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New insights into the regulation of the *Saccharomyces cerevisiae UGA4* gene: two parallel pathways participate in carbon-regulated transcription

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The *Saccharomyces cerevisiae UGA4* gene, which encodes the γ-aminobutyric acid (GABA) and δ-aminolaevulinic acid (ALA) permease, is well known to be regulated by the nitrogen source. Its expression levels are low in the presence of a rich nitrogen source but are higher when a poor nitrogen source is used. In addition, GABA can induce *UGA4* expression when cells are grown with proline but not when they are grown with ammonium. Although vast amounts of evidence have been gathered about *UGA4* regulation by nitrogen, little is known about its regulation by the carbon source. Using glucose and acetate as rich and poor carbon source respectively, this work aimed to shed light on hitherto unclear aspects of the regulation of this gene. In poor nitrogen conditions, cells grown with acetate were found to have higher *UGA4* basal expression levels than those grown with glucose, and did not show *UGA4* induction in response to GABA. Analysis of the expression and subcellular localization of the transcription factors that regulate *UGA4* as well as partial deletions and site-directed mutations of the *UGA4* promoter region suggested that there are two parallel pathways that act in regulating this gene by the carbon source. Furthermore, the results demonstrate the existence of a new factor operating in *UGA4* regulation.

INTRODUCTION

The yeast *Saccharomyces cerevisiae* is widely used as a model organism to study the function of mammalian proteins. The GATA family of DNA-binding proteins, responsible for regulating globin gene expression (Weiss & Orkin, 1995) and a diverse set of developmental functions in animal cells (Kelley *et al.*, 1993; Laverriere *et al.*, 1994), is one of the gene families shared by *S. cerevisiae* and higher eukaryotes. Yeast GATA proteins Gln3, Gat1/Nil1, Dal80/Uga43 and Gzf3/Deh1 are the main regulators of nitrogen catabolism gene expression that are responsible for the phenomenon known as nitrogen catabolite repression (NCR). The expression of the *UGA4* gene, which encodes the γ-aminobutyric acid (GABA) and δ-aminolaevulinic acid (ALA) permease in *S. cerevisiae*, depends on GABA induction and NCR (Andre *et al.*, 1993; Bermudez Moretti *et al.*, 1996). Induction of this permease requires at least two positive-acting proteins, the specific Uga3p factor and the pleiotropic Dal81p/Uga35p factor (Andre *et al.*, 1995; Bricmont *et al.*, 1991). These factors act through a 19 bp CG-rich upstream activating sequence, UAS_{GABA}. The promoter region of *UGA4* also contains four adjacent repeats of the heptanucleotide 5′-CGAT(A/T)AG-3′, which constitute a UAS_{GATA} element. This element can potentially confer high levels of expression in the absence of inducer. Nevertheless, the basal expression levels of the *UGA4* gene in uninduced cells grown with a poor nitrogen source such as proline are low since there is a strong repression mechanism involving Dal80p/Uga43p, a pleiotropic regulatory factor (Andre *et al.*, 1995; Bricmont *et al.*, 1991; Cunningham *et al.*, 1994). Gln3p, another GATA transcription factor, upregulates the expression of *UGA4* in the presence of inducer by competing with Dal80p/Uga43p for binding to the UAS_{GATA} sequence (Coffman *et al.*, 1997; Soussi-Boudekou *et al.*, 1997). The outcome of this competition influences basal levels of transcription. The role of the other positive and negative GATA factors, Gat1 and Gzf3 respectively, in *UGA4* regulation has been poorly studied.

NCR is superimposed on the above regulation: in cells grown with a rich nitrogen source such as ammonium or glutamine, Ure2p, a pre-prionic cytoplasmic protein, prevents nuclear localization of Gln3p by retaining it in

Abbreviations: ALA, δ-aminolaevulinic acid; GABA, γ-aminobutyric acid; NCR, nitrogen catabolite repression; UAS, upstream activating sequence.

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the cytoplasm, consequently preventing its activity (Kulkarni *et al.*, 2001).

Gat1 was thought to act in a way similar to Gln3, but whereas a Gln3–Ure2 complex has been isolated from cells in which NCR-sensitive transcription is repressed, a similar Gat1–Ure2 complex has not yet been reported (Tate *et al.*, 2006).

Many reports have been published assessing the relationship between nuclear localization and phosphorylation levels of Gln3; emerging results show a lack of correlation between these parameters. However, Gln3 intracellular localization and NCR do correlate well (Tate *et al.*, 2005). It must be noted that much less is known about Gat1 phosphorylation and localization in response to nutrient availability (Kulkarni *et al.*, 2006).

