Glucose Signaling Pathway and Growth Conditions Regulate Gene Expression in Retrotransposon Ty2

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Z. Naturforsch. **64c**, 526–532 (2009); received January 30/March 5, 2009

Gene expression in the yeast retrotransposon Ty2 is regulated at transcriptional and translational levels. In this study, we have shown that the transcription of Ty2 is partially dependent on the membrane-bound glucose sensors Gpr1p and Mth1p in *Saccharomyces cerevisiae*. Transcription of Ty2 decreased approx. 3-fold in the *gpr1*, *mth1* yeast mutant. Moreover, our results revealed that the transcription of Ty2 fluctuates during the growth stages of *S. cerevisae*. Both transcription and the frameshift rate of Ty2 rapidly dropped when the stationary stage yeast cells were inoculated into fresh medium. There was an instant activation of Ty2 transcription and a high level expression during the entire logarithmic stage of yeast growth. However, the transcription of Ty2 decreased 2-fold when the yeast cultures entered the stationary stage. The frameshift rate in Ty2 also varied depending on the growth conditions. The highest frameshift level was observed during the mid-logarithmic stage. It decreased up to 2-fold during the stationary stage. Furthermore, we have found that the frameshift rate of Ty2 diminished at least 5-fold in slowly growing yeasts. These results indicate that the transcription and the frameshift efficiency are coordinately regulated in the retrotransposon Ty2 depending on the growth conditions of *S. cerevisiae*.

Key words: Frameshift, Ty Elements, Glucose Sensing

Introduction

Ty elements of the yeast *Saccharomyces cerevisiae* belong to the retrotransposon family of the eukaryotic mobile genetic elements (Cameron *et al.*, 1979). Ty elements transpose via an RNA intermediate using a similar strategy as in vertebrate retroviruses (Boeke *et al.*, 1985). Five different families of Ty elements, Ty1 through Ty5, have been characterized in *S. cerevisiae* cells. Ty1 is the high-copy element from which up to 30 copies per haploid yeast genome can be found. Ty2–Ty5 are low-copy elements that are present as 1–5 copies per haploid yeast genome (Kim *et al.*, 1998). Recently, Ty elements are re-classified based on their genomic organization. In the new system, Ty1, Ty2, Ty4 and Ty5 are classified within the pseudoviridae family of the retrovirales order. Since Ty3 has a different genomic structure, it has been categorized within the metaviridae genus (Capy, 2005).

Gene expression is regulated at transcriptional and translational levels in Ty2. The transcriptional regulatory region of Ty2 is located within the first 1-kbp region in a highly compact fashion, which contains overlapping activator and repressor binding sites (Liao *et al.*, 1987; Farabaugh *et al.*, 1989, 1993; Türkel and Farabaugh, 1993). Translation of Ty mRNAs occurs at the cytoplasm to generate proteins that are essential for the formation of virus-like particles. Ty mRNAs have two overlapping protein-coding regions known as TYA and TYB. These reading frames are analogous to the retroviral gag and pol polypeptides, respectively. The TYB polypeptide is translated by +1 translational frameshifting as fusion protein with TYA (Belcourt and Farabaugh, 1990; Farabaugh, 1996). Post-translational cleavage of TYA generates nucleocapsid proteins that are required for the formation of TY virus-like particles (Ty-VLP). Post-translational proteolytic cleavage of TYB yields three different proteins that show protease (PR), integrase (IN), and reverse transcriptase/RNAse H (RT/RH) activities (Roth, 2000).

Ty elements do not encode any regulatory factors for their gene expressions. Hence the gene expression of Ty elements is totally dependent on the yeast-encoded transcription and translation factors. It is known that the activities of certain transcription and translation factors are regulated upon the growth conditions of *S. cerevisiae* cells (Schneper *et al.*, 2004; De Virgilio and Loewith, 2006). Previously, we showed that the transcription of Ty2 is activated by high levels of glucose (Türkel and Arik, 2007). In the present study, the effects of Gpr1p and Mth1p, two membrane-bound sensors that are necessary for glucose signalling, on the Ty2 transcription have been analyzed. In addition, the effects of different growth conditions on the transcription and translational frameshift rate in pseudovirus Ty2 have been investigated in the yeast *S. cerevisiae*.

