Allantoin pathway gene expression in *Saccharomyces cerevisiae* responds to two different environmental stimuli. The expression of these genes is induced in the presence of allantoin or its degradative metabolites and repressed when a good nitrogen source (e.g. asparagine or glutamine) is provided. Three types of cis-acting sites and trans-acting factors are required for allantoin pathway gene transcription as follows: (i) UAS\textsubscript{NTR} element associated with the transcriptional activators Gln3p and Gat1p, (ii) URS\textsubscript{GATA} element associated with the repressor Dal80p, and (iii) UIS\textsubscript{ALL} element associated with the Dal82 and Dal81 proteins required for inducer-dependent transcription. Most of the work leading to the above conclusions has employed inducer-independent allantoin pathway genes (e.g. *DAL5* and *DAL3*). The purpose of this work is to extend our understanding of these elements and their roles to inducible allantoin pathway genes using the *DAL7* (encoding malate synthase) as a model. We show that eight distinct cis-acting sites participate in the process as follows: a newly identified GC-rich element, two UAS\textsubscript{NTR}, two UIS\textsubscript{ALL}, and three URS\textsubscript{GATA} elements. The two GATA-containing UAS\textsubscript{NTR} elements are coincident with two of the three GATA sequences that make up the URS\textsubscript{GATA} elements. The remaining URS\textsubscript{GATA} GATA sequence, however, is not a UAS\textsubscript{NTR} element but appears to function only in repression. The data provide insights into how these cis- and trans-acting factors function together to accomplish the regulated expression of the *DAL7* gene that is observed in vivo.

Inducible nitrogen catabolic genes in *Saccharomyces cerevisiae* respond to at least two different types of signals. One is a dominant, general signal that monitors the overall nitrogen supply of the cell. The availability of readily transported and/or metabolized nitrogen sources in the environment contains a zinc finger motif homologous to the mammalian regulatory signals and the cell’s detection of and response to them. Genes encoding proteins involved in the transport and metabolism of poor nitrogen sources are NCR-responsive, i.e. they are expressed at low levels under conditions of high NCR and at higher levels under conditions of low NCR; the latter is also referred to occasionally as derepression (1, 2). The second signal is pathway-specific and derives from the presence of a particular nitrogen source (e.g. allantoin or one of its metabolites) (3). Responses to these two physiological signals are integrated at transcription resulting in fine regulation of catabolic gene expression that ranges from high to low as needed to exploit most effectively the prevailing environmental nitrogen supplies for the needs of the cell.

Allantoin catabolic pathway gene expression has been a useful model with which to study the nature of nitrogen regulatory signals and the cell’s detection of and response to them (see Ref. 4 for a comprehensive review of the allantoin pathway literature; shorter literature reviews covering contributions from the range of investigators in the field of yeast GATA factors and nitrogen regulation *per se* appear in the introductions of Refs. 5 and 6). These studies identified three types of cis-acting elements and trans-acting factors associated with allantoin pathway genes (7). The cis-acting element mediating NCR-sensitive transcriptional activation is UAS\textsubscript{NTR} (UAS Nitrogen-Regulated) (9, 10), consisting of two separated dodecanucleotides each with the sequence GATAAA at its core (10). UAS\textsubscript{NTR} has been shown to be both necessary and sufficient for NCR-sensitive *DAL* gene transcription (11); Gln3p and Gat1p are required for this transcription (12). The Magasanik group (13, 14) reported that antibody against a Gln3p peptide containing the GATA family zinc finger motif precipitated a synthetic DNA fragment containing seven repeats of a 32-bp *GLN1* promoter fragment containing a GATA sequence from crude extract; this led them to suggest that Gln3p bound to GATA sequences. Their observation was consistent with the finding that the deduced Gln3p sequence contains a zinc finger motif homologous to the mammalian GATA-binding family of transcription factors (15). By using electrophoretic mobility shift assays (EMSAs), direct binding of Gln3p to UAS\textsubscript{NTR} sequences has been demonstrated (16, 17). Multiple UAS\textsubscript{NTH} homologous sequences are situated upstream of all allantoin pathway genes, and these sequences have been shown to account for NCR-sensitive, Gln3p-dependent expression of the inducer-independent *DAL5* and *DAL3* genes (8, 10, 11, 18, 38).

