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Full Length Research Paper

A glimpse of the yeast *Saccharomyces cerevisiae* responses to NaCl stress

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The budding yeast *Saccharomyces cerevisiae* has been widely used in fermentation and brewing industries. In industrial fermentation processes, the raw material often attaches certain NaCl or Na^+ into bioreactor. High salinity represents an osmotic stress and specific ion toxicity for yeast cell. Protective biochemical reactions range from the synthesis of osmolyte to altered ion transportation, signal transduction, transcriptional response and translational response. This review will focus on responses of the yeast *S. cerevisiae* and mechanisms of adaptation to NaCl stress and offer a glimpse at the mechanisms of ion homeostasis, regulation of HOG (High Osmosis Glycerol) signaling pathway and the genomic expression response of the cells against the stress.

Key words: Genomic expression response, HOG (High Osmosis Glycerol) signaling pathway, *Saccharomyces cerevisiae*, saline stress.

INTRODUCTION

Recently, growing attention has been devoted to the conversion of biomass into fuel ethanol due to high price and environmental problems caused by fossil fuel. The budding yeast *Saccharomyces cerevisiae* which has distinct advantages in terms of ethanol yields, high ethanol tolerance and salinity tolerance has been widely used in the fermentation industries to convert the bio-mass into ethanol. In industrial fermentation processes, the raw material often attaches certain NaCl or Na^+ into bioreactor, which produces higher Na^+ concentration than normal one. The NaCl stress usually affects yeast cells growth and productivity of target products because the cells cultivated in culture medium with high NaCl concentration both expose to specific ion toxicity and to osmotic stress by decreasing the water activity. Low water activity limits yeast growth and reduces the yields of target production (Rahman, 1999). Adaptation to change in intracellular salinity is a critical event for cell survival and the ability to survive after a saline shock must be an intrinsic property of cell (Dhar et al., 2011). Faced with the salinity stress, yeast cells should deal with two problems. One is to develop ion detoxification mechanisms and the other is to acquire the tolerance to osmotic stress. Upon encountering the saline stress, yeast cells display a complex array of stress responses including ion transportation, signal transduction, transcriptional response and translational response (Hirasawa et al., 2006). The yeast strains that can grow well under saline stress conditions are useful for the industrial production of target products. For the construction of industrial useful and saline stress tolerance yeast strains, it is necessary to investigate the adaptive mechanisms to salinity stress. What's more, yeast cells also have been considered as an excellent model for the study of the mechanisms underlying tolerance to saline stress because it has been shown that fungi and higher plants not only have similar ion transport

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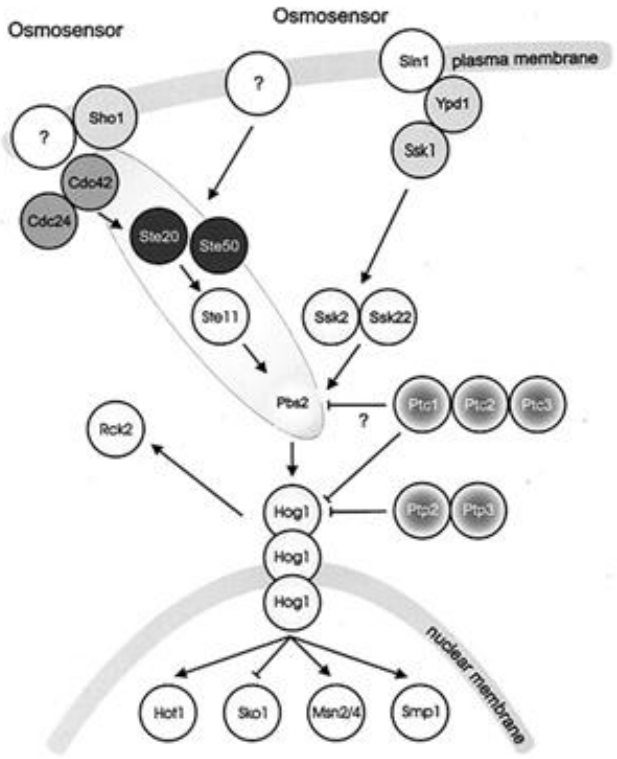