Material and Methods

Yeast strains and plasmids

The *S. cerevisiae* strains used in this research were: BY4741 (*MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0*), W303-1A (*MATa, ura3-1, his3-11,15, leu2-3, 112, trp1-1, ade 2-1, can1-100*), CJM479 (*MATa, ura3-1, his3-11,15, leu2-3, 112, trp1-1, ade 2-1, can1-100, gpr1::kanMX4, mth1::TRP1*). *S. cerevisiae* strain BY4741 was purchased from EUROSCARF (University of Frankfurt, Germany) and used in our research as a standard *S. cerevisae* strain. The yeast strains CJM479 and W303-1A are isogenic except for the *gpr1* and *mth1* mutations (Belinchon and Gancedo, 2007).

The plasmids YEp917-555 (Ty2-555) and YEp917-754 (Ty2-754) are 2-μM-*URA3*-based shuttle vectors containing a fusion of the first 555- or 754-bp region of Ty2 element to the *E. coli* lacZ gene, respectively (Farabaugh *et al.*, 1993; Türkel and Farabaugh, 1993). The plasmid carrying the Gpd1-lacZ fusion gene is also a 2-μM-*URA3*-based expression vector (Rep *et al.*, 1999). It was used as a control fusion gene in this research. Ty2 frameshift and Ty2 frame fusion reporter plasmids are 2-μM-*URA3*-based shuttle vectors (Clare *et al.*, 1988; Belcourt and Farabaugh, 1990). In Ty2 frameshift plasmids, the Ty2 frameshift site is fused to the *E. coli* lacZ gene in the +1 reading frame. Therefore, translation of the TYA-lacZ fusion protein in this expression vector depends on the efficient frameshift event in the +1 direction at the frameshift site of Ty2. Ty2 frame fusion construct does not contain the frameshift site. In this expression vector, translation of the TYA-lacZ fusion protein does not require a frameshift and takes place at zero frames (Clare *et al.*, 1988; Belcourt and Farabaugh, 1990). Plasmids were transformed into the competent yeast cells as described previously using the lithium acetate method (Ito *et al.*, 1983). It is known that these expression vectors can be stably maintained and their copy numbers do not drastically alter in various yeast transformants under selective growth conditions (Liao *et al.*, 1987; Farabaugh *et al.*, 1993).

Growth conditions

S. cerevisiae cells were cultivated in YPAD (1% yeast extract, 2% Bacto peptone, 40 mg/L adenine sulfate, 2% glucose) medium for transformation. To determine the effects of glucose sensors Gpr1p and Mth1p, plasmids carrying the Ty2-555-lacZ, Ty2-754-lacZ, and Gpd1-lacZ gene fusions were transformed into W303-1A and CJM479 strains of *S. cerevisiae*. To test the effects of different growth stages on the Ty2 transcription and frameshift efficiency, Ty2-754-lacZ, frameshift and frame fusion constructs were transformed into strain BY4741 of *S. cerevisiae*. The yeast transformants were plated on synthetic complete dextrose medium without uracil (Sc-Ura, +2% glucose) (Rose *et al.*, 1990). 9–12 transformant colonies were randomly selected for each plasmid and patched on Sc-Ura dextrose plates to use in liquid culture inoculations.

Transformants of *S. cerevisiae* strains W303-1A and CJM479 were grown in Sc-Ura medium supplemented with 2% glucose (w/v) to the logarithmic stage as described, and then harvested for β-galactosidase assays (Türkel and Farabaugh, 1993). To determine the effects of different growth stages on the Ty2 transcription and frameshift efficiency, yeast transformants were grown in 20 mL of Sc-Ura dextrose liquid medium at 30 ºC in an incubator shaker (130 rev/min) for 24 h to obtain saturated pre-cultures. After that, yeast transformants were inoculated into 250 mL of fresh Sc-Ura dextrose medium from saturated pre-cultures. Initial cell densities of the cultures were adjusted to OD_{600} = 0.2 to 0.25. Yeast cultures were incubated at 30 ºC in a shaker, and samples were removed at the time intervals given in Fig. 1 and 2 for β-galactosidase assays. OD_{600} values of each yeast sample were determined to prepare the growth curves.

