ABSTRACT

Msn2p and its homolog Msn4p in yeast Saccharomyces cerevisiae are transcription factors which regulate the genes in response to general stresses including heat to protect cell damage. They bind specifically to stress-response elements (STREs) and activate the transcription of its target genes. In this work, we have focused on the role of Msn4p in heat stress of natural thermotolerant yeast strain. Based on DNA microarray data of S. cerevisiae, we searched for genes that are regulated by Msn4p under high temperature growth by computational analysis using YEASTRACT. Among 303 up-regulated genes (≥2-folds), 56 genes contained STREs at their upstream promoter. Investigation of yeast disruptants in those 56 genes revealed that 17 disruptants showed high temperature sensitive growth phenotype. By real-time PCR analysis, the change in expression level along growth phase at high temperature of Msn4 was not observed whereas expression of UBI4 significantly increased in both natural thermotolerant and laboratory strains. In addition, the expression level of NTH1, known as Msn4p-regulated gene, also elevated. These observations suggest that higher expression of UBI4 at early-log phase could support the heat resistance phenotype in both of laboratory and thermotolerant strains.

Keywords: Msn4, heat stress, thermotolerant yeast, gene expression, real-time PCR
INTRODUCTION

During ethanol fermentation process, yeast cells are exposed to several types of stresses such as increase in temperature, osmotic pressure and ethanol concentration. Therefore, yeast cells have to reprogram their gene expression appropriately in order to maintain their metabolic activity to survive in stress conditions. High temperature fermentation by thermotolerant yeast can reduce cooling cost and the risk of contamination by performing the fermentation under high temperature condition, and can be applied for SSF (simultaneous saccharification fermentation).

In S. cerevisiae, heat response is governed by the transcription factors Hsf1p, Msn2p and Msn4p (Trott and Morano, 2003). Msn2/Msn4p are related to each other and activated in stress conditions, which results in translocation from the cytoplasm to the nucleus. Msn2/Msn4p binds to the stress-response elements (STREs), 5'-CCCCT-3', which are located in the promoter region of their target genes (Martinez-Pastor et al., 1996). MSN4 expression is itself Msn2/Msn4p dependent and induced by heat and other stresses, while MSN2 expression is constitutive (Gasch et al., 2000, Causton et al., 2001).

C3723-8B, natural thermotolerant strain (Choowong et al., 2012) was used as a model to investigate the special mechanisms that allow it to grow at high temperature. We have found the genes CDC19 and RSP5 encoding pyruvate kinase and ubiquitin ligase, respectively, which support the Htg 
phenotype of C3723 thermotolerant strain (Suthee et al, 2012 and Hosein et al, 2011). To search for genes that are responsible for growth at high temperature, we employed DNA microarray approach. This research aims to elucidate the expression of MSN4 and genes under its regulation (UBI4 and NTH1) in thermotolerant C3723-8B and thermosensitive BY4743 strains in different growth phase through the comparison of their expression between 30°C and higher temperatures of 41 °C (C3723-8B) and 40°C (BY4743).

MATERIALS AND METHODS

A. Growth Condition

S. cerevisiae strains were cultured in nutrient rich broth (Yeast Extract Peptone Dextrose, YPD) at 30 °C and 41 °C or 40 °C with shaking at 180 rpm for 18 – 24 h. Samples were taken for OD660 measurement and collected for total RNA extraction at appropriate growth phases.

B. DNA Microarray Data Analysis

DNA microarray data was analyzed using SGD, GOTermMAPP and YEASTRACT (Dário et al, 2011) for the biological process and for identifying transcriptional regulators of up-regulated genes.

C. Screening for Heat Responsive Genes under Regulation of Msn4p

S. cerevisiae BY4742 were cultured in YPD broth for 24 h and 2.5 µl of 10-fold serial dilution were dropped on YPD agar, and incubated for 3 days at 30, 37, 39 and 40 °C.

