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Original Research

Hydrogen peroxide-induced oxidative stress upregulates *ght5* gene belonging to hexose transporters in *Schizosaccharomyces pombe*

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Abstract: Hydrogen peroxide is an agent that triggers oxidative stress. Glucose, which is a source of carbon and energy has a regulatory role in many metabolic processes such as growth rate, fermentation capacity and stress response. *Schizosaccharomyces pombe* has eight hexose transporters with a different affinity for glucose and/or related monosaccharides. In *S. pombe,* Ght5 is a glucose transporter with high-affinity. We aimed to investigate the effects of H_2O_2-induced oxidative stress on hexose transporters using glucose repression-resistant mutant strains (*ird5* and *ird11*) of *S. pombe*. We analyzed the percentage of glucose consumption in *S. pombe* wild-type and mutant cells under stressed and non-stressed conditions. Then we compared the expression levels of the genes encoding hexose transporters under the same conditions. We confirmed that the glucose consumption efficiencies of the mutants were slower than the wild-type as in earlier study under non-stressed condition. The percentage of glucose consumption reduced by approximately two-fold in *ird11* and wild-type, but not change in *ird5*, under a stressed condition. There is no difference between cells shape and size of *S. pombe* strains under stressed and non-stressed conditions. Under stress-induced condition, the expression levels of *ght3, ght4* genes in *ird11* and wild-type, and *ght4, ght6* genes in *ird5* decreased, but that of *ght5* gene remarkably increased in only wild-type. We suggested that oxidative stress caused by H_2O_2 leads to upregulation of the *ght5* gene in *S. pombe*.

Key words: Oxidative stress; Hexose transporters; *ght5*; *Schizosaccharomyces pombe*; Glucose repression.

Introduction

In *S. pombe*, glucose is detected via G protein-coupled receptor (GPCR); the transcription of target genes are also regulated via cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) (1-4). Glucose detection decreases some cellular process such as respiration, various stress response pathways, gluconeogenesis and the uptake of alternative carbon sources; while activates glucose transport, glycolysis, and related catabolic processes (4-8).

There are two main pathways triggered by an excess of hydrogen peroxide (H_2O_2) for generating stress response in *S. pombe* (9). Pap1 is activated by moderate oxidative levels, while mitogen-activated protein kinase (MAPK), Sty1/MAPK, the pathway is activated by higher oxidant levels (10). In addition, a new Pap1–Oxs1 pathway in the oxidative stress response in *S. pombe* for diamide has been added recently (11). Oxs1 is the nucleoplasmic High-Mobility Group protein and working cooperatively with Pap1 to regulate transcription.

Glucose transporters are a wide group of membrane proteins called Major Facilitator Superfamily that facilitate the transport of glucose across the plasma membrane (12-15). In many organisms including *S. pombe*, glucose controls its metabolism by regulating the quantity, types, and activity of glucose transporters, both at the transcriptional and posttranslational levels (16). Each of these transporters has a different affinity for glucose and/or related monosaccharides (17, 18). Ght1, Ght2, Ght5 and Ght8 are directly involved in glucose uptake. But Ght3 and Ght4 responsible for gluconate uptake, while Ght6 for fructose. Ght1, Ght3, Ght4 and Ght5 represents the high-affinity glucose transporters of *S. pombe*. In addition, while Ght2 and Ght8 have moderate glucose affinity (17, 18), Ght7 has no signal peptide and almost exclusively does not exist outside the process of meiotic sprouting (19).

Earlier we reported that both the resistance to glucose repression in *S. pombe ird5* and *ird11*mutants might lead to resistance to oxidative stress, and glucose signaling and glucose utilization might be regulated apart from each other (20, 21). Here, we aimed to find whether the effects of oxidative stress induced H_2O_2 on hexose transporters using glucose repression-resistant mutant strains (*ird5* and *ird11*) of *S. pombe*.

Materials and Methods

Yeast strain, growth media and conditions

S. pombe wild-type (972h$^-$) and glucose repression mutants (*ird5*, *ird11*, (22)) used in the present study. *S. pombe* strains were cultured either in standard rich medium (repressed condition), containing 0.5% (w/v) yeast extract, 3% (w/v) glucose (YEL) or in standard mini-

Table 1. The list of primers used qRT-PCR.

