CHAPTER I
INTRODUCTION

At present, global consumption of fossil fuel such as petroleum and gasoline are increasing, leading to an increase of greenhouse gas emission, which causes several environmental problems including global climate change. Thus, in order to decrease the fossil fuel consumption and the environmental problems, the demand for environmental friendly-energy such as ethanol is increasing (Hill et al., 2006).

The yeast S. cerevisiae has been widely used in fermentation process for the production of alcoholic beverages and ethanol fuel. This process is the conversion of sugar, usually glucose, into two carbon dioxide gas (CO_2) and ethanol under the condition without oxygen. During fermentation, yeast cells are usually exposed to several environmental changes such as high ethanol concentration, high osmolarity, and oxidative stress (Auesukaree et al., 2009). Among these, the high ethanol concentration is a major stress that affects vitality and viability of yeast cells (Fujita et al., 2006). Recently, many genes involved in cell wall compositions and cell wall biosynthesis have been found to be required for ethanol tolerance, suggesting the role of cell wall during ethanol stress (Auesukaree et al., 2009; Fujita et al., 2006; Ma and Liu, 2010; Takahashi et al., 2001; Teixeira et al., 2009). Furthermore, cell wall remodeling has been demonstrated to play an important role in protecting yeast cells against ethanol stress (Teixeira et al., 2009).

Cell wall is an important organelle for maintaining cell integrity. They are found in various organisms such as plants, bacteria, algae, and fungi including budding yeast (Popolo et al., 1997). Yeast cell wall is composed of four major components, i.e. β-1,3-glucan, β-1,6-glucan, chitin, and mannoproteins (Klis, 1994; Lesage and Bussey, 2006; Smits et al., 1999). When yeast cells are exposed to cell wall stress caused by cell wall perturbing agents, such as calcofluor white and congo red, or cell wall degrading enzyme, several changes in cell wall structure is induced. These include (1) increasing β-glucan and chitin contents, (2) changing the

relationship between the polysaccharides in the cell wall, (3) increasing the cell wall proteins, and (4) re-localizing the important proteins from the cell wall construction to the lateral cell wall (Arroyo et al., 2009).

Furthermore, several genes encoding signaling components of the pathway, one of the mitogen-activated protein kinase (MAPK) pathway, has been found to be essential for ethanol tolerance (Auesukaree et al., 2009; Fujita et al., 2006; Ma and Liu, 2010; Takahashi et al., 2001). These include MID2 and WSC1 encoding sensors of the cell wall integrity pathway, ROM2 encoding GDP/GTP exchange protein (GEP) for Rho1p and Rho2p, BCK1 encoding MAPK kinase kinase (MAPKKK), and MPK1 encoding MAPK. In addition, STE3 encoding receptor for a factor pheromone and AKR1 encoding negative regulator involved in pheromone signaling pathway, the another MAPK pathway, have also been reported to be required for ethanol tolerance (Auesukaree et al., 2009; Fujita et al., 2006). It is therefore possible that not only the cell wall integrity pathway but also the other MAPK pathways are involved in signal transduction in response to ethanol stress.

The MAPK pathway is one of the important signaling pathways that yeast cells use to transduce the signals of environmental stresses (Chen and Thorner, 2007; Gustin et al., 1998; Levin, 2005). 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 (Gustin et al., 1998; Qi and Elion, 2005; Saito and Tatebayashi, 2004; Widmann et al., 1999). The budding yeast S. 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 signaling pathway, and the high-osmolarity glycerol (HOG) pathway (Gustin et al., 1998; Qi and Elion, 2005). The cell wall integrity pathway plays an essential role in response to cell wall damages caused by cell wall perturbing agents, cell wall degrading enzyme, and heat shock (de Nobel et al., 2000; Gray et al., 1997; Harrison et al., 2004). The filamentation-invasion pathway mediates a filamentous growth during nutrient starvation (Gimeno and Fink, 1994). The pheromone signaling pathway transduces a pheromone signal of opposite mating type to mediate several mating responses (Bardwell, 2004; Buehrer and Errede, 1997; Elion, 2000; Rensing and Ruoff, 2009;

Schwartz and Madhani, 2004). The high-osmolarity glycerol (HOG) pathway mediates signal transduction in response to osmotic stress, leading to a production of glycerol functioning as an osmolyte (Gustin et al., 1998; Hohmann, 2002; O'Rourke et al., 2002; Westfall et al., 2004).