The cis-acting element mediating inducer responsiveness of the allantoin pathway genes is the dodecanucleotide element,UIS\textsubscript{ALL} (Upstream Induction Sequence) (7, 19). The inducer to which proteins associated with the UIS\textsubscript{ALL} respond is the last intermediate of the allantoin pathway, allophanate, or its non-metabolized analogue, oxalurate (20). The allophanate-inducible *DAL* genes contain one or two copies of UIS\textsubscript{ALL} (7, 21). The *DAL81/DURL/UGA35* and *DAL82/DURM* gene products are required for this inducer responsiveness (22-25). Dal82p has
been shown to be the UIS\textsubscript{ALL}, DNA-binding protein whose binding to DNA is independent of inducer (21). Whereas Dal82p appears to be a pathway-specific regulatory element, Dal81p functions more broadly, being required for induced expression of the UGA (γ-amino-butyrate catabolic pathway) and AGP1 (tryptophan uptake) genes as well as those of the allantoin pathway (26, 27).

A third type of cis-acting element and cognate transcription factor down-regulate DAL gene expression; they are URS\textsubscript{GATA} and Dal80p, respectively (28, 29). The DAL80 locus was first identified genetically (28). Mutations in this gene increase allantoin pathway-inducible gene expression in the absence of inducer to the same level observed in a wild type strain grown with inducer. This observation led to the suggestion that Dal80p functions to reduce inducible gene expression when inducer is absent (28). Dal80p was subsequently found to perform a similar function for the inducible UGA genes (33, 34). However, Dal80p also down-regulates inducer-independent DAL gene expression 2–20-fold (18), suggesting a more general physiological function than simply maintaining inducible gene expression at low levels. Gln3p and Dal80p were proposed to be opposing regulators of most NCR-sensitive nitrogen catabolic genes (30). This proposal has been subsequently supported by data from other laboratories (31). The deduced amino acid sequence of Dal80p contains a zinc finger motif homologous to the one in Gln3p (32, 34). Prompted to test the implication of this homology, we demonstrated Dal80p to be a DNA-binding protein whose optimal binding site, URS\textsubscript{GATA}, consists of two GATAAG-containing sequences separated 15–35 bp, oriented tail-to-tail or head-to-tail (29). The requirement of two GATA-containing sequences in a Dal80p-binding site correlates with the fact that Dal80p molecules have been recently shown to dimerize via a leucine zipper motif in their C-terminal domains using two-hybrid assays (35). The structural similarity of UAS\textsubscript{NTR} and URS\textsubscript{GATA} sites led to the suggestion that Dal80p-binding sites might be Gln3p-binding sites as well (18). This suggestion was supported by the demonstration that the GATA sequences of the UGA4 and DAL3 UAS\textsubscript{NTR} bind Gln3p as well. The fact that Gln3p can bind to a single GATA sequence while Dal80p cannot explains why some genes, such as DAL5, are Gln3p-dependent but largely immune from regulation by Dal80p (4).

The bulk of our understanding of allantoin pathway gene regulation and the role played by Dal80p down-regulating Gln3p transcriptional activation has been derived from studies with inducer-independent genes such as DAL5 and DAL3 (4). Our objective here is to extend these studies to the inducible allantoin pathway genes whose expression is not only NCR-sensitive like that of DAL5 and DAL3, and Dal80p-regulated like that of DAL3, but also inducer-responsive. The inducible DAL7 gene is the one we investigated. The results obtained delineate which of the many DAL7 upstream sequences that are homologous to known transcription factor binding sites actually participate in DAL7 expression. They further identify a cis-acting site not previously known to mediate allantoin pathway gene transcription and suggest a crude picture of how Dal80p, Gln3p, and Dal82p might work together to regulate inducer-dependent DAL7 expression.