In order to test the effects of different carbon sources on the frameshift of Ty2, yeast transformants were cultivated in Sc-Ura (10 mL) media supplemented with different carbon sources as shown in Table II. For nutritional upshift experiments, first yeast transformants were grown in liquid Sc-Ura (10 mL) media supplemented with 2% glycerol and 2% sodium lactate up to the logarithmic stage. 4 h prior to harvest, aliquots of cultures (5 mL each) were shifted to Sc-Ura medium supplemented with 2% glucose.

In order to determine the growth rates of the yeast transformants, yeast cells were first precultured up to the stationary stage in Sc-Ura (10 mL) media supplemented with one of the carbon sources shown in Table II. Then, the yeast transformants were inoculated into 50 mL of fresh Sc-Ura medium containing different carbon sources. Initial cell densities of the yeast cultures were adjusted to A_{600} = 0.2 to 0.25. Yeast cultures were incubated at 30 °C in an incubator shaker, and samples were taken every 2 h. Duplication times of the yeast strains were calculated from the growth curves that were obtained by plotting the A_{600} values of the samples versus time.

Enzyme assays

Yeast cells were harvested at the given times, washed with 1 mL of sterile distilled water, and then re-suspended in 200 μL of yeast cell breaking buffer. Yeast transformants were permeabilized with 20 μL of 0.1% SDS and 20 μL of chloroform (Guarente, 1983). β-Galactosidase assays were done in triplicate and units are given in nmol of ONPG (2-Nitrophenyl β-D-galactopyranoside) cleaved per min per mg of protein in permeabilized yeast cells. Protein concentrations in the permeabilized cell lysates were determined by the Lowry assay as described (Lowry *et al.*, 1951). Frameshift rates were calculated as the percentage of the ratio of β-galactosidase activities expressed from the Ty2 frameshift vector to the β-galactosidase activities expressed from the frame fusion vector. Yeast cultures were cultivated in triplicate. All experiments are repeated at least once under the same growth conditions. The numbers (β-galactosidase units) given in the figures and tables are the mean values of at least 15–18 independent β-galactosidase assays. Standard deviations in these assays were less than 15%. It was previously shown that the β-galactosidase levels expressed from Ty2-lacZ or Gpd1-lacZ gene fusions are correlated to the Ty2 and Gpd1 mRNA levels (Farabaugh *et al.*, 1993; Rep *et al.*, 1999).

Results

Effects of the glucose sensors Gpr1p and Mth1p on the transcription of Ty2

Transcription of Ty2 is activated by high levels of glucose (Türkel and Arik, 2007). Therefore, we wanted to investigate if the glucose-controlled transcriptional activation of Ty2 is also dependent on the membrane-bound sensor proteins Gpr1p and Mth1p. Ty2-lacZ gene fusions were transformed into the wild-type and the isogenic *gpr1*, *mth1* double mutant yeast strains. As shown in Table I, transcription from Ty2-555 gene fusion yielded 808 units of β-galactosidase activity in the wild-type yeast. However, transcription of Ty2-555 gene fusion in the *gpr1*, *mth1* double mutant yeast strain significantly decreased and yielded an approx. 3-fold lower (296 units) β-galactosidase activity (Table I). Since Ty2-754 gene fusion contains the negative regulatory region of Ty2, its transcription is always lower than that of the Ty2-555 gene fusion in wild-type yeast cells (Farabaugh *et al.*, 1989, 1993). Transcription from Ty2-754 gene fusion yielded 320 units of β-galactosidase activity in the wild-type yeast cells. In addition to the drop of the transcription of Ty2-555, Ty2-754 transcription also decreased in the *gpr1*, *mth1* double mutant, resulting in 190 units of β-galactosidase activity (Table I). The transcription of control gene fusion Gpd1-lacZ produced high levels of activity in the wild-type yeast as expected. However, its transcription increased approx. by 50% in the *gpr1*, *mth1* double mutant. It is already known that the transcription of the *GPD1* gene is regulated by glucose

Table I. Effects of Gpr1p and Mth1p on the Ty2 transcription (± standard deviation).

