Two mutually exclusive regulatory systems inhibit UAS_{GATA}, a cluster of 5'-GAT(A/T)A-3' upstream from the *UGA4* gene of *Saccharomyces cerevisiae*

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ABSTRACT

The *S.cerevisiae* Uga43(Dal80) protein down-regulates the expression of multiple nitrogen pathway genes. It contains a zinc-finger motif similar to the DNA-binding domain of the vertebrate GATA family of transcription factors; this domain is known to direct binding to 5'-GATA-3' core sequences. The inducible *UGA4* gene, which encodes the specific γ -aminobutyrate permease, undergoes strong repression by Uga43p. This study shows that the 5' region of *UGA4* contains a UAS element made of four directly repeated 5'-CGAT(A/T)AG-3' sequences. This element, called UAS_{GATA}, can potentially confer to the *UGA4* gene high-level expression in the absence of inducer, but this potential activity is inhibited by two distinct repression systems. One system is Uga43p-dependent; it operates in cells grown on a poor nitrogen source. The other is the nitrogen repression system, which relies on Ure2p and glutamine and operates when a good nitrogen source is present. Nitrogen repression also blocks the synthesis of Uga43p, making the two repression systems mutually exclusive. Previous studies have shown that expression supported by 5'-GATA-3'-containing UAS elements requires Gln3p, another global nitrogen regulatory factor containing a GATA zinc-finger domain. Although Gln3p contributes to UAS_{GATA} activity, evidence suggests that a second factor can potentially direct expression through UAS_{GATA}. Expression conferred by this putative factor is subject to both Uga43p- and Ure2p-mediated repression. The role of UAS_{GATA} in the expression of the *UGA4* gene is discussed in relation to its sensitivity to the two distinct repression systems.

INTRODUCTION

The *UGA4* gene of *Saccharomyces cerevisiae* encodes a specific permease for γ -aminobutyrate, which yeast cells can use as a

nitrogen source (2–3). In the absence of γ -aminobutyrate, the *UGA4* gene is hardly expressed, while γ -aminobutyrate in the culture medium has a strong inducer effect (3). Induction of this permease and of two enzymes (Uga1p and Uga2p, each of which catalyzes a step of γ -aminobutyrate catabolism) requires at least two functional positive-acting proteins, Uga3p and Uga35p (3–5). Uga3p behaves like an inducer-specific factor, while Uga35p(Dal81p/Durlp) is pleiotropic, being required also for allophanate-triggered induction of a number of genes involved in the utilization of ornithine, allantoin and urea (6–8). Both Uga3p and Uga35p contain a Cys₆-Zn₂ binuclear zinc finger (9–11) that serves as a DNA-binding domain for several fungal *trans*-acting factors including Gal4p (12). The Uga3p and Uga35p proteins act through a GC-rich upstream activating sequence (UAS_{GABA}), essential to γ -aminobutyrate-induced expression; this UAS_{GABA} element is also sufficient to confer to a reporter gene some degree of γ -aminobutyrate-inducible expression (13).

The *UGA43* gene encodes another regulatory protein which affects expression of the *UGA4* gene. Mutations in *UGA43* were originally isolated because they caused constitutive expression of *UGA4* (5). Further experiments showed that *UGA43* and *DAL80*, a gene responsible for down-regulation of several allophanate-inducible genes (7), are one and the same gene (14,15) and that Uga43p(Dal80p) actually down-regulates, to various extents, a number of other nitrogen-pathway genes exhibiting various patterns of regulation (16). The deduced sequence of pleiotropic Uga43p includes 269 amino acids and contains at least two motifs, a leucine zipper and a GATA zinc finger, both of which are essential to Uga43p function (14,15). The GATA zinc finger, characterized by a metal-binding region of the form Cys-X₂-Cys-X₁₇-Cys-X₂-Cys, is a DNA-binding domain identified in a number of proteins of very diverse eukaryotic organisms, including yeast, filamentous fungi, plants and animals. This domain directs binding to 5'-GATA-3' core sequences, although both the nucleotides directly flanking the 5'-GATA-3' central core and the number and relative proximity of 5'-GATA-3' sequences appear to affect the efficiency of binding (17–19). The Uga43 protein expressed in bacteria binds *in vitro* to a pair of

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5'-GATAA-3' core sequences separated by at least 15 nucleotides (18).