**EXPERIMENTAL PROCEDURES**

*Strains and Media.—*The strains used in this work are listed in Table I. When correlating data from this and other work, it is important to recognize that the fold induction (induced level divided by the basal level of activity) for and Dal80p regulation of DAL7 expression is highly strain-dependent. It ranges from as much as 20–30-fold in strains such as RH218 (7) to as little as 2–4-fold in the case of strains derived from

### TABLE I  
Yeast strains used in this work

| Strain   | Genotype   |
|----------|------------|
| Σ1278b   | MATa       |
| TCY1     | MATa lys2 ura3 |
| TCY17    | MATa lys2 ura3 dal80::hisG-URA3-hisG |
| M1682–19b| MATa ura3–52 trp1-889 |

\*J. Daugherty and T. G. Cooper, unpublished observations.*
tively acting DAL7 elements that participate in physiologically relevant heterologous reporter gene expression, UASNTR and UISALL (7). However, these studies were unable to address adequately how these cis-acting elements and the trans-acting proteins associated with them cooperate to mediate inducer-dependent, Dalp80-regulated DAL7 expression. The inadequacy derived, in part, from a lack of knowledge about the biochemical function of Dal80p and clear identification of which of the cis-active element homologous sequences actually participated in DAL7 expression (7). When early 5′ deletion data (derived from a DAL7 lacZ fusion plasmid (7)) are analyzed from the perspective of sequences homologous to those of known cis-active elements, there are instances in which single deletions potentially removed more than one cis-acting element. The region between positions −290 and −221, relative to the ATG, is not only an area where our information is incomplete but also one in which existing deletions have the greatest effect upon expression (7).

To characterize further the regions most responsible for DAL7 expression, we cloned a synthetic fragment, containing sequences from −320 to −199, into a heterologous expression vector (plasmid pHJ98, see “Experimental Procedures”; sequential 5′ deletions were then used to delineate the cis-acting elements (Fig. 2). The first two deletions, to −300 and −266, had little effect upon β-galactosidase production (Fig. 2, plasmids pHJ98, pHJ95, and pHJ68). This argues that the UASNTR homologous sequence between positions −291 and −286 (5′-ATTATC-3′) does not demonstrably function as a UAS element in this fragment. However, deletion of the next 12 bases (to position −254) decreased reporter gene expression 3-4-fold in the presence or absence of inducer (plasmid pHJ72). This region contained a sequence homologous to UISALL and has been shown to bind Dal82p (21). These results argue that this UISALL sequence participates in reporter gene expression.

When the above data and those published earlier (7) are considered together, the only region of plasmid pHJ68 not analyzed is between positions −244 and −230. To remedy this, we synthesized a mutant form of plasmid pHJ68 in which a GC-rich sequence, 5′-CCGCGG-3′, at positions −240 to −235 was mutated. This particular sequence was chosen because it is a GC-rich inverted repeat, both characteristics of multiple cis-acting elements. There are instances in which single deletions potentially removed more than one cis-acting element. The region between positions −290 and −221, relative to the ATG, is not only an area where our information is incomplete but also one in which existing deletions have the greatest effect upon expression (7).

To characterize further the regions most responsible for DAL7 expression, we cloned a synthetic fragment, containing sequences from −320 to −199, into a heterologous expression vector (plasmid pHJ98, see “Experimental Procedures”); sequential 5′ deletions were then used to delineate the cis-acting elements (Fig. 2). The first two deletions, to −300 and −266, had little effect upon β-galactosidase production (Fig. 2, plasmids pHJ98, pHJ95, and pHJ68). This argues that the UASNTR homologous sequence between positions −291 and −286 (5′-ATTATC-3′) does not demonstrably function as a UAS element in this fragment. However, deletion of the next 12 bases (to position −254) decreased reporter gene expression 3-4-fold in the presence or absence of inducer (plasmid pHJ72). This region contained a sequence homologous to UISALL and has been shown to bind Dal82p (21). These results argue that this UISALL sequence participates in reporter gene expression.

When the above data and those published earlier (7) are considered together, the only region of plasmid pHJ68 not
Fig. 3. Expression of wild type and mutant DAL7 promoter fragments (synthetic oligonucleotides) cloned into heterologous expression vector plasmid pNG15. Wild type sequences of the various cis-acting elements appear as capital letters in the figure and those of the mutant alleles in lowercase letters. Transformants of parent strain M1682-19b were grown and β-galactosidase assayed as described in Fig. 2.

Fig. 4. Expression of wild type and mutant DAL7 promoter fragments described in Fig. 3 in wild type (TCY1) and gln3Δ (RR91) strains growing in minimal YNB medium containing 2% glucose and 0.1% γ-aminobutyric acid (GABA) as sole carbon and nitrogen sources. Plasmids and assay conditions were as described in Fig. 3.

