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Cross Regulation of Four GATA Factors That Control Nitrogen Catabolic Gene Expression in *Saccharomyces cerevisiae*

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Nitrogen catabolic gene expression in *Saccharomyces cerevisiae* has been reported to be regulated by three GATA family proteins, the positive regulators Gln3p and Gat1p/Nil1p and the negative regulator Dal80p/Uga43p. We show here that a fourth member of the yeast GATA family, the Dal80p homolog Deh1p, also negatively regulates expression of some, but not all, nitrogen catabolic genes, i.e., *GAP1*, *DAL80*, and *UGA4* expression increases in a *deh1*Δ mutant. Consistent with Deh1p regulation of these genes is the observation that Deh1p forms specific DNA-protein complexes with GATAA-containing *UGA4* and *GAP1* promoter fragments in electrophoretic mobility shift assays. Deh1p function is demonstrable, however, only when a repressive nitrogen source such as glutamine is present; *deh1*Δ mutants exhibit no detectable phenotype with a poor nitrogen source such as proline. Our experiments also demonstrate that GATA factor gene expression is highly regulated by the GATA factors themselves in an interdependent manner. *DAL80* expression is Gln3p and Gat1p dependent and Dal80p regulated. Moreover, Gln3p and Dal80p bind to *DAL80* promoter fragments. In turn, *GAT1* expression is Gln3p dependent and Dal80p regulated but is not autogenously regulated like *DAL80*. *DEH1* expression is largely Gln3p independent, modestly Gat1p dependent, and most highly regulated by Dal80p. Paradoxically, the high-level *DEH1* expression observed in a *dal80*::*hisG* disruption mutant is highly sensitive to nitrogen catabolite repression.

GATA family DNA-binding proteins are present in organisms from *Saccharomyces cerevisiae* to humans (3, 12, 21, 26, 29, 35, 36, 49, 57, 62, 66). These proteins contain one or more characteristic C_4 zinc finger motifs and bind to DNA sequences with the sequence GATA at their core (3, 5, 22, 24, 31, 32, 35, 36, 62, 64). The three-dimensional structure of the C_4 zinc finger has previously been reported (41). In well studied cases, multiple GATA family proteins with related DNA-binding specificities and regulatory functions have been found (62, 64, 65). *S. cerevisiae* has four such GATA factors, Gln3p, Gat1p/Nil1p, Dal80p/Uga43p, and a fourth GATA protein introduced here, the Dal80p homolog Deh1p (YJL110c) (29b, 45). The GATA family proteins of *Aspergillus nidulans* (*areA*), *Neurospora crassa* (*nit2*), and *Penicillium chrysogenum* (*Nre*) function similarly to Gln3p of *S. cerevisiae* (29a, 30a, 41b). Homologs of yeast Gat1p, Dal80p, and Deh1p, however, have not yet been reported for filamentous fungi.

In yeast as in higher eucaryotes, the GATA factors possess related functions. In *S. cerevisiae*, they are responsible for regulated expression of nitrogen catabolic genes (6, 9, 11, 12, 13, 16, 18, 20, 21, 25, 40, 42, 51–53, 56, 58, 63). When preferentially used nitrogen sources (asparagine, glutamine, or ammonia in some strains) are available to cells, genes encoding permeases and enzymes required for the uptake and degradation of poor nitrogen sources (proline or allantoin) are expressed at low levels. When only poor nitrogen sources are available, these genes are expressed at much higher levels. This physiological control is designated nitrogen catabolite repression (NCR) (14, 15, 59) and is the means by which *S. cerevisiae* selectively utilizes various nitrogen sources (14, 15, 59). Control of NCR-sensitive gene expression in *S. cerevisiae* is accomplished by GATA family transcriptional activation factors (Gln3p and Gat1p) which bind to *cis*-acting elements, UAS_{NTR} (5, 6, 22–24, 44, 53). UAS_{NTR}, a dodecanucleotide element with the sequence GATAA at its core (6), has been shown to be the binding site for Gln3p (5, 24) and to mediate NCR-sensitive transcription (6, 17). The binding site for Gat1p has not yet been determined, but the presence of a GATA-type zinc finger in Gat1p suggests that it contains a GATA sequence as well (12).

NCR-sensitive transcription supported by Gln3p and Gat1p is downregulated by Ure2p when cells are grown in the presence of a good nitrogen source (2, 10, 11, 19, 20, 27, 28, 63). This conclusion is supported by observations that (i) mutation of Ure2p results in increased expression of some, but not all, NCR-sensitive genes when a good nitrogen source is present (10) and (ii) *gln3p* mutations are epistatic to *ure2* mutations (20). A comparison of the deduced Ure2p sequence to sequences in databases reveals a limited homology to glutathione *S*-transferase, an observation interpreted as suggesting that its biochemical function is one of posttranslational modification (19). More recently, Ure2p has also been suggested to bind to Gln3p directly (4). However, the actual biochemical role of Ure2p in NCR has not been determined. Wickner has clearly demonstrated that Ure2p is a member of the prion family of proteins (61). Preliminary structure-function mapping of Ure2p indicates that the N-terminal region is responsible for prion formation and that the portion of Ure2p required for its role in NCR is restricted to the C terminus (61). The role of Ure2p as a negative regulator in NCR is not, however, unique. Downregulation of NCR-sensitive gene expression mediated by either Gln3p or Gat1p has also been shown to occur normally in *ure2* deletion mutants (9–13).

Downregulation of NCR-sensitive gene expression is also accomplished by the action of GATA family member Dal80p, as evidenced by the observation that the deletion of *DAL80*

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TABLE 1. Strains used in this work

Strain	Genotype
S. cerevisiae	
TCY1	*MATα lys2 ura3*
RR91	*MATα ura3 lys2 gln3::hisG*
RJ71	*MATα lys2 ura3 gat1Δ::hisG URA3 hisG*
RJ715	5-FOA-resistant derivative of RJ71
RJ72	*MATα lys2 ura3 gln3Δ::hisG gat1Δ::hisG URA3 hisG*
JCY37	*MATα lys2 ura3 gln3Δ::hisG ure2Δ::URA3*
JCY55	*MATα lys2 ura3 gat1Δ::hisG ure2Δ::URA3*
JCY125	*MATα lys2 ura3 ure2Δ::URA3*
JCY62	*MATα lys2 ura3 dal80::hisG gat1Δ::hisG URA3 hisG*
JCY625	5-FOA-resistant derivative of JCY62
TCY17	*MATα lys2 ura3 dal80::hisG*
EGY48	*MAT***a** *3lexAop::LEU2 ura3 trp1 his3*
JCY23	*MATα lys2 ura3 dal80::hisG ure2Δ::URA3*
RR92	*MATα lys2 ura3 gln3Δ::hisG dal80::hisG*
JCY48	*MATα lys2 ura3 gln3Δ::hisG dal80::hisG ure2Δ::URA3*
RJ81	*MATα ura3 deh1Δ::hisG URA3 hisG*
RJ815	5-FOA-resistant derivative of RJ81
RJ82	*MATα ura3 deh1Δ::hisG URA3 hisG dal80::hisG*
E. coli	
HB101	*supE44 hsdS20* ($r_B m_B$) *recA13 ara-14 proA2 lacY1 galK2 rpsl20 xyl-5 mtl-1*
DH5α	F′ *endA* [*hsdS20* ($r_k^- m_k^+$) *supE44 thi-1 recA*] *gyrA* (Nal$^+$) *re*[*A*] Δ(*lacZYA-argF*)*U169* [φ80dlacΔ(*lacZ*)M15]

results in increased expression levels of many NCR-sensitive genes when proline is provided as a nitrogen source. Although *dal80* and *ure2* mutations exhibit related phenotypes, i.e., increased gene expression, the physiological roles of Dal80p and Ure2p are distinct. Ure2p is not a GATA factor and is required for NCR of gene expression. Dal80p, on the other hand, is not demonstrably associated with NCR, i.e., the NCR sensitivity of nitrogen catabolic gene expression is not diminished or otherwise affected by *DAL80* deletion (8, 10–13, 25). The Dal80p binding site, URS_{GATA}, consists of a pair of GATA-containing sequences oriented tail-to-tail or head-to-tail 15 to 35 bp apart (22). As might be expected from these characteristics, there is significant functional overlap in the Gln3p and Dal80p binding sites, UAS_{NTR} and URS_{GATA}, respectively (22, 23). However, it is important to emphasize that the overlap is by no means complete. *DAL5* expression, for example, is Gln3p and Gat1p dependent but is only minimally Dal80p regulated (12, 21, 25).

One of the most intriguing characteristics of the yeast GATA family genes is the regulation of their expression by other GATA family members. Both *GAT1* and *DAL80* contain GATA sequences in their promoters (12, 21). *DAL80* and *GAT1* expression is both Gln3p dependent and Dal80p regulated (12, 21). It is not known, however, whether *DAL80* expression and *GAT1* expression are regulated by Gat1p. If *DAL80* expression was Gat1p dependent, the *GAT1* and *DAL80* genes would be reciprocally regulated in addition to being dependent upon Gln3p (5, 12, 24).

An open reading frame (ORF) of unknown function (YJL110c), whose cognate gene we designated *DEH1* (for *DAL80* homolog), exhibits significant homology to the Dal80p, Gat1p, and Gln3p proteins (12, 29a, 45, 51). A related shorter sequence (*NIL2*) was reported by Stanbrough, though no phenotype could be demonstrated when it was deleted (51). Although Deh1p is similar to all of the yeast GATA family proteins, the greatest homology is between it and Dal80p (both proteins possess GATA-type zinc finger and leucine zipper motifs [12, 29a, 45]), raising the possibility that Deh1p also functions as a negative regulator of NCR-sensitive gene expression. We observed multiple GATA sequences upstream of *DEH1*, further raising the possibility that this gene, like *DAL80* and *GAT1*, is regulated by other members of the yeast GATA family proteins (12).

The observations mentioned above point to a number of

FIG. 1. (Top) Oligonucleotides used for EMSAs. Only a single strand of double-stranded DNA fragments is shown. (Bottom) Nucleotide sequences of portions of the *DAL80* and *DEH1* upstream regions, indicating the positions of GATA sequences. The numbers between GATA sequences indicate the numbers of nucleotides between them. Arrows indicate the positions and orientations of GATA sequences.

