA for all ligations was treated with calf alkaline phosphatase (Boehringer Mannheim, Indianapolis, Ind.) as described for blunt-ended molecules (40), restriction fragments were isolated from SeaPlaque low-melting-point (LMP) agarose (FMC BioProducts, Rockland, Maine), and ligations were performed in the agarose as described previously (13).

BAL-31 deletions. Deletions of the upstream region of *GDH2* were made from both the 5′ end and the 3′ end. The 5′ deletions were made in two sets, one designed to yield widely spaced endpoints and the other designed to concentrate them in the region of the carbon upstream activation sequence (UAS) element. The first set was made by linearizing 60 μg of plasmid pSM15 (Fig. 1) with restriction enzyme *Bam*HI, precipitating the plasmid, resuspending it in a volume of 600 μl of 1× BAL-31 buffer (as described previously [40], with the addition of spermidine to a final concentration of 3 mM), and incubating the mixture at 30°C with 10 U of BAL-31 enzyme. At 2, 4, 6, 8, 12, and 15 min, 100-μl portions of the reaction mixture were removed and added to tubes containing 200 μl of phenol-chloroform (1:1) to stop the reactions. The DNA was then extracted with chloroform, precipitated, resuspended, and digested with *Sal*I enzyme. The reaction mixtures were loaded onto a 0.6% LMP agarose gel, and the deleted fragments from each time point were excised from the gel. Plasmid pSLFΔ178K was digested with *Sma*I and *Xho*I, the ends were dephosphorylated by calf alkaline phosphatase, and the large fragment was isolated from a 0.6% LMP agarose gel. DNA fragments from each time point of the BAL-31 reaction were mixed with the pSLFΔ178K vector, and ligations were done in the gel. Ligation mixtures were transformed into *Escherichia coli* XL-1 Blue (Stratagene), and minipreps (33) were performed on cultures from the transformant colonies. Recombinant plasmids with inserts of the appropriate sizes were sequenced to determine their endpoints, and some were transformed into yeast strain PM38 to assay for UAS activity. The second set of 5′ deletion constructs was made exactly as the first except that plasmid pSM17 (Fig. 1) was used instead of pSM15, and it was linearized with the enzyme *Eco*RI before the BAL-31 digests. Deletions starting from the 3′ end of the upstream region of *GDH2* were made in the same manner as the 5′ end deletions, with the following modifications. Plasmid pSM16 (Fig. 1) was digested with enzyme *Sal*I, BAL-31 reactions were done for the six time points, and DNA from the reactions was digested with restriction enzyme *Dra*I. The deleted fragments were ligated into *Sma*I-digested pSLFΔ178K, and the orientation of the fragments in the recombinant plasmids was determined by the position of the *Bgl*II site of the *GDH2* DNA with respect to the *Xho*I site of the vector DNA.

Mutagenesis. Strain PS100 was mutagenized to about 10% survival as described by Miller and Magasanik (41). Cells of

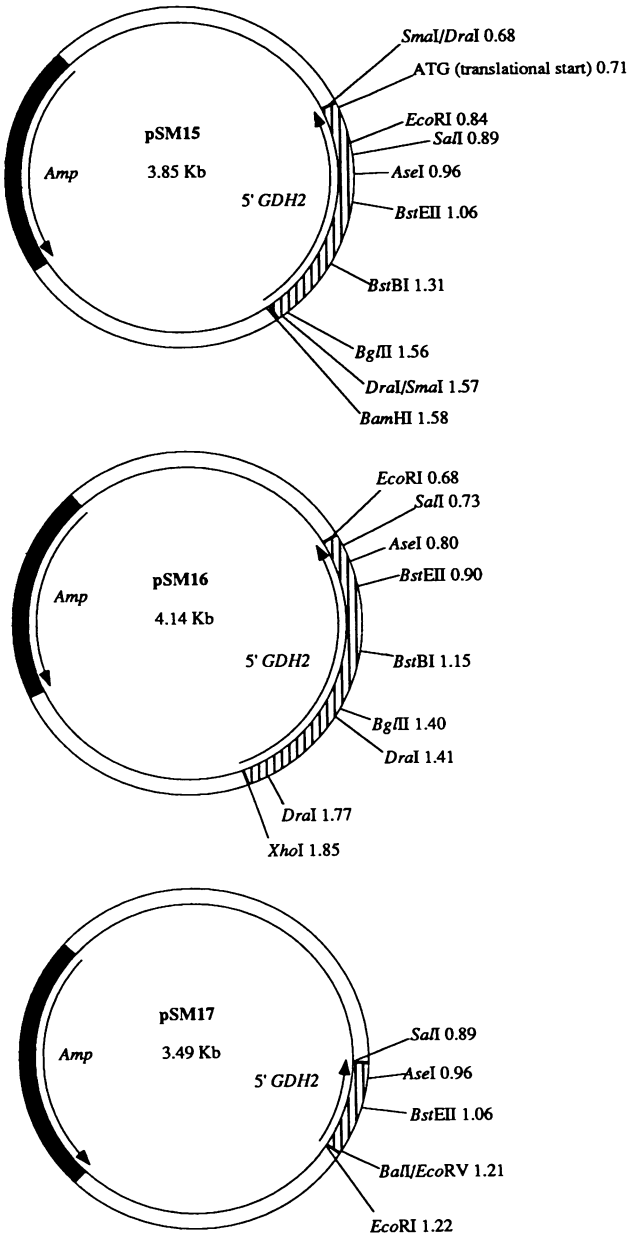


FIG. 1. Plasmids used for constructing deletions. BAL-31 deletions of the upstream region of *GDH2* were made by linearizing plasmids pSM15, pSM16, and pSM17 with *Bam*HI, *Sal*I, and *Eco*RI, respectively, before exonuclease treatment, as described in Materials and Methods. Striped portions of the plasmids indicate *GDH2* sequence cloned into Bluescript KS$^-$ vector; arrows indicate orientation of inserts, from 5′ (tail) to 3′ (head), with respect to their orientation within the *GDH2* gene.

the mutagenized strain were spread onto minimal plates containing glutamine as a nitrogen source and either 2 or 0.1% glucose as a source of carbon and energy.