Up to 5-fold less activity was observed with each of the mutant plasmids when asparagine was provided in place of proline as the nitrogen source (Fig. 3), indicating that reporter gene expression supported by plasmid pJD68 and its derivatives was NCR-sensitive. However, this NCR sensitivity was less than that observed when the intact gene (39) or a DAL7-lacZ fusion plasmid were analyzed (7); the earlier work was performed with strains that are different from the ones used here. Occurrences of the lowest NCR sensitivities (plasmids pJD92, pJD155, and pJD154) were coincident with those in which there was the least activity with proline as nitrogen source. In this regard, plasmids pJD92 and pJD154 generated a puzzling result. The UASNTR element has been demonstrated by several laboratories to be the one upon which NCR sensitivity depends (7, 18, 19). Therefore, one would a priori have expected that expression supported by plasmids pJD92 and pJD154 to be as NCR-sensitive as wild type plasmid pJD68, or more so because (i) their UASNTR elements are intact and (ii) elements supporting NCR-insensitive expression have been inactivated by mutation thereby decreasing their contribution to the overall results (Fig. 3). We cannot presently determine whether this lack of NCR sensitivity derived from a requirement of functional UISALL and GC-rich elements for the UASNTR elements to carry out their normal function or, alternatively, that the mutant constructs in plasmids pJD92 and pJD154 supported insufficient expression to obtain a meaningful result. We tend to favor the latter explanation.

Next we compared reporter gene expression supported by the collection of plasmids used in Fig. 3 in wild type and gln3Δ mutant strains (Fig. 4). γ-Aminobutyric acid was used as the nitrogen source in this experiment because even though proline is the nitrogen source (among those we normally use) generating the least NCR, gln3 mutant strains grow very poorly when provided with proline (13). Therefore, note that all values derived from cells growing with γ-aminobutyric acid as nitrogen source were 2–4-fold lower than with proline (compare Figs. 3 and 4). The Gln3p dependence of reporter gene expression was about 5-fold for most constructs (Fig. 4). Plasmids pJD156 and pJD155, both of which contain mutations in the UASNTR elements, on the other hand, yielded opposite results. The Gln3p dependence of plasmid pJD156 increased approximately 2-fold relative to wild type, whereas that of plasmid pJD155 decreased by about the same amount. The source of this behavior has not been identified, although the effect may be due to the participation of Gat1p in transcription supported by one or both of the UASNTR sites.

In sum, the data presented above suggest that at least five putative cis-acting elements participate in gene transcription supported by plasmid pJD68. Two observations, however, were unexpected. (i) These constructs supported lacZ expression that was less inducer-responsive than that supported by the entire DAL7 promoter (7); this derived from the elevated basal levels of activity in the constructs. (ii) The high basal level activity was largely dependent upon the GC-rich element. This is best seen when comparing the results with plasmids pJD68 and pJD92 (Fig. 3). Although mutating the GC-rich element dramatically decreased the overall levels of transcription, that which remained was far more inducer-responsive (Fig. 2).

Dal80p-binding Sites Upstream of DAL7—Dal80p has been shown to play a pivotal role in allophanate/oxalurate-induced allantoin pathway gene expression. This is evidenced by the observation that dal80 mutants exhibit a significant high level of DAL expression in the absence of inducer as when the wild type is assayed with inducer present (28). Searching for cis-acting sites required for Dal80p-regulated expression using the approach described in Figs. 2–4 is ill-advised because Dal80p...
Specific Dal80p-DNA complexes are indicated by arrows on the autoradiogram. Lanes F and lane G were treated with increasing amounts of the unlabeled oligonucleotide as indicated above.