A number of nitrogen-regulated genes of *S.cerevisiae* contain several 5'-GATA-3' sequences in their upstream regions. In wild-type cells grown on a poor nitrogen source like proline, i.e. under conditions of nitrogen derepression, oligonucleotides containing at least two sequences with 5'-GATAA-3' at their central cores and inserted upstream from a reporter gene have been shown to act as upstream activating sequences (UAS) (20,21). Likewise, DNA segments isolated either from *GLN1*, the gene encoding glutamine synthetase, or *GDH2*, the gene encoding NAD-dependent glutamate dehydrogenase, and containing, respectively, the 5'-GATAAGATAA-3' or the 5'-GAT-TAGATTA-3' decanucleotide, could also stimulate reporter gene transcription. This occurred in cells shifted from growth on glutamine (as the nitrogen source) to growth on glutamate, the former being considered a 'better' nitrogen source because it supports faster growth (22,24). Activation of transcription, under these conditions, required a functional Gln3p factor (24,25). This factor contains a GATA zinc-finger domain (23) and is known to up-regulate the expression of several nitrogen-regulated genes, including *GLN1* and *GDH2* (24). Antibody raised against Gln3p has been shown to co-immunoprecipitate a DNA fragment containing several repeats of the sequence 5'-GATAAGATAA-3' (23). Under conditions of nitrogen repression, i.e. when cells are provided with a good nitrogen source like ammonium, glutamine or asparagine, the UAS elements with 5'-GAT(A/T)A-3' at their central cores lose their ability to activate transcription (22,24,26). The *URE2*(*GDHCR*) gene product, a protein essential to nitrogen repression of all nitrogen-repressible genes tested so far (27), is required to repress expression directed by the 5'-GAT(A/T)A-3'-containing regions of the *GLN1* and *GDH2* genes (22,24).

In this study, we show that the 5'-flanking region of the γ -aminobutyrate-inducible gene *UGA4* contains a UAS element made of four directly repeated 5'-CGAT(A/T)AG-3' sequences. This element, called UAS_{GATA}, can potentially confer high levels of expression in the absence of inducer. In fact, however, this potential activity is maintained at a low level by two mutually exclusive repression systems, one involving Uga43p and one involving Ure2p and glutamine. Under conditions of nitrogen derepression, it is the former system which is active, while in the presence of a nitrogen source supporting optimal growth, synthesis of Uga43p is inhibited and the second system takes over to keep UAS_{GATA} inactive (Fig. 1). Our evidence further suggests that a second positive factor, distinct from Gln3p, can potentially activate transcription through UAS_{GATA}.

MATERIALS AND METHODS

Strains and media

The *S.cerevisiae* strains used in this study (Table 1) are isogenic with the wild type Σ 1278b (*MATa*) except for the mutations mentioned. Cells were grown on minimal medium (28) with 0.1% proline (conditions of nitrogen derepression) or 10 mM (NH₄)₂SO₄ (conditions of nitrogen repression) as the sole source of nitrogen. Yeast cells treated with lithium acetate (29) were transformed according to (30).

Figure 1. The UAS_{GATA} element of the *UGA4* gene is subject to two mutually exclusive negative controls. Schematic representation of the negative controls affecting UAS_{GATA} in cells grown under conditions of nitrogen repression or nitrogen derepression. The closed drawn lines represent operating negative regulations, the open drawn lines represent non-operating regulations.

