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Gat1p, a GATA Family Protein Whose Production Is Sensitive to Nitrogen Catabolite Repression, Participates in Transcriptional Activation of Nitrogen-Catabolic Genes in *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae **cells selectively use nitrogen sources in their environment. Nitrogen catabolite repression (NCR) is the basis of this selectivity. Until recently NCR was thought to be accomplished exclusively through the negative regulation of Gln3p function by Ure2p. The demonstration that NCR-sensitive expression of multiple nitrogen-catabolic genes occurs in a *gln3*Δ *ure2*Δ *dal80*::*hisG* triple mutant indicated that the prevailing view of the nitrogen regulatory circuit was in need of revision; additional components clearly existed. Here we demonstrate that another positive regulator, designated Gat1p, participates in the transcription of NCR-sensitive genes and is able to weakly activate transcription when tethered upstream of a reporter gene devoid of upstream activation sequence elements. Expression of *GAT1* is shown to be NCR sensitive, partially Gln3p dependent, and Dal80p regulated. In agreement with this pattern of regulation, we also demonstrate the existence of Gln3p and Dal80p binding sites upstream of *GAT1*.**

Saccharomyces cerevisiae cells selectively scavenge nitrogenous compounds from their environment. This selectivity is accomplished by nitrogen catabolite repression (NCR), the designation given to the physiological process through which transcription of genes encoding proteins needed for the uptake and degradation of poorly used or nonrepressing nitrogen sources (e.g., proline and allantoin) is maintained at low levels when more readily used or repressing nitrogen sources (e.g., asparagine and glutamine) are available (15, 16, 56). To date, three global nitrogen regulatory factors (Gln3p, Ure2p, and Dal80p) have been shown to participate in the regulated transcription of nitrogen-catabolic genes; this transcription is mediated through the *cis*-acting nitrogen-regulated upstream activation sequence (UAS_{NTR}) element containing the sequence 5′-GATAAG-3′ at its core (2, 3, 6, 8, 12, 13, 17–24, 26, 30, 34, 36–39, 43–46, 58, 59).

Gln3p binds to UAS_{NTR} elements in the 5′ flanking sequences of NCR-sensitive genes (3, 25) and is required for their transcription when readily used nitrogen sources are absent (18). In keeping with this physiological function, Gln3p supports transcriptional activation when tethered by LexAp upstream of a reporter gene devoid of UAS elements (25). Ure2p has been proposed to be a negative regulator of Gln3p function according to a model derived from (i) Lacroute's initial finding that a wild-type *URE2* locus is required for ammonia repression (later referred to as NCR) (27, 28, 30), (ii) the demonstration that *gln3* mutations are epistatic to *ure2* mutations (21), and (iii) the finding that the Ure2p functions through UAS_{NTR} (12). Dal80p has been proposed to be a DNA-binding protein that represses Gln3p-mediated transcription perhaps by competing with Gln3p for binding to some but not all GATAAG sequences (2, 8, 19, 22–24, 26). Consistent with this suggestion are the observations that (i) *dal80* mutants overexpress multiple genes whose transcription is Gln3p dependent (2, 8, 19, 22–24, 26), (ii) Dal80p binds to a subset of UAS_{NTR} elements designated URS_{GATA}s (23, 24), and (iii) Dal80p behaves as a weak repressor protein when fused to LexA (52a). Although Dal80p is judged to be a repressor of some Gln3p-dependent transcription, it is not required for NCR. *DAL5* (which encodes allantoate permease) expression, for example, is only slightly Dal80p regulated but is fully sensitive to NCR (15, 16, 40). In addition, all NCR-sensitive, Dal80p-regulated genes remain fully NCR sensitive in *dal80*-null mutants (2, 12, 16, 19, 26).

The above data collectively suggested the following working model of NCR-responsive regulation exerted on the transcription of nitrogen-catabolic genes (16, 26). Nitrogen-catabolic gene expression is activated by Gln3p, which competes with Dal80p for binding to some, but not all, UAS_{NTR} sites. The outcome of this competition influences transcriptional activation for inducer-independent genes and basal-level transcription for inducer-dependent genes. NCR is superimposed on the above regulation; availability of preferred nitrogen sources in some way mediates the ability of Ure2p to prevent Gln3p from performing its biochemical function in UAS_{NTR}-mediated transcription. Hence, NCR of gene expression is the failure of nitrogen-catabolic genes to be activated at high levels when readily used nitrogen sources are available.

Not only are the structural genes of nitrogen catabolism regulated by Gln3p and Dal80p, but the expression and operation of these regulatory proteins are, in turn, also regulated. Gln3p operation, rather than *GLN3* steady-state mRNA levels, is thought to be regulated by Ure2p in response to the quality of nitrogen source available (12, 13, 20–24, 26). However, the biochemical mechanism by which Ure2p regulates Gln3p function has not been demonstrated (20). More clearly understood are the regulatory relationships that exist between the Gln3

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and Dal80 proteins. Multiple UAS_{NTR}/URS_{GATA} elements are situated in the 5′ flanking region of *DAL80* (2, 19, 22–24, 26). In agreement with the presence of these elements, it has been shown that *DAL80* expression is Gln3p dependent and autogenously regulated by Dal80p itself; i.e., Dal80p inhibits *DAL80* transcription (2, 22). The dependence of Dal80p production upon Gln3p operation and the intracellular level of Dal80p results in a regulatory network that is highly responsive to the quality of the nitrogen source available. At the same time, the ratio of Gln3p to Dal80p is maintained reasonably constant by autogenous repression of *DAL80* expression (16).

The above model of nitrogen-catabolic gene regulation accounted for most early data. Recently, however, inconsistencies between expectation and observation have become increasingly apparent, thereby challenging the exclusive role of the Ure2p→Gln3p→UAS_{NTR} pathway for delivery of the NCR signal to the transcription apparatus (12, 13, 26, 59). First, if NCR is mediated exclusively through Gln3p, then the response of a given gene's expression to deletion of *GLN3* should be equivalent to its NCR sensitivity. However, *UGA1* expression, which is NCR sensitive, was found to be Gln3p independent (26). A similar correlation was expected for deletion of *URE2*; if Ure2p is the exclusive route for transmitting NCR control (via Gln3p), then deletion of *URE2* should result in the abolishment of NCR sensitivity. Variations ranging from nearly complete NCR sensitivity to insensitivity were observed among the responses of nitrogen-catabolic gene expression to *URE2* deletion (12). It was, therefore, concluded that the environmental signal→Ure2p→Gln3p→UAS_{NTR}→gene expression control pathway was not exclusive (12, 26). Finally, we demonstrated the presence of NCR-sensitive expression of four nitrogen-catabolic genes in a *gln3*Δ single mutant, a *gln3*Δ *ure2*Δ double mutant, and a *gln3*Δ *ure2*Δ *dal80*::*hisG* triple mutant (13). The triple mutant lacked all known regulators of NCR-sensitive transcription, indicating the existence of a Gln3p-independent, NCR-sensitive transcription system. A similar conclusion has been reached by Stanbrough and Magasanik (51a) and Xu et al. (59).

The objective of the present work was to identify and characterize the component(s) supporting NCR-sensitive transcription in the triple mutant mentioned above. Data presented below demonstrate that *S. cerevisiae* contains a Gln3p homolog, designated Gat1p, that is responsible for Gln3p-independent, NCR-sensitive transcription of multiple nitrogen-catabolic genes. *GAT1* expression itself is also NCR sensitive, Dal80p regulated, and partially Gln3p dependent. These findings explain several of the inconsistencies noted above in our current understanding of NCR-sensitive nitrogen-catabolic gene transcription. Preliminary reports of this work have already appeared (10, 11, 14).

MATERIALS AND METHODS

Strains. *S. cerevisiae* and *Escherichia coli* strains used in this work are listed in Table 1. Plasmids pEG202 (32), pNKY51 (26), and pHP41 (41) have been described earlier. All yeast strains used are Σ1278b derivatives.