The radioactive probe was DNA fragment DAL3-5, containing a well-end-points indicated relative to the ATG) that were used as competitors. The radioactive probe was DNA fragment DAL3-5, containing a well-characterized Dal80p-binding site (29). Lanes E—A and I—M contained increasing amounts of the unlabeled oligonucleotide as indicated above the autoradiogram. Lanes F and H have no added unlabeled competitor, and lane G has no protein extract added to the reaction mixture. Specific Dal80p-DNA complexes are indicated by arrows.

binds to some of the same sequences as the transcriptional activator, Gln3p (18). Therefore, each deletion and substitution mutation would potentially generate two changes as follows: transcriptional activation and repression would both decrease. We avoided this complication by using an EMSA rather than comparing reporter gene expression in wild type and dal80p mutant strains. To identify Dal80p-binding site(s) upstream of DAL7, we synthesized several large DNA fragments containing promoter sequences previously shown to participate in control of Dal80p expression (Ref. 7 and Figs. 2–4 of present work) and used them as competitors for binding of a well-characterized Dal80p-DNA probe (DAL3–5) have been described elsewhere (29). DNA fragment DAL7-1 (nucleotides –183 to –122) was also able to compete with DAL3-5 DNA for Dal80p binding but less well than the DAL7-2 fragment (lower panel, lanes G–M). DNA fragment DAL7-4 (nucleotides –265 to –196) was an ineffective competitor of DAL3-35 DNA for Dal80p binding (Fig. 6, lanes G–M); DNA fragment DAL3-3 (29) was used as the positive control (lanes A–G). The result with fragment DAL7-4 was not unexpected because only one of the sequences contained GATAA (the other was GATAG) and they are oriented head-to-head.

The DAL7-2 and DAL7-1 fragments each contained three GATA sequences, two contiguous GATAs (schematic at the top of Fig. 5, elements D and E) at one end of each fragment and a single GATA at the other (elements C or F). Based on earlier work defining the Dal80p-binding site (24), we predicted that the two GATA sequences at the 3′ end of DNA fragment DAL7-2, or the 5′ end of fragment DAL7-1 (Fig. 5, elements D and E) would perhaps be too close to one another to function as a strong Dal80p-binding site. If this were true, we hypothesized that either one or both of them, in combination with a single GATA sequence at the opposite end of the fragment, formed the Dal80p sites observed with the DAL7-2 and DAL7-1 fragments. To test this, we prepared variants of the DAL7-1 and DAL7-2 fragments in which each of the three GATA sequences was mutated; these fragments were then tested as competitors of the DAL3-5 DNA fragment for Dal80p binding (Figs. 7 and 8).

As shown in Fig. 7, fragment DAL7-8 (containing elements C and D) and DAL7-9 (containing elements D and E) were both competitors of the DAL3-5 fragment for Dal80p binding, with DAL7-9 being somewhat better than DAL7-8 (Fig. 7, upper panel, compare lanes A–G with G–M). The effectiveness of fragments DAL7-9 and DAL7-8 as competitors correlates with data from the DAL3 gene demonstrating that two GATA sequences oriented tail-to-tail bind Dal80p better than a head-to-tail orientation (29). We also expected that DNA fragment DAL7-10 would compete less well than fragment DAL7-9 because the GATA sequences it contained were only 8 bp apart (elements D and E, oriented tail-to-tail); this was confirmed experimentally (Fig. 7, compare data with DNA fragments, DAL7-7, 7-8, and 7-10). A smaller DNA fragment, containing only the closely spaced GATAs (elements D and E), behaved similarly to fragment DAL7-10 (DAL7-11, Fig. 7, lower panel, lanes G–M).

We next assayed the GATA sequences contained on DNA fragment DAL7-1; recall that fragment DAL7-1 was less effective competitor than fragment DAL7-2 (Fig. 5). In contrast to the data obtained with mutant alleles of fragment DAL7-2, the only DAL7-1 mutant allele that retained high effectiveness...
as a DAL3-5 competitor was the one in which the isolated 3'-GATA (element F) was mutated (Fig. 8, lower panel, lanes G–M, fragment DAL7-1). Mutation of either of the two closely spaced GATA sequences (elements D or E) significantly decreased the ability of the mutant fragments to act as effective competitors (Fig. 8, upper panel, lanes G–M, and lower panel, lanes A–G). The DAL7-7 and DAL7-1 fragments were similar competitors of the DAL3-5 fragment for binding to Dal80p (compare Fig. 8, lower panel, lanes G–M with Fig. 5, lower panel, lanes G–M). In other words, the closely spaced GATA sequences that were in common with fragment DAL7-2 (elements D and E) were more effective competitors than either of them paired with the isolated GATAG at the 3' end of fragment DAL7-1 (element F) and accounted for the overall ability of fragment DAL7-1 to serve as competitor. Whereas this observation might seem somewhat at odds with data derived from assaying fragment DAL7-2, the effectiveness of a Dal80p-binding site is determined not only by spacing and orientation of the GATA but also by their specific sequences. Although the GATA sequences of DNA fragment DAL7-1 possess acceptable orientations and spacings, one of the two GATA elements possesses the sequence GATAG. Together, these data identify the GATA elements C—D, C—E, and D—E (Fig. 8) as Dal80p-binding sites, URS\_GATA\_8, upstream of DAL7.