TOR1/2 are phosphatidylinositol kinase-related proteins, which are inhibited by rapamycin and sense nutrients, specifically carbon and nitrogen quality, regulating gene expression. These kinases are, at least in part, involved in Gln3 and Gat1 phosphorylation (Bertram *et al.*, 2002). It has been reported that the activation of Gln3 and Gat1 is not identical, that they control distinct (although overlapping) sets of genes and that low-quality carbon or nitrogen activates Gln3 or Gat1 differentially (Crespo *et al.*, 2002; Kuruvilla *et al.*, 2001; Shamji *et al.*, 2000). Moreover, the phosphorylation state of Ure2, the anchor protein of the positive GATA factors in the cytoplasm when carbon and nitrogen nutrients are abundant (Beck & Hall, 1999), responds not to the nitrogen availability but rather to the carbon source (Kuruvilla *et al.*, 2002).

Evidence indicates that not only nitrogen but also carbon nutrient quality controls the expression of some NCR genes (Shamji *et al.*, 2000) and that Gln3 and Gat1 localization and/or phosphorylation depend on the quality of the available carbon and/or nitrogen source (Bertram *et al.*, 2002; Crespo *et al.*, 2002; Kulkarni *et al.*, 2006; Tate *et al.*, 2005).

Since the *UGA4* gene is tightly regulated and some of the transcription factors participating in its regulation (i.e. Gln3 and Gat1) have been linked to NCR gene expression in response to the quality of the carbon source, the aim of our work was to get further insights into the regulation of this gene by the carbon source. We analysed the expression of *UGA4* and the role of the GATA factors and the target DNA sequences on the *UGA4* promoter involved in the regulation of the *UGA4* gene.

METHODS

Strains and media. The *Saccharomyces cerevisiae* strains used in this study, isogenic to the wild-type Σ1278b, were 23344c (*MATα ura3*), 30505b (*MATα ura3 gln3*Δ), 30078c (*MATα ura3 uga43*Δ), CD17 (*MATα ura3 uga35*Δ), 26790a (*MATα ura3 uga3*Δ), 34411c (*MAT***a** *ura3 nil1*° (*Kan*^{R}) *gln3*°) and 32164b (*MATα ura3 nil1*° (*Kan*^{R})). These strains were kindly provided by Professor S. Vissers (Université Libre de Bruxelles, Belgium). Cells were grown in minimal medium containing 0.17 % Difco yeast nitrogen base (YNB without amino acids and ammonium sulfate) with 2 % glucose or 2 % potassium acetate as carbon source, and 10 mM proline or 10 mM ammonium sulfate as nitrogen source. Yeast strains were transformed using the methodology described by Gietz & Woods (2002).

Construction of fusion plasmids. All procedures for manipulating DNA were standard ones (Sambrook *et al.*, 1997). 5′-Regulatory regions and part of the coding regions of the *UGA4* (−583 to +15, with respect to the ATG initiation codon), *UGA43* (−848 to +48), *GZF3* (−790 to +21), *GLN3* (−826 to +44), *GAT1* (−787 to +48), *UGA35* (−393 to +51) and *UGA3* (−261 to +24) genes were fused in-frame to the *lacZ* gene lacking its first seven codons, in the plasmid YEP357 (Myers *et al.*, 1986). This plasmid carries the *URA3* selectable marker complementing the uracil auxotrophy of the yeast strains used. DNA fragments were generated by PCR amplification using Σ1278b genomic DNA as template. Primers used in these constructions are listed in Table 1. All fusion plasmids were verified by DNA sequence analysis. *Escherichia coli* DH5α was used to amplify and maintain the plasmids.

Two nested 5′ deletions of *UGA4* called $UAS_{GATA}\Delta$ (i.e. without the UAS_{GATA} sequence) and UASΔ (i.e. without the UAS_{GATA} and UAS_{GABA} sequences) (see Table 1 and Fig. 5) fused to the *lacZ* reporter gene were also generated using the strategy described above.

In vitro site-specific mutagenesis was carried out according to Strachan & Read (1999). Briefly, two PCRs, using primers mut1 and F-UGA4 or primers mut2 and R-UGA4 and the fusion plasmid carrying *UGA4-lacZ* as template, were performed. Primers mut1 and mut2 (Table 1) contain the specific pre-determined mutation (Fig. 5a), located in a central segment. After the two products were combined, denatured and allowed to reanneal, the DNA polymerase extended the 3′ end of heteroduplexes with recessed 3′ ends. Thereafter, a full-length product (UAS_{GABA}mut) with the introduced mutation in a central segment was amplified by PCR using the outer primers F-UGA4 and R-UGA4 and then it was fused to the *lacZ* gene in YEP357.