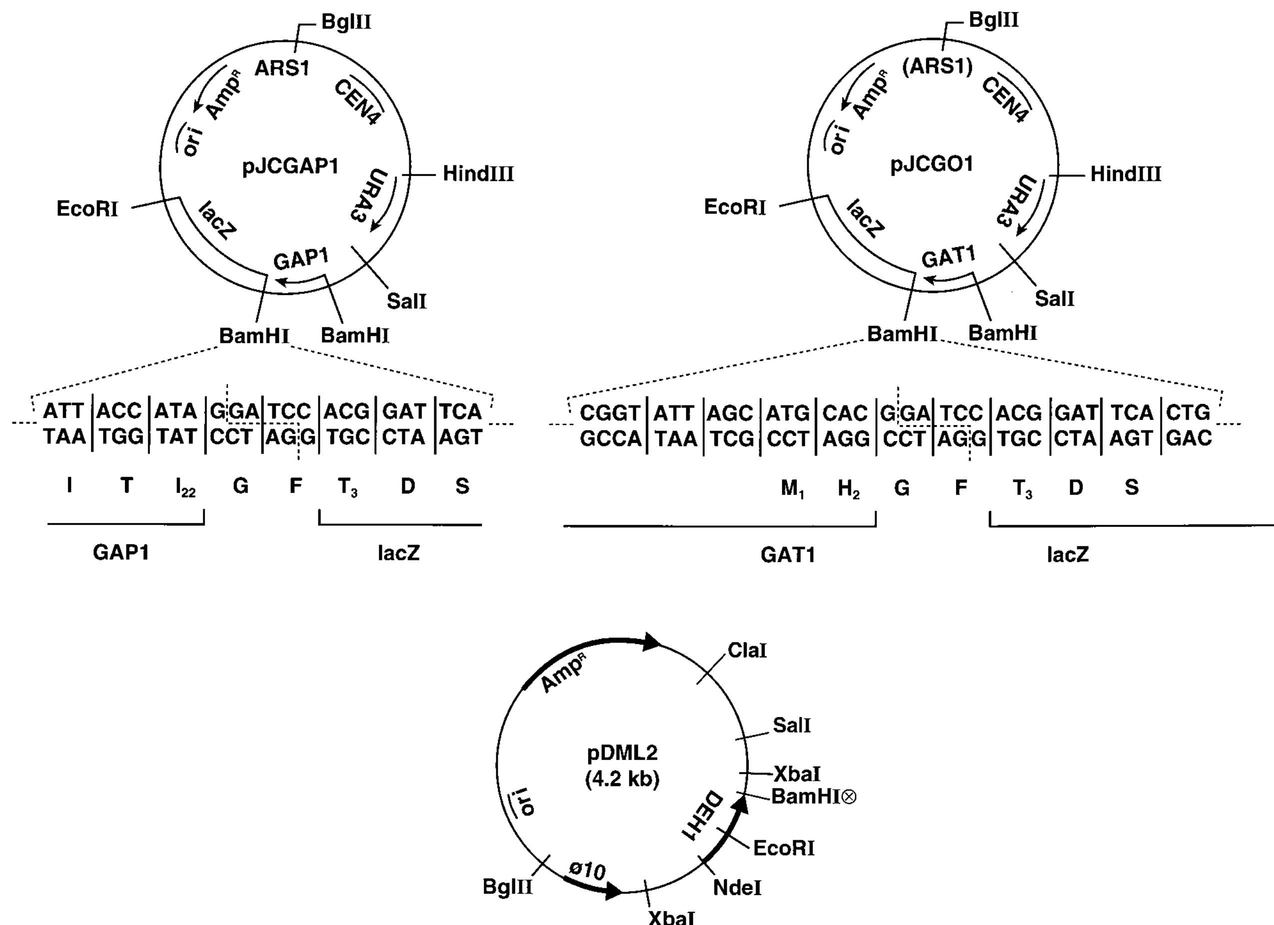

FIG. 2. Maps of the *GAP1-lacZ* and *GAT1-lacZ* fusion and DEH1 expression plasmids used in this work. ori, origin.

important but unanswered questions about GATA family gene function and expression. Is the autogenously regulated *DAL80* gene also regulated by Gat1p? Is *GAT1* expression autogenously regulated, as previously reported for *DAL80* (21)? Is Gat1p able to mediate transcriptional activation exclusively through the GATAA-containing UAS_{NTR} element? Is Deh1p a GATA-binding, negative regulator of NCR-sensitive gene expression, including expression of *DAL80* and *GAT1*? How is *DEH1* gene expression regulated? Our objective in this work was to answer these questions, thereby extending analysis of the Deh1p and Dal80p GATA family proteins and the regulated expression of their cognate genes.

MATERIALS AND METHODS

Cloning of *DEH1*. To clone the *DEH1* gene, a synthetic oligonucleotide (5′-TGATTCGGTACCTCGGATGGATTAGAGTCAGTCGTCCGTGAAGAAG TGGG-3′) homologous to the YJL110c or *NIL2* gene (29b, 45, 51) was radiolabeled on its end with ^{32}P. The probe was used to screen a YCp50-based yeast genomic library (ATCC 37415) (46) by standard protocols. One of seven clones isolated from approximately 60,000 colonies analyzed (plasmid pTSC525) was studied further.

Construction of *DEH1* deletion strains. The plasmid used for deletion of the *DEH1* gene was constructed as follows (47). A 4.1-kb *Cla*I-*Xba*I fragment from plasmid pTSC525 was subcloned into plasmid pBS(KS$^+$) (Strategene), yielding plasmid pTSC534. Plasmid pTSC534 was digested with *Eco*RI, the extension sequences were filled in with Klenow fragment, and a *Bgl*II linker (10-mer) was ligated into it. The resulting plasmid was digested with *Bgl*II, the 1.3-kb *Bgl*II-*Bgl*II fragment containing *DEH1* was deleted, and the plasmid was reclosed to yield plasmid pTSC538. Plasmid pTSC538 was linearized by digestion with *Bgl*II. The 3.8-kb *Bam*HI-*Bgl*II, *hisG-URA3-hisG* fragment from pNKY51 (1) was ligated into the linear form of plasmid pTSC538 to yield plasmid pRR346. Plasmid pRR346 was subsequently digested with *Sal*I and *Xba*I, and the 6.6-kb fragment was isolated and used for transformations. Wild-type strain TCY1 and *DAL80* disruption strain TCY17 (Table 1) were transformed with the linear DNA, and recombinants were selected for growth on selective media. Genomic Southern blot analysis was used to verify chromosomal deletions.

EMSAs. Electrophoretic mobility shift assays (EMSAs) were performed as described earlier (22–24); total crude protein extract, when present in reaction mixtures, was present at 1 μg per reaction mixture. The DNA fragments used as probes are shown in Fig. 1. Highly sheared calf thymus DNA was used in all reaction mixtures as nonspecific competitor DNA (final concentration, 200 μl/ml).

Northern blot analysis. Strains were grown in Wickerham's minimal medium (60) with 2% glucose and a nitrogen source supplied at a concentration of 0.1%. Uracil (20 mg/liter), L-glutamine (60 mg/liter), and L-lysine (40 mg/liter) were added as auxotrophic requirements when needed. Cultures were grown to mid-log phase (55 to 65 Klett units), as measured with a Klett-Summerson photoelectric colorimeter (green filter), and total RNA was prepared by the method of Carlson and Botstein (7). Poly(A)$^+$ RNAs were isolated by oligo(dT)-cellulose (Pharmacia) affinity chromatography, resolved in 1.4% agarose (SeaKem; FMC Bioproducts)–2% formaldehyde–morpholinepropanesulfonic acid (MOPS) gels, and transferred to GeneScreen Plus Nylon 66 membranes (Dupont, New England Nuclear Research Products) by capillary action with 10× SSC (1.5 M NaCl, 0.15 M sodium citrate). The membranes were probed with synthetic oligonucleotides radiolabeled by the polynucleotide kinase reaction (New England Biolabs, Inc., or Boehringer Mannheim) or with double-stranded DNA radiolabeled by random priming (Boehringer Mannheim or Promega). Hybridization conditions included 50% deionized formamide, 1 M NaCl, and 1% sodium dodecyl sulfate at 42°C for 12 to 20 h. Membrane washes were carried out as described earlier (12). All synthetic oligonucleotides used as probes for Northern blot analysis in this work have been described earlier.

Construction of *lacZ* fusion plasmids. Plasmid pJCG01 (Fig. 2) containing 695 bp upstream of *GAT1* fused to *lacZ* was prepared as follows. A 695-bp *GAT1* fragment was prepared by PCR amplification with oligonucleotides JC001

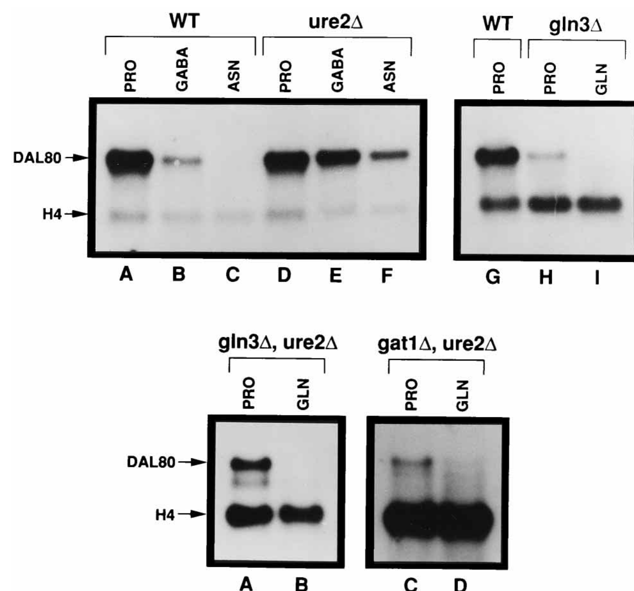

FIG. 3. (Top) Northern blot analysis of *DAL80* steady-state mRNA levels in wild-type (WT), *gln3*Δ, and *ure2*Δ strains with glutamine (GLN), asparagine (ASN), GABA, or proline (PRO) as the sole nitrogen source. (Bottom) Northern blot analysis of *DAL80* steady-state mRNA levels in *gln3*Δ *ure2*Δ and *gat1*Δ *ure2*Δ double mutants with glutamine or proline as the sole nitrogen source. Wild-type strain TCY1 (lanes A to C and G), *ure2*Δ strain JCY125 (lanes D to F), *gln3*Δ strain RR91 (lanes H and I), *gln3*Δ *ure2*Δ strain JCY37 (lanes A and B), and *gat1*Δ *ure2*Δ strain JCY55 (lanes C and D) cells were used in this experiment. Poly(A)$^+$ RNA (10 μg per lane) immobilized on nylon membranes was hybridized with randomly primed plasmid pTSC319 DNA for the measurement of *DAL80* mRNA. A 5′- and ^{32}P-labeled synthetic oligonucleotide complementary to the histone H4 gene (48) was used as a control to determine loading efficiency.