Gene fusion	β-Galactosidase activity[a]	
	Wild-type strain[b]	*gpr1*, *mth1* mutant
Ty2-555-lacZ	808 ± 48	296 ± 41
Ty2-754-lacZ	320 ± 37	190 ± 8
Gpd1-lacZ	1023 ± 79	1520 ± 62

[a] β-Galactosidase activities are expressed in nmol of ONPG cleaved per min per mg of protein in permeabilized yeast cells.

[b] Wild-type and *gpr1*, *mth1* double mutant strains are W303-1A and CJM479, respectively.

repression (Albertyn *et al.*, 1994). Thus, it appears that the decrease in glucose signaling due to *gpr1*, *mth1* mutations in the double mutant yeast strain leads to a partial derepression of the *GPD1* transcription.

Effects of different growth stages on the Ty2 transcription

In order to examine whether gene expression in Ty elements is affected by the growth stages, we have analyzed the transcript levels of Ty2-754-lacZ gene fusion throughout the entire growth stages of the yeast cells. We have used only Ty2-754-lacZ gene fusion in this assay since it contains all of the regulatory sites required for the regulated expression of retrotransposon Ty2 (Farabaugh *et al.*, 1993). When the yeast transformants were inoculated into fresh medium from the stationary stage pre-cultures, a sudden drop was observed in the transcription of Ty2. Later, Ty2 transcription remained at low levels (20–30 units) during the first 4 h of growth which correspond to the lag phase of the yeast cultures (Fig. 1). After the lag stage, transcription of Ty2 increased steadily during the log stage and reached to the maximal level at the end of the log phase. However, it is clear that the magnitude of the increase in transcription declined after the first 12 h of growth (Fig. 1). While there was a rapid increase in transcription during the mid-logarithmic stage (*e.g.* from 10–12 h after inoculation), which corresponds to approx. 150 units/h, it took 12 h (from 12 to 24 h after inoculation) to obtain the same level of increase in the transcription of Ty2. Once the yeast cultures reached the maximal level of cell density under our growth conditions, transcription of Ty2 also reached its highest level and then began to decrease gradually. It remained at low levels throughout the post-diauxic and stationary stage of the yeast cultures (Fig. 1). These results suggest that there is a transcriptional regulation for pseudovirus Ty2 expression which is driven by a growth stage-dependent mechanism.

Effects of different growth stages on the frameshift efficiency

Frameshift rates of Ty2 were determined in different growth stages of the *S. cerevisiae* cells to see if there are any similarities to the transcription pattern. When the yeast transformants were transferred to fresh medium, the frameshift rate decreased slightly (from 25% to 21%) and remained at that level for a short period of time (Fig. 2). The frameshift rate started to increase at the lag stage of the yeast cultures and continued to increase with approx. the same rate throughout the logarithmic stage. However, unlike the transcription pattern of Ty2, the frameshift rate

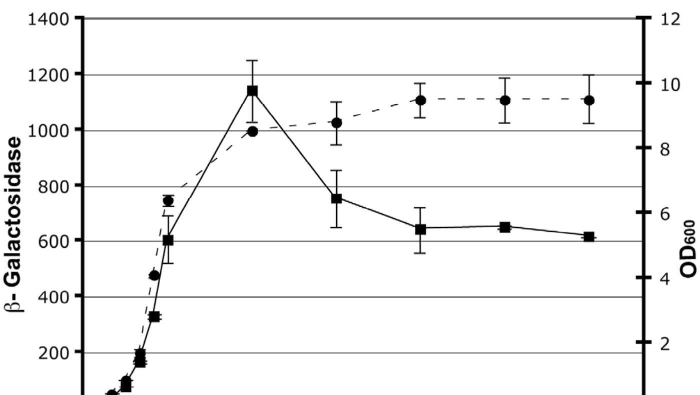

Fig. 1. Transcription of Ty2 at different growth stages of *S. cerevisiae*. Yeast transformants were grown in the selective medium at 30 °C in an incubator shaker. Samples were removed at the indicated times and OD_{600} values of the yeast samples were determined spectrophotometrically. The vertical bars represent the standard deviations. Closed circles indicate the growth curve; closed rectangles indicate the level of Ty2 transcription in the yeast transformants.

