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UGA4 gene encoding the γ-aminobutyric acid permease in *Saccharomyces cerevisiae* is an acid-expressed gene

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Abstract

Background and aims: biological processes in all organisms are controlled by environmental conditions, however, information concerning the molecular responses to external pH is scarce. In this work we studied the pH response of *UGA4* gene encoding δ-aminolevulinic acid and γ-aminobutyric acid permease in *Saccharomyces cerevisiae*. *Methods*: we analyzed the effect of pH on the expression of *UGA4* gene measuring β-galactosidase activity in cells carrying a *UGA4::lacZ* fusion gene. *Results*: results indicate that *UGA4* expression is higher at acidic pH. The expression of *UGA3* and *UGA35* genes, which encode two positive transcription factors, is not regulated by external pH, while the expression of *UGA43* gene encoding a repressor of *UGA4* transcription is dependent on pH. Using a strain lacking Uga43p we clearly showed that the effect of ambient pH on *UGA4* expression is not a secondary effect of the pH regulation on *UGA43*. We have also demonstrated that the effect of pH can only be detected when *UGA4* gene is not subject to a strong repression by Uga43p nor to GABA induction. *Conclusion*: here, we demonstrate that *UGA4* is an acid-expressed gene. This regulation is probably mediated by Rim101p through the consensus site 5′-GCCARG-3′ at 237 bp preceding the *UGA4* coding sequence (201).

Keywords: δ-aminolevulinic acid; γ-aminobutyric acid; *UGA4* gene; *Saccharomyces cerevisiae*; Ambient pH; Transcriptional regulation

1. Introduction

There is a continuous cell response to changes in their immediate environment. Many micro-organisms are able to adapt themselves to a wide pH range, therefore, extracellular pH is a key signal. A conserved pH response pathway has been identified in the fungi *Aspergillus nidulans* [1], *Penicillium chrysogenum* [2], *Yarrowia lipolytica* [3], *Candida albicans* [4] and *Saccharomyces cerevisiae* [5]. In this pathway, the Zn finger transcription factor Rim101p/PacC, from *S. cerevisiae* and *A. nidulans*, stimulates the expression of alkaline response genes and represses that of acid-expressed genes [6]. PacC consensus sites tend to be less frequent in acid-expressed genes than in alkali-expressed genes [6–9]. Recently, Espeso and

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Arst [10] studying the acid-expressed *gabA* gene, encoding γ-aminobutyric acid (GABA) permease in *A. nidulans* found that PacC acts as a repressor by preventing the binding of the positively acting transcription factor IntA. The target site of this factor, which mediates ω-amino acid induction of *gabA* gene, is a 19 bp CG-rich upstream sequence. This sequence contains the entire region responsible for repression of GABA permease synthesis at alkaline pH, with the overlap of PacC and IntA sites [10].

Rim101p/PacC activity is controlled by proteolytic processing [11,12]. Unlike PacC cleavage, which is completely dependent upon external alkaline pH, Rim101p cleavage occurs under both acidic and alkaline growth conditions [11]. Lamb and collaborators [5] have studied gene induction by alkaline pH and its relationship to the RIM101 pathway in *S. cerevisiae*. However, little is known about acid-expressed genes in this organism.

In *S. cerevisiae*, GABA is incorporated into the cells through three permeases [13]. Expression of *UGA4* gene, which encodes the specific GABA permease, is subject to GABA induction and nitrogen catabolite repression. Induction of this permease requires at least two positive acting proteins, the specifc Uga3p factor and the pleiotropic Uga35p factor (i.e. Dal81p/DurLp) [14,15]. 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 an 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 the Uga43p (i.e. Dal80p), the pleiotropic regulatory factor [14,16].

Moreover, besides the nitrogen source, we have shown that the composition of the cell growth media, also regulates *UGA4* gene expression [17,18] and under certain conditions this expression is independent on the presence of GABA.

Here we show that *UGA4*, as many other genes in ascomycetes, is a pH response gene. We demonstrate that *S. cerevisiae UGA4* gene is an acid-expressed gene such as the *gabA* gene encoding GABA permease in *A. nidulans* [10].