The activation of the cell wall integrity pathway is mediated through the plasma membrane sensors (Mid2p, Wsc1p, Wsc2p, and Wsc3p) (Philip and Levin, 2001; Verna et al., 1997), which then transduce a signal to activate the guanine nucleotide exchange factor (GEF) Rom2p. The activated Rom2p trigger the activation of the small GTPase Rho1p, which in turn activates the protein kinase C Pkc1p (Bickle et al., 1998; Harrison et al., 2004). The activated Pkc1p phosphorylates MAPKKK Bck1p, which in turn phosphorylates a redundant MAPKK Mkk1p and Mkk2p, leading to the phosphorylation of MAPK Mpk1p (Chen and Thorner, 2007; de Nobel et al., 2000; Gustin et al., 1998; Levin, 2005). The activated MAPK Mpk1p activates transcription factors Rlm1p and SBF (Swi4p-Swi6p cell cycle box binding factors) complex to promote the expression of cell wall-related genes and cell cycle-regulating genes at the G1/S phase, respectively (de Nobel et al., 2000; Gray et al., 1997; Kim et al., 2008).

Recently, the cross-talk between the cell wall integrity pathway and the HOG pathway in response to zymolyase-mediated cell wall stress has been suggested (Bermejo et al., 2008; Fuchs and Mylonakis, 2009; Garcia et al., 2009). The HOG pathway, which is normally used to transduce a signal of hyperosmotic stress (Gustin et al., 1998; Hohmann et al., 2007), contains two signaling branches, Sln1p and Sho1p branches, that are activated by different mechanisms. The transmembrane Sln1p that functions as an osmosensor forms a complex with Ypd1p and Ssk1p. Under hyperosmotic stress condition, Sln1p is inhibited and Ypd1p and Ssk1p are in dephosphorylated forms, leading to the activation of the redundant MAPKKK Ssk2p and Ssk22p. These activated MAPKKKs phosphorelate MAPKK Pbs2p, which then phosphorylates MAPK Hog1p. On the Sho1p branch, Ste20p activates MAPKKK Ste11p, which in turn phosphorylates MAPKK Pbs2p to phosphorylate Hog1p (Gustin et al., 1998; Hohmann, 2002; Hohmann et al., 2007; O'Rourke et al., 2002; Posas and Saito, 1997; Saito and Tatebayashi, 2004). The activation of the HOG pathway leads to the production of glycerol as a compatible solute to increase the total intracellular

concentration, resulting in osmotic stabilization (Hohmann et al., 2007; O'Rourke et al., 2002; Westfall et al., 2004). Recently, in addition to its role in maintaining osmotic balance, the Sho1p branch of the HOG pathway has been shown to be involved in cross-signaling with the cell wall integrity pathway in response to a cell wall degrading enzyme, β-1,3 glucanase (Fuchs and Mylonakis, 2009; Garcia et al., 2009). Therefore, not only a linear activation of the cell wall integrity pathway but also a lateral interaction with the HOG pathway is essential for recovery of damaged cell wall (Harrison 2004). However, it is still unknown whether the cross-talk between these MAPK cascades is required during ethanol stress.

In this study, to determine the role of the MAPK pathways during ethanol stress, we investigate the growth of the yeast deletion mutants lacking genes encoding components of the MAPK pathways under ethanol stress condition. We next investigate cell wall remodeling during ethanol stress by using β-1,3 glucanase sensitivity test to monitor the cell wall alterations. We perform real-time quantitative PCR to examine the expression of cell wall-related genes during ethanol stress. To investigate the role of cross-talk between the cell wall integrity pathway and the HOG pathway during ethanol stress, we examine the effect of hyperosmolarity on cell growth and cell wall remodeling during ethanol stress. The understanding of the adaptation process during ethanol stress should be helpful for development of ethanol tolerant yeast strain producing higher ethanol yield.