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Role of the GATA factors Gln3p and Nil1p of *Saccharomyces cerevisiae* in the expression of nitrogen-regulated genes

(nitrogen regulation/transcriptional activators/glutamine/glutamate)

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ABSTRACT **We have isolated the *NIL1* gene, whose product is an activator of the transcription of nitrogen-regulated genes, by virtue of the homology of its zinc-finger domain to that of the previously identified activator, the product of *GLN3*. Disruption of the chromosomal *NIL1* gene enabled us to compare the effects of Gln3p and of Nil1p on the expression of the nitrogen-regulated genes *GLN1*, *GDH2*, and *GAP1*, coding respectively for glutamine synthetase, NAD-linked glutamate dehydrogenase, and general amino acid permease. Our results show that the nature of GATAAG sequences that serve as the upstream activation sequence elements for these genes determines their abilities to respond to Gln3p and Nil1p. The results further indicate that Gln3p is inactivated by an increase in the intracellular concentration of glutamine and that Nil1p is inactivated by an increase in intracellular glutamate.**

The presence of glutamine in the growth medium of *Saccharomyces cerevisiae* prevents the expression of *GLN1* and of *GDH2*, the structural genes for glutamine synthetase and the NAD^+-linked glutamate dehydrogenase (1). In a medium containing glutamate as a source of nitrogen, the expression of these genes requires the product of *GLN3*, Gln3p, a protein containing a zinc finger domain homologous to that of GATA factors of avian and mammalian cells, capable of binding to the sequences 5′-GATAAGATAAG-3′ and 5′-GATTAGATTAG-3′ located upstream of *GLN1* and *GDH2*, respectively (1, 2). Deletion of these sequences greatly reduces the expression of these genes. The sequence 5′-GATAA-3′ is also found upstream of other nitrogen-regulated genes and is apparently responsible for the activation of the expression of these genes by Gln3p. Another protein, the product of *URE2*, disables Gln3p in response to an increase in the intracellular concentration of glutamine. Mutants lacking Ure2p express Gln3p-activated genes in the presence of glutamine (1).

Among the genes whose transcription can be activated by Gln3p is *GAP1*, the structural gene for the general amino acid permease. This gene is not expressed in media containing glutamine, and the lack of Gln3p greatly diminished its expression in a medium containing glutamate as the source of nitrogen. Nevertheless, the lack of Gln3p did not prevent strong expression of *GAP1* in media containing ammonia or urea as source of nitrogen. Apparently, a transcription factor other than Gln3p, which we have named Nil1p, is responsible for the activation of transcription of *GAP1* in media containing these sources of nitrogen (3). Elimination of Ure2p enabled Gln3p, but not Nil1p, to activate the transcription of *GAP1* in the presence of glutamine (4).

Examination of the DNA sequence revealed the presence of five GATAAG sequences within the 650-bp region preceding the translational start site of *GAP1*. This region was analyzed by making partial deletions from the 5′ and 3′ ends, fusing the remainder to *CYC1–lacZ*, and examining the β-galactosidase levels in wild-type and *gln3* mutant cells carrying plasmids containing these fusions during growth with glutamate or urea as nitrogen source. This analysis revealed that the region of DNA extending from 380 to 650 bp from the translational start site of *GAP1* was adequate for full expression of this gene. This region contains two GATAAG elements centered on positions −546 and −466, as well as the sequence TTGTT centered on position −599 and the sequence TTGGT centered on position −455; these sequences had previously been shown to play an auxiliary role in the activation of the expression of *GLN1* and *GDH2*, respectively, by Gln3p (1). Finally, a binding site for Abf1p was found centered on position −525. The response to Gln3p was found to require at least a single GATAAG and a single TTGG/TT site. These two sites, as well as the Abf1p binding site, were also required for a response to Nil1p (4).