2. Materials and methods

2.1. Strain and growth conditions

The *Saccharomyces cerevisiae* strains 23344c (*ura3*) and 30078b (*ura3 uga43Δ*) used in this study are isogenic with the wild type Σ1278b (*Matα*) [19]. These strains were kindly supplied by S. Vissers from the Université Libre de Brussels, Belgium.

Minimal medium 1 (MM1) used was as described by Vavra and Johnson [20]. In 1 l, it contained 1 g $MgSO_4 \cdot 7H_2O$, 4 g KH_2PO_4, 12 g $NaH_2PO_4 \cdot H_2O$, 10 ml vitamin solution (40 mg biotin, 1 g calcium pantothenate, 20 g myo-inositol, 8.8 g thiamine, 2.4 g pyridoxine and 500 mg nicotinamide per litre) and 10 ml of trace metal solution, 1.73 g $ZnSO_4 \cdot 7H_2O$, 0.722 g $FeSo_4 \cdot 7H_2O$ and 100 mg $CuSO_4 \cdot 5H_2O$ per litre. Final pH is four. Carbon and nitrogen sources were 2% glucose and 10 mm proline, respectively, in MPM1. It was sterilized at 121 °C for 20 min.

Minimal medium 2 (MM2) was described by Jacobs et al. [21]. The following solutions, earlier sterilized were mixed. One litre of medium 165 contained 0.7 g $MgSO_4 \cdot 7H_2O$, 1 g KH_2PO_4, 0.4 g $CaCl_2 \cdot 2H_20$, 0.5 g NaCl, 1 g K_2SO_4, 10.5 g citric acid H_2O. This medium was adjusted to pH 6 with addition of KOH. The trace metal solution (1 ml in 1 l of medium 165) contained per litre, 10 mg H_3BO_3, 1 mg $CuSO_4 \cdot 5H_2O$, 2 mg KI, 4 mg $Na_2MoO_4 \cdot 2H_2O$, 14 mg $ZnSO_4 \cdot 7H_2O$, 10 g citric acid. H_2O, 400 mg $MnSO_4 \cdot H_2O$ and 5 g $FeCl_3 \cdot 6H_2O$. Vitamin solution (10 ml in 1 l of medium 165) contained per litre 250 mg D-biotin, 100 mg thiamin. HCl, 1 g inositol, 200 mg calcium pantothenate and 100 mg pyridoxine HCl. Glucose was added at 3 g for 100 ml final volume by addition of 30% sterile glucose solution to a mixture of the other three solutions. Proline was added at a final concentration of 10 mM (MPM2).

2.2. DNA preparation, manipulation and transformation

Yeast transformation was performed as already described by Gietz and Schiestl [22]. The procedures for growth and transformation of *Escherichia coli* and for preparation of plasmid DNA were as reported by Sambrook et al. [23].

2.3. Plasmids

The *UGA4::lacZ*, *UGA43::lacZ*, *UGA3::lacZ* and *UGA35::lacZ* fusion genes were constructed by replacing the *Eco*RI–*Hind*III fragment of plasmid YEp357 [24] with PCR-amplified fragments spanning nucleotides −583 to +15, −848 to +48, −261 to +24 and −393 to +41 with respect to the ATG initiation codons of *UGA4*, *UGA43*, *UGA3* and *UGA35*, respectively. PCR were performed using genomic DNA of strain Σ1278b. The YEp357 [24] carries the *URA3* selectable marker, thus, complementing the uracil auxotrophy of the yeast strains used. The structures of restriction endonuclease joints and PCR product were verified by DNA sequence analysis. *E. coli* JM109 strain was used to amplify and maintain the plasmids.

2.4. β-Galactosidase assay

A 10 ml culture of exponentially growing cells (A570 nm, 0.3–0.7) was collected by centrifugation and resuspended in 2 ml Z buffer [25]. The β-galactosidase activity measured according to Miller [25], is expressed as Miller units. Each transformant was assayed in duplicate and the reporter β-galactosidase activities are the average of at least two separate, randomly selected transformants from the same transformation event.