reached its highest level (39%) 8 h after inoculation. The frameshift rate declined slowly after the mid log stage and reached its lowest level (15%) at the stationary stage of the yeast cultures. There was a 2.5-fold decrease in the frameshift rates of the Ty elements when the highest and the lowest levels of frameshift rates were compared (Fig. 2). There were clear differences as well as similarities between the levels of transcription and frameshift with respect to the different growth stages of *S. cerevisiae.* Transcription of Ty2 increased up to the late logarithmic stage of the yeast cultures. In contrast to the transcription pattern, the frameshift rate did not increase after the mid logarithmic stage. In fact, it began to decrease after the mid logarithmic stage of the yeast cultures (Figs. 1 and 2). These results propose that the frameshift rate also occurs at optimum levels, if the cells are dividing at the highest rate.

Effects of the growth rate on the frameshift efficiency

In order to obtain yeast cultures that have different growth rates, yeast transformants were cultivated in a Sc-Ura medium containing different carbon sources. The frameshift rate of Ty2 in glucose- or sucrose-grown yeast cells was determined as 27% and 24%, respectively. However, the growth of yeast cells in non-fermentable carbon source containing media such as glycerol/lactate resulted in an approx. 4-fold decrease (6%) in the frameshift rate of Ty2 (Table II). Accordingly, shifting of the yeast cells from the glycerol/lactate medium to high level glucose medium led to a more than 3-fold increase (from 6% to 21%) in the frameshift rate of Ty2. Duplication times of the yeast cells in glucose- or sucrose-containing medium were calculated as 120 min under our growth conditions. However, duplication times of the yeast cells in glycerol/lactate medium increased to 480 min. These results indicate that high levels of fermentable carbon sources like glucose or sucrose, that lead to faster growth and shorter duplication times, also increase the frameshift rate of yeast retrotransposon Ty2 (Table II).

Table II. Frameshift efficiency in the pseudovirus Ty2 changes depending on the growth rates of *S. cerevisiae* (± standard deviation).

Carbon source	Td[a] [min]	Frameshift rate (%)
2% Glucose	120 ± 10	27 ± 4
2% Sucrose	120 ± 10	24 ± 2
2% Glycerol/lactate	480 ± 10	6 ± 1
Gly/lact to 2% glucose	120 ± 10	21 ± 3

[a] Duplication time.

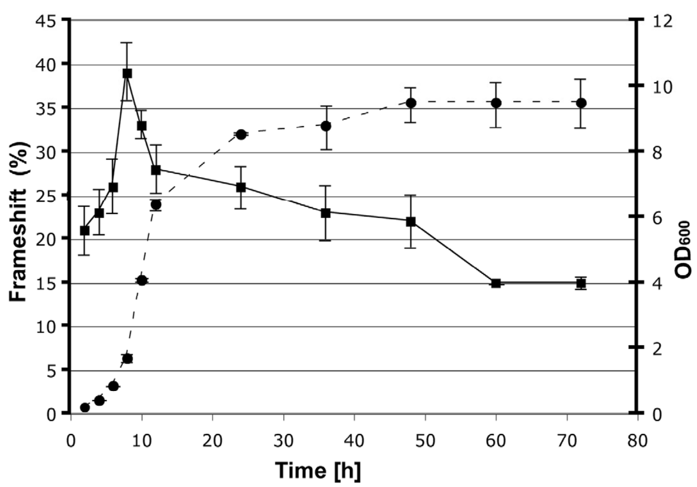

Fig. 2. Frameshift rates of Ty2 at different growth stages of *S. cerevisiae*. Yeast transformants were grown in selective medium at 30 °C in an incubator shaker. Samples were removed at the indicated times and OD_{600} values of the yeast samples were determined spectrophotometrically. Standard deviations are indicated as vertical bars. Closed circles indicate the growth curve; closed rectangles indicate Ty2 frameshift levels in the yeast transformants.