Effects of Promoter Mutations in the Context of a Complete DAL7 Promoter—The data described above in combination with earlier studies (7) yield a reasonably consistent picture of the cis-acting elements functioning in DAL7 expression. However, none of the mutations used in any of the DAL7 studies has ever been analyzed in the context of an intact DAL7 promoter. Although the contribution of cis-acting elements contained on small promoter fragments to the overall lacZ expression supported in a heterologous expression vector can be determined with certainty, their relation(s) to DAL7 expression in situ cannot be as confidently deduced. This problem was addressed by systematically mutating the putative cis-acting elements in an intact, wild type DAL7 promoter (Fig. 9). In each case, substitution mutations were used to ensure that the relative positions of the remaining promoter elements were not otherwise altered. Furthermore, the plasmids were CEN-based to eliminate conceivable “dose response” or transcription factor “titration” effects. Mutation of the 5’-most GATA sequence (plasmid pRR373) resulted in 25% less transcription in the presence of inducer compared with wild type (Fig. 9, plasmid pRR373 versus plasmid pRR352). These data correlate with those in Figs. 2 and 3, demonstrating that this element participates in DAL7 expression. Although inducer responsiveness is decreased in this mutant plasmid, it is still present, arguing that a single UIS element is sufficient to elicit this physiological response,

FIG. 7. Competition EMSAs between DNA fragment DAL3-5 and mutant fragments derived from the DAL7-2 fragment for Dal80p binding. Reactions conditions and lane designations were as described in Fig. 6. The top portion of the figure diagrams the DAL7 promoter and the oligonucleotide fragments used in the experiment. X indicates the mutated elements (see Table III).

FIG. 8. Competition EMSAs between DNA fragment DAL3-5 and mutant DNA fragments derived from the DAL7-1 fragment for Dal80p binding. All parameters were as described in Fig. 7.
albeit at a more modest level than when two UIS\textsubscript{ALL} elements are present.

Mutation of the next GATA sequence (plasmid pRR375) yielded an unexpected result (Fig. 9). Based on data derived from the isolated promoter fragments (plasmids pJD68 and pJD156, Fig. 3), one would have expected mutation of this element to have a negative effect on reporter gene expression. However, in the context of the entire DAL7 promoter, it did not appear to participate in transcription (Fig. 9, plasmid pRR375). A similar surprise was observed in the case of GC-rich element. We had concluded from data derived with isolated fragments that this element played a central role in transcription, because it was the one that generated the most drastic decreases in transcription when mutated (plasmids pJD68 and pJD92, Figs. 2 and 3). Mutating the element in an otherwise intact promoter, however, decreased induced expression by only half (Fig. 9, plasmid pRR371); one can conclude that it is a participant in DAL7 transcription but probably not the central one. The elements downstream of the GC-rich element that are most important to DAL7 expression are the UIS\textsubscript{NTR} and UIS\textsubscript{ALL} elements; the direction of the arrow indicates the orientation of the sequence. UIS and GC designate UIS\textsubscript{ALL} and GC-rich elements, respectively. The maps are drawn approximately to scale. Filled boxes containing white letters or symbols indicate mutant alleles of the designated element (see Table II). Coordinates above the map are relative to the ATG of the coding sequence.

DISCUSSION

The data presented above document the participation of eight cis-acting elements in the regulated expression of DAL7 (Fig. 11). Transcriptional activation depends upon a GC-rich element, two UIS\textsubscript{ALL}, and two GATA-containing UIS\textsubscript{NTR} elements. Transcriptional repression (i.e., Dal80p-binding), on the other hand, depends upon three URS\textsubscript{GATA} elements; two of the three GATA sequences associated with the URS\textsubscript{GATA} elements are also common to the DAL7 UIS\textsubscript{NTR}.