Table 1. Yeast strains

Strain	Genotype	Reference
23344c	<i>MATa, ura3</i>	M. Grenson
30078c	<i>MATa, ura3, uga43Δ</i>	(15)
30505b	<i>MATa, ura3, gln3Δ</i>	This study
30495a	<i>MATa, ura3, uga43Δ, gln3Δ</i>	This study
26854a	<i>MATa, ura3, ure2(gdhCR)</i>	(38)
26882b	<i>MATa, ura3, gln1^{ts}</i>	(39)
26992c	<i>MATa, ura3, uga43Δ, ure2(gdhCR)</i>	This study
26988d	<i>MATa, ura3, uga43Δ, gln1^{ts}</i>	This study

Plasmids

The isolation of nested deletions upstream from the *UGA4-lacZ* gene has been described (13). Construction of the *UGA4-lacZ* gene with specific deletion of UAS_{GATA} has also been described (13). To isolate the UAS_{GATA}-*CYC1-lacZ* gene, we synthesized two complementary oligonucleotides flanked by *Xho*I sites, which were then hybridized and ligated into *Xho*I-restricted plasmid pLG670Z (31) bearing a UAS-lacking *CYC1-lacZ* gene. The two oligonucleotides used were

5'-tcgagCTTATCGCTAATCGCTTATCGCTTATCGc-3' and

5'-tcgagCGATAAGCGATAAGCGATTAGCGATAAGc-3'.

The *UGA43-lacZ* fusion gene was isolated by inserting into plasmid YCpAJ150 (3) an *Eco*RI-*Eco*72I fragment encompassing the first 30 codons of *UGA43/DAL80* and 894 bp its 5' region.

Isolation of a *gln3Δ* deletion mutant

A 12 kb *Hind*III-*Hind*III DNA fragment containing the *GLN3* gene was isolated by hybridization of a plasmid library representing the genome of strain Σ 1278b with an oligonucleotide deduced from the published sequence of the *GLN3* gene (23). The identity

Table 2. Deletion analysis of the upstream region of *UGA4* and identification of UAS_{GATA}

A

β -galactosidase
(nmoles $\cdot$ min⁻¹ $\cdot$ mg prot.⁻¹)

UGA43⁺

uga43 Δ

1		≤ 10	2380
2	- 679	≤ 10	1990
3	- 581	≤ 10	3050
4	- 542	≤ 10	3975
5	- 514	≤ 10	3990
6	- 429	≤ 10	640
7	- 418	≤ 10	80
8	- 406	≤ 10	≤ 10
9	- 391	≤ 10	≤ 10
10		≤ 10	≤ 10

B

UAS_{GATA}

UAS_{GABA}

-444	AGGTACTCTTATCGCTAATCGCTTATCGCTTATCGCTGCGCCAAAAACCGCCGCGGCCAATTCGA	-380
	TCCATGAGAAATAGCGATTAGCGAATAGCGAATAGCGACGCGCTTTTGGCGCGCCGCTTAAGCT	
	-429	-418
		-406
		-391

(A) 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} element. Cells bearing the low-copy-number plasmids carrying the *UGA4-lacZ* gene with the represented 5' deletions were grown on minimal proline medium. The reported β -gal activities are averages of the values obtained in two or three independent experiments. Variations were $\leq 15\%$. (B) Partial sequence of the 5' region of *UGA4* showing the relative position of the UAS_{GATA} and UAS_{GABA} elements.

of the cloned DNA fragment was checked by restriction analysis and partial sequencing. The *gln3Δ* mutant obtained by deleting the chromosomal *GLN3* gene was isolated by the allele-replacement method (32). An integrative plasmid was first constructed by inserting into *Aat*II- and *Bam*HI-restricted plasmid pFL44 (33) a 0.5 kb *Aat*II-*Dra*I segment covering the 5' flanking region of *GLN3* (positions -730 to -241) joined to a 1 kb *Asu*II-*Bgl*III segment covering the last 123 codons and the 3'-flanking region of *GLN3*. The resulting plasmid was linearized with *Hpa*I and used to transform a *ura3/ura3* diploid strain. Several *Ura*⁺ transformants were subsequently grown on rich medium and plated on minimal medium containing 5-fluoroorotic acid as a selective agent (34). *Ura*⁻ clones were selected and analyzed by Southern blotting using the 1 kb *Asu*II-*Bgl*III DNA segment as a probe. A heterozygous *gln3Δ/GLN3*⁺ strain exhibiting the expected chromosomal deletion was selected. After sporulation, a *gln3Δ* haploid strain was isolated and used in the β -galactosidase assays.