Discussion

Growth rate and growth stage have a large impact on the gene expression in the yeast *S. cerevisiae*. Specific sets of genes are either repressed or activated depending on the growth patterns of the yeast cells (Schneper *et al.*, 2004; De Virgilio and Loewith, 2006). Ty elements cannot transpose intercellularly. Therefore, being obligatory genetic entities within the *S. cerevisiae* cells, Ty elements are also subjected to physiological changes resulted from different growth rates and growth stages. Gene expression in the pseudovirus Ty2 elements is controlled at transcriptional and translational levels.

Transcription of Ty2 largely depends on the transcription factor Gcr1p complex and is activated by a high glucose level (Türkel *et al.*, 1997; Türkel and Arik, 2007). Results presented in the present study connect the glucose induction of Ty2 transcription to one of the membrane-bound glucose sensor proteins Gpr1p. Gpr1p is involved in the activation of cAMP-dependent protein kinase A in response to glucose (Gancedo, 2008). Mth1p interacts with glucose sensors and transmits the glucose signals to cytoplasmic complexes. Hence, lack of Gpr1p and Mth1p leads to a decrease in glucose signaling. We think that glucose signaling activates the Gcr1p complex, which in turn activates the Ty2 transcription. It is known that the Gcr1p complex is a phosphoprotein (Zeng *et al.*, 1997). Its transcription is autoregulated in response to glucose and participates to nutrient-responsive gene expression (Sasaki *et al.*, 2005; Barbara *et al.*, 2007).

Our results revealed that both transcription and frameshift in the Ty2 element is regulated in a growth stage-dependent manner. There is a direct positive correlation between the increased transcription of Ty elements and formation of Ty virus-like particles. This was already shown by activating Ty transcription with a GAL4-dependent strong promoter in yeast cells (Boeke *et al.*, 1985; Garfinkel *et al.*, 1985). We think that the growth stage and growth rate-dependent regulation of Gcr1p activities may lead to the growth stage-dependent regulation of Ty2 transcription. Regenberg *et al.* (2006) found in their microarray analysis that the transcription of *GCR1* is regulated in a growth rate-dependent manner.

Translation of Ty mRNA, which gives the structural and enzymatic parts of the Ty virus-like particles, is an important stage in the Ty elements' life cycles. Translation of TYB, which is synthesized as fusion protein with TYA, depends on programmed frameshifting at the +1 direction. Our results indicate that the frameshift rate in Ty2 mRNA also changes in a growth stage- and growth rate-dependent manner. Previously Stahl *et al.* (2004) reported that the translational accuracy and frameshift rate of Ty2 and human immune deficiency virus type-1 (HIV-1) alter at different points of the growth stages of *S. cerevisiae*. Our results are also in agreement with these data. Frameshift events take place during the elongation stage of translation. It is known that the phosphorylation of translation elongation factors alter the ribosomal fidelity in the elongation process (Farabaugh and Vimaladithan, 1998). It was shown that the transcriptions of elongation factor genes are also regulated in a growth rate-dependent manner (Regenberg *et al.*, 2006). Thus, it is conceivable that the growth stage- and growth rate-dependent modifications of the elongation factors result in variations in the frameshift rate of Ty2 in different growth stages.

Previously we have shown that the transcription of Ty2 is activated by high levels of glucose (Türkel and Arik, 2007). Results of the present study indicate that the glucose-dependent activation of Ty2 transcription is exerted through the membrane-bound glucose sensors Gpr1p and Mth1p. Moreover, our results clearly demonstrate that the transcription and frameshift rate are collinear in pseudovirus Ty2, and they are tightly regulated depending on the growth conditions of *S. cerevisiae*.

Acknowledgements

We thank Dr. P. J. Farabaugh (University of Maryland, Baltimore, MD, USA) for kindly providing Ty2 frameshift and frame fusion plasmids and Dr. J. M. Gancedo (CSIC, UAM, Madrid, Spain) for providing the yeast strain CJM479. This work was supported by research grants from The Scientific and Technological Research Council of Turkey (TUBİTAK, Project no. 104T307) and from the Uludag University research fund (Project no. 2004-39).

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