Transformations. Yeast transformations were carried out by the lithium acetate method of Ito et al. (34). Strain PH6 was constructed by replacing the *HXK2* gene of PM38 with a disrupted copy (obtained from D. Botstein) by the method of Rothstein (53). Strain PH9 was constructed in the same way by transforming PM38 with a fragment from plasmid

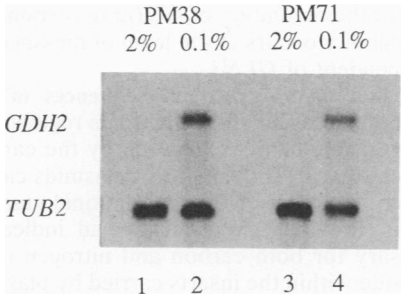


FIG. 2. Northern analysis of *GDH2* regulation in response to the carbon source. Cells were grown with glutamine and either 2 or 0.1% glucose as indicated above the lanes. Lanes 1 and 2, strain PM38 (*GLN3*); lanes 3 and 4, strain PM71 (*gln3Δ5::LEU2*). Approximately equal amounts of poly(A)$^+$ RNA were loaded into each lane. The blot was probed with both a *GDH2* fragment and a *TUB2* fragment (to standardize the amount of RNA loaded), as explained in Materials and Methods.

pJS22 (56). Bacterial transformations were done as described by Maniatis et al. (40).

Preparation and analysis of RNA. Total yeast RNA was purified essentially by the method of Carlson and Botstein (5). Poly(A)$^+$ RNA was then prepared in batch as described by Maniatis et al. (40). RNA was separated on a 1% agarose formaldehyde gel (36) and transferred to GeneScreen (NEN DuPont, Boston, Mass.) as described by Coschigano and Magasanik (11). ^{32}P-labeled probes (2.6-kb *Sal*I-*Sal*I fragment for *GDH2* [41] and 0.9-kb *Sal*I-*Asp* 718 fragment for *TUB2* [obtained from J. Thomas]) were made by nick translation with a kit from Boehringer Mannheim (50), and hybridization was carried out as described by Clark-Adams and Winston (10).

DNA sequencing. Dideoxy DNA sequencing was performed by the method of Sanger et al. (55), using [α-^{35}S]dATP. Sequenase enzyme (modified T7 polymerase) and reagents were obtained in a Sequenase kit from U.S. Biochemical Corp. (Cleveland, Ohio). The BAL-31 fusion junctions and the inserts of constructs pL15-1, pL15-6, and pL15-7 were sequenced by using primers made to hybridize to DNA upstream of the *Sma*I site and downstream of the *Xho*I site in the pSLFΔ178K vector (primers were obtained from Operon Technologies, Alameda, Calif.).

RESULTS

Northern (RNA) analysis of NAD-GDH. Nitrogen-regulated expression of NAD-GDH depends on the product of the *GLN3* gene and is repressed by the presence of glutamine in the growth medium (43). We found that the level of NAD-GDH was greatly increased by growing the cells in a glutamine-containing medium with 0.1% glucose, rather than 2% glucose, as the source of carbon. To determine whether this increase in the level of NAD-GDH in response to carbon limitation resulted from an increase in the abundance of mRNA made from its structural gene *GDH2*, we determined the intracellular level of *GDH2*-specific mRNA. Strains PM38 (wild type) and PM71 (*gln3*) were grown with glutamine as the nitrogen source and either 2 or 0.1% glucose as the carbon source, and the mRNA was isolated and examined by Northern analysis (Fig. 2). In both strains, there was significantly more *GDH2* message when strains were grown with 0.1% glucose than when they were grown with 2% glucose as the source of carbon (Fig. 2). This finding

indicates that the regulation of *GDH2* in response to carbon source availability occurs at the level of message abundance and is independent of *GLN3*.

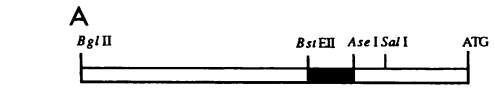
Deletion analysis of upstream sequences of *GDH2*. To determine which portion of the upstream region of the *GDH2* gene is responsible for its regulation by the carbon source, we constructed BAL-31 deletions of plasmids carrying DNA derived from upstream of the translational start site of the *GDH2* gene. Previous experiments had indicated that all sites necessary for both carbon and nitrogen regulation of the gene reside within the inserts carried by plasmids pSM15 and pSM16 and that transcription of the *GDH2* message begins about 160 bp downstream from the *Sal*I site from which the 3′ deletions were initiated (data not shown). Plasmids pSM15, pSM16, and pSM17 (Fig. 1) were linearized with restriction enzymes to allow digestion of *GDH2* sequence by the exonuclease BAL-31, as described in Materials and Methods, and the deleted fragments were cloned into the *CYC1-lacZ* fusion vector pSLFΔ178K (20). This vector contains the TATA box and transcriptional start site sequences from the *CYC1* gene fused to the *E. coli lacZ* gene and requires a UAS for expression of β-galactosidase in yeast cells. While expression of *CYC1* is glucose repressed, all sequences known to affect carbon regulation of the gene have been removed from vector pSLFΔ178K (20) so that the remaining *CYC1* sequence on the plasmid would not be expected to interfere with measurements of the carbon regulation of *GDH2*. Constructs having a 5′ deletion endpoint at bp −338 (with respect to the translational start of *GDH2*) or further upstream retained the ability to express β-galactosidase when carried by yeast strains grown with low (0.1%) glucose, whereas a construct that had lost an additional 9 bp of *GDH2* DNA past bp −338 had only about 10% of wild-type UAS activity (Fig. 3A). Deletion fragments made by digests initiated from the *Sal*I site of pSM16 having 3′ endpoints at bp −186, −236, and −279 (with respect to the translational start site of *GDH2*) were able to activate transcription under glucose-limiting conditions, but a fragment with its 3′ endpoint at bp −309 did not activate transcription specifically under this condition (Fig. 3B). Thus, the entire carbon source-sensitive UAS of *GDH2* (UAS_c) is contained within the 101-bp *Bst*EII-*Ase*I restriction fragment (Fig. 3).