(5′-GTCGACCGGATCCACCCCTGATAAAA-3′) and JC002 (5′-AGCAAA GGAAAGGATCCGTGCATGCTAATA-3′) as primers, plasmid pTSC542 (12) as template, and PWO DNA polymerase (Boehringer Mannheim). The resulting PCR product was isolated, digested with *Bam*HI, and ligated into plasmid pVAN2 digested with *Bam*HI. Plasmid pVAN2 is a derivative of plasmid pHP41 (41a) in which the 1.3-kb *Sma*I-*Bam*HI fragment containing the upstream region of *CYC1* has been replaced with a *Bam*HI linker. The *lacZ* fusion site is located 7 bp downstream of the *GAT1* ATG. Plasmid pJCGAP1 (Fig. 2), containing 683 bp upstream of *GAP1* fused to *lacZ*, was prepared as follows. The 683-bp *GAP1* fragment was prepared by PCR amplification with oligonucleotides JC022 (5′-CATGGGATCCGTCGGCGACTCATT-3′) and JC023 (5′-GTTAGAAACTC GGATCCTATGGTA-3′) as primers and chromosomal DNA (strain TCY1) as template. The resulting PCR product was isolated, digested with *Bam*HI, and ligated into plasmid pHP41 digested with *Bam*HI. Plasmid pJCD52 was constructed by ligating the 2.6-kb *Sal*I-*Sac*I fragment of plasmid pRR30, containing the *DAL5* upstream region and part of the *lacZ* gene (44), into plasmid pHP41 digested with *Sal*I and *Sac*I (41a).

Construction of the pT7-DEH1 plasmids used for production of Deh1p in *Escherichia coli*. A T7-*DEH1* fusion plasmid was constructed to permit the production of Deh1p in *E. coli* (22, 55). To this end, plasmid pTSC534 was digested with *Eco*RI and *Bgl*II and the 1.37-kb *Eco*RI-*Bgl*II fragment containing the 3′ end of *DEH1* was isolated and ligated into the backbone of plasmid pT7-7 (22) (digested with *Bam*HI and *Eco*RI) to yield the 3.9-kb plasmid pDML1. A 342-bp *Nde*I-*Eco*RI DNA fragment containing the 5′ end of *DEH1* was prepared by PCR and ligated into plasmid pDML1 digested with *Nde*I and *Eco*RI to yield the 4.2-kb plasmid pDML2 (Fig. 2). The structures of the restriction endonuclease joints and PCR product were verified by restriction site and DNA sequence analysis.

β-Galactosidase assays. Strains TCY1 (wild type) and RJ815 (*deh1*Δ) were transformed by the method of Ito et al. (33) and spread on selective medium. Positive transformants were restreaked, patched, and grown to an A_{600} of 0.4 to 0.9 in YNB medium (0.17% yeast nitrogen base without amino acids or ammonium sulfate) (Difco Laboratories) supplemented with glucose and proline (Pro) or glutamine (Gln) as the sole nitrogen source at final concentrations of 2.0 and 0.1%, respectively. L-Lysine was provided at a concentration of 40 mg/liter when needed. Assays were performed by the method of Guarente and Mason (30) and Rai et al. (44), and β-galactosidase activity (in Miller units) was determined as described earlier (37).

RESULTS

Regulation of *DAL80* expression by Gln3p, Gat1p, and Ure2p. Three GATA family proteins, Gln3p, Gat1p, and Dal80p, have been shown to be responsible for regulating NCR-sensitive gene expression (8, 12, 13, 16, 18, 20, 21, 25). In addition, Gln3p and Dal80p bind to the UAS_{NTR} and URS_{GATA} elements in the promoters of nitrogen catabolic genes (5, 22–24). Dal80p and Gln3p have also been shown to be principal regulators of *GAT1* expression, and both proteins bind to UAS_{NTR} and URS_{GATA} elements contained on DNA fragments from the *GAT1* promoter (12). The autogenous regulation and NCR sensitivity of *DAL80* expression raised the interesting possibility that Dal80p production and Gat1p production are reciprocally regulated. We assessed this possibility by analyzing *DAL80* expression in various nitrogen regulation-deficient mutant strains. *DAL80* expression, as determined by Northern blot analysis of steady-state mRNA levels, was found to be NCR sensitive (Fig. 3, upper panel,

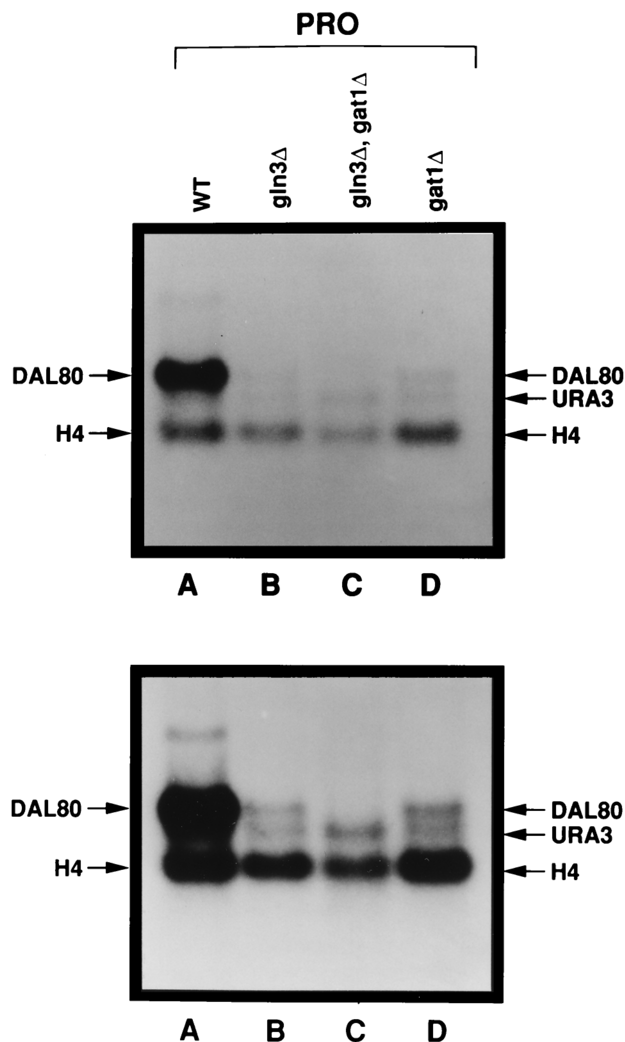

FIG. 4. Northern analysis of *DAL80* steady-state mRNA levels in wild-type (WT; strain TCY1; lanes A) and *gln3*Δ (strain RR91; lanes B), *gln3*Δ *gat1*Δ (strain RJ72; lanes C), and *gat1*Δ (strain RJ71; lanes D) mutant cells cultured in minimal glucose-proline (PRO) medium. Blotting procedures and probes are described in the legend to Fig. 3. In the lower panel is an overexposure of the autoradiogram in the upper panel to visualize the low levels of *DAL80* mRNA present in mutant strains.

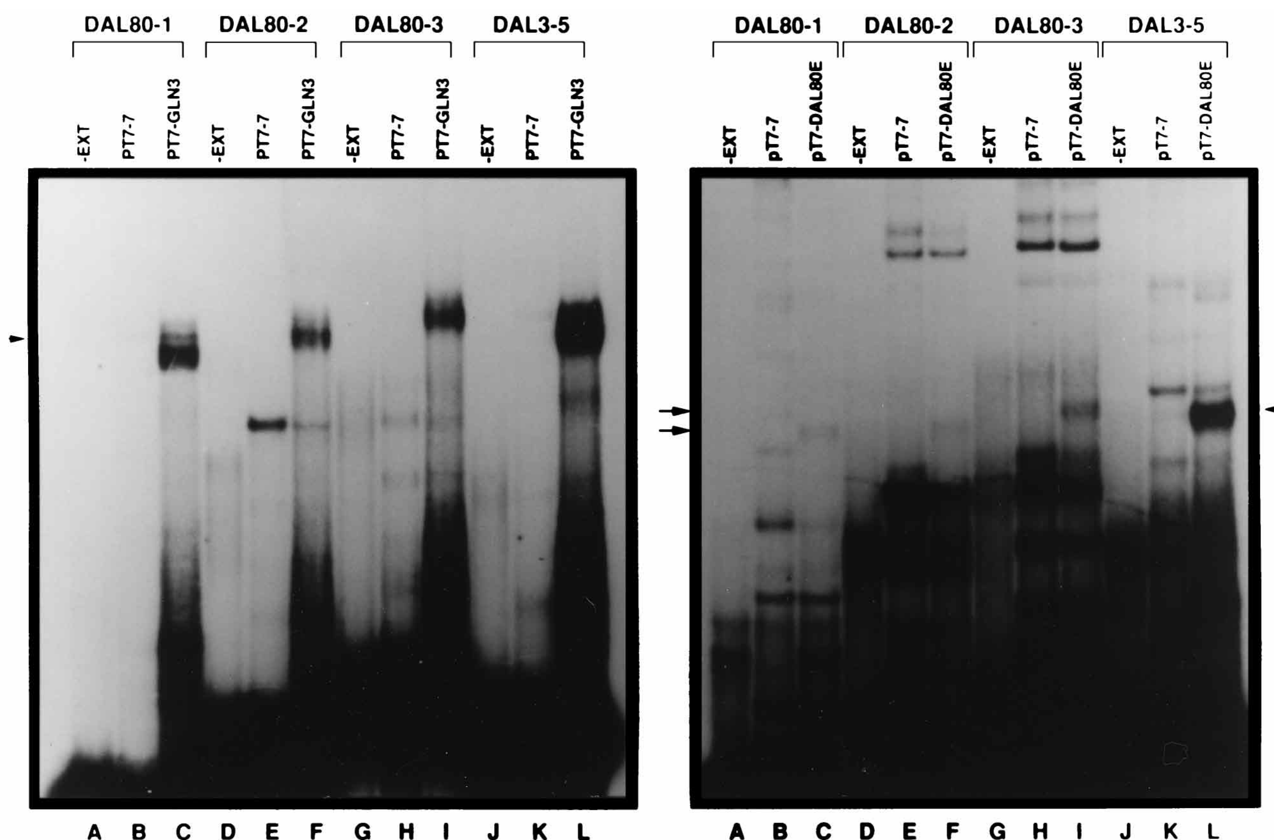

FIG. 5. EMSA of Gln3p and Dal80p binding to DNA fragments containing GATAA sequences from the *DAL80* and *DAL3* upstream regions. Lanes A, D, G, and J, no protein extract (−EXT); lanes B, E, H, and K, reaction mixtures with extracts from *E. coli* cells containing expression vector plasmid pT7-7; and lanes C, F, I, and L, reaction mixtures with extracts from cells carrying plasmid pT7-GLN3 (left panel) or pT7-DAL80 (right panel). Arrows designate Gln3p (left panel)- and Dal80p (right panel)-specific DNA complexes.

