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FEMS Yeast Research 5 (2005) 323–329

FEMS Yeast Research

www.fems-microbiology.org

# *YAP1*-mediated *KNQ1* expression in *Kluyveromyces lactis*

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Received 16 July 2004; received in revised form 26 August 2004; accepted 18 November 2004

First published online 8 December 2004

**Abstract**

The b-Zip transcription factor Yap1p plays an important role in oxidative stress response and multidrug resistance in *Saccharomyces cerevisiae*. We have previously demonstrated that the *KNQ1* gene, encoding a multidrug transporter of the major facilitator superfamily in *Kluyveromyces lactis* and containing two potential Yap1p response elements in its promoter, is a putative transcriptional target of *Kl*Yap1p, the structural and functional homologue of *Sc*Yap1p. In this work, we provide evidence that *KlYAP1* controls the expression of the *KNQ1* gene. Using a $P_{KNQ1}$-*gusA* fusion construct we showed that the expression of *KNQ1* is induced upon cell treatment with the oxidizing agents $H_2O_2$ and menadione and that this induction is mediated by *Kl*Yap1p. These results were confirmed by Northern-blot analysis showing that the expression of *KNQ1* is responsive to hydrogen peroxide and dependent on the presence of *Kl*Yap1p. The role of *KlYAP1* in the control of *KNQ1* expression was further demonstrated by EMSA experiments and drug resistance assays. These results clearly demonstrate the involvement of the *Kl*Yap1p transcription factor in the control of *KNQ1* gene expression.


*Keywords:* *Kluyveromyces lactis*; Transcriptional regulation; β-Glucuronidase; Multidrug permease

## 1. Introduction

Among the molecular mechanisms that cells have evolved to protect themselves from injuries caused by environmental exposure to toxic compounds, multidrug resistance (MDR) plays a significant role. This ubiquitous biological phenomenon is often associated with the overproduction of membrane transporters of the ATP-binding cassette (ABC) or the major facilitator (MFS) superfamily [1,2]. Various transcription factors have been shown to regulate the expression of genes encoding ABC or MFS proteins [3]. There are two major families of transcription factors involved in MDR: (1) the bZip protein family (Yap family), and (2) zinc cluster proteins. Yap1p is the best-characterized member of the bZip family and is an important regulator in the stress response [4–6]. Whereas in *S. cerevisiae* the Yap family comprises eight different transcription factors displaying partially overlapped but also specific targets [7], in other yeast species like *Schizosaccharomyces pombe* (Pap1p) [8], *Candida albicans* (Cap1p) [9] and *Kluyveromyces lactis* (*Kl*Yap1p) [10] their numbers are rather limited. Zinc cluster or binuclear zinc cluster proteins form a family of transcription factors found exclusively in fungi. These proteins have been shown to be involved in various processes in the cell, including regulation of primary and secondary metabolism, drug resistance, and meiotic development [11]. Several zinc cluster proteins, Pdr1p, Pdr3p, Pdr8p, Yrr1p, Yrm1p have been shown to positively control the expression of genes involved in MDR [12–14].

Recently, we have described the first *Kluyveromyces lactis* multidrug permease Knq1p, and have shown that it is the structural and functional homologue of

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1567-1356/$22.00 
doi:10.1016/j.femsyr.2004.11.004

*Saccharomyces cerevisiae* Atr1p [15]. The *ATR1* gene encodes the MFS-MDR determinant required for resistance to the competitive inhibitor of histidine biosynthesis, 3-aminotriazole, and the DNA-damaging agent 4-nitroquinoline-N-oxide [16]. The expression of *ScATR1* gene is controlled by two different bZip transcription factors, Yap1p and Gcn4p. Gcn4p is involved in regulating the transcription of genes encoding enzymes involved in the biosynthesis of amino acids and nucleotides, while Yap1p transcriptionally controls the expression of loci involved in oxidative stress response and drug resistance. As we have shown, the expression of *KlKNQ1* was transiently induced by hydrogen peroxide like that of the *ScATR1* gene [15]. Moreover, the *KlKNQ1* promoter was found to contain two putative Yap1p response elements (YRE) at positions −360 and −395. Therefore, it is highly probable that *Kl*Yap1p plays a role in the oxidative stress response of *KlKNQ1*. In this work, we provide evidence that *KNQ1* is the target gene of *Kl*Yap1p.