β -galactosidase assays

β -galactosidase assays were performed as previously described (3) on exponentially growing cells having reached the state of balanced growth (27). Enzyme activities are expressed in nmoles of *o*-nitrophenol formed/min/mg of protein.

RESULTS

UAS_{GATA}, a cluster of 5'-GAT(A/T)A-3' motifs, is inhibited by Uga43p

In cells grown on proline as the sole source of nitrogen, i.e. under conditions of nitrogen derepression, deletion of the *UGA43* gene results in high, constitutive expression of the *UGA4* gene (Table 2, line 1). The Uga43p factor most likely exerts repression through 5'-GATA-3' core sequences, since this factor contains a GATA zinc-finger domain and binds *in vitro* to DNA segments containing several copies of this sequence (14,15,18). The

Table 3. Assays of β -galactosidase activities in wild-type and mutant strains grown on minimal medium with proline or ammonium ions as sole nitrogen source

N°	Gene on plasmid	N source	β -galactosidase (nmoles . min ⁻¹ . mg prot ⁻¹)							
			Wild-type	<i>uga43Δ</i>	<i>ure2</i>	<i>ure2</i> , <i>uga43Δ</i>	<i>gln1^{ts}</i>	<i>gln1^{ts}</i> <i>uga43Δ</i>	<i>gln3Δ</i>	<i>gln3Δ</i> , <i>uga43Δ</i>
1	<i>UAS_{GATA}-CYC1-lacZ</i>	Proline	15	720	≤ 10	175	15	1960	≤ 10	320
2		NH ₄ ⁺	≤ 10	≤ 10	25	165	≤ 10	45	25	≤ 10
3	<i>CYC1-lacZ</i>	Proline	≤ 10	≤ 10	≤ 10	15	≤ 10	≤ 10	15	≤ 10
4		NH ₄ ⁺	15	20	≤ 10	15	≤ 10	≤ 10	20	≤ 10
5	<i>UGA4-lacZ</i>	Proline	≤ 10	2040	≤ 10	1770	≤ 10	2560	≤ 10	320
6		NH ₄ ⁺	≤ 10	≤ 10	≤ 10	570	≤ 10	60	≤ 10	≤ 10
7	<i>UGA43-lacZ</i>	Proline	920	3630	870	3600	950	4340	n.d.	n.d.
8		NH ₄ ⁺	≤ 10	30	490	1140	380	390	n.d.	n.d.

Cells contain a low-copy-number plasmid bearing the *UGA4-lacZ*, *UGA43-lacZ*, *CYC1-lacZ* or *UAS_{GATA}-CYC1-lacZ* fusion gene. The indicated β -gal activities are averages of values obtained in two or three independent experiments. Variations were ≤ 15%.

upstream region of the *UGA4* gene contains nine copies of the pentanucleotide 5'-GAT(A/T)A-3' (or its reverse complement 5'-T(A/T)ATC-3') in the region spanning positions -470 to -71 from the translation initiation codon (13) (Table 2). Four of these copies, three 5'-GATAA-3' and one 5'-GATTA-3', are clustered in a 28 nt region situated just upstream from *UAS_{GABA}*, the *UAS* element essential to induction by γ -aminobutyrate (Table 2B). The four pentanucleotides are flanked by C and G, resulting in four directly repeated 5'-CGAT(A/T)AG-3'. The five remaining sequences are downstream from *UAS_{GABA}*, there are spaces between them, and they are not flanked by C and G. In an attempt to identify *cis*-acting sequences mediating negative control by *Uga43p*, we introduced a series of centromere-based plasmids, containing nested deletions in the 5'-flanking region of the *UGA4-lacZ* gene, into wild-type and *uga43Δ* cells and tested the effect of each deletion by measuring β -galactosidase (β -gal) activity in cells grown on proline medium (Table 2A). In these experiments, we failed to identify any upstream repressing sequences (URS) whose deletion produced an effect similar to that observed upon deletion of *UGA43*. What we did find was the following: while *UGA4-lacZ* plasmids with deletions extending to -514 supported high constitutive expression of the reporter gene in *uga43Δ* cells (Table 2, lines 1-5), the next three deletions caused progressive reduction of constitutive expression. When the sequence was deleted to position -406, the resulting *UGA4-lacZ* gene was expressed at undetectable levels in wild-type and *uga43Δ* cells (Table 2, lines 6-8). Further deletions in the upstream region flanking the *UGA4* gene did not alter this expression pattern any further. The successive deletions leading to loss of constitutive expression of *UGA4-lacZ* in *uga43Δ*

