The Role of the MAPK Pathways in Response to Ethanol Stress in *Saccharomyces cerevisiae*

Nisarut Udom*, Choowong Auesukaree.
Department of Biology, Faculty of Science, Mahidol University, 10400
**Corresponding Author:* sccar@mahidol.ac.th

Abstract

During fermentation process, yeast cells are often exposed to ethanol. Although the toxicity of ethanol to budding yeast *Saccharomyces cerevisiae* has widely been studied, the ethanol stress signaling is still unknown. The growth of the mutants lacking genes involved in the mitogen activated protein kinase (MAPK) pathways was examined under ethanol stress condition. Our results revealed that the *STE3*, and *AKR1* genes involved in the pheromone signaling pathway, and the *BCK1, MPK1, SWI4*, *SWI6*, and *MBP1* genes involved in the cell wall integrity pathway, were required for tolerance to ethanol. In addition to the mutants of the cell wall integrity pathway, the *Δste3* and *Δakr1* mutants were sensitive to a cell wall perturbing agent, calcoflour white, suggesting the role of these signaling molecules in maintaining cell wall during ethanol stress.

Keywords: ethanol, MAPK pathway, cell wall integrity pathway, pheromone signaling pathway, *Saccharomyces cerevisiae*

1. Introduction

The yeast *Saccharomyces cerevisiae* has been widely used in alcoholic fermentation and in fuel ethanol production. During fermentation, yeast cells are usually exposed to several environmental changes such as high ethanol concentration, high osmolarity, and oxidative stress [1]. Among these, the high ethanol concentration is a major stress that affects vitality and viability of yeast cells [4]. To deal with several types of damage caused by increased ethanol concentration, yeast cells should have developed appropriate mechanisms for adaptation. Cells constantly respond to their external environment, leading to intracellular changes for cell survival [3]. A number of signaling pathways responsible for such transmission have been identified and characterized. One of the most important signaling pathways that yeast cells use to transduce the signals of environmental stresses is the mitogen activated protein kinase (MAPK) pathways [6]. The budding yeast *Saccharomyces cerevisiae* contains at least four MAPK cascades that respond to different physiological stimuli: the cell-wall integrity pathway, the filamentous/invasive growth pathway, the pheromone response pathway, and the high osmolarity glycerol pathway. The key control responsible for MAPK signaling is composed of three sequentially activating kinases; a MAPK kinase kinase (MAPKKK) phosphorylates and activates a MAPK kinase (MAPKK), which then activates a MAPK [3] (Fig. 1). The understanding of the adaptation process during high ethanol concentration is important for development of ethanol tolerant yeast strain that may produce higher ethanol yield. In this study, we investigated the growth of the yeast deletion mutants lacking genes encoding components of the MAPK pathways under ethanol stress condition. To investigate the role of the MAPK pathways during cell wall stress, we examined the growth of these deletion mutants in YPD media containing calcofluor white, a cell wall perturbing agent.

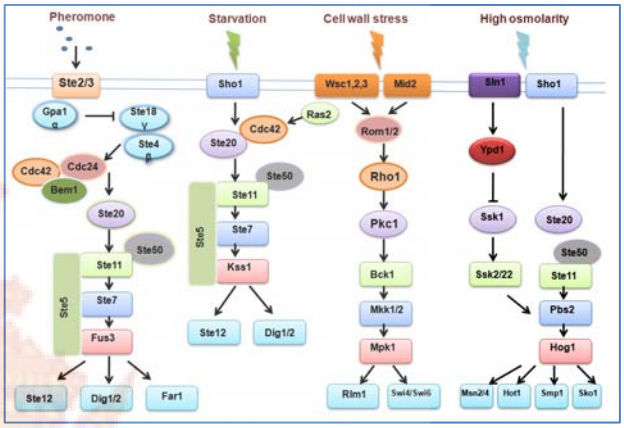

Figure 1. The MAPK cascades of *Saccharomyces cerevisiae*. At least four pathways, which are important for stress signaling and cell adaptation for survival, were identified.