That elimination of the GATAAG elements affected the activation of transcription by both Gln3p and Nil1p suggested that both of these activators contain the zinc finger domain required for the recognition of GATA elements. We now present evidence that not only Gln3p but also Nil1p can recognize a sequence composed of seven repeated GATAA-GATAAG sequences. We describe the isolation of the *NIL1* gene and the effect of eliminating this gene on the expression of *GAP1*, *GLN1*, and *GDH2*.

MATERIALS AND METHODS

Strains and Media. Strain P40-2c (*MATα ura3-52 leu2-312 ade2-102 gln3::LEU2*) of *S. cerevisiae* (5) was used for the isolation of the *nil1* and *nil2* mutant strains described in this paper. Strains MS207 and MS201 have the same genotype as strain P40-2c but contain in addition *nil1::hisG* and *nil2::hisG*, respectively. For the experiments comparing the effects of the loss of Gln3p and Nil1p, an isogenic set of strains was constructed in the PM38 background (6) with strain MS218 (*MATα ura3-52 LEU2*) serving as wild-type strain and strains PM71, MS221, and YDR21 with the additional markers (*leu2-3,112 gln3::LEU2*), (*nil1::hisG*), and (*leu2-3,112 gln3::LEU2 nil1::hisG*), respectively. The plasmids carrying the 7×GATAAG element (6), the *GLN1* and *GDH2* upstream activation sequence (UAS)-N elements (pL53-4 and pL24, respectively; ref. 7), *GLN1* from −659 to +1 (6), *GDH2* from −863 to −130 (7), and *GAP1* from −749 to −205 (4) fused to *CYC1–lacZ* have been described. Plasmid pPM4 carries *GLN3* (8) and plasmid pMS26-1 carries *NIL1* (Fig. 1). The cultivation of the cells in media with different sources of nitrogen has been described (3, 6).

Genetic and Biochemical Methods. DNA transformation, isolation of plasmids, and the assays for β-galactosidase and general amino acid permease have been described (3). β-Galactosidase levels were measured in at least three separate

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Abbreviation: UAS, upstream activation sequence.

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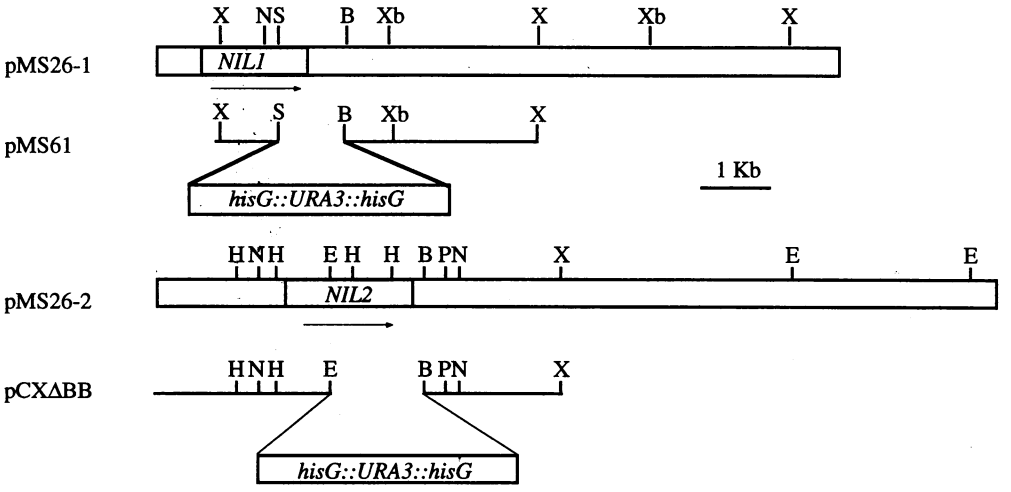

FIG. 1. Restriction maps of *NIL1* and *NIL2* and of insertions inactivating these genes. B, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; N, *Eco*NI; P, *Pva* II; S, *Stu* I; X, *Xho* I; Xb, *Xba* I.

cultures and the average values reported agree within 10%. Southern blot hybridizations were performed by a published method (9). Minipreparations of double-stranded plasmids purified by alkaline lysis were subjected to DNA sequence analysis by the dideoxy chain termination method (10) with a modified T7 DNA polymerase (United States Biochemical) according to the directions of the supplier.