Figure 1. Outline of the HOG pathway (Source: Stefan, 2002).

systems at their plasma membranes (Serrano et al., 1997), but also share similar cationic detoxification mechanisms (Gaxiala et al., 1999) and, most probably, signal transduction pathways (Lee et al., 1999). Several mechanisms of tolerance to salinity stress have been studied in different yeast species. Because of industrial interest, baker yeast (*S. cerevisiae)* is the most commonly used model system. This review will focus on responses of the yeast *S.cerevisiae* and mechanisms of adaptation to salt stress and offer a glimpse at the mechanisms of ion homeostasis, regulation of HOG (High Osmosis Glycerol) signaling pathway and the genomic expression response of the cells against NaCl stress.

The Na^+ transport and homeostasis

High intracellular concentration of Na^+ produces the specific problem of cationic toxicity by deactivation of some enzymes or interfering with cationic sites involved in binding K^+, Ca^{2+} or Mg^{2+}. Maintaining a low intracellular concentration of Na^+ and Na^+ homeostasis is primordial importance in the tolerance of yeast cells to NaCl stress and the plasma membrane transport systems play a key role in increasing the efflux of Na^+ to maintain a suitable intracellular Na^+ concentration when the cells are under a high Na^+ concentration environmental condition (Serrano, 1996). There is a fundamental difference between the transport mechanism of animal cells and yeast cells to exclude Na^+ from cytosol. In animal cells, the Na^+ gradient is generated by Na^+, K^+-ATPase that deposits K^+ inside the cell by its stoichiometry of 3 M Na^+ efflux per 2 M K^+ influx (Serrano et al., 1999). *S. cerevisiae* actively extrudes Na^+ ions primary through the plasma membrane P-type Na^+-ATPase encoded by the gene PMR2 to maintain high intracellular concentration of essential K^+ and low intracellular concentration of toxic Na^+ (Alejandro et al.,1995). In addition, under high NaCl stress, lots of Na^+ floods into cytosol and the cytosolic Ca^{2+} level is elevated. The highly intracellular Ca^{2+} level is generated by the plasma membrane P-type Ca^{2+}-ATPase encoded by the gene PMR1. The *pmr1* mutation confers salt tolerance through continuous activation of calcineurin and enhanced PMR2 expression (Shi et al., 2001). It has been suggested that the intracellular Ca^{2+} level plays an important role in the saline stress response.

HOG signaling pathway involved in osmoadaptation

The NaCl stress has two harmful effects on *S. cerevisiae*. In addition to producing toxicity to cellular metabolism, the highly cytosolic Na^+ level induces the cells to lose the turgor pressure due to osmotic stress. *S. cerevisiae* has an intrinsic property to adapt to the condition through the introduction of lots of signaling pathways, such as protein kinase and the HOG MAP kinase pathway, against the osmotic shock (Wu et al., 2010). By far the best-understood signaling pathway is HOG signaling pathway that regulates the glycerol synthesis to restore turgor-pressure to resistant the osmotic changes. The architecture of the HOG pathway (Figure 1) (Ota and Varshavsky, 1993) has been illuminated through several clever genetic screens. The HOG system is of two branches, namely Sln1 p and Sho1 branch. The two branches operate independently of each other. It is suggested that blocking Sln1p branch or Sho1 branch, the Hog1p is still phosphorylated rapidly too resist to high osmolarity. It has been proposed that the two branches have different sensitivities to respond over a wide range of osmotic pressure (Table 1). The data suggests that Sln1p branch is more sensitive than Sho1 branch upon an osmotic shock. How do the two branches of HOG pathway regulate the glycerol synthesis to respond the osmolarity changes?

Regulation via the Sln1p branch

Sln1p is a protein encoded by gene *SLN1* and is located in plasma membrane. It is of 1,220 amino acids and organized into four distinct regions (Figure 2): (i) an N-terminal section; (ii) a linker region; (iii) a histidine kinase; and (iv) a receiver domain (Ota and Varshavsky, 1993). Sln1p is described as a sensor and is the only sensor histidine kinase in the *S cerevisiae* proteome, while other fungi or higher plant may have several ones. Sln1p is a

Table 1. Different sensitivities to saline stress between Sln1p and Sho1p branch.