2. Materials and Methods

Yeast strains and growth conditions

S. cerevisiae BY4742 wild-type strain and its isogenic mutants lacking genes encoding components of the MAPK pathways were used in this study. YPD medium (1% yeast extract, 2% Bacto peptone, 2% glucose) was used as a rich medium. Routinely, cells were grown overnight in liquid media with shaking at 200 rpm at 30°C for 12 hours.

Growth of the mutants lacking genes encoding components of the MAPK pathways during ethanol and calcofluor white stresses

The effect of ethanol and cell wall stresses on cell growth was monitored by spot

susceptibility test. To investigate the growth of the mutants lacking genes encoding components of the MAPK pathways under ethanol and cell wall stresses, the *S. cerevisiae* wild-type strain (BY4742) and its isogenic deletion mutants were precultivated in YPD liquid media with shaking at 200 rpm at 30°C for 12 hours. The overnight culture was diluted to an initial $O.D._{600}$ of 1.0 with sterile water and cells were then serially diluted 10-fold. Aliquots (3 µl) were spotted onto YPD agar plate and YPD agar plate containing 12% ethanol or 100 µg/ml calcofluor white. Growth was monitored after incubation at 30°C for 2 days.

3. Results

The growth of the deletion mutants lacking genes encoding components of the MAPK pathways under ethanol stress condition was examined by spot susceptibility test. We found that the mutants lacking *STE3* gene encoding a transmembrane receptor for yeast pheromone and *AKR1* gene encoding a negative regulator of the pheromone signaling pathway, were sensitive to 12% ethanol (Fig.2). However, the other deletion mutants lacking downstream components of the pheromone signaling pathway were not sensitive to ethanol (data not shown). These results suggested that only upstream components of the pheromone signaling pathway, namely, Ste3p and Akr1p, were required for ethanol tolerance. Furthermore, our results revealed that the mutants lacking conponents of the cell wall integrity pathway, i.e. the *Δbck1* mutant lacking MAPKKK, the *Δmpk1* mutant lacking MAPK, the *Δswi4, Δswi6 and Δmbp1* mutants lacking the transcription cofactors of the cell wall integrity pathway, were sensitive to 12% ethanol stress (Fig. 2). These results suggested that the cell wall integrity pathway is important for sensing cell wall stress caused by ethanol for recovery from cell wall damage. Since a pheromone-activated signaling pathway is known to contribute to mediate many of mating responses including the remodeling of cell wall [2] and the mutants lacking sensors of the cell wall integrity pathway did not show significant growth defect (data not shown), it is possible that ethanol stress may be sensed by Ste3p and the signal is then transduced to activate the cell wall integrity pathway.

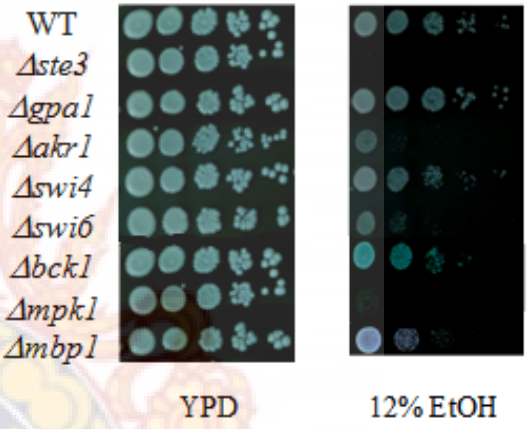

Figure 2. Growth of the mutants lacking genes encoding components of the MAPK pathways on YPD agar plate containing 12% ethanol.