Screening with Degenerate Oligonucleotides. The technique has been described (11). The library used was a *Sau*3A1 partial restriction digest of yeast DNA cloned into the *Bam*HI site of Ycp50. Bacterial transformants were distributed in a suspension in Luria–Bertani (LB) medium on 140-mm nitrocellulose filters in a Buchner funnel at a density of 2000–4000 colonies per filter. Ten master filters were incubated on LB ampicillin plates overnight. The master filters were replicated onto duplicate secondary filters the next day. After overnight incubation on LB ampicillin plates, the replica filters were transferred to LB chloramphenicol (50 μg/ml) plates for 10 hr. The filters were processed for hybridization by placing them on 3 MM paper soaked with 0.5 M NaOH for 5 min, then on paper soaked with 1 M Tris Cl (pH 7.5) for 5 min, and finally on paper soaked with 0.5 M Tris Cl, pH 7.5/1.25 M NaCl for 5 min. The filters were then dried under vacuum at 80°C for 1 hr. Hybridization of the filters with the labeled oligonucleotide probe was done exactly as described (11). The degenerate probe mixture used [5′-CTNTGYAAYGCNTGYGGNCT-NTTY-3′ (Biopolymer Laboratory, Massachusetts Institute of Technology)] was labeled by the use of polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP according to suppliers' directions. The hybridization solution (20 ml) was 6× standard saline citrate (SSC)/1× Denhardt's solution/0.05% sodium pyrophosphate containing yeast tRNA (100 μg/ml) and 25 pmol of labeled oligonucleotide. Hybridization was carried out for 72 hr at 48°C and the filters were washed in 6× SSC/0.05% sodium pyrophosphate at 60°C for 30 min. Approximately 25,000 colonies were screened on duplicate filters to yield 30 primary selections. Colonies in the region of each hybridization signal were picked and rescreened with the same probe mixture to yield 17 secondary selections. Fourteen of these were placed in groups on the basis of restriction digests and Southern blots. One group (2 clones) had a 1.2-kb *Pvu* II–*Eco*RI fragment which hybridized to the probe and was exactly the same size as a *Pvu* II–*Eco*RI fragment from the *GLN3* clone pPM4. A second group (2 clones) had a 1.4-kb *Pvu* II–*Eco*RI fragment which hybridized to the probe. A 60-base fragment of DNA sequence was obtained from these clones by using as primer a 17-base degenerate oligonucleotide encoding the amino acid sequence TPLWR(R). This sequence matched the sequence of *DAL80* exactly and was located about 60 bp from the conserved metal finger region. A third group (4 clones) had a 1.6-kb *Pvu* II–*Eco*RI fragment which hybridized to the probe. A fourth group (6 clones) had a very large *Pvu* II–*Eco*RI fragment which hybridized to the probe. All of the fourth group of clones had at least three *Eco*RI–*Pvu* II–*Eco*NI restriction fragments in common and were identified as *NIL1*. The members of the third set of clones were named *NIL2* and confirmed as a group by hybridizing a Southern blot with a 1.6-kb *Pvu* II–*Eco*RI fragment from one of them.

Construction of *NIL1* and *NIL2* Null Alleles. A 4.6-kb *Xho* I fragment of *NIL1* clone 26-1, pMS26-1, was ligated into the *Xho* I site of pBluescript KS(+) (Stratagene). A *Bam*HI linker was ligated to the *Stu* I site of *NIL1* and then the plasmid was digested with *Bam*HI and *Bgl* II. The 3.8-kb *Bgl* II–*Bam*HI *hisG*::*URA3*::*hisG* fragment from pNKY51 (12) was ligated into this vector to yield pMS61. Disruption of the *NIL1* locus was accomplished by transformation with the *Xho* I fragment. A *gln3 nil1* double mutant was created by disruption of *NIL1* in the *gln3 GAP1* strain P40-2c. The *ura3* marker was recovered in strain MS207 by 5-fluoroorotic acid selection (13). Construction of a *GLN3 nil1* strain was complicated by the fact that the wild type strains normally used in this laboratory are *leu2* and do not have Gap1p activity unless very high concentrations of leucine are added to the medium. This effect is posttranscriptional, since *leu2* and *LEU2* strains regulate the expression of *GAP1–lacZ* reporters identically in response to the nitrogen source. The *ura3 leu2* strain PM38 was transformed with *Eco*RV-cut Yip351 (*LEU2*) and selected on minimal medium without leucine, yielding strain MS218, which has high Gap1p activity when grown with urea as the nitrogen source. *NIL1* was disrupted in this strain as above; the *ura3* derivative was designated MS221.

