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RESEARCH ARTICLE

P. Roig · D. Gozalbo

Candida albicans UBI3 and *UBI4* promoter regions confer differential regulation of invertase production to *Saccharomyces cerevisiae* cells in response to stress

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Abstract *Candida albicans* ubiquitin genes *UBI3* and *UBI4* encode a ubiquitin-hybrid protein involved in ribosome biogenesis and polyubiquitin, respectively. In this work we show that *UBI3* and *UBI4* promoter regions confer differential expression consistent with the function of their encoded gene products. Hybrid genes were constructed containing the *SUC2* coding region under the control of *UBI3* or *UBI4* promoters in the yeast vector pLC7. Invertase production in *Saccharomyces cerevisiae* transformants was differentially regulated: the *UBI4* promoter was induced by stress conditions (thermal upshift and/or starvation) whereas the *UBI3* promoter conferred constitutive invertase production in growing yeast cells. These results indicate that the *UBI4* promoter is regulated by stress-response signaling pathways, whereas the *UBI3* promoter is controlled according to the requirement for protein synthesis to support cell growth.

Keywords *Candida albicans* · Ubiquitin genes · Invertase · *Saccharomyces cerevisiae* · Promoter gene fusion · Heterologous expression

Introduction

Two ubiquitin genes, *UBI3* and *UBI4*, have been described in *Candida albicans*. *UBI3* encodes a ubiquitin-hybrid protein involved in ribosome biogenesis, and *UBI4* encodes polyubiquitin [19, 20, 22]. Different mechanisms regulate the expression of these genes; *UBI3* is expressed coordinately with members of the translational apparatus according to the requirements of protein synthesis and cellular growth rate, whereas *UBI4* expression is enhanced by stress conditions [19]. Regulatory mechanisms such as the control of mRNA stability may be involved in the regulation of gene expression, especially in the case of *UBI3* and ribosomal protein genes in which a stress (heat)-induced mRNA-decay mechanism may participate in their regulation [13, 17, 19]. The main control of gene expression, however, occurs at the level of transcription from 5′ upstream non-coding promoter sequences.

We studied the regulation of transcription from the promoter region of *UBI3* and *UBI4* *C. albicans* genes. Most *C. albicans* genes, under the control of their own promoter sequences, are functional when expressed in *S. cerevisiae*. In addition, complementation of *S. cerevisiae* mutant phenotypes has been widely used to show the function of *C. albicans* cloned genes [2,15], including *UBI3* [19] and *UBI4* [unpublishing results]. Based on these findings, we constructed hybrid genes consisting of the coding region of the glucose-repressible *S. cerevisiae* *SUC2* gene coding for external (periplasmic) invertase [10,16] under the control of the *C. albicans* *UBI3* and *UBI4* promoter regions. The introduction of these genes into a *S. cerevisiae* strain defective in invertase (Suc^-) allows expression from *UBI3* and *UBI4* promoters to be studied by measuring invertase activity in whole yeast cells.

Materials and methods

Strains and growth conditions

Saccharomyces cerevisiae SEY2101 strain (*MATα ura3-52 leu2-3 leu2-112 ade2 SUC2Δ9*) [5] was used as recipient strain for plasmids pLC7 [3], pIN3, and pIN4 (see below). This strain was cultured in rich medium (YPD: 1% yeast extract, 2% peptone, 2% glucose) at 28 °C, and transformants were cultured at 28 °C in SD medium (0.67% yeast nitrogen base without amino acids, 2% glucose, 1% agar for plates) supplemented with leucine (30 mg l^{-1}) and adenine (20 mg l^{-1}). When indicated (for invertase assays), transformants were cultured in modified YPD medium (4% glucose) or in YPS medium (1% yeast extract, 2% peptone, 2% sucrose) supplemented

P. Roig · D. Gozalbo (✉)
Departament de Microbiologia i Ecologia,
Facultat de Farmàcia, Universitat de València,
Av. Vicent Andrés Estellés, s/n, 46100 Burjassot, Spain
E-mail: daniel.gozalbo@uv.es
Tel.: +34-963983026
Fax: +34-963864299

with antimycin A (2 ppm). Starvation assays were carried out according to Gil et al. [9].

Escherichia coli strain DH5α [12] was used for plasmid propagation. Transformants were grown at 37 °C in LB medium (0.5% yeast extract, 1% tryptone, 0,5% NaCl) supplemented with ampicillin (100 μg ml^{-1}).

