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SHORT COMMUNICATION

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Expression of the *UGA4* gene encoding the δ-aminolevulinic and γ-aminobutyric acids permease in *Saccharomyces cerevisiae* is controlled by amino acid-sensing systems

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Abstract In yeasts, several sensing systems localized to the plasma membrane which transduce information regarding the availability and quality of nitrogen and carbon sources and work in parallel with the intracellular nutrient-sensing systems, regulate the expression and activity of proteins involved in nutrient uptake and utilization. The aim of this work was to establish whether the cellular signals triggered by amino acids modify the expression of the *UGA4* gene which encodes the δ-aminolevulinic (ALA) and γ-aminobutyric (GABA) acids permease. In the present paper, we demonstrate that extracellular amino acids regulate *UGA4* expression and that this effect seems to be mediated by the amino acid sensor complex SPS (*SSY1*, *PTR3*, *SSY5*).

Keywords *Saccharomyces cerevisiae* · Gene expression · *UGA4* gene · Sensor systems

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Introduction

In *Saccharomyces cerevisiae*, δ-aminolevulinic acid (ALA) and γ-aminobutyric acid (GABA) are imported into the cells through the Uga4 permease (Grenson et al. 1987; Bermúdez Moretti et al. 1996). GABA is also imported through the general amino acid permease (Gap1p) and the proline-specific permease (Put4p) (Grenson et al. 1987).

Expression of the *UGA4* gene depends on the GABA induction and nitrogen catabolite repression (NCR). Induction of this permease requires at least two positive acting proteins, the specific Uga3p factor and the pleiotropic Dal81p/Uga35p factor (Bricmont et al. 1991; André et al. 1995). 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′-CGA-T(A/T)AG-3′, which constitute an UAS_{GATA} element. This element can potentially confer high levels of expression in the absence of inducer. Nevertheless, this potential activity is inhibited in uninduced cells grown under conditions of nitrogen derepression, by a strong repression mechanism, involving Dal80p/Uga43p, a pleiotropic regulatory factor (André et al. 1995; Cunningham et al. 1994). Gln3p, another GATA transcriptional factor, acts in the presence of inducer up-regulating the expression of *UGA4* 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. NCR is superimposed on the above regulation; availability of preferred nitrogen sources mediates the ability of Ure2p, a preprionic cytoplasmic protein, to prevent nuclear localization of Gln3p, and consequently, its activity (Kulkarni et al. 2001). Tor1/2 signal transduction pathway is involved in this regulation (Cooper 2002).

Several sensing systems localized to the plasma membrane transduce information regarding the availability and quality of nitrogen and carbon sources and work in parallel with intracellular nutrient-sensing systems regulating the expression and activity of proteins involved in nutrient uptake and utilization. Among the extracellular sensing complexes, there are two glucose sensors, *SNF3* and *RGT2* (Özcan et al. 1996); a G-protein coupled receptor that is activated by the presence of fermentable sugars, *GPR1* (Kraakman et al. 1999; Lorenz et al. 2000); a high affinity ammonium transporter, *MEP2* (Marini et al. 1997), that may function as an ammonium sensor (Lorenz and Heitman 1998); a general amino acid permease, *GAP1*, that may also act as an amino acid sensor (Donaton et al. 2003); and a multicomponent amino acid sensor complex known as SPS sensor (*SSY1*, *PTR3*, *SSY5*), which contains a transmembrane Ssy1p protein together with peripheral plasma membrane Ptr3p and Ssy5p proteins (Didion et al. 1998; Iraqui et al. 1999; Klasson et al. 1999; Forsberg and Ljungdahl 2001; Bernard and André 2001).

The aim of this work was to determine whether the *UGA4* gene, encoding the ALA and GABA permease, could be included among the genes that are under the control of amino acid-sensing systems.

Materials and methods

Strains and growth conditions

The *Saccharomyces cerevisiae* strains 23344c (*ura3*), 30629c (*ura3 gap1Δ::kanMX2*) and 30995b (*ura3 ssy1Δ::kanMX2*) used in this study are isogenic with the wild type Σ1278b (Béchet et al. 1970) except for the mutations mentioned. These strains were kindly supplied by S. Vissers from the Université Libre de Brussels, Belgium. Strains transformed with YEp357 plasmid (Myers et al. 1986) containing the *UGA4::lacZ* fusion gene were also used. The *UGA4::lacZ* fusion gene was constructed by replacing the *Eco*RI-*Hin*dIII fragment of plasmid YEp357 with a PCR amplified fragment spanning nucleotides −583 to +15 with respect to the ATG initiation codon of *UGA4* (YEp *UGA4::lacZ*) (Correa García et al. 2000). Selective medium was minimal medium without uracil.

