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A potential protective role for thiamine in glucose-driven oxidative stress

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ABSTRACT. The relationship between glucose repression and the oxidative stress response was investigated in *Schizosaccharomyces pombe* wild type cells (*972h*$^-$) and glucose repression resistant mutant type cells (*ird11*). We aimed to reveal the mechanism of simultaneous resistance to glucose repression and oxidative stress in *ird11* mutants. Compared to the wild type, the expression of the *sty1* gene was not altered in the *ird11* mutant under normal growth conditions, but decreased after exposure to H_2O_2. This effect was clearly explained by the immunoblotting results, which showed elevated levels of a much more stable phosphorylated form of Sty1 mitogen-activated protein kinase in the *ird11* mutant. Increased *ght3* gene expression levels were also found, which may play a role in protecting the *ird11* mutant from the deleterious effects of oxidative stress. In addition, decreased expression levels of glycolytic enzyme enolase- and thiamine synthesis/transport-related genes were detected. This might have resulted from the flux redirection toward mitochondrial respiration, which would enhance NADPH generation to prevent the high reactive oxygen species accumulation that is generated by respiration. Some evidence supported a flux shift toward fermentation as well as respiration. We conclude that a defect in the glucose-sensing signaling pathway in

ird11 mutants likely causes erroneous low glucose-sensing signaling and high ATP production. This most likely occurs because high glucose availability in the medium induces an impairment in the respiratory chain and fermentation balance in these cells, which might explain the glucose repression and oxidative stress resistance in *ird11* compared to the wild type.

Key words: Oxidative stress; Glucose metabolism; Glucose transport; Thiamine; Hydrogen peroxide; *Schizosaccharomyces pombe*

INTRODUCTION

Glucose is an essential source of carbon and energy for many organisms. In eukaryotes, glucose is mainly assimilated through the respiration in mitochondria by being reduced to CO_2 and H_2O, thereby generating ATP for maximum energy yield in the presence of oxygen. However, the Crabtree-positive yeast species, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, undergo aerobic fermentation in which glucose is predominantly fermented to ethanol even in the presence of oxygen (Walker, 1998; Flores et al., 2000).

In *S. pombe*, glucose is sensed by a heterotrimeric G protein-coupled receptor, which generates a downstream signal through a cAMP-dependent protein kinase A (PKA) pathway (Hoffman, 2005). Subsequently, glucose uptake and glycolysis is induced (Rolland et al., 2001) and the use of alternative carbon sources, glyconeogenetic pathways, and respiration in mitochondria are repressed, eventually resulting in the accumulation of reactive oxygen species (ROS) (Carlson, 1999). Some of the genes related to glucose sensing and signaling are *git3*, *gpa2*, *git5*, *git11*, *git1*, *git7*, *git10* (Byrne and Hoffman, 1993; Nocero et al., 1994), and *cyr1*, encoding adenylate cyclase, *pka1*, encoding the PKA catalytic subunit, and *cgs1*, encoding the PKA regulatory subunit (Maeda et al., 1994). Heiland et al. (2000) identified six genes (*ght1-ght6*) encoding monosaccharide transporters. Among these, Ght1, Ght2, and Ght5 have substrate specificity for D-glucose, whereas the specificity of Ght6 is to D-fructose. Furthermore, the activity of Ght3 is optimal at low glucose concentration (0.2%), 2% D-gluconate, or 2% maltose. Recently, it was reported that glucose depletion or respiratory growth on non-fermentable carbon sources leads to an increased oxidative stress response (Chen et al., 2003; Madrid et al., 2004; Kim et al., 2006; Chen and Runge, 2009) and extends life span in *S. pombe* (Lin et al., 2002; Roux et al., 2009, 2010).