Plasmid constructions

Plasmid pIN3 contains the coding region of the *S. cerevisiae SUC2* gene under the control of the *C. albicans UBI3* promoter. The 5′ upstream non-coding region of *UBI3* was PCR-amplified from plasmid pPR3, which contains the *C. albicans UBI3* gene previously characterized [19]. For the amplification, two primers were used (F3: GCAC*GAATTC*TTATCTAAGCGAGAGTAAGTCTTGGC, and R3: CGTG*AAGCTT*GCTTGTCGGCAAATAGCTATGCTTC) containing engineered *Eco*RI and *Hin*dIII sites (italics), respectively. These primers hybridize at positions –451 to –426 (F3) and –34 to –10 (R3) relative to the *UBI3* translational start site. PCR amplifications were carried out according to standard protocols previously detailed [14]. The PCR-amplified DNA fragment was purified, digested with *Eco*RI and *Hin*dIII, and ligated to pLC7 digested with the same restriction enzymes. The resulting plasmid (pIN3) contains the *SUC2* coding region under the control of the *UBI3* promoter sequence, which replaces the *SUC2* promoter contained within an *Eco*RI *Hin*dIII DNA fragment in pLC7 [3].

With a similar strategy, the promoter region of the *C. albicans UBI4* gene was amplified by PCR from plasmid pPR4, which contains the *C. albicans UBI4* gene [19], using two synthetic engineered oligonucleotides as primers (F4: GGTC*GAATTC*CAATTGCTAATAAGATCGAAGAGG, which anneals at positions –553 to –530 relative to the translational start site, and R4: GCTG*AAGCTT*GTAGATTTGTATATATATGTTAGTTGACG, which anneals at positions –6 to –34), and subcloned into the *Eco*RI/ *Hin*dIII sites of pLC7 to construct pIN4. This plasmid contains the *SUC2* coding region for external invertase under the control of the *UBI4* promoter.

Saccharomyces cerevisiae transformation

S. cerevisiae SEY2101 was transformed with pLC7, pIN3 or pIN4 basically as described by Elble [4]. Transformants carrying pLC7, pIN3 or pIN4 were isolated on selective media lacking uracil. Selected Ura^+ transformants were analyzed for genetic markers, and uracil prototrophy was found to be conferred by the plasmid: after two successive overnight cultures in liquid YPD, about 30% yeast cells did not grow on plates of selective medium, which suggests the instability of the Ura^+ character.

Determination of invertase activity

External (periplasmic) invertase activity was determined in intact whole cells according to a method previously described [16]. One unit of invertase activity is defined as the amount of enzyme that hydrolyzes 1 μmol of sucrose per minute of reaction. Relative activity is expressed as milliunits (mU) per mg (dry weight) of cells.

Results

Thermal upshift causes increased invertase production from the *UBI4* promoter but not from the *UBI3* promoter

To study the response of *UBI3* and *UBI4* promoters to thermal uspshift, invertase activity was measured in exponentially growing yeast cells transformed with pIN3 and pIN4 respectively, following a temperature upshift from 28 to 42 °C. The invertase activity of pIN3 transformants increased parallel to cell growth, the activity per cell remaining constant (about 90 mU mg^{-1} dry weight of cells in YPD-antimycin) (Fig. 1), which indicates that the *UBI3* promoter is not responsive to thermal stress and that it is constitutively expressed during cell growth.

The results were similar when cells were grown in YPS-antimycin (results not shown), although invertase activity was slightly higher (about 120 mU mg^{-1} dry