β-Galactosidase assays. Cells were grown on the indicated media to OD_{570} 0.5–0.9, then harvested and transferred to the same fresh medium with or without 0.1 mM GABA. An aliquot (10 ml) of each culture was collected by centrifugation after 2 h incubation at 30 °C and resuspended in 2 ml buffer Z (Miller, 1972). β-Galactosidase activity measured according to Miller (1972) was expressed as Miller units. Results are shown as mean ± standard deviation of duplicates within a representative assay. At least duplicate assays for each of two independent transformants were performed. The deviation of these values from the mean was less than 15 %.

Fluorescence microscopy. 23344c cells transformed with the plasmid pRR482 (kindly provided by Dr T. Cooper, University of Tennessee, Memphis, USA), containing the full-length coding sequence of Gln3 fused to GFP under the *GAL1* promoter, were grown on a plate of ammonium-glucose medium. Cells were picked from the plate, transferred to fresh liquid medium containing 4 % galactose and 10 mM proline as the nitrogen source and incubated for 30 min. Then cells were collected, washed and transferred again to the indicated fresh medium. After a 90 min incubation, cells from a 5 ml culture were fixed with 70 % ethanol in 50 mM Tris/HCl pH 7.5. After 1 h of incubation, an overnight RNase treatment was performed and nuclei were stained with a 50 μg ml^{-1} propidium iodide solution in PBS. Analysis was carried out by using a laser-scanning confocal microscope (Fluoview FV300 BS61; Olympus). Images were acquired simultaneously into acquisition channels with the FLUOVIEW FV300 (version 3.3) acquisition/analyser program. Photographs were imported to Adobe Photoshop 8.0 for image management.

Table 1. Oligonucleotide primers

Bold type letters represent the restriction sites.

Primer	Sequence (5′–3′)
F-UGA4	CGCG**GAATTC**GACAATTTCTTCAATCATTGAAATG
R-UGA4	CCCC**AAGCTT**CATACTCATTGTTAGTAATAATAAATTATAAGACCT
F-UGA43	CGCG**GAATTC**TTGTTGTAGAGCCGGTGCAT
R-UGA43	CCCC**AAGCTT**AGCTGAAAGTGTAGGCGA
F-GZF3	CCG**GAATTC**CTGTATCCTTGGTC
R-GZF3	CGC**GGATCC**AGTTGTAGCCTG
F-GLN3	CGCG**GAATTC**CTGATCGAGATCCTCC
R-GLN3	CCCC**AAGCTT**CAGCAGGTCGTACAGCT
F-GAT1	CCG**GAATTC**AGCATTTTTCACG
R-GAT1	CCC**AAGCTT**GAACAGAACAGGG
F-UGA35	CGCG**GAATTC**GAGCTCCCACAGTGATGTAG
R-UGA35	CCCC**AAGCTT**GCTCTTCGTAGGCGATGC
F-UGA3	CGCG**GAATTC**ACGTTTTGCGGGCATCACGT
R-UGA3	CCCC**AAGCTT**CAGCTTCTCCACGCCATAATT
F-UAS$_{GATA}$Δ	CGCG**GAATTC**CCAAAAACCGCCGGCGGCAATTT
F-UASΔ	CGCG**GAATTC**TTCGAGATGTTTTATGTCATGTTGG
mut1	ACATAAAACATCTCGAAATTATTACTAATGGTTTTTGGCGCACGA
mut2	TCGTGCGCCAAAAACCATTAGTAATAATTTCGAGATGTTTTATGT

RESULTS

Nitrogen and carbon sources regulate *UGA4* gene expression

β-Galactosidase activity was measured in wild-type cells (strain 23344c) transformed with a plasmid carrying the 5′-regulatory region and part of the coding region of *UGA4* fused in-frame to *lacZ* (YEP357 *UGA4*::*lacZ*) grown with different nitrogen and carbon sources. Both basal and GABA-induced *UGA4* expression were analysed. When glucose, a glycolytic substrate, was used in the presence of the poor nitrogen source proline, the basal expression level was low and significant GABA induction was detected, whereas in the presence of ammonium, a rich nitrogen source, both basal and GABA-induced levels were repressed (Fig. 1). These were the expected results since *UGA4* is an inducible gene and it is subject to NCR (Talibi *et al.*, 1995). When the carbon source was acetate, a gluconeogenic substrate, basal expression levels in cells grown in derepressed conditions (i.e. proline as nitrogen source) were higher than those observed in cells from glucose-proline medium and there was no significant GABA induction. NCR was still observed when acetate was the carbon source. These results show that *UGA4* is not only regulated by the nitrogen source, as has been extensively described, but also by the carbon source.