A *Cla* I–*Xba* I fragment of the *NIL2* clone pMS26-2 was ligated into *Cla* I–*Xba* I-digested pBluescript KS(+). In the deletion plasmid pCXΔBB, the 1.3-kb *Eco*RI–*Bgl* II fragment was replaced with the 3.8-kb *Bgl* II–*Bam*HI *hisG*::*URA3*::*hisG* cassette. The disruption allele was integrated into the chromosome of yeast by transformation of the *Cla* I–*Xba* I fragment. The deletion was transformed into strain p40-2c. All chromosomal disruptions were confirmed by Southern hybridization.

RESULTS

Activation of Transcription by Gln3p and Nil1p. In the case of *GAP1*, GATAAG elements are essential for the activation of transcription by both Gln3p and Nil1p (4). We therefore

compared the response of UAS elements to activation of transcription by these two activators (Table 1). To assess activation by Gln3p, the wild-type strain pM38 was grown with glutamate as the source of nitrogen, and to assess activation by Nil1p the *gln3* mutant strain PM71 was grown with urea as the source of nitrogen. The UAS consisting of seven repeats of GATAAGATAAG responded well to both activators. The UAS-N of *GLN1* was more effectively activated by Gln3p than by Nil1p, and the UAS-N of *GDH2* was responsive to Gln3p but barely responsive to Nil1p.

Isolation of the *NIL1* Gene. That Nil1p appeared to be able to recognize the sequence GATAAG suggested that this gene contained the coding sequence for the characteristic zinc finger region common to GATA factors of yeasts, fungi, and avian and mammalian cells. We therefore searched for genes containing this characteristic sequence.

The zinc finger region of Gln3p contains the amino acid sequence LCNACGLF, which is fully conserved in the corresponding nitrogen-responsive activators of *Neurospora crassa* and *Aspergillus nidulans* encoded respectively by *NIT2* and *AREA*; with the exception of the first and last amino acids of this octamer, the sequence is also found in the mammalian and avian GATA factors (8). We therefore used a degenerate oligonucleotide mixture (CTNTGYAAYGCNTGYGGNCTNTTK) encoding this peptide to screen a yeast genomic library in *Escherichia coli* by hybridization as described in *Materials and Methods*. We obtained 17 plasmids carrying the coding sequence of this peptide that could be placed in four groups on the basis of restriction analysis and Southern blots. One group of clones contained *GLN3*, and another group had the nucleotide sequence corresponding to the previously described *DAL80* gene, a repressor of some Gln3p-activated promoters (14). The sequence of a third group of clones matched not only the sequence coding for the fully conserved octamer but also the sequence coding for the conserved hexamer TPLWRR of the zinc finger. The fourth group of clones contained a gene coding for the fully conserved octamer sequence and for the sequence TPLWRK, with lysine rather than arginine in the last position of the hexamer. We sequenced the zinc finger regions of these two genes and subjected them to restriction analysis. We used this information to insert a 3.8-kb *hisG*::*URA3*::*hisG* cassette into these genes (*Materials and Methods* and Fig. 1). We substitutively transformed the *gln3 ura3* strain P40-2c with these plasmids, selecting for uracil independence, thus disrupting the corresponding chromosomal gene. We found that the disruption of the gene corresponding to the third group of clones had no effect on the ability of the transformed cells to activate the transcription of *GAP1*; on the other hand, the deletion of the gene corresponding to the fourth group of clones resulted in total inability to produce the permease, identifying this gene as *NIL1*. We call the other gene, whose function remains unknown, *NIL2*. The results summarized in Table 2 demonstrate that either Gln3p or Nil1p is required for the formation of the general amino acid permease.