In *S. pombe*, the multistep phosphorelay system and the mitogen-activated protein kinase (MAPK) pathway govern the transcriptional regulation in response to oxidative stress, which is generated by the accumulation of ROS caused by both the incomplete reduction of O_2 during respiration and exposure to a variety of chemicals and metals. This cascade includes a conserved set of three protein kinases (MAPKKK, MAPKK, and MAPK), which triggers phosphorylation of Sty1 after exposure to stress, leading to nuclear localization of Sty1 (Marshall, 1994). MAPK Sty1 is a major regulator in the oxidative stress response, and is similar to mammalian p38 and HOG1 in *S. cerevisiae* (Shieh et al., 1997). Phosphorylated Sty1 phosphorylates two transcription factors, Atf1 and Pap1, to activate and to make them more stable; Atf1 is constitutively localized in the nucleus and is active even in absence of Sty1 (Wilkinson et al., 1996; Lawrence et al., 2007; Sansó et al., 2008). Atf1 can bind to a b-ZIP

small transcription factor, Pcr1, thus forming a heterodimer that can bind to stress response elements, and induces a set of genes that defend against some environmental stressors. Stress-response genes can be activated in both a Pcr1-dependent and Pcr1-independent manner by the binding of Atf1 to related DNA elements (Reiter et al., 2008). These transcription factors activate or induce the expression of anti-oxidant genes such as cytoplasmic catalase (*ctt1*), glutathione peroxidase (*gpx1*), thioredoxinreductase (*ttr1*), thioredoxin (*trx2*), neutral trehalaz ($ntp1^{+}$), glutathionreductase (*pgr1*), and superoxide dismutase SOD (*sod1*) (Feldmann, 2010).

In this study, the mechanism(s) of protection from the damaging effects of oxidative stress were investigated by using the resistant glucose repression *S. pombe* mutant (*ird11*) (Kig et al., 2005), which is affected by glucose signaling in a different manner than that caused by glucose deprivation (Palabiyik et al., 2012).

During our investigation into the possible relationship between glucose repression and the oxidative stress response, we made the unexpected discovery that these cells display the same characteristics as diabetic cells in response to glucose since the regulatory mechanisms of glucose usage are highly conserved between *S. pombe* and human cells. Although the glucose concentration in standard culture media for yeast is approximately 20-30 times higher than that in normal human blood, *S. pombe* cells optimally divide at the same conditions (Pluskal et al., 2011). Chen and Runge (2009) revealed that calorie restriction (0.1-0.3% glucose) promotes efficient cell cycle exit and extends longevity. For this reason, *S. pombe* cells may be used as a model organism to better understand the damaging effects of high glucose-induced oxidative stress in diabetic patients.

MATERIAL AND METHODS

Chemicals and reagents

Anti-phospho-p38 MAPK (Tyr182) polyclonal antibody and goat anti-rabbit IgG (horseradish peroxidase-conjugated) antibody were purchased from Thermo Scientific (USA). The ECL-Plus Western Blotting Detection system was purchased from Amersham (Piscataway, NJ, USA). Other reagents were obtained from Sigma-Aldrich (USA).

Yeast strains and media

Wild type *S. pombe* Lindner Liquifacience (972 h^{-}) and the glucose-repression-resistant mutant (*ird11*) (Kig et al., 2005) were used in this study. A selective medium consisting of 0.5% yeast extract, 3% sucrose, and 400 µg/mL 2-deoxy-D-glucose was prepared for the *ird11* mutant, whereas the wild type was cultured in standard rich medium (YEL).

RNA isolation

S. pombe cells were grown to the logarithmic phase in YEL at 30°C. The cultures of each strain were divided into two groups: a control and test group. For test groups, the 2 mM optical density $(OD)_{50}$ level, which was determined previously (Pekmez et al., 2008) in cells cultured in our department, was applied for 1 h. Total RNA was extracted from cells using the High Pure RNA Isolation Kit (Roche) in accordance with manufacturer instructions.