lanes A to C), and Gln3p dependent (lanes G and H). Further, the NCR sensitivity of *DAL80* expression was diminished but not eliminated in *ure2* deletion strains (Fig. 3, upper panel, lanes D to F). To determine whether *DAL80* expression was affected by the loss of Gat1p, we performed similar assays with *gat1* and/or *gln3* deletion mutants (Fig. 4). *DAL80* expression required both Gln3p and Gat1p functions (Fig. 4, lanes A, B, and D). The loss of either protein reduced *DAL80* expression to a barely detectable level, and it was virtually undetectable in a *gln3 gat1* double deletion mutant (Fig. 4, lower panel, lane C). The residual *DAL80* expression observed in *gln3* deletion strains was NCR sensitive (Fig. 3, upper panel, lanes H and I), and this NCR sensitivity remained even when *URE2* was deleted (Fig. 3, lower panel). These data and those reported earlier demonstrate that *DAL80* expression and *GAT1* expression are both Gln3p dependent and reciprocally regulated (12, 21). Moreover, the expression of both genes is negatively regulated by one (or more) uncharacterized protein(s) that appears to function analogously to Ure2p (12).

Gln3p and Dal80p bind to GATAA sequences upstream of *DAL80*. The observed Gln3p dependence and autogenous regulation of *DAL80* predicted that both Gln3p and Dal80p should bind to the *DAL80* upstream region. We tested this prediction by measuring Gln3p and Dal80p binding to three DNA fragments from the *DAL80* upstream region by EMSAs. The three fragments we used for these assays were chosen because they contain multiple GATAA sequences (Fig. 1). We incubated each radioactive DNA fragment in the absence of cell extract and with extracts derived from *E. coli* cells containing either expression vector plasmid pT7-7 (54) or plasmid pT7-GLN3, in which the *GLN3* coding sequence was cloned downstream of the T7 promoter in plasmid pT7-7 (Fig. 5). For each *DAL80*-derived fragment tested, incubation with extract from cells containing plasmid pT7-GLN3 resulted in the formation of a DNA-protein complex that was not present under the other two reaction conditions (Fig. 5, left panel). To test whether Gln3p binding to *DAL80* upstream sequences was GATA sequence specific, we performed a competition EMSA (Fig. 6). The radioactive probe used for this experiment was *UGA4* fragment UGA4-17. Gln3p binding to this DNA has been previously characterized in detail and shown to occur through the GATA sequences it contains (24). Similarly, characterized DNA fragments PUT-2M (24) and DAL3-5 (22, 24) were used as negative (no competition) and positive control DNA fragments, respectively (Fig. 6, lanes C and B, respectively). As shown in Fig. 6, lane A, DNA fragment DAL80-3 was an effective competitor for Gln3p binding to UGA4-17 DNA. The results of these experiments support the contention that Gln3p binds to GATA sequences in the *DAL80* upstream region.

The degree of autogenous regulation of *DAL80* expression similarly predicted that Dal80p might also bind, albeit more poorly, to sequences upstream of *DAL80*. Therefore, we repeated the experiment described above but used an extract from *E. coli* cells containing the *DAL80* gene expressed from

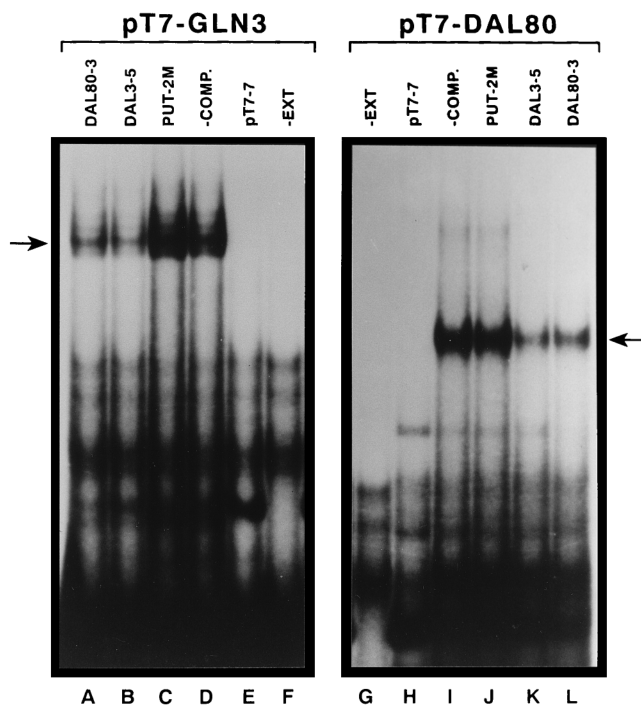

FIG. 6. Competition of *DAL80* promoter (Dal80-3; lanes A and L), GATAA-containing *DAL3* (Dal3-5; lanes B and K), and GATAA mutant (Put-2M; lanes C and J) fragments with a *UGA4* (UGA4-17) fragment containing three tandem GATAAG sequences for binding to Gln3p and Dal80p. The conditions for these EMSAs were the same as those for the EMSAs of Fig. 5. Competitor DNAs were present at a final concentration of 1 μg per reaction mixture. Crude cell extract (−EXT; lanes F and G) or competitor (−Comp.; lanes D and I) was omitted from the reaction mixtures indicated. The sources of protein for these experiments were *E. coli* cells containing plasmids pT7-GLN3 (left) and pT7-DAL80 (right). Vector plasmid pT7-7 was substituted for one of these plasmids in the reaction mixtures analyzed in lanes E and H. Arrows indicate Gln3p (left)- and Dal80p (right)-specific DNA complexes.

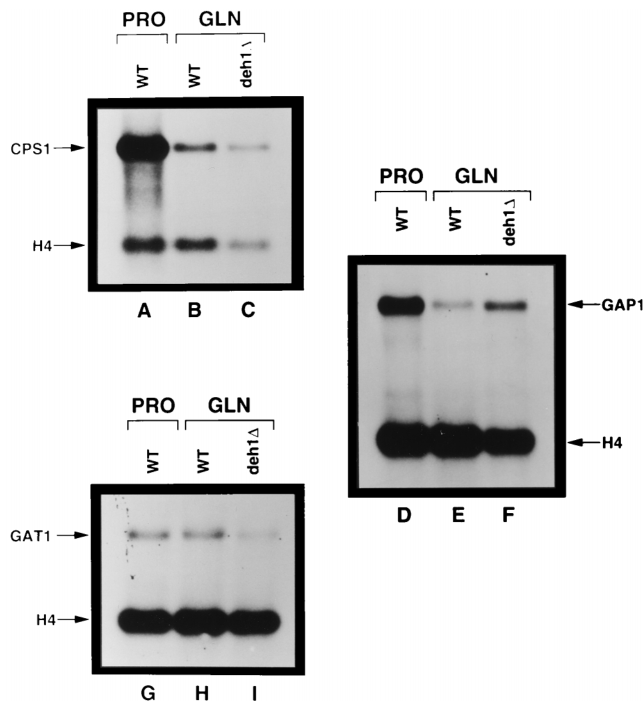

FIG. 7. Northern analysis of *CPS1*, *GAP1*, and *GAT1* mRNA levels in wild-type (WT; strain TCY1) and *deh1*Δ (strain RJ81) mutant cells cultured in minimal-proline (PRO) and -glutamine (GLN) media. Poly(A)$^+$ RNAs (10 μg per lane) immobilized to a nylon membrane were hybridized with 5′-labeled oligonucleotides complementary to *CPS1* sequence −112 to −162 (50) or with randomly primed plasmid pPL257 (34, 36a) or plasmid pTSC550 (12), containing DNA complementary to *GAP1* and *GAT1*, respectively. A 5′- and ^{32}P-labeled synthetic oligonucleotide complementary to the histone H4 gene (48) was used as a probe to determine loading efficiency.

the T7 promoter of plasmid pT7-7 (plasmid pT7-DAL80E). An altered mobility complex that formed only with extract from cells containing plasmid pT7-DAL80E was observed with each *DAL80* DNA fragment (Fig. 5, right panel). To test whether Dal80p binding to *DAL80* upstream sequences was GATA sequence specific, we performed a competition EMSA similar to the one whose results are shown in Fig. 6 (left panel). The radioactive probe used in this experiment was *UGA4* fragment UGA4-17, the GATA sequences of which have been previously shown to bind Dal80p (23). As before, DNA fragments Put-2M (24) and Dal3-5 (22, 24) were used as negative (no competition) and positive control pasmids, respectively (Fig. 6, lanes J and K). As shown in Fig. 6, lane L, DNA fragment DAL80-3 was an effective competitor for Dal80p binding to UGA4-17 DNA. The results of these experiments support the contention that Dal80p binds to GATA sequences in the *DAL80* upstream region. The DNA-protein complexes observed with *E. coli*-produced Dal80p were much weaker than those with Gln3p. This correlates with the fact that *DAL80* expression is much less strongly regulated by Dal80p than by Gln3p (Fig. 5 in reference 21) (Fig. 3, lanes G and H).

TABLE 2. *lacZ* expression supported by nitrogen catabolic gene-*lacZ* fusion plasmids[a]

Strain	Pertinent genotype(s)	β-Galactosidase activity[b]	
		GAT1 (pJCG01)	*DAL80* (pTSC572)
TCY1	Wild type	29	4,435
TCY17	*dal80::hisG*	99	12,493
JCY625	*gat1*Δ *dal80::hisG*	100	1,283
RJ715	*gat1*Δ	100	1,130
RR91	*gln3*Δ	1	71
RR92	*gln3*Δ *dal80::hisG*	3	54
RJ725	*gln3*Δ *gat1*Δ	4	9
JCY745	*gln3*Δ *gat1*Δ *dal80::hisG*	4	5

[a] Transformants were grown in YNB medium with 0.1% proline as the nitrogen source (see Materials and Methods).
[b] β-Galactosidase activity was measured in Miller units (37).

