Cis-* and *trans*-acting elements determining induction of the genes of the γ -aminobutyrate (GABA) utilization pathway in *Saccharomyces cerevisiae

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ABSTRACT

In *S.cerevisiae*, γ -aminobutyrate (GABA) induces transcription of the *UGA* genes required for its utilization as a nitrogen source. Analysis of the 5' region of the *UGA1* and *UGA4* genes led to the identification of a conserved GC-rich sequence (UAS_{GABA}) essential to induction by γ -aminobutyrate. Alone, this UAS_{GABA} element also supported some levels of reporter gene transcription in the presence of γ -aminobutyrate. To be effective, UAS_{GABA} requires two positive-acting proteins that both contain a Cys₆-Zn₂ type zinc-finger motif, namely pathway-specific Uga3p and pleiotropic Uga35p(Dal81p/DurLp). Further analysis of the *UGA4* gene revealed that Gln3p, a global nitrogen regulatory protein containing a GATA zinc-finger domain, is required in order to reach high levels of γ -aminobutyrate-induced transcription. The Gln3p factor exerts its function mainly through a cluster of 5'-GAT(A/T)A-3'(UAS_{GATA}) situated just upstream from UAS_{GABA}. The role of Gln3p is less predominant in *UGA1* than in *UGA4* gene expression. We propose that tight coupling between the UAS_{GABA} and UAS_{GATA} elements enables the cell to integrate, according to its nitrogen status, the induced expression levels of *UGA4*.

INTRODUCTION

In *Saccharomyces cerevisiae*, the *UGA1* (γ -aminobutyrate:2-oxoglutarate aminotransferase), *UGA2* (succinate semialdehyde dehydrogenase) and *UGA4* (γ -aminobutyrate-specific permease) genes are involved in the use of γ -aminobutyrate (GABA) as a nitrogen source. Expression of all three *UGA* genes is induced in the presence of γ -aminobutyrate (1–6). Mutations in one of two positive regulatory genes, *UGA3* or *UGA35*, abolish this induction (1,3). The Uga3p and Uga35p proteins both contain a Cys₆-Zn₂ type zinc-finger motif and other features found in transcriptional regulatory factors (5,7,8). The Cys₆-Zn₂ motif has been found in at least 20 proteins, all of fungal origin, including

the well-studied Gal4p transcriptional activator. It has been demonstrated, by X-ray crystallographic analysis of a dimeric Gal4p domain bound to the 17 bp upstream activating sequence (UAS_{GAL}), that the Cys₆-Zn₂ motif establishes direct contacts with DNA (9). The Uga3p factor acts as a specific regulator of γ -aminobutyrate-inducible genes. Uga35p, in contrast, is pleiotropic (4,7): its encoding gene is identical to the *DURL* and *DAL81* genes, originally identified as positive regulatory elements of allophanate-inducible genes (10,11), which include the *CAR2* gene of ornithine catabolism (12), the *DUR* genes of urea utilization (10) and some of the *DAL* genes of allantoin utilization (11). In the allophanate-induction process, the pleiotropic Uga35p(Dal81p/DurLp) acts in conjunction with another, inducer-specific protein, DurMp(Dal82p), which specifically controls the expression of allophanate-inducible genes (4,10,13,14). The Uga35p(Dal81p/DurLp) and DurMp(Dal82p) factors act through a dodecanucleotide sequence (UIS; upstream induction sequence) located in the 5'-flanking region of allophanate-inducible genes (15).

The upstream regions of several nitrogen pathway genes of *S.cerevisiae* contain multiple copies of the sequence 5'-GAT(A/T)A-3' (18). This feature is also shared by several nitrogen-regulated genes of the filamentous fungi *Neurospora crassa* and *Aspergillus nidulans* (16). Several promoter DNA fragments containing at least two copies of this sequence behave like upstream activating sequences (UAS) that lose their activity under conditions of nitrogen repression, i.e. when cells use a nitrogen source supporting optimal growth (17,18). In *S.cerevisiae*, stimulation of expression via these UAS elements requires a functional *GLN3* gene product (18,19). The Gln3p factor up-regulates the expression of several nitrogen utilization genes (18,20–22) and contains a motif, called the GATA zinc-finger, which is known to direct binding to 5'-GATA-3' core sequences. Similarly, both the NIT2 protein of *N.crassa* and the AREA protein of *A.nidulans* contain a GATA zinc-finger domain and up-regulate the expression of many genes, encoding proteins involved in various nitrogen-catabolic pathways (16,34). Another GATA zinc-finger regulatory protein, Uga43p(Dal80p), down-regulates to a variable extent the expression of several *S.cerevisiae* nitrogen pathway genes (3,4,11,22–24). In mutants

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lacking a functional *UGA43(DAL80)* gene, the *UGA4* gene encoding the γ -aminobutyrate-specific permease, is expressed at high constitutive levels (3,6).