Northern analysis. Strains were grown in Wickerham's minimal medium (57) with glucose and a nitrogen source at final concentrations of 0.6 and 0.1%, respectively. Uracil (20 mg/liter), L-glutamine (30 mg/liter), and L-lysine (20 mg/liter) were added to cover auxotrophic requirements of the strains as needed. Cultures were grown to mid-log phase (55 to 65 Klett units on a Klett-Summerson colorimeter equipped with a green filter), and total RNA was prepared according to the method of Carlson and Botstein (7). Poly(A)$^+$ RNAs (prepared as described earlier [13]) were resolved in 1.4% agarose gels (SeaKem; FMC Bioproducts) prepared in 2% formaldehyde–0.4 M morpholinepropanesulfonic acid (MOPS) (pH 7.2). The separated RNAs were transferred to GeneScreen Plus Nylon 66 membranes (Dupont, New England Nuclear Research Products) by capillary action with 1.5 M NaCl–0.15 M sodium citrate (10× SSC). The membranes were hybridized with oligonucleotides radiolabelled by the polynucleotide kinase reaction (New England Biolabs or Boehringer Mannheim) or with plasmid DNAs radiolabelled by random priming (Boehringer Mannheim or Promega). Hybridization conditions included 50% deionized formamide, 1 M NaCl, and 1% sodium dodecyl sulfate (SDS) at 42°C for 12 to 20 h. Membranes were washed twice with 2× SSC at room temperature for 5 min, twice with 2× SSC containing 1% SDS at 60°C for 30 min, and twice in 0.2× SSC at room temperature for 30 min. For *DAL5*, *UGA4*, and *GAP1*, we used plasmids pRR20 (43–46), pUGA4-2 (24), and pPL257 (35), respectively, as hybridization probes. For histone H4, *PUT1*, *GAT1*, and *UGA1*, we used the synthetic oligonucleotides 5′-ATACCTTGGATGTTATCTCTTAGAATCTTTCTGTGACGCTTGGC ACCACC-3′ (positions 90 to 40) (50), 5′-AGCGATATAACCTGGAGCAATG TCAGTGATTGGCTTAGATTCCAGCTGGC-3′ (positions 579 to 529) (55), 5′-GTGGCGAGTGTTAGAGCGGAGGTATTGTGGGATGGAAGGTG AGGCAATCT-3′ (positions 1,293 to 1,243) (this work), and 5′-TTGAACGCT AATTCATTGGCATCTGCACCGGAAAGCCCTGACCAGACGTG-3′ (positions 431 to 382) (1), respectively, as probes.

It is important to emphasize that rigorous comparisons of the quantities of various RNA species are possible only if the RNA species compared are present on the same blot; each experiment was designed as a self-contained unit, taking this limitation into account. Widely varying amounts of gene expression are often observed in experiments like those described in this article. This variation derives from the nature of the gene being analyzed as well as the particular mutant strain and growth conditions used. When small amounts of expression were expected, autoradiograms were exposed for longer times. In some cases, hybridization to the standard histone H4 probe was carried out separately from that in which the test gene expression was assayed. This permitted the use of optimal exposures for each signal assayed and hence the most accurate assessment of each experimental question posed. It is for these reasons that comparisons between blots must be limited to the conclusions derived from each one.

β-Galactosidase assays. Strains were transformed by the method of Ito et al. (33) and plated on selective medium. Positive transformants were purified by restreaking, patched, and grown to a cell density of A_{600} of 0.4 to 0.8 in 0.17% yeast nitrogen base medium without amino acids or ammonium sulfate (YNB; Difco Laboratories) supplemented with glucose (2.0%) and proline (Pro) or glutamine (Gln) as the sole nitrogen source at a final concentration of 0.1%. L-Lysine and L-glutamine were also provided at concentrations of 20 and 30 mg/liter, respectively. Assays were performed by the method of Guarente and Mason (31) as described by Rai et al. (43).

EMSAs. The electrophoretic mobility shift assay (EMSA) was performed essentially as described earlier, except that 1 μg of total crude extract protein was used in the binding reactions (23).

Cloning the *GAT1* gene. To clone the *GAT1* gene, a radioactive oligonucleotide, 5′-GTTGGCGAGTGTTAGAGCGGAGGTATTGTGGGATGGAAGG TGAGGCAATCT-3′ (see Results), was used to screen a YCp50-based yeast genomic library (ATCC no. 37415) constructed by Rose et al. (48) using standard methods. Four positive clones were recovered from approximately 60,000 colonies analyzed. One clone, plasmid pTSC531, was studied further (Fig. 1). An 8.8-kb *Asp*718-*Eco*RI fragment, derived from plasmid pTSC531, was cloned into plasmid pBS(KS$^+$), yielding plasmid pTSC536, and this plasmid was used as the parental fragment for DNA sequencing and further DNA manipulations. DNA sequence analysis was performed as described earlier (29).

Construction of a *gat1* deletion strain and *lexA-GAT1* fusion plasmids. The chromosomal *GAT1* gene was deleted (positions 810 to 1035) by the one-step replacement method of Rothstein (49). Plasmid pRR336 (Fig. 1) was digested with *Eco*RI and *Xba*I, and the linear 8.1-kb fragment was isolated and used for the gene replacement procedures in wild-type (TCY1) and *gln3*Δ (RR91) strains (17). Genomic Southern blot analysis was used to verify the structures of all chromosomal deletions. As reported elsewhere, the *E. coli*-produced Gln3p we used in this work was truncated to about two-thirds of its normal length by

TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype
TCY1	*MAT*α *lys2 ura3*
RR91	*MAT*α *ura3 lys2 gln3*::*hisG*
RJ71	*MAT*α *lys2 ura3 gat1*Δ::*hisG URA3 hisG*
RJ72	*MAT*α *lys2 ura3 gln3*Δ::*hisG gat1*Δ::*hisG URA3 hisG*
JCY37	*MAT*α *lys2 ura3 gln3*Δ::*hisG ure2*Δ::*URA3*
JCY55	*MAT*α *lys2 ura3 gat1*Δ::*hisG ure2*Δ::*URA3*
JCY125	*MAT*α *lys2 ura3 ure2*Δ::*URA3*
TCY17	*MAT*α *lys2 ura3 dal80*::*hisG*
EGY48	*MAT*α *3lexAop*::*LEU2 ura3 trp1 his3*
JCY23	*MAT*α *lys2 ura3 dal80*::*hisG ure2*Δ::*URA3*
RR92	*MAT*α *lys2 ura3 gln3*Δ::*hisG dal80*::*hisG*
JCY48	*MAT*α *lys2 ura3 gln3*Δ::*hisG dal80*::*hisG ure2*Δ::*URA3*