Whereas the deletion construct with its 5′ endpoint at bp −401 of the *GDH2* sequence allowed expression of β-galactosidase during growth with low glucose, it did not permit expression during growth with 2% glucose when glutamate was the nitrogen source (Fig. 3A). Conversely, deletions initiated from the *Sal*I site and extending just past the *Bst*EII site of pSM15 eliminated carbon source activation but did not hinder nitrogen source activation when these fragments were cloned into pSLFΔ178K (Fig. 3B). These results are consistent with the observation that the *gln3* mutation, which abolishes nitrogen source control of *GDH2*, does not affect regulation of *GDH2* by the carbon source (Fig. 2; see also Table 5). It might also be noted that the 3′ deletion fragments with endpoints at bp −236, −279, −309, and −360 were able to drive transcription under repressing growth conditions (2% glucose) to a significantly greater degree than were any of the 5′ deletion fragments or the 3′ deletion fragment with its endpoint at bp −186 (Fig. 3). We believe that the 3′ deletion fragments able to function as activation sites in cells grown with 2% glucose have lost a DNA element which acts as a repression site (42).

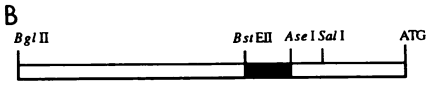
Isolation of mutants defective in carbon control of *GDH2*. We sought to isolate mutants with altered carbon regulation of *GDH2* by mutagenizing the wild-type strain PS100 and screening either for colonies with high levels of β-galactosidase when grown on high-glucose plates or for colonies with low levels of β-galactosidase when grown on low-glucose plates. This yeast strain contains plasmid pL15-6, which was constructed by inserting two copies of *GDH2* UAS_c upstream of the *CYC1-lacZ* fusion in vector pSLFΔ178K. A plasmid containing multiple copies of the UAS was used to ensure higher expression of the fusion and to decrease the likelihood of isolating the unwanted class of mutants having an impaired promoter element. About 20,000 colonies were initially screened on 2% glucose plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal), and about 20,000 colonies were also screened on 0.1% glucose X-Gal plates. Promising candidates were then grown in liquid culture and assayed for β-galactosidase activity. None of the strains giving rise to blue colonies on the 2% glucose plates had significantly higher enzyme levels than the parent strain grown with the same carbon source, but seven mutants giving rise to white or light blue colonies on 0.1% glucose X-Gal plates had a similar low level of β-galactosidase activity when grown on 0.1% glucose medium. These seven strains, along with the parent strain, were then grown in 25-ml cultures with 0.1% glucose as the carbon source, and cell extracts were made and assayed for NAD-GDH activity. All of the seven mutants assayed had NAD-GDH levels significantly lower than that of the parent and therefore appear to contain chromosomal mutations affecting the regulation of *GDH2*.

Growth with 0.1% glucose presumably reflects the ability of the cell to obtain energy by respiration of the ethanol derived from the initial fermentation of the glucose. A pleiotropic regulatory mutation preventing activation of *GDH2* by growth with nonfermentable carbon sources might also prevent activation of genes required for utilization of these poor carbon sources. Of the seven mutants, six were petites in that they failed to grow in liquid YEP-ethanol or YEP-acetate medium whereas they grew as well as the parent on YEP-glucose. The other mutant grew normally on YEP-ethanol and YEP-acetate media. Each of the seven mutant strains was crossed to wild-type strain 736-11D, and the diploids were assayed for NAD-GDH activity. All seven diploids were normal for the carbon regulation of *GDH2*, indicating that the seven mutations are recessive. Diploids from each cross were sporulated, and haploid progeny of both mating types were obtained to perform complementation tests among the seven mutants. Between three and eight tetrads were examined for each cross. The petite growth phenotype segregated 2:2 in each tetrad for all six petite mutants, indicating that all have nuclear mutations. In addition, the petite growth phenotype and the defect in *GDH2* regulation cosegregated for each of the six crosses, so that in each mutant one mutation caused both phenotypes. The six petite mutants failed to complement each other for their growth phenotype, whereas the nonpetite mutant complemented each of the others both for the growth phenotype and for production of β-galactosidase. We have named the loci defined by these mutations *rgc1* (nonpetite) and *rgc2* (petite), for regulation of *GDH2* by carbon source.

Comparison of carbon regulation of *GDH2* with that of *CYC1* and *SUC2*. Two of the most extensively studied genes whose expression is subject to repression by glucose are *SUC2* and *CYC1*, which encode invertase and iso-1-cytochrome *c*, respectively (51, 58). To determine whether the *rgc1* and *rgc2* mutations affect regulation of *CYC1* UAS2, the UAS of *CYC1* responsible for carbon regulation (27), we



	ß-Galactosidase Units		
	2% Glucose Glutamine	0.1% Glucose Glutamine	2% Glucose Glutamate
-811	<0.2	126 ± 25	164 ± 23
-674	<0.2	86 ± 16	ND
-573	<0.2	79 ± 9	ND
-483	<0.2	108 ± 20	ND
-401	<0.2	136 ± 9	0.7 ± 0.2
-351	<0.2	55 ± 5	ND
-338	<0.2	78 ± 2	ND
-329	<0.2	13 ± 3	ND
-261	<0.2	<0.2	ND



	ß-Galactosidase Units		
	2% Glucose Glutamine	0.1% Glucose Glutamine	2% Glucose Glutamate
-186	0.9 ± 0.2	88 ± 17	101 ± 11
-236	3.6 ± 0.8	142 ± 26	ND
-279	8.1 ± 1.0	72 ± 17	ND
-309	22 ± 4	14 ± 1	ND
-360	25 ± 5	9.6 ± 0.2	307 ± 33

FIG. 3. Deletion analysis of the *GDH2* gene. (A) 5′ deletion analysis; (B) 3′ deletion analysis. The thin shaded boxes represent the portion of *GDH2* DNA remaining after the BAL-31 digests, and the numbers immediately to the left (A) or to the right (B) of the boxes are the deletion endpoints of the fragments. The deletion endpoint refers to the last base pair of *GDH2* sequence remaining at the BAL-31-digested end of the fragment with respect to its native distance from the translational start of *GDH2*. Cells of wild-type strain PM38 carrying the indicated plasmids were grown in minimal medium supplemented with either 2 or 0.1% glucose and with 0.1% glutamine as the nitrogen source, or with 2% glucose and 0.1% glutamate as the nitrogen source. All cultures were also supplemented with leucine to satisfy the requirements of the strain. β-Galactosidase assays were performed in triplicate for each deletion construct; both the average and the range (represented as a ± value) reported except when the assay value was <0.2 U. ND, not determined.