In this report, we have undertaken to analyse the *cis*-acting sequences that determine induction by γ -aminobutyrate of the *UGA1* and *UGA4* genes. A conserved 19 bp sequence, UAS_{GABA} , was identified and shown to be essential to γ -aminobutyrate-induced transcription. This UAS_{GABA} element is also sufficient to support reporter gene expression in the presence of γ -aminobutyrate. The inducer-specific Uga3 and pleiotropic Uga35(Dal81/DurL) proteins both exert their positive functions through UAS_{GABA} . Further analysis of the *UGA4* gene additionally showed that UAS_{GABA} acts in conjunction with a cluster of 5'-GAT(A/T)A-3' core sequences (UAS_{GATA}) which also contributes considerably to promoting high-level γ -aminobutyrate-induced transcription. Up-regulation through UAS_{GATA} is Gln3p-dependent. In the accompanying paper, André *et al.* demonstrate that the UAS_{GATA} element is subject to two distinct and separate negative controls, one of which is exerted by Uga43p(Dal80p).

MATERIALS AND METHODS

Strains, growth conditions and methods

The *S.cerevisiae* strains used in this study (Table 1) are isogenic with the wild-type $\Sigma 1278b$ (25) except for the mutations mentioned. Cells were grown on minimal medium (26) with 0.1% proline (conditions of non-induction) or 0.1% proline + 0.1% γ -aminobutyrate (conditions of induction) as nitrogen sources. Yeast cells treated with lithium acetate (27) were transformed according to Sherman *et al.* (28). The *E.coli* strain used was JM109. All procedures for manipulating DNA relied on standard methods (29,30). Plasmid constructs were locally sequenced by the dideoxy chain termination method (31) with T7 DNA polymerase (Pharmacia LKB) and, as the template, alkali-denatured plasmid DNA. Site-directed mutagenesis was carried according to the 'Altered Sites *in vitro* Mutagenesis System' of Promega.

Table 1. Yeast strains

Strain	Genotype	Reference
$\Sigma 1278b$	<i>MATa</i>	(25)
23344c	<i>MATa, ura3</i>	M. Grenson
26970a	<i>MATa, ura3, uga3Δ</i>	(49)
CD17	<i>MATa, ura3, uga35Δ</i>	(7)
30078c	<i>MATa, ura3, uga43Δ</i>	(24)
30505b	<i>MATa, ura3, gln3Δ</i>	(41)

Plasmids

The YCpUGA1-lacZ gene was isolated by inserting into the filled-in *HindIII* site of plasmid YCpAJ152 (6) a blunt-ended *HincII*-*TthIII* fragment comprising the first 19 codons of *UGA1* and 0.72 kb of upstream sequences. The inserted *UGA1* fragment is flanked at the 5' end by the polylinker of the pGem7Zf(+) plasmid (Promega) contained between the *ApaI* and *HindIII* sites.

Plasmid YCpUGA4-lacZ has been described (6). To isolate the nested deletions upstream from the *UGA1-lacZ* and *UGA4-lacZ* genes, the YCpUGA1-lacZ and YCpUGA4-lacZ plasmids restricted by *SphI* and *HindIII* were treated with Exonuclease III as instructed for the 'Nested Deletions' kit (Pharmacia LKB). The YCpUGA3-lacZ plasmid was constructed by inserting into the filled-in *HindIII* site of plasmid YCpAJ150 (6) a blunt-ended *EcoRV*-*AsuII* fragment comprising the first 13 codons of *UGA3* and 0.9 kb of upstream sequences. To isolate *CYC1-lacZ* genes with upstream insertions, we started from pLG670Z plasmid (32). The upstream DNA regions of the *UGA1* gene inserted upstream from *CYC1-lacZ* were obtained by PCR amplification. The amplified DNA fragments flanked by *XhoI* sites were restricted by *XhoI* and ligated into the unique *XhoI* site situated in the 5' region of *CYC1-lacZ*. For the *CYC1-lacZ* upstream insertions of the 19 bp UAS_{GABA} elements, we used annealed synthetic oligonucleotides flanked by *XhoI* sites. The ΔUAS_{GATA} -UGA4-lacZ construct described in Table 5 is identical to the centromere-based *UGA4-lacZ* plasmid except that the promoter region was specifically deleted of the sequence 5'-CTTATCGCTAATCGCT-TATCGCTTATCG-3' (positions -438 to -411) corresponding to the UAS_{GATA} element. To isolate this construct, a 1 kb *HindIII*-*BamHI* fragment comprising the *UGA4* promoter region and upstream plasmid sequences was inserted into the pALTER phagemid (Promega). Single-stranded DNA was produced and the UAS_{GATA} sequence was deleted by site-directed mutagenesis using the 'Altered Sites *in vitro* Mutagenesis System' (Promega). The accuracy of the mutated plasmid was checked by complete re-sequencing of the *UGA4* upstream region.

