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Interaction of the GATA Factor Gln3p with the Nitrogen Regulator Ure2p in *Saccharomyces cerevisiae*

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We used cells carrying plasmids causing the overproduction of Gln3p, Ure2p, or both of these proteins to elucidate the ability of Ure2p to prevent the activation of gene expression by Gln3p in cells growing in a glutamine-containing medium. Our results indicate that Ure2p probably does not interfere with the binding of the GATA factor Gln3p to GATAAG sites but acts directly on Gln3p to block its ability to activate transcription.

In *Saccharomyces cerevisiae*, the expression of a number of genes whose products enable the organism to use a variety of compounds as sources of nitrogen is activated by the product of the *GLN3* gene, Gln3p. This zinc finger protein exerts its effect by binding to GATAAG sequences located upstream of the regulated genes (1). The product of another gene, *URE2*, prevents the activation of the regulated genes by Gln3p in cells growing with glutamine as the source of nitrogen (5, 6).

We have cloned the *GLN3* and *URE2* genes on plasmids, enabling us to overproduce the products of these genes (4, 7). We now present the results of experiments with cells that overproduce either one or both of the products of these genes. Our experiments suggest that Ure2p acts directly on Gln3p, converting it to a form that is unable to activate transcription.

As previously reported, overproduction of Gln3p results in very slow growth, with a mass doubling time of more than 10 h in minimal media (7). We examined the effects of Ure2p on the growth inhibition exerted by Gln3p. To this end, we transformed strain PM38 (*MAT*α *leu2-3,112 ura3-52*) with plasmid pPM49 (2 μm *GAL10-GLN3* [7]). We also transformed this strain with plasmids carrying the *URE2* gene fused to *GAL10* as well as the *LEU2* gene in order to create strains that overproduce both Gln3p and Ure2p. The high-copy-number plasmid pLE-8 was constructed by inserting a 1.7-kb *Stu*I-*Sal*I fragment of p8 containing the *GAL10-URE2* fusion into the *Bam*HI site of YEp13 (2, 3). The resulting plasmid has a *LEU2* selectable marker and the *GAL10* upstream activating sequence driving the *URE2* gene. The single-copy centromere plasmid pLC-8 (YCp*GAL10-URE2*) was constructed by replacing the *URA3* fragment of the p8 plasmid with the *Sal*I-*Xho*I *LEU2*-containing fragment of YEp13.

The results presented in Fig. 1 show that overproduction of Gln3p in the strain carrying the *GLN3* gene fused to *GAL10* causes strong growth inhibition and that growth is restored by the overproduction of Ure2p resulting from the simultaneous presence of the plasmid carrying *URE2* fused to *GAL10.*

The effect of Ure2p is also demonstrated by the ability of Ure2p to depress the ability of overproduced Gln3p to activate the synthesis of glutamine synthetase. As shown in Table 1, overproduction of Gln3p enables the cell to produce glutamine synthetase during growth with glutamine as the source of nitrogen, but this increase in the level of the enzyme is largely prevented by the simultaneous overproduction of Ure2p.

We have previously shown that Ure2p blocks neither the

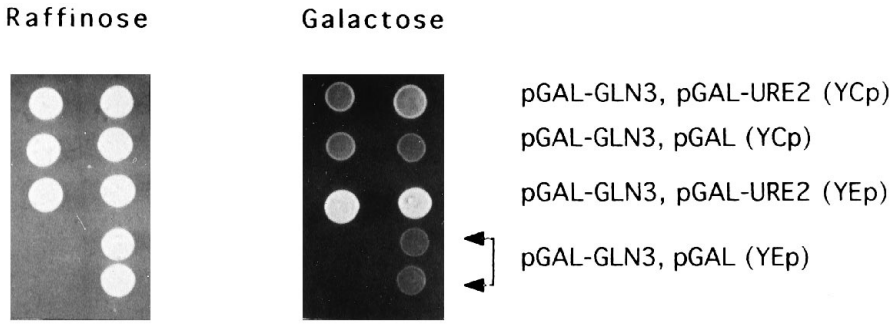

FIG. 1. Suppression of *GLN3* lethality by overexpression of *URE2*. Cultures with the indicated plasmids were grown on raffinose, and equal numbers of cells ($>10^5$) were spotted on raffinose and galactose plates. The plates were incubated at 30°C for 2 days. Pictures were taken with a Polaroid camera. pGAL-GLN3 is pPM49 (6), pGAL-URE2 (YCp) is pLC-8, pGAL-URE2 (YEp) is pLE-8, YCp is YCp50-LEU2, and YEp is YEp13. The single-copy centromere plasmid YCp50-LEU2 was constructed by replacing the *URA3* fragment of plasmid YCp50 with the *Sal*I-*Xho*I *LEU2*-containing fragment of YEp13.