Some of the proteins associated with the three types of cis-acting elements have been identified as follows: UIS\textsubscript{ALL} elements bind Dal82p (21), which is required for induction of allantoic pathway gene expression (22, 40). The GATA-containing UIS\textsubscript{NTR} elements bind Gln3p and are also thought to bind Gat1p as well (12, 14, 16, 42). Although Gat1p-binding remains to be experimentally demonstrated, the protein contains a GATA-binding zinc finger motif similar to the one in Gln3p (12, 36, 43, 44), and both proteins are known to be
transcriptional activators through which NCR operates (16). We speculate that the role of the UIS\textsubscript{ALL}-Dal82p complex may be to facilitate, or stabilize, Gln3p and/or Gat1p binding. Supporting this idea is the observation that a UIS\textsubscript{ALL} site placed close to a UAS\textsubscript{NTR} site, containing point mutations in it, will suppress those mutations (19); suppression requires Dal82p but not Dal81p or inducer. Whether this interaction is at the level of a Gln3p-Dal82p complex or further along the pathway of preinitiation complex formation is not known at present. It is interesting, however, that the distance between the functional 5’ UIS\textsubscript{ALL} and UAS\textsubscript{NTR} elements is the same as between the 3’ UIS\textsubscript{ALL} and UAS\textsubscript{NTR} elements (Fig. 11).

It should be noted that UIS\textsubscript{ALL} and Dal82p do not appear to be unique in their ability to function synergistically with Gln3p and/or Gat1p; the GC-rich element and the protein(s) presumed to be associated with it also appear to function in this capacity. Similar relationships have been observed between the UAS\textsubscript{NTR} and the Rap1p and Abf1p sites in the CAR1 promoter (36), the GATA element and the Put3p site of the PUT1 promoter (45), and the UAS\textsubscript{NTR} and Abf1p sites of CAR2 promoter.3

Finally, we turn to the URS\textsubscript{DATA} elements that bind Dal80p, the repressor protein responsible for maintaining inducible DAL gene expression at low basal levels in the absence of inducer, and for down-regulating those genes (e.g. DAL3) whose expression is not inducer-dependent. Only three of six possible DAL7 GATA sequences appear to participate in Dal80p binding. Although the two 5’-most GATA elements (A and B) possess the appropriate orientation and spacing, they did not demonstrably bind Dal80p; the 3’ of these two GATA elements possess the sequence TAGAT. Binding of Dal80p to the two GATA elements at positions B and C did not occur because they are oriented head-to-head. Dal80p, on the other hand, does bind to the three GATA sequences at positions C, D, and E (Fig. 11). To the extent that the ability of a DNA fragment to serve as an effective competitor in an EMSA is a reflection of its binding affinity, Dal80p binding to these sites is, in order of decreasing affinity, C–E, C–D, and D–E (Fig. 11). We found earlier that a tail-to-tail orientation was the most favorable for Dal80p binding (29), a conclusion that correlates with the present data. It also makes sense that the D–E-binding site is the weakest site because the spacing is at the limit of the requirements determined in the DAL3 work (29). Finally, Dal80p binding to the D–F and E–F element pairs does not occur even though the orientation and spacing requirements are met, presumably because the F element does not possess the sequence GATAA but GATAG. Although participating as a Dal80p-binding site, the E element does not demonstrably function in transcriptional activation. To our knowledge, this is the first example of a GATA sequence that possesses this characteristic.

This work identifies a new cis-acting participant in DAL7 expression, the GC-rich sequence. Whereas this element plays an important role in transcription supported by the DAL7 promoter fragment contained in plasmid pJD68, its role appears somewhat less prominent or masked when assayed in the context of the intact DAL7 promoter. Earlier results may be consistent with the suggestion that the GC-rich sequence participates in gene expression in that the footprint covering the UIS\textsubscript{ALL} sequence extends to the GC-rich sequence as well (Fig. 4 and see Ref. 19). We are not, however, suggesting that available data point to the existence of an interaction between proteins associated with the GC-rich and UIS\textsubscript{ALL} elements. Detailed analysis of the CAR1 gene (encoding arginase) also identified a positively acting GC-rich sequence similar to the one identified here (36). Whether these sequences represent the same cis-acting element or are just coincidentally similar sequences is not clear.