Cells were grown in the minimal buffered (pH6.1) medium previously described by Jacobs et al. (1980). Carbon and nitrogen sources were 3% glucose and 10 mM proline, respectively. Medium was supplemented with amino acids as described previously (Sherman 1991).

β-Galactosidase assay

An aliquot (10 ml) of a culture of exponentially growing cells ($A_{570\ \mathrm{nm}}$: 0.7–1.0) was collected by centrifugation and resuspended in 2 ml buffer Z (Miller 1972). β-galactosidase activity measured according to Miller (1972) was expressed as Miller units. At least duplicate assays for each of the two independent transformants were performed for each value reported. The deviation of these values from the mean was less than 15%.

Results and discussion

Since basal level of *UGA4* expression is low and it is induced in the presence of GABA, we decided to examine whether GABA-dependent *UGA4* induction was modulated by amino acids. For this purpose, β-galactosidase activity from cells under different conditions and carrying YEp *UGA4::lacZ* was measured. When GABA was added to *wild type* cells grown in the presence of amino acids (Fig. 1a, up triangles), the induction of *UGA4* was significantly lower than when it was added to cells grown without amino acids (Fig. 1a, circles) or with amino acids but transferred to fresh amino acid-free medium before the addition of GABA (Fig. 1a, squares). Similar curves were obtained using citrulline or leucine instead of all amino acids (data not shown). These results clearly show that the presence of amino acids affected induction of the *UGA4* gene producing a delay but not an abolishment of this induction. Interestingly, the induction observed after adding GABA was similar in cells grown without amino acids (Fig. 1a, circles) or with amino acids but transferred to fresh amino acid-free medium (Fig. 1a, squares), suggesting that the amino acids present in the medium but not intracellular amino acid pools were affecting *UGA4* induction and that there was a very rapid response to external amino acid depletion. The rapid recovery of the induction rate implicates post-translational changes rather than de novo synthesis of proteins. This is in agreement with the conformational changes of SPS sensor previously reported (Forsberg and Ljungdahl 2001).

To establish which one of the two amino acid sensors postulated so far was responsible for the observed effect on *UGA4* expression we carried out β-galactosidase assays using a *gap1Δ* strain (Fig. 1b) and an *ssy1Δ* strain (Fig. 1c). The effect of amino acids on *UGA4* induction was identical in *gap1Δ* (Fig. 1b) and *wild type* (Fig. 1a) cells. In contrast, the levels of *UGA4* induction were similar in *ssy1Δ* cells grown in the presence (Fig. 1c, up triangles) or absence (Fig. 1c, circles, squares) of amino acids. These results clearly indicate that in the absence of a functional SPS sensor, the presence of amino acids did not affect the GABA induction. All these results together prompted us to postulate that the effect of amino acids on *UGA4* expression would be mediated by SPS sensor rather than GAP1.

In this work, we demonstrate that GABA-dependent *UGA4* expression is repressed by extracellular amino acids and that the sensing system involved in this