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TABLE 1. Glutamine synthetase activity in *GLN3*- and *URE2*-overexpressing cells[a]

Overexpressing extract	Activity of:	
	Transferase (μmol/min/mg)[b]	Synthetase (nmol/min/mg)[c]
None	0.04 ± 0.01	0.05 ± 0.01
GAL-URE2 (pLE-8)	0.04 ± 0.01	0.05 ± 0.01
GAL-GLN3 (pPM49)	0.16 ± 0.04	0.32 ± 0.07
GAL-GLN3 (pPM49) *GAL-URE2* (pLE-8)	0.06 ± 0.02	0.13 ± 0.03

[a] Mid-exponential-phase cultures were grown in glutamine-raffinose minimal medium. Galactose was added to 3%, and cells were harvested after 3 h of incubation. Glutamine transferase and synthetase activities were determined as described by Mitchell and Magasanik (8). Each value indicated is the average (± the standard deviation) of at least three determinations.

[b] Activity units are micromoles of μ-glutamyl hydroxamate per minute per milligram of protein.

[c] Activity units are nanomoles of μ-glutamyl hydroxamate per minute per milligram of protein.

TABLE 2. Immunoprecipitation of GATAAG DNA fragment by *GLN3*- and *URE2*-overexpressing extracts[a]

Overexpressing plasmids[b]	Serum	% (± SD) of counts precipitated[c]
pGAL-GLN3 (pPM49), YEp13	Preimmune	1.2 ± 0.2
pKP15, YEp13	Anti-Gln3	2.2 ± 0.84
pKP15, pGAL-URE2 (pLE-8)	Anti-Gln3	2.5 ± 2.13
pGAL-GLN3 (pPM49), YEp13	Anti-Gln3	20.4 ± 11.59
pGAL-GLN3 (pPM49), pGAL-URE2 (pLE-8)	Anti-Gln3	21.7 ± 12.44

[a] The strain used in these experiments was PM38 (6). Cells were grown to mid-log phase in glutamine-raffinose minimal medium. Galactose was added to 3%, and cells were left for an additional 3 h before being harvested. Immunoprecipitations were performed as described previously (7). In brief, total cell lysates were incubated with radioactively labelled DNA fragments to allow binding to occur. The multiple GATAAG site DNA fragment was a 224-bp fragment consisting of a sevenfold-repeated 32-bp oligomer containing the GATAAG motif, which is the Gln3 binding site (1, 7). *GLN3* antiserum was then added to the binding-reaction mixtures, which were subsequently immunoprecipitated with fixed *Staphylococcus aureus* Cowan I strain. Radioactive counts in the pellet were determined. All experiments were repeated at least twice.

[b] Overexpressing plasmids are described in the text.

[c] Approximately 10^6 cpm was added to each reaction mixture.

transcription of *GLN3* nor the translation of *GLN3*-specific RNA (4, 7). We were now able to show that the intracellular concentration of the overproduced Gln3p monomer is not reduced by the overproduction of Ure2p. By using Western blot (immunoblot) analysis (as described in reference 7) to determine the presence of Gln3p monomer, we found that the overproduction of Ure2p had no significant effect on the amount of Gln3p that was overproduced (Fig. 2).

We considered the possibility that Ure2p could interfere with the binding of Gln3p to the GATAAG sites making up the nitrogen-responsive upstream activating sequence. We had previously used the immunoprecipitation of a complex consisting of Gln3p and GATAAG-containing DNA to demonstrate the ability of overproduced Gln3p in cell extracts to bind to this DNA (7). We used the same method to determine whether overproduced Ure2p prevents the binding of Gln3p to this DNA. The results presented in Table 2 show that this is not the case. The same fraction of radioactive DNA was precipitated by the Gln3p-specific antiserum whether or not the cell extract contained overproduced Ure2p in addition to overproduced Gln3p. However, these results do not exclude the possibility that Ure2p weakens the affinity of Gln3p for its binding sites on the DNA and that our method is not sensitive enough to detect this effect.

