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Regulation of Nitrogen Assimilation in *Saccharomyces cerevisiae*: Roles of the *URE2* and *GLN3* Genes

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Mutations in the *GLN3* gene prevented a normal increase in the NAD-glutamate dehydrogenase and glutamine synthetase levels in glutamate-grown *Saccharomyces cerevisiae* cells, whereas mutations in the *URE2* gene resulted in high levels of these enzymes in glutamate- and glutamine-grown cells. A *ure2 gln3* double mutant had low levels of glutamate dehydrogenase and glutamine synthetase in cells grown on glutamate and glutamine; thus, *gln3* mutations were epistatic to the *ure2* mutations. The results suggest that the *GLN3* product is capable of promoting increases in enzyme levels in the absence of a functional *URE2* product and that the *URE2* product antagonizes the *GLN3* product. The *URE2* and *GLN3* genes were also found to regulate the level of arginase activity. This regulation is completely independent of the regulation of arginase by substrate induction. The activities of glutamate dehydrogenase, glutamine synthetase, and arginase were higher in cells grown on glutamate as the nitrogen source than they were in cells grown under a nitrogen-limiting condition. It had previously been shown that the levels of these enzymes can be increased by glutamine deprivation. We propose that the *URE2-GLN3* system regulates enzyme synthesis, in response to glutamine and glutamate, to adjust the intracellular concentration of ammonia so as to maintain glutamine at the level required for optimal growth.

Glutamate and glutamine play the role of amino donors in the flow of nitrogen into all organic compounds. In *Saccharomyces cerevisiae* there are three enzymes of central importance that are involved in the metabolism of glutamate and glutamine: NADPH-glutamate dehydrogenase (NADP-GDH), NAD-GDH, and glutamine synthetase. NADP-GDH functions in the biosynthesis of glutamate from ammonia, while NAD-GDH degrades glutamate to ammonia and glutamine synthetase catalyzes the synthesis of glutamine from glutamate and ammonia. The activities of NAD-GDH and glutamine synthetase vary depending on what nitrogen source is present in the growth medium (11, 14, 15, 20). Their activities are high when glutamate is the nitrogen source and low when glutamine is the source. The activities of many catabolic enzymes also vary depending on the nitrogen source. The activities of these enzymes are also increased in response to the presence of their substrate (reviewed in references 4 and 25). These catabolic enzymes catalyze the degradation of nitrogen compounds. The final product of degradation is either ammonia or glutamate, which can give rise to ammonia by the activity of NAD-GDH. The activities of some of these enzymes are also increased when glutamate rather than glutamine or ammonia is the source of nitrogen. We examined the relationship of the regulation of glutamine synthetase with that of NAD-GDH and of arginase, a catabolic enzyme subject to nitrogen regulation.

Mutants lacking glutamine synthetase activity were first isolated by Dubois and Grenson (11), and the structural gene encoding glutamine synthetase was shown to be at the *GLN1* locus by Mitchell and Magasanik (19). No structural gene mutation for NAD-GDH has been isolated.

Regulatory mutations affecting the expression of NAD-GDH and glutamine synthetase have been isolated. Drillien et al. (8) isolated mutants (*ure2-1* and [URE3]) that had increased NAD-GDH and glutamine synthetase activities in cells grown in a normally repressing medium such as one containing glutamine as the nitrogen source. Legrain et al. (15) suggested that the *URE2* gene product is involved in inactivation of glutamine synthetase. Drillien et al. (8) and Grenson et al. (13) isolated mutants (altered in *ngl3*) that had decreased NAD-GDH activity but had normal levels of the other enzymes. No characterization of these mutants has been reported. Mitchell and Magasanik (20) reported a regulatory mutation, *gln3*, that results in low levels of NAD-GDH and glutamine synthetase in cells grown on glutamate. NAD-GDH and glutamine synthetase activities normally vary approximately 50- and 150-fold, respectively, from low levels found in cells grown on glutamine. This response requires the product of the *GLN3* gene and is blocked by the presence of glutamine.

In a selection designed to increase the activity of amino acid permeases, we obtained several classes of mutants with altered regulation of NAD-GDH. Some of the mutations were found to be allelic to the *ure2-1* mutation (8). We determined the relationship between *ure2* mutations and *gln3* mutations with respect to their control of the expression of various enzymes.