Real-time polymerase chain reaction (RT-PCR)

RT-PCR was performed with the Stratagene FastStart SYBR Green Master Kit (Roche) using the synthetic first-strand cDNA as a template following manufacturer instructions. Briefly, the reaction mixtures, consisting of 25 µL FastStart SYBR Green Master, 0.2 µM of each forward and reverse primers, 0.1 µg cDNA, were filled up to 50 µL with ultrapure nuclease-free water. The PCR conditions were as follows: 95°C for 10 min (pre-incubation), followed by 40 cycles of 95°C for 10 s, 53°C for 10 s, and 72°C for 20 s. The final step included a gradual temperature increase from 55 to 95°C at a rate of 1°C/10 s to enable melting curve data collection. A non-template control was run, and serial dilutions (1, 1:10, and 1:100) of the reference gene, actin (*act1*), and the target genes were included in every assay. Amplification specificity of each reaction was verified by melting curve analysis. Expression levels were normalized against the reference gene, *act1*. Relative gene expression levels were determined according to the method of Pfaffl (2001).

DNA microarray

For DNA microarray analysis, preparation of double stranded cDNA was carried out using the SuperScript Double-Stranded cDNA Synthesis Kit according to the NimbleGene Arrays User's Guide (Invitrogen). Fragmentation and Cy3 labeling, hybridization, washing, staining, scanning, and data normalization steps were carried out by GenMar (Izmir, Turkey). Finally, bioinformatic analysis was performed using the DNASTAR software, as recommended by NimbleGene.

Cell disruption

Exponentially grown wild type and *ird11* mutant cells under the assay conditions were harvested by centrifugation at 4000 *g* for 5 min at 4°C, and resuspended in 200 µL lysis buffer, containing 50 mM Tris buffer, pH 6.8, 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), 10% glycerol, 100 mM dithiothreitol, and protease inhibitor cocktail. The cells were disrupted with 0.5-mm acid-washed glass beads (Sigma) for 10 min at 60-s intervals, interspersed with periods of cooling in an ice-bath. Cellular debris was removed by centrifugation at 20,000 *g* for 5 min at 4°C. The supernatant was collected and protein concentrations of cell-free crude extracts were determined by the Bradford assay.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyses

For SDS-PAGE and Western blot analysis, protein extracts were denatured in sample buffer containing 25 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 10% 2-mercaptoethanol, and 0.002% bromphenol blue, and boiled for 3 min. Fifty micrograms protein per well were analyzed by 10% SDS-PAGE. After electrophoresis, proteins were transferred onto polyvinyldifluoride membranes using a BIO-RAD Semi-Dry apparatus. Membranes were blocked with 5% bovine serum albumin Fraction V (in Tris-buffered saline with Tween) at room temperature for 1 h. Primary antibody against phospho-p38 MAPK was used at 1:500 for 1 h at room

temperature. Peroxidase-conjugated goat anti-rabbit secondary antibody was used at 1:100 for 1 h at room temperature. Protein bands were detected by incubating the membranes with ECL-plus. Quantitative protein levels were evaluated with the ChemiDoc MP and ImageLab 4.0.1 software (BIO-RAD).

Statistical analysis

Data are reported as means ± SD with N denoting the number of experiments. Statistical comparisons were conducted using the one-way analysis of variance (ANOVA) module of the GraphPad Prism 5 statistical software. Differences in mean values were considered to be significant when $P < 0.05$.

RESULTS

Activation of Sty1 in oxidative stress-resistant mutant of *S. pombe* (*ird11*)

We compared the expression level of the *sty1* gene encoding MAPK Sty1p, which increases the expression of various stress-responsive genes through the activation of the Atf1p and Pap1p transcription factors in *S. pombe* (Madrid et al., 2004), both in *ird11* and wild type cells. In this study, no significant increase was observed in *sty1* expression in *ird11* under the non-stressed condition, whereas the expression level of *sty1* decreased in *ird11* under the stressed condition. The expression level of *sty1* was significantly increased in wild type cells under the stressed condition (Figure 1). Phosphorylated Sty1 was analyzed by immunoblotting with anti-phospho-p38 (Thr^{182}) in wild type and *ird11* mutant cells treated with and without H_2O_2 (Figure 2). The level of phosphorylated Sty1-MAPK increased under the non-stressed condition, whereas the level of this protein decreased dramatically under the stressed condition in *ird11*.