These results suggest that Ure2p either directly or indirectly blocks the ability of Gln3p to activate transcription. We reasoned that the direct interaction of Ure2p with Gln3p would result in the formation of a complex containing both proteins. We examined this possibility by treating cell extracts containing either Gln3p or both Gln3p and Ure2p with anti-Gln3p serum, precipitating the antibody-containing complexes with protein A beads, collecting the precipitate, and examining the denatured protein contained in the precipitate for the presence of Gln3p and Ure2p by Western blot analysis. The 100-μl binding-reaction mixtures contained 4 mM Tris-HCl (pH 8.0), 40 mM NaCl, 4 mM $MgCl_2$, 5% glycerol, and 100 μg of protein from total cell extracts. A 5-μl portion of either preimmune, anti-Gln3p, or anti-Ure2p serum was added to each of the reaction mixtures (3, 7). After incubation on ice for 1 h, 40 μl of protein A-agarose beads (Sigma Chemical Co., St. Louis, Mo.) was added to each of the reaction mixtures, and incuba-

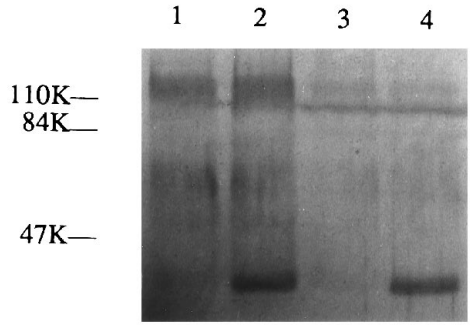

FIG. 2. Overexpression of *URE2* does not affect the level of Gln3p in cells. Cultures carrying both, one, or none of the constructs were grown to mid-exponential phase, and galactose was added to 3%. Cell extracts were made after 3 h of incubation with galactose. Equal amounts of protein were loaded in each lane. To determine the relative levels of Gln3p and Ure2p, both anti-Gln3p and anti-Ure2 polyclonal antibodies were added to the same blot. Lane 1, YEp *GAL-GLN3* (pPM49) and YEp13; lane 2, YEp*GAL-GLN3* (pPM49) and YEp-*GAL-URE2* (pLE-8); lane 3, YEp24 and YEp13; lane 4, YEp24 and YEp*GAL-URE2* (pLE-8). The deduced molecular weights of the Gln3p and Ure2p polypeptides are 79,256 and 40,226, respectively.

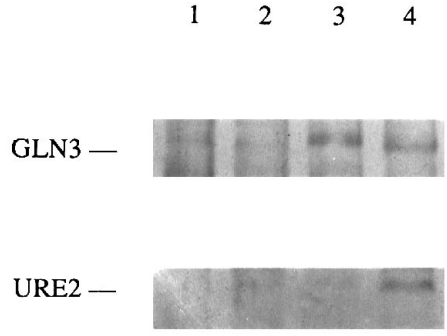

FIG. 3. Coimmunoprecipitation of Gln3p and Ure2p with anti-Gln3p serum. Strains carrying plasmids with *GAL10* promoter-controlled *GLN3* or *URE2*, or both, were grown to mid-exponential phase, and galactose was added to a final concentration of 3%. After a 3-h incubation, extracts were made, and anti-Gln3p serum was then added to the extracts. After 30 min of incubation, the complexes were precipitated with protein A beads. The whole precipitates were boiled in the loading buffer and loaded on a 7.5% polyacrylamide gel for SDS-PAGE. Lane 1, vectors only; lane 2, YEp24 and YEp*GAL-URE2* (pLE-8); lane 3, YEp*GAL-GLN3* (pPM49) and YEp13; lane 4, YEp*GAL-GLN3* (pPM49) and YEp*GAL-URE2* (pLE-8).