Effect of transcription factors acting through the UAS$_{GATA}$ element in the *UGA4* promoter

We decided to study if the molecular mechanisms by which *UGA4* expression is regulated by carbon source involve the GATA factors.

Firstly, the expression of the four GATA factors in wild-type cells grown with either glucose or acetate was determined using β-galactosidase assays. These experiments were performed using proline as the nitrogen source since expression values obtained with ammonium were too low to detect any differences (data not shown). A high expression of *UGA43* and a low expression of *GZF3* were

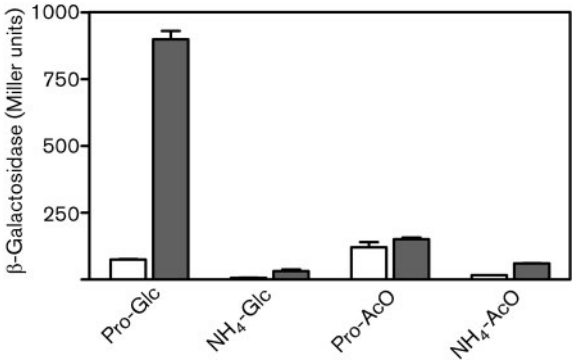

Fig. 1. Effect of different carbon and nitrogen sources on *UGA4* expression. β-Galactosidase activity was determined in extracts from wild-type cells (strain 23344c) carrying the *UGA4*::*lacZ* fusion gene. Cells grown on minimal medium containing proline and glucose (Pro-Glc), ammonium and glucose (NH_4-Glc), proline and acetate (Pro-AcO) or ammonium and acetate (NH_4-AcO) as nitrogen and carbon sources were collected and transferred to the same fresh media containing (filled bars) or not (open bars) 0.1 mM GABA. After a 2 h incubation samples were taken and β-galactosidase activity was measured. Results are shown as mean ± SD of duplicates within a representative assay.

Table 2. Effect of different carbon sources on expression of GATA factors

β-Galactosidase activity was determined in extracts from wild-type cells (strain 23344c) carrying the *UGA43*::*lacZ*, *GZF3*::*lacZ*, *GLN3*::*lacZ* or *GAT1*::*lacZ* fusion. Cells grown in minimal medium containing proline as the nitrogen source and glucose or acetate as the carbon source were transferred to the same fresh media, and after a 2 h incubation samples were taken and β-galactosidase activity was measured. Results are shown as mean ± SD of duplicates within a representative assay.

Carbon source	β-Galactosidase activity (Miller units)			
	UGA43	*GZF3*	*GLN3*	*GAT1*
Glucose	680.5 ± 27.5	85.7 ± 23.5	223.9 ± 33.2	8.0 ± 1.8
Acetate	538.9 ± 66.4	79.1 ± 26.4	29.7 ± 4.7	2.7 ± 0.8

Table 3. Effect of the repressor Uga43 on *UGA4* regulation by carbon source

β-Galactosidase activity was determined in extracts from wild-type (strain 23344c) and *uga43*Δ (strain 30078c) cells carrying the *UGA4*::*lacZ* fusion. Cells grown on minimal medium containing proline and glucose or acetate were collected and transferred to the same fresh media containing or not 0.1 mM GABA. After a 2 h incubation samples were taken and β-galactosidase activity was measured. Results are shown as mean ± SD of duplicates within a representative assay.

Genotype	Carbon source	β-Galactosidase activity (Miller units)	
		−GABA	+GABA
Wild-type	Glucose	75.1 ± 2.8	898.9 ± 44.4
	Acetate	133.0 ± 26.3	151.4 ± 11.0
*uga43*Δ	Glucose	1363.8 ± 154.9	1576.2 ± 410.3
	Acetate	185 ± 25.5	131.3 ± 28.5

observed and they did not vary with the carbon source (Table 2). Thus, changes in expression of the negative GATA factors do not explain the observed variation in *UGA4* expression.

The expression level of the positive GATA factor *GAT1* was very low in both growth conditions tested. The expression of the other positive GATA factor, *GLN3*, in the presence of glucose was significantly higher than when acetate was used. Thus, there is a correlation between GABA induction of *UGA4* gene (Fig. 1) and *GLN3* expression (compare Fig. 1 and Table 2).

