CHARPTER III
LITERATURE REVIEW

3.1 Eukaryotic model: Saccharomyces cerevisiae

S. cerevisiae or budding yeast is a simple unicellular organism commonly known as baker's yeast or brewer's yeast. This budding yeast has been used for baking and brewing since ancient time. S. cerevisiae belongs to

Kingdom: Fungi

Phylum: Ascomycota

Subphylum: Saccharomycotina

Class: Saccharomycetes

Order: Saccharomycetales

Family: Saccharomycetaceae

Genus: Saccharomyces

Species: S. cerevisiae

S. cerevisiae is an ideal eukaryotic model for molecular genetics research because it shares common basic cellular activities, such as replication, recombination, cell division, and metabolism, with those of higher eukaryotes, including mammalian cells (Barnett, 2003). In addition, the other advantages of using yeast in research include rapid growth rate, easy and inexpensive cultivation, versatile DNA transformation system, and diverse techniques for molecular genetic analysis (Barnett, 2003). S. cerevisiae was the first eukaryote whose genome has been completely sequenced and found to be composed of approximately 13,000,000 base pairs and 6,275 genes (Garrels, 2002). Budding yeast can exist in a haploid state or a diploid state but generally proliferates in a diploid state (Bardwell, 2004). Haploid yeast cells exist in one of two mating types, a or α, (genotypes MATa and MATα, respectively), which exhibit the different cell surface receptors (Ste2p and Ste3p) for pheromone response (Bardwell, 2004). Haploid cells can mate with the opposite mating to form diploid

cells (Fig. 3.1). To undergo this mating process, MATα cells secrete α-factor pheromone and exhibit a-factor receptor required for response to α-factor whereas MATa cells secrete a-factor pheromone and exhibit α-factor receptor required for response to a-factor. This response to pheromone finally leads to an induction of a series of physiological changes in preparation for mating (Bardwell, 2004; Elion, 2000). Although the diploid cells can undergo a simple lifecycle of mitosis and growth, they initiate sporulation under starvation or stressful conditions, resulting in a production of four haploid spores (Gustin et al., 1998). Interestingly, cells in the diploid phase have been shown to be more resistant to extreme environmental conditions than haploid cells (Anderson et al., 2004; Herskowitz, 1988).

Yeast is a "facultative anaerobe" that is able to survive and grow under conditions with and without oxygen. Under aerobic condition, yeast cells convert energy from nutrients such as sugar into ATP using oxygen as an oxidizing agent. Whereas, under anaerobic condition, yeast cells undergo fermentation that convert glucose to ethanol and carbon dioxide to generate energy for cellular metabolism (Jeffries, 2005; Piskur et al., 2006).

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + H_2O \text{ ---------- > } \underset{\text{(Carbon dioxide gas)}}{2CO_2} + \underset{\text{(Ethanol)}}{2C_2H_5OH}$$

Therefore, the yeast fermentation process has been widely used for the production of alcoholic beverages and bioethanol. Furthermore, yeast is able to utilize and ferment a wide range of sugar such as sucrose, glucose, galactose, maltose, and maltotriose ((D'Amore et al., 1988). Although ethanol is a toxic organic compound that reduce growth rate of organism, yeast exhibits relatively higher tolerance to ethanol (up to 20% (v/v)) than other microorganisms (D'Amore et al., 1988). However, damages in yeast cells and reduced fermentation activity have been observed when ethanol concentration was higher than 10% (Alexandre et al., 2001; Dinh et al., 2008; Ma & Liu, 2010).

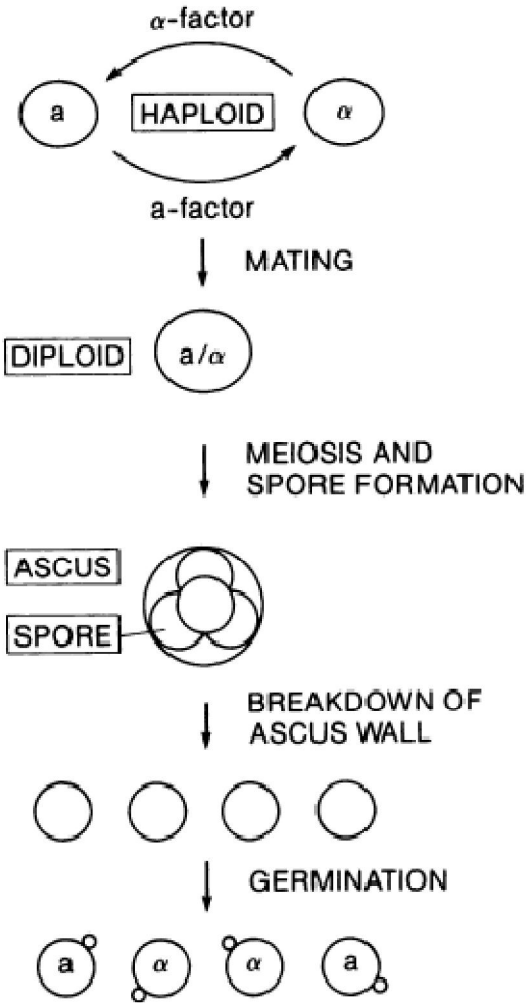

Figure 3.1. Life cycle of S. cerevisiae. There are three cell types of S. cerevisiae (a, α, and a/α). This diagram shows the mating of haploids yields a diploid, and meiosis of a diploid yields haploid cells. One a/α cell produces the four haploid spores (Herskowitz, 1988).

3.2 The effects of ethanol on S. cerevisiae

At present, global consumption of fossil fuel such as petroleum and gasoline are increasing, leading to increased greenhouse gas emission that is a major cause of global warming and climate change. A large interest on eco-friendly energy such as ethanol is rising with an aim to reduce fossil fuel consumption (Hill et al., 2006). Currently, ethanol is a major alternative fuel which provides about 1.8% of the global consumption of transport fuel (Bringezu et al., 2009). One of the several means of increasing ethanol production is an improving fermentation efficiency of yeast to produce higher ethanol yield.