To investigate the role of Ste3p and Akr1p in regulating cell wall remodeling during ethanol stress, we examined the growth of the *Δste3* and *Δakr1* mutants and the mutants of the cell wall integrity pathway in the presence of calcofluor white, a cell wall perturbing agent. Our result revealed that, in addition to the mutants lacking components of the cell wall integrity pathway, the *Δste3* and *Δakr1* mutants of the pheromone signaling pathway were sensitive to calcofluor white, suggesting the role of these signaling molecules in maintaining cell

wall structure during ethanol stress (Fig. 3). Additionally, these findings further suggested the cross-talk between the pheromone signaling pathway and the cell wall integrity pathway during ethanol stress. The interaction between the different MAPK pathways has been recently reported. For instance, the cross-talk between the cell wall integrity pathway and the high osmolarity glycerol pathway was found to be important for stabilizing cell during hypertonic condition [5].

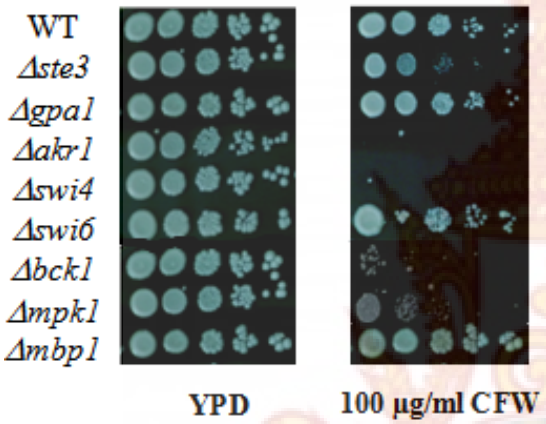

Figure 3. Growth of the mutants of genes encoding components of MAPK pathways on YPD agar plate containing 100 µg/ml calcofluor white.

4. Conclusions

In conclusion, our results revealed that the *STE3* and *AKR1* genes involved in the pheromone signaling pathway, and the *BCK1*, *MPK1*, *SWI4*, *SWI6*, and *MBP1* genes involved in the cell wall integrity pathway, were required for tolerance to ethanol. In addition to the mutants of the cell wall integrity pathway, the *Δste3* and *Δakr1* mutants were sensitive to a cell wall perturbing agent, calcoflour white, suggesting the role of these signaling molecules in maintaining cell wall during ethanol stress.

5. Acknowledgements

This work was supported by the grant from the Center for Environmental Health, Toxicology and Management of Chemicals (ETM) and Mahidol University.

6. References

[1]. Auesukaree, C., Damnernsawad, A., Kruatrachue, M., Pokethitiyook, P., Boonchird, C., Kaneko, Y., and Harashima, S. (2009). Genome-wide identification of genes involved in tolerance to various environmental stresses in *Saccharomyces cerevisiae*. *J Appl Genet*, 50: 301-310.

[2]. Bardwell, L. (2005). A walk-through of the yeast mating pheromone response pathway. *Peptides*, 26: 339-350.

[3]. Chen, R. E., and Thorner, J. (2007). Function and regulation in MAPK signaling pathways: lessons learned from the yeast *Saccharomyces cerevisiae*. *Biochim Biophys Acta*, 1773: 1311-1340.

[4]. Fujita, K., Matsuyama, A., Kobayashi, Y., and Iwahashi, H. (2006). The genome-wide screening of yeast deletion mutants to identify the genes required for tolerance to ethanol and other alcohols. *FEMS Yeast Res*, 6: 744-750.

[5]. Garcia, R., Rodriguez-Pena, J. M., Bermejo, C., Nombela, C., and Arroyo, J. (2009). The high osmotic response and cell wall integrity pathways cooperate to regulate transcriptional responses to zymolyase-induced cell wall stress in *Saccharomyces cerevisiae*. *J Biol Chem*, 284: 10901-10911.

[6]. Levin, D. E. (2005). Cell wall integrity signaling in *Saccharomyces*

cerevisiae. *Microbiol Mol Biol Rev*, 69: 262-291.