Table 1. Activation of transcription by Gln3p and Nil1p

Plasmid	β-Galactosidase activity units			
	PM38 in Gln	PM71 in Gln	PM38 in Glu	PM71 in urea
No UAS	1	4.7	1	2
7×GATAAG	10	4	301	611
GLN1 UAS-N	3	0.2	255	64
GDH2 UAS-N	1	0.5	240	11

The UAS element 7×GATAAG consists of seven sequential repeats of the GATAAGATAAG sequence found upstream of the *GLN1* gene. *GLN1* UAS-N is the *Sal* I–*Ava* II fragment from *GLN1* and contains in addition to the GATAAGATAAG element the sequence TTGTT. *GDH2* UAS-N is the *Rsa* I–*Bst*EII fragment of *GDH2* and contains in addition to the GATTAGATTAG element the sequence TTGGT. Strain PM38 is the wild type and was grown in media with glutamine (Gln) or glutamate (Glu) as nitrogen source. Strain PM71 is the *gln3* mutant and was grown in media with glutamine or urea.

The determination of the DNA sequences coding for the zinc fingers of Nil1p and Nil2p enabled us to locate these genes on recently sequenced yeast chromosomes. The *NIL1* gene is on chromosome VI, whose sequence was recently reported by Y. Murakami *et al.* (19) (GenBank accession no. D50617) and the *NIL2* gene is on chromosome X, whose sequence was reported by Rasmussen (20) (GenBank accession no. X85021). A comparison of the derived sequences of the proteins encoded by *NIL1, GLN1, DAL80*, and *NIL2* shows considerably greater similarity between Nil1p and Gln3p than between either of these proteins and the products of *DAL80* and *NIL2*. For example, both Nil1p and Gln3p (8) contain a highly acidic region comprising the first 160 amino acid residues which is not found in the other two proteins. The presence of such a region could indicate a transcriptional activation domain (15). In addition, there are several homologous regions in both Nil1p and Gln3p in the region between amino acid residues 60 and 308. The region with the greatest homology, which is very rich in asparagine, is shown in Fig. 2. This figure also illustrates the extensive homology in the zinc finger regions of the four proteins.

Activation of Transcription by Gln3p and Nil1p. We determined the abilities of strains defective in *GLN3, NIL1*, or both to grow in media containing various sources of nitrogen. We found that in all cases loss of either one of the two genes resulted in a relatively small decrease in the growth rate. The loss of both genes severely reduced the rate of growth, except when glutamine served as the source of nitrogen. The results suggest that Gln3p and Nil1p play a role in the uptake of nitrogen compounds.

We used fusions of *CYC1–lacZ* to the region upstream of the promoters of *GLN1, GDH2*, and *GAP1* to explore the roles of Gln3p and Nil1p in the activation of transcription of these genes in media containing various sources of nitrogen. The plasmids containing the fusions were placed in wild-type cells and in mutants lacking Gln3p, Nil1p, or both activators. Growth with glutamine as nitrogen source prevented expression of these genes in all four strains; the β-galactosidase activity was ≤2% of that in cells grown on glutamate. Further, the lack of both Gln3p and Nil1p prevented activation of the expression of these genes irrespective of the source of nitrogen. Finally, *GDH2* could be activated by Gln3p, but not by Nil1p, as shown by the lack of any significant effect of the *nil1* mutation on the expression of this gene, and by the lack of its expression in the *gln3* mutant (data not shown).

