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Nitrogen Regulation of *Saccharomyces cerevisiae* Invertase

Role of the URE2 *Gene*

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Abstract

The regulation of extracellular enzymes is of great biotechnological interest. We studied the regulatory role of the *URE2* gene on the periplasmic invertase of *Saccharomyces cerevisiae*, because its periplasmic asparaginase is regulated by the URE2/GLN3 system. Enzymatic activity was measured in the isogenic strains P40-1B, the *ure2* mutant P40-3C, and the P40-3C strain transformed with the pIC-CS plasmid carrying the *URE2* gene. The assays were performed using midlog and stationary phase cells and nitrogen-starved cells from these growth phases. During exponential growth, the level of invertase in both wild-type and *ure2* mutant cells was comparable. However, the invertase activity in *ure2* mutant cells from stationary phase was sixfold lower than in the wild-type cells. When P40-3C cells were transformed with the pIC-CS plasmid, the wild-type phenotype was restored. On nitrogen starvation in the presence of sucrose, the invertase activity in wild-type cells from midlog phase decreased three times, whereas in stationary cells, the activity decreased eight times. However, invertase activity doubled in *ure2* mutant cells from both phases. When these cells were transformed with the aforementioned plasmid, the wild-type phenotype was restored, although a significant invertase decrease in stationary cells was not observed. These results suggested that the URE2 protein plays a role in invertase activity.

Index Entries: *Saccharomyces cerevisiae*; nitrogen regulation; *URE2*; invertase; asparaginase.

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Introduction

Saccharomyces cerevisiae is commonly used as a model to investigate the eukaryotic regulatory network and its response to environmental signals. Much information has been accumulated in relation to the response of *S. cerevisiae* to metabolizable carbohydrates *(1–7)*, as compared to the regulation on uptake and metabolism of nitrogen sources, although regulatory systems and proteins have been identified *(8)*. *S. cerevisiae* is able to use selectively a variety of compounds as nitrogen sources. When preferred sources such as glutamine and ammonium are available, *S. cerevisiae* represses the expression of nitrogen-regulated genes. This mechanism involves the protein URE2, which is a negative transcription regulator. When the cells are nitrogen starved, the nitrogen catabolic genes are activated via a mechanism involving the transcriptional activator GLN3 *(8–25)*.

The URE2/GLN3 system regulates the expression levels of glutamate dehydrogenase; glutamine synthetase *(9,10)*; the precursor of the soluble vacuolar proteinase B *(11)*; and permeases such as general amino acid permease, proline permease, and ammonium permeases (PUT4, GAP1, MEP2) *(12–14)*. Recently, we reported that the regulatory system URE2/GLN3 regulates an enzyme with peripheral functions, the yeast periplasmic asparaginase II *(15)*.

Asparaginase II is encoded by the *ASP3* gene. Its formation is dependent on the functional *GLN3* gene, and the response to nitrogen availability is under the control of the *URE2* gene product *(15)*. The level of asparaginase is higher in cells from midlog growth phase and declines in the stationary phase. The decrease in the enzyme activity during stationary phase is dependent on the URE2 because in *ure2* mutants the enzyme is 30 times higher *(16)*.

The periplasmic invertase encoded by hydrolysis of the *SUC2* gene of sucrose into glucose and fructose and is repressed by glucose. The regulation of this gene, which is not inducible by carbon source, has been extensively studied, because it seems to be less complex than other glucose-repressed genes. Several studies have pointed out that the products of the genes *SNF1* and *SNF4* are needed to promote the derepression of *SUC2* in the absence of glucose *(26,27)*. The protein complex SNF2/SNF5/SNF6 appears to play a direct role in the activation of *SUC2* transcription *(28)*, and MSN1 seems to increase the rate of *SUC2* expression only under derepressive conditions *(29)*. On the other hand, MIG1 binds to the *SUC2* promoter, repressing its expression *(30)*, and the protein complex CYC8/TUP1 would indirectly inhibit transcription in the presence of glucose *(31,32)*.