MATERIALS AND METHODS

Organism and culture conditions. The strains used are listed in Table 1. The wild-type strain, Σ1278b, was the parent strain for all the other strains except for MA96-sp6 and MA96-sp17, which were derived from FL100. Growth conditions and media were as described previously (5). Nitrogen sources were used in the following concentrations (except where noted otherwise): ammonium sulfate (0.2%), glutamate (0.1%), glutamine (0.1%), proline (0.1%), and arginine (0.1%). The doubling times for the wild type growing on ammonia, glutamine, glutamate, and proline are approximately 130, 130, 160, and 210 min, respectively.

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TABLE 1. Strain list

Strain	Genotype
Σ1278b[a]	*MATα*, wild type
D175-1A	*MATα ure2-2 ura-24*
D176-1D	*MATα ure2-3 ura-24 ade1 pro3-66*
D176-2A	*MATα ure2-3 gln3-1 ura-24 ade1*
727-8D[b]	*MATa gln3-1 ade2-102 ura3-77*
MA96-sp6[c]	*MATa ure2-1*
MA96-sp17[c]	*MATα ure2-1*
D182-3A	*MATa gln3-1 ade2-102*
D182-3B	*MATα ure2-1 ura3-77*
D182-3C	*MATa*, wild type
D182-3D	*MATα ure2-1 gln3-1 ade2-102 ura3-77*

[a] Obtained from M. Grenson.
[b] Obtained from A. Mitchell.
[c] Obtained from F. Lacroute.

Enzyme assays. NAD-GDH and glutamine synthetase were assayed in cell extracts (19). Transferase activity of glutamine synthetase was assayed as described previously (19). Measurement of NAD-GDH, based on the method of Doherty (7), was by 2-ketoglutarate-dependent oxidation of NADH. A 1-ml assay mixture contained 0.1 M Tris (pH 8.0), 2.5 mM 2-ketoglutarate, 50 mM NH_4Cl, and 2.5 mM disodium NADH at 25°C. The decrease in A_{340} was monitored with a Zeiss PM6 recording spectrophotometer, directly subtracting a blank reaction from which 2-ketoglutarate was omitted. Arginase was assayed in permeabilized cells as described previously (24). In all cases, enzyme activities were determined on duplicate cell extracts for the experiments described in the tables, where the average of the activities is recorded. In many cases the enzyme activities were measured in cell extracts of the same strains grown in the same medium at different stages of this study. The lowest and highest activities (range) are also recorded in the tables.

Unpublished experiments using the wild-type strain and various mutant segregants obtained in the course of routine genetic crosses showed that the presence of L-proline (500 mg/liter) or uridine (40 mg/liter) in the growth medium, irrespective of the major nitrogen source, had no effect on the activities of glutamine synthetase, NAD-GDH, and arginase. Addition of adenine sulfate (30 mg/liter) lowered the level of NAD-GDH by approximately one-half in the wild-type strain, but not in *ure2* mutants; it did not affect the activities of glutamine synthetase and of arginase.

Isolation of *ure2* mutants. The strains used to isolate *ure2* mutants were proline auxotrophs (*pro3-66*; 1). These strains have an absolute requirement for proline but fail to grow on rich medium YP (containing 1% yeast extract and 2% Bacto-Peptone [Difco]). From previous work we knew that the permease activity for proline is low in cells grown in YP medium, and so, by selecting spontaneously arising colonies on YP plates, we found mutants with altered permease activities (5). We also found two additional independent spontaneous mutants (RM2 and RM27) which were found not to complement the *ure2-1* mutants (isolated by Drillien and Lacroute [9]) for regulation of NAD-GDH and were therefore designated *ure2-2* and *ure2-3*, respectively. Strains RM2 and RM27 were outcrossed to wild type before further analysis was done. The phenotype of the *ure2-3* allele was the same as the phenotype of the *ure2-1* allele with respect to the effect on NAD-GDH, glutamine synthetase, NADP-GDH, and arginase. The levels of arginase and of NAD-GDH in the *ure2-2* mutant were not increased in cells grown on ammonia, in contrast to strains with the *ure2-1* allele. Glutamine synthetase activity was also lower in the *ure2-2* mutant than in the *ure2-1* mutant when glutamine was the nitrogen source. Thus the *ure2-2* mutant appears to retain more function than the *ure2-1* mutant.