The abilities of Gln3p and of Nil1p to activate transcription of *GAP1* and *GLN1* are compared in Table 3. Nil1p was most effective in activating the expression of GAP1 in media containing ammonia or urea as nitrogen source but was quite ineffective in the medium containing glutamate as nitrogen source. On the other hand, Gln3p was quite ineffective as an activator in the ammonia-containing medium but much more effective as an activator than Nil1p in the medium containing glutamate as the source of nitrogen; further, in cells growing with ammonia as the source of nitrogen, elimination of Gln3p resulted in markedly increased expression of the regulated

Table 2. Gap1p permease activity of *gln3* and *gln3 nil1* strains

Strain	Genotype	Gap1p, unit(s)
P40-2c	*gln3*	24
MS207	*gln3 nil1*	0.5
MS207/pPM4	*gln3 nil1/GLN3*	28
MS207/pMS26-1	*gln3 nil1/NIL1*	40

Proline served as source of nitrogen.

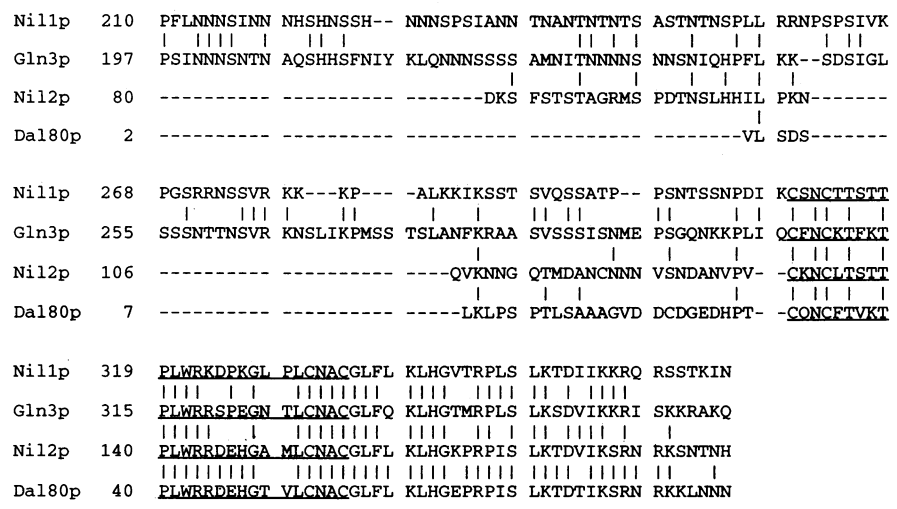

FIG. 2. Comparison of the homologous amino acid sequences of Nil1p, Gln3p, Nil2p, and Dal80p. The putative zinc fingers are underlined.

gene. It appears, therefore, that when glutamate serves as nitrogen source, Gln3p alone is able to activate transcription, but that Nil1p is the preferred activator in cells growing with ammonia as nitrogen source; in this case Gln3p may be present in an inactive form and compete with Nil1p for binding to the same GATAAG sites.

Nil1p was much less effective as an activator of the expression of *GLN1*. It can be seen that in cells growing with glutamate as nitrogen source, activation depends on the presence of Gln3p: the gene is not expressed in the *gln3 NIL1* mutant. Nevertheless, *GLN1* is somewhat better expressed during growth on glutamate in the *GLN3 NIL1* strain than in the *GLN3 nil1* mutant, suggesting that Nil1p can cooperate with Gln3p to activate transcription of *GLN1*. This cooperative effect is even more evident in cells growing on the other sources of nitrogen. In cells growing with proline as the source of nitrogen, Gln3p plays the more important role, but in cells growing with urea as the source of nitrogen, the contributions of the two activators are approximately equal.

Our results show that activation of transcription of *GLN1, GDH2*, and *GAP1* in the absence of glutamine depends on either Gln3p or Nil1p. Elimination of both activators leads to the total lack of response. However, the three genes differ in their responses to these two activators: *GDH2* responds only to Gln3p, *GLN1* prefers Gln3p, and *GAP1* prefers Nil1p. Furthermore, the nitrogen source of the growth medium affects the abilities of Gln3p and of Nil1p to activate transcription differently: Gln3p is active and Nil1p largely inactive when glutamate is the source of nitrogen; but Nil1p is active and Gln3p is largely inactive when ammonia serves as the source of nitrogen.