## 2. Materials and methods

### 2.1. Strains and media

Table 1 lists the yeast strains used in this study. *Escherichia coli* strain *XL-1 Blue* was used for plasmid amplification and preparation. *K. lactis* strains were maintained on either YEPD (Bactopeptone 1%, yeast extract 1% and glucose 2%) or minimal medium (mixture of inorganic salts, vitamins and 2% of a carbon source) [18]. The nutrients, essential for auxotrophic strains, were added at 40 μg $ml^{-1}$. The media were solidified with a 2% Bactoagar (DIFCO). The liquid yeast cultures were grown on a shaker at 28 °C. *E. coli* strains were cultivated in LB (Luria-Bertani) broth or on LB plates, supplemented with ampicillin (0.1 mg $ml^{-1}$). Resistance to metabolic inhibitors used was tested on solid minimal glucose medium supplemented with the required auxotrophic nutrients. 3-Aminotriazole (Serva) used in this study was dissolved in sterile water. 4-Nitroquinoline-N-oxide (Sigma) was dissolved in acetone.

### 2.2. Drug resistance assays

Drug resistance was determined by spot assay. Yeast strains were grown to the early stationary phase in liquid minimal medium ($5 \times 10^7$ cells $ml^{-1}$) and 10 μl of three independent clones were spotted onto solid minimal medium supplemented with various concentrations of the specific chemical compound being tested. Qualitative growth differences among the transformants were recorded following the incubation of the plates at 28 °C for three up to five days.

### 2.3. Recombinant DNA, plasmids and transformation procedures

Standard techniques were used for generating recombinant DNAs as described by Sambrook et al. [19]. Both plasmid minipreps and large-scale preparations of plasmid DNA from *E. coli* were done by the alkaline-lysis method. *K. lactis*/*E. coli* shuttle vector pRS306K (2 μm *URA3 ARS1 KARS2 ori Amp*$^r$) was used as empty vector for yeast cell transformations. The *KNQ1* and *YAP1* genes were cloned in the YRp14/∇SUP11KARS2 vector [20]. *K. lactis* strains were transformed by electroporation [21] using a Bio-Rad gene pulser at 1.0 kV, 25 μF, 400 Ω in 0.2 cm cuvettes. Plasmid DNA was extracted from yeast cells according to Ward [22]. *E. coli* cells were transformed by electroporation [23]. The $P_{KNQ1}$-*gusA* fusion gene was constructed as follows. A 0.8 kb *Hin*dIII–*Bgl*II fragment (−679 to +102) from plasmid p23 containing the cloned *K. lactis KNQ1* gene [15] was subcloned into pBluescript KS (+) cut with *Hin*dIII–*Bam*HI. The 0.8 kb *Sal*I–*Xba*I fragment from pBluescript KS (+) was integrated into the plasmid KEpKHT3GUS (9.3 kb) to fuse the first 34 amino acids of *K. lactis KNQ1* to the N-terminus of bacterial *gusA* [24,25].

### 2.4. β-Glucuronidase reporter assay

Enzyme activity was measured in crude cell extracts prepared by glass bead disruption. Cells transformed with a $P_{KNQ1}$-*gusA* chimeric construct were grown in 50 ml minimal medium at 30 °C to the late-logarithmic phase. The cells were harvested for 5 min at 3000 rpm at 4 °C. The cell pellet was washed with 20 ml β-glucuronidase (GUS) extraction buffer (10 mM sodium phosphate, pH 7.0, 10 mM $Na_2EDTA$, 10 mM dithiothreitol, 0.1% sodium lauryl sarcosine, 0.1% Triton X-100). The final pellet was resuspended in 500 μl GUS extraction buffer. An equal volume of ice-chilled, acid-washed glass beads (0.45 mm diameter) was added

Table 1
Yeast strains used in this study

| Strain | Genotype | Reference |
|---|---|---|
| *Kluyveromyces lactis MW179-1D* | *MATα lac4-8 uraA1-1 leu2 metA1-1 Ade*$^-$ *trp1* Rag$^+$ (pKD1$^+$) | [10] |
| *Kluyveromyces lactis MW179-1D*/Δ *yap1* | *MATα klyap1::LEU2 lac4-8 uraA1-1 leu2 metA1-1 Ade*$^-$ *trp1* Rag$^+$ (pKD1$^+$) | [10] |
| *Kluyveromyces lactis* JA6 | *MATa ade-600 adeT600 trp1-11 ura3-12* | [17] |