TABLE 2. Effects of *rgc* mutations on *GDH2* UAS_c and on *CYC1* UAS2

Strain	Plasmid[b]	β-Galactosidase value (U) for strain grown with carbon source indicated[a]			
		2% glucose	Galactose	Raffinose	0.1% glucose
PM38 (*RGC*)	pLGΔ-265	3.0 ± 0.4	17 ± 2	50 ± 6	84 ± 13
PM38 (*RGC*)	pL15-1	2.0 ± 0.8	0.6 ± 0.2	9.0 ± 2	36 ± 3
PM38 (*RGC*)	pL15-7	0.4 ± 0.3	0.8 ± 0.3	17 ± 1	126 ± 20
PS101 (*rgc1*)	pLGΔ-265	3.0 ± 0.4	19 ± 5	71 ± 30	73 ± 2
PS101 (*rgc1*)	pL15-1	2.4 ± 0.3	1.1 ± 0.5	2.2 ± 0.6	1.2 ± 0.6
PS101 (*rgc1*)	pL15-7	0.3 ± 0.1	0.4 ± 0.2	1.8 ± 0.1	1.4 ± 0.7
PS102 (*rgc2*)	pLGΔ-265	4.7 ± 0.2	28 ± 1	87 ± 7	ND[c]
PS102 (*rgc2*)	pL15-1	2.9 ± 0.1	1.3 ± 0.2	3.9 ± 1.4	ND
PS102 (*rgc2*)	pL15-7	0.4 ± 0.1	0.5 ± 0.2	1.6 ± 0.1	ND

[a] β-Galactosidase assays were carried out as described by Guarente (25). Cells were grown in minimal media with the indicated carbon source and with glutamine serving as the nitrogen source. Cultures were also supplemented with leucine to satisfy the nutritional requirements of the strains.

[b] Plasmid pLGΔ-265 contains the carbon-regulated UAS2 of *CYC1* driving a *CYC1-lacZ* fusion. Plasmid pL15-7 contains *GDH2* UAS_c in the native orientation driving the same fusion; pL15-1 contains UAS_c inserted in the opposite orientation.

[c] ND, not determined because of inadequate growth.

tested the expression of a *CYC1-lacZ* fusion plasmid driven by either *CYC1* UAS2 (pLG-Δ265) or *GDH2* UAS_c (pL15-1 and pL15-7). In the wild-type strain, expression of the *CYC1-lacZ* fusion driven by *GDH2* UAS_c was highest with 0.1% glucose as the carbon source, lower with raffinose, and lowest with either 2% glucose or galactose (Table 2). These results are consistent with the NAD-GDH activity levels in cells grown with these carbon sources (see Table 5). It should also be noted that plasmids pL15-1 and pL15-7, in which the *Bst*EII-*Ase*I fragment (containing UAS_c) is cloned into the vector in opposite orientations, gave rise to similar levels of β-galactosidase activity under the various conditions, the result anticipated for a UAS element (26). As expected, expression from *GDH2* UAS_c was greatly reduced with either raffinose or 0.1% glucose as the carbon source in the *rgc1* strain and with raffinose as the carbon source in the *rgc2* strain. In contrast, expression of the *CYC1-lacZ* fusion driven by *CYC1* UAS2 was not affected by the *rgc1* mutation in cells grown with raffinose, galactose, or low glucose as the carbon source (Table 2). Thus, *rgc1* does not significantly affect regulation of *CYC1*. Expression of the fusion driven by *CYC1* UAS2 was also unaffected by the *rgc2* mutation for cells grown with either raffinose or galactose as the carbon source, again in contrast to the *GDH2* UAS_c-driven fusion. Therefore, the two classes of mutations which prevent activation of transcription by *GDH2* UAS_c have no effect on the activity of *CYC1* UAS2.

It should be noted that a mitochondrial petite derived from parent strain PS100 by exposure to ethidium bromide had some of the same properties with respect to the regulation of NAD-GDH as did *rgc2* strains. A mitochondrial petite grown with 2% glucose as the carbon source and shifted for 2 h to a medium with ethanol as the carbon source or to a medium with no carbon source had the same low level of NAD-GDH activity as did a *rgc2* strain grown under the same conditions. Furthermore, we found that the parent strain itself did not increase NAD-GDH levels when shifted from high-glucose medium to medium with no carbon source (data not shown). These results suggested that any strain unable to respire, such as a petite, might have the regulatory defect exhibited by *rgc2* strains and made clear the importance of testing other petite strains for the ability to express NAD-GDH under conditions of cell growth. Since the *rgc2* strains grew with raffinose as the carbon source, but unlike the wild type were not able to express the *GDH2-CYC1-lacZ* fusion when grown with this carbon source, we sought to determine whether other petites could express NAD-GDH when utilizing raffinose. We attempted to test the mitochondrial petite strain derived from strain PM38 for derepression of fusion constructs driven by *GDH2* UAS_c and *CYC1* UAS2, but this strain was unable to utilize raffinose. However, strains carrying either *hap3* or *hap4* mutations, which are nuclear petites because of their inability to express *CYC1*, were able to grow on raffinose and were not defective for the expression of a *lacZ* fusion driven by *GDH2* UAS_c (see below and Table 3). Therefore, the defect in *rgc2* strains which specifically prevents transcriptional activation through *GDH2* UAS_c cannot be attributed solely to the inability of the cells to respire. It is not entirely clear why the mutant screen which yielded the *rgc2* strains did not also yield other petite strains, but apparently under the conditions of the screen these other petite strains were able to express the *GDH2-lacZ* fusion.