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). The high ethanol concentration affects several cellular metabolisms, leading to reductions of cell vitality and viability. These include disturbance of several transport systems such as those for glucose, maltose, ammonium, and amino acid, causing cell leakage of nucleotides, amino acids, and potassium (Piper, 1995; Salmon, 1989), inhibition of the crucial glycolytic enzymes such as pyruvate kinase and hexokinase (Pascual et al., 1988), induction of the reactive oxygen species (ROS) production (Du & Takagi, 2007; Landolfo et al., 2008), damages in mitochondrial DNA (Costa et al., 1997), changes in plasma membrane fluidity (Ingram, 1976; Ma & Liu, 2010; Quintas et al., 2000; You et al., 2003), and activation of plasma membrane H^+-ATPase (Rosa & Sa-Correia, 1991) (Figure 3.2).

Plasma membrane seems to be one of the main target organelle for ethanol because this compound is possible to intrude into the hydrophobic interior of the membrane lipid bilayer. This intrusion causes severe effects on lipid organization, bilayer stability, membrane permeability, and lipid composition (Weber and de Bont, 1996), resulting in an increased fluidity of plasma membrane and loss of membrane integrity (D'Amore et al., 1988; You et al., 2003). In addition, increase of membrane fluidity caused by ethanol has led to increased passive proton influx, resulting in a loss of proton motive force important for maintaining a gradient concentration across membrane (Sikkema et al., 1995; Meyrial et al., 1997; Quintas et al., 2000).

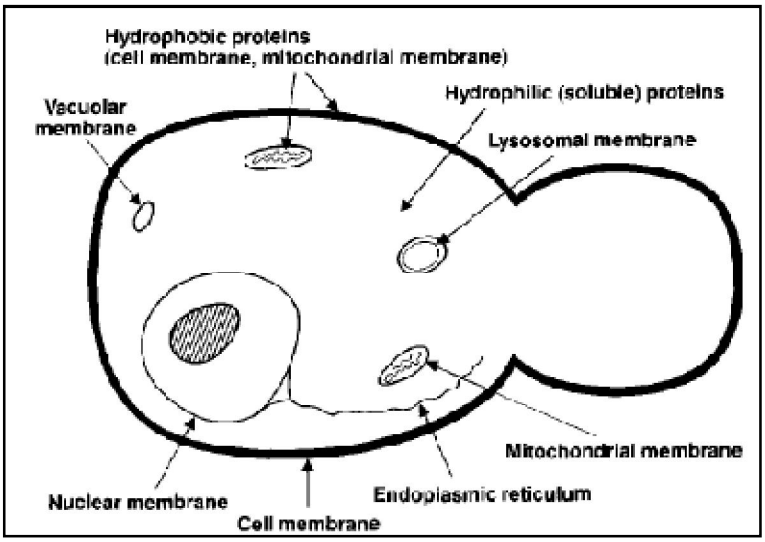

Figure 3.2 The possible target sites of ethanol in yeast cells (D'Amore et al., 1990).

3.3 The cell wall construction

Cell wall is an important organelle for maintaining cell integrity, which are found in several species such as plants, bacteria, algae, and fungi including budding yeast (Popolo et al., 1997). Cell wall is a dynamic structure that acts as the strongest mechanical component for cell protection. Thus this organelle is thought to be a key defense to encounter various stresses. The yeast cell wall has four major functions as follows:

1. Stabilization of internal osmolarity and regulation of water influx, which protect cell swollen.
2. Offering efficient protection against mechanical damages caused by physical stresses.
3. Maintaining cell shape during cell morphogenesis process.
4. Functioning as a scaffold for external glycoproteins, such as glycosylphosphatidylinositol (GPI) proteins and PIR (protein with internal repeats) proteins (Klis et al., 2006), which are important for controlling the permeability of the cell wall.

Cell wall is composed of four major components, i.e. β-1,3-glucan, β-1,6-glucan, chitin, and mannoproteins, which are interconnected to form two layers (Cid et al., 1995; Klis, 1994; Lesage & Bussey, 2006; Smits et al., 1999). β-1,3-glucan and mannoproteins are the major components and β-1,6-glucan and chitin are the minor components (Table 3.1). The inner layer contains approximately 80-90% of β-1,3-glucan chains with a small amount of β-1,6-glucan branches. β-1,6-glucan acts as a backbone for linkage with the other components of cell wall by covalent bond, leading to microfibrils formation (Bowman & Free, 2006; Cabib et al., 2001; Firon et al., 2004; Klis et al., 2006). β-1,3 glucan is also covalently linked to chitin to make up the inner cell wall, producing the main rigidity of cell wall (Inoue et al., 1996; Utsugi et al., 2002). Although only a small amount of chitins are contained in the cell wall, this component plays an important role in maintaining the integrity of cell wall during stress. Chitin content has been found to be increased reach up to 20% during cell wall stress (Cid et al., 1995; Popolo et al., 1997). In addition, a defect in chitin synthesis resulted in disordered and dysfunctional cell wall (Bowman & Free, 2006). Furthermore, the increased cross-linking of proteins to a β-1,6-glucan-chitin complex

serves as a rescue mechanism to compensate for a reduction of β-1,3-glucan content (Kapteyn et al., 1997). On the other hand, the outer layer of cell wall contains two classes of glycoprotein, i.e. glycosylphosphatidylinositol (GPI) proteins and PIR proteins (Klis et al., 2006; Lesage & Bussey, 2006). GPI-dependent cell wall proteins (GPI-CWPs) are linked to β-1,3 glucan through a β-1,6 glucan chain wherease PIR proteins (PIR-CWPs) are directly linked to β-1,3 glucan (Klis et al., 2006; Lesage & Bussey, 2006). This outer layer is important for the protection of the inner layer and cell-cell recognition during sexual reproduction of yeast cell (Cabib et al., 2001; Cid et al., 1995; Klis et al., 2006; Lesage & Bussey, 2006; Levin, 2005; Lipke & Ovalle, 1998; Popolo et al., 2001) (Fig. 3.3)