mutant cells coincide with the progressive removal or truncation of the repeated 5'-CGAT(A/T)AG-3' sequences located just upstream from *UAS_{GABA}* (Table 2B). In a further experiment, we specifically deleted by site-directed mutagenesis the 28 nt region encompassing the four repeated sequences. The result was the complete loss of constitutive expression in *uga43Δ* mutant cells (Table 2, line 10). These results indicate that the repeated 5'-CGAT(A/T)AG-3' motifs are essential to the high constitutive expression of *UGA4* in *uga43Δ* mutant cells. Next, an oligonucleotide corresponding to the four repeated 5'-CGAT(A/T)AG-3' sequences was inserted upstream from the *CYC1-lacZ* reporter gene lacking the regulatory sites for *CYC1* and carried on a low-copy-number plasmid. The β -gal activity assays performed on cells containing this plasmid construct clearly showed that this cluster of 5'-CGAT(A/T)AG-3' sequences (called *UAS_{GATA}*) can strongly enhance reporter gene expression in cells lacking a *UGA43* gene product (Table 3, line 1). Taken together, these results clearly show that *Uga43p* prevents the *UAS_{GATA}* element from activating *UGA4* gene expression in the absence of inducer. Previous experiments have shown that multiple 5'-GATA-3' core sequences from the *DAL5* upstream region act cooperatively when directing reporter gene expression (20). The same is true of the repeated 5'-CGAT(A/T)AG-3' sequences of *UAS_{GATA}*, since constitutive expression in *uga43Δ* mutant cells was at each step reduced by a factor of 8-10 when two (-429), three (-418) or all four (-406) of these sequences were removed or truncated (Table 2, lines 6-8). This suggests that the repeated 5'-CGAT(A/T)AG-3' sequences act synergistically rather than additively when directing expression.

Gln3p does not entirely account for expression conferred by UAS_{GATA}

UAS_{GATA}-supported expression most likely involves at least one positive-acting protein that binds to 5'-GAT(A/T)A-3' sequences. The protein encoded by the *GLN3* gene, a GATA zinc-finger protein, is a good candidate for this function, since Gln3p does up-regulate the expression of many nitrogen pathway genes and since it acts through upstream sequences with 5'-GAT(A/T)A-3' at their central cores (24). To investigate the role of Gln3p in the activity of UAS_{GATA}, we isolated a yeast strain deleted specifically of its chromosomal *GLN3* gene (see Materials and Methods) and investigated UAS_{GATA}-supported reporter gene expression in this *gln3Δ* mutant strain grown under conditions of nitrogen derepression (Table 3, line 1). Albeit lower by about half than in [*uga43Δ*, *GLN3*⁺] cells, UAS_{GATA}-driven expression remained high in [*uga43Δ* *gln3Δ*] double mutant cells (Table 3, line 1). Likewise, constitutive expression of the *UGA4-lacZ* gene was lower in [*uga43Δ*, *gln3Δ*] cells than in [*uga43Δ*, *GLN3*⁺] cells but nevertheless quite high (Table 3, line 5). This shows that while Gln3p contributes to the high level of UAS_{GATA}-supported expression observed when repression by Uga43p is relieved, it does not entirely account for it. We therefore interpret these data as pointing to the existence of a positive factor, distinct from Gln3p, which can potentially act through UAS_{GATA} to activate transcription. This process is nevertheless inhibited by Uga43p, since UAS_{GATA} supports much higher levels of expression in [*gln3Δ*, *uga43Δ*] cells than in [*gln3Δ*, *UGA43*⁺] cells (Table 3, line 1).