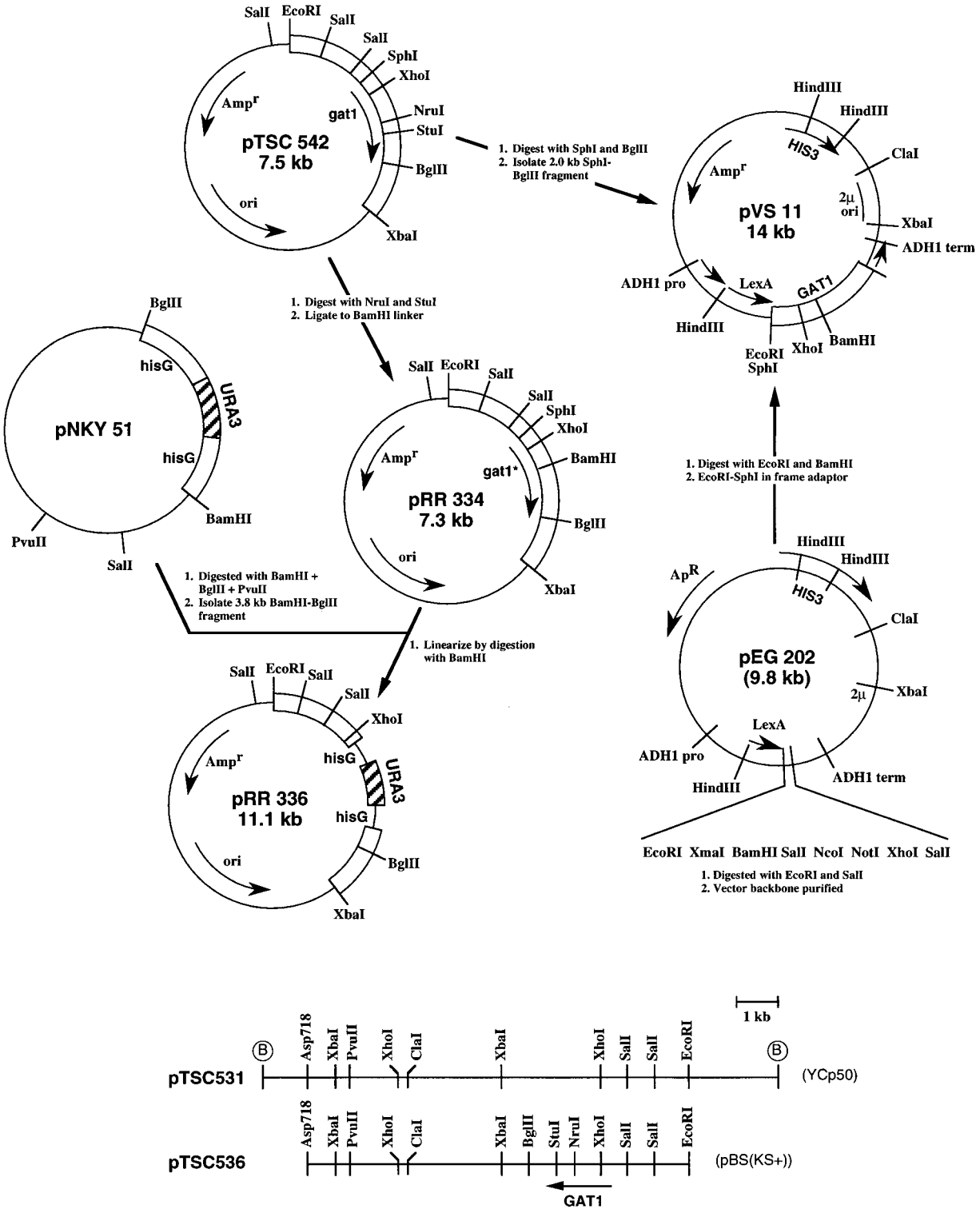

FIG. 1. Strategy for construction of various *GAT1* plasmids used in this work and restriction endonuclease maps of two plasmids isolated from a genomic bank containing the *GAT1* gene. The *GAT1* coding region and the direction of transcription are indicated.

proteolysis. This truncation of the C-terminal portion of Gln3p does not, however, destroy its ability to bind to UAS_{NTR} elements (25).

A *lexA-GAT1* fusion plasmid was constructed by insertion of a 2.0-kb *Sph*I-*Bgl*II fragment from plasmid pTSC542, containing the *GAT1* coding sequence, into plasmid pEG202 (32) digested with *Eco*RI and *Bam*HI (Fig. 1). This construction required addition of an *Eco*RI-*Sph*I in-frame adaptor prior to ligation. The adaptor was prepared by annealing the oligonucleotides 5′-CCAGCGTA GTCTGGGACGTCGTATGGGTAG-3′ and 5′-AATTCTACCCATACGACG TCCCAGACTACGCTGGCATG-3′ (Fig. 1).

Nucleotide sequence accession number. The GenBank accession number for *GAT1* is U27344. *GAT1* is open reading frame YFL021W in the chromosome VI sequence of the genome project (40).

FIG. 2. Nucleotide sequence of the *GAT1* gene determined by using the Sequenase version II protocol (U.S. Biochemical) and synthetic oligonucleotides as primers (29). Arrows, positions of UAS_{NTR}-homologous sequences upstream of *GAT1*.

RESULTS

Isolation and nucleotide sequence of *GAT1*. We previously demonstrated NCR-sensitive gene expression in *gln3*Δ single, *gln3*Δ *ure2*Δ double, and *gln3*Δ *dal80*::*hisG ure2*Δ triple mutants (13). Since these mutants lacked Gln3p, we postulated the existence of another positive regulator that performed a similar function. Three observations provided insight into the nature of this putative regulator: (i) the only *cis*-acting element known to mediate NCR-sensitive transcription is UAS_{NTR}, which consists of two separated copies of a dodecanucleotide with the sequence 5′-GATAAG-3′ at its core (6, 46), (ii) the patterns of regulation observed for NCR-sensitive gene expression in single, double, and triple mutants with defects in the three global nitrogen regulatory genes suggested that the unidentified transcription factor was strongly regulated by Dal80p (13), and (iii) during Southern blot analysis of yeast genomic DNA (60) and Northern blot analysis of *DAL80* mRNA (9) using *DAL80* sequences as probes, DNA fragments and RNA species containing *DAL80*-homologous sequences other than those of *DAL80* or *GLN3* were observed. These observations raised the possibility that the unidentified transcription factor might contain a GATA zinc finger and its cognate gene would possess UAS_{NTR}-homologous sequences in its upstream region. Several years ago Stanbrough reported a 488-bp sequence of a DNA fragment identified on the basis of its hybridization with a degenerate oligonucleotide encoding a conserved portion of the Gln3p and Dal80p zinc finger motifs (51). Unfortunately, neither the DNA fragment nor the gene from which it was derived was studied further (51). However, since the partial open reading frame contained a zinc finger motif homologous to the two motifs possessed by Gln3p and Dal80p, we considered it a possible candidate to be part of the gene for which we were searching. Therefore, using a portion of the Stanbrough sequence as a probe, we isolated a 12-kb DNA fragment containing the zinc finger sequences mentioned above from the YCp50 genomic bank of Rose (ATCC 37415) (48). Restriction maps of this fragment (plasmid pTSC531) and a subclone containing an 8.8-kb DNA fragment (plasmids pTSC536) used for most of our experiments are shown in Fig. 1.

The nucleotide sequence of the gene we designate *GAT1* was determined as described in Materials and Methods (Fig. 2). Inspection of the *GAT1* sequence revealed that it contained a 1,530-bp open reading frame encoding a protein of 510 amino acids with a calculated molecular weight of 56,334 and a pI of 8.56. In agreement with our expectations, the *GAT1* 5′

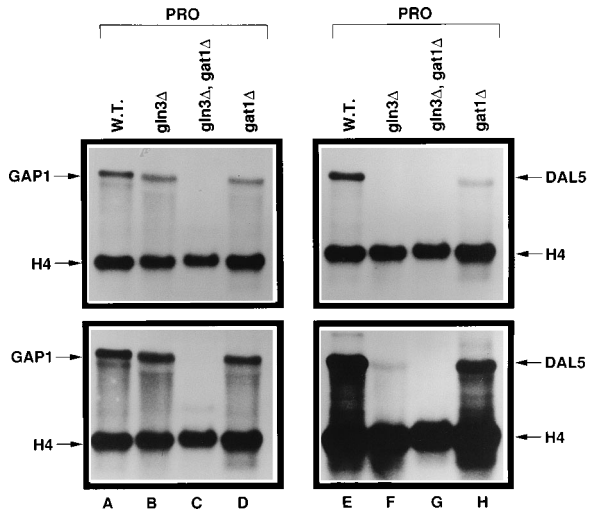

FIG. 3. Northern analysis of *GAP1* and *DAL5* steady-state mRNAs obtained from strains with the indicated genotypes. Wild-type (W.T.) strain TCY1 (lanes A and E), *gln3*Δ strain RR91 (lanes B and F), *gln3*Δ *gat1*Δ strain RJ72 (lanes C and G), and *gat1*Δ strain RJ71 (lanes D and H) were grown with proline as the sole nitrogen source as described in Materials and Methods. Poly(A)$^+$ RNA (10 μg per lane) immobilized to a nylon membrane was hybridized with randomly primed plasmid pPL257 (34) or pRR20 (45), containing DNA complementary to *GAP1* or *DAL5*, respectively. The end-labelled synthetic oligonucleotide complementary to the H4 gene was used as a probe to determine loading efficiency. The lower panels are overexposures of the autoradiograms above.

flanking sequence contains multiple sequences homologous to the positively and negatively acting UAS_{NTR} and URS_{GATA} elements, the binding sites of Gln3p and Dal80p, respectively.

Requirement of Gat1p for expression of nitrogen-catabolic genes. To determine whether Gat1p participated in transcriptional regulation of nitrogen-catabolic gene expression, we constructed a set of strains in which *GLN3* (RR91), *GAT1* (RJ71), or both *GAT1* and *GLN3* (RJ72) were deleted (see Materials and Methods) and used them in a series of Northern blot analyses. We chose to analyze the expression of a variety of previously studied and highly NCR-sensitive nitrogen-catabolic genes. This permitted us to gain some insight into the physiological specificity of Gat1p participation in catabolic nitrogen control. Steady-state *GAP1* (encoding the general amino acid permease) mRNA levels in *gln3*Δ or *gat1*Δ single mutants were about half of that in wild-type strain TCY1 (Fig. 3, lanes A, B, and D). In contrast, *GAP1* mRNA in the *gln3*Δ *gat1*Δ double mutant was below the level of detection (Fig. 3, lane C). *GAP1* mRNA also could not be detected (lane C) in an autoradiogram of this gel that was significantly overexposed to visualize even small amounts of mRNA (Fig. 3).