Gene		Oligonucleotides
ght2	Forward:	5′- GGCTGCACTTTTGGTGGTAT-3′
	Reverse:	5′- ACGAGAACCGACAGCTGAAT-3′
ght3	Forward:	5′- CTGTAGGCGATCGAAAGCTC-3′
	Reverse:	5′- GCCCCAAGAACAGCAGTAAG-3′
ght4	Forward:	5′- TGGTGCGGATAATGACGATA-3′
	Reverse:	5′- CCAAGTCGGGTGTGATTCTT-3′
ght5	Forward:	5′- TTTGGTGGTCTTTTCGTCCT-3′
	Reverse:	5′- CCAACAGCTGCGTAAATGAA-3′
ght6	Forward:	5′- TGGTGGTGTTTGGCAATCTA-3′
	Reverse:	5′- CACAATCATAACGGCACCAG-3′
ght7	Forward:	5′- CGGTACCGGTATGGATTCAC-3′
	Reverse:	5′- AGGAGAACGTCGTCCAAAGA-3′
ght8	Forward:	5′- TTCGCTGCCTGTAATTTGTG-3′
	Reverse:	5′- TAGCGCCGGAGAGATACAGT-3′
act1	Forward:	5′-AGATTCTCATGGAGCGTGGT-3′
	Reverse:	5′-TCAAAGTCCAAAGCGACGTA-3′

mal medium (derepressed condition), containing 0.5% (w/v) glucose (MML), at 28 °C on a rotary shaker at 180 rpm. *S. pombe ird* mutants were selected on selection medium, containing 0.5% (w/v) yeast extract, 3% (w/v) sucrose, 400 µg/mL 2-deoxy-D-glucose (2-DOG) (22). Cultures were prepared in 50 mL of YEL/MML in 250 mL Erlenmeyer flasks. The cells (10^6 cells/mL) were incubated on an orbital shaker until their mid-log phase at 30°C at180 rpm . Each culture was split into two tubes (20 mL culture in 100 mL Erlenmeyer flasks) as the experimental and the control groups. In the experimental group, 2 mM H_2O_2 (Sigma H1009) was added to the medium and incubated for 1 h (20). The control group was not exposed to oxidative stress. Both groups were washed at least twice with sterile distilled water. The pellet of each group was used to determine the percentage of glucose consumption and to isolate RNA.

Glucose consumption

After washing twice with distilled water, the experimental and control group of each *S. pombe* strains were resuspended in liquid medium containing 0.5% (w/v) yeast extract and 0.5% (w/v) glucose. Each cell suspension was incubated on a rotary shaker at 30°C and 180 rpm for 3 hours (22). The amount of glucose at the end of the incubation was measured by glucose oxidase-peroxidase system (GOD-POD assay) using a commercial kit ("Glucose Calorimetric Assay Kit", Cayman).

RNA isolation and quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from experimental and control groups using a High Pure RNA Isolation Kit (Roche) following the manufacturer's instructions. Single strand cDNA was synthesized using Transcriptor High Fidelity cDNA Synthesis Kit (Roche) according to manufacturer's protocol as previously reported (23-27). qRT-PCR was performed with FastStart SYBR Green Master using Stratagene Technology (Stratagene, La Jolla, CA, USA). *S. pombe* beta-actin (*act1*) gene, as the reference gene, was used to normalize the expression levels (28). A non-template control, and serial dilutions (1, 1:10, and 1:100) of the reference gene (*act1*) and the target genes were added in each assay. The amplification specificity of each reaction was confirmed by melting curve analysis. Relative gene expression levels were calculated by Pfaffl's method (29). Table 1 shows the oligonucleotides of target genes and the reference gene used in this study.

Statistical analysis

Data are reported as means ± standard deviation for at least two experiments . Statistical comparisons were made using one-way analysis of variance (ANOVA) at GraphPad Prism 6 software. Differences in mean values were considered to be significant when $P<0.05$.

Results

Cell physiology under oxidative stress

In order to test the effect of hydrogen peroxide on glucose uptake, first we analyzed the percentage of glucose consumption in *S. pombe ird* mutants and wild-type under stressed and non-stressed conditions; and then we compared the expression levels of the genes encoding hexose transporters in these *S. pombe* cells under the same conditions.

Under optimal condition (non-stressed condition), we calculated that the glucose consumption efficiencies of the mutants were slower than that of the wild-type (Table 2). Under the stressed condition, the percentage of glucose consumption reduced by approximately

Table 2. The percentage of glucose consumption in H_2O_2- treated / untreated *S. pombe* strains (*ird5, ird11* and WT), exponentially growing in rich media containing 3% glucose.