Assays of β -galactosidase activity

Measurements of β -gal activity in crude extracts of yeast cells were carried out as previously described (6). Enzyme activities are expressed in nmoles of *o*-nitrophenol formed/min/mg of protein. Protein concentrations were measured by the method of Lowry *et al.* (33), with bovine serum albumin as the standard. All β -gal assays were performed in exponentially growing cells having reached the state of balanced growth (34).

Nucleotide sequence accession number

The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDBJ nucleotide sequence data bases under accession numbers X80073 (*UGA4*) and X80074 (*UGA1*).

RESULTS

Expression of *UGA1-lacZ* and *UGA4-lacZ* fusion genes

The *UGA1* gene encoding γ -aminobutyrate:2-oxoglutarate aminotransferase and the *UGA4* gene encoding the γ -aminobutyrate-specific permease have been previously cloned and sequenced (6,35). Examination of the two promoter regions (accession numbers X80073 and X80074) revealed that both contain several 5'-GAT(A/T)A-3' sequences. Nine copies were found between positions -430 and -71 of *UGA4* and seven between positions -470 and -178 of *UGA1* (Table 3). A sequence resembling URS1, an upstream repressing sequence that down-regulates expression of many genes in *S.cerevisiae* (36,37), was also found upstream from *UGA1* (5'-AGCCGCGGG-3'; -384 to -376) and from *UGA4* (5'-AACC GCCGG-3'; -401 to -393). A

sequence similar to the binding site for ABF1 is located between positions -570 and -588 of *UGA4*. Other sequences identified in the promoter regions of several nitrogen pathway genes, including the URS and UIS elements of allophanate-inducible genes (38) and the 5'-TT(T/G)TTT-3' sequence identified upstream from the *GDH1* and *GDH2* genes (39,40), were not found in the upstream region of either *UGA1* or *UGA4*.

To facilitate the measurement of *UGA1* gene expression levels, we constructed a *UGA1-lacZ* fusion and inserted it into a derivative of the centromere-based plasmid YCp50. We introduced the resulting plasmid, YCpUGA1-lacZ, into wild-type and mutant cells and measured β -galactosidase (β -gal) activity in cells grown in the absence or presence of γ -aminobutyrate (Table 2). The values were compared with those obtained in the same experiment with a similar *lacZ* construct bearing a promoter region derived from the *UGA4* gene (6). In wild-type cells, *UGA1-lacZ* was induced ~30-fold in response to the presence of γ -aminobutyrate. The induced levels were drastically reduced in cells with chromosomal deletions of either the *UGA3* or the *UGA35* gene. The more drastic effect was observed in *uga3* Δ mutant cells. Basal expression measured in the absence of γ -aminobutyrate was also significantly reduced in *uga3* Δ and *uga35* Δ mutant cells. These data agree with the results of the Northern analysis of *UGA1* transcripts as well as with the measured γ -aminobutyrate aminotransferase activity levels (3,4). Basal and induced expression levels were, respectively, about 6 and 20 times higher for *UGA1-lacZ* than for *UGA4-lacZ*.

Table 2. Expression of the *UGA1-lacZ* and *UGA4-lacZ* genes in wild-type and mutant strains

Strain	β -galactosidase (nmol/min/mg protein)			
	<i>UGA1-lacZ</i>		<i>UGA4-lacZ</i>	
	-GABA	+GABA	-GABA	+GABA
Wild-type	230	6530	≤ 10	1430
<i>uga3</i> Δ	80	180	≤ 10	≤ 10
<i>uga35</i> Δ	80	670	≤ 10	≤ 10
<i>uga43</i> Δ	770	8030	2630	2920

Cells were grown on minimal proline medium with (+GABA) or without (-GABA) 0.1% γ -aminobutyrate. The reported β -gal activities are averages of the values from two independent experiments. Variations were $\leq 15\%$. The values obtained with the *UGA4-lacZ* construct are similar to those previously reported (6).