Nitrogen repression of UAS_{GATA}: role of Ure2p and glutamine

The above results show that in *uga43Δ* cells grown on a poor nitrogen source like proline, i.e. under conditions of nitrogen derepression, the UAS_{GATA} element of the inducible *UGA4* gene confers high levels of constitutive expression. In cells grown on a good nitrogen source such as ammonium ions, however, i.e. under conditions of nitrogen repression, expression of the *UGA4-lacZ* gene was almost undetectable in both the wild type and the *uga43Δ* mutant strain (Table 3, line 6). This suggests that UAS_{GATA}-supported expression is sensitive to nitrogen repression. Accordingly, reporter gene expression from a UAS_{GATA}-*CYC1-lacZ* construct was much lower in *uga43Δ* mutant cells grown on ammonium medium than in cells of this same strain grown on proline medium (Table 3, lines 1 and 2). It thus appears, as previously shown for the 5'-GAT(A/T)A-3'-containing UAS elements identified upstream from the *DAL5*, *DAL7*, *GLN1* and *GDH2* genes (24), that the UAS_{GATA} element of the *UGA4* gene is subject to nitrogen repression. This nitrogen repression was largely relieved, whether the construct used was *UGA4-lacZ* or UAS_{GATA}-*CYC1-lacZ*, in *uga43Δ* mutant cells bearing an additional mutation in the *URE2* gene (Table 3, lines 2 and 6). It was also somewhat relieved in *uga43Δ* mutant cells partially starved of glutamine by means of a thermosensitive mutation in *GLN1*, the gene for glutamine synthetase (Table 3, lines 2 and 6). These results indicate that nitrogen repression of UAS_{GATA}-supported expression requires a functional *URE2* gene, while glutamine or a derivative acts as an effector of this repression. Expression of the *UGA4-lacZ* and UAS_{GATA}-*CYC1-lacZ* genes was also much lower in [*uga43Δ*, *gln3Δ*] cells

grown on ammonium medium than in ones grown on proline medium (Table 3, lines 2 and 6). This shows that the expression conferred by the uncharacterized factor assumed to act through UAS_{GATA} in the absence of Uga43p is very sensitive to nitrogen repression.

In conclusion, the above experiments demonstrate the existence of two repressor systems capable of reducing the activity of the UAS_{GATA} element of the inducible *UGA4* gene. One is Uga43p-dependent and operates under conditions of nitrogen derepression; the other is Ure2p- and glutamine-dependent and operates under conditions of nitrogen repression.

Repression by Uga43p and repression by Ure2p are mutually exclusive regulatory systems

Previous Northern analyses have shown that *UGA43* transcripts are much more abundant in cells grown on proline or urea than in cells grown on ammonium ions or glutamine (14,15). This indicates that *UGA43* gene expression is subject to nitrogen repression. High accumulation of *UGA43* transcripts was found to require a functional *GLN3* product. Furthermore, *UGA43* transcripts were detected in greater quantity in *uga43* mutant cells, indicating that Uga43p negatively controls the expression of its own gene (14,15). The 5'-flanking region of the *UGA43* gene contains multiple copies of the 5'-GAT(A/T)A-3' sequence, some of which are clustered, and it seems likely that at least some of these sequences play an important role in the expression of *UGA43* (14,15).

To further characterize the regulated expression of the *UGA43* gene, we constructed a *UGA43-lacZ* fusion gene in a low-copy-number plasmid and introduced the plasmid into the wild-type and several mutant strains. In agreement with the results of the Northern analyses, β-gal assays revealed high expression of *UGA43-lacZ* in proline-grown wild-type cells and 4-fold higher expression in *uga43Δ* mutant cells (Table 3, lines 7 and 8). In cells grown on ammonium medium, *UGA43-lacZ* expression was strongly reduced. The much higher expression levels observed in *ure2* and *gln1^{ts}* mutant cells under these conditions show that Ure2p and glutamine participate in nitrogen repression of the *UGA43* gene.