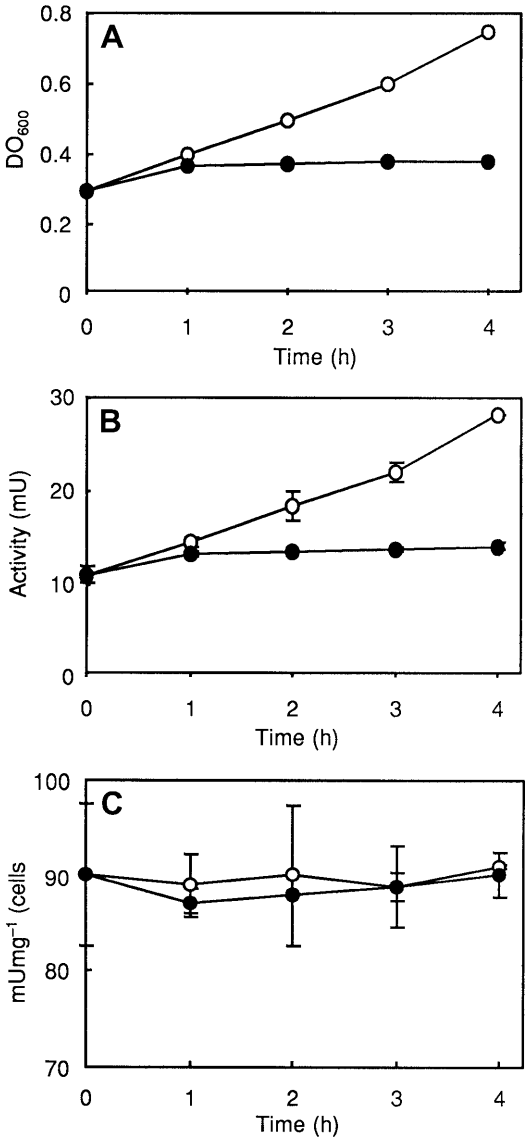

Fig. 1A–C. Effect of temperature upshift on invertase production in *Saccharomyces cerevisiae* SEY2101 strain carrying pIN3. An exponentially growing culture of pIN3 yeast transformant in YPD (4% glucose)-antimycin was shifted, at the indicated OD_{600}, to 42 °C (●) or maintained at 28 °C, as a control (○). At the indicated times, the OD_{600} of the cultures (**A**) and the invertase activity in cells from 0.5-ml aliquots were measured. Activity is expressed as total milliunits (mU) in the assayed sample (**B**), and relative activity as mU mg^{-1} dry weight of cells (**C**). Data are mean values of triplicate determinations ± standard deviations

weight of cells), probably due to a higher stability of the invertase mRNA in the absence of glucose [16]. In addition, *UBI3* appears to be a strong promoter, as it directs invertase production levels higher than those induced by the *SUC2* promoter in the presence of sucrose as fermentable carbon source (60 mU mg^{-1} dry weight of cells; results not shown). pIN4 transformants showed a different pattern of invertase production (Fig. 2). The temperature upshift induced invertase production, despite the inhibition of cell growth (the relative invertase activity per cell in YPD increased about four-fold after 4 h of thermal upshift: from 0.4 to 1.5 mU mg^{-1} dry weight of cells). In control cultures at 28 °C, basal invertase production was proportional to cell mass. These results indicate that the *UBI4* promoter is weakly expressed in growing cells (200-fold weaker than the *UBI3* promoter), and that it is induced by stress conditions such as temperature upshift. Invertase production by pIN4 transformants was not sufficient to support growth with sucrose as the only fermentable carbon source.

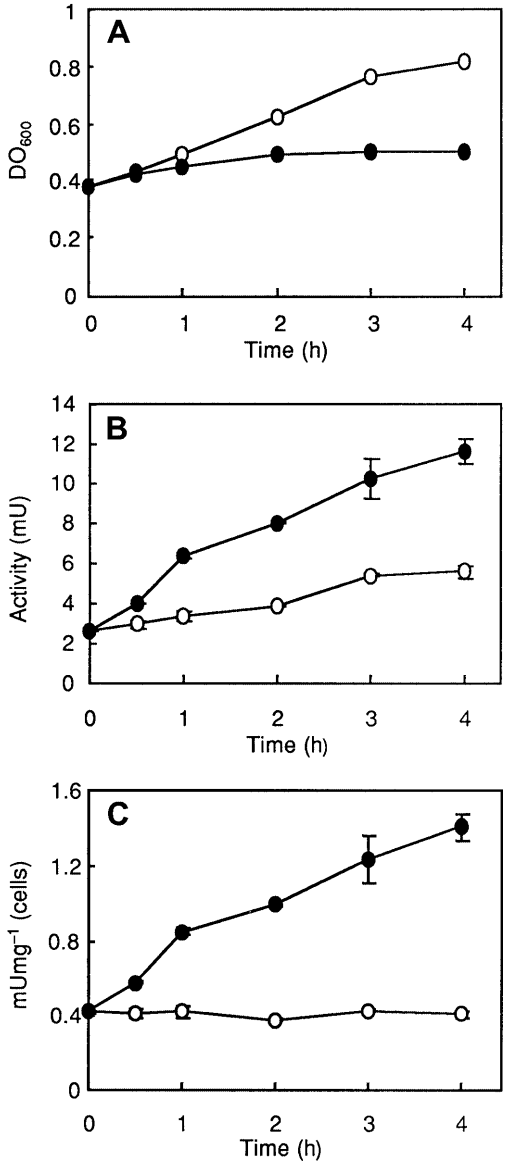

Fig. 2A–C. Effect of temperature upshift on invertase production in *S. cerevisiae* SEY2101 strain carrying pIN4. An exponentially growing culture of pIN4 yeast transformant in YPD (4% glucose)-antimycin was shifted, at the indicated OD_{600}, to 42 °C (●) or maintained at 28 °C, as a control (○). At the indicated times, the OD_{600} of the cultures (**A**) and the invertase activity in cells from 10-ml aliquots were measured. Activity is expressed as total mU in the assayed sample (**B**), and relative activity as mU mg^{-1} dry weight of cells (**C**). Data are mean values of triplicate determinations ± standard deviations