The *UGA43* gene encodes a protein which down-regulates the expression of multiple nitrogen pathway genes (3,4,11,22). Previous experiments have shown that in *uga43* Δ mutant cells grown in the absence of γ -aminobutyrate, the *UGA4* gene is constitutively expressed at levels 200–300 times as high as in wild-type cells grown under the same conditions (6) (Table 2). Basal expression of *UGA1-lacZ* is also higher in *uga43* Δ mutant cells, but the levels are only about three times as high as in the wild-type (Table 2). This suggests that the repressive effect of Uga43p on *UGA1* gene expression is moderate. It is also detectable, albeit moderate, in Northern blot experiments and in γ -aminobutyrate aminotransferase assays previously carried out

Table 3. Deletion and mutational analysis of the upstream region of the *UGA1* and *UGA4* genes and identification of *cis*-acting sequences required for induction by γ -aminobutyrate

		β -galactosidase (nmol . min ⁻¹ . mg prot. ⁻¹)	
		- GABA	+ GABA
A	1 - 718	390	6370
	2 - 602	520	9870
	3 - 580	590	9140
	4 - 458	610	9820
	5 - 396	410	9840
	6 - 356	140	310
	7 - 327	180	310
	8 - 250	200	500
	9 - 186	560	570
	10 - 718	40	80
	11 - 718	40	270
		COO → AAA	
B	12	≤ 10	1220
	13 - 679	≤ 10	1380
	14 - 581	≤ 10	1880
	15 - 542	≤ 10	1580
	16 - 514	≤ 10	1740
	17 - 429	≤ 10	1380
	18 - 418	≤ 10	1380
	19 - 406	≤ 10	850
	20 - 391	≤ 10	≤ 10
	21 - 328	≤ 10	≤ 10
	22 - 222	≤ 10	≤ 10
	23	≤ 10	≤ 10

Cells bearing the low-copy-number plasmids carrying the *UGA1-lacZ* genes (lines 1–11) or the *UGA4-lacZ* genes (lines 12–23) with the indicated 5' deletions were grown on minimal proline medium with (+GABA) or without (-GABA) 0.1% γ -aminobutyrate. The black vertical lines indicate the relative positions of the 5'-GAT(A/T)A-3' sequences. The open box indicates the position of the UAS_{GABA} elements. The reported β -galactosidase activities are averages of the values obtained in two or three independent experiments. Variations were $\leq 15\%$.

on *uga43* mutant strains (4,22). The *UGA1-lacZ* and *UGA4-lacZ* genes are induced by γ -aminobutyrate to higher levels in the *uga43* Δ mutant strain, suggesting that Uga43p still exerts some repression, though limited, under conditions of induction.

A conserved, 19 bp, GC-rich sequence essential to γ -aminobutyrate-induced transcription

To analyze the *cis*-acting sequences involved in expression of the *UGA* genes, we first constructed a series of deletions in the upstream regions of both *UGA1* and *UGA4* (Table 3). The effects of these deletions were tested by measuring β -gal activities in wild-type cells grown in the presence or absence of γ -aminobutyrate. *UGA1-lacZ* genes with upstream deletions extending from -718 to -396 supported high inducible β -gal activity, while removal of another 40 bp (to -356) led to drastic reduction of induced expression (Table 3, lines 1–6). In similar experiments with the *UGA4-lacZ* fusion gene, genes with deletions to -514 supported highly inducible β -gal activity (Table 3, lines 12–16). The next three deletions together caused a ~3-fold reduction of

5'-AAAAGCCGCGGGCGGGATT-3' UGA1 (-387 to -369)
 5'-AAAAACCGCCGCGGCAAT-3' UGA4 (-403 to -385)

Figure 1. Sequence of the UAS_{GABA} elements of the *UGA1* and *UGA4* genes.

induced expression. These deletions include four clustered 5'-GAT(A/T)A-3' core sequences (Table 3, lines 17–19). The next deletion, removing another 15 bp, completely abolished induced expression (Table 3, line 20). By comparing this 15 bp sequence with the 40 bp sequence essential to induction of *UGA1*, we were able to identify a similar 19 bp sequence (Fig. 1). This sequence, truncated by the 15 bp deletion causing loss of *UGA4-lacZ* expression, contains a GC-rich central core of 11–12 nucleotides flanked by AT-rich segments. The central core of the sequence from *UGA4* contains a perfect, 10 bp palindrome, 5'-CCGCCGCGG-3'. The corresponding region in the 19 bp sequence from *UGA1* is an imperfect palindrome differing by a single nucleotide from the *UGA4* palindrome. Interestingly, both GC-rich central cores somewhat resemble the URS1 element (5'-AGCCGCCGA-3') situated upstream from many *S.cerevisiae* genes and through which transcription is down-regulated. To test the role of the 19 bp sequence in *UGA1* and *UGA4* gene expression, we deleted it by site directed mutagenesis. β -gal activity assays showed that deletion of this 19 bp sequence abolishes γ -aminobutyrate-induced expression of both genes (Table 3, lines 10 and 23). Furthermore, substitution of AAA for the CGG triplet situated at the 3' end of the GC-rich sequence of *UGA1* also severely reduces γ -aminobutyrate-induced expression (Table 3, line 11). These data demonstrate that the conserved 19 bp sequences identified upstream from the *UGA1* and *UGA4* genes are essential to γ -aminobutyrate-induced expression.