2.5. Significance of data

In every experiment for each point duplicates were run and the values presented are the average of three experiments. The deviation of these values from the mean was less than 15%.

3. Results

In this work we studied the effect of ambient pH on *UGA4* gene using *S. cerevisiae* cells grown on two different media, MPM1 and MPM2 (the composition of which is described in Section 2). In cells from MPM2 the expression of *UGA4* is very low unless cells were preincubated with GABA. However, in earlier studies, in cells grown in MPM1, high basal levels of *UGA4* expression are detected even in the absence of GABA [18]. A very important difference between these two media is that the pH of MPM1 is four and that of MPM2 is six.

To determine whether or not external pH is affecting *UGA4* gene expression β-galactosidase activity was measured in wild type cells carrying a *UGA4::lacZ* fusion gene. In cells grown in MPM1 at different pHs, *UGA4* gene expression is significantly higher at pH 4 than at pH 6, earlier GABA induction (Table 1). High levels of β-galactosidase activity are found in GABA induced cells from MPM1 at both pHs.

Interestingly, very low basal levels of expression are detected in cells grown in MPM2 at any pH assayed. After GABA induction, β-galactosidase activity was higher and similar at both pHs, indicating that, as expected *UGA4* gene expression is induced by GABA and that this induction in MPM2 is independent on pH.

Regulation of ambient pH on *UGA4* gene could be direct, acting a pH response protein such as

Table 1
lacZ expression driven by *UGA4* promoter in wild type cells

	GABA induction	β-galactosidase activity (Miller units)	
		PH 4	pH 6
MPM1	−	64.1 ± 5.2	8.1 ± 1.2
MPM1	+	225.1 ± 19.7	178.1 ± 14.6
MPM2	−	11.8 ± 1.5	12.0 ± 1.5
MPM2	+	70.5 ± 6.1	84.9 ± 7.9

β-galactosidase activity was measured in 23344c strain cells carrying *UGA4::lacZ* fusion gene grown on MPM1 or MPM2 at the indicated pHs. For GABA induction, cells were incubated with 0.1 mM GABA for 1 h before harvesting.

Table 2
lacZ expression driven by *UGA3*, *UGA35* and *UGA43* promoters in wild type cells

	β-galactosidase activity (Miller units)			
	MPM1		MPM2	
	pH 4	pH 6	pH 4	pH 6
UGA3::lacZ	33.1 ± 2.5	30.0 ± 2.6	28.3 ± 3.4	29.5 ± 2.6
UGA35::lacZ	12.0 ± 1.8	15.3 ± 1.3	13.4 ± 1.8	18.0 ± 1.4
UGA43::lacZ	125.7 ± 8.0	23.2 ± 2.9	320.5 ± 18.3	345.0 ± 19.1

β-galactosidase activity was measured in 23344c strain cells carrying *UGA3::lacZ*, *UGA35::lacZ* or *UGA43::lacZ* fusion genes grown on MPM1 or MPM2 at the indicated pHs.

Rim101p on the 5′-upstream regulatory region of the gene, or indirect, if pH is regulating the expression of one or more of the transcription factors that are involved in *UGA4* gene control.

Using *UGA3::lacZ*, *UGA35::lacZ* and *UGA43::lacZ* constructs, the effect of ambient pH on the expression of *UGA3*, *UGA35* and *UGA43* genes was studied. The expression of *UGA3* and *UGA35* genes, which encode two positive transcription factors, is not regulated by external pH. In contrast, the expression of *UGA43* gene depends on pH in cells grown in MPM1 but not in MPM2, and it is significantly higher at acidic pH (Table 2).

Since pH effect is similar on both *UGA4* and *UGA43* genes (Table 1 lane 1 and Table 2 lane 3) and because Uga43p is a negative regulatory factor of *UGA4*, the effect of pH on *UGA4* is not due to the effect of pH on *UGA43*.