β-1,3 glucan is a major component of the cell wall whose synthesis and assembly are required for protection against cell lysis. Glucan synthase (GS) is a protein complex involved in β-1,3 glucan synthesis. GS is composed of two homologous catalytic subunits, Fks1p and Fks2p, and a regulatory subunit, small GTPase Rho1p (Klis et al., 2006; Lesage & Bussey, 2006; Lipke & Ovalle, 1998). The components of GS are localized on the plasma membrane at the site of cell wall remodeling (Qadota et al., 1996). In addition to GS, β-1,3-glucanosyltransferase encoded by GAS1 and chitin synthase encoded by CHS1 and CHS2 are also involved in cell wall remodeling (Popolo & Vai, 1999). The deletion of FKS1 and GAS1 genes resulted in reduced levels of β-1,3 glucan components but increase of chitin content and expression of the alternative subunit of glucan synthase, Fks2p (Adams, 2004; de Nobel et al., 2000; Lesage & Bussey, 2006). The mechanisms of cell wall remodeling in response to cell wall stress are as follows (Arroyo et al., 2009);

1. Increasing β-glucan and chitin contents to generate rigid wall.
2. Changing the relationship between the polysaccharides in cell wall.
3. Increasing expression of cell wall genes.
4. Re-localizing the important cell wall proteins to the lateral cell wall.

During ethanol stress, yeast cell wall composition and structure are supposed to be important factors contributing cell protection. Many genes involved in cell wall composition and biosynthesis have been found to be required for ethanol tolerance such as KRE6 encoding a β-glucan synthase required for β-1,6-glucan biosynthesis, and WSC1 encoding a sensor-transducer for cell wall stress, and MPK1

encoding a MAPK protein of the cell wall integrity (CWI) pathway (Teixeira et al., 2009). It is therefore possible that ethanol induces cell wall remodeling through an activation of the CWI pathway. However, the characterization of this activation during ethanol stress has not been clarified.

Table 3.1 Major components of S. cerevisiae cell walls (Klis et al., 2006).

Macromolecule	% of wall mass
Mannoproteins	30-50
β-1,3 glucan	30-45
β-1,6 glucan	5-10
chitin	1.5-6

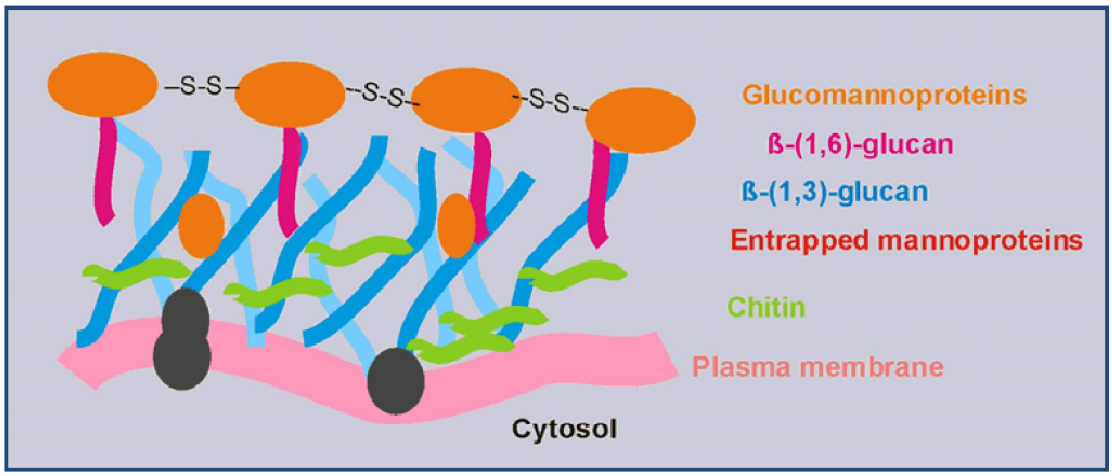

Figure 3.3 The components of cell wall and the relationship between cell wall components in S. cerevisiae (Klis et al., 2006).

3.4 Mechanisms of ethanol tolerance in S. cerevisiae

To deal with damages caused by increased ethanol concentration, yeast cells should have developed appropriate mechanisms for adaptation to ethanol stress (Querol et al., 2003; Zhao & Bai, 2009). During ethanol stress, the expression of genes involved in wide range of functional categories of cell has been found to be increased. These include genes involved in protein biosynthesis, amino acid metabolism, nucleotide metabolism, transport, cell cycle and growth, lipid metabolism, fatty acid and ergosterol metabolism, membrane and cell wall organization, proline biosynthesis, and tryptophan biosynthesis (Stanley et al., 2010) (Table 3.2). In addition, several genes involved in cell cycle and DNA processing, protein fate, cellular transport mechanisms, and transcription have also been found to be required for ethanol tolerance (Auesukaree et al., 2009; Fujita et al., 2006) (Table 3.3).

It has been suggested that ethanol may intrude into the plasma membrane, resulting in increased membrane fluidity (D'Amore et al., 1990; Ma & Liu, 2010). To cope with this effect, it has been shown that, in response to high ethanol concentration, yeast cells altered the compositions of their plasma membranes to maintain membrane fluidity (D'Amore et al., 1990; You et al., 2003). The level of unsaturated fatty acids (UFAs), such as palmitoleic acid (9z-C16:1) and oleic acid (9z -C18:1), and ergosterol, increased in response to high ethanol concentration (Daum et al., 1998; Ingram, 1976; Swan & Watson, 1998; You et al., 2003). In addition, an alteration in cell wall compositions has also been suggested to be another important mechanism for protecting yeast cells against ethanol stress (Ogawa et al., 2000). Several genes involved in cell wall compositions and cell wall biosynthesis have been found to be required for tolerance to ethanol stress (Table 3.3) (Auesukaree et al., 2009; Fujita et al., 2006). Furthermore, it has been reported that ethanol stress induced cell wall remodeling process, possibly leading to increased resistance to ethanol stress (Teixeira et al., 2009). It is therefore likely that this process may also play an important role in ethanol tolerance in yeast cells.