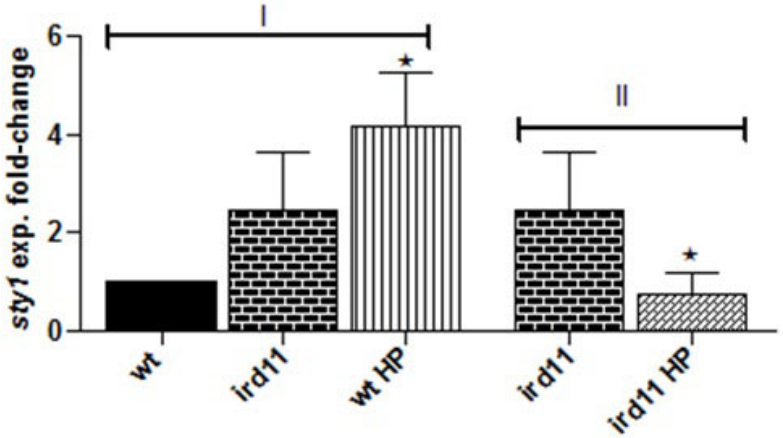

Figure 1. Expression level of the *sty1*gene relative to *act1* gene. Expression levels of the *sty1*gene in wild type and *ird11* mutant cells treated with H_2O_2 or untreated, after growth in rich media containing 2% glucose (repressed media), were determined according to the Pfaffl method. Significant differences between: (I) untreated wild type and untreated *ird11* and treated wild type cells, (II) untreated and treated *ird11* cells were evaluated by the Student *t*-test. *$P < 0.05$. wt = wild type; HP = treated with H_2O_2.