The 19 bp GC-rich sequence is a UAS conferring Uga3p- and Uga35p-dependent γ -aminobutyrate induction

To test the ability of the *UGA1* and *UGA4* 19 bp sequences to activate transcription, we inserted each one upstream from the *CYC1-lacZ* reporter gene and measured the level of expression. The results are shown in Table 3. Clearly, each sequence acts like an upstream activating sequence driving γ -aminobutyrate-dependent transcription. We have called these sequences UAS_{GABA}. The UAS_{GABA} of the *UGA1* gene produced a three times greater effect than did the UAS_{GABA} of the *UGA4* gene. In both cases, UAS_{GABA}-directed expression was abolished in *uga3* Δ mutant cells and drastically reduced in *uga35* Δ mutant cells. Hence, both Uga3p and Uga35p appear to exert their positive functions through UAS_{GABA}. The UAS_{GABA} of the *UGA4* gene worked equally well in both orientations. Two adjacent copies gave rise to an eight times higher expression level than did a single copy, indicating that several UAS_{GABA} elements can potentially act in synergy.

Uga35p is not required for expression of a *UGA3-lacZ* gene

The data presented above show that functional *UGA3* and *UGA35* gene products are essential to UAS_{GABA}-mediated activation of γ -aminobutyrate-induced transcription. While Uga3p specifically regulates expression of γ -aminobutyrate-inducible genes,

Figure 2. Expression of a *UGA3-lacZ* gene in wild-type and *uga35* Δ mutant cells. Cells of strains 23344c [*ura3*] (open squares) and CD17 [*uga35* Δ , *ura3*] (closed squares) were grown on minimal medium containing proline and γ -aminobutyrate as nitrogen sources. The β -galactosidase activity (expressed in nmol of *o*-nitrophenol formed/min/ml of culture) was measured as a function of cellular mass in exponentially growing cultures. The values are averages of two independent assays. Variations were $\leq 20\%$.

Uga35p (Dal81p/DurLp) is pleiotropic, since it is also required for the expression of allophanate-induced genes. A possible explanation for the requirement of Uga35p in UAS_{GABA}-mediated transcription could be that the pleiotropic factor is required for expression of the *UGA3* gene. To test this hypothesis, we constructed a *UGA3-lacZ* fusion gene in a low-copy-number plasmid and measured the resulting β -gal activity in the wild type and a *uga35* Δ strain. The results (Fig. 2) show that expression of the *UGA3-lacZ* gene in the presence of γ -aminobutyrate is not abolished in *uga35* Δ mutant cells. On the contrary, it is actually higher in the mutant strain. These data indicate that the non-inducibility of the *UGA* genes in *uga35* Δ mutant cells is not attributable to impaired expression of the *UGA3* gene. In fact, Uga35p seems to down-regulate, directly or indirectly, *UGA3* expression. The opposite hypothesis, i.e. that Uga3p is essential to the expression of the *UGA35* gene, can be discarded since allophanate-induced expression of the *DUR* genes, a process requiring a functional *UGA35* gene product, is not affected by mutations in the *UGA3* gene (4).

Role of Gln3p and of 5'-GAT(A/T)A-3' sequences in γ -aminobutyrate-induced transcription

The first 430 nucleotides preceding the translation initiation codon of *UGA4* contains nine copies of the pentanucleotide 5'-GAT(A/T)A-3' (or its reverse complement 5'-T(T/A)ATC-3'). Four of them are clustered in a 28 nucleotide region just upstream from the UAS_{GABA} element and are flanked by C and G, resulting in four directly repeated 5'-CGAT(A/T)ATG-3' motifs. The five remaining sequences are situated downstream from UAS_{GABA} and are not clustered. In the accompanying paper (41), experiments are presented showing that the repeated 5'-CGAT(A/T)ATG-3' constitute a UAS element, called UAS_{GATA}, which can potentially direct high-level transcription. In the absence of γ -aminobutyrate, however, the potential activity of UAS_{GATA} is inhibited by two distinct factors: Uga43p, when

Table 4. Properties of the UAS_{GABA} elements from the UGA1 and UGA4 genes

Insertion upstream from <i>CYC1-lacZ</i>	Wild-type		<i>uga3Δ</i>		<i>uga35Δ</i>	
	- GABA	+ GABA	- GABA	+ GABA	- GABA	+ GABA
UAS _{GABA} UGA1	80	4040	20	35	40	460
UAS _{GABA} UGA4	15	1520	≤ 10	≤ 10	≤ 10	≤ 10
UAS _{GABA} UGA4	15	1570	≤ 10	≤ 10	≤ 10	20
(UAS _{GABA}) ₂ UGA4	55	11490	≤ 10	n.d.	≤ 10	70
no insert	15	35	≤ 10	≤ 10	≤ 10	≤ 10