Protein content. Protein concentration was determined by the method of Lowry et al. (17).

Genetics. Standard genetic methods were used (22).

RESULTS

***URE2* and *GLN3* control of NAD-GDH and glutamine synthetase.** Recessive mutations of the *URE2* and *GLN3* genes appear to have opposite phenotypes (8, 9, 20); thus, it was of interest to examine the relationship of the *ure2* and *gln3* mutations. Strains MA96-sp17 (*ure2-1*) and 727-8D (*gln3-1*) were crossed, the resulting diploid was allowed to sporulate, and the meiotic products were subjected to tetrad analysis. Table 2 shows the NAD-GDH and glutamine synthetase activities of a representative tetrad. The *gln3* mutation appeared in two spores of each of six four-spored tetrads analyzed (scored by low glutamine synthetase activity), whereas the *ure2* phenotype (high NAD-GDH activity) was apparent in only one spore in every tetrad analyzed. The two *gln3* segregants shown in Table 2 were crossed to a wild-type strain, the diploids were allowed to sporulate, and tetrads were analyzed for the presence of a *ure2* segregant. The cross between strain D182-3D and a wild-type strain (*URE2 GLN3*) produced a meiotic segregant that had a *ure2* phenotype, showing that D182-3D contained the *ure2-1* mutation. Furthermore, the *gln3-1* strains D182-3A and D182-3D were crossed to *ure2-1* strains of the opposite mating type. Only the diploid cells resulting from the cross with strain D182-3D had the *ure2* phenotype. It is thus apparent that the *gln3-1* mutation is epistatic to the *ure2-1* mutation in regard to the expression of NAD-GDH and glutamine synthetase activities.

***GLN3* control of arginase.** The *GLN3* gene product was found to be required for increasing arginase activity during a

TABLE 2. *GLN3* and *URE2* epistasis

Strain	Genotype	Enzyme sp act (nmol/min per mg of protein) with indicated nitrogen source[a]			
		NAD-GDH		Glutamine synthetase	
		Gln + Glt	Glt	Gln + Glt	Glt
D182-3C	*URE2 GLN3*	5 (3–7)	90 (79–95)	0.04 (0.02–0.06)	1.55 (1.50–1.60)
D182-3A	*URE2 gln3-1*[b]	1 (1–2)	15 (12–19)	0.03 (0.01–0.05)	0.14 (0.10–0.18)
D182-3B	*ure2-1 GLN3*[b]	553 (490–601)	982 (884–1100)	0.62 (0.58–0.64)	0.83 (0.75–0.91
D182-3D	*ure2-1 gln3-1*	10 (8–13)	26 (20–30)	0.04 (0.03–0.05)	0.07 (0.05–0.09)

[a] Cells were grown in minimal glucose (2%) medium containing either glutamine (Gln) plus glutamate (Glt) or just glutamate as the nitrogen source, plus adenine sulfate and uridine (each at 30 mg/liter) to supplement requirements.
[b] The strains D182-3A and D182-3B are phenotypically the same as the parent strains, 727-8D and MA96-sp6, respectively.

TABLE 3. *GLN3* control of arginase

Strain	Genotype	Rate of arginase synthesis[a] (10^3 nmol of urea/min per Klett unit)		
		Preshift	After nutritional shift:	
			To glutamate[b]	+ Homoarginine[c]
Σ1278b	*URE2 GLN3*	0.4	1.1 (1.0–1.3)	5.3 (5.0–5.6)
727-8D	*URE2 gln3-1*	0.3	0.3 (0.2–0.3)	6.6 (6.2–6.9)
D176-1D	*ure2-3 GLN3*	0.6	2.1 (1.9–2.3)	6.0 (5.8–6.1)
D176-2A	*ure2-3 gln3-1*	0.4	0.2 (0.2–0.2)	5.8 (4.3–6.4)

[a] The differential rate of arginase synthesis was measured after nutritional shifts and is equal to $\Delta E/\Delta K$, where ΔE is the difference in total enzyme activity and ΔK is the difference in Klett value over the period of the shift.