We also sought to determine whether the regulatory factors known to be required for derepression of *CYC1* might be required for expression of *GDH2*. The *CYC1* gene UAS2 requires the products of the *HAP2*, *HAP3*, and *HAP4* genes for activation when grown with carbon sources other than glucose (21, 30, 48). Strains defective for the *HAP2*, *HAP3*, and *HAP4* gene products are unable to respire (21, 27, 30), but we were able to test the ability of *GDH2* UAS_c to drive a *CYC1-lacZ* fusion in strains lacking the *HAP3* or *HAP4* gene products by growing the cells on raffinose. As expected, the *hap3* and *hap4* strains failed to express a *CYC1-lacZ* fusion driven by *CYC1* UAS2 (Table 3), but they

TABLE 3. Regulation of UAS_c in conjunction with *HAP3* and *HAP4*

Plasmid[a]	β-Galactoside activity (U)[b]		
	BWG1-7A (wild type)	SHY40 (*hap3*)	SLF401 (*hap4*)
pL15-7	12 ± 2	18 ± 1	21 ± 11
pLGΔ-265	36 ± 12	2.1 ± 0.1	1.9 ± 0.1
pSLFΔ178K	0.6 ± 0.2	1.4 ± 0.1	1.8 ± 0.4

[a] Plasmid pL15-7 contains *GDH2* UAS_c in the native orientation driving a *CYC1-lacZ* fusion. Plasmid pLGΔ-265 contains the carbon-regulated UAS2 of *CYC1* driving the same fusion. Plasmid pSLFΔ178K is a control plasmid with no UAS.

[b] Determined as described by Guarente (25). Cells were grown on raffinose-glutamine medium supplemented with adenine and Casamino Acids.

TABLE 4. Regulation of NAD-GDH in conjunction with *HXK2* and *SSN6*

Strain	Sp act					
	Glucose-glutamine		Raffinose-glutamine		0.1% glucose-glutamine	
	NAD-GDH[a]	Invertase[b]	NAD-GDH	Invertase	NAD-GDH	Invertase
PM38 (wild type)	1.4 ± 0.5	<0.1	9.1 ± 0.5	1.7 ± 0.1	33.7 ± 1.0	1.3 ± 0.1
PH6 (*hxk2*)	1.3 ± 0.4	0.3 ± 0.1	5.6 ± 0.2	1.6 ± 0.1	28.3 ± 2.0	1.1 ± 0.1
PS101 (*rgc1*)	1.4 ± 0.3	<0.1	3.6 ± 0.5	3.0 ± 0.1	7.8 ± 0.2	1.3 ± 0.1
PH9 (*ssn6*)	6.6 ± 1.6	7.5 ± 0.1	7.2 ± 0.6	6.7 ± 0.1	NG[c]	NG

[a] Expressed as nanomoles per minute per milligram of protein.
[b] Expressed as micromoles of glucose generated per minute per milligram of protein (23).
[c] NG, no growth.

were able to express the *GDH2-lacZ* fusion to the same level as the wild type. Additionally, it has recently been found that the presence of the *HAP4* gene, driven by the constitutive *ADH1* promoter, on a multicopy plasmid in an otherwise wild-type strain allows derepression of a *CYC1-lacZ* fusion driven by *CYC1* UAS2 in yeast strains grown with 2% glucose (59). We tested *CYC1-lacZ* fusions driven by either *GDH2* UAS_c or *CYC1* UAS2 in a strain bearing the multicopy plasmid containing the *HAP4* gene and found that *CYC1* UAS2 activated transcription during growth with 2% glucose, whereas *GDH2* UAS_c did not (data not shown). These results and the data presented in Table 2 demonstrating normal regulation of *CYC1* UAS2 in *rgc1* and *rgc2* strains suggest that the regulatory pathways of *GDH2* and of *CYC1* are distinct.

Expression of the gene encoding invertase, *SUC2*, is known to be altered by a number of unlinked, pleiotropic mutations, including those at the *SSN6* and *HXK2* loci; invertase expression is elevated, to different degrees, in *ssn6* and *hxk2* strains grown on 2% glucose. To explore the possibility that *GDH2* might also be regulated by *HXK2* and *SSN6*, and that *RGC1* might regulate *SUC2*, we grew wild-type, *hxk2*, *ssn6*, and *rgc1* strains with glucose, raffinose, or 0.1% glucose as the carbon source. On high glucose, all strains had low NAD-GDH activities (Table 4). The *ssn6* strain did have roughly fivefold-higher NAD-GDH activity than the others, but this level was only one-fifth that of the wild-type strain grown on low glucose. In contrast, invertase levels were five- to sixfold higher in the *ssn6* strain grown with 2% glucose or with raffinose than in the wild-type strain grown with low glucose. Thus, the elevation in NAD-GDH activity caused by the *ssn6* mutation is small compared with the increase in invertase activity caused by *ssn6* in cells grown with either high glucose or raffinose (Table 4). Interestingly, NAD-GDH levels in the *ssn6* strain were nearly identical whether the cells were grown with raffinose or with 2% glucose as the carbon source, whereas wild-type cells had sevenfold-greater activity when grown with raffinose rather than with 2% glucose. This suggests that perhaps the *SSN6* product is involved in a regulatory pathway responsible for the modest elevation in NAD-GDH levels that occurs during weakly activating conditions, such as growth of cells with raffinose, but that the more dramatic increase in enzyme levels observed in cells grown with limiting amounts of glucose depends on a pathway which is independent of *SSN6*. Invertase expression was not altered in the *rgc1* strain, compared with the wild type, for any of the carbon sources tested, whereas NAD-GDH levels were significantly lower in the *rgc1* strain than in the wild type for growth with raffinose or with low glucose. Therefore, the *RGC1* product is not involved in the regulation of invertase, providing further evidence that carbon regulation of NAD-GDH is largely independent of the invertase regulatory pathway.