It has been extensively described that *UGA4* expression is, at least in part, the result of the competition between positive and negative GATA factors for the binding to the UAS_{GATA} element (Andre *et al.*, 1995; Cunningham *et al.*, 1994). Gat1 and Gzf3 regulate *UGA4* expression in the presence of a preferred nitrogen source (Coffman *et al.*, 1997; Soussi-Boudekou *et al.*, 1997) while Gln3 and Uga43 are the main GATA factors acting on the UAS_{GATA} element of the *UGA4* promoter in our experimental conditions, i.e. proline-glucose medium. To determine if the absence of GABA induction of *UGA4* gene in cells grown on acetate is due to a strong binding of the repressor Uga43 to UAS_{GATA}, *UGA4* expression was measured in cells deficient in this negative factor. As expected, *UGA4* levels were high in *uga43*Δ cells grown on glucose even in the absence of GABA; however, in *uga43*Δ cells grown on acetate, values were similar to those obtained in wild-type cells (Table 3). These data clearly show that the effect of carbon source on *UGA4* does not depend on Uga43.

One reason for the low expression of *UGA4* in acetate-grown cells could be that *GLN3* levels, even in the absence of Uga43, are not enough to allow *UGA4* GABA induction. On the other hand, it is well known that the activity of the positive GATA factors depends on their intracellular localization; thus, another reason for *UGA4* expression profiles in acetate could be a cytoplasmic accumulation of Gln3. Therefore, subcellular localization of the fusion protein Gln3-GFP was determined by fluorescence microscopy of cells grown with different carbon sources (Fig. 2). As expected, in wild-type cells grown on glucose, Gln3 was mainly observed in the nucleus. In acetate-grown cells, GFP

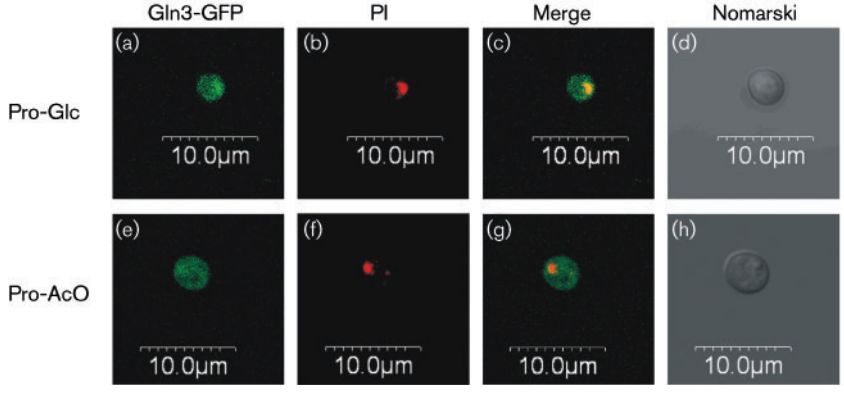

Fig. 2. Gln3 localization in cells grown on different carbon sources. Subcellular localization of Gln3-GFP was determined by confocal microscopy in the wild-type strain (23344c) transformed with pRR482. After 30 min of 4% galactose induction, cells were transferred and incubated for 90 min in different minimal media containing proline and glucose (a–d) or proline and acetate (e–h). Nuclear staining was performed with propidium iodide (PI) as described in Methods. Representative images are shown.

fluorescence was preferentially seen in the nucleus, although some fluorescence was detected in the cytoplasm. In cells incubated in glucose or acetate the protein Gln3-GFP was overexpressed. As expression of this fusion protein is driven by *GAL1*, when cells were transferred to glucose the shut-off of the promoter was probably much more drastic than in acetate. In this case, the amount of Gln3-GFP in acetate-grown cells would be higher than in glucose-grown cells. In any case, since the fluorescence was mainly observed in the nucleus, the absence of induction of *UGA4* gene in acetate medium can not be attributed to the absence of Gln3 in the nucleus. Although the Gln3 localization patterns were slightly different, the observation of a large number of cells from independent experiments, where several Gln3-GFP expression levels were assayed, led us to the belief that carbon source may regulate Gln3 intracellular localization.

To further analyse the role of the GATA factors in carbon regulation, *UGA4* expression was measured in mutant strains deficient in the *GLN3* and/or *GAT1* genes. *UGA4* expression was almost undetectable in a *gln3*Δ strain irrespective of the carbon source, whereas the deletion of *GAT1* did not seem to affect *UGA4* expression (Fig. 3a, b). Interestingly, in the double mutant *gln3*Δ *gat1*Δ the expression levels of *UGA4* were similar to those observed in wild-type cells. This apparent discrepancy was explained by analysing the expression of each negative GATA factor in these strains (Fig. 4a–d). The data show that in *gln3*Δ cells there was significant expression of the negative factor *UGA43* while in *gln3*Δ *gat1*Δ cells almost no expression of both negative factors was observed, allowing the recovery of *UGA4* expression levels. In other words, the absence of the two activators is compensated by the simultaneous absence of the two repressors. It must be noted that in the double mutant strain (*gln3*Δ *gat1*Δ), when both positive GATA factors were absent and the expression of the negative ones was negligible, the expression profiles of *UGA4* were significantly different depending on the carbon source: *UGA4* levels were lower, and no GABA induction was observed, in cells from acetate compared to those from glucose (Fig. 3).