When steady-state *DAL5* (encoding allantoate permease) mRNA levels were assayed in the experiment described above, quantitatively different results were obtained (Fig. 3, lanes E to H). *DAL5* mRNA in the *gln3*Δ strain RR91, rather than being about half of the wild-type level, was barely detectable (Fig. 3, lanes E and F). The small amount of *DAL5* mRNA in the *gln3*Δ strain is best seen in an overexposed autoradiogram (Fig. 3). *DAL5* mRNA in a *gat1*Δ culture (strain RJ71) was much less abundant than the wild type but was, nonetheless, clearly present (Fig. 3, lane H). Here, as noted earlier, the effects of deleting *GAT1* were more severe on *DAL5* mRNA levels than on those of *GAP1*. In the *gln3*Δ *gat1*Δ double mutant (strain RJ72) no *DAL5* mRNA signal could be detected even in overexposed autoradiographs (Fig. 3, lane G).

The expression patterns of *PUT1* (encoding proline oxidase) and *UGA1* (encoding γ-aminobutyric acid [GABA] aminotransferase) were different from those of *DAL5* and *GAP1*. For the experiment with *PUT1*, GABA rather than proline was used as nitrogen source to avoid the complication of induced *PUT1* transcription mediated by PUT3p (4, 55). *PUT1* expression was dramatically less in a *gln3*Δ strain than in the wild type (Fig. 4a, lanes A and B), as we previously reported (12, 13, 26). The striking feature of these data was that *PUT1* expression occurred at the same low level regardless of the mutant assayed, i.e., *gln3*Δ (lane B) and *gat1*Δ (lane D) single mutants or the *gln3*Δ *gat1*Δ double mutant (lane C). For *UGA1*, the *gat1*Δ mutant possessed the strongest phenotype, i.e., decrease in *UGA1* mRNA compared with the wild type, though none of the mutant phenotypes were particularly pronounced or informative. There was, in fact, a higher level of *UGA1* mRNA in the double mutant than in any of the other strains assayed (Fig. 4a, lanes E to H).

We also investigated the requirement of Gat1p for *UGA4* (encoding GABA permease) expression. As shown in Fig. 4b, *UGA4* expression exhibited a strong dependence upon both Gln3p and Gat1p (lanes A, B, and D). There is a barely detectable level of *UGA4* expression in the *gat1*Δ mutant (lane D), but it is below the level of detection in both lanes B and C, containing mRNAs from the *gln3*Δ single and *gln3*Δ *gat1*Δ double mutants, respectively. *UGA4* expression and *DAL5* expression exhibit similar requirements for Gln3p and Gat1p, but whereas *UGA4* expression is highly responsive to *DAL80* deletion, *DAL5* expression is not (2, 19, 24, 26, 54).

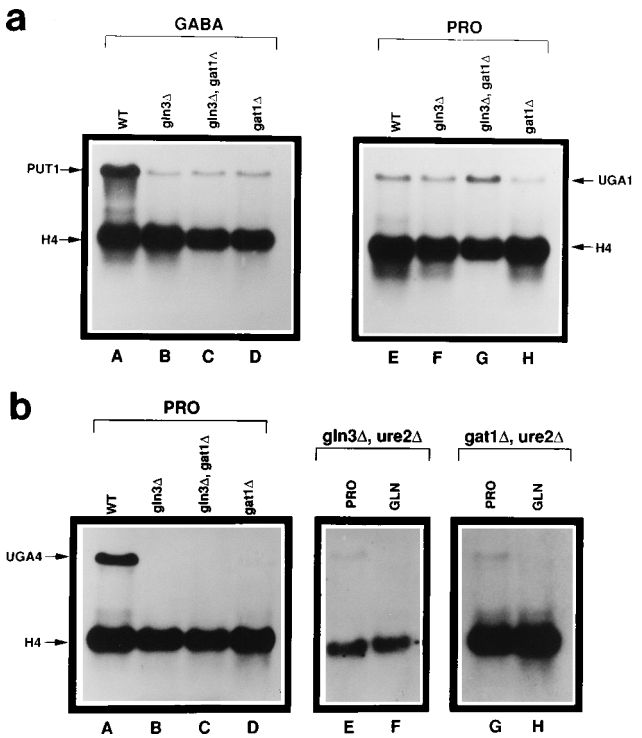

FIG. 4. Northern analysis of *PUT1*, *UGA1*, and *UGA4* steady-state mRNAs obtained from strains with the indicated genotypes. The experimental format of this experiment was the same as that described in the legend to Fig. 3 except for lanes E to H in panel b, in which *gln3*Δ *ure2*Δ and *gat1*Δ *ure2*Δ double-mutant strains JCY37 and JCY55, respectively, were used for RNA preparations. The nitrogen source used in each case is indicated. The nylon membrane containing the resolved RNA species was hybridized with an end-labelled synthetic oligonucleotide complementary to the *PUT1* gene or *UGA1* gene or with randomly primed *UGA4*-containing plasmid pUGA4-2 (24), and H4 was assayed as a control to determine loading efficiency. WT, wild type.

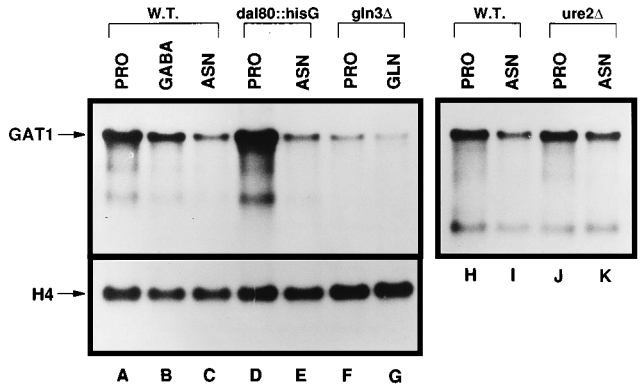

FIG. 5. Effects of *dal80*-, *gln3*-, or *ure2*-null mutations on steady-state *GAT1* mRNA levels. Lanes: A, B, C, H, and I, wild-type (W.T.) strain TCY1; D and E, strain TCY17 (*dal80*::*hisG*); F and G, strain RR91 (*gln3*Δ); J and K, strain JCY125 (*ure2*Δ). These strains were grown with the indicated nitrogen sources as described in Materials and Methods. Poly(A)$^+$ RNA (10 μg per lane) immobilized on a nylon membrane was probed with randomly primed *GAT1*-containing plasmid pTSC550. Hybridization to H4 sequences was assayed as a control to determine loading and transfer efficiencies.

Together, these data argue that Gln3p and Gat1p both participate in nitrogen-catabolic gene expression, though the extent to which they are required for a particular gene's expression is specific to that gene. In some cases (e.g., *DAL5* and *UGA4*) expression exhibits a stronger requirement for Gln3p than for Gat1p, whereas in others (e.g., *GAP1*) about equal requirements are observed for the two proteins.

Regulation of *GAT1* expression. The multiple UAS_{NTR} and URS_{GATA}-homologous sequences between positions −245 and −205 upstream of *GAT1* prompted us to determine whether *GAT1* expression was regulated in a manner typical of nitrogen-catabolic genes, i.e., whether it was (i) NCR sensitive, (ii) Gln3p dependent, (iii) increased in a *dal80*Δ mutant growing with proline as nitrogen source, and (iv) increased in a *ure2*Δ mutant, relative to the wild type, growing with asparagine or glutamine as nitrogen source. As shown in Fig. 5, wild-type cells provided with a repressive nitrogen source (asparagine) contained less *GAT1* mRNA than when the cells were provided with a nonrepressive nitrogen source (proline) instead (lanes A and C). NCR sensitivity of *GAT1* expression in a wild-type strain, however, was significantly less than those observed for most nitrogen-catabolic genes (26). *GAT1* expression also significantly increased in response to disruption of *DAL80* (Fig. 5, lanes A versus D). Note that, although the level of *GAT1* mRNA in a *dal80*Δ mutant was much greater than that in the wild type when proline was provided as sole nitrogen source (lanes D and A), the levels were the same when asparagine was provided instead (lanes E and C). This is consistent with the proposed role of Dal80p as a competitive repressor binding to UAS_{NTR}/URS_{GATA} sites upstream of *GAT1* (24, 26). The NCR sensitivity of *GAT1* expression argued that it might possess a Gln3p requirement. This Gln3p requirement is observed by comparing the levels of *GAT1* expression in the wild-type (TCY1) and *gln3*Δ mutant (RR91) strains growing with proline as nitrogen source (Fig. 5, lanes A and F, respectively). *GAT1* expression in a *gln3*Δ mutant provided with proline, a nonrepressive nitrogen source, was greater than when glutamine, a repressive nitrogen source, was provided instead (Fig. 5, lanes F and G), i.e., Gln3p-independent *GAT1* expression was still moderately NCR sensitive. When we assayed the effects of a *ure2*Δ mutation on *GAT1* expression in cells provided with asparagine as nitrogen source, there was only a slight increase of *GAT1* mRNA in the *ure2*Δ mutant; it was too small relative to that for the wild type (less than twofold) to be convincing (Fig. 5, lanes H to K).