	Lowest NaCl concentration	Maximal Hog1p phosphorylation time
Sln1p	100 mM NaCl	After 1 min
Sho1p	300 mM NaCl	After 5 min

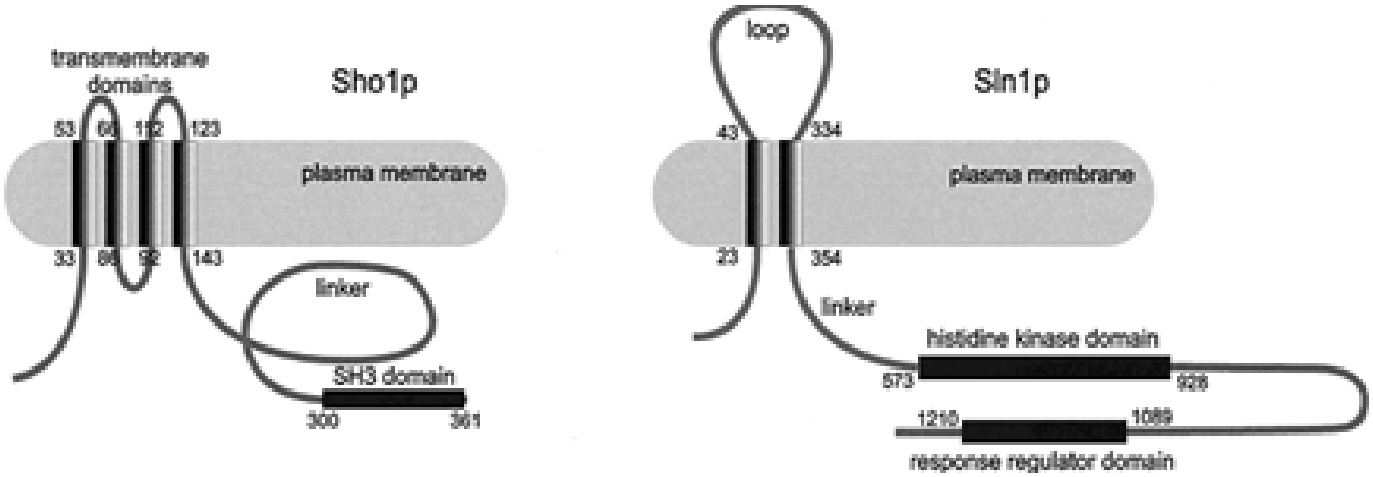

Figure 2. Topology of Sln1p and Sho1p.Numbers indicates amino acid positions (Source: Stefan, 2002).

negative regulator of HOG pathway and the Sln1p histidine kinase is activated by hypo-osmolarity and inhibited by hyper-osmolarity, as indicated by the fact that deletion of *SLN1* is lethal because of pathway over activation (Maeda et al., 1994). Under low osmolarity, Sln1p constantly autophosphorylates itself on His 576. This phosphate is then transferred to Asp1144 of Sln1p receiver domain. Subsequently, the phosphate group is transferred to Ypd1p on His64 and further to Ssk1p on Asp554 (Janiak et al., 2000; 1999). The Sln1p-Ypd1p-Ssk1p phosphorylation system has been demonstrated convincingly by analysis of truncated and mutated protein both in vivo and vitro (Posas et al., 1996). Suffering from a hyper-osmolarity, the histidine kinase activity of Sln1p drops transiently and the the phosphorylation sate of Ypd1p decreases because the phosphate group comes from the Sln1p receiver domain. Ssk1p is then dephosphorylation and the downstream kinase Ssk2p/Ssk22p is actived by dephosphorylated Ssk1p. Eventually, Ssk2p phosphorylates itself probably on Thr1460 rather than is phosphorylated by dephosphorylated Ssk 1p (Posas and Satio, 1998). As such, the phosphorylay in itself of the Sln1p branch acts on an effective feedback regulation to HOG pathway.