GAT1 expression is regulated by Dal80p but not by Gat1p. The regulation of *DAL80* expression and that of *GAT1* expression are similar in several ways. The expression of both genes is NCR sensitive, Gln3p dependent, and Dal80p regulated. Since *DAL80* expression is Gat1p dependent (12), it might be predicted that *GAT1* expression is also Gat1p dependent, i.e., *GAT1* expression might be autogenously regulated. We tested this prediction by assaying *lacZ* expression supported by a *GAT1-lacZ* fusion plasmid in wild-type and mutant yeast strains; a *DAL80-lacZ* fusion plasmid was also analyzed for purposes of comparison. As shown in Table 2, *GAT1-lacZ* and *DAL80-lacZ* expression increased approximately threefold over that of the wild type when the *DAL80* gene was disrupted (compare the results for strains TCY1 and TCY17). Similarly, expression of both *lacZ* fusion genes was strongly decreased when *GLN3* was deleted (compare the results for strains TCY1 and RR91). However, expressions of the *GAT1* and *DAL80*

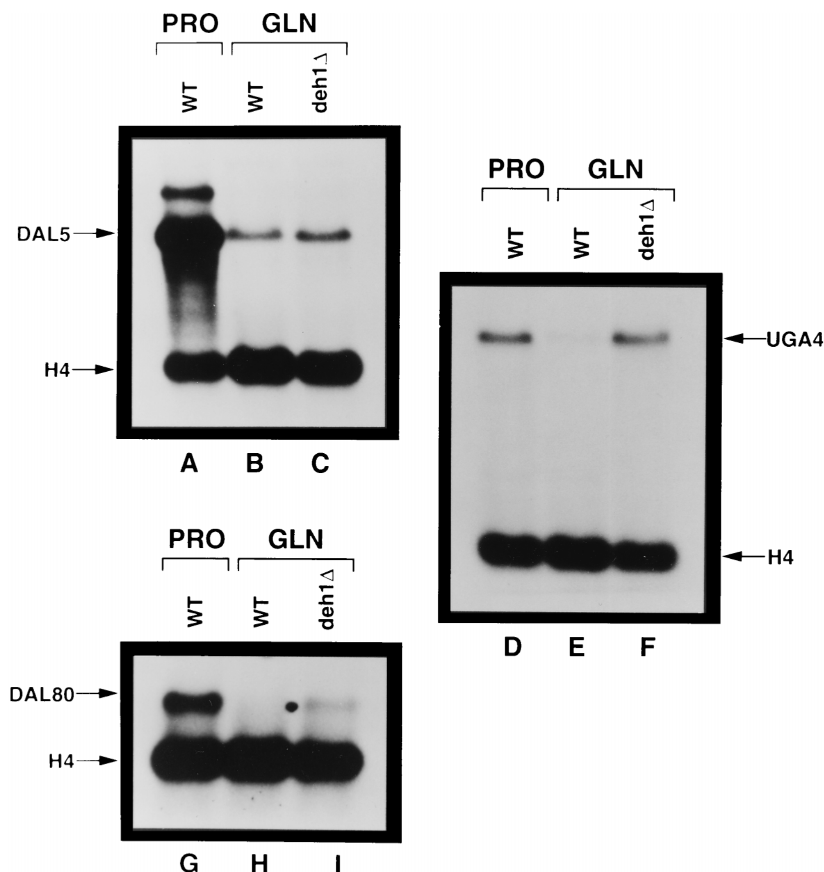

FIG. 8. Northern analysis of *DAL5*, *UGA4*, and *DAL80* steady-state mRNA levels in wild-type (WT; strain TCY1) and *deh1*Δ (strain RJ81) mutant cells cultured in minimal-glutamine (GLN) or -proline (PRO) medium. Poly(A)$^+$ RNAs (10 μg per lane) were hybridized with randomly primed plasmids, pRR20 (42), pUGA4-2 (23, 56, 58), and pTSC319 (21, 43), that contain DNA complementary to *DAL5*, *UGA4*, and *DAL80*, respectively. A 5′- and ^{32}P-labeled synthetic oligonucleotide complementary to the histone H4 gene (48) was used as a probe to determine loading efficiency.

genes responded differently to deletion of *GAT1* (compare the results for strains TCY1 and RJ715). *DAL80-lacZ* expression decreased nearly fourfold in the *gat1* deletion mutant, while *GAT1-lacZ* expression increased over threefold relative to that of the wild type (Table 2; compare the results for strains TCY1 and RJ715). In other words, *GAT1-lacZ* expression responded in the same way to deletions of *GAT1* and *DAL80* (compare the data for strains TCY1, TCY17, and RJ715). The differences in *GAT1-lacZ* and *DAL80-lacZ* expression in response to deletion of *GAT1* were even more striking when the experiment was performed in a *dal80* disruption background. Under these conditions, deletion of *GAT1* decreased *DAL80-lacZ* expression nearly 10-fold while *GAT1-lacZ* expression was not affected (Table 2; compare the results for strains TCY17 and JCY625). The fact that *DAL80* expression is highly Gat1p dependent and *GAT1* expression is Gat1p independent (Table 2) is consistent with the observation that strains TCY17, JCY625, and RJ715 transformed with plasmid pJCG01 produce the same level of β-galactosidase. These data suggest that in contrast to *DAL80* expression, *GAT1* expression is not autogenously regulated. *GAT1* expression and *DAL80* expression are, however, reciprocally regulated.

A fourth GATA family protein regulates nitrogen catabolic gene expression. A fourth GATA family protein sequence was deduced from the sequence of one (*NIL2*) of two isolated DNA fragments that cross-hybridized to a DNA fragment encoding a GATA zinc finger homologous to those of Dal80p and Gln3p (51). When we inspected the nucleotide sequence reported for the putative gene, we noticed that it contained an ORF of more than 70 amino acids in its upstream regulatory region. Therefore, we isolated a genomic DNA fragment (plasmid pTSC534) containing the putative ORF, as described in Materials and Methods, and determined its sequence 330 bp upstream and 90 bp downstream of the starting ATG reported by Stanbrough for *NIL2* (51). The sequence we obtained resulted in an ORF that was 73 amino acids longer than the one previously reported (51) (data not shown). Just after we completed our sequencing, Rasmussen (45) reported ORF YJL110c (*GZF3*) as part of the yeast genome sequencing project (29b). Our sequence and that of Rasmussen were identical and differed from Stanbrough's sequence at position −211. The *NIL2* sequence contained a thymidine at that position, resulting in the formation of an amber stop codon (51). The Rasmussen sequence and ours contained a cytosine, resulting in the formation of a glutamine codon (29b, 45, 54a). The amino acid sequence of this ORF was most homologous to Dal80p (12, 29b, 45), leading us to designate its cognate gene *DEH1* for *DAL80* homolog (12).

The sequence similarities of Dal80p and Deh1p raised the possibility that the two proteins are also functionally related. Therefore, we constructed a *deh1*Δ mutant and measured its nitrogen catabolic gene expression. Deletion of *DEH1* did not demonstrably affect *DAL5*, *GAP1*, *UGA4*, *CPS1*, or *DAL80* expression when proline was used as the nitrogen source (data