We recently analyzed the responses of multiple nitrogen-catabolic genes to single, double, and triple null mutations in genes encoding the global nitrogen regulatory proteins Ure2p, Gln3p, and Dal80p (13). These experiments not only provided indications that Gln3p was not unique in its ability to support NCR-sensitive transcription, but they also provided clues about how the Gln3p-independent, NCR-sensitive transcription system was regulated. Since we wanted to compare the responses of *GAT1* expression with those observed with other NCR-sensitive genes (12, 13, 26), we performed a similar set of experiments with *GAT1*. In agreement with the assay results of genes such as *GAP1*, *CAN1*, and *DAL5*, disruption of the *DAL80* locus in a *ure2*Δ background resulted in an increase in *GAT1* expression when cells were provided with proline or glutamine (Fig. 6, lanes A to D). Also analogous to the responses of the nitrogen-catabolic genes mentioned above, *GAT1* expression was NCR sensitive in the *gln3*Δ *dal80*::*hisG* double and *gln3*Δ *dal80*::*hisG ure2*Δ triple mutant strains (Fig. 6, lanes E to H). The magnitude of *GAT1* expression sensitivity to NCR was higher in a Dal80$^-$ background (Fig. 6, lanes E

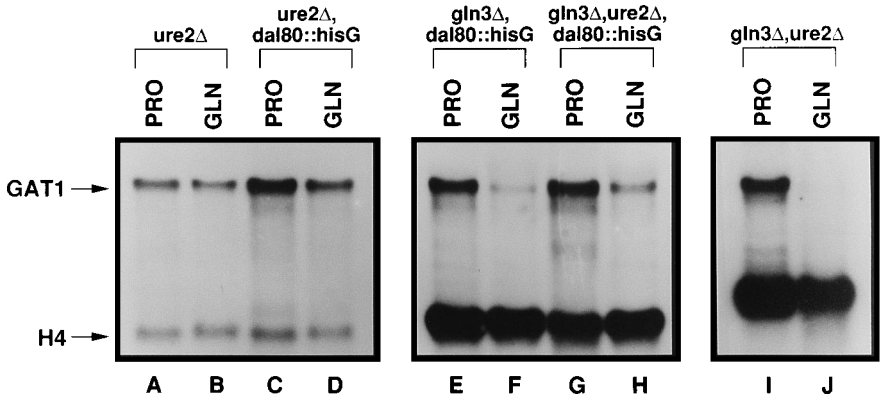

FIG. 6. Effects of multiple regulatory mutations on steady-state levels of *GAT1* mRNA. Lanes: A and B, strain JCY125 (*ure2*Δ); lanes C and D, strain JCY23 (*ure2*Δ *dal80*::*hisG*); lanes E and F, strain RR92 (*gln3*Δ *dal80*::*hisG*); lanes G and H, strain JCY48 (*gln3*Δ *ure2*Δ *dal80*::*hisG*); lanes I and J, strain JCY37 (*gln3*Δ *ure2*Δ). The strains were grown with proline (PRO) or glutamine (GLN) as described in Materials and Methods. Poly(A)$^+$ RNA (10 μg per lane) immobilized on a nylon membrane was hybridized with randomly primed *GAT1*-containing plasmid pTSC550; H4 was assayed as a control to determine loading and transfer efficiencies.

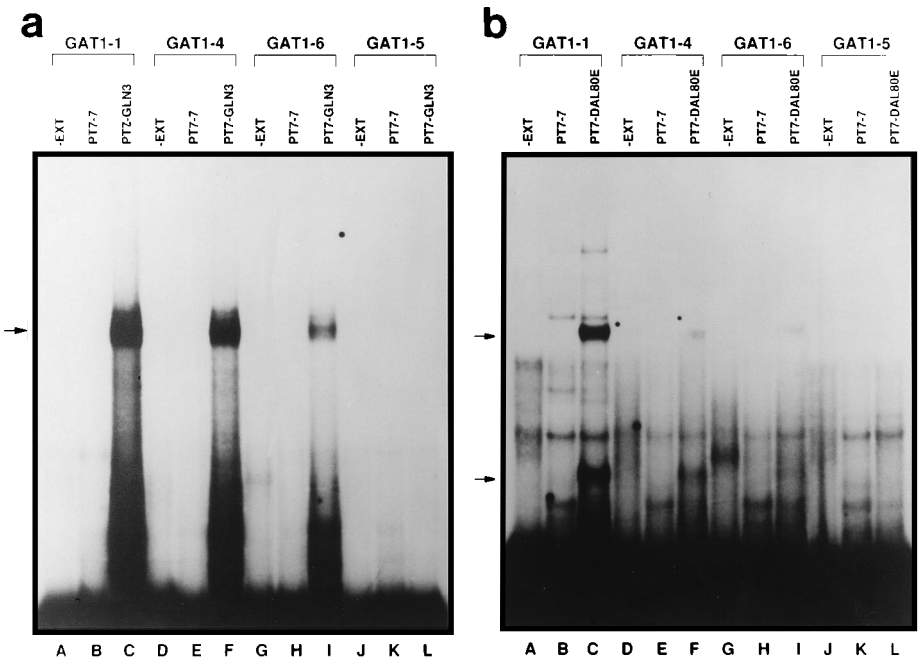

FIG. 7. Binding of Gln3p and Dal80p, produced in *E. coli*, to *GAT1* upstream sequences. (a) EMSA of *GAT1* DNA fragments with Gln3p; (b) EMSA of *GAT1* DNA fragments with Dal80p. Lanes A, D, G, and J, no added protein extract (−EXT) lanes B, E, H, and K, extracts from cells harboring plasmid pT7-7; lanes C, F, I, and J, protein extracts from cells expressing either Gln3p (pT7-Gln3) or Dal80p (pT7-DAL80E). The sequences of the wild-type and mutant *GAT1* DNA fragments were as follows: GAT1-1, tcgacACAACGGGCTGATAAGGGAGAAGATAAGATAAGATAACAAATCATTGCGTCCGACg; GAT1-4, tcgacACAACGG GCTggatccGGAGAAGATAAGATAAGATAAGATAACAAATCATTGCGTCCGACg; GAT1-5, tcgacACAACGGGCTggtaccGGAGAActagactagactagactagagAA ATCATTGCGTCCGACg; GAT1-6, tcgacACAACGGGCTGATAAGGGAGAActagactagacttaaGATAACAAATCATTGCGTCCGACg (all of the DNA fragments were double stranded, even though only one strand is given; added nucleotides [at the termini] that can be used for cloning purposes that were not part of the *GAT1* sequences and mutant nucleotides [within the sequences] are lowercased).

and F, and 5, lanes D and E) than it was in a Dal80$^+$ background (Fig. 5, lanes A, C, and F to I). We also noted that *GAT1* expression in a *gln3*Δ *ure2*Δ double mutant was surprisingly strong (Fig. 6, lanes I and J) compared with responses observed with *gln3*Δ and *ure2*Δ single mutants (Fig. 5, lanes F, G, J, and K, and 6, lanes A and B).

In sum, *GAT1* expression responded to genetic and physiological alterations of nitrogen metabolic control in a manner similar in most respects to those observed for the permease genes mentioned above, including NCR-sensitive expression in the triple mutant. Therefore, in contrast to the constitutive expression of *GLN3*, *GAT1* expression responded to the quality of the nitrogen source and was regulated much like a typical nitrogen-catabolic gene.