Regulation of *GDH2* expression by different sources of carbon. The fact that growth on low glucose increased the level of NAD-GDH suggested that providing the cells with carbon sources supporting slower growth than glucose might also increase the level of the enzyme. The results of experiments determining the level of NAD-GDH in wild-type, *gln3*, and *rgc1* strains are summarized in Table 5. It can be seen that in cells grown with 2% glucose, substitution of glutamate for glutamine increased the level of the enzyme approximately 25-fold (experiments 1 and 2). This increase, as previously reported (46), required a functional *GLN3* gene, but the *rgc1* mutation did not affect this response. The increase in the level of NAD-GDH resulting from the substitution of ethanol, acetate, or 0.1% glucose for 2% glucose in glutamine-containing medium was not affected by the *gln3* mutation but was greatly reduced by the *rgc1* mutation (experiments 3 to 5). Substitution of 2% galactose or 2% fructose for 2% glucose as the source of carbon did not result in an increased level of NAD-GDH (experiments 7 and 8), but substitution of 2% raffinose allowed a small increase in the level of the enzyme (experiment 6).

We also sought to determine whether the nitrogen source might affect the carbon control of NAD-GDH activity in a *GLN3*-independent manner. Cells of strain PM71 (*gln3*) were grown with ethanol as the carbon source and with various nitrogen sources (Table 6). When grown with either glutamine or glutamate as the nitrogen source, cells had the highest levels of NAD-GDH, but with ammonia as nitrogen source the enzyme level was four- to sevenfold lower (compare experiments 1 and 2 with experiment 3). Since ammonia did not prevent an increase in activity in the presence of glutamine (compare experiments 1 and 4), am-

TABLE 5. Regulation of NAD-GDH by carbon source

Expt	Medium[a]	NAD-GDH sp act (nmol/min/mg of protein)		
		PM38 (wild type)	PM71 (*gln3*)	PS101 (*rgc1*)
1	G-Gln	1.1 ± 0.3	1.5 ± 0.1	0.5 ± 0.3
2	G-Glt	24.9 ± 0.3	2.5 ± 0.4	24.1 ± 0.2
3	0.1% G-Gln	22.4 ± 0.7	31.6 ± 2.7	3.4 ± 0.8
4	Et-Gln	36.9 ± 0.6	32.6 ± 0.3	6.6 ± 0.4
5	Ace-Gln	31.5 ± 1.4	33.6 ± 1.3	8.9 ± 0.6
6	Raf-Gln	4.5 ± 0.6	6.7 ± 0.5	3.1 ± 0.3
7	Fru-Gln	2.6 ± 0.5	2.5 ± 0.4	1.6 ± 0.3
8	Gal-Gln	0.5 ± 0.4	2.7 ± 0.7	<0.1

[a] Abbreviations: G, glucose; Et, ethanol; Ace, acetate; Raf, raffinose; Fru, fructose; Gal, galactose, Gln, glutamine; Glt, glutamate.

TABLE 6. Nitrogen source requirement in carbon regulation[a]

Expt	Nitrogen source[b]	Sp act			
		NAD-GDH[c] (no plasmid)	β-Galactosidase (U)[d]		
			pL15-7	pLGΔ-265	pSLFΔ178K
1	Gln	36.0 ± 2.5	415 ± 65	165 ± 23	2.2 ± 1.2
2	Glt	22.5 ± 3.7	304 ± 28	91 ± 16	1.1 ± 0.1
3	N	5.4 ± 2.6	47 ± 3	64 ± 10	0.7 ± 0.3
4	Gln + N	33.3 ± 2.4	493 ± 27	172 ± 41	1.3 ± 0.8
5	N + Leu	17.6 ± 1.0	176 ± 32	130 ± 8	0.4 ± 0.1
6	N + Ser	12.6 ± 2.3	117 ± 19	98 ± 10	0.7 ± 0.1
7	N + Val	10.6 ± 1.1	79 ± 26	94 ± 23	0.5 ± 0.2

[a] All assays were carried out in strain PM71 (*gln3*) with the indicated plasmid grown on ethanol as the carbon source. Plasmid pL15-7 contains *GDH2* UAS_c in the native orientation driving a *CYC1-lacZ* fusion. Plasmid pLGΔ-265 contains the carbon-regulated UAS2 of *CYC1* driving the same fusion. Plasmid pSLFΔ178K is a control plasmid with no UAS.

[b] Abbreviations: Gln, glutamine; Glt, glutamate; N, ammonia; Leu, leucine; Ser, serine; Val, valine. Leu, Ser, and Val were added to the media to a final concentration of 2 mM, 1/10 the ammonia concentration.

[c] Expressed as nanomoles per minute per milligram of protein.

[d] Determined as described by Guarente (25).

monia cannot simply be inhibitory to a system of activation which is dependent only on the primary carbon source. Instead, it is possible that full carbon activation of NAD-GDH requires nitrogen sources which upon utilization provide the cell with an auxiliary carbon source. Consistent with this view is the fact that small amounts of leucine, serine, or valine were also able to cause modest (2- to 3-fold) increases in NAD-GDH activity when ammonia was the primary nitrogen source (present in 10-fold excess) (experiments 5 to 7). Experiments 1 through 7 were repeated using cells of strain PM71 carrying plasmids with *lacZ* fusions driven by *GDH2* UAS_c (pL15-7), by *CYC1* UAS2 (pLGΔ-265), or by no UAS (pSLFΔ178K) to determine whether the effect of the nitrogen source on carbon activation of NAD-GDH is transcriptional (Table 6). Cells with plasmid pL15-7 gave rise to β-galactosidase levels which mirrored the NAD-GDH levels, indicating that the nitrogen source effect is primarily, if not completely, transcriptional and dependent only on UAS_c. In contrast, cells with plasmids pLGΔ-265 and pSLFΔ178K gave rise to β-galactosidase levels which fluctuated minimally for the various nitrogen sources, so the effect seen for *GDH2* UAS_c is not observed for *CYC1* UAS2.