The sensitivity of *UGA43* expression to nitrogen repression suggests that the two repression systems acting upon UAS_{GATA}, i.e. the Uga43p-dependent and the Ure2p-dependent system, must be mutually exclusive, meaning that one is operational when the other is not (Fig. 1). According to this model, the UAS_{GATA} element should remain inactive in *ure2* or *gln1^{ts}* mutant cells grown on ammonium medium, as repression by Uga43p should take over from defective nitrogen repression. The results presented in Table 3 confirm this prediction: when grown on ammonium medium, *ure2* mutant cells with a functional *UGA43* product hardly expressed the UAS_{GATA}-*CYC1-lacZ* and *UGA4-lacZ* genes (Table 3, lines 2 and 6); in contrast, *ure2* cells grown under the same conditions but lacking the *UGA43* gene displayed much higher expression levels (Table 3, lines 2 and 6). Likewise, cells partially released from nitrogen repression by means of a *gln1^{ts}* mutation exhibited very low expression of the UAS_{GATA}-*CYC1-lacZ* genes and *UGA4-lacZ*, while further deletion of the *UGA43* gene resulted in slight derepression of both genes (Table 3, lines 2 and 6). Taken together, these results demonstrate that release from nitrogen repression results in

derepressed synthesis of Uga43p which in turn inhibits UAS_{GATA} (Fig. 1).

DISCUSSION

In *S. cerevisiae*, a number of DNA segments containing sequences with 5'-GAT(A/T)A-3' at their central cores have been shown to behave like upstream activating sequences whose activity relies on Gln3p and is sensitive to nitrogen repression (22,25,26,35). In this study, we show that four adjacent repeats of the heptanucleotide 5'-CGAT(A/T)AG-3', situated in the promoter region of the γ -aminobutyrate-inducible *UGA4* gene, constitute a UAS element. This UAS_{GATA} element can potentially confer high levels of expression in the absence of inducer, but this potential activity is inhibited, in uninduced cells grown under conditions of nitrogen derepression, by a strong repression mechanism. This repression involves Uga43p, a negative regulatory protein known to bind to 5'-GATAA-3'-containing sequences (18). In the absence of a functional Uga43p, the 5'-CGAT(A/T)AG-3' repeats of UAS_{GATA} behave like cooperatively acting positive regulatory sequences supporting high levels of constitutive expression.

The 5'-GATA-3'-containing UAS elements responsible for constitutive expression of the *DAL5* gene do not appear to be very sensitive to inhibition by Uga43p, since expression of *DAL5* is only minimally derepressed in *uga43/dal80* mutant strains (14,16). Furthermore, a UAS element of the *DAL5* gene, consisting of two 5'-GATAA-3' core sequences oriented head to tail and separated by 47 nucleotides, is reported to support similar levels of reporter gene expression in *UGA43/DAL80*⁺ and *uga43/dal80* Δ strains (14). Saturation mutagenesis experiments performed on this Uga43p-insensitive UAS led investigators to define the so-called UAS_{NTR} element, i.e. two copies of the dodecanucleotide 5'-TTNCTGATAAGG-3', as the minimal functional upstream activating sequence (21). The UAS_{GATA} element of the *UGA4* gene does not conform with the UAS_{NTR} consensus sequence. A possible explanation could lie in the fact that the original and mutagenized UAS sequences used to define UAS_{NTR} were tested for their ability to activate reporter gene expression in the presence of a functional Uga43p. This means that at least some of the mutagenized UAS elements having apparently lost their ability to activate expression might in fact have become sensitive to inhibition by Uga43p. The apparent insensitivity of some UAS elements with 5'-GAT(A/T)A-3' at their central cores to inhibition by Uga43p might mean that this inhibition involves specific neighboring sequences and/or a particular orientation or spacing of the 5'-GAT(A/T)A-3' core sequences. In the UAS_{GATA} element, all four 5'-GAT(A/T)A-3' sequences are flanked by C and G and oriented head to tail. Clearly, further experiments will be needed to determine the specific properties of UAS_{GATA} that make it highly sensitive to inhibition by Uga43p.