Moreover, the addition of isoleucine, methionine, phenylalanine, and inositol, has been found to result in ethanol tolerance (Hu et al., 2005; Kelley et al., 1988; Takagi et al., 2005). This may be due to the activity of these amino acids and sugar in enhancing the stability of plasma membrane to antagonize the increased

membrane fluidity (Ding et al., 2009; Ma & Liu, 2010). Furthermore, the expression of several genes encoding heat shock proteins such as HSP12, HSP26, HSP30, HSP42, HSP78, HSP82, and HSP104, were strongly induced under ethanol stress condition (Ma & Liu, 2010; Piper, 1995; Stanley et al., 2010), suggesting the role of heat shock proteins (HSPs) in ethanol tolerance. HSPs is a group of chaperone proteins functioning in protecting and refolding structural proteins during a variety of stresses including ethanol stress (Piper, 1995; Swan & Watson, 1998; Vianna et al., 2008). In addition to HSP genes, the expression of genes involved in trehalose metabolism has been found to be induced during ethanol stress to prevent the denaturation and aggregation of misfolded proteins (Alexandre et al., 2001; Ma & Liu, 2010; Singer & Lindquist, 1998).

Furthermore, many genes encoding the components of mitogen-activated protein kinase (MAPK) signaling pathway, such as AKR1, BCK1, MID2, MPK1, ROM2, STE3, and WSC1, have been found to be required for ethanol tolerance (Auesukaree et al., 2009; Fujita et al., 2006; Ma & Liu, 2010; Takahashi et al., 2001; Teixeira et al., 2009). It is therefore possible that yeast may transduce the signal of ethanol stress through the MAPK pathways.

Table 3.2 Genes required for ethanol tolerance whose expression has been reported to be induced during ethanol stress (Stanley et al., 2010).

Gene	Description
HSP12, 26, 30, 42, 78, 82, 104	Heat shock proteins (HSP)
CTT1	Cytosolic catalase T, has a role in protection from oxidative damage
DDR2	Multi-stress response protein
SSA4	Member of the HSP70 family
YRO2	Putative protein of unknown function
TDH1	Glyceraldehyde-3-phosphate dehydrogenase
TSL1	Large subunit of trehalose 6-phosphate synthase
TPS1	Synthase subunit of trehalose-6-phosphate synthase
ALD4	Mitochondrial aldehyde dehydrogenase
GLK1	Glucokinase, catalyses the phosphorylation of glucose
YGP1	Cell wall-related secretory glycoprotein
HOR7	Protein of unknown function; induced under hyperosmotic stress
PYC1	Pyruvate carboxylase isoform
DAK1	Dihydroxyacetone kinase, required for detoxification of dihydroxyacetone (DHA); involved in stress adaptation
YER053C, YDR516C YBR139W	Products have unknown function
HXK1	Hexokinase isoenzyme 1, a cytosolic protein that catalyses phosphorylation of glucose during glucose metabolism
PGK1	3-phosphoglycerate kinase, enzyme in glycolysis and gluconeogenesis
SPI1	GPI-anchored cell wall protein involved in weak acid resistance
CYC7	Cytochrome c isoform 2, expressed under hypoxic conditions

Table 3.3 Classification of genes whose deletion confer ethanol sensitivity (Fujita et al., 2006).

Cell cycle and DNA processing (20)	*ANC1, ARD1, BFR1, BIK1, BNI1, BUB1, CNM67, CTF4, ELM1, GRR1, HEX3, HPR1, HTL1, POL32, RAD27, RSC2, SHP1, SHS1, UME6, VID21*
Protein fate (20)	*ALG6, DOA4, GIM4, GIM5, LHS1, MFT1, NAT1, NAT3, PAC10, PFD1, PPM1, PRE9, RAD6, TOM37, UMP1, VPS36, VPS41, YME1, YND1, YTA7*
Cellular transport mechanisms (17)	*AKR1, APN1, ATP15, BRO1, CLC1, FEN2, FPS1, GTR1, ISA2, LUV1, SHE4, SNF7, SNF8, STP22, TRS33, VPS20, VPS28*
Transcription (16)	*CAF16, CST6, CTK3, DHH1, ELP2, ELP6, IKI3, KCS1, PAF1, PAT1, RPB9, SNT309, SRB2, SWI3, TSR2, YAP3*
Biogenesis of cellular components (15)	*BEM1, BEM4, BUD27, CWH36, FZO1, HOC1, MID2, NUP120, NUP133, RMD7, SAC6, SMI1, SSD1, TPM1, YIL090W*
Vacuolar function (14)	*MEH1, TRP1, VAC14, VMA1, VMA2, VMA3, VMA4, VMA6, VMA8, VMA10, VMA12, VMA13, VMA16, VMA21*
Metabolism (6)	*CDS1, CSG2, ERG28, IDP1, TCO89, TRP4*
Signal transduction (5)	*ARG82, BCK1, FAB1, RAS2, SLT2*
Protein synthesis (3)	*ASC1, RPL13B, RPS6A*
Cell rescue, defense, and virulence (3)	*KTI12, SLG1, SOD2*
Unknown function (18)	*YBL006C, YDR008C, YDR149C, YDR433W, YEL044W, YGR196C, YHR167W, YKL037W, YKL118W, YLR315W, YLR322W, YLR331C, YLR368W, YML095C-A, YMR003W, YNL080C, YNL133C, YOR258W*