Gln3p and Dal80p are able to bind UAS_{NTR}/URS_{GATA}-homologous sequences upstream of *GAT1*. The Gln3p requirement demonstrated for *GAT1* expression in Fig. 5, lanes A and F, suggested that *GAT1* would be expected to possess one or more Gln3p binding sites in its upstream region. *GAT1* sequences between positions −245 and −205 (Fig. 2, arrows) exhibit strong homology to sites previously shown to bind Gln3p and Dal80p produced in *E. coli* (23–25). There is a GATAAG sequence separated by 7 bp from four consecutively repeated GATAA sequences (Fig. 2, arrows); this arrangement of tandem GATAA sequences resembles those in *DAL3* and *UGA4* (2, 23, 24). To ascertain whether Gln3p was able to bind the *GAT1* UAS_{NTR}-homologous sequences, we prepared a 70-bp double-stranded oligonucleotide (GAT1-1) and used it in an EMSA for Gln3p binding. As shown in Fig. 7a (lanes A to C), an altered-mobility species was observed when the DNA probe was incubated with an *E. coli* extract derived from a strain carrying an epitope-tagged version of Gln3p (plasmid pT7-GLN3E) (lane C). This species was not observed when the extract was derived from cells carrying only the T7 vector (plasmid pT7-7) (Fig. 7a, lane B) or when no extract was added to the complexation mixture (lane A). A *DAL3-5* DNA fragment previously shown to contain a strong Gln3p binding site (25) was used as the positive control for this experiment. As expected, an altered-mobility species similar to the one in lane C was also observed (data not shown).

To determine whether it was the UAS_{NTR}-homologous sequences contained on DNA fragment GAT1-1 that were responsible for Gln3p binding, we prepared and assayed several *GAT1* fragments in which one or more of the GATA sequences were mutated. In DNA fragment GAT1-4, the isolated 5′-most GATAAG sequence was mutated. As shown in Fig. 7a (lanes D to F), this mutation reduced Gln3p binding by about one-third to one-half of that observed with fragment GAT1-1. Mutation of the 5′ three of the four consecutively repeated GATAA sequences (fragment GAT1-6) resulted in a dramatic decrease in Gln3p binding (Fig. 7a, lanes G to I). Mutation of all five GATAA sequences on DNA fragment GAT1-1 (fragment GAT1-5) resulted in total elimination of Gln3p binding (Fig. 7a, lanes J to L). In sum, these data argue that Gln3p will bind to *GAT1* upstream sequences homologous to UAS_{NTR}.

Similarly, increased *GAT1* expression observed when the *DAL80* locus was disrupted (Fig. 5, lanes A and D) suggested that *GAT1* would also be expected to possess one or more Dal80p binding site(s) in its upstream region in addition to the Gln3p sites just described. Therefore, DNA fragments used in the above experiment were also tested for their ability to bind Dal80p produced in *E. coli* (23). As shown in Fig. 7b (lanes A to C), an altered mobility species was observed when the wild-

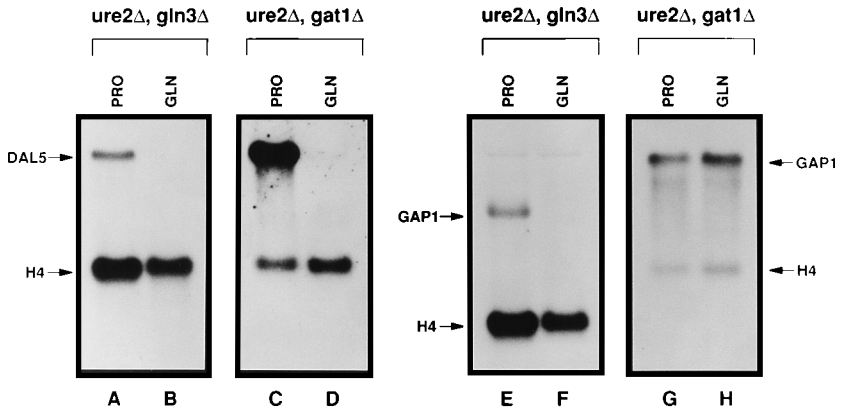

FIG. 8. Effect of a *ure2* deletion in a *gln3*Δ or *gat1*Δ background on *DAL5* (lanes A to D) and *GAP1* (lanes E to H) mRNA levels. Lanes: A, B, E, and F, strain JCY37 (*ure2*Δ *gln3*Δ); C, D, G, and H, strain JCY55 (*ure2*Δ *gat1*Δ). The strains were grown with proline or glutamine as the sole nitrogen source as described in Materials and Methods. Poly(A)$^+$ RNA (10 μg per lane) immobilized to a nylon membrane was probed as described for Fig. 3 and 4.

type GAT1-1 DNA fragment was incubated with an *E. coli* extract derived from a strain carrying an epitope-tagged version of Dal80p (plasmid pT7-DAL80E) (Fig. 7b, lane C). This species was not observed when the extract was derived from cells carrying only the T7 vector (plasmid pT7-7) (Fig. 7b, lane B) or when no extract was added to the reaction mixture (Fig. 7b, lane A). The DAL3-5 DNA fragment, previously shown to contain a strong Dal80p binding site (23), was again used as the positive control for this experiment. As expected, an altered-mobility species similar to the one in lane C was observed (data not shown). We have not thus far been able to determine why there are so many additional signals when Dal80p binding is assayed compared with Gln3p assays.

Dal80p complex formation with mutant *GAT1* fragments yielded results that were quite different from those for Gln3p. A prominent DNA-protein complex was observed only with the wild-type GAT1-1 fragment (Fig. 7b, lanes A to C). The GAT1-4 and GAT1-6 DNA fragments yielded complexes that were barely detectable, and no detectable complex was observed with DNA fragment GAT1-5 (Fig. 7b, lanes D to L). These data argue that the *GAT1* upstream sequences homologous to URS_{GATA} bind Dal80p and that at least two GAT AAG sequences are required for strong binding. They are also consistent with previous reports suggesting that Dal80p strongly binds only to a subset of UAS_{NTR} sequences (24) and, hence, its binding characteristics are more restrictive than those of Gln3p.

The existence of a GATA zinc finger motif in Gat1p and the requirement of Gat1p for NCR-sensitive expression of genes whose transcription has been previously shown to be mediated by the 5′-GATAAG-3′-containing UAS_{NTR} element raised the possibility that Gat1p was capable of binding to GATAAG sequences. Therefore, we cloned the coding sequence of *GAT1* downstream of the T7 promoter in the same way as that previously used for *DAL80* and *GLN3* (23, 25). The structure of the construction was verified by DNA sequence analysis and with the plasmid used to transform *E. coli* BL21(DE), containing T7 polymerase. To our dismay, no transformants could be obtained, even though a lawn of transformants were obtained when *E. coli* BL21, which cannot express T7 genes, was used as the recipient (52, 53). These observations suggested that expression of *GAT1* in *E. coli* was highly toxic to the cell. Therefore, we included either plasmid pLysS or plasmid pLysE in the transformation; the Lys gene contained in these plasmids has been reported to strongly repress T7 polymerase function (52, 53). We expected that tighter control of T7 gene expression prior to the time of its induction would permit the cells to survive. Unfortunately, it did not, and transformants could not be obtained except with control plasmids. Therefore, it is not possible to ascertain at this time whether Gat1p binds to GATAAG-containing sequences.