Synthetic oligonucleotides corresponding to the UAS_{GABA} element of *UGA1* (5'-AAAAGCCGCGGGCGGGATT-3') or *UGA4* (5'-AAAACCGCCGCGGCAAT-3') were inserted in single or double copies upstream from the *CYC1-lacZ* gene deleted of its own regulatory sequences and carried on a high-copy-number plasmid. Wild-type and mutant cells bearing these plasmids were grown on minimal proline medium with (+GABA) or without (-GABA) 0.1% γ -aminobutyrate. The reported β -gal activities are averages of the values obtained in two or three independent experiments. Variations were $\leq 15\%$.

cells are grown under conditions of nitrogen derepression, and Ure2p, when cells are grown under conditions of nitrogen repression. To determine whether the UAS_{GATA} element participates in γ -aminobutyrate-induced transcription of *UGA4*, we assayed the expression of a mutagenized *UGA4-lacZ* gene, lacking only its UAS_{GATA} element, in wild-type cells grown in the absence or presence of γ -aminobutyrate (Table 5). Induced expression was reduced by about 80% in this experiment. Accordingly, the successive deletions from positions -514 to -406 that led to complete removal of UAS_{GATA} together caused an ~60% reduction of induced *UGA4-lacZ* transcription (Table 3, lines 16–19). Thus, while the inducible UAS_{GABA} element plays an essential role in γ -aminobutyrate-induced transcription, the UAS_{GATA} element situated just upstream from it also contributes considerably to producing high levels of induced expression. The fact that deletion of UAS_{GATA} causes a 60% reduction of γ -aminobutyrate-induced expression in one *UGA4-lacZ* construct (Table 2, lines 17–19), and an 80% reduction in another (Table 5A), might be due to the totally different sequences that precede the UAS_{GABA} element in the two plasmids. The major contribution of UAS_{GATA} to expression of the *UGA4* gene prompted us to test the role of the pleiotropic, positive-acting factor Gln3p (Table 5). Assays of *UGA4-lacZ* expression in a strain deleted of the chromosomal *GLN3* gene showed that γ -aminobutyrate-induced expression is reduced by ~80% (Table 5). Hence, in addition to Uga3p and Uga35p, Gln3p also contributes considerably to γ -aminobutyrate-induced expression of the *UGA4* gene. In contrast, deletion of the *GLN3* gene did not further significantly reduce the residual level of induced expression exhibited by the *UGA4-lacZ* fusion gene lacking the UAS_{GATA} element. This indicates that Gln3p exerts its positive function mainly through UAS_{GATA}.

The upstream region of the *UGA1* gene also contains several 5'-GAT(A/T)A-3' sequences. In the *gln3Δ* mutant strain, γ -aminobutyrate-induced expression of the *UGA1-lacZ* gene was

Table 5. Role of Gln3p and 5'-GAT(A/T)A-3' sequences in the expression of the UGA1 and UGA4 genes

Plasmid	Strains	β -galactosidase (nmol . min ⁻¹ . mg prot. ⁻¹)	
		- GABA	+ GABA
A	<i>UGA4-lacZ</i> Wild-type	≤ 10	1060
	<i>gln3Δ</i>	≤ 10	130
	Δ UAS _{GATA} <i>UGA4-lacZ</i> Wild-type	≤ 10	170
	<i>gln3Δ</i>	≤ 10	140
	<i>UGA1-lacZ</i> Wild-type	220	6020
	<i>gln3Δ</i>	50	3230
B	pDT3 Wild-type	70	5030
	pDT8	≤ 10	20
	pDT7	15	3310
	no insert	15	20

(A) Wild-type and *gln3Δ* cells contain centromere-based plasmids carrying the indicated genes. (B) Wild-type cells contain a high-copy-number plasmid carrying the *CYC1-lacZ* reporter gene under the control of the original 94 bp upstream region of UGA1 (-395 to -302) (plasmid pDT3) or a derivative lacking the 19 bp UAS_{GABA} (marked by the open box) (plasmid pDT7). The reported β -galactosidase activities are averages of the values obtained in three independent experiments. Variations were $\leq 15\%$.

~50% lower than in the wild type. In further experiments, we focused on a 94 bp upstream region (-395 to -302) including UAS_{GABA} and which supports high levels of reporter gene transcription in the presence of γ -aminobutyrate (Table 5, plasmid pDT3). As expected, deleting the UAS_{GABA} element from this region resulted in complete loss of reporter gene expression (Table 5, plasmid pDT8). Downstream from UAS_{GABA} (-344 to -335), there is a 5'-GATTAGATTA-3' sequence identical to a decanucleotide located within UAS_N, a positive *cis*-acting sequence lying upstream from the NAD-linked glutamate dehydrogenase *GDH2* gene. The positive function of the latter sequence depends on Gln3p (40). Specific deletion of this element slightly but significantly reduced gene expression, both in the presence and in the absence of inducer (Table 5, plasmid pDT7). These results indicate that Gln3p and at least one other sequence, a 5'-GATTAGATTA-3' decanucleotide downstream from UAS_{GABA}, contribute, to some limited extent, to *UGA1* gene expression.