Strains	Glucose Consumption (%)
WT C	93.8 ±2.2
WT HP	47.2±7.4
ird5 C	46.86±4.1
ird5 HP	44.58±8.7
ird11 C	59.15±1.3
ird11 HP	28±4.2

WT: wild-type; C: untreated with H_2O_2; HP: treated with H_2O_2.

two-fold in both *ird11* and wild-type compared to the non-stressed condition. However, a similar percent glucose consumption in *ird5* under both stressed and non-stressed conditions was observed.

In order to understand whether there is any effect of H_2O_2-induced oxidative stress on the cell-shape and size, we imaged the cells with DAPI staining. We did not observe any difference between cells shape and size of *S. pombe* strains under stressed and non-stressed conditions (Data not shown).

The effect of oxidative stress on the expression levels of hexose transporters

To check possibility effects of glucose starvation on transcriptional regulation of hexose transporters in *S. pombe*, we compared the levels of *ght* genes expression in *ird5* and *ird11* mutants and wild-type. We found that the expression level of *ght3* gene was elevated in the wild-type under derepressed condition compared to the repressed condition, while the expression levels of *ght3* and *ght6* genes were increased in *ird5* under the same condition (Figure 1).

Under stress-induced condition, the expression levels of *ght3*, *ght4* genes in both *ird11* and wild-type, and *ght4, ght6* genes in *ird5* decreased. On the other hand, the expression level of *ght5* gene remarkably increased in only wild-type (Figure 2).

We observed that the expression levels of the only *ght5* gene among these genes remarkably increase in *ird* mutants compared with wild-type grown in optimal condition. (Figure 3).

Discussion

It is known that glucose repression decreases oxidative stress response, while the deficiency of glucose repression increases oxidative stress response, and the percentage of glucose consumption is also an effect on glucose repression (7, 30, 31).

The fact that glucose consumption efficiencies of *ird11* and wild-type under stressed condition reduced by approximately two-fold compared to non-stressed condition but not change in ird5, supports our previous results related to *ird5* and *ird11* studies (20, 21). Increased oxidative stress response might result from decreased glucose uptake because it is known that glucose depletion causes resistance to oxidative stress in *S. pombe* (32, 33).

High glucose concentration might result an increase in the cell size and in ROS generation a mitochondrial-independent way (34). The fact that there is no difference between cells shape and size of *S. pombe* strains under stressed and non-stressed conditions might be due to the lower intracellular oxidation levels in *ird11* according to that of the wild-type. (20).

It is known that glucose controls its metabolism by regulating the quantity, types, and activity of glucose transporters, both at the transcriptional and posttranslational levels (16). Therefore, we found that the expression level of *ght3* gene was elevated in the wildtype under derepressed condition compared to the repressed condition, while that of *ght3* and *ght6* genes were increased in *ird5* under the same condition. Upregulation of these genes encoding hexose transporters with high affinity of glucose (17) is an expected situation. However, the fact that no significant increase was observed in any *ght* genes expression in *ird11* under derepressed condition might arise from resistance to glucose repres-

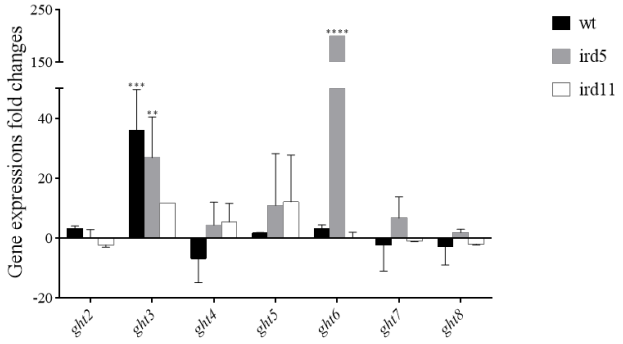

Figure 1. Expression profiles in derepressed condition (0.5% glucose, w/v) of hexose transporter genes (*ght2-ght8*) in wild-type, *ird5* and *ird11* mutants. Relative expression for each gene in each *S. pombe* strains grown in low glucose media comparing to each strain grown in glucose-rich media (3% glucose, w/v) was normalized relative to *act1* gene by using the Pfaffl method. Each fold change was evaluated by Bonferroni's multiple comparisons test. **P<0.01; ***P<0.001; ****P<0.0001. wt: wild-type.