[b] Cells were grown in minimal medium containing glucose (2%), uridine (30 mg/liter), adenine sulfate (30 mg/liter), and ammonium sulfate (0.2%). Proline (0.5%) was present in the medium for strain D176-1D to supplement a requirement. When cells were in mid-exponential phase, two 15-ml samples were taken by filtration. One sample was suspended in arginase assay buffer, and the other sample was suspended in 15 ml of fresh minimal medium containing glucose, uridine, adenine sulfate, proline for strain D176-1D, and glutamate (0.1%) before being assayed.

[c] Homoarginine (10 mM) was added to a growing culture without filtration.

nutritional shift from ammonia to glutamate as the sole nitrogen source. Table 3 shows the differential rates of arginase synthesis in a wild-type strain and strains with *ure2* and *gln3* mutations after a shift from medium containing ammonia to one containing glutamate. The differential rate (measured over a 90-min period) did not increase in the *gln3-1*-bearing strains, whereas there was about a threefold increase in the *GLN3*-bearing strains. The *gln3-1* mutation was epistatic to the *ure2-3* mutation for this induction. The differential rate in the doubly mutant strain D176-2A was about 5-fold below that of the wild-type strain and 10-fold below that of the *ure2-3*-bearing strain. The ability of homoarginine, a nonmetabolizable analog of arginine (24), to induce arginase in the ammonia-containing medium was not affected by the mutations in *GLN3* and *URE2* (Table 3).

Induction by glutamate. It has been reported that *ure2* mutant cells grown with ammonia as the source of nitrogen have higher levels of arginase than correspondingly grown cells of the wild type (11). However, a different result was observed in a strain carrying the *ure2-2* mutation: cells of this strain grown with ammonia as the source of nitrogen had the same low level of arginase as correspondingly grown cells of the wild type. The effects of the *ure2-2* mutation became apparent in cells grown with glutamate as the source of nitrogen. In this case the levels of arginase and of NAD-GDH (not shown) in the cells carrying the *ure2-2* mutation were much higher than those in cells of the wild type (Table 4). This observation allowed us to determine whether the increased arginase level reflected the substitution of the poorer nitrogen source glutamate (doubling time, 160 min) for the good nitrogen source ammonia (doubling time, 130 min), or induction by glutamate. We found that the addition of glutamate to the ammonia-containing medium did not decrease the growth rate, but increased the level of arginase in the *ure2-2*-bearing mutant to the level found in cells grown with glutamate as the sole nitrogen source. This addition did not increase the level of NAD-GDH (not shown). The addition of glutamate to cells of the wild type growing on ammonia also increased the arginase level, but to a lesser extent. These results suggest that induction by glutamate, rather than deprivation of nitrogen, is responsible for the effect on arginase. We obtained additional evidence for this view by using proline, which is exclusively converted to glutamate, as the source of nitrogen. The growth with proline as the source of nitrogen (doubling time, 210 min) was much slower than growth with glutamate as the source of nitrogen (160 min), a reflection of the relatively slow conversion of exogenous proline to endogenous glutamate. We found that cells of the wild type had the same low arginase level whether grown with ammonia or proline as the source of nitrogen, a level considerably lower than that of wild-type cells grown with glutamate or glutamate plus ammonia as sources of nitrogen. In the case of the *ure2-2* mutant, the level of arginase was higher in cells grown with proline than in cells grown with ammonia as the source of nitrogen, but not nearly as high as in cells grown in the presence of glutamate with or without ammonia. These results confirm that arginase is induced by glutamate and suggest that the *ure2-2* mutation has made the cell more sensitive to this induction. The highest levels of arginase were found in cells grown in media containing arginine.

We carried out similar experiments to explore the role of glutamate in the regulation of glutamine synthetase and NAD-GDH. We compared the levels of these enzymes in cells of the wild type and of a mutant carrying the *ure2-3* allele, grown with glutamine (doubling time, 130 min), glutamate (doubling time, 160 min), or proline (doubling time, 210 min) as the source of nitrogen. The enzyme levels in cells grown with proline as the source of nitrogen were higher than those of cells grown with glutamine as the source of nitrogen, but they were not as high as those of cells grown with glutamate as the source of nitrogen. The effect was particularly striking in the case of glutamine synthetase in wild-type cells: the level of the enzyme in cells grown with proline as the source of nitrogen was only one-fifth that of cells grown with glutamate (Table 5).