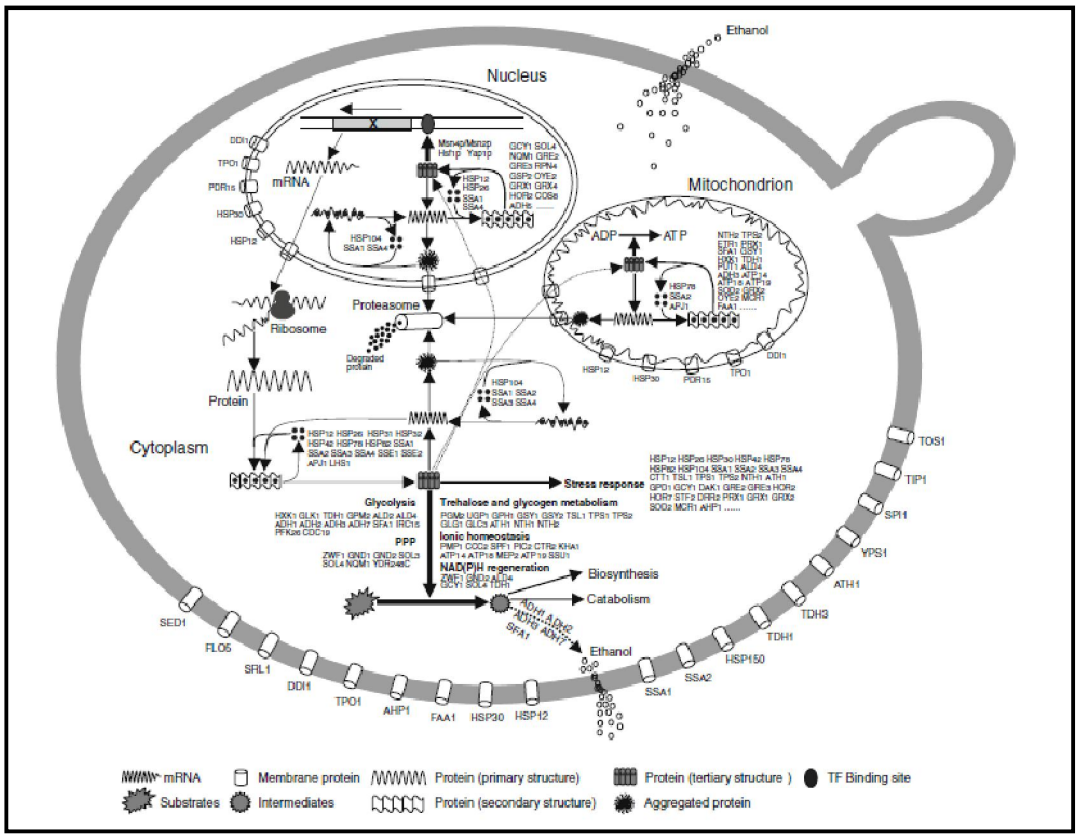

Figure 3.4 Mechanisms of ethanol tolerance in S. cerevisiae (Ma & Liu, 2010) .

3.5 Mitogen-activated protein kinase (MAPK) signaling pathways

Eukaryotic cells, from yeast to mammalian cells, have developed appropriate mechanisms to encounter the harmful effects of environmental stresses. Cell constantly responds to changes in its environment, leading to intracellular changes for cell survival (Chen & Thorner, 2007). 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 MAPK pathways (Chen & 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 & Elion, 2005; Saito & 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 (CWI) pathway, the filamentous/invasive growth pathway, the pheromone signaling pathway, and the high osmolarity glycerol (HOG) pathway (Gustin et al., 1998; Qi & Elion, 2005) (Fig. 3.5).

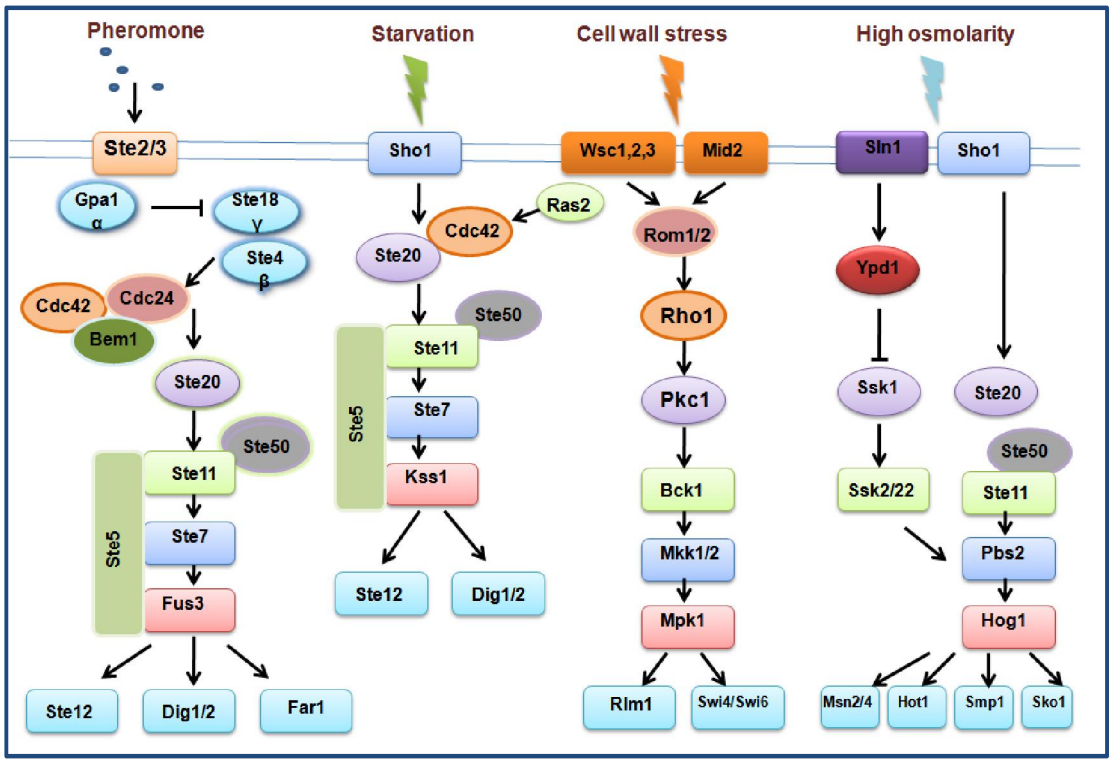

Figure 3.5 The MAPK cascades of S. cerevisiae. At least four pathways, which are important for stress signaling and cell adaptation for survival, were identified.