A previous study indicated that the *URE2* gene plays no regulatory role in invertase activity in *S. cerevisiae* cells collected at the exponential growth phase *(12)*. In the present study, we investigated the regulatory role of the *URE2* gene on invertase using *S. cerevisiae* cells from exponential and stationary growth phases and the corresponding nitrogen-starved cells. The invertase activity was measured using the isogenic strain P40-1B, the

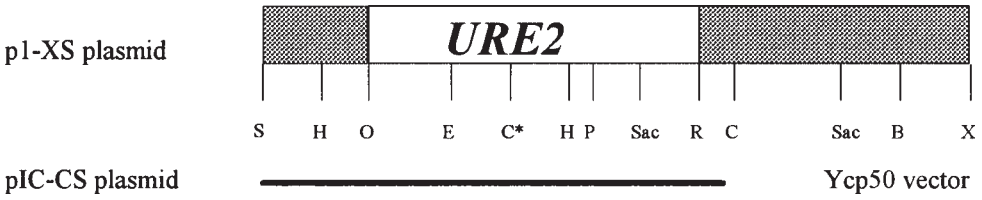

Fig. 1. pIC-CS plasmid. The *Cla*/*Sal*I fragment of p1-XS was inserted into the *Cla*/*Sal*I site of YCp50 *(10)*. C*, *Cla*I blocked by dam methylation.

ure2 mutant P40-3C, and the P40-3C strain transformed with the pIC-CS plasmid carrying the *URE2* gene. Owing to the glucose-repressive effect on invertase, the cells were always grown on sucrose.

Materials and Methods

Yeast Strains, Plasmid, and Growth Conditions

The *S. cerevisiae* wild-type strain P40-1B (*MATa ura3-52 leu2-3,112 his4-619*), the *ure2* mutant P40-3C (*MATα ura3-52 leu2-3,112 ade2-102 ure2Δ11::LEU2*), and the P40-3C transformed with a centromeric plasmid pIC-CS (relevant markers: Amp, URA3, ARS1/CEN4, URE2) (Fig. 1) were used. Transformation was carried out by the lithium acetate method *(33)*. *Escherichia coli* DH5α was used for plasmid amplification. Yeast strains were cultivated in 500-mL shake flasks containing 100 mL of growth medium (3.0% sucrose, 0.5% $[NH_4]_2SO_4$, and 0.1% yeast extract) and amino acids as supplement whenever required. Cultures were incubated at 29°C and 160 rpm.

Nitrogen Starvation Conditions

Cells were harvested at the relevant growth phase and centrifugated for 5 min at 3000 rpm. The cell pellet was washed three times with 50 m*M* potassium phosphate buffer (pH 7.0), suspended in a 3% sucrose solution in the same buffer, and incubated at 29°C and 160 rpm for 3 h.

Enzymatic Activity Assays

Invertase and asparaginase were measured using fresh and nitrogen-starved cells from the relevant growth phases. Invertase activity was measured at 30°C under agitation in reaction mixtures containing 5 mg/mL of cell suspension, 300 m*M* sucrose solution, and 50 m*M* NaF in 50 m*M* sodium acetate buffer (pH 5.0) *(34)*. After 8 min, the enzyme reaction was stopped by filtration using a 0.45-μ Millipore membrane. The sucrose hydrolysis was measured by determining glucose concentration using a Glucose Analyser (Beckman II). One unit of invertase activity corresponded to the production of 1 μmol of glucose/min. Enzyme concentration was expressed per milligram of cell dry wt (U/mg dry wt). For the asparaginase II activity

assay, L-asparagine was added to a cell suspension (optical density = 600 nm of 0.6) to a final concentration of 500 m*M* in a 20 m*M* phosphate buffer (pH 7.4). After incubating for 30 min, a 0.5-mL sample was withdrawn and filtered to separate the cells and stop the reaction. Ammonia concentration on the supernatant was measured spectrophotometrically by coupling to glutamate dehydrogenase (Boehringer Mannhein, Indianapolis, IN). The enzyme activity was expressed as Δμg of ammonia/mL *(15)*.