DISCUSSION

In cells grown on a poor nitrogen source like proline or urea, expression of the *UGA* genes is strongly induced upon addition of γ -aminobutyrate to the culture medium (1–6). In this study, we identified a conserved, 19 bp, GC-rich sequence which we called UAS_{GABA} and which is essential to γ -aminobutyrate-induced transcription. This UAS_{GABA} element is also sufficient to enable some levels of reporter gene transcription in the presence of γ -aminobutyrate. We also found that two adjacent copies act synergistically rather than additively in this respect. To perform its function, UAS_{GABA} requires the presence of two distinct positive regulatory proteins, namely inducer-specific Uga3p and, to a slightly lesser extent, pleiotropic Uga35p(Dal81p/DurLp). The pleiotropic Uga35p factor is not required for expression of

Figure 3. Model of γ -aminobutyrate-induced transcription of the yeast *UGA4* gene. High-level γ -aminobutyrate-induced transcription of *UGA4* involves two adjacent UAS elements, UAS_{GABA} and UAS_{GATA}. In the presence of γ -aminobutyrate, the Uga3p and Uga35p factors act through UAS_{GABA} (i) to support by themselves some levels of transcriptional activation (ii) to somehow recruit the positive function of the global nitrogen regulatory factor Gln3p, which competes with the Uga43p repressor for binding to UAS_{GATA}.

the *UGA3* gene. Conversely, Uga3p does not seem to be required for expression of the *UGA35* gene, since mutations in *UGA3* do not alter the expression of some Uga35p-regulated genes (4). Although one cannot exclude the possibility that Uga3p and Uga35p operate through a regulatory cascade at the post-translational level, these data are consistent with the hypothesis that Uga3p and Uga35p act jointly at the level of UAS_{GABA}. The Uga3p and Uga35p factors both contain a Cys₆-Zn₂ type zinc finger motif (5,7,8). More than 20 different regulatory proteins, all of fungal origin, contain this motif, including the well-studied Gal4p transcriptional activator. X-ray crystallographic analysis of the N-terminal domain of Gal4p bound to DNA has shown that dimeric Gal4p interacts with a dyad-symmetrical 5'-CGG...CGG-3' sequence (9). Other members of the Cys₆-Zn₂ family (Put3p, Ppr1p) have been shown to bind to sites consisting of identical nucleotide triplets (5'-CGG...CCG-3') but differing by the spacing between the triplets (43). The central core of the 19 bp UAS_{GABA} element contains a GC-rich decanucleotide which is perfectly (5'-CCGCCGGCGG-3'; *UGA4* gene) or imperfectly (5'-CCGC GGGCGG-3'; *UGA1* gene) palindromic. The two core sequences contain the triplets 5'-CCG...CGG-3', a motif similar to though different from the 5'-CGG...CCG-3' motif recognized by Gal4p. The two triplets are spaced by four nucleotides. A similar 5'-CCG...CGG-3' motif can be found in the sequences recognized by another Cys₆-Zn₂-containing protein, namely Mal63p (45). In this case however, the two triplets are spaced by 10 nucleotides. It seems unlikely that the nucleotide triplets and the distance separating them are the sole elements accounting for the specific regulatory properties of UAS_{GABA}. Indeed, the widely conserved decanucleotide core sequence 5'-CCGGNNCCGG-3' shared by the binding sites for Leu3p, a Cys₆-Zn₂-containing protein that activates genes involved in branched-chain amino acid biosynthesis (46–48), contains the 5'-CCGN₄CGG-3' motif identical to that found in UAS_{GABA}. As mentioned above, the ability of UAS_{GABA} to confer inducibility by γ -aminobutyrate requires functional

Uga3p and Uga35p factors. Previous studies have shown that mutations affecting the Cys₆-Zn₂ domain of Uga3p abolish its activity (49). In addition, a clear case of interallelic complementation between two uninducible *uga3* mutants has been reported, suggesting that Uga3p might associate to form oligomers (5). It is therefore reasonable to suppose that Uga3p binds as a dimer to UAS_{GABA}. The pleiotropic function of Uga35p(Dal81p/DurLp) raises the question of its function in UAS_{GABA}-directed transcription. In *uga35Δ* mutant cells, the UAS_{GABA} element still supports low levels of γ -aminobutyrate-induced reporter gene transcription (Table 3). This suggests that Uga3p by itself can activate transcription in response to γ -aminobutyrate and that Uga35p potentiates this activation process. This is reminiscent of the recent suggestion that Gal4p could act *in vivo* in conjunction with a pleiotropic factor (9,50–52).