3.5.1 The pheromone signaling pathway

The pheromone signaling pathway is essential for mediating mating response (Bardwell, 2004; Chen & Thorner, 2007; Gustin et al., 1998). The budding yeast cells exist as either haploid, which have two mating types, a and α (MATa and MATα), or diploid cells (MATa/MATα). Haploid cells of the opposite mating type (a or α) can mate to form a diploid cell. This process is initiated by the release of different pheromones, which bind to specific cell surface receptors of the opposite mating-type cell. The a-factor secreted by a-cell binds to Ste3p receptor on the α-cell, whereas the α-factor released from α-cell binds to Ste2p receptor on the a-cell (Bardwell, 2004; Chen & Thorner, 2007; Gustin et al., 1998). The pheromone signals are transduced through the pheromone signaling pathway from cell surface to nucleus to mediate several mating responses (Bardwell, 2004; Chen & Thorner, 2007; Gustin et al., 1998). The association of pheromone with cell surface receptor activates a heterotrimeric G protein, leading to the dissociation of G protein α subunit (Gpa1p) from the G protein βγ subunits (Ste4p and Ste18p, respectively). Free Gβγ trigger the activation of a downstream signaling cascade through the guanine nucleotide exchange factor Cdc24p, the protein kinase Ste20p, and the kinase scaffold protein Ste5p, which in turn activate a MAPK cascade comprised of Ste11p (a MAPKKK), Ste7p (a MAPKK), and Fus3p (a MAPK) through a multistep phosphorylay. Finally, the phosphorylated Fus3p activates the transcription factor Ste12p to induce expression of mating-related genes such as AFR1, AGA1, ASG7, CHS1, FUS1, and FUS2. In addition, the phosphorylated Fus3p also induced the association of Far1p with Cdc28p, the cyclin dependent kinase, leading to cell cycle arrest at G1 phase (Bardwell, 2004; Buehrer & Errede, 1997; Elion, 2000; Rensing & Ruoff, 2009; Schwartz & Madhani, 2004).

3.5.2 Cell wall integrity (CWI) pathway

Cell walls are found in several organisms such as plants, bacteria, algae, and fungi including budding yeast (Lesage & Bussey, 2006). Cell wall, an external envelope organelle, is essential for cell shape determination, prevention of cell lysis, and protection against various stresses (Popolo et al., 1997). The CWI pathway plays an important role in maintaining the integrity of cell wall in response to various cell wall damages (de Nobel et al., 2000; Harrison et al., 2004). When yeast cells are exposed to several cell wall stresses caused by cell wall perturbing agents or cell wall degrading enzyme, yeast cells remodel this structure in order to protect the cells from cell wall damages (de Nobel et al., 2000; Gray et al., 1997; Harrison et al., 2004). The cell wall stress is sensed by the plasma membrane sensor proteins composed of Mid2p, Wsc1p, Wsc2p, and Wsc3p (Philip & Levin, 2001; Verna et al., 1997), which then transduce a signal to activate the guanine nucleotide exchange factor (GEF) Rom2p. The activated Rom2p stimulates the GTP loading of the small GTPase Rho1p (Bickle et al., 1998; Harrison et al., 2004), leading to the interaction with protein kinase C Pkc1p. The activated Pkc1p triggers a phosphorylay of MAPK module, in which MAKKK Bck1p phosphorylates a redundant MAPKK Mkk1p and Mkk2p, thereby leading to the phosphorylation of the MAPK Mpk1p (Chen & Thorner, 2007; de Nobel et al., 2000; Gustin et al., 1998; Levin, 2005). The activated MAPK Mpk1p activates transcription factors Rlm1p and SBF complex composed of Swi4p and Swi6p, which consequently promote expression of their target genes (Gray et al., 1997; Kim et al., 2008). The Rlm1p regulates the expression of genes involved in cell wall biogenesis (Jung et al., 2002), whereas SBF complex controls the expression of cell cycle-regulating genes at the G1/S phase involved in budding and cell-wall biosynthesis (de Nobel et al., 2000; Iyer et al., 2001; Levin, 2005).

3.5.3 High-osmolarity glycerol (HOG) pathway

To adapt to hyperosmotic conditions, the yeast transduces a signal via the high-osmolarity glycerol (HOG) pathway to maintain osmotic equilibrium in the cell (Gustin et al., 1998; Hohmann, 2002). This pathway contains two independent branches, Sln1 and Sho1 branches, that activated by different mechanism. The Sln1 branch is controlled by the osmosensor Sln1p, which form a membrane-bound protein complex and phosphorelay system with Ypd1p and Ssk1p. Under isotonic conditions, this osmosensor is active and inactivates the other components of downstream cascade. When cells are exposed to hyperosmotic stress, Sln1p is inhibited, thereby allowing the unphosphorylated Ypd1p and Ssk1p to phosphorylate the redundant MAPKKK Ssk2p and Ssk22p. These activated MAPKKK phosphorelate MAPKK Pbs2p, which in turn phosphorylates MAPK Hog1p. On the Sho1p branch, Ste20p activates MAPKKK Ste11p, which then phosphorylates MAPKK Pbs2p, thereby resulting in the phosphorylation of MAPK Hog1p (Gustin et al., 1998; Hohmann, 2002; Hohmann et al., 2007; O'Rourke et al., 2002; Posas & Saito, 1997; Saito & Tatebayashi, 2004). Hog1p induces the expression of several genes such as GPD1 encoding glycerol-3-phosphate dehydrogenase, and GPP1 and GPP2 encoding glycerol-3-phosphatase (Hohmann, 2002, 2009). (Fig. 3.6) The increased expression of these genes leads to the increased production of glycerol functioning as a compatible solute, resulting in osmotic stabilization (Hohmann et al., 2007; O'Rourke et al., 2002; Westfall et al., 2004).