These results support the view that the increase in the level of enzymes subject to regulation by the products of *URE2* and *GLN3* is a response not to a deficiency of nitrogen, but rather to increased availability of glutamate.

TABLE 4. Arginase induction by glutamate[a]

Nitrogen source[b]	Sp act[a] (μmol of urea/h per mg of protein) of strain:	
	Σ1278b (*URE2*)	D175-1A (*ure2-2*)
Ammonia	7 (4–8)	7 (4–18)
Ammonia + glutamate	24 (24–25)	142 (142–142)
Glutamate	44 (43–46)	126 (90–172)
Proline	8 (6–9)	30 (20–35)
Arginine	236 (185–362)	163 (160–166)
Arginine + glutamate	230 (215–246)	375 (359–391)

[a] Measured during steady-state growth.

[b] Cells were grown in minimal medium containing glucose (2%) plus the indicated nitrogen source. Uridine (40 mg/liter) was added to the D175-1A medium to supplement an auxotrophy.

TABLE 5. Effect of nitrogen source and *URE2* product on NAD-GDH and glutamine synthetase

Nitrogen source[a]	Sp act (nmol/min per mg of protein)			
	Σ1278b (*URE2*)		D176-1D (*ure2-3*)	
	NAD-GDH	Glutamine synthetase	NAD-GDH	Glutamine synthetase
Glutamine	8.0 (7.2–9.2)	0.01 (0.01–0.02)	395 (350–410)	1.18 (1.00–1.33)
Glutamate	118 (90–146)	1.24 (1.12–1.37)	989 (970–1120)	1.43 (1.21–1.56)
Proline	67 (33–102)	0.20 (0.20–0.21)	816 (790–850)	0.87 (0.81–0.93)

[a] Cells were grown in minimal medium containing glucose (2%) plus the indicated nitrogen source, plus uridine (40 mg/liter), adenine (30 mg/liter), and proline (0.05%).

DISCUSSION

Our results indicate that the regulation of the synthesis of glutamine synthetase, NAD-GDH, and arginase can be mediated by the products of *GLN3* and *URE2*. These gene products in turn appear to respond to changes in the levels of glutamine and glutamate.

The product of *GLN3* has previously been shown to be required for the great increase in the levels of glutamine synthetase and NAD-GDH that occurs when glutamate replaces glutamine or ammonia as the sole nitrogen source (20). We have now shown that the increase in the level of arginase in response to the substitution of glutamate for ammonia as the source of nitrogen also requires the product of *GLN3*. On the other hand, induction of arginase by homoarginine, and presumably arginine, does not require the *GLN3* product (Table 3).

The *URE2* product may be an antagonist of the *GLN3* product: it appears to prevent the activation of the formation of glutamine synthetase, NAD-GDH, and arginase when glutamine or ammonia is substituted for glutamate as the source of nitrogen. Thus, as previously reported (8, 9) and confirmed here with newly isolated *URE2* mutants, mutations in this gene result in high levels of these enzymes in cells grown with glutamine or ammonia as the source of nitrogen. Furthermore, the phenotype resulting from the mutation in *GLN3* is epistatic to that resulting from a mutation in *URE2*.

So far the effect of the *gln3* mutation has only been directly shown to affect the formation of glutamine synthetase, NAD-GDH, and arginase. There are, however, several as-yet-unidentified polypeptides whose intracellular level increases when wild-type cells, but not *gln3* mutant cells, are shifted from a medium containing glutamine to one containing glutamate as the source of nitrogen (20). One of these polypeptides has the unusually high molecular weight of 204,000, characteristic of the subunit of the urea carboxylase-allophanate hydrolase complex (23). The level of this enzyme has been shown to be greatly increased as a result of the *ure2* (here called *gdhCR*) mutation (10, 16). On this basis we may add this enzyme to the list of those whose synthesis is subject to regulation by the products of *GLN3* and *URE2*.

