GABA induction of the *Saccharomyces cerevisiae* UGA4 gene depends on the quality of the carbon source: Role of the key transcription factors acting in this process

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**A R T I C L E   I N F O**

Article history:
Received 19 March 2012
Available online 14 April 2012

Keywords:
UGA4
Carbon regulation
GATA factors
Dal81
Uga3
Kinases

**A B S T R A C T**

Yeast cells are able to adapt their metabolism according to the quality of both carbon and nitrogen sources available in the environment. *Saccharomyces cerevisiae* UGA4 gene encodes a permease capable of transporting γ-aminobutyric acid (GABA) into the cells. Yeast uses this amino acid as a nitrogen source or as a carbon skeleton that enters the tricarboxylic acid cycle.

The quality of the carbon source modulates UGA4 expression through two parallel pathways, each one acting on different regulatory elements, the UASGATA and the UASGABA. In the presence of a fermentable carbon source, UGA4 expression is induced by GABA while in the presence of a non-fermentable carbon source this expression is GABA-independent.

The aim of this work was to study the mechanisms responsible for the differences in the profiles of UGA4 expression in both growth conditions.

We found that although the subcellular localization of Gln3 depends on the carbon source and UGA4 expression depends on Tor1 and Snf1, Gln3 localization does not depend on these kinases. We also found that the phosphorylation of Gln3 is mediated by two systems activated by a non-fermentable carbon source, involving the Snf1 kinase and an unidentified TORC1-regulated kinase.

We also found that the activity of the main transcription factors responsible for UGA4 induction by GABA varies depending on the quality of the carbon source. In a fermentable carbon source such as glucose, the negative GATA factor Dal80 binds to UGA4 promoter; only after the addition of the inducer, the positive factors Uga3, Dal81 and Gln3 interact with the promoter removing Dal80 and leading to gene induction. In contrast, in the non-fermentable carbon source acetate the negative GATA factor remains bound to UGA4 promoter in the presence or absence of GABA, the positive factors are not detected bound in any of these conditions and in consequence, UGA4 is not induced.

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1. Introduction

The budding yeast *Saccharomyces cerevisiae* cells sense the amount and quality of external nutrients through multiple interconnected signaling networks, which allow them to adapt readily and appropriately to changes in nutritional states. Carbon and nitrogen are the two most basic nutrient sources for cellular organisms. In response to the quality of carbon and nitrogen, cells can regulate the expression of genes involved in different metabolic pathways, particularly those involved in utilization and transport of the available nutrients.

The UGA4 gene that encodes the γ-aminobutyric acid (GABA) and δ-aminolevulinic acid permease in *S. cerevisiae*, is regulated by both nitrogen [1,2] and carbon [3] sources and is inducible by GABA [4,5]. Expression of this permease requires at least two positive acting proteins, the specific Uga3 factor and the pleiotropic Dal81 factor [6,7]. These factors interact with UGA4 promoter in response to GABA through a 19 bp CG-rich upstream activating sequence, named UASGATA [8,9]. The promoter region of UGA4 also contains four adjacent repeats of the heptanucleotide $5'$-CGAT(A/T)AG-3', which constitute a UASGATA element. The GATA factors Gln3, Gat1 and Dal80 are known to act on this element [1,2,6,10–12]. The role of the other negative GATA factor Gzf3 has not been demonstrated yet on UGA4 regulation.

We previously demonstrated that the quality of the carbon source modulates UGA4 expression through two parallel pathways: one of them acting on the Gln3 target sequence, the UASGATA element, and the other acting on the UASGABA element of UGA4 gene [3].

Gln3 activity depends on both its subcellular localization and its phosphorylation [13] and this phosphorylation depends on TORC1.
(target of rapamycin) activity [14]. Moreover, Snf1, a yeast homolog of AMP-activated protein kinase (AMPK), is required for metabolic adaptation in response to reduced levels of available glucose, the preferred carbon source. When glucose is unavailable, alternative carbon sources are used for the production of metabolic energy [15]. Snf1 is activated by glucose limitation by phosphorylation of the threonine 210 and by inactivation of Tor kinases [16,17]. Gln3 phosphorylation and subcellular localization are regulated by glucose availability via the SNF1/AMPK pathway [18].

Based on these findings, the aim of this work was to establish the molecular mechanisms of the two proposed pathways involved in carbon regulation of UGA4 gene. We found that in glucose Uga3 and Dal81 factors are recruited to UGA4 promoter in response to GABA and that Gln3 and Dal80 binding to this promoter is also modulated by the inducer. In contrast, under non-fermentable conditions, these factors do not respond to GABA and, in consequence, no induction of UGA4 is observed. Results here presented suggest that the two pathways regulating UGA4 expression by carbon source act on two different DNA elements but in a coordinated way.