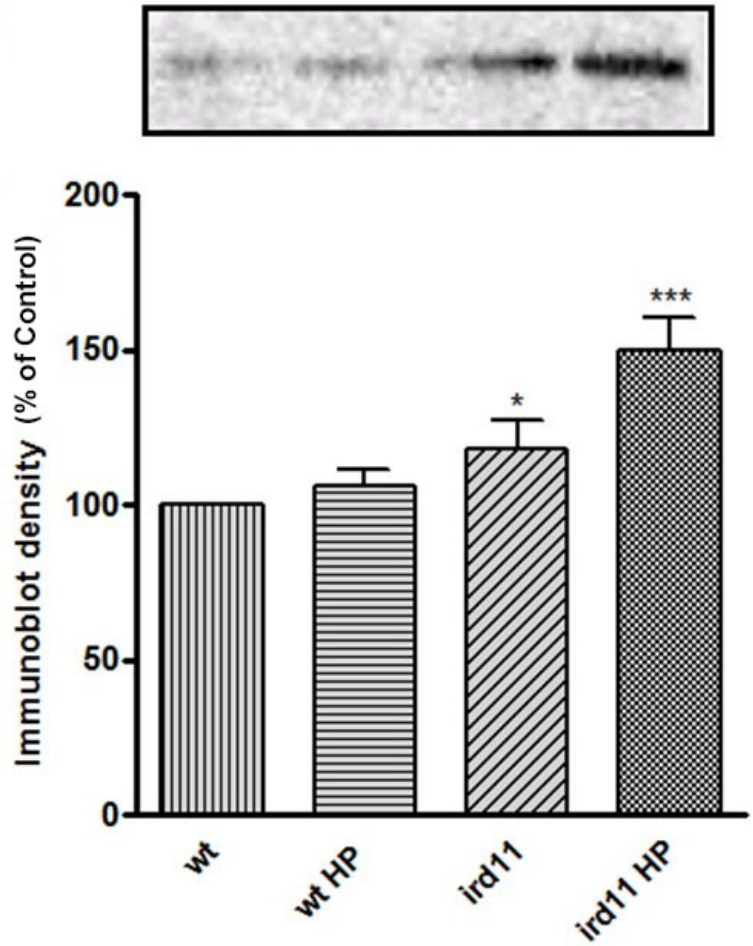

Figure 2. Detection of phosphorylated Sty1 by immunoblotting. Phosphorylated Sty1 protein was analyzed by immunoblotting with anti-phospho-p38 (Thr^{182}) as described in "experimental procedures" in wild type and *ird11* mutant cells treated with H_2O_2 or untreated, after grown in rich media containing 2% glucose (repressed media). All results were evaluated by the Student *t*-test: *P < 0.05; ***P < 0.0001. wt = wild type; HP = treated with H_2O_2.

Scanning of the glucose repression- and oxidative stress response-related genes in microarray data sets

Microarrays were used to follow the global changes in gene expression before and after exposure to H_2O_2 in *ird11* and wild type cells. In these cells, the expression levels of genes induced or repressed more than 2-fold were analyzed. Comparing *ird11* and wild type (*ird11*C/wtC), 88 genes were upregulated and 84 genes were downregulated. After exposure to H_2O_2, 152 genes were upregulated and 58 were downregulated (wtHP/wtC). In addition, by comparing *ird11* cells before and after stress conditions (*ird11*HP/*ird11*C), the upregulation of 28 genes and the downregulation of 91 genes were observed.

In order to investigate a possible relationship between glucose repression resistance and stability against oxidative stress in the *ird11* mutant, genes related to glucose sensing and signaling, carbohydrate metabolism, and the stress response were scanned in a microarray assay. There were no significant changes in the expression levels of genes related to glucose sensing through the cAMP signaling pathway, whereas decreased expression levels for 4 genes and increased expression of 1 gene related to overall signaling pathways were observed. Expression levels of two hexose transporters, Ght8 (located in the mitochondrial membrane) and Ght3, decreased by approximately 2-fold and increased by 5-fold, respectively. These findings confirmed the results of a previous study, which showed that changes in the *ght3* expression level were induced by low glucose concentration (0.2%), 2% D-glutathione, or 2% maltose (Heiland et al., 2000). Among the genes related to carbohydrate metabolism, downregulation of 8 genes along with upregulation of only 1 gene were detected. Furthermore, the upregulation of 7 genes and the downregulation of 1 gene that play roles in the purine and pyrimidine biosynthesis pathways were found. The GenBank accession numbers, description, and fold change in each gene are shown in Table 1.

Table 1. Changes in expression of glucose repression- and oxidative stress response-related genes.