The levels of glutamine synthetase, NAD-GDH, and arginase are low in wild-type cells grown with glutamine or ammonia as the source of nitrogen and are greatly increased by substituting glutamate as the source of nitrogen (Tables 4 and 5; 11, 12, 15, 20). On the other hand, the substitution of proline as the source of nitrogen results in a small increase in the level of NAD-GDH and in still smaller increases in the levels of glutamine synthetase and of arginase. Similar results have been reported for allophanate hydrolase: in this case too, the levels of the enzyme were higher in cells using glutamate rather than proline as the source of nitrogen (16). Proline serves as a source of nitrogen by virtue of its exclusive conversion to glutamate. The cells grow considerably more slowly on proline than on glutamate, apparently reflecting a limitation in their ability to transport proline into the cell and to convert it to glutamate. Consequently, we may assume that the intracellular level of glutamate is higher in cells using glutamate than in those using proline as the source of nitrogen and that this increased intracellular concentration of glutamate is responsible for the increased level of the *GLN3-URE2*-controlled enzymes. This view receives additional support from the observation that the addition of glutamate to cells grown with ammonia as the source of nitrogen results in an increase in the level of arginase (Table 4). This effect is particularly striking in the case of the *ure2-2* allele. The mutation, in contrast to other *ure2* mutations, does not result in an increase of the arginase level in ammonia-grown cells, but addition of glutamate causes a 20-fold increase in the enzyme level to that found in cells grown with glutamate as the source of nitrogen.

In addition to the substitution of glutamate for glutamine or ammonia, the level of *GLN3-URE2*-controlled enzymes could also be elevated by starvation for glutamine. The levels of arginase, NAD-GDH, and allophanate hydrolase were increased greatly by growing a glutamine-requiring mutant with ammonia as the major source of nitrogen in a glutamine-limited chemostat (10). Similarly, the level of NAD-GDH was very high in a leaky glutamine auxotroph during its slow growth with ammonia as the sole source of nitrogen (20). This partial glutamine deprivation resulted in an approximately eightfold increase in NAD-GDH when the leaky glutamine auxotroph was grown with glutamate as the sole source of nitrogen (20). The increase in the NAD-GDH level corresponds to the increase resulting from the *ure2* mutation in cells using glutamate as a source of nitrogen (see Table 5).

On the basis of these results, we propose that the expression of the genes coding for *GLN3-URE2*-controlled enzymes is activated by glutamate and repressed by glutamine. In cells utilizing ammonia as a source of nitrogen, the formation of glutamine is catalyzed by glutamine synthetase and that of glutamate is catalyzed by the NADP-linked GDH. The intracellular level of glutamine in these cells is apparently sufficiently low to allow an adequate synthesis of glutamine synthetase (18), but sufficiently high to repress NAD-GDH and arginase almost fully (10). The fact that addition of glutamate to these cells does not alter the growth rate, but causes an increase in arginase, suggests that activation by glutamate can partly overcome repression by glutamine.

We can attempt to gain an understanding of the physiological significance of the *GLN3-URE2*-dependent regulation by comparing the enzymes of nitrogen metabolism subject to

this control with related enzymes that are not subject to this control. Arginase catalyzes the conversion of arginine to urea and ornithine; the urea is converted to CO_2 and ammonia by the urea carboxylase-allophanate hydrolase enzyme complex (reviewed in references 4 and 25). Both arginase and the enzyme complex are subject to *GLN3-URE2* regulation. Ornithine is converted to glutamate by way of proline; neither ornithine aminotransferase, which catalyzes the formation of the proline precursor Δ^1-pyrroline-5-carboxylate (6), nor the two enzymes required for the conversion of proline to glutamate are subject to *GLN3-URE2* control (2). The enzymes subject to this control, but not the others, are required for the formation of ammonia from arginine. Similarly, NAD-GDH is required for the formation of ammonia from glutamate, and finally, glutamine synthetase is required for the utilization of ammonia for the synthesis of glutamine. The *GLN3-URE2*-dependent regulation of enzyme synthesis in response to glutamine and glutamate may therefore principally serve to adjust the intracellular concentration of ammonia so as to maintain glutamine at the level required for optimal growth. Arginine is present in relatively high concentrations in the vacuolar pool of the yeast cell as a potential intracellular source of ammonia (reviewed in reference 4).