We believed that this carbon regulation of NAD-GDH might suggest a physiological role for the enzyme under certain growth conditions. To examine this possibility, we compared a wild-type strain and one deleted for *GDH2*. Both strains were able to grow with any of a number of nonfermentable carbon sources supplying the energy, but we found no difference in the rate of growth of the two strains during utilization of any carbon source. We next considered the possibility that cells could use glutamine or glutamate as a source of both carbon and nitrogen. Perhaps cells with a functional *GDH2* gene would be able to convert glutamate to α-ketoglutarate for use as a carbon source, while the strain lacking *GDH2* would be unable to carry out this reaction. Neither strain grew when either glutamine or glutamate was supplied at 0.5% (to serve as the sole carbon and nitrogen source). Since a richer medium seemed to cause a greater induction than minimal medium (cells grown in YEP-acetate-glutamine medium had higher levels of NAD-GDH than those grown in minimal acetate-glutamine medium; data not shown), we compared the two strains in YEP with glutamine or glutamate at 1% or leucine at 0.5% (to supply carbon), hoping once again to find a growth phenotype, but there was no growth difference (data not shown).

DISCUSSION

In *S. cerevisiae*, synthesis of a number of enzymes involved in the catabolism of carbon sources is repressed during growth with glucose (for a review, see reference 4). Carlson and coworkers isolated mutants unable to derepress the *SUC2* gene (6), which encodes invertase, the enzyme responsible for the catabolism of sucrose to yield fructose and glucose (51). Some of these mutants carried lesions at the *SNF1* locus (also known as *CCR1* [9]), which encodes a protein kinase (8) apparently necessary for the derepression not only of *SUC2* (6) but also of other genes repressed by glucose, such as *CYC1* (iso-1-cytochrome *c*), *COX6* (cytochrome oxidase) (61), *ADH2* (alcohol dehydrogenase) (9), *GAL10* (UDP-glucose-4-epimerase), and *MAL1* (maltase) (6). These workers also isolated a suppressor of the *snf1* mutation at a locus termed *ssn6* (7), which allows derepression of *SUC2*, *GAL10*, *MAL1*, *CYC1*, and *COX6* during growth on glucose, as well as the derepression of a number of other genes not regulated by the carbon source (7, 56, 61). Earlier it had been shown that mutants lacking hexokinase isozyme PII, encoded by the *HXK2* gene (37), are also defective for glucose repression of invertase, maltase, iso-1-cytochrome *c*, and UDP-glucose-4-epimerase (19, 39). Whether or not the effects of the *hxk2* mutation are entirely mediated by the regulatory pathway defined by the *SNF1* and *SSN6* gene products remains to be determined, but it appears that the *SNF1*, *HXK2*, and *SSN6* gene products are all involved in a global system that determines carbon regulation of a large number of genes (4).

Within this global system, subsets of genes may also be controlled by specific regulatory factors. One such subset includes *CYC1*, *COX6*, and *COR2*, which encode portions of the electron transport apparatus and require the products of the *HAP2*, *HAP3*, and *HAP4* genes for derepression by growth with nonfermentable carbon sources (15, 61). The *HAP2* and *HAP3* gene products form a heteromeric DNA-binding complex which recognizes the UAS2 element of *CYC1*, the UAS responsible for carbon regulation of the gene, and along with the *HAP4* gene product activate transcription of *CYC1* during growth with carbon sources other than glucose (21, 29, 47). It has recently been reported that the *KDG1* gene, whose product is part of the α-ketoglutarate dehydrogenase complex, a tricarboxylic acid (TCA) cycle enzyme required for respiration, is also under *HAP* control (49). In addition, other genes encoding TCA cycle proteins, such as *ACO1* (also known as *GLU1*), which codes for the mitochondrial aconitase (22); *LPD1*, which codes for lipoamide dehydrogenase, a subunit of both pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (52); and the gene which encodes the iron-sulfur subunit of succinate dehydrogenase (38), are regulated by UAS elements which have homology to *CYC1* UAS2 and so may be under the control of the *HAP* genes. The *CIT1* gene, which encodes the mitochondrial form of the TCA cycle enzyme citrate synthase (60), also contains an element with homology to *CYC1* UAS2 and appears to require HAP2, HAP3, and HAP4 for derepression in the absence of glucose (54).

Since one of the products of the NAD-GDH reaction is α-ketoglutarate, a TCA cycle intermediate, activation of *GDH2* in the absence of fermentable carbon sources might be expected also to require the *HAP* gene products. How-

ever, we have found that expression of *GDH2* is independent of the *HAP2*, *HAP3*, and *HAP4* gene products; thus, *GDH2* is not coregulated with a number of genes encoding proteins required for respiration. A comparison of the DNA element defined by deletion analysis as the carbon-responsive site in the *GDH2* gene with the consensus HAP2-HAP3 binding site in *CYC1* UAS2 (20) supports this claim, as there is no homology between the two sites. The system of carbon control that regulates *GDH2* also appears to be independent of the system of glucose repression affected by *hxk2* mutations and to be at least partially independent of the pathway of glucose repression mediated by the *SSN6* product. Whereas glucose-repressed genes generally escape repression in *SSN6* or *HXK2* strains, repression of *GDH2* expression is only partially released in a *ssn6* strain and is not at all released in a *hxk2* strain.

We isolated two classes of mutants unable to activate *GDH2* by growth with nonfermentable carbon sources. The first class, *rgc1*, was represented by a single allele, and appears to affect specifically the regulation of *GDH2*, as *CYC1* UAS2 and invertase expression were not altered. In *rgc1* strains grown with 0.1% glucose as the carbon source, NAD-GDH levels are at least 4-fold lower than in the wild type and expression of a UAS_c-driven *CYC1-lacZ* fusion is decreased more than 50-fold in the *rgc1* strain. This discrepancy between NAD-GDH levels and UAS_c-driven β-galactosidase levels in the *rgc1* strain could be the result of posttranscriptional regulation of the NAD-GDH enzyme, or it may be that a second, as yet unidentified *cis*-acting element regulating the *GDH2* gene responds to factors distinct from *RGC1*. Since the level of NAD-GDH measured in the *rgc1* strain grown with limiting glucose was similar to that observed for the *ssn6* strain grown with 2% glucose or with raffinose, it is tempting to speculate that the *SSN6/SNF1* regulatory pathway might be responsible for the modest expression of NAD-GDH in the *rgc1* strains. Although nothing is known about the *RGC1* gene product, the properties of the *rgc1* mutant suggest it might be a transcription factor specific for activation of *GDH2*.