Regulation via the Sho1p branch

Sho1p is a protein located at the places on the cell surface where cell growth and cell expansion occur, such as the bud neck and growing bud. It is of 367 amino acids consisting of four domains (Figure 2): (i) N-terminal part; (ii) a linker domain; (iii) transmembrane domains; and (iv) an SH3 domain for protein-protein interaction. Although Sho1 has been described as an osmo-sensor as well as Sln1p, in fact, it is not an osmo-sensor itself. However, the Sho1p branch indeed meditates the HOG pathway activation upon a hyper-osmotic shock and it is suggested that there may be an unidentified osmo-sensor. Exactly, Sho1p plays a role as an anchor protein rather than a genuine osmo-sensor. Sho1p associated with other proteins could monitor subtle osmotic changes during the cell growth and formats a complex protein rapidly and transiently at the places of cell growth (Ratii et al., 2000). At least Sho1p and Pbs2p are involved in the event and the two proteins interact via a proline-rich region around position 96 in the N- terminal domain of Pbs2p and an SH3 domain of Sho1p (Maeda, 1995). In addition, a complex system also exists not necessarily at the same time, the PAK Ste20p, the rho-like G-protein Cdc42p, and the ste11p as well as ste50p (Posas and Satio, 1997; Jansen et al., 2001).How do the complex model work? It may be interpreted as follows (Resier, 2000). Sho1p is anchored at the places of cell growth or cell expansion. The Cdc42p makes a role as marker and marks the place where the new cell material is accumulated. However, Cdc42p is not necessary for Sho1p localization but a necessary signaling for Ste20p and

activates it. Eventually, Ste20p is activated, the ste11p is phosphorylated and Pbs2p is phosphorylated. Interestingly, the whole process took place too rapidly to be observed in wild-type cell unless with a mutant.

Regulation downstream of HOG pathway

Pbs2p is a cyto-plasmic protein and appears to be specifically excluded from nucleus (Resier et al., 1999). Pbs2p is activated and phosphorylated on Ser514 and Thr518 by any of three phosphorylated protein Ssk2p/Ssk22p and ste11p. Dual phosphorylation on the conserved Thr174 and Tyr176 activates the MAP kinase Hog1p (Schuller, 1994) and causes a rapid and marked concentration of Hog1p to appear in nucleus and can be observed with less than 1 min after an osmolarity upshift. The catalytic activity hardly affects the yield of Hog1p in nucleus. The Gsp1p, a G-protein, is required to enhance the Hog1p concentration level in nucleus by importing Hog1p into nucleus. Both the phosphorylation of Hog1p and nuclear localization are rapid and transient. For example, when an osmotic shock is given with 0.4 M NaCl, phosphorylation of Hog1p peaks within 1 min and disappears within about 30 min (Vandenbol et al., 1993). An interesting appearance is that the more severe the osmotic shock, the longer time for Hog1p is taken to be phosphorylated. There is a lag phase after osmotic shock. By far the mechanism has been not revealed yet and more work is required to make interesting event clearer.