2. Methods

2.1. Strains and media

The S. cerevisiae strains used in this study, isogenic to the wild type Σ1278b, are listed in Table 1. All the strains generated in this study were constructed using the PCR-based gene-deletion strategy [19,20] or modified versions of it [21]. All the parental strains are listed in Table 1 and all primers used for PCR reactions are listed in Supplementary material Table S1.

Cells were grown in minimal medium containing 0.17% Difco yeast nitrogen base (YNB without amino acids and ammonium sulfate) containing 2% glucose or 2% potassium acetate as carbon source and 10 mM proline as nitrogen source.

All yeast transformations were carried out using the lithium method [22].

2.2. Cell extracts and immunoblotting

Cells expressing tagged versions of Uga3 or Dal81 proteins or wild type cells transformed with a plasmid from the Movable ORFs collection containing the open reading frame of GLN3 gene [23] were grown on the indicated media and were harvested by centrifugation. Protein extraction was immediately carried out as already described [24]. Briefly, total proteins were prepared by lysing yeast cells in 1.85 N NaOH-7.5% β-mercaptoethanol on ice for 10 min, followed by precipitation with trichloroacetic acid (TCA) at a final concentration of 8%. The TCA pellets were neutralized with 1 M unbuffered Tris and resuspended in sodium dodecyl sulfate (SDS) loading buffer. Proteins were separated on a 7% SDS–PAGE, transferred to PVDF membranes and proteins were detected using the rat monoclonal anti-HA antibody (anti-HA high affinity 3E10 Roche) or the rat monoclonal anti-α-tubulin (YOL1/34 Santa Cruz) and with the secondary goat anti-rat antibody IgG conjugated with horseradish peroxidase (Santa Cruz). Chemiluminescence immunodetection was performed (FUJIFILM LAS-1000 Reader) and immunoreactive bands were analyzed by digital imaging.

2.3. Fluorescence microscopy

Cells transformed with the CEN-based plasmid pRS416-GLN3-GFP (kindly provided by Dr. T. Cooper, University of Tennessee, Memphis, USA) [25], containing the full-length GLN3 gene fused to GFP were fixed with 70% ethanol in 50 mM Tris/HCl pH 7.5 for 60 min. Nuclei were stained incubating cells with 50 μg/ml DAPI (4′,6-Diamidino-2-phenylindole) in 50 mM Tris/HCl pH 7.5 for 60 min. Cells were washed twice with 50 mM Tris/HCl pH 7.5. Analysis was carried out by using fluorescence microscopy. Gln3-GFP localization was manually scored in 200 or more cells in multiple randomly chosen fields from each image taken [26].

2.4. Quantitative RT-PCR

RT-qPCR experiments were performed according to Cardillo et al. [27]. cDNAs were quantified by RT-PCR using an Opticon Monitor 3 (Bio-Rad) with the primers F-qRT-UGA4 and F-TBP qPCR. Expression values correspond to the ratio of concentrations of UGA4 over TBP1 specific mRNAs determined in each sample and represent the mean ± SEM of three independent experiments.

2.5. Chromatin immunoprecipitation assays

Chromatin immunoprecipitation (ChIP) experiments were performed according to Cardillo et al. [9]. Normal mouse IgG (Santa Cruz) or monoclonal anti HA antibody (HA probe (F-7), Santa Cruz) were used. Real time quantitative PCR was carried out in an Opticon Monitor 3 (BioRad) with primers that amplified promoter regions of UGA4 (F/R-UGA4qPCR). A pair of primers that amplified a region located 2.5 Kb downstream of UGA4 promoter was used as an unbound control. ChIP DNA was normalized to input DNA and calculated as a signal to noise ratio over an IgG control ChIP. The ΔΔCT method was used to calculate fold change of binding to the promoter of interest [28]. Results are expressed as the mean ± SEM of three independent experiments.

