Effects of Various Physiological Stresses on Transcription of the SUC2 Gene in the Yeast Saccharomyces cerevisiae

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Abstract: Physiological conditions in the growth habitat of yeast cells dramatically changes the gene expression pattern. In this study, it was shown that transcription of the SUC2 gene, which encodes the cytoplasmic and secreted enzyme invertase, is modulated according to the environmental conditions in the yeast Saccharomyces cerevisiae. Hyperosmotic stress and oxidative stress repressed the transcription of the SUC2 gene up to 50%. However, preconditioning of the yeast cells prevented the negative effects of osmotic stress on SUC2 gene transcription. These results clearly show that, apart from the control of invertase activity and its secretion, osmotic stress and oxidative stress also represses transcription of the SUC2 gene in the yeast S. cerevisiae.

Key Words: Saccharomyces cerevisiae, SUC2, invertase, osmotic stress, oxidative stress, transcriptional repression.

Introduction

The SUC2 gene of Saccharomyces cerevisiae encodes both the secreted and the cytoplasmic form of the invertase enzyme that is required for the hydrolysis of sucrose (1). The secreted extracellular form of the enzyme is highly glycosylated while the cytoplasmic form is not. Both enzymes are translated from mRNAs transcribed from the single SUC2 gene (2, 3). However, the secreted enzyme contains the amino terminal signal peptide region for the intracellular
modifications and secretions. The exact molecular mechanism for the differential translations of both enzymes from the SUC2 mRNAs are not known. Expression of the SUC2 gene is regulated by glucose repression (4). It is repressed by the Mig1p-Ssn6p-Tup1p complex that directly binds to the promoter region of the SUC2 gene in growth media containing a high level of glucose (2%w/v) (5, 6). Its transcription is derepressed upon phosphorylation of the Mig1p complex by Snf1 protein kinase complex in low glucose (0.1%) or in growth media containing sucrose (7, 8).

Physiological stress in the growth habitat of living organisms may lead to a series of physiological events that results in cell shrinkage, cell burst, or DNA damage. Heat shock, osmotic stress and oxidative stress are examples of physiological conditions which dramatically affect gene regulation in S. cerevisiae. In response to these types of physiological conditions, the expression of specific sets of genes is activated or repressed to adjust to new growth conditions at the cellular level.

S. cerevisiae accumulates glycerol up to molar concentrations in response to hyperosmotic stresses such as saturating concentrations of sugar or salt in its growth media (9). The transmembrane proteins Sln1p and Sho1p are the osmo-sensors in Saccharomyces cerevisiae (10). These osmo-sensor proteins activate the HOG1 MAP-kinase signal transduction pathway, which leads to the increased synthesis of glycerol as an osmoprotectant solute (11, 12). Hyperosmotic stress also reduces glucose and glycerol consumption rates, as well as the ethanol production rate, but increase the trehalose content of the cell (13, 14). Additionally, transcriptions of distinct sets of genes (hyperosmolarity-responsive -HOR- genes) are activated (15-17). Some of the hyperosmolarity-responsive genes are previously characterized genes with normal cellular functions, namely, glycerol-3-phosphate dehydrogenase-1 (GPD1), glucokinase-1 (GLK1), heat-shock protein (HSP12), P-type (Na⁺, Li⁺, K⁺) ATPase (ENA1), Catalase T (CTT1) and one of the hexose transporter genes (HXT1).

Oxidative stress is generated indirectly in the final stages of the respiratory chain or externally by reactive oxygen species such as superoxide anions, hydrogen peroxide and hydroxyl radicals present in the growth environment. These types of reactive oxygen species also repress the expression of the subset of genes, but they activate the oxidative stress response pathway (18, 19). These genes include the following; γ-glutamylcysteine synthetase (GSH1), which is required for glutathion synthesis (20, 21); thioredoxin genes (TRX1 and TRX2) (22), and glucose-6-phosphate dehydrogenase (G6PDH) (18). After activation of the oxidative stress response pathway, reactive oxygen species are eliminated non-enzymatically mostly by Glutathion (GSH) or enzymatically by catalase (Ctt1p) or superoxide dismutase (Sudp) (18).

The effects of physiological factors such as heat and ionic conditions on invertase activity and its secretion have been reported previously (22, 23). Heat shock, which interferes with protein folding and secretion, reduces invertase activity in S. cerevisiae (23). Ionic conditions also change the enzymatic activities of invertase by modulating its Km or Vmax values for its substrate sucrose (22).
In this study, the effects of oxidative and hyperosmotic stresses on the transcriptional regulation of the SUC2 gene of S. cerevisiae were investigated. It was shown that transcription of the SUC2 gene is repressed by both oxidative stress and hyperosmotic stress when the yeast cells are grown in glucose derepressed conditions. However, preconditioning of the yeast cells overcomes the negative effects of the hyperosmotic stress on the SUC2 gene promoter and restores SUC2 gene expression at least to the fully derepressed level. These results show that, apart from the enzymatic activities and the secretion of the invertase, physiological stresses also down regulate the transcription of SUC2 gene.