Although deletion of UAS_{GABA} led to complete loss of γ -aminobutyrate-induced transcription of the *UGA4* gene, another element, called UAS_{GATA} and located just upstream from UAS_{GABA}, was shown to play a determining role in producing high levels of induced expression. This UAS_{GATA} element contains four contiguous 5'-CGAT(A/T)AG-3' sequences and its deletion led to ~80% reduction of γ -aminobutyrate-induced expression. In addition to UAS_{GATA}, the upstream region of the *UGA4* gene contains five other copies of the pentanucleotide 5'-GAT(A/T)A-3' [or its reverse complement 5'-T(T/A)ATC-3']. These elements are located downstream from the UAS_{GABA} element and are not clustered. Whether these sequences also contribute to γ -aminobutyrate-dependent expression of *UGA4* remains to be investigated. The positive function of UAS_{GATA} in induced transcription of *UGA4* was shown to be largely dependent on Gln3p. This pleiotropic factor up-regulates the expression of a large number of inducible and constitutive nitrogen-regulated genes (18). In the case of the *UGA1* gene, the Gln3p factor and at least one 5'-GAT(A/T)A-3'-containing sequence (5'-GATTAGATTA-3')

located downstream from UAS_{GABA} also contribute to γ -aminobutyrate-induced transcription, but their contributions to the regulation of this gene are lesser than to the regulation of the *UGA4* gene. In the accompanying paper, we show that in cells grown on a poor nitrogen source like proline, UAS_{GATA} is potentially able to produce high levels of expression in the absence of γ -aminobutyrate. Under such conditions, however, the potential activity of UAS_{GATA} is inhibited provided that a functional Uga43p factor is present. As both Gln3p and Uga43p have been shown to bind to several 5'-GATA-3' core sequences *in vitro* (21,42), a simple hypothesis is that Gln3p and Uga43p compete for binding to UAS_{GATA}. In the absence of γ -aminobutyrate, Uga43p would efficiently prevent Gln3p from binding, while in the presence of the inducer, the Uga3p and Uga35p factors acting through UAS_{GABA}, in addition to promoting some levels of transcriptional activation on their own, would somehow facilitate binding of Gln3p to the adjacent UAS_{GATA}, enabling *UGA4* to be induced at much higher levels (Fig. 3).

Induction by allophanate of the *DAL7* gene also relies on tight cooperation between an inducer-responsive element (UIS; upstream induction sequence) and 5'-GATA-3'-containing, Gln3p-dependent UAS elements (15,38). In this latter system, however, the UIS element by itself does not activate transcription, and Uga43p(Dal80p) is proposed to act through a proximal upstream repressing sequence (URS) that prevents expression of *DAL7* in the absence of allophanate. Like UAS_{GABA}, the UIS element requires, in order to function, a functional Uga35p(Dal81p/DurLp), which in this case acts in conjunction with DurMp(Dal82p). In this respect, it is noteworthy that the UIS and UAS_{GABA} elements, although different, share 5'-AAAA-3' and 5'-GCG-3' motifs. High-level expression of the *GDH2* gene in the presence of glutamate is also determined by a two-component system involving an inducer-responsive sequence (UAS_G) and a 5'-GATTAGATTA-3'-containing element (UAS_N) whose positive function requires the presence of Gln3p (40). Interestingly, the glutamate-dependent UAS_G element is a GC-rich, partially palindromic sequence containing the motif 5'-GGCN₇GCC-3'. The identity of the positive acting factor(s) acting through UAS_G remains, however, unknown (18). The mechanisms underlying induction by γ -aminobutyrate of the *UGA* genes is also reminiscent of several inducible nitrogen pathway genes in filamentous fungi, like those involved in nitrite and nitrate assimilation, where both a global activator (areA, NIT2) containing a GATA zinc-finger domain and binding to 5'-GATA-3' core sequences acts in tight conjunction with pathway-specific regulatory proteins containing a Cys₆-Zn₂ domain (*nit-4*, *nirA*) (16). In all these systems, the global activator binding to 5'-GATA-3' core sequences (Gln3p, areA, NIT2) is most active in cells grown under conditions of nitrogen limitation, its action being prevented when a nitrogen source supporting optimal growth is available (16,18,34). Hence, tight coupling between this global factor and inducer-responsive elements most likely allows integration of the expression levels of inducible nitrogen pathway genes according to nitrogen source availability in the growth medium. Further experiments will be needed to elucidate the molecular mechanisms underlying the integrated functioning of the UAS_{GABA} and UAS_{GATA} elements of the *UGA4* gene.

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