Table 3. Activation of transcription of *GAP1* and *GLN1* by Gln3p and Nil1p in response to the nitrogen source

Nitrogen source	Relative β-galactosidase activity							
	GAP1–lacZ plasmid				*GLN1–lacZ* plasmid			
	WT	*gln3*	*nil1*	*gln3 nil1*	WT	*gln3*	*nil1*	*gln3 nil1*
Glutamate	100	19	85	<0.1	100	1	62	2
Proline	89	87	64	<0.1	89	4	24	1
Ammonia	47	137	11	<0.1	37	5	12	1
Urea	110	130	38	NG	66	19	16	NG

The wild-type strain (WT) was MS218 and the *gln3*, *nil1*, and *glu3 nil1* mutants were strains PM71, MS221, and YDR21, respectively. The plasmids carried *GAP1–CYC1–lacZ* or *GLN1–CYC1–lacZ*. The β-galactosidase levels of cells grown with glutamate as nitrogen source (701 units for *GAP1–lacZ* and 516 units for *GLN1–lacZ*) were set at 100 to relate the other values to them. The relative β-galactosidase activity in cells grown on glutamine was <2. NG, no growth.

DISCUSSION

We have identified *NIL1*, whose product is a transcription factor for nitrogen-regulated genes. This product is responsible for the activation of transcription of *GAP1*, the gene coding for the general amino permease (3, 16), in cells lacking the previously identified transcription factor for nitrogen-regulated genes, the product of *GLN3* (8, 17).

Gln3p and Nil1p share highly homologous zinc finger regions with the previously identified Dal80p (14) and with Nil2p, also identified in this study (Fig. 2). Gln3p and Nil1p, but not the other two proteins, also have a highly acidic amino-terminal region, a feature characteristic of transcriptional activators (15). The lack of such a region in Dal80p is in accord with its role as inhibitor, rather than activator, of transcription (14), and by analogy Nil2p, whose function remains unknown, may play a similar role.

Previous work in this laboratory had shown that the activation of transcription of *GLN1* and of *GDH2* by Gln3p (6, 7) and of *GAP1* by either Gln3p or Nil1p (4) depends on the presence of GATA sequences located upstream of the transcriptional start sites of these genes and had demonstrated the binding of Gln3p to the GATAAGATAAG and GATTAGATTAG sites located upstream of *GLN1* and *GDH2*, respectively (2, 8). That Nil1p can activate transcription of a fusion of seven separated repeats of GATAAGATAAG to *CYC1–lacZ* (Table 1) demonstrates its ability to recognize a GATAAG element.

The transcription of *GLN1, GDH2*, and *GAP1* is prevented by the presence of glutamine in the growth medium (reviewed in ref. 1). All three genes are well expressed in media containing glutamate as nitrogen source and to varying degrees in media containing instead proline, ammonia, or urea. Mutants lacking either Gln3p or Nil1p retain the ability to express these genes in some of these media, but mutants lacking both transcription factors have lost this ability. A comparison of the conditions in which the target genes could be expressed in cells lacking either Gln3p or Nil1p showed that both the nature of the UAS elements associated with these genes and the nitrogen source determined the abilities of Gln3p and Nil1p to serve as activators of transcription.

The transcription of *GDH2* can be activated in cells growing with glutamate or proline, but not with ammonia or urea, as the source of nitrogen and can be activated by Gln3p, but not by Nil1p. The only nitrogen-responsive UAS of *GDH2* contains the sequence GATTAGATTAG and, as shown in Table

1, responds to Gln3p, but hardly at all to Nil1p. On the other hand, the transcription of *GLN1* can be activated by either Gln3p or Nil1p (Table 3), and the major nitrogen-responsive UAS of *GLN1*, which has the sequence GATAAGATAAG, responds to Nil1p, though not as well as to Gln3p (Table 1); however, Nil1p is much more effective as an activator of *GAP1* transcription (Table 3). Apparently the UAS elements of the three genes differ in their sensitivity to Gln3p and Nil1p. They all can respond to Gln3p, but the GATTAGATTAG element of *GDH2* is unresponsive to Nil1p and the fused GATAA-GATAAG element of *GLN1* does not respond to Nil1p as well as the two separated GATAAG elements found upstream of *GAP1* (4).