Materials and Methods

Yeast Strain and Growth Conditions

The S. cerevisiae strain used in this study is YST102 (MATa leu2-3, 112 his3 trp1-289 ura3-52). Yeast cells were grown in YPD media for transformation. Transformants were grown in synthetic complete (SC) media supplemented with the proper amino acids and with either 0.1% or 2% glucose (24). Tryptophane or Uracil was omitted (SC-Trp or SC-Ura) from the growth media to maintain the selection for SUC2::lacZ or HIS4::lacZ plasmids. First, transformants were grown at 30˚C with constant shaking at 250 rpm to stationary phase. Then, 100 µl of saturated cultures were transferred to 10 ml of fresh media and grown until the pre-logarithmic stage (OD600: 0.6-0.7). Then, some of the cells were harvested and transferred to fresh SC-Trp or SC-Ura media supplemented with 0.1% or 2% glucose and 0.2 mM H2O2 or 1M NaCl as oxidative and hyperosmotic stress-inducing agents respectively (15, 21). Transformants were further incubated under these conditions for 90 minutes at 30˚C with constant shaking at 250 rpm, then harvested for β-galactosidase assays. For preconditioning experiments, transformants were prepared as described for stress induction except that cells were incubated in 0.25 M NaCl for 30 minutes under the same conditions and then shifted to hyperosmotic stress conditions as described (25).

Plasmids and Genetic Methods

The structures and constructions of the plasmids used in this study have been described previously (4, 26). In brief, the SUC2::lacZ fusion plasmid is a Trp-Ars based yeast expression vector. It contains 516 bp of the promoter region of the SUC2 gene extending from -384 bp to -900 bp (4). The HIS4::lacZ fusion plasmid is the same as pFN8X-n (26). It was used as a negative control in our experiments, as its expression is expected to be independent of the oxidative and hyperosmotic stresses.

Plasmids were transformed into yeast cells as described previously with lithium acetate (27).

β-Galactosidase Assays

Yeast transformants were grown under normal or stress induced growth conditions in triplicate. At the end of the growth period, cells (10 ml) were harvested and washed once with 1ml of ice-cold distilled sterile water and then resuspended in 200 µl of breaking buffer. Cells were permeabilized with 20 µl of 0.1% SDS and 20 µl of chloroform. β-galactosidase assays were done in triplicate as described previously (28). Standard error in these assays was less than 10%. β-galactosidase activities of transformants are given in Miller units.
Results

Repression of the transcription of SUC2 gene caused by hyperosmotic and oxidative stress

Expression of SUC2 gene is activated by the glucose derepression mechanism. In the presence of high glucose in the growth habitat of the yeast S. cerevisiae, transcription of SUC2 is repressed by the Mig1p complex. In low glucose, transcription of SUC2 is derepressed by the Snf1p protein kinase complex upon phosphorylation of the Mig1p-Ssn6p-Tup1p complex.

SUC2 encodes the invertase enzyme, which is required for the hydrolysis of sucrose. Negative effects of several physiological factors, such as heat shock and ionic conditions, on invertase activity have been reported previously (22, 23). In this study, the direct effects of hyperosmotic stress and oxidative stress on the transcriptional regulation of SUC2 were investigated in both glucose-repressed and glucose-derepressed growth conditions. A SUC2::lacZ fusion gene was used in the quantitation of the effects of osmotic stress and oxidative stress on the transcriptional regulation of SUC2. Previously, it was clearly shown that the promoter region of the SUC2 gene is able to exert glucose-dependent regulation of heterologous genes on the expression vector used in this study (4).

Hyperosmotic stress repressed the transcription of the SUC2::lacZ fusion gene by about 44% under glucose-derepressed growth conditions. Transcription of the SUC2::lacZ fusion decreased from 82 units to 46 units upon direct exposure of yeast transformants to hyperosmotic stress conditions (Table 1 - compare line 1 and 2). Oxidative stress also repressed the transcription of the SUC2::lacZ gene under glucose-derepressed growth conditions. Hydrogen peroxide-triggered oxidative stress decreased the transcription of the SUC2::lacZ fusion gene from 82 units to 42 units, which resulted in a 49% decrease in the transcription rate of this gene (Table 1, line 3).

Transcription of the HIS4::lacZ fusion gene was not affected by the physiological stress conditions tested in this study. Its transcription remained at about the same level (35-36 units) in both sets of growth conditions (Table 1).

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>SUC2::lacZ</th>
<th>HIS4::lacZ</th>
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</thead>
<tbody>
<tr>
<td>1- Normal Growth</td>
<td>82</td>
<td>35</td>
</tr>
<tr>
<td>2- Hyperosmotic Stress</td>
<td>46</td>
<td>36</td>
</tr>
<tr>
<td>3- Oxidative Stress</td>
<td>42</td>
<td>35</td>
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<tr>
<td>4- Hyperosmotic Stress</td>
<td>75</td>
<td>36</td>
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Table 1. Effects of hyperosmotic and oxidative stresses on the SUC2 gene transcription under glucose derepressed growth conditions.