Does Ure2p negatively regulate Gat1p function in the same way it regulates Gln3p? Ure2p is proposed to be a nitrogen source-responsive negative regulator of Gln3p operation (21). In view of this proposal, our objective was to determine whether or not deletion of *URE2* permitted Gat1p-mediated transcription to occur in the presence of a readily used nitrogen source as previously reported for Gln3p. Since we had not identified a nitrogen-catabolic gene whose expression requires only either Gat1p or Gln3p but not both proteins, we constructed *gln3*Δ *ure2*Δ (JCY37) and *gat1*Δ *ure2*Δ (JCY55) double-mutant strains. In strain JCY55 the effects of Ure2p on Gln3p operation could be assayed, and in strain JCY37 the effects of Ure2p on Gat1p operation could be assayed. The mutant strains were each grown in glucose-proline and glucose-glutamine media, and the cultures were used to prepare poly(A)$^+$ RNA. Figure 8 (lanes A to D) depicts the outcome of this experiment for *DAL5* expression. It is important to recall that *DAL5* steady-state mRNA levels in *ure2*Δ single-mutant cultures provided with glutamine as nitrogen source were less than those observed when proline was provided as nitrogen source (Fig. 2, lanes J and K, in reference 12 and Fig. 6, lanes I and J, in reference 13). In other words, loss of Ure2p did not result in the acquisition of complete NCR insensitivity. The *gat1*Δ *ure2*Δ double mutant (JCY55) contained a high level of *DAL5* mRNA when proline was the nitrogen source, but none was detectable when glutamine was provided instead (Fig. 8, lanes C and D). When the same experiment was performed with strain JCY37 (*gln3*Δ *ure2*Δ), again *DAL5* mRNA could be detected only when proline was the nitrogen source (Fig. 8, lanes A and B). The *DAL5* mRNA level in proline-grown *ure2*Δ *gln3*Δ cells, however, was significantly less (estimated relative to the H4 mRNA level) than that in the *gat1*Δ *ure2*Δ double mutant (JC55) (lane A); this result was expected from the data presented in Fig. 3. If either Gln3p or Gat1p was eliminated along with Ure2p by mutation, *DAL5* transcription was repressed when glutamine was the nitrogen source. These data demonstrate that elimination of Ure2p and its negative regulation of *DAL5* expression when glutamine was used as nitrogen source was insufficient to permit Gln3p-dependent

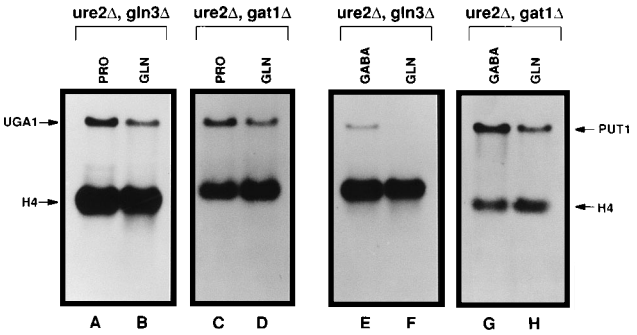

FIG. 9. Effect of a *ure2* deletion in a *gln3*Δ or *gat1*Δ background on steady-state levels of *UGA1* (lanes A to D) and *PUT1* (lanes E to H) mRNAs. The format and conditions of the experiment were the same as for Fig. 8, except that GABA was used in place of proline where indicated.

DAL5 transcription to occur in the absence of Gat1p. Stated in another way, deletion of *GAT1* suppressed the phenotype of a *ure2*Δ mutation (*DAL5* expression with asparagine or glutamine as nitrogen source). As these results are interpreted, it is also important to keep in mind that *DAL5* expression exhibited a greater requirement for Gln3p than Gat1p (Fig. 3).

We determined whether the phenotypes of *gln3*Δ *ure2*Δ and *gat1*Δ *ure2*Δ double mutants were general or restricted to *DAL5*. *GAP1* expression was decreased about 50% when either *GLN3* or *GAT1* was deleted (Fig. 3). As shown in Fig. 8, *GAP1* expression differed from that of *DAL5*. In a *gat1*Δ *ure2*Δ double mutant the levels of *GAP1* expression with proline and glutamine as nitrogen sources were about the same (Fig. 8, lanes G and H), i.e., GAP1 expression was not NCR sensitive in the *ure2*Δ *gat1*Δ double mutant. When the experiment was repeated with a *gln3*Δ *ure2*Δ double mutant, *GAP1* expression was observed with proline as nitrogen source but not with glutamine, i.e., in this mutant *GAP1* expression remained NCR sensitive. In a *ure2*Δ mutant, Gat1p function is not essential for loss of NCR sensitivity of *GAP1* expression.

Steady-state *UGA1* and *PUT1* mRNA levels were also analyzed in the double-mutant strains. *UGA1* expression occurred in both the *ure2*Δ *gln3*Δ (JCY37) and the *ure2*Δ *gat1*Δ (JCY55) double-mutant strains, and similar levels of expression were observed regardless of the nitrogen source used (Fig. 9, lanes A to D). In contrast, *PUT1* expression was observed when GABA was provided as nitrogen source to a *ure2*Δ *gln3*Δ double mutant but not when glutamine was used instead (Fig. 9, lanes E and F), i.e., *PUT1* expression was still NCR sensitive. Deletion of *URE2* and *GAT1* resulted in loss of NCR sensitivity, i.e., *PUT1* expression was observed regardless of the nitrogen source provided (Fig. 9, lanes G and H); the response was similar to that observed with *GAP1*.

UGA4 expression was highly sensitive to deletion of either *GLN3* or *GAT1*. The *UGA4* mRNA signal was below detection in a *gln3*Δ mutant and barely detectable in a *gat1*Δ mutant; no signal could be detected in the double mutant (Fig. 4b, lanes A to D). As shown in Fig. 4b, only minimal expression was observed when either the *ure2*Δ *gln3*Δ or the *ure2*Δ *gat1*Δ double mutant was provided with proline as nitrogen source (lanes E to H). When glutamine was provided to these strains in place of proline, no expression could be detected at all, indicating that residual expression in proline medium was still NCR sensitive. In sum, the characteristics of *UGA4* expression were similar to those of *DAL5* (Fig. 8).

The above results with various nitrogen-catabolic genes prompted us to inquire about the expression of *GAT1* in a *gln3*Δ *ure2*Δ double mutant. *GAT1* mRNA was produced in a *gln3*Δ *ure2*Δ double-mutant strain (JCY37) but only when proline was the nitrogen source; mRNA could not be detected in cultures growing in glucose-glutamine medium (Fig. 6, lanes I and J). In contrast, *GAT1* mRNA was present in a *ure2*Δ single-mutant strain (JCY125) provided with either proline, asparagine, or glutamine as nitrogen source (Fig. 5, lanes J and K, and Fig. 6, lanes A and B).

Gat1p supports transcriptional activation. As will be discussed later, the Gat1 and Gln3 proteins share significant homology. This observation and the recent report that Gln3p is able to activate transcription when tethered upstream of a reporter gene lacking UAS elements (25) raised the possibility that Gat1p might also possess that capability. Consequently, we constructed a *GAT1-lexA* fusion plasmid (pVS11) and used it to transform strain EGY48 containing reporter plasmid p1840. Plasmid p1840 contained *lacZ* fused downstream of a *CYC1* core promoter in which one *lexA* binding site replaced the UAS elements (5, 32). As shown in Fig. 10, *lexA* alone (expression from plasmid pEG202) was able to support only minimal β-galactosidase production. A *lexA-GAT1* fusion plasmid (pVS11) supported 20- to 30-fold-greater *lacZ* expression (Fig. 10). The ability of Gat1p to support transcriptional activation was, however, only a small fraction of that observed (2,263 to 2,333 U of β-galactosidase produced) when an analogous *GLN3-lexA* plasmid (pVS32) was assayed in the same way (Fig. 10). Although transcriptional activity of a DNA binding (*lexA*)-tagged protein cannot be a priori identified with its function in vivo, a number of yeast transcription factors have been shown to retain their pattern of physiological responses and protein-protein interactions when assayed as *lexA* fusions.

The proposed role of Gat1p in NCR warranted inquiry into whether its ability to activate transcription responded to the quality of nitrogen source available. When a lexA-Gat1p fusion was assayed in association with reporter plasmid pSH18-34 with four *lexA* operators preceding the minimal *CYC1* promoter upstream of a *lacZ* gene, the level of expression it supported increased two- to threefold when cells were grown with proline as sole nitrogen source compared with cells utilizing a preferred nitrogen source (glutamine). When the less sensitive reporter plasmid p1840 was used, the difference was less prominent.

Growth phenotype of a *gat1*Δ mutant. To ascertain whether a *gat1*-null mutant possessed a demonstrable growth phenotype, we grew strain RJ71 in Wickerham's minimal medium with allantoin, proline, citrulline, or ammonia as sole nitrogen source under conditions similar to those used in the Northern blot experiments. Growth of wild-type and *gat1*Δ mutant cells was the same whether proline, citrulline, or ammonia was provided as sole nitrogen source. When allantoin was used as sole nitrogen source, however, the doubling time of the *gat1*Δ cells increased by 1 h, from 270 min for the wild type to 324 min for the mutant.