In conclusion, although the expression levels and probably the intracellular localization of Gln3 are modulated by the carbon source, indicating that this factor may be involved in carbon regulation of *UGA4*, our results suggest that this regulation is also mediated by other factors.

Effect of transcription factors acting through the UAS_{GABA} element in the *UGA4* promoter

The transcription factors Uga3 and Uga35 regulate *UGA4* expression, acting through the UAS_{GABA} element. Thus, the expression of these factors in wild-type cells was analysed in order to establish their involvement in *UGA4* regulation by carbon source. *UGA35* levels did not vary and were very low on both carbon sources (less than 25 Miller units) whereas *UGA3* levels in glucose medium were three times higher than those measured in acetate medium (160 Miller units in glucose and 53 Miller units in acetate).

Results of *UGA4* expression in strains deficient in *UGA35* and *UGA3* were not reproducible although always low. It must be noted that these strains do not grow well in minimal media. Thus, the involvement of Uga3 in the effect of the carbon source on *UGA4* gene (i.e. GABA induction in glucose but not in acetate) could not be determined by this methodology.

Role of the UAS elements in the response of *UGA4* to carbon source

In order to establish the DNA sequences participating in carbon regulation of *UGA4*, modifications in the promoter

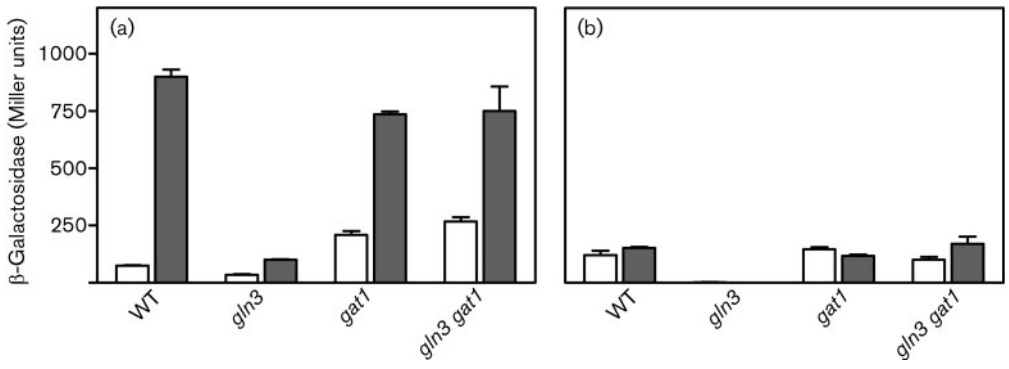

Fig. 3. Effect of different carbon sources on *UGA4* expression in cells deficient in the GATA factors Gln3 and Gat1. β-Galactosidase activity was determined in extracts from wild-type (strain 23344c), *gln3*Δ (strain 30505b), *gat1*Δ (strain 32164b) and *gln3*Δ *gat1*Δ (strain 34411c) cells carrying the *UGA4*::*lacZ* fusion. Cells grown in minimal medium containing proline as the nitrogen source and glucose (a) or acetate (b) as the carbon source were collected and transferred to the same fresh media containing (filled bars) or not (open bars) 0.1 mM GABA. After a 2 h incubation, samples were taken and β-galactosidase activity was measured. Results are shown as mean ± SD of duplicates within a representative assay.