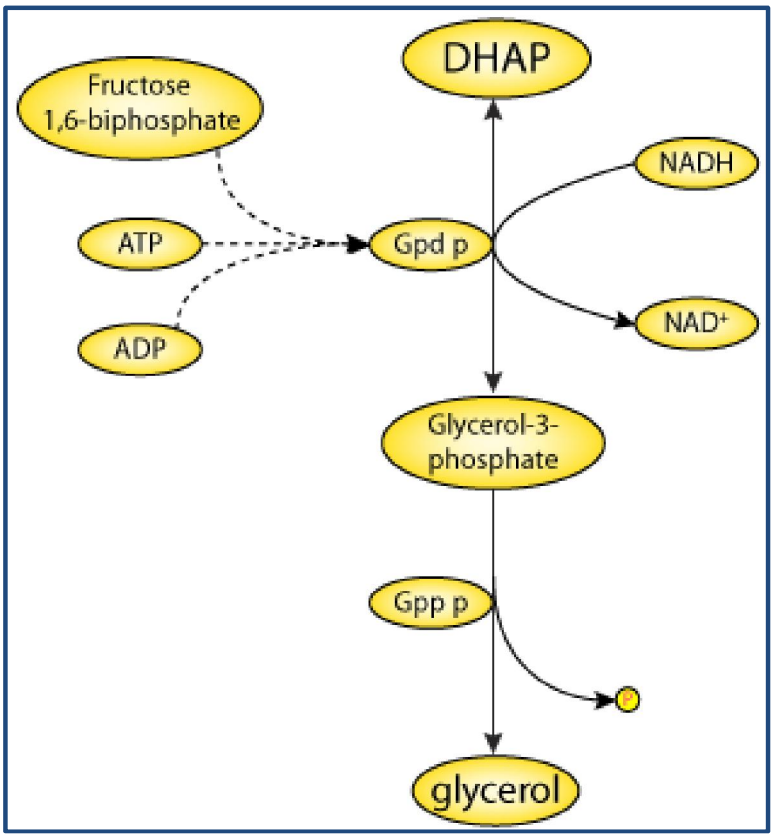

Figure 3.6 The glycerol synthesis pathway in S. cerevisiae. Glycerol is synthesized by the reduction of dihydroxyacetonephosphate (DHAP) to glycerol in two steps. This synthesis pathway is catalyzed by NAD-dependent glycerol-3-phosphate dehydrogenase (Gpdp) and glycerol-3-phosphatase (Gppp) (Hohmann, 2009).

3.5.4 The filamentation-invasion pathway

A nutrient limitation induced pseudohyphal growth in diploids and invasive growth in haploids. Pseudohyphal cells have an elongated morphology, an altered cell cycle and budding pattern, and enhanced substrate invasion (Gimeno & Fink, 1994). These filamentous growth responses are mediated by the filamentation-invasion pathway. This pathway shares the same signaling components with the pheromone signaling pathway and the Sho1 branch of the high-osmolarity glycerol (HOG) pathway, including Ste20p, Ste11p, and Ste7p (Bao et al., 2004; Breitkreutz & Tyers, 2002). However, in the filamentation-invasion pathway, Ste20p activation requires the functions of two small GTP binding proteins, Ras2p and Cdc42p. The complex of Cdc42p-Ste20p transmits a signal to the MAPK cascade composed of MAPKKK Ste11p, MAPKK Ste7p, and MAPK Kss1p. The phosphorylated Kss1p regulates the transcription factor Ste12p to induce expression of filamentation-related genes such as ADE1, CWP1, GFA1, and HAL1 genes, leading to initiation of pseudohyphal growth (Breitkreutz & Tyers, 2002; Chen & Thorner, 2007).

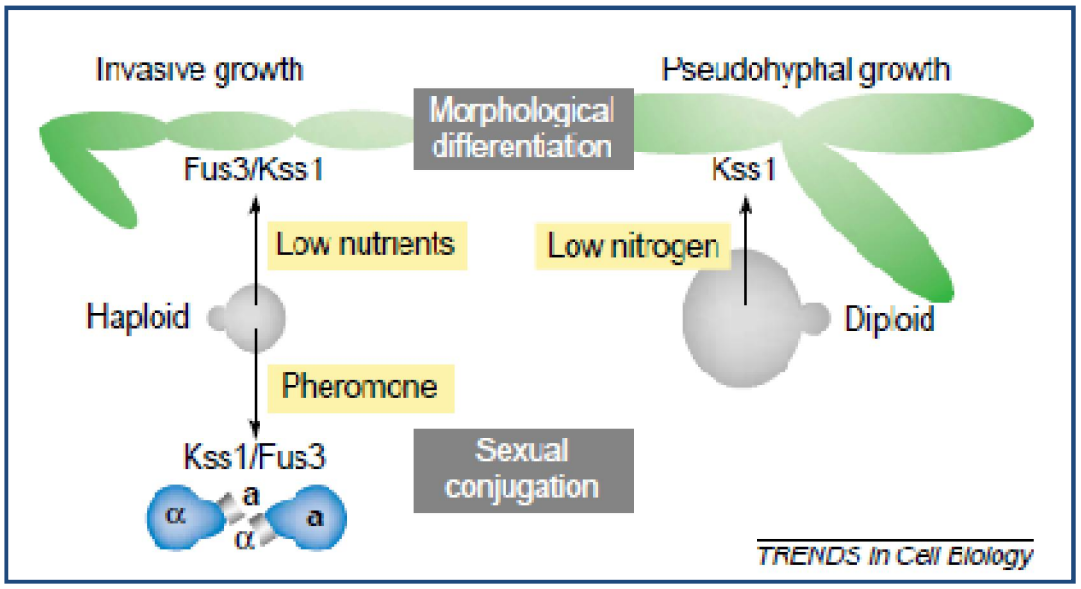

Figure 3.7 Pseudohyphal growths in S. cerevisiae. Cells are round or oval when grown in a rich medium. Cells elongate and form pseudohyphae when they are exposed to a low-nutrient medium. Haploid cells exposed to pheromone of the opposite mating type are arrested in G1 and extend a projection to their mating partner (Breitkreutz & Tyers, 2002).

3.6 The cross-talk between the MAPK pathways

When cells are exposed to stress that have diverse effects, it would be difficult for cells to withstand the harmful effects by a single signaling pathway. For cell survival, more than one signaling pathway or the cross-talk between signaling pathways may play an essential role in an efficient response to these stimuli. The reconstruction of cell wall is considered as one of key defenses against stresses. Although this process is mainly regulated by the cell wall integrity (CWI) pathway, the cross-talks with the other pathways have been reported.

The coordination of the CWI pathway with the pheromone signaling pathway has been shown to be essential for mating projection (Buehrer & Errede, 1997). During cell division, cell wall has to increase its flexibility, allowing budding process. This cross-talk is suggested to be mediated by two possible distinct mechanisms. Pkc1p, an upstream component of CWI pathway, may be activated by Ste20p, the components of pheromone signaling pathway, or by Ste12p, the transcription factor of the pheromone signaling pathway, in response to mating signal, leading to the reconstruction of cell wall (Buehrer & Errede, 1997).