Transcriptional response to saline stress

Upon NaCl shock, the intercellular ion uptake and efflux balance is broken and the metabolic system is disrupted. The cells must rapidly respond to the drastic stressful shift and adjust the global cellular organization to maintain the cells' normal functions. Although some responses of *S. cerevisiae* to saline stress have been detected, many details and a great deal information still have been completely unknown. Since the global genomic map of the yeast has been successfully displayed and the development of DNA microarray or chip analysis, the global analysis allows research fellow to gain more information of the cellular regulation, coordination and diverse responses when the cells suffer from a saline shock. DNA microarray is a useful and powerful tool to assess a variety of cellular features such as transcriptional expression, polysome association (Kuhn et al., 2001) and the location of DNA-binding protein and give a integrated view of how the cells work upon a sudden environmental changes. To date, a number of research staff used the DNA microarray to measure the relative transcript level of candidate genes in yeast genome and obtain a view of gene expression changes when the cells were shifted from standard growth condition to a medium supplied with a highly Na^+ concentration. The yeast cells were cultured to mid-log phase in complete media containing 1 M NaCl for 0, 10, 30 and 90 min and the genomic expression levels of about 10% of the yeast ORFs (Open reading frame) were affected by the high salinity (Jaqueline and Hans, 2001). The number of *O-Ring Face Seal* (ORFs) increased more than 2-fold from 107 (10 min) to 354 (90 min) and more than 3-fold from 87 (10 min) to 165 (30 min), and the up-regulation ORFs contained ORFs for ribosomal proteins synthesis, nucleotide biosynthesis and ORFs related to signal of HOG pathway (Jaqueline and Hans, 2001). Hirasawa (2006) compared the transcriptional responses to saline stress of the laboratory strain of *S. cerevisiae* (FY834) and brewing one (IFO2347) with DNA microarray. The brewing strain shows better tolerance to NaCl stress than the laboratory one. Genomic expression dynamically was affected in both yeast strains and just the degree of change in genomic expression level in the laboratory strain was larger than that in the brewing one. The over expressed genes were involved in many proteins related to many functional pathways against the salinity up-shift. The *GDP1*, *ENA2* and *ENA5* were induced to higher expression level of brewing strain than laboratory one upon saline shock. The product of *GDP1* is involved in glycerol biosynthesis and *ENA2*/ *ENA5* is related to P-type ATPase Na^+ pump. This phenomenon is one of probable reasons for brewing strain with higher saline stress tolerance. In addition, many other genes were also up-regulation including *CUP1-1*, *CUP1-2*, *BTN2*, *HSP26* as well as *HSP12*. Those genes are also induced to over-express by other stressful environmental changes. *CUP1-1* and *CUP1-2* shows an up-regulation when strains experience starvation and the proteins encoded by *CUP1-1*and *CUP1-2* are involved in metabolism. *HSP26* and *HSP12* are responsibility for heat shock (Haslbeck et al., 1999) and *BTN2* is related to regulation of pH. From the DNA microarray analysis (Table 2), it is suggested that both the transcript expression of HOG pathway gene and the expression of sodium ion efflux pump gene are enhanced under high Na^+ concentration. Interestingly, there are also many overlap over expression genes-encoding enzymes involved in carbohydrate metabolism or heat shock proteins. The relationship between the over expression and the adjustment of cells to saline stress is unrealized and more work is required.

Perspectives

The yeast *S. cerevisiae* responses to NaCl stress is a complex system. Although some information of mechanism of sodium ion efflux and the regulation of HOG pathway has been obtained through several clever genetic screens, some details are still understood. The

Table 2. Listing of some upregulated ORFs in saline stress response of *S. cerevisiae.*

Gene	NaCl concentration (M)	Shock time (min)	Function of genne product	Fold induction
STL1	1	60	Sugar transporter-like protein	496
	0.4	10		89.8
GLK1	0.4	10	Glucokinase	52.4
	1	60		78.6
HXT5	1	60	Hexose transporter	96.9
	0.4	10		6.6
GDP1	1	60	Glycerol-3-phosphate dehydrogenase	80.6
	0.4	10		76.4
	1	10		39.1
YGP1	1	10	Secreted glycoprotein	25.6
DAK1	0.4	10	Dihydroxyacetone	9.4
GDP2	0.4	10	Glycerol-3-phosphatedehydrogenase	16.3
ENA2	1	60	P-type ATPase Na^+ pump	37.0
ENA5	1	60	Na^+ ATPase	36.5
ENA1	1	60	P-type ATPase Na^+ pump	34.3
SSA1	1	10	Heat shock protein of HSP70 family	3.1
SSA4	1	60	Heat shock protein of HSP70 family	24.9
SSE2	1	90	Heat shock protein of HSP70 family	4.9
HSP12	1	30	Heat shock protein	62.7
	1	60		19.6
HSP26	1	60	Heat shock protein 26	72.2
	1	90		7.6
	0.4	10		26.3

(Source: Hirasaw et al., 2006; Jaqueline and Hans, 2001; Francesc et al., 2000).

genomic expression studies with Deoxyribonucleic acid (DNA) microarray have contributed to our understanding of yeast responses to saline shock and there is still a great deal to learn about the certain functions of the over-expression genes. The DNA microarray is a useful and powerful tool to understand the global genomic expression of cells and can contribute research staff to realize the global regulation mechanism of cells against the saline stress. A huge amount of data is acquired via DNA microarray so an effective methodology is required to handle these data and to extract useful information. After that, the useful candidate genes were selected and the function was analyzed. Finally, we actually constructed ideal industrially useful yeast strains.

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