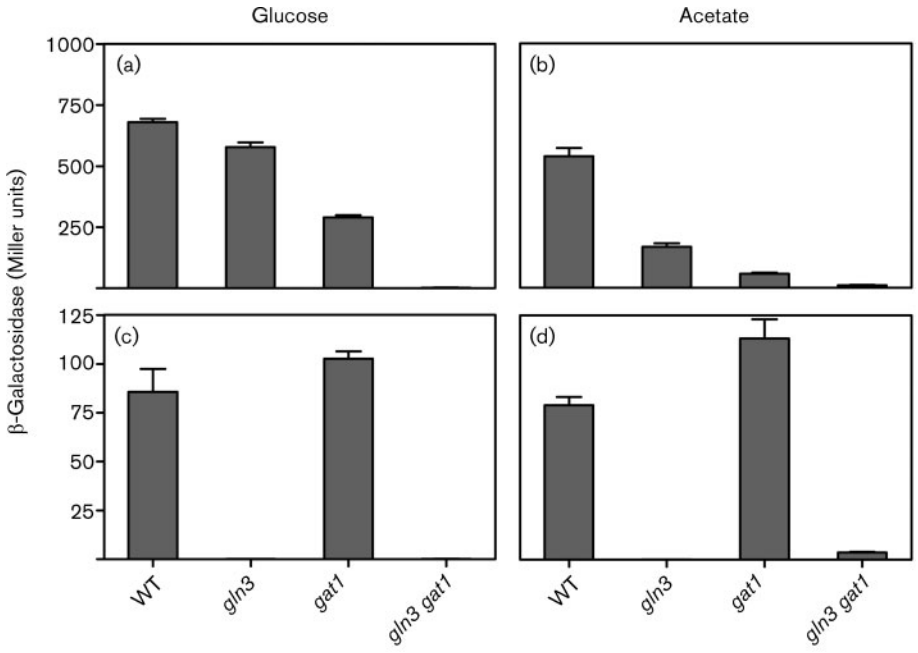

Fig. 4. Effect of different carbon sources on *UGA43* and *GZF3* expression in cells deficient in the GATA factors Gln3 and Gat1. β-Galactosidase activity was determined in extracts from wild-type (strain 23344c), *gln3*Δ (strain 30505b), *gat1*Δ (strain 32164b) or *gln3*Δ *gat1*Δ (strain 34411c) cells carrying the *UGA43*::*lacZ* (a, b) or *GZF3*::*lacZ* (c, d) fusion genes. Cells grown in minimal medium containing proline as the nitrogen source and glucose (a, c) or acetate (b, d) as the carbon source were collected were transferred to the same fresh media, and after a 2 h incubation, samples were taken and β-galactosidase activity was measured. Results are shown as mean ± SD of duplicates within a representative assay. The deviation of these values from the mean was less than 15 %.

were introduced and cloned upstream of *lacZ*. Expression driven by a 5′ deletion of the *UGA4* promoter lacking the UAS_{GATA} element in wild-type cells was lower than when the whole promoter was used but the effect of the carbon source remained unchanged (Fig. 5a, b, c). These results indicate that other elements besides GATA participate in this regulation. No differences between wild-type cells grown with glucose or acetate were observed in *lacZ* expression driven by a 5′ deletion of the *UGA4* promoter lacking both UAS_{GATA} and UAS_{GABA} elements (Fig. 5a, b, d). Taken together these results indicate that the *UGA4* DNA sequence –406 to –385 is involved in carbon regulation. Expression levels driven by UAS_{GABA}mut were high with both glucose and acetate, indicating that this element is responsible for carbon regulation (Fig. 5a, b, e). Moreover, a repressor seems to be acting on the native UAS_{GABA} element (compare Fig. 5b, e).

DISCUSSION

Data presented in this work provide clear evidence that the quality of the carbon source regulates *UGA4* expression and that this regulation is the result of at least two parallel pathways. Different elements present in the 5′ regulatory region of *UGA4* are involved in this regulation. *UGA4* expression is regulated by carbon source through Gln3 acting on the UAS_{GATA} element and through another factor, as yet unknown, acting on the UAS_{GABA} element.

Kuruvilla *et al.* (2002), dissecting glucose signalling in yeast, found a set of genes regulated by carbon source but *UGA4* was not one of them. Moreover, this research group postulated that carbon and nitrogen quality signal preferentially through the multiprocessing Tor proteins to Gat1 and Gln3, respectively (Kuruvilla *et al.*, 2001). Our results do not agree with these findings since the activity of Gat1 on *UGA4* does not seem to depend on the carbon source, wheras Gln3 activity is clearly regulated by carbon quality.

We found that in the presence of the rich carbon source glucose *UGA4* expression is low and a significant increase of this expression is observed when the inducer GABA is added. In contrast, *UGA4* expression in cells grown on the non-fermentable carbon source acetate is higher than that observed in glucose-grown cells and it is not dependent on the presence of GABA. We conclude that the expression of the GATA factor Gln3 is also modulated by the carbon source. Moreover, the slight changes in the subcellular localization patterns observed suggest that Gln3 activity could be also regulated by carbon source at this level. Thus, *UGA4* expression must be affected by the different patterns