Recently, several studies have demonstrated the cross-talk between the CWI pathway and the HOG pathway in response to zymolyase-mediated cell wall stress (Bermejo et al., 2008; Fuchs & Mylonakis, 2009; Garcia et al., 2009), suggesting that the HOG pathway is not only important for cell survival under hyperosmotic conditions but also involved in adaptation to cell wall stress. Both MAPK pathways have been shown to be required for signaling cell wall stress caused by zymolyase through the Sho1p branch of the HOG pathway and some essential components of the CWI pathway, i.e. Pkc1p, MAPKKK Bck1p, and the redundant MAPKK Mkk1/Mkk2, but not the other upstream elements of the CWI pathway. Although the connection between these MAPK pathways during cell wall stress caused by zymolyase has been reported, the cross-talk between MAPK cascades during ethanol stress has not been characterized.

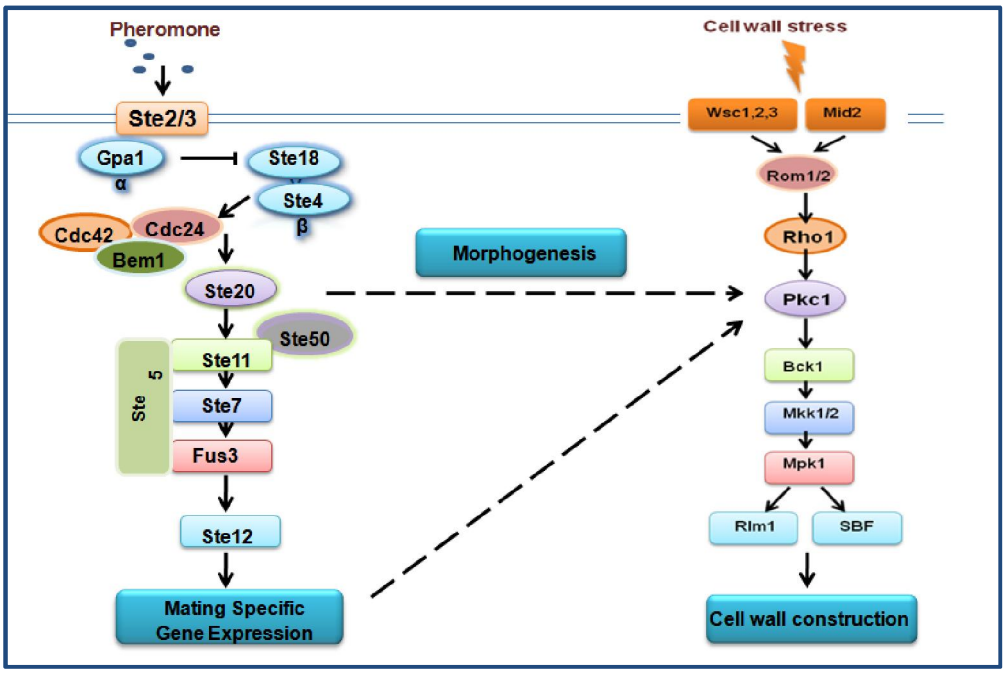

Figure 3.8 Model for pheromone-induced activation of the cell wall integrity (CWI) pathway. Signaling from the pheromone pathway to cell wall integrity pathway is achieved via two distinct mechanisms: one via Ste20-induced morphological change and the other via Ste12-mediated new gene transcription (Buehrer & Errede, 1997).

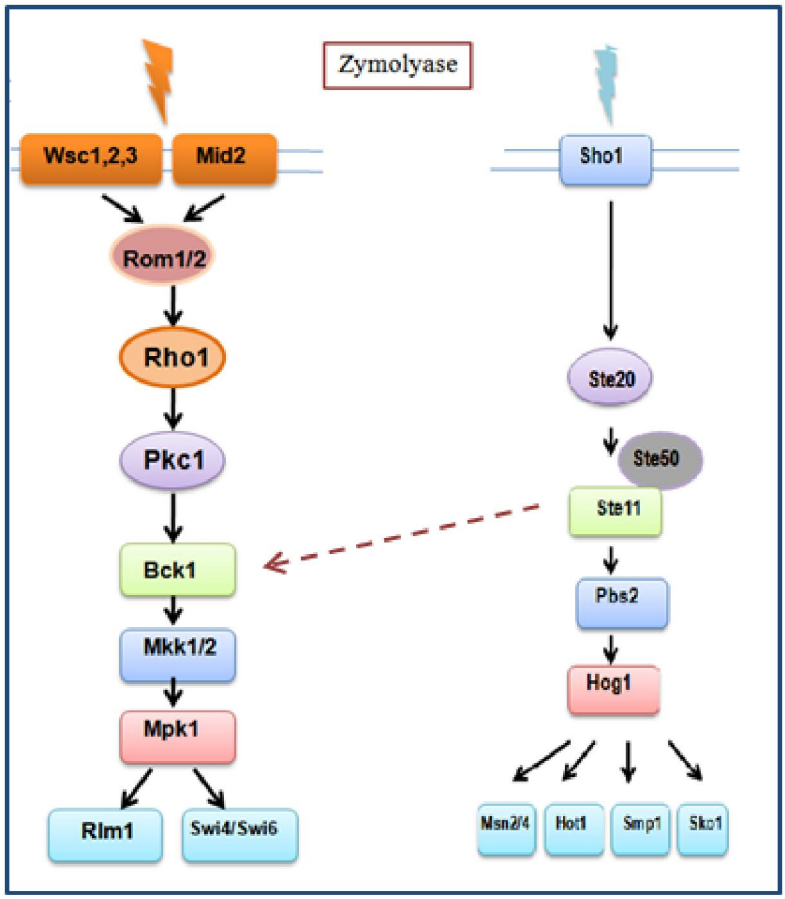

Figure 3.9 Model for the cross-talk between the CWI and HOG pathways in response to zymolyase-mediated cell wall stress. The activation of the CWI pathway depends on the Sho1p branch of the HOG pathway. Thus, a sequential activation of two MAPK pathways is required for cellular adaptation to cell wall damage caused by zymolyase (Arroyo et al., 2009; Bermejo et al., 2008).