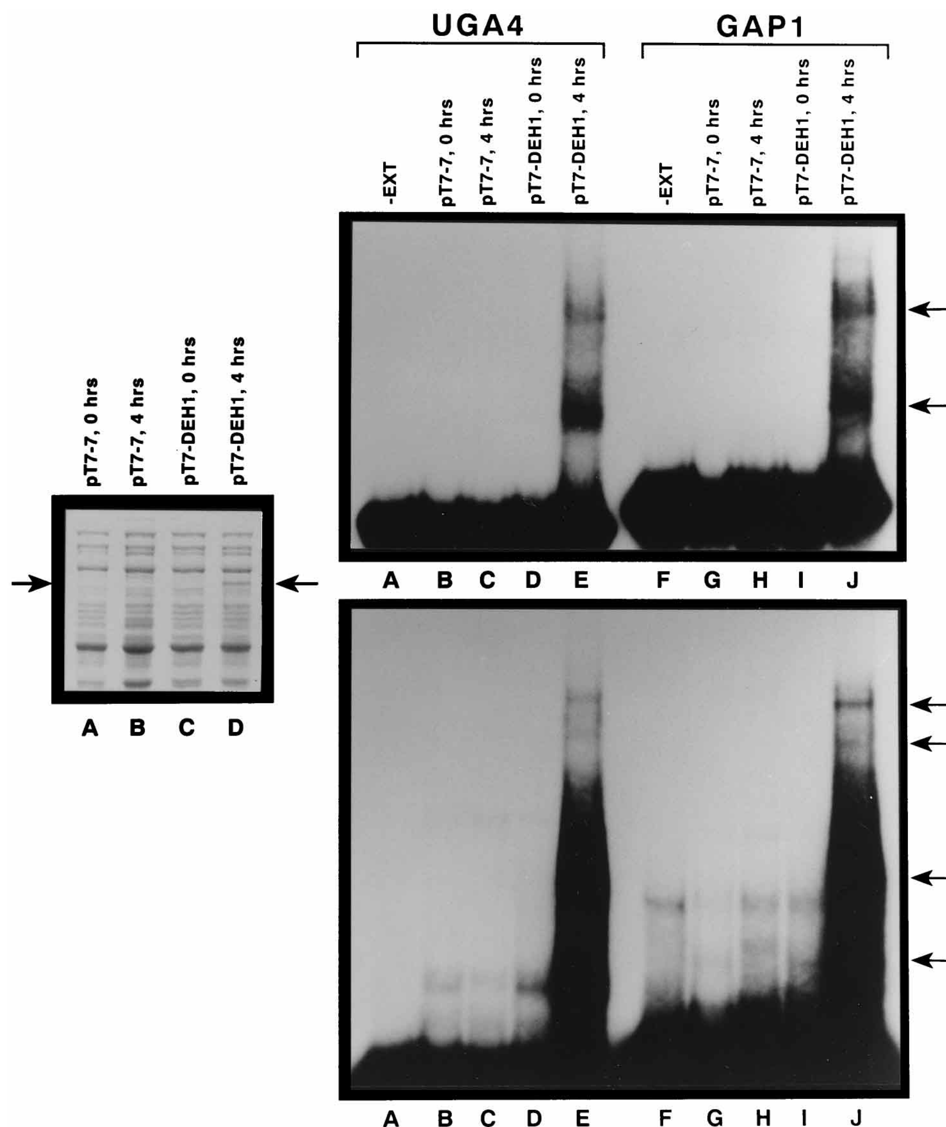

FIG. 9. *DEH1* expression in *E. coli* cells (left) and binding of Deh1p to *UGA4* (UGA4-17) and *GAP1* DNA fragments (right). (Left) The proteins contained in crude cell extracts, prepared from *E. coli* cultures expressing *DEH1* for 0 (pT7-DEH1, 0 hrs) or 4 (pT7-DEH1, 4 hrs) h, were resolved in a sodium dodecyl sulfate–10% polyacrylamide gel and stained with Coomassie brilliant blue (lanes C and D). A similar experiment was performed with extracts of *E. coli* cells containing the expression vector devoid of any *S. cerevisiae* gene (pT7-7, 0 hrs [lane A] and pT7-7, 4 hrs [lane B]). (Right) EMSA analysis of the protein preparations of the left panel analyzed for the ability to bind to GATAA-containing promoter fragments from Deh1p-regulated genes, *UGA4* and *GAP1*. Arrows indicate the positions of Deh1p-specific DNA-protein complexes. A longer exposure of the autoradiogram in the upper right panel is depicted in the lower right panel to visualize small amounts of two slowly migrating DNA-protein complexes (arrows). The radioactive *UGA4* EMSA probe (UGA4-17) has been described previously (23). The *GAP1* probe (Fig. 1) included sequences from *GAP1* positions −379 to −324. The proteins used in these assays were prepared by the procedure described in Materials and Methods with minor modifications. *E. coli* cultures (100 ml), grown to a cell density (A_{600}) of 0.5 in Luria-Bertani–AMP (20 mg/100 ml), were induced with IPTG (isopropyl-β-D-thiogalactopyranoside) (1 mM [final concentration]), harvested, and resuspended in 2 ml of sonication buffer. Cells were broken by 12 to 14 cycles of sonication (12 s on and 12 s off). Each EMSA reaction mixture contained 6 μg of protein. −EXT, no protein extract.

not shown). Deletion of *DEH1* also did not further increase the expression levels of these genes in a *dal80*Δ mutant growing in minimal-proline medium beyond the levels observed with the *dal80* deletion mutant (data not shown). However, there was an effect of deleting *DEH1* on the expression levels of some genes when glutamine was used as the nitrogen source. *GAP1* expression and *UGA4* expression increased in *deh1*Δ strain RJ81 relative to those of the wild type (Fig. 7 and 8, lanes D to F). Similarly, *DAL80* expression increased modestly in a *deh1*Δ mutant (Fig. 8, lanes G to I) and *DAL5* expression increased slightly (Fig. 8, lanes A to C), but the expression levels of representative NCR-sensitive genes (*GAT1* and *CPS1*) did not increase (Fig. 7, lanes A to C and G to I). *PUT4* expression in cells with glutamine as the nitrogen source was similarly unaffected by deletion of *DEH1* (data not shown).

Deh1p binds to GATAA sequences upstream of *GAP1* and *UGA4*. The deduced amino acid sequence of Deh1p (12) and the increased expression of some nitrogen catabolic genes upon deletion of *DEH1* raised the possibility that Deh1p binds to the upstream regions of Deh1p-regulated genes. We tested this expectation by measuring Deh1p binding to *GAP1* and *UGA4* promoter fragments by EMSAs. At least one of the DNA fragments tested, the one from *UGA4*, was chosen because it contained only GATAA sequences. The protein used

in this assay was derived from an *E. coli* strain expressing *DEH1* from the T7 promoter (see Materials and Methods for plasmid constructions). As shown in Fig. 9, a unique protein species was observed on a Coomassie brilliant blue-stained gel (left panel, lane D) of proteins from *E. coli* cells that had expressed *DEH1* for 4 h. This species was not observed prior to the onset of *DEH1* expression (Fig. 9, left panel, lane C) or at any time in cells that did not contain the *DEH1* gene (left panel, lanes A and B). When the *E. coli* extract analyzed in Fig. 9, left panel, lane D, was tested in the EMSA, two slowly migrating complexes were observed with both the *UGA4* and *GAP1* DNA fragments (upper right panel, lanes E and J). When the autoradiogram depicted in the upper right panel of Fig. 9 was overexposed, two additional and far more slowly migrating species were observed with the two DNA probes (lower right panel, lanes E and J). None of these DNA-protein complexes were observed unless Deh1p was produced (Fig. 9, right panels, lanes A to D and F to I).

To ascertain whether the Deh1p binding observed (Fig. 9) was GATAA sequence dependent, we determined whether the small *UGA4* DNA fragment containing only GATAA sequences (UGA4-17) was an effective competitor of the *GAP1* fragment for Deh1p binding. As shown in Fig. 10, all four EMSA bands observed (Fig. 9) could be effectively competed by the *UGA4* fragment (lanes A to G). A *PUT2* fragment (PUT-2M) containing a mutated GATA sequence was unable to serve as an effective competitor of the *GAP1* fragment for Deh1p binding (Fig. 10, lanes G to M). Together, these data are consistent with the argument that Deh1p, like Gln3p and Dal80p, is able to bind to DNA fragments containing GATAA sequences.

The regulation of *DEH1* expression. The sequence similarities of Deh1p and Dal80p and their shared characteristic of negatively regulating nitrogen catabolic gene expression prompted us to determine whether their cognate genes were also similarly regulated. The presence of multiple GATA sequences upstream of *DEH1* certainly pointed toward this possibility (Fig. 1). Therefore, we determined steady-state *DEH1* mRNA levels in wild-type and mutant yeast strains with repressing (asparagine or glutamine) and nonrepressing (proline) nitrogen sources. As shown in Fig. 11, *DEH1* expression was only modestly NCR sensitive in wild-type cells, i.e., the *DEH1* mRNA levels in cells with proline versus asparagine as the nitrogen source differed no more than twofold (lanes A to C). This observation correlated with a similarly small effect of deleting *GLN3* on *DEH1* expression (Fig. 11; compare lanes A and F and lanes H and I). *DEH1* expression, on the other hand, did exhibit measurable Gat1p dependence (Fig. 11; compare lanes H and K). The strongest regulation, however, was exerted by Dal80p (Fig. 11, lanes A and D). *DEH1* expression significantly increased in a *dal80* disruption mutant, and this high-level expression, in contrast to that observed in the wild type, was fully NCR sensitive (Fig. 11, lanes A and C to E).

Gat1p participates in UAS_{NTR}-mediated transcription. Although the expression of many nitrogen catabolic genes is highly Gln3p and Gat1p dependent, there are exceptions. For example, the effect of deleting either *GLN3* or *GAT1* on *GAP1* expression is twofold or less (11, 12, 25, 52). This observation raised the question of whether Gat1p would support transcription from UAS_{NTR}. This question was answered by measuring *lacZ* reporter gene expression supported by a *UGA4* promoter fragment containing only the three tandem GATAA sequences (plasmid pTSC491) (23). *lacZ* expression from this plasmid was highly Gln3p dependent (Table 3; compare the results obtained with strains TCY1 and RR91), as observed earlier (23). However, the effect of *GAT1* deletion on expression was less than twofold. We repeated this experiment in a *dal80*::*hisG* background, which removes the effects of Dal80p binding to the *UGA4* UAS_{NTR} element and Dal80p regulation of *GAT1* expression. Under these conditions, β-galactosidase production supported by the *UGA4* plasmid decreased 13-fold upon deletion of *GAT1* (Table 3; compare the results for strains TCY17 and JCY625).

The results (Table 3) obtained with a small *UGA4* DNA fragment containing only the tandem GATAA sequences differed from those observed with a DNA probe for *UGA4* gene expression (Fig. 12, lanes A to D). When steady-state levels of *UGA4* mRNA were determined by Northern blot analysis, *UGA4* expression was Gat1p dependent (Fig. 12, lanes A and B). In contrast, the 44-bp *UGA4* DNA fragment (23) exhibited a Gat1p requirement of less than twofold (strains TCY1 and RJ715) (Table 3). To ascertain whether this difference was derived from assaying β-galactosidase production instead of mRNA, we compared *DAL3* expression, as assayed by Northern blot analysis, with β-galactosidase production supported by a *DAL3-lacZ* fusion plasmid. The patterns of *DAL3* expression

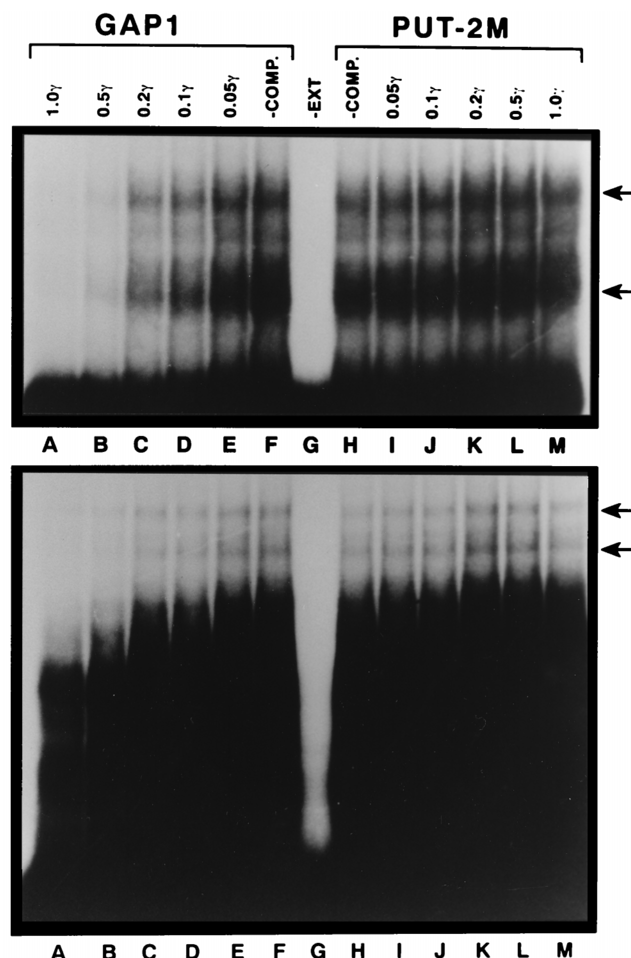

FIG. 10. Competition of GATAA-containing *GAP1* (lanes A to G) and GATAA mutant (Put-2M) DNA fragments with a *UGA4* promoter fragment (UGA4-17) containing three tandem GATAAG sequences for Deh1p binding. The concentration of competitor used (in micrograms [γ]) in each reaction mixture is indicated above each lane. The assay conditions were the same as those described in the legend to Fig. 9. Arrows indicate the positions of Deh1p-specific DNA-protein complexes. In the lower panel is an overexposure of the autoradiogram in the upper panel to visualize the small amounts of two more slowly migrating, Deh1p-specific DNA-protein complexes (arrows).