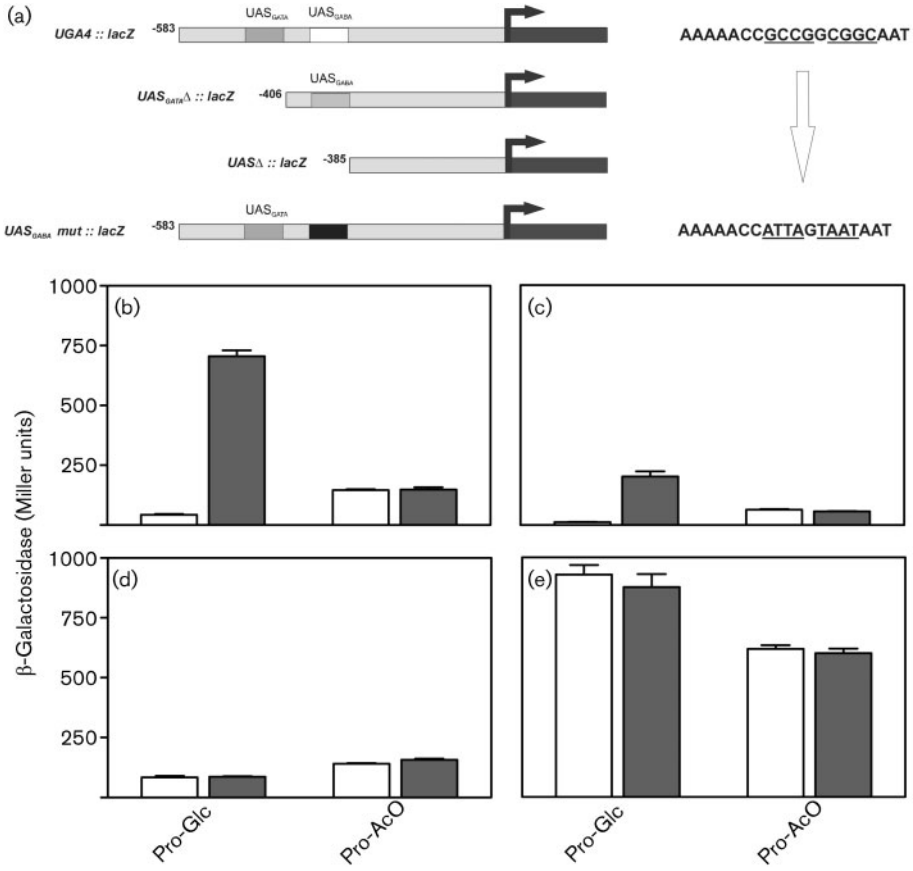

Fig. 5. Participation of the UAS elements in *UGA4* regulation by carbon source. (a) Scheme of the fusion genes used. (b–e) β-Galactosidase activity was determined in extracts from wild-type (23344c strain) cells carrying the *UGA4*::*lacZ* (b), $UAS_{GATA}\Delta$::*lacZ* (c), *UASΔ*::*lacZ* (d) or $UAS_{GABA}mut$::*lacZ* (e) fusion genes. Cells grown in minimal medium containing proline as the nitrogen source and glucose or acetate as the carbon source were collected and transferred to the same fresh media containing (filled bars) or not (open bars) 0.1 mM GABA. After a 2 h incubation samples were taken and β-galactosidase activity was measured. Results are shown as mean ± SD of duplicates within a representative assay. The deviation of these values from the mean was less than 15 %.

of Gln3 expression and it probably responds to changes in Gln3 localization too. However, there is another pathway participating in the regulation of *UGA4* by carbon source since in the absence of the four GATA factors or in the absence of the UAS_{GATA} element, *UGA4* still responds to the carbon source. Our results also show that the UAS_{GABA} element is the target of factors acting in response to the quality of the carbon source since expression driven by the promoter with a mutated UAS_{GABA} becomes independent of the carbon source. Moreover, results using this mutated promoter clearly show that there is a negative factor acting on UAS_{GABA} and its activity depends on the carbon source. It is known that, to be effective, UAS_{GABA} requires two positive-acting proteins, namely pathway-specific Uga3p and pleiotropic Uga35p (Dal81p/DurLp) (Talibi *et al.*, 1995). Thus two possibilities may arise in the light of this new evidence. The first one is that Uga3 and/or Uga35 may have a negative influence on *UGA4* transcription. Our results argue against a direct negative function of these factors since *UGA4* expression levels detected in cells deficient in Uga3 or Uga35, although not reproducible, were always lower than those in wild-type cells. The second possibility would be the action, not yet described, of another transcription factor on UAS_{GABA}. Even though the participation of Leu3 in *UGA4* transcription has not been reported, its consensus site for the binding to DNA overlaps the UAS_{GABA} element, suggesting that this factor may be the negative factor operating in *UGA4* regulation. Moreover, Boer *et al.* (2005) recently reported the impact of the *LEU3* deletion on transcriptional regulation of nitrogen metabolism in cells under different growth conditions; they found an increase in *UGA4* and *UGA3* transcription. The results presented in this work and preliminary results recently obtained in our laboratory together with data from Boer *et al.* (2005) support our present hypothesis which is that the interaction of Uga3 and Uga35 with others factors (protein or effector molecules) results in the formation of a repressor complex

that acts on UAS_{GABA} in a way that depends on the carbon source.

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