In addition, the *GLN3-URE2* system appears to play a role in the regulation of the formation of transport systems for nitrogen compounds. A *ure2* mutant was selected by virtue of its ability to overcome the inhibition of ureidosuccinate uptake by ammonia (8, 9); similarly, the *ure2* mutants isolated in the present study were selected for their ability to overcome the inhibition of proline uptake by ammonia. It has been shown that the levels of the permease for allantoate, responsible also for the uptake of ureidosuccinate (4), and the level of the general amino acid permease are increased in *ure2* mutants (here called *gdhCR*) grown with ammonia as source of nitrogen (25), and the *gln3* mutation prevents the increase in the level of the general amino acid permease that is seen in wild-type cells grown with glutamate rather than glutamine as the source of nitrogen (W. E. Courchesne, Ph.D. dissertation, Massachusetts Institute of Technology, Cambridge, 1985). However, as will be discussed below, the *GLN3-URE2* system is not the principal regulator of the permeases.

Our hypothesis concerning the principal role of the products of *GLN3* and *URE2* agrees with the suggestion by Cooper that the "*URE2* gene product directly affects only the enzymes associated with the interconversion of ammonia and glutamate" and is not involved in nitrogen catabolite repression (4). Nitrogen catabolite repression is defined as the mechanism responsible for the decrease in the levels of certain enzymes in cells grown on a good nitrogen source such as ammonia or glutamine (4, 25). Generally, the addition of ammonia to cells utilizing a poor nitrogen source greatly reduces the rate of formation of inducible enzymes responsible for the conversion of the compound serving as nitrogen source to ammonia or glutamate. In the case of arginine (5), proline (2), and ornithine (6) it has been shown that this inhibition results from the exclusion of the amino acid serving as inducer of the degradative enzymes. The general amino acid permease and the high-affinity proline permease are inactivated by ammonia, which thus prevents the entry of amino acids into the cell (reviewed in reference 25). A mutation (*per1*) that blocks this ammonia-dependent inactivation permits arginine and homoarginine to induce arginase in the ammonia-containing medium (5). As mentioned earlier, we have shown that the induction of arginase does not require the product of *GLN3* (Table 3). The levels of arginase and of proline oxidase can be increased by incubating the uninduced cells in a medium devoid of any source of nitrogen (5). In such nitrogen-deprived cells, protein synthesis depends on the utilization of amino acids presumably stored in the vacuolar pools, and it has been shown that the release of arginine and proline from these pools is responsible for induction of these enzymes (3, 24). There are, however, other proteins whose formation does not require a specific inducer whose synthesis is controlled in response to the availability of a nitrogen source. Although the formation of the general amino acid permease can be controlled by the products of *GLN3* and *URE2* in response to the substitution of glutamate for glutamine as the source of nitrogen, the much greater increase in the levels of this permease and of the specific proline permease that results from using proline as the source of nitrogen or from subjecting the cells to complete nitrogen deprivation does not depend on a functional *GLN3* gene (Courchesne, Ph.D. dissertation). On the other hand, the level of glutamine synthetase, an enzyme whose synthesis is primarily regulated by the *GLN3-URE2* products, increased rapidly in cells shifted from the glutamine to the glutamate medium (20), but not at all in cells shifted from the glutamine medium to one containing no source of nitrogen (A. P. Mitchell and B. Magasanik, unpublished data).

In conclusion, our results suggest that the transcription of the structural genes for glutamine synthetase, NAD-GDH, arginase, urea carboxylase-allophanate hydrolase, and perhaps other enzymes, is activated by the product of *GLN3* in response to an increase in the intracellular level of glutamate and a decrease in the intracellular level of glutamine. The inability of *GLN3* to activate the expression of these genes in cells with an excess of glutamine depends on a functional product of *URE2*. The principal role of *GLN3-URE2* control appears to be the maintenance of glutamine at an intracellular concentration optimal for growth. It has been shown that the expression of *GLN1*, the structural gene for glutamine synthetase, can also be activated in the absence of *GLN3* product by starvation for histidine through the product of *GCN4* and by starvation for adenine (21). Similarly, the expression of the structural gene for arginase, *CAR1*, can be activated in the absence of the *GLN3* product through induction by arginine or homoarginine. Finally, the *GLN3* product can activate expression of *GAP1*, the structural gene for the general amino acid permease, but a much greater activation of the expression of this gene and of *PUT4*, the structural gene for the specific proline permease, is achieved independently of the *GLN3* product by subjecting the cells to nitrogen starvation. The regulation of enzyme synthesis in response to changes in the nitrogen source in *S. cerevisiae* may actually result from the activities of several independent control systems responding to different metabolic signals.

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