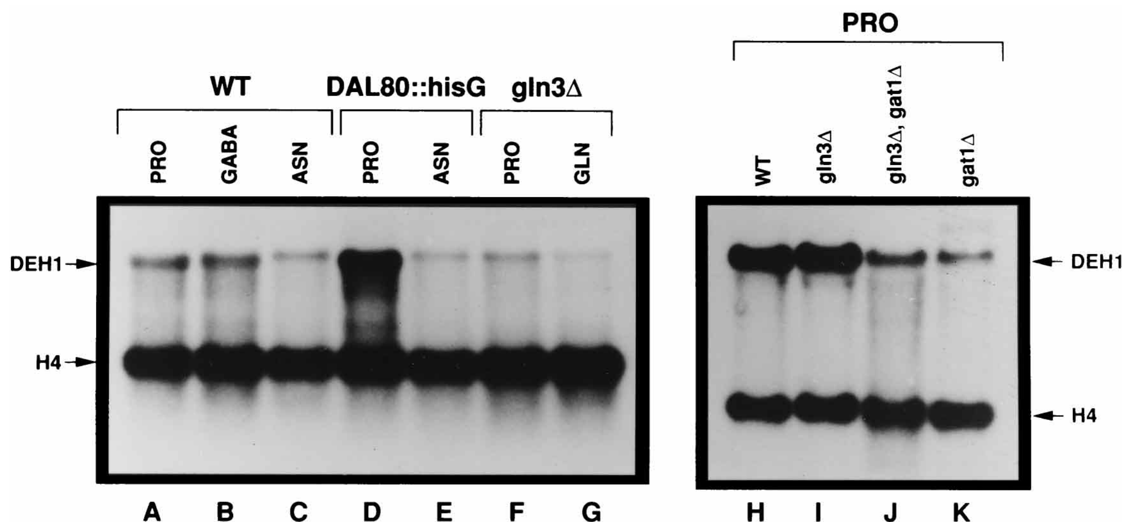

FIG. 11. Northern analysis of *DEH1* steady-state mRNA levels in wild-type cells (WT; strain TCY1; lanes A to C and H) and *dal80* disruption (*DAL80::hisG*; strain TCY17; lanes D and E), *gln3*Δ (strain RR91; lanes F, G, and I), *gln3*Δ *gat1*Δ (strain RJ72; lane J), and *gat1*Δ (strain RJ71; lane K) mutant cells growing in minimal glucose-proline (PRO), -asparagine (ASN), and -glutamine (GLN) media. Poly(A)$^+$ RNA (10 μg per lane) was hybridized with end-labeled oligonucleotides complementary to the *DEH1* gene (lanes A to G) or randomly primed plasmid pRR346 (lanes H to K), which contains the entire *DEH1* coding region. A 5′- and ^{32}P-labeled synthetic oligonucleotide complementary to the histone H4 gene (48) was used as a probe to determine loading efficiency.

observed by the two assay methods were similar (Fig. 12, lanes E to H, and Table 3 [plasmid pTSC435]). This result argued that the overall *UGA4* promoter likely contains *cis*-acting elements beyond the three tandem GATA sequences that influence steady-state levels of *UGA4* mRNA.

DISCUSSION

Data presented here and earlier (11, 12, 16, 18, 21, 25, 38, 40, 42, 51–53) demonstrate that nitrogen catabolic gene expression in *S. cerevisiae* is regulated by an intricate, cross-regulatory system involving four GATA family transcription factors, Gln3p, Gat1p, Dal80p, and Deh1p (12, 13). Both Gln3p and Gat1p are required for maximal expression of NCR-sensitive genes. In contrast, Dal80p and the newly characterized Deh1p possess characteristics of negative regulators. Our observations highlight the complex relationships that exist among these four proteins and their cognate genes. The most straightforward genetic model to describe the coordinated regulation of NCR-sensitive gene expression is that pairs of positively and negatively acting GATA factors antagonize each other's operation, with the resulting activation or repression potential ultimately dictated by nitrogen source quality and its influence on transcription supported by Gln3p and Gat1p (Fig. 13).

Deh1p appears to be most closely related to Dal80p; both proteins possess GATA zinc finger and leucine zipper motifs; both Gln3p and Gat1p lack the latter motif. Dal80p and Deh1p are also similar to *Schizosaccharomyces pombe* Gaf2p (Fig. 14), which may provide some insight into the latter's function (direct submission to the database). Likewise, Gln3p and Gat1p are more similar to one another than to Dal80p and Deh1p and to the more distantly related Ash1, Srd1, Yle4, and Yph8 proteins (Fig. 14). Our data extend the *DAL80-DEH1* homology to include their functions by demonstrating that Deh1p negatively regulates expression of the NCR-sensitive genes, *GAP1*, *UGA4*, and *DAL80* (Fig. 7 and 8), and binds to GATAA-containing promoter fragments of Deh1p-regulated genes (Fig. 9 and 10). Like *DAL80* expression, *DEH1* expression is partially Gat1p dependent and Dal80p regulated (Fig. 11). For these reasons, the cognate gene was designated a *DAL80* homolog (Fig. 14) (12, 29b, 45). The distinguishing characteristic between Dal80p and Deh1p functions is that Deh1p appears to operate detectably only when glutamine is used as the nitrogen source, i.e., when Dal80p production is severely repressed. The regulation of *DEH1* also differs from that of *DAL80* in that Gln3p, which is a strong positive regulator of *DAL80*, has little effect on *DEH1* expression.

Since Gln3p, Gat1p, Deh1p, and Dal80p all possess GATA-type zinc finger motifs (12, 18, 21, 38, 45), it's not surprising that these four proteins regulate the expression of genes containing related GATA sequences in their promoters. However, their specificities of operation are by no means congruent. Each of these four proteins has been shown to regulate a particular set of NCR-sensitive genes (12, 18, 21, 24, 25, 38). For example, both Gln3p and Gat1p are required for *DAL5* activation, even though *DAL5* expression is nearly independent of Dal80p and Deh1p regulation (12, 16, 21). *GAT1* expression, on the other hand, is Gln3p dependent and Dal80p

TABLE 3. *lacZ* expression supported by a *DAL3-lacZ* fusion plasmid or a *UGA4* fragment cloned into a heterologous expression plasmid[a]

Strain	Pertinent genotype(s)	β-Galactosidase activity[b]	
		DAL3 (pTSC435)	*UGA4* (pTSC491)[c]
TCY1	Wild type	513	399
TCY17	*dal80::hisG*	4,950	3,972
JCY625	*gat1*Δ *dal80::hisG*	2,590	298
RJ715	*gat1*Δ	1,624	268
RR91	*gln3*Δ	3	8
RR92	*gln3*Δ *dal80::hisG*	16	29
RJ725	*gln3*Δ *gat1*Δ	2	27
JCY745	*gln3*Δ *gat1*Δ *dal80::hisG*	2	22

[a] Transformants were grown in YNB medium with 0.1% proline as the nitrogen source (see Materials and Methods).
[b] β-Galactosidase activity was measured in Miller units (37).
[c] The *UGA4* DNA fragment covered positions −446 to −401 (56, 58). Plasmid pTSC491 has previously been described (23).

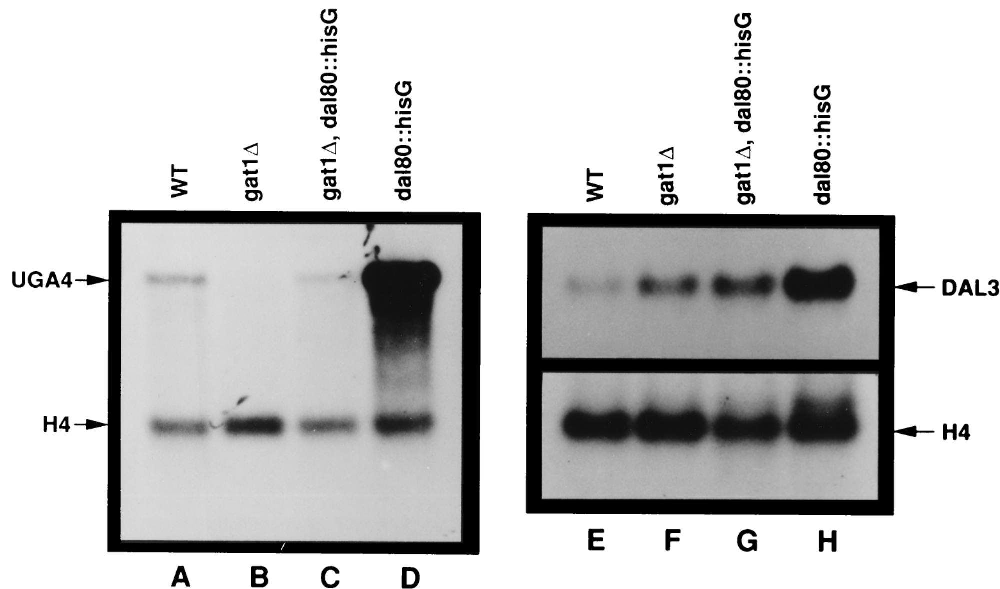

FIG. 12. Northern analysis of *UGA4* (left) and *DAL3* (right) steady-state mRNA levels in wild-type (WT; strain TCY1; lanes A and E) and *gat1*Δ (strain RJ71; lanes B and F), *gat1*Δ *dal80*::*hisG* (strain JCY62; lanes C and G), and dal80::hisG (strain TCY17; lanes D and H) mutant cells cultured in minimal-proline medium. RNA was isolated as described in Materials and Methods. End-labeled oligonucleotides complementary to *DAL3* and randomly primed plasmid pUGA4-2 DNA were used as hybridization probes. A 5′- and ^{32}P-labeled synthetic oligonucleotide complementary to the histone H4 gene (48) was used as a probe to determine loading efficiency.

regulated (12) but is not influenced by either Gat1p or Deh1p (Fig. 7 and Table 2). *DEH1* expression is nearly Gln3p independent, reasonably Gat1p dependent, and Dal80p regulated (Fig. 11). The molecular basis for these overlapping but distinct specificities of operation is not yet understood, but EMSA analyses of Gln3p and Dal80p binding to GATA sequences indicate that both nucleotide sequence and GATA element number and orientation are important determinants of DNA binding specificity (22, 24). We anticipate that similar determinants specify the DNA binding of Gat1p and Deh1p as well.