D. Gene Expression Analysis by Quantitative Real-time PCR

Total RNA was extracted by hot acidic phenol method. A 1 µg of total RNA was purified and converted to cDNA by using QuantiTect® Reverse Transcription Kit (QIAGEN®, Germany). The expression level of genes was examined by using SYBR® Premix Ex Taq™ (TliRNaseH Plus) (Takara, Japan) in 7300 Real-Time PCR System (Applied Biosystem, USA). A standard curve was determined with cDNA from mid-log phase of S. cerevisiae at 30°C. ACT1 was used as an endogenous control and data was calculated by relative standard curve method.
RESULTS AND DISCUSSION

A. DNA microarray analysis of S. cerevisiae BY4742 at 40°C and identification of genes involved in heat response

The DNA microarray data of S. cerevisiae BY4742 grown at 40 °C revealed that out of 5,868 genes, expression level of 303 genes were up-regulated by more than 2 folds under high temperature as compared with that at 30°C. Among 303 up-regulated genes, the computational analysis using YEASTRACT showed that 71 genes are under Msn4p regulation. By investigation of those 71 genes, only 56 genes contain STREs at their upstream promoter. This result suggested that Msn4p was directly or indirectly involved in their transcriptional regulation supported by the previous studies which showed that Msn2/4p could regulate the gene expression of GRE1 even it does not contain any STREs. As in this case the researcher observed the PDS sequences at its upstream promoter which similar to STREs binding site so it could be recognized as STREs (Garay-Arroyo and Covarrubias, 1999). Screening of thermosensitive phenotype using S. cerevisiae BY4742 in single 56 genes disruption revealed that 17 disruptants exhibited thermosensitive phenotype at least equal to S. cerevisiae BY4742. Among those, UBI4 was subjected to gene expression analysis since it was reported to be involved in stress response and it is the representative of protein turn-over process. UBI4 encodes polyubiquitin which function as molecular specific chaperone in response to heat stress (Parsell and Lindquist, 1993) NTH1 was used as positive control since it is known as Msn4p-regulated gene.

B. Gene expression in S. cerevisiae C3723-8B and BY4743

Gene expression of MSN4 and two genes under its regulation, UBI4 and NTH1, were examined at early-, mid- and late-log phases. The results revealed that the expression of MSN4 at high temperature in natural isolated thermotolerant C3723-8B (41 °C) and diploid laboratory BY4743 (40 °C) strains displayed no significant change (≤2-folds) when compared to its expression at 30°C (data not shown) (Fig. 1a). This suggested that the activation of MSN4 was not regulated at transcriptional level as in the case of its homologue gene, MSN2 which activated by hyperphosphorylation resulting in translocation from cytoplasm into nucleus (Garreau et al, 2000). For NTH1, expression was not significantly different between high temperature and 30°C (≤2-folds). However, the expression in C3723-8B under high temperature growth was insignificantly higher than that in BY4743 strain (Fig. 1b). This gene encodes neutral trehalase which is required for trehalose degradation (Kopp et al, 1993). Interestingly, the expression level of UBI4 in both C3723-8B and BY4743 significantly increased (≥2-folds) when cells were exposed to high temperature (Fig. 1c) and it seems to be higher in C3723-8B strain than BY4743. UBI4 is known as a stress response gene which is involved in ubiquitination and it is known as stress-inducible gene (Finley et al, 1987). The previous studies investigated that Ubi4p affected to free ubiquitin when yeast cells were exposed to heat stress. This protein is required for protein degradation and turnover (Berry et al, 2011).

C. Phenotype of ubi4 disruptant in response to heat stress

Quantitative real-time PCR analysis showed significant change in UBI4 expression upon exposure to high temperature stress. Therefore, we analyzed growth behavior of ubi4 disruptant (Fig. 2). ubi4 disruptant showed high temperature sensitive growth phenotype as compared with parental strain BY4742. This suggests that Ubi4p plays important roles in high temperature resistance in BY4742 genetic background. In contrast to the observation in previous report, Δubi4 disruptant showed no different growth in comparison to wild type strain when treated with heat shock at 40 °C (Berry et al, 2011).
Figure 1 Expression of genes under Msn4p regulation in response to high temperature in \emph{S. cerevisiae} C3723-8B and BY4743 strains. Cells were grown in YPD broth for 2 h (early-log phase), 5 h (mid-log phase) and 8 h (late-log phase) at indicated temperature. Transcription level of \emph{MSN4} (a), \emph{NTH1} (b) and \emph{UBI4} (c) were normalized by \emph{ACT1} transcription levels. Each bars show average value with standard deviation of three replication for two independent experiments.

Figure 2 Growth of \emph{S. cerevisiae} BY4742 \textDelta ubi4 disruptant and its parent strain. Growth was monitored in nutrient-rich broth at 40 °C.

CONCLUSION

Our results showed that the expression of \emph{MSN4} did not significantly change in thermotolerant strain. The slightly increased expression of \emph{NTH1} and higher expression of \emph{UBI4} in thermotolerant strain might correlate with its heat resistance phenotype in \emph{S. cerevisiae}. The biological function related to heat resistance of \emph{UBI4} should be further elucidated.

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