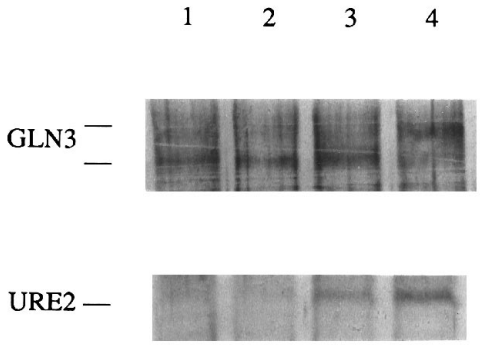

FIG. 4. Coimmunoprecipitation of Gln3p and Ure2p in Ure2p-overexpressing extracts. A strain carrying a plasmid with *GLN3* under the control of its own promoter (pPM7) was transformed with the YEp*GAL-URE2* (pLE-8) construct. *URE2* expression was controlled by growth on different carbon sources. Extracts were made, and Gln3p-Ure2p complexes were immunoprecipitated with anti-Gln3 serum. Equal amounts of protein were loaded in each lane. Lane 1, cells used were grown on glucose; lane 2, cells used were grown on raffinose, lane 3, cells used were grown on raffinose plus galactose for 3 h; lane 4, cells used were grown on galactose. The positions of Gln3p and Ure2p are indicated.

tion on ice was continued for an additional 30 min. The beads were pelleted by a 30-s centrifugation in an Eppendorf microcentrifuge. The pellets were washed twice in the 1× binding buffer (1). After addition of the loading buffer (1), the samples were boiled and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 7% polyacrylamide gel. The gel contents were transferred to a nitrocellulose membrane. Strips of nitrocellulose were cut horizontally according to the molecular weight standards (Bio-Rad), and each strip was incubated with antiserum to Gln3 or Ure2. The membranes were then developed as described previously (7). The results illustrated in Fig. 3 show that the addition of anti-Gln3p serum to the cell extracts containing both Gln3p and Ure2p resulted in the precipitation of not only Gln3p but also Ure2p. Similar experiments (data not shown) using anti-Ure2p serum were not successful; neither Ure2p nor Gln3p was found in the precipitate. It is possible that the Ure2p-specific antibody recognizes only denatured Ure2p and not native Ure2p, since denatured TrpE-Ure2p was used as the antigen to produce anti-Ure2p serum.

We repeated the experiment using Gln3p produced by a plasmid (pPM7) carrying *GLN3* with its native promoter (7) and Ure2p produced by a plasmid (pLE8) carrying the *GAL10-URE2* fusion. Growth of the cells in different media permitted us to vary the intracellular concentration of Ure2p while the concentration of Gln3p remained constant. The results of this experiment, illustrated in Fig. 4, show that in extracts of cells grown on glucose or raffinose with a low concentration of Ure2p there was no significant coprecipitation of Gln3p and Ure2p. Growing the cells on raffinose, followed by the addition of galactose to induce formation of Ure2p during an additional 3-h period of growth, resulted in the coprecipitation of Ure2p with Gln3p. Interestingly enough, growth on galactose for the entire 10-h period, which presumably resulted in the highest intracellular concentration of Ure2p, not only increased the amount of Ure2p in the Gln3p-Ure2p complex but also brought about a change in the Gln3p subunit to a more slowly migrating form. However, this alteration did not account for the inability of Gln3p to activate transcription, since in the experiment summarized in Table 2 the cells had been exposed to galactose for only 3 h, a period inadequate to cause the change in the mobility of the Gln3p subunit, but had responded to the presence of Ure2p by a sharp reduction in *GLN1* expression.

In summary, our results indicate that Ure2p acts directly on Gln3p to disable its ability to activate transcription. Our results do not allow us to decide whether the effect of Ure2p results from a stoichiometric interaction with Gln3p or from a catalytic alteration of Gln3p, since in either case the interaction of the two proteins would result in the formation of a complex. We favor the view that Ure2p acts catalytically on Gln3p because, as previously shown in diploid cells, the gene dosage of *GLN3*, but not that of *URE2*, affects the level of glutamine synthetase and of NAD^+-linked glutamate dehydrogenase and that, based on the level of β-galactosidase in *GLN3-lacZ* and *URE2-lacZ* fusion plasmids, the intracellular concentration of Gln3p is four times higher than that of Ure2p (4).

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