To study the regulation of *UGA4* by ambient pH without the influence of Uga43p, β-galactosidase activity was measured in a mutant strain lacking Uga43p but carrying the *UGA4::lacZ* construct. Results presented in Table 3 clearly show that the same effect of ambient pH on *UGA4* expression is observed in cells, with or without Uga43p, grown in MPM1. These data also indicate that in *uga43Δ* cells the expression of *UGA4* gene is always derepressed. When comparing *UGA4* expression in cells with and without Uga43p (23344c–30078b strains) it appears that the higher the Uga43p levels, the stronger the derepression (see also Table 2 lane 3).

4. Discussion

Environmental pH exerts broad control over cell metabolism. Yeast cells grow more rapidly on acid media than in neutral or alkaline media. One effect of external alkalinity is the disruption of membrane proton gradients which normally supply energy for nutrient and ion transport [26]. Consequently, cells are capable of responding to nutrient and ion limitation and several of their transporters are encoded by alkaline expressed genes [5]. Here we demonstrate that *S. cerevisiae UGA4* gene encoding δ-aminolevulinic acid (ALA) and GABA permease [27] responds to external pH. However, this response is only expressed under certain growth conditions (MPM1), while pH effect does not appear to be significant when cells are grown in other medium such as MPM2. Therefore, *UGA4* response to pH would be dependent on the presence of at least some other component of the culture medium. Changes in the composition of the medium lead to different levels of the negative regulatory factor Uga43p and this is probably the reason why the effect of pH on *UGA4* gene is only seen under certain conditions. When there are high levels of Uga43p (MPM2), repression of *UGA4* gene is so strong that this could overlap the pH effect. In contrast, when the expression of *UGA43* is relatively low (MPM1), the effect of pH can be detected.

In analogy, GABA induction could also be overlapping the effect of external pH since, in GABA induced cells the expression of *UGA4*

Table 3
lacZ expression driven by *UGA4* promoter in cells lacking Uga43 repressing factor

	β-galactosidase activity (Miller units)			
	MPM1		MPM2	
	pH 4	pH 6	pH 4	pH 6
23344 (*wt*)	64.0 ± 5.2	8.1 ± 1.2	8.1 ± 1.1	8.3 ± 1.1
30078b (*uga43Δ*)	92.7 ± 6.3	32.5 ± 2.9	156.8 ± 10.3	219.5 ± 14.3

β-galactosidase activity was measured in 23344c and 30078b strain cells carrying *UGA4::lacZ* fusion gene grown on MPM1 or MPM2 at the indicated pHs.

reaches high values independently on pH and medium composition.

It is worth to note that expression of *UGA4* after GABA induction is significantly higher in cells grown on MPM1 than in cells grown on MPM2. This is probably due to the different expression of *UGA43* in each medium. Moreover, *UGA4* expression in GABA induced cells lacking Uga43p is high coming up to similar values in both media at any pH (data not shown).

UGA4 is an acid-expressed gene, such as *gabA* gene encoding GABA permease in *A. nidulans* [10]. Ambient pH regulation of *gabA* is mediated by PacC that acts as a repressor by preventing the binding of the positively acting transcription factor IntA. The proposed consensus binding site for Rim101p/PacC is 5′-GCCARG-3′ (where R is purine) [10]. Searching for potential binding sites in *UGA4* 5′-regulatory region using the MatInsepector V2.2 software [28] we found one possible binding site at −237 to −242 positions relative to the ATG *UGA4* gene. The UAS_{GABA} GC-rich sequence responsible for GABA induction of *UGA4* gene is at positions −385 to −403. In contrast with PacC regulation of *A. nidulans gabA* gene, binding sites for Rim101p and positive transcription factors, Uga3p and Uga35p, in *UGA4* promoter are not overlapping each other. This is in good agreement with results here presented, since GABA induction of *S. cerevisiae UGA4* gene is not prevented at any pH.

Some ambient pH response genes have been shown to be independent on the RIM101 pathway in *S. cerevisiae* [5] and *C. albicans* [29]. So, further studies should be carried out to confirm whether or not pH response of *UGA4* gene is dependent on Rim101p.

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