In the case of Gln3p it is well established that the signal responsible for its inactivation is an increase in the intracellular level of glutamine (reviewed in ref. 1). That Gln3p is a good activator of transcription in cells grown with glutamate as source of nitrogen shows that these cells are glutamine limited. The ineffectiveness of Nil1p as an activator of *GLN1* and *GAP1* transcription in these cells indicates that some intracellular component other than glutamine is responsible for keeping Nil1p in an inactive state. Ure2p, which transduces the glutamine signal to Gln3p, does not affect Nil1p (4), a fact in good accord with the view that Nil1p responds to a signal other than glutamine.

In cells growing in minimal media all cellular nitrogen originates from the amino nitrogen of glutamate or the amido nitrogen of glutamine. The nitrogen source permitting most rapid growth is glutamine, which serves as an excellent source of both intracellular glutamine and glutamate (1). Cells grow somewhat slower with either glutamate or ammonia as the source of nitrogen. In the former case the cells are deficient in glutamine but not glutamate, since the synthesis of glutamine depends on the generation of ammonia from glutamate by the NAD-linked glutamate dehydrogenase (18). In the latter case the cells are somewhat deficient in glutamate but not in glutamine, since the synthesis of glutamate depends on the reductive amination of 2-oxoglutarate by the NADP-linked glutamate dehydrogenase, but the resulting glutamate can readily react with ammonia in a reaction catalyzed by glutamine synthetase to produce glutamine. The only product of proline is glutamate and the only nitrogenous product of urea is ammonia: the slower growth rate of the cells on these sources of nitrogen therefore suggests that the resulting cells are deficient in both glutamine and glutamate. However, since the growth rate can be determined by only a single deficiency, these cells presumably oscillate between glutamate-restricted and glutamine-restricted growth.

The results of the experiments presented in Table 3 are compatible with the view that the intracellular signal resulting in the inactivation of Nil1p is a high intracellular concentration of glutamate. In the case of *GAP1*, which is sensitive to both Gln3p and Nil1p, growth with glutamate as the source of nitrogen (glutamate high, glutamine low) results in activation by Gln3p, but not by Nil1p; growth on ammonia (glutamate low, glutamine high) results in activation by Nil1p and not by Gln3p; in cells grown on proline and urea, where both glutamine and glutamate are low, the transcription can be activated by either Gln3p or Nil1p. A similar pattern is seen in the activation of *GLN1* expression, except that Gln3p is the much more effective activator and Nil1p alone is incapable of full activation of *GLN1* transcription. It is of particular interest that in cells growing with proline or urea as nitrogen source, both Gln3p and Nil1p are required for optimal expression of *GLN1*. This behavior presumably reflects the oscillatory growth of the cells in these media: they are alternately starved for glutamate and glutamine and effective expression of *GLN1* depends on activation in both conditions, in the former by Nil1p and in the latter by Gln3p.

The difference in the sensitivities of *GDH2*, *GLN1*, and *GAP1* to activation by Nil1p is in good accord with the physiological roles of the products of these genes. Since the role of the NAD-dependent glutamate dehydrogenase is the generation of ammonia from glutamate in the growth medium (18), it would be inappropriate to have the expression of this gene activated by Nil1p in response to a deficiency of glutamate. The sensitivity of *GLN1* to activation by Nil1p may reflect the potential role of glutamine synthetase, the product of *GLN1*, in the synthesis of glutamate (18). Finally, the great sensitivity of *GAP1* to Nil1p can be accounted for by the role of its product, the general amino acid permease, in the uptake of proline and arginine, which are potential sources of glutamate.

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