Results and Discussion

Our results showed that, in accordance with the literature *(12)*, during exponential growth the level of invertase activity in both wild-type and P40-3C strains was comparable. However, considering stationary cells, the invertase activity of the *ure2* mutant was sixfold lower in comparison with that of the wild type. Comparing the asparaginase activity of the *ure2* mutant with that of the wild-type strain, the mutant presented an activity 2.5-fold higher in midlog cells and 80-fold higher in stationary cells. The insertion of the pIC-CS plasmid carrying the *URE2* gene into the P40-3C cells restored the patterns of invertase and asparaginase activities displayed by the wild-type strain (Table 1). These data confirm a major effect of the URE2 on asparaginase and indicate that this regulatory protein also affects invertase activity. Interestingly, at the stationary phase, in which carbon-limiting conditions are expected, in wild-type cells, URE2 plays a positive role concerning invertase activity that is related to the carbon metabolism and a repressive effect toward asparaginase activity that is related to the nitrogen metabolism.

Considering the foregoing and to understand better the regulatory role of URE2, wild-type and *ure2* mutant cells from midlog and stationary phases were submitted to nitrogen starvation in the presence of sucrose. The invertase activity in wild-type cells decreased three- and eightfold in midlog and stationary phase cells, respectively. However, a twofold increase was observed in the *ure2* mutant cells from both growth phases (Fig. 2). For asparaginase activity, nitrogen starvation in wild-type cells caused an increase of 3- and 150-fold in midlog and stationary phase cells, respectively. These data for asparaginase activity indicated clearly that URE2 is more active in stationary cells. On nitrogen starvation, the *ure2* mutant cells, which were already derepressed for this enzyme, displayed an overall threefold increase in activity in both growth phases (Fig. 3). When the *ure2* cells were transformed with the pIC-CS plasmid carrying the *URE2* gene, the wild-type phenotype for both enzymes, on nitrogen starvation, was restored, although a significant decrease in invertase in stationary cells was not observed (Figs. 2 and 3).

Our results indicated that invertase, which is typically related to carbon metabolism, was under direct or indirect URE2 regulation. URE2 acted on invertase and asparaginase in an opposite fashion in response to

Table 1
Yeast Invertase and Asparaginase II Activities Measured in Cells at Midlog and Stationary Growth Phases

	Enzymatic activities			
	Invertase (U/mg dry wt)		Asparaginase (U/mg dry wt)	
Yeast strain	Midlog cells	Stationary cells	Midlog cells	Stationary cells
P40-1B	0.17	0.39	1.58	0.05
P40-3C	0.16	0.06	3.96	4.00
P40-3C (pIC-CS)	0.12	0.41	1.96	0