The second class of mutants isolated in our screen designed to yield strains unable to activate *GDH2* in response to the carbon source, *rgc2* mutants, have a nuclear mutation which renders them unable to respire. This growth phenotype complicated our analysis of regulation of *GDH2* in these strains. While *GDH2* expression is not increased in *rgc2* strains which are shifted from media containing 2% glucose to media with ethanol as the carbon source, we found that mitochondrial petites isolated by exposure of our wild-type strain to ethidium bromide were also unable to activate *GDH2* in cells grown in this way. These results suggested that induction of *GDH2* by the carbon source requires cell growth and that the inability of *rgc2* strains to induce *GDH2* might be a general effect of the petite growth phenotype of the strains. However, we found that *hap3* and *hap4* strains, which are also unable to respire, express *GDH2* normally when grown on raffinose. A diploid made by crossing the *rgc2* strain to a *hap4* strain grew normally with ethanol as the carbon source, demonstrating that these two loci are not allelic, and it is unlikely that *hap3* is allelic to *rgc2* because *GDH2* expression is differently affected by the two mutations. The demonstration that not all petite strains are unable to express NAD-GDH argues that the mere inability to respire is not sufficient to prevent expression of *GDH2*. It remains to be determined whether the specific respiratory defect caused by the *rgc2* mutation prevents activation of *GDH2* indirectly by altering the level of a carbon metabolite within the cell, or whether the mutation affects transcription of *GDH2* more directly.

Glucose repression of *GDH2* appears to be tightly regulated in comparison with that of other genes not requiring a specific inducer for activation. Whereas *CYC1* UAS2 activates transcription weakly during growth with galactose and strongly during growth with raffinose, *GDH2* UAS_c does not activate transcription in response to galactose and activates it only weakly in response to raffinose. Our strains grew noticeably faster with galactose as the carbon source than with raffinose, perhaps because the organism may rely more heavily on respiration when growing with raffinose than with galactose. This could explain why both *CYC1* and *GDH2* are derepressed more during growth with raffinose than with galactose. It is possible that utilization of glutamate as a source of energy is only a measure of last resort so that derepression of *GDH2* is effected only when very poor carbon sources are available to the cell, explaining why *GDH2* is induced not at all by galactose and only slightly by raffinose.

Induction of NAD-GDH levels by respiratory growth also differs from that of other proteins in that the presence of an amino acid in the growth medium is necessary for full induction. NAD-GDH levels were found to be slightly higher in cells grown in ethanol-ammonia medium than in 2% glucose-glutamine medium, but they were highest when the nitrogen source was glutamate or glutamine and the carbon source was ethanol, acetate, or 0.1% glucose. This observation is consistent with the fact that both glutamate and glutamine are potential sources of α-ketoglutarate whereas ammonia is not. High levels of NAD-GDH would best serve the cell when its substrate, glutamate, was available from the environment, as otherwise generation of α-ketoglutarate from glutamate would be futile. Supplementing cells with a small amount of an amino acid in the presence of ammonia brought about an increase in the levels of NAD-GDH and β-galactosidase (in cells containing a *lacZ* fusion driven by the *GDH2* UAS_c). Since addition of serine, which is not a direct precursor to α-ketoglutarate, to the growth medium also improved expression of NAD-GDH (Table 6), it is possible that any nitrogen source which can also serve as a source of carbon improves expression. Thus, it appears that maximal expression of NAD-GDH requires the presence of an amino acid in the growth medium of cells utilizing nonfermentable carbon sources and that this increased expression is due to increased transcription.

It is somewhat surprising that we could not demonstrate a role for NAD-GDH in carbon metabolism, given the regulation of *GDH2* by the carbon source. The NAD-GDH of *Neurospora crassa* has also been reported to be carbon regulated, but no physiological role for the regulation was found in this organism either (14). We felt that the most likely purpose for elevated NAD-GDH levels would be to provide the cell with α-ketoglutarate as a carbon source or to compensate for NADP-GDH, which synthesizes glutamate from α-ketoglutarate and ammonia (24) and thus siphons α-ketoglutarate from the TCA cycle. However, if the cell were to benefit in either way from activation of NAD-GDH during growth with nonfermentable carbon sources, the advantage must be subtle since we could not measure a difference in growth rate between a wild-type strain and one deleted for the *GDH2* gene (lacking NAD-GDH activity) when the strains were grown with limiting glucose or with nonfermentable carbon sources. The possibility remains, of course, that the carbon regulation of *GDH2* is a remnant of ancestor yeast strains which relied upon NAD-GDH to serve

a more important role in the catabolism of amino acids so that they could be used as a source of energy. Some bacteria and fungi are known to be able to use amino acids as a source of both nitrogen and carbon (31). It has been shown that the fungus *Aspergillus nidulans* requires NAD-GDH to use glutamate as a carbon source (35) and that *N. crassa* can use glutamine as a sole source of nitrogen and carbon if the strain is deficient for NADP-GDH (to prevent the recycling of α-ketoglutarate to glutamate) and partially deficient for glutamine synthetase (to ensure that some glutamate is available for NAD-GDH to convert to α-ketoglutarate) (3). Possibly a similar set of circumstances would enable a *S. cerevisiae* strain to use glutamine or another amino acid as the sole source of nitrogen and carbon.

ACKNOWLEDGMENTS

We thank Paul Sugiono and the members of the Magasanik laboratory, especially Patricia Minehart, for helpful suggestions and discussion and for critical reading of the manuscript. We thank D. Botstein, M. Carlson, P. Sugiono, S. Forsburg, and J. Thomas for strains and plasmids. We also thank P. Sugiono and M. Rosenkrantz for communicating results prior to publication. P.W.C. and S.M.M. contributed equally to this work.

This work was supported by Public Health Service research grant GM-07446 to B.M. P.W.C. and S.M.M. were supported by National Institutes of Health training grants.

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