What is the role of UAS_{GATA}? In cells grown under conditions of nitrogen derepression, addition of β -aminobutyrate to the culture medium triggers high-level induction of the *UGA4* gene. In the accompanying paper, Talibi *et al.* (13) show that while UAS_{GABA} is essential to β -aminobutyrate induction and sufficient to confer some inducible reporter gene expression, the UAS_{GATA} element situated just upstream from it also contributes greatly to induction. The contribution of UAS_{GATA} to induction by β -aminobutyrate is dependent on Gln3p. Specific deletion of UAS_{GATA}, or deletion of the *GLN3* gene, caused induced

expression to decrease by about 80%. In the presence of β -aminobutyrate, UAS_{GABA} thus seems capable not only of supporting some degree of transcription but also of somehow recruiting the positive function of Gln3p acting through the adjacent UAS_{GATA} (13). Whatever the recruitment mechanism involved, the major contribution of UAS_{GATA} to induction of *UGA4* implies that inhibition of UAS_{GATA} by Uga43p must be at least partially relieved when the inducer is present.

On the other hand, the activity of UAS_{GATA} is also sensitive to nitrogen repression. This repression requires Ure2p and glutamine. A difference between Ure2p-dependent inhibition of UAS_{GATA} activity (under conditions of nitrogen repression) and Uga43p-dependent repression of this activity (under conditions of nitrogen derepression) might lie in the ability of Ure2p to prevent UAS_{GATA} from contributing to β -aminobutyrate-induced transcription. This would result in variable levels of *UGA4* induction according to the nitrogen status of the cell.

As previously demonstrated in Northern analyses, our expression assays with the *UGA43-lacZ* construct show that *UGA43* is sensitive to nitrogen repression. The repression exerted by Uga43p is thus sensitive to nitrogen repression (Fig. 1). The existence of this cascade regulation appears in the fact that, in cells grown on ammonium medium, inhibition of UAS_{GATA} becomes Uga43p-dependent if the cells carry a mutation that suppresses nitrogen repression. In the wild-type, a shift from a good to a poor nitrogen source relieves Ure2p-mediated nitrogen repression. This leads to high-level synthesis of Uga43p, which in turn takes over the job of repressing gene expression (Fig. 1). As mentioned above, however, Uga43p-mediated repression seems more specific than nitrogen repression, since some genes like *DAL5*, containing 5'-GAT(A/T)A-3' UAS elements and exhibiting high expression levels under conditions of nitrogen derepression, respond very little to *uga43* mutations. These genes most probably contain specific arrangements of 5'-GAT(A/T)A-3' UAS elements which cannot be strongly inhibited by Uga43p. The *UGA43* gene itself provides an intermediate case, that of a gene whose expression is greatly derepressed in cells grown on a poor nitrogen source and nevertheless subject to moderate repression by Uga43p. An extreme situation is represented by the *UGA4* gene, which is not expressed at all in cells grown on a poor nitrogen source in the absence of inducer. In this case, the UAS_{GATA} element is very sensitive to inhibition by Uga43p, but it can nevertheless contribute greatly to expression once a specific inducer is supplied (13).

We conclude that a major physiological role of Uga43p is to conditionally inhibit the activity of specific upstream-activating 5'-GAT(A/T)A-3' sequences once nitrogen repression of this activity has been relieved.

How does Uga43p prevent UAS_{GATA} from activating transcription in the absence of inducer? A simple hypothesis would be that Uga43p binds to UAS_{GATA}, thereby preventing the subsequent binding of a positive-acting factor such as Gln3p. The Gln3p factor seems to account for contribution of UAS_{GATA} to induced expression (13). In the absence of inducer, however, Gln3p does not entirely account for the derepressed activity exhibited by UAS_{GATA} in *uga43* Δ mutant cells. We therefore conclude that under such conditions, a positive-acting factor other than Gln3p is capable of supporting UAS_{GATA}-activated expression. The action of this uncharacterized positive-acting element appears to be sensitive to nitrogen repression. Like Gln3p and Uga43p, this

factor likely contains a GATA zinc-finger domain for binding to 5'-GAT(A/T)A-3' sequences.

During preparation of this manuscript, a report by the group of T. G. Cooper (36) was published in which the authors show that the cluster of 5'-GAT(A/T)A-3' sequences of *UGA4* (referred to here as UAS_{GATA}) acts like a UAS mediating both Uga43p(Dal80p)-responsive regulation and *in vitro* binding of Uga43p(Dal80p).

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