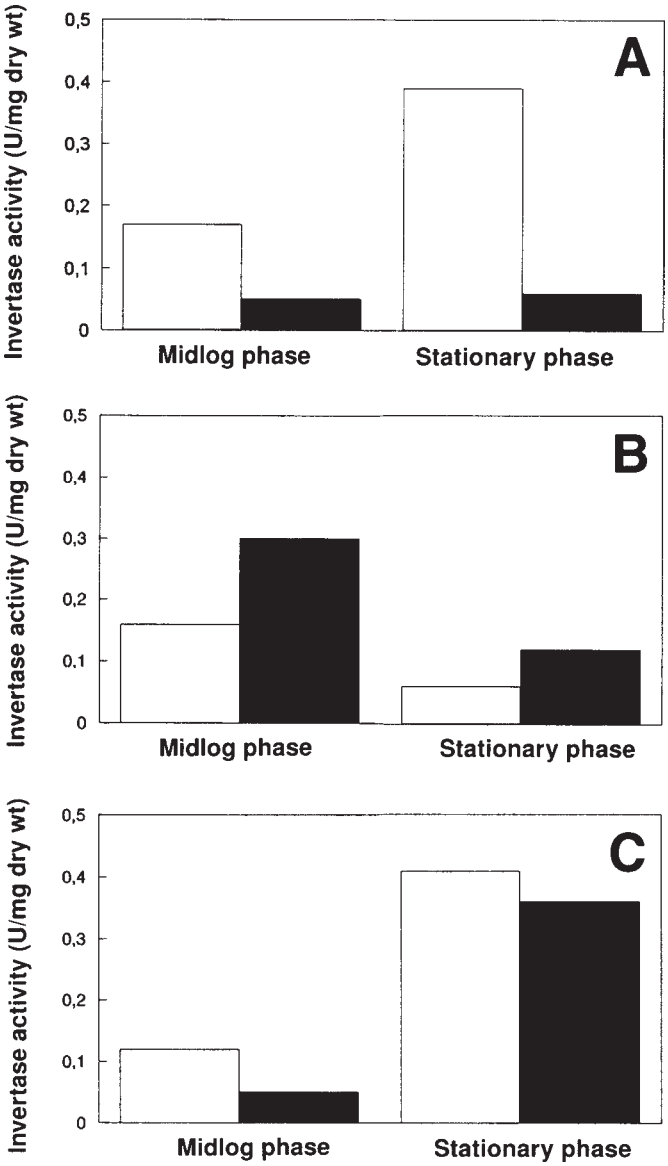

Fig. 2. Invertase activity measured at the midlog and stationary phases (□) and in nitrogen-starved cells from both phases (■). Wild-type strain P40-1B **(A)**, *ure*2 mutant P40-3C **(B)**, and P40-3C (pIC-CS) **(C)** were used.

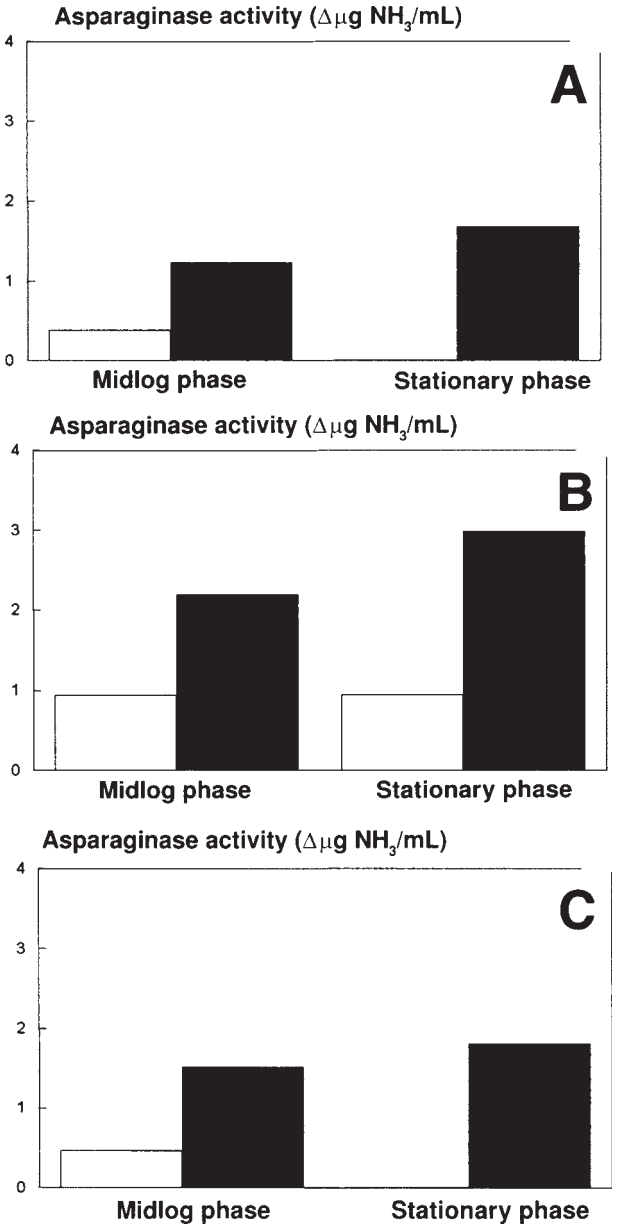

Fig. 3. Asparaginase II activity measured at the midlog and stationary phases (□) and in nitrogen-starved cells from both phases (■). Wild-type strain P40-1B **(A)**, *ure2* mutant P40-3C **(B)**, and the transformed P40-3C (pIC-CS) **(C)** were used.

carbon or nitrogen limitation. In conditions of nitrogen starvation in the presence of a carbon source, URE2 had a negative effect on invertase activity whereas asparaginase was derepressed. In cells from the stationary phase, which were under carbon limitation, the URE2 activated invertase and repressed asparaginase.

Conclusion

The data presented herein for invertase and asparaginase II, using wild-type and *ure2* mutant cells of *S. cerevisiae*, indicated that the protein URE2 would have a pleiotropic role, regulating both enzymes in accordance with the cells' nutritional needs and the external availability of carbon and nitrogen.

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