3. Results and discussion

To further analyze the mechanisms of the regulation of UGA4 by carbon source proposed by Luzzani et al. [3], in the first place we studied the phosphorylation state and subcellular localization of Gln3. The subcellular localization of the fusion protein Gln3-GFP determined by fluorescence microscopy was classified in three categories, cytoplasmic, nuclear-cytoplasmic and nuclear, according to Tate et al. [26]. A representative collection of standard images
Fig. 1. Effect of the carbon source on the subcellular localization and on the phosphorylation levels of Gln3. (A) Wild type (23344c), tor1Δ, snf1Δ, and double mutant snf1Δ tor1Δ cells transformed with the plasmid pRS416-GLN3-GFP, were grown on minimal medium with glucose as the carbon source. Harvested cells were transferred to fresh medium containing glucose or acetate and incubated for 30 min. Then, cells were fixed and nuclei were stained with DAPI. Images were processed and colored using the ImageJ software. (B) and (C) Wild type (23344c), tor1Δ TOY01, snf1Δ CELY01 and snf1Δ tor1Δ (CELY02) cells transformed with the plasmid pRS416-GLN3-GFP, were grown on minimal medium with 2% raffinose, induced with 1% galactose for 1 h and incubated in minimal medium with glucose or acetate in the presence or absence of 200 ng/ml rapamycin for 30 min. Total cell extracts were prepared and immunoblotting was carried out with anti-HA antibody.
of their target genes and that the balance of this competition leads to different expression levels [12]. We recently reported that the binding of Gln3 and Dal80 to \( \text{UGA4} \) promoter in glucose responds to GABA in an opposite way: Dal80, but not Gln3, interacts with DNA in the absence of GABA while Gln3, but not Dal80, interacts with DNA in the presence of GABA [9]. So, we decided to also compare the binding of Dal80 in both carbon sources. In glucose, Dal80-HA bound to \( \text{UGA4} \) promoter in the absence of the inducer and its binding decreased after the addition of GABA (Fig. 3B) as we already reported [9]. In acetate, this negative GATA factor was detected bound to \( \text{UGA4} \) promoter even in the presence of the inducer (Fig. 3B) in agreement with the fact that Gln3-HA was not detected bound (Fig. 3A).

Luzzani et al. [3] proposed a second mechanism responsible for the regulation by carbon source of \( \text{UGA4} \) gene which would act on the UAS\( \text{GABA} \) element. \( \text{UGA4} \) induction depends on Uga3 and Dal81 transcription factors that act through this element [5,7]. Since the main difference between expression in glucose and acetate is that the induction in acetate is significantly lower than in glucose (Fig. 2A), we decided to analyze the binding dynamics of Uga3 and Dal81 to \( \text{UGA4} \) promoter in both carbon sources. In glucose, both factors were detected bound to DNA only after the addition of the inducer (Fig. 3C and D) as we already reported [8]. In contrast, HA-Uga3 and HA-Dal81 binding was impaired in acetate (Fig. 3C and D). As this observation could be due to a decrease in the expression of these factors in acetate, we analyzed by Western...
blot their expression. We found that Uga3 and Dal81 levels were similar in both carbon sources (Fig. 3E), confirming that the binding ability of these factors depended on the carbon source. These results correlated with the very low induction observed in this condition (Fig. 2A). It must be noted that different forms of both Uga3 and Dal81 proteins were detected. Although the mechanisms that lead to the activation of Uga3 and Dal81 in response to GABA are still unknown, it has been proposed that Uga3 activation may occur through a post-translational modification since levels of expression of this factor remain unaffected in the presence of the inducer [32]. Moreover, in silico analysis of UGA3 and DAL81 coding sequences yielded many putative phosphorylation sites [33]. Taken together, all these evidences suggest that the different forms of both Uga3 and Dal81 proteins detected could be a consequence of different phosphorylation states. Further research must be done in order to demonstrate this hypothesis.

In summary, in glucose, the negative GATA factor Dal80 is bound to UGA4 promoter repressing its expression. When GABA is added, the two factors responsible for induction, Uga3 and Dal81, and the non-phosphorylated positive GATA factor Gln3, which competes with Dal80 for binding to the UASGATA element, bind to the promoter producing the induction of UGA4. In acetate, Dal80 interacts with UGA4 promoter whereas the highly phosphorylated Gln3, Uga3 and Dal81 factors do not. This interaction accounts for the absence of GABA induction.

Results presented here do not allow us to determine whether the phosphorylation of Gln3 in acetate or its less nuclear distribution impairs its interaction with UGA4 promoter. However, recently we showed that in glucose the binding dynamics in response to GABA of Dal80, and consequently of Gln3, are modulated by Uga3 and Dal81 transcription factors [9]. These findings resemble those observed in acetate where we do not detect binding of Uga3 and/or Dal81 and at the same time we do not detect changes in the binding of the GATA factors after the addition of GABA. All together these observations suggest that the impaired binding of Gln3 in acetate could be a consequence of the lack of function of Uga3 and Dal81, although we can not discard an effect of the phosphorylation status of Gln3 on its binding, and that consequently could affect Dal80 binding.

In conclusion, the quality of the carbon source regulates UGA4 expression by two parallel but connected pathways. The role in these pathways of other transcription factors known to regulate UGA4, such as Gat1 [12] and Leu3 [8], needs to be analyzed.

Acknowledgments

This research was supported by University of Buenos Aires (UBA) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. We thank Dr. T. Cooper (University of Tennessee, Memphis) and Dr. J. Ariño (Universidad Autónoma de Barcelona, Barcelona) for kindly providing the plasmids used here.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbrc.2012.04.047.

References