DISCUSSION

Over several years, numerous observations have pointed to a major inconsistency with the prevailing model of nitrogen regulation, i.e., Gln3p was unique in its ability to support NCR-sensitive expression of nitrogen-catabolic genes in *S. cerevisiae* (12, 13, 26). In this report, a transcription factor designated Gat1p was identified and characterized. Like Gln3p, it possesses a GATA zinc finger motif, is required for NCR-sensitive gene expression, and exhibits the potential for weak transcriptional activation. Although both Gln3p and Gat1p are required for full inducer-independent expression of the genes we tested,

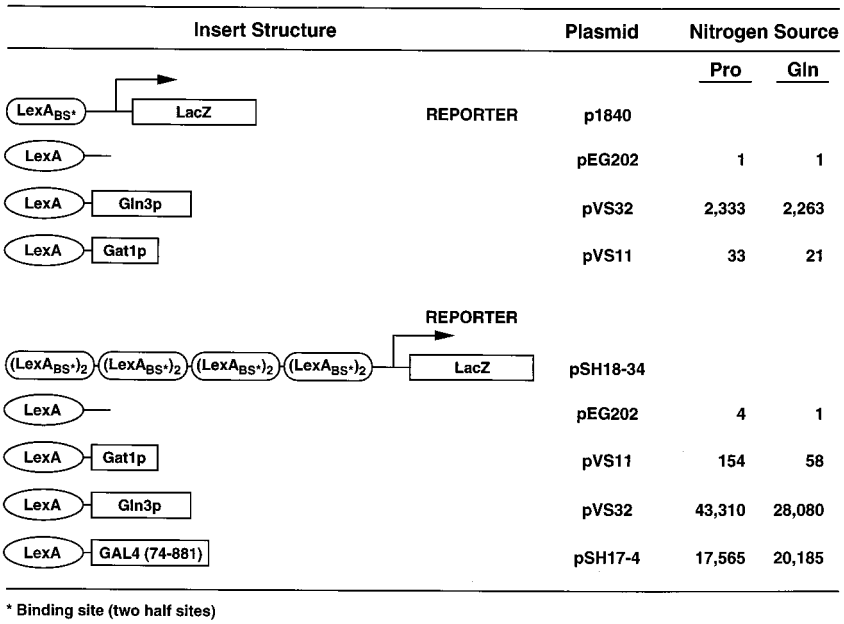

FIG. 10. Transcriptional activation supported by Gln3p and Gat1p. Transcriptional activities of LexA-Gat1p and LexA-Gln3p fusions were assayed by measuring the levels of β-galactosidase produced in cells (strain EGY48) transformed with plasmids pVS11 and pVS32, respectively. Cultures used for these measurements were grown in the presence of either proline or glutamine as nitrogen source. Plasmid pEG202 was used as a control to assess the level of basal activation derived from the binding of LexA alone. Reporter plasmids p1840 and pSH18-34 contained, respectively, one and four *lexA* binding sites upstream of the *lacZ* gene.

the extent to which the two transcription factors are individually required is gene specific. *DAL5* expression, for example, exhibited a greater requirement for Gln3p than Gat1p, while GAP1 expression was about equally dependent upon both proteins. The diversity of responses to deletion of *GLN3* or *GAT1* argues that their products participate to different degrees in the transcription of the various nitrogen-catabolic genes. These results are consistent with the suggestion that Gln3p and Gat1p probably possess overlapping as well as distinct binding specificities, as hypothesized earlier (16). Although Gat1p has not yet been shown to bind DNA, the presence of the zinc finger motif homologous, but not identical, to those of the GATA family of DNA-binding proteins including Dal80p and Gln3p (Fig. 11) is certainly consistent with such a capability.

The regulation of *GAT1* expression parallels that of *DAL80*; it is largely NCR sensitive, Gln3 dependent, and Dal80p regulated (22). These characteristics correlate with the patterns of UAS_{NTR} and URS_{GATA} elements in the *GAT1* promoter; they are similar to those previously reported for *DAL3*, *DAL80*, and *UGA4* (2, 23, 24). When control of GAT1p production is incorporated into our current view of nitrogen-catabolic gene regulation, the picture in Fig. 12 emerges. A balance occurs between activation and repression of nitrogen-catabolic gene expression because not only is the level of transcriptional repressor Dal80p positively regulated by Gln3p operation, so too is its transcriptional activation mediated by the Gln3p analog Gat1p. Additionally, *GAT1* is negatively regulated by Dal80p. By these mechanisms basal-level nitrogen-catabolic gene expression is tightly regulated as a result of the specific *cis*-acting elements situated in individual promoters, yet the genes are simultaneously and exquisitely responsive to nitrogen source quality.

Although we have learned much about the role Gat1p plays in nitrogen-catabolic gene expression and the regulated expression of *GAT1*, we have not yet been able to experimentally demonstrate the physiological regulatory advantage that accrues to a cell as a result of Gat1p's participation in the regulation of nitrogen-catabolic gene expression. A possible interaction that may be involved in this physiological function is the one between Gln3p and Gat1p. This is pertinent because previous studies of promoter sequences participating in NCR-sensitive, Gln3p-dependent transcription have shown that a single GATAAG sequence is alone insufficient for transcriptional activation (42, 43, 46). Two separated GATAAG sequences must be present as in the *DAL5*, *DAL3*, *UGA4*, and *DAL7* genes (16). Alternatively, there must be one GATAAG sequence and another nearby sequence that can serve as the target for a second transcription factor such as we have shown for *PUT1* and *GLN1* promoter fragments (43). The question these observations raise is whether Gln3p can combine with

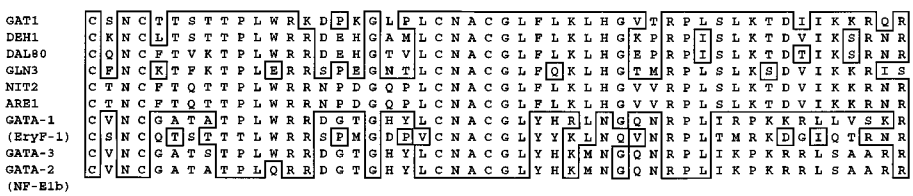

FIG. 11. Homology of deduced amino acid sequences in proximity to the zinc finger motif of a number of GATA family DNA-binding proteins. *DEH1* is the *DAL80* homolog reported by Rasmussen (47).

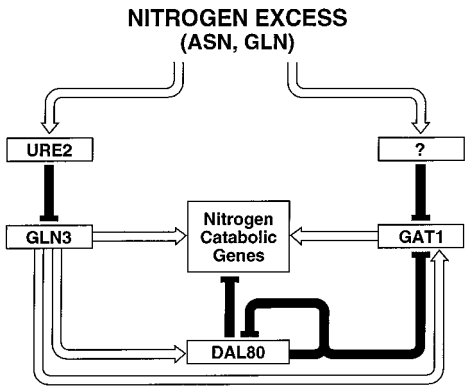

FIG. 12. Working model of the regulatory circuit for activation and repression of nitrogen-catabolic genes in *S. cerevisiae*. Arrows, positive regulation; closed bars, negative regulation.

Gat1p to form a productive transcriptional complex. One observation may be interpreted to favor the existence of such a Gln3p-Gat1p combination: the mutant data obtained with *DAL5* and *UGA4* expression (Fig. 3 and 4). Deletion of either *GLN3* or *GAT1* resulted in dramatic losses of *DAL5* and *UGA4* mRNAs. In other words, the contributions of Gln3p and Gat1p to *DAL5* and *UGA4* expression are more than additive.

The experiments described in this work also contribute to our knowledge of Ure2p operation. Three graded responses of gene expression were observed following deletion of the *URE2* and *GAT1* genes or the *URE2* and *GLN3* genes. The first pattern is represented by *UGA1* expression. *UGA1* was expressed at similar levels in both double mutants, and that expression was not affected by the nature of the nitrogen source provided; the levels of expression were the same whether glutamine or proline was provided. The second pattern is represented by *PUT1* and *GAP1* expression. Here deletion of *URE2* in a Gat1$^+$ genetic background was insufficient to permit expression of *PUT1* and *GAP1* when glutamine was provided as nitrogen source. However, when *URE2* was deleted in a Gln3$^+$ background, expression of the *PUT1* and *GAP1* genes was observed in glutamine-containing medium. The third pattern was that of *DAL5* and *UGA4*, in which deletion of *URE2* was insufficient to permit expression when glutamine was provided as nitrogen source regardless of whether Gln3p or Gat1p was present. These data suggest that, in analogy to the situation with Gln3p, Ure2p is not a unique regulator, i.e., NCR sensitivity is not abolished when Ure2p is lost. There appear to be more than one protein with a function analogous to that of Ure2p.

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