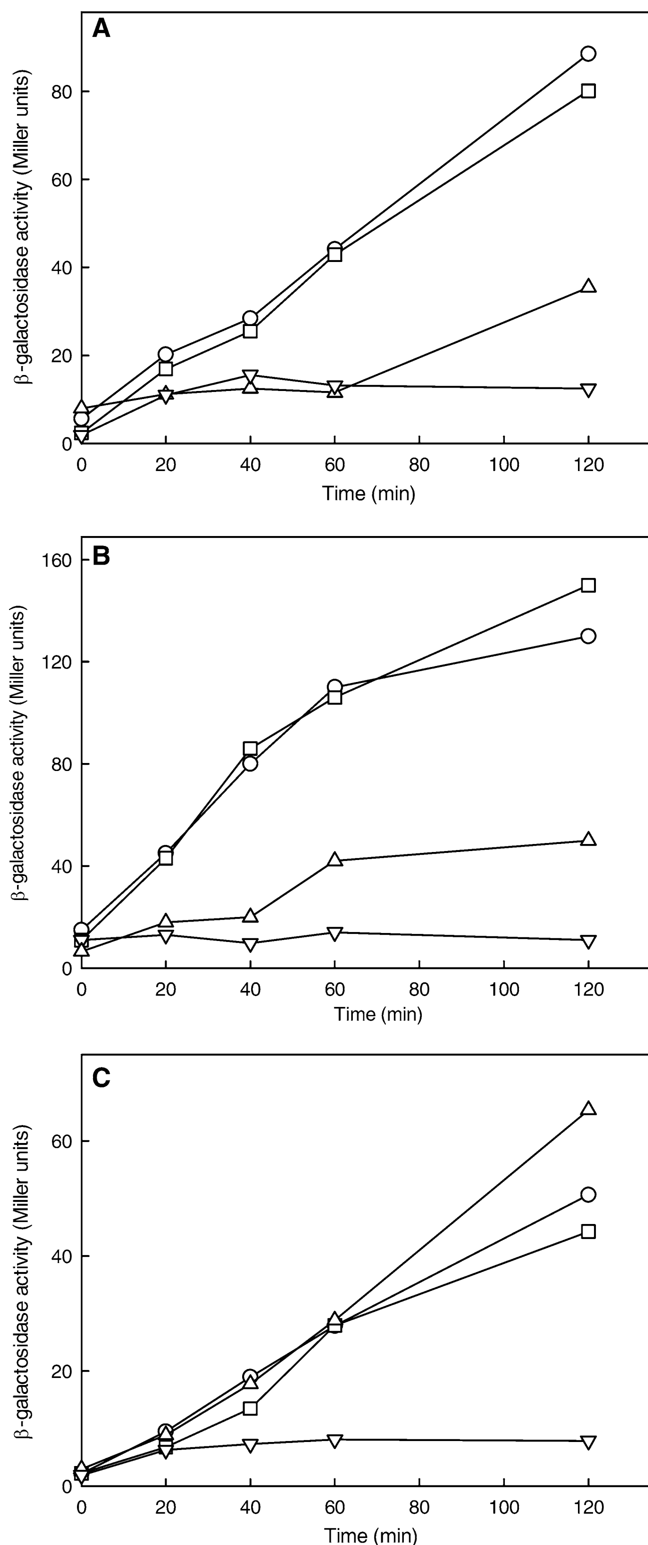

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Fig. 1 Effect of amino acids on *UGA4* expression in *S. cerevisiae* cells. β-galactosidase activity was measured in *wild type* (**a**), *gap1*Δ (**b**) and *ssy1*Δ (**c**) cells carrying the *UGA4::lacZ* fusion gene. 0.1 mM GABA was added (time = 0) to cells grown on minimal medium (*circles*), minimal medium supplemented with amino acids but transferred to fresh amino acid-free medium before GABA addition (*squares*) and minimal medium supplemented with amino acids (*up triangles*). Cells from minimal medium supplemented with amino acids and transferred to fresh amino acid-free medium without the addition of GABA were used as control (*down triangles*). The amino acid mix was that described by Sherman (1991) without uracil. Samples were taken out from cultures at the indicated times for β-galactosidase activity measurements

response is SPS. Interestingly, unlike a number of other genes regulated by SPS such as those encoding other amino acid permeases (*AGP1*, *BAP2*, *BAP3*, *GNP1*, *TAT1* and *TAT2*), the peptide transporter *PTR2* and arginase (*CAR1*) (Forsberg and Ljungdahl 2001), *SSY1* is not fully required for the induction of *UGA4*. However, it does significantly contribute to the high levels of expression seen in the *wild type* grown in the absence of amino acids. Our results support the idea postulated by Abdel-Sater et al. (2004) who predicted that the addition of amino acids leads not only to the induction of Ssy1-regulated genes but also to the down-regulation of other genes under the regulation of Dal81p/Uga35p such as allophanate-inducible DUR genes.

Genome-wide expression analysis reports, which showed that SPS sensor regulates the transcription of several amino acid permease genes as well as other nitrogen metabolizing genes (Forsberg et al. 2001; Kodama et al. 2002), did not include *UGA4* among those genes modulated by the SPS sensor. This is not a discrepancy since in their working conditions the *UGA4* inducer, GABA, was not present. Genome wide expression analysis has revealed many genes affected by SPS sensor, but many other genes apart from *UGA4* might have failed to be detected although being under this regulation. On the other hand, there is still a need for further research to elucidate the transduction pathway involved in the regulation of each particular gene.

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