A model describing how the above cis-acting elements and trans-acting factors accomplish regulated expression of DAL and perhaps other inducible nitrogen catabolic genes was proposed some time ago (7, 36, 46). According to this model, NCR-sensitive, inducer-independent transcriptional activation is mediated by UAS\textsubscript{NTR}, Gln3p, and the homologous Gat1p. Dal80p limits the interaction of GATA-containing UAS\textsubscript{NTR} elements with Gln3p by competing with it for binding to the GATA elements. We speculate that, when it is possible to perform the experiments, the same will be found to be true for Gat1p binding. In other words, the positively acting Gln3p/Gat1p contribution to DAL gene transcription is balanced by the negative action of Dal80p; the metaphor of a see-saw was used in the original formulation of this model (7, 36, 46). The implication is that the effect of Dal80p impeding Gln3p and/or Gat1p binding is greater than its facilitation by Dal82p in the absence of inducer, and hence the DAL genes are expressed at only basal levels. Dal82p and Dal81p, associated with the cis-acting element UIS\textsubscript{ALL}, shift the balance in the direction of elevated expression in an inducer-dependent manner (7). This hypothesis makes the prediction that gene expression should be influenced by the intracellular levels of both Dal80p and Gln3p/Gat1p. Recent evidence has shown this to occur.4

This model of inducer-independent transcriptional repression acting in opposition to inducer-independent activation, with the balance being shifted by inducer-dependent transcriptional activation, has also been proposed to account for NCR-sensitive, inducer-dependent regulation of the CAR genes (36, 46). Although the cis- and trans-acting elements are different in the case of the CAR genes, their operation follows the same formal model as the allantoin pathway genes (36). Published studies of the NCR-sensitive, inducible UGA genes similarly fit this perspective (30).

Finally, GATA sequences situated contiguously head-to-tail or tail-to-tail are a rather common feature of NCR-sensitive gene promoters that respond to Dal80p regulation. In addition to DAL7, such contiguous GATA elements are found in many promoters, e.g. DAL3, DUR1, DUR3, UGA4, CAN1, GAP1, and MEF2. They are rarely found in NCR-sensitive genes that are not Dal80p-regulated (e.g. DAL5). These correlations and this

3 H.-D. Park, R. Rai, S. Scott, R. Dorrington, and T. G. Cooper, manuscript in preparation.

4 T. Cunningham and T. G. Cooper, manuscript in preparation.
work suggest that this array of contiguous GATA sequences is responsible for Dal80p regulation of Gln3p/Gat1p in the case where it exists. In this regard, as more nitrogen catabolic promoters are dissected in detail, it will be interesting to assess how widely GATA sequences that participate in Dal80p binding but not transcriptional activation occur, as is the case for the E element in Fig. 11.

In a technical context, this work has pointed out one of the limitations of heterologous expression vector systems in identifying promoter elements and analyzing their functions. We identified three instances in which the characteristics of a potential cis-acting site carried on a cloned promoter fragment are quite different than when the same site is contained within an intact promoter. For example, transcription supported by the promoter fragment carried in plasmid pJD68 appears less NCR-sensitive than when the entire promoter is assayed (10). This behavior may be explained by suggesting that (i) the GC-rich element is responsible for a greater proportion of the transcription supported by plasmid pJD68 than by an intact promoter, and (ii) the GC-rich mediated transcription is not NCR-sensitive. An analogous situation occurs when assessing the contribution of the GC-rich element to gene expression. When the GC-rich element is carried on plasmid pJD68, it is the major contributor to transcriptional activation. In contrast, this element seems to play a more limited role when present in the native promoter. Finally, the UAS_{NTA}-homologous GATA sequence at positions −249 to −245 (element B) appears to contribute to reporter gene transcription supported by plasmid pJD156 in that β-galactosidase decreased 53% when it was mutated (Fig. 4, plasmid pJD156). However, when the sequence was mutated in an otherwise native DAL7 promoter, a much more modest effect was observed (Fig. 9, plasmid pRR375).

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