One of the most fascinating and potentially important characteristics of Gln3p, Gat1p, Dal80p, and Deh1p functions is their influence on the expression of one another's genes. Each gene displays a distinct pattern of regulation, depending on the quality of the nitrogen source available and the presence or absence of the other three regulatory gene products. *DAL80* expression exhibits the most complex pattern of regulation and the one most similar to those of genes encoding nitrogen catabolic pathway enzymes and permeases. It is Gln3p and Gat1p dependent and Dal80p and Deh1p regulated (Fig. 13). Transcriptional regulation of the remaining GATA family genes is more restricted. *GAT1* expression is Gln3p dependent and Dal80p regulated but not Gat1p or Deh1p regulated (Fig. 13). *DEH1* expression is largely Gln3p independent, modestly Gat1p dependent, and most highly regulated by Dal80p (Fig. 13). *GLN3* expression is minimally, if at all, regulated in response to the nitrogen source (38, 39). Instead, Gln3p operation has been proposed to be principally regulated through its DNA binding and/or its ability to support transcriptional activation (12, 24, 38). This regulation appears to involve the participation of Ure2 and one or more other uncharacterized proteins (Fig. 13) (9–11, 19, 20, 41c). We suggest that the presence of regulatory loops controlling the expression of genes that encode proteins responsible for the transcription of nitrogen catabolic genes results in the ability of cells to respond finely to a variety of disparate environmental stimuli and facilitates a smooth transition from one response to another.

The integration of Deh1p function into the model of nitrogen regulation depicted in Fig. 13 is a challenging problem. The most straightforward interpretation of our observations is that Dal80p downregulates NCR-sensitive gene expression under conditions of nitrogen limitation and that Deh1p appears to perform this function under conditions of nitrogen excess. The sensitivity of *DEH1* expression to Dal80p regulation is consistent with this interpretation. There should be little need for Deh1p when Dal80p levels are high, i.e., under conditions of nitrogen limitation. Conversely, when Dal80p levels are lowest, i.e., under conditions of nitrogen excess, Deh1p levels are high (Fig. 11). These correlations were, in fact, observed experimentally here. Disruption of *DAL80* affected *DEH1* expression more than did any other genetic or environmental

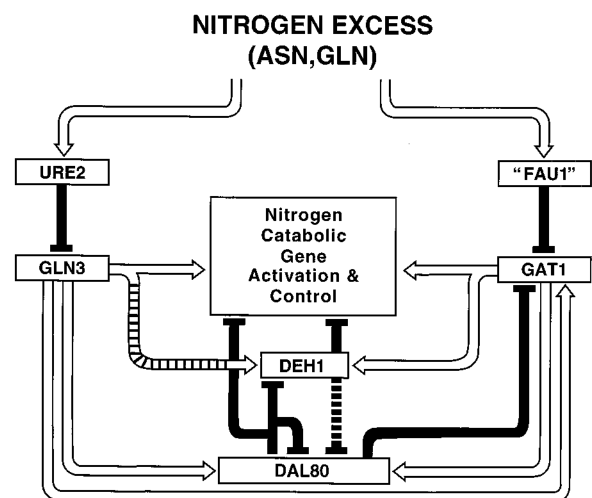

FIG. 13. Working model of the regulatory circuit for activation and repression of nitrogen catabolic genes in *S. cerevisiae*. Open arrows indicate positive regulation; closed bars indicate negative regulation.

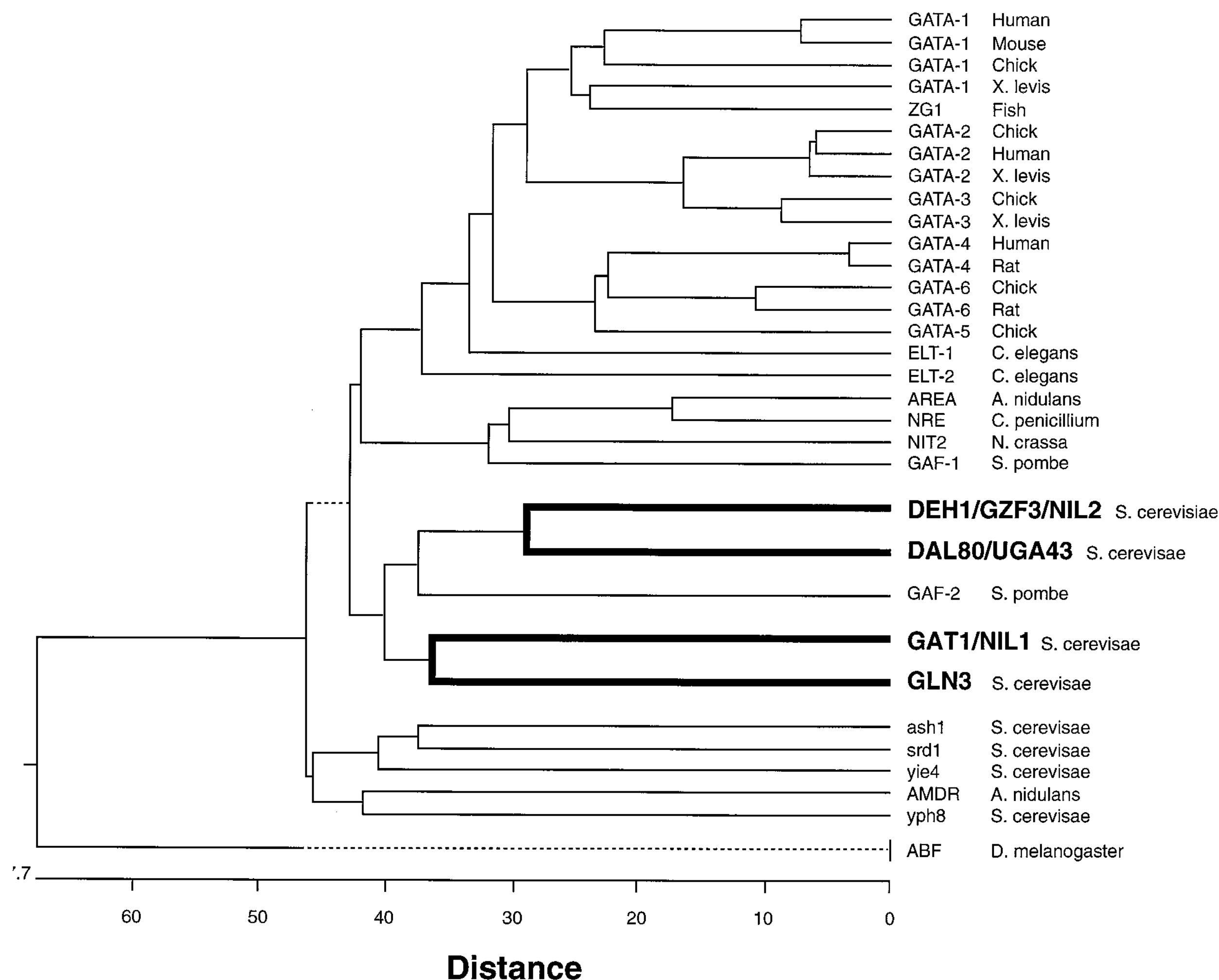

FIG. 14. Phylogenetic tree of eucaryal GATA-type zinc finger proteins. This phylogenetic tree was assembled by the CLUSTAL method (30b) (DNASTAR and PAM250 residue weight table) with polypeptide sequences retrieved from the SWISSPROT database. Non-GATA C_4 zinc finger-related proteins (Ash1, Srd1, Yie8, and Yph8) and a member of the fungal C_6 binuclear cluster family (AMDR) were also added to the pool of sequences.

factor (Fig. 11). *DEH1* expression also responded to Gat1p levels in a way similar to that of *DAL80* (Fig. 11).

A corollary of the interpretation given above, however, is that Deh1p directly participates in implementation of NCR of nitrogen catabolic gene expression. While this interpretation of the data is supported by some experimental evidence, several exceptional observations are not easily rectified with expectation. If Deh1p plays an important direct role in maintaining NCR-sensitive gene expression at a low level when cells are provided with a good nitrogen source, why are the expression levels of some NCR-sensitive genes independent of Deh1p regulation? Another experimental result that is difficult to understand is the NCR sensitivity of *DEH1* expression observed in a *dal80* disruption mutant (Fig. 11, lanes D and E). Here, the level of *DEH1* expression was lowest at just the time when one would expect it to be the highest, i.e., when *DEH1* was performing its suggested function of maintaining nitrogen catabolic gene expression at a low level when glutamine was provided as the nitrogen source. An alternative interpretation of these data is that the response of *DEH1* expression to the loss of Dal80p derives from Gat1p being a strong regulator of *DEH1* expression. In other words, the high-level, NCR-sensitive *DEH1* expression observed in a *dal80* deletion mutant may result from the elevated expression of *GAT1* that occurs in the absence of Dal80p. The high-level NCR sensitivity of *DEH1* expression would in turn derive from the high-level NCR sensitivity of *GAT1* expression and operation of Gat1p.

The role of Deh1p function in NCR and its relation to Ure2p function is important but poorly understood. The first biochemical role suggested for Ure2p, participation in posttranslational modification of Gln3p, was based on its homology to glutathione *S*-transferase (19). More recently, Ure2p and Gln3p have been precipitated with anti-Gln3p antibodies (4). Although the reciprocal experiment did not work as expected, i.e., Gln3p could not be precipitated with Ure2p antibodies, it was suggested that Ure2p binds directly to Gln3p and thereby regulates its operation (4). Moreover, Ure2p function no longer appears to be unique (10–13). Downregulation of Gln3p- or Gat1p-dependent transcription can be convincingly demonstrated in *ure2* deletion mutants (12). Phenotypically, *deh1* mutations most closely resemble *ure2* mutations. However, the requirement of Deh1p for complete downregulation of some, but not all, nitrogen catabolic genes when glutamine is provided as the nitrogen source and its relation to previously suggested Ure2p biochemical functions is currently an enigma.

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