β-Galactosidase activities were expressed as Miller units.
Effects of preconditioning and glucose repression on the stress-induced repression of the SUC2 gene.

Preconditioning of yeast cells to lower doses of stress inducing agents is known to increase the survival rate and resistance to higher doses of stress inducing agent (16, 29). In the present study, the effects of preconditioning on the hyperosmotic stress-dependent repression of SUC2::lacZ fusion gene transcription was investigated. Yeast transformants were grown in growth media containing 0.25 M NaCl in low glucose then shifted to hyperosmotic growth conditions (1 M NaCl) as described in Materials and Methods. Unexpectedly, hyperosmotic stress-dependent repression of the SUC2::lacZ transcription was prevented by preconditioning. Transcription of the SUC2::lacZ fusion gene remained at a level (75 units) comparable to the normal growth conditions (Table 1, line 4). Transcription of the negative control gene HIS4::lacZ was not affected, by the preconditioning as expected indicating that release from the hyperosmotic stress-dependent repression is not a global response but is specific to the SUC2::lacZ gene in this case.

Transcription of SUC2 is subject to glucose repression. Hence, the effects of the hyperosmotic stress and also the oxidative stress on the SUC2::lacZ fusion gene were investigated under glucose repressed growth conditions. Transcription of the SUC2::lacZ fusion decreased to 25 units under glucose-repressed growth conditions as expected (Table 2, line 1). Growth of glucose repressed yeast transformants under hyperosmotic or oxidative stress conditions did not lead to any further decrease in the transcription level of the SUC2::lacZ fusion gene (Table 2, lines 2-3). Its transcription remained at the same low levels (22-27 units) in normal or stress-induced growth conditions in glucose-repressed yeast transformants. Transcription of the HIS4::lacZ gene was not affected by glucose repression nor by the stress conditions as expected (Table 2).

Discussion

Environmental conditions such as heat, ionic conditions and extracellular carbon sources affect the synthesis and the secretion of the invertase enzyme in S. cerevisiae. It has previously been shown that high extracellular glucose concentration represses the transcription of the SUC2 gene through the global repressor protein Mig1p complex (30, 31). The direct effects of hyperosmotic stress and oxidative stress on SUC2 gene transcription was analyzed in this study.

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<td>27</td>
<td>36</td>
</tr>
<tr>
<td>3- Oxidative Stress</td>
<td>24</td>
<td>35</td>
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</tbody>
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*β-Galactosidase activities were expressed as Miller units.

Table 2. Effects of hyperosmotic and oxidative stresses on the SUC2 gene transcription under glucose repressed growth conditions.
Since the transcription of SUC2 is regulated by glucose repression-derepression mechanisms, the effects of hyperosmotic stress and oxidative stress on SUC2 transcription were analyzed both in glucose-repressed and derepressed growth conditions.

Hyperosmotic stress activates the high osmolarity glycerol (HOG) MAP-Kinase signal transduction pathway. Transcriptional activation or derepression of SUC2 requires phosphorylation of the Mig1p complex by the Snf1 protein kinase complex. The physiological activities of the Snf1p complex are also regulated according to nutrient availability and stress conditions, and is probably mediated by the intracellular AMP/ATP ratio (32, 33). Lack of complete derepression of the SUC2 gene transcription under osmotic or oxidative stress conditions suggests that the Mig1p complex is not fully dissociated from the SUC2 promoter. It is conceivable that the HOG1 signal transduction pathway interferes with the functions of the Snf1 protein kinase under stress induced conditions in glucose-derepressed yeast cells.

It was also previously shown that osmotic stress activates the cytoplasmic protein phosphatase calcineurine in yeast cells (34). In the case of glucose-derepressed but osmotically stressed yeast cells, calcineurine may also interfere with Snf1p activities by dephosphorylating the Mig1p complex. This may lead to the partial derepression of SUC2 transcription in yeast cells grown in glucose derepressed but stress-induced conditions.

Direct repression of SUC2 transcription by stress-induced transcription factors is also possible. It has recently been shown that the stress-induced transcriptional repressor protein Xbp1 is involved in the direct repression of several genes in yeast cells under stress-induced growth conditions (35). The Xbp1 gene encodes a DNA-binding repressor protein. Putative binding sites for Xbp1 have been identified within the regulatory region of the SUC2 promoter. However, direct effects of Xbp1 on the SUC2 gene have not been determined yet.

The preconditioning of yeast cells for osmotic stress essentially prevented the negative effects of osmotic stress on SUC2 gene transcription. It has been reported that Hog1p protein kinase is phosphorylated at maximal levels if the yeast cells have been exposed to lower doses of osmocytotoxic inducing agents. SUC2 transcription was restored to almost fully derepressed levels in preconditioned yeast transformants grown under hyperosmotic conditions in glucose derepressed media. This result suggests that phosphorylation of Hog1p is essential for the transcriptional derepression of SUC2 in preconditioned yeast cells.

References


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