Starvation increases invertase production from the *UBI4* promoter but not from the *UBI3* promoter

Invertase activity was also measured after starvation of exponentially growing yeast transformed with pIN3 and pIN4 (Fig. 3). After 4 h of starvation, either at 28 or 42 °C, levels of invertase were constant in pIN3 transformants, indicating the absence of expression from the *UBI3* promoter under these conditions. In addition,

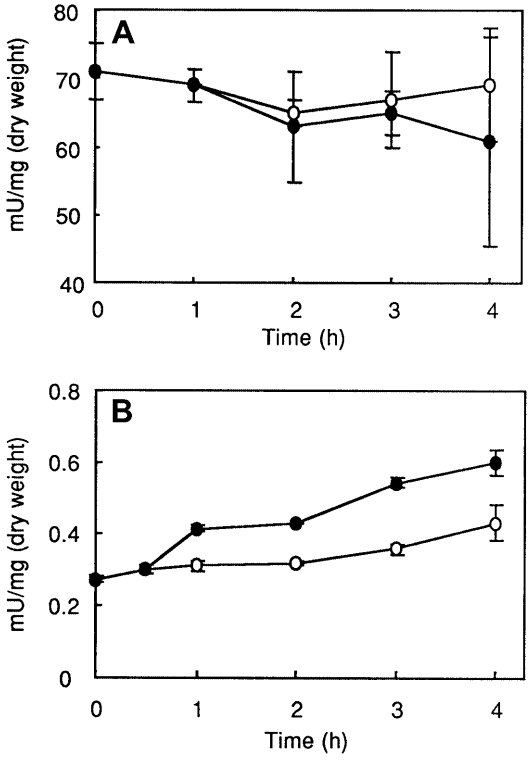

Fig. 3A, B. Effect of starvation on invertase production in *S. cerevisiae* SEY2101 strain carrying pIN3 or pIN4. Yeast cells from exponentially growing cultures of pIN3 (**A**) and pIN4 (**B**) transformants in YPD (4% glucose)-antimycin were collected by centrifugation, resuspended in one volume of prewarmed sterile water, and incubated at 28 °C (○) or 42 °C (●). Periodically, invertase activity was determined in cells from 10-ml (pIN4) or 0.5-ml (pIN3) aliquots. Data represent the relative activity (mU mg^{-1} dry weight of cells) as mean values of three determinations ± standard deviations

pIN4 transformants increased their invertase activity at 28 °C, and particularly at 42 °C (2.5-fold after 4 h of starvation).

Discussion

Our results show that the *C. albicans UBI4* promoter is poorly expressed in growing *S. cerevisiae* cells and that it is induced under stress conditions, particularly by temperature upshift, consistent with the role of polyubiquitin in the yeast stress-response system [7, 8, 13]. In addition, the *C. albicans UBI3* promoter confers constitutive high expression in growing yeast cells, and stress conditions negatively regulate its expression, consistent with its involvement in ribosome biogenesis [6, 18, 19]. This pattern of expression from *UBI* promoters in *S. cerevisiae*, as determined by invertase production, agrees with that described in *C. albicans* cells, as determined by Northern blot analysis: *UBI3* mRNA decreased to undetectable levels after thermal upshift or starvation, whereas *UBI4* mRNA levels were not affected by starvation and increased transiently following thermal shifts [19]. In addition, our results demonstrate the validity of the yeast *SUC2* as a reporter gene, with the advantage of easy quantification of its expression by assaying invertase activity in intact cells.

Note that, within the *UBI4* promoter sequence, the motif of the stress response element (STRE) [21] is present twice, at positions –114 (CCCCT) and –130 (AGGGG) relative to the translational start site. Furthermore, the sequence TAATTGGT, which matches the consensus motif thought to bind the Hap2/Hap3/Hap4 complex (TNA/GTTGGT) [11], is found at position –255. The STRE may regulate induction of *C. albicans* polyubiquitin gene in response to stress conditions such as starvation and thermal upshift, probably via different signaling pathways, whereas the HAP complex binding sequence may participate in the induction of *UBI4* gene in response to oxidative stress, as reported in *S. cerevisiae* [21, 23, 24]. Similar motifs have been found in the upstream promoter sequence of the polyubiquitin gene of *Kluyveromyces lactis* [1]. Although many biological processes and mechanisms are highly conserved between *S. cerevisiae* and *C. albicans*, there are also differences that may account for the observed low level of expression from the *UBI4* promoter.

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