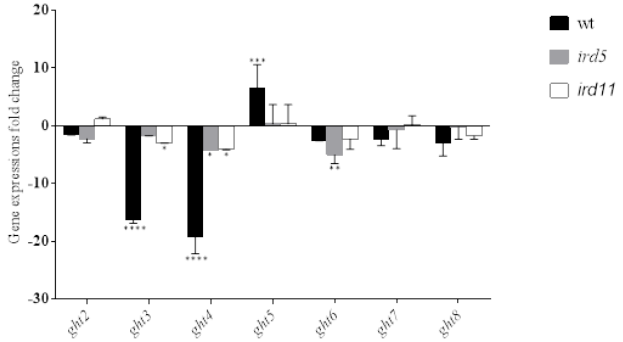

Figure 2. Expression profiles in hydrogen peroxide-induced condition of hexose transporter genes (*ght2-ght8*) in wild-type, *ird5* and *ird11* mutants. Relative expression for each gene in each *S. pombe* strains treated with 2mM H_2O_2 for one hour comparing to untreated strains was normalized relative to *act1* gene by using the Pfaffl method. Each fold change was evaluated by Bonferroni's multiple comparisons test. *P<0.05; **P<0.01;***P<0.001; ****P<0.0001. wt: wild-type.

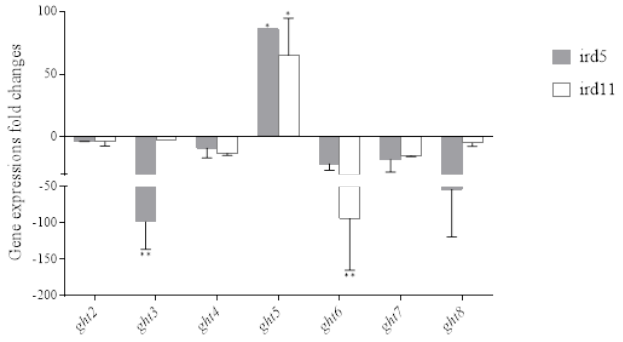

Figure 3. Expression profiles of *ght2-ght8* genes in *S. pombe ird5* and *ird11* grown in rich media relative to wild-type. Relative expression for each glucose transport gene in *S. pombe* mutants compared to wild-type was normalized relative to *act1* gene by using the Pfaffl method. Each fold change was evaluated by Bonferroni's multiple comparisons test. *P<0.05; **P<0.01;***P<0.001; ****P<0.0001. wt: wild-type.

sion in *ird11* (20).

The fact that the expression levels of *ght3, ght4* genes in *ird11* and wild-type, and *ght4, ght6* genes in *ird5* under stress-induced condition decreased, but that of *ght5* gene remarkably increased in only wild-type are correlated with the results obtained for the percentage of glucose consumption. It has been recently reported that *ght5* is transcriptionally repressed by glucose through the activation of the zinc finger protein Scr1 and is activated by glucose starvation through the inactivation of Scr1 with Ssp1 kinase and the Sds23 phosphatase inhibitor (18). Similarly, we have recently observed the reduced transcription of *ght5* gene by increasing iron stress in *S. pombe* wild-type (35).

Concerning the upregulation of only *ght5* gene in wild-type under stress condition, we have raised the question of how the expression profiles of hexose transporters in *ird* mutants compared with wild type grown in optimal condition. As expected, we observed that the expression levels of the only *ght5* gene among these genes remarkably increase in *ird* mutants, indicating that *ght5* might be related to stress response, alongside the glucose transport. A recent study in *S. pombe* have drawn attention to the interaction between a transcription factor, Pap1 and oxidative stress transcription coactivator, Oxs1, and asserts that they regulate transcription of the *ght5* gene (11). In the study, Ght5, as a glucose transporter, was associated with stress response for the first time. In spite of the fact that the *ght5* gene is positively associated with Pap1 and negatively with Oxs1 (11), earlier we found that the expression level of *pap1* gene is elevated in *S. pombe* wild-type and *ird* mutants under H_2O_2-induced oxidative stress (20, 21). This difference between studies is thought to be due to the substance used as a stress source. In the former study (11), oxidative stress was induced by diamide, and in the latter ones (20, 21) by H_2O_2.

As a result, it is known that stress response pathways are suppressed by glucose repression (36, 37). In this study, under H_2O_2-induced oxidative stress, despite the presence of glucose, the upregulation of only *ght5* gene among all genes encoding hexose transporters and slowing down the percentage of glucose consumption brings to mind the link between glucose repression and stress response via *ght5*.

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Interest conflict
There is no conflict of interest.

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