Function	SEQ_ID	Name	wtHP/wtC	*ird11*C/wtC	*ird11*HP/*ird11*C
Signalling	SPAC4G8.13c	Calcineurin responsive transcription factor Prz1	0.396	0.496	1.771
	SPBC25B2.02c	M-factor transporter Mam1	0.348	0.451	1.342
	SPBP16F5.03c	SAGA complex phosphatidyl-inositol pseudokinase Tra1	0.811	0.488	1.267
	SPAC1006.09	MAP kinase kinase kinase Win1	0.727	0.456	1.687
	SPBC17A3.06	Phosphoprotein phosphatase	2.086	2.02	0.618
Hexose transporter	SPAC1F8.01	Hexose transporter Ght3	0.236	4.999	0.209
	SPCC548.06c	Hexose transporter Ght8	1.712	0.352	1.714
Carbohydrate metabolism	SPAC22F8.05	Alpha, alpha-trehalose-phosphate synthase	1.032	0.461	1.630
	SPAC513.02	Phosphoglycerate mutase family	2.304	0.482	3.446
	SPAC513.05	Alpha-mannosidase	0.808	0.285	2.213
	SPAC13F5.03c	Mitochondrial glycerol dehydrogenase Gld1	0.941	0.476	1.527
	SPBC8E4.04	Aldo/ketoreductase involved in pentose catabolism	0.902	0.475	1.698
	SPBPB21E7.01c	Enolase	1.138	0.367	2.340
	SPBC3E7.12c	Chitin synthase regulatory factor Chr1	0.322	0.442	1.718
	SPAC5H10.06c	Alcohol dehydrogenase Adh4	0.856	0.463	1.377
	SPBC354.12	Glyceraldehyde 3-phosphate dehydrogenase Gpd3	1.072	0.785	2.0162
	SPCC1259.09c	Pyruvatedehydrogenase protein x component, Pdx1	0.542	0.441	1.305
	SPAC1039.11c	Alpha-glucosidase	3.101	4.983	0.487
	SPBPB2B2.12c	UDP-glucose 4-epimerase/aldose 1-epimerase Gal10	1.361	2.224	1.309
	SPAC186.09	Pyruvatedecarboxylase	4.974	2.248	1.219
Purine/pyrimidine	SPCC1672.03c	Guaninedeaminase	1.126	0.486	1.429
	SPAC23A1.03	Adeninephosphoribosyltransferase	0.863	2.473	0.427
	SPAPYUG7.04c	DNA-directed RNA polymerase II complexsubunit Rpb9	0.313	1.393	0.460
	SPBC19C2.03	DNA-directed RNA polymerase I, II and III subunit Rpc10	0.853	1.384	0.478
	SPAC22A12.05	DNA-directed RNA polymerase III complexsubunit Rpc11	1.079	1.486	0.440
	SPBC19C2.03	DNA-directed RNA polymerase I, II and III subunit Rpc10	0.853	1.384	0.478
	SPAC19G12.04	Ureidoglycolatehydrolase	1.397	2.068	0.374

Expression levels of induced and repressed thiamine-related genes in microarray data sets

In this study, we found that the *thi3* gene, which encode no message in thiamine (Nmt1), was downregulated by 33.5-fold in *ird11* mutant cells compared to wild type cells,

whereas it was upregulated by approximately 31-fold in *ird11* cells after exposure to H_2O_2.

Thiamine biosynthesis and transport genes are directly influenced by the level of the thiamine pool available in the cytosol and the medium. Thiamine, as an enzymatic cofactor, regulates carbohydrate metabolism. In these microarray data sets, the 7 genes involved in thiamine metabolism were downregulated in *ird11* compared to the control group. All of these genes were upregulated in *ird11* after exposure to H_2O_2. Of these, the *thi3*/*mt1* gene was dramatically downregulated in *ird11* compared to the wild type, and was upregulated in *ird11* after exposure to H_2O_2. The list of genes and their relative expression changes in *ird11* under normal conditions and after exposure to H_2O_2 are shown in Table 2. Of these, the *thi3*-related gene was confirmed by quantitative RT-PCR (Figure 3). In qRT PCR, the level of *thi3* gene expression decreased by approximately 8-fold in *ird11* compared to the wild type, and it increased by approximately 120-fold in *ird11* after exposure to H_2O_2.

Table 2. Changes in expression of thiamine-related genes.

Function	SEQ_ID	Name	wtHP/wtC	*ird11*C/wtC	*ird11*HP/*ird11*C
Thiamine related genes	SPCC1223.02	"No message in thiamine", Nmt1 (thi3)	0.879	0.029	31.256
	SPBC26H8.01	Thiazole biosynthesis enzyme	1.291	0.221	3.867
	SPAC17A2.01	High-affinity import carrier for pyridoxine, pyridoxal, and pyridoxamine Bsu1	0.410	0.156	5.538
	SPBP8B7.18c	TENA/THI family protein	0.560	0.359	2.314
	SPBP4G3.02	Acid phosphatase Pho1	1.035	0.206	2.475
	SPCC18B5.05c	Phosphomethylpyrimidine kinase	0.715	0.453	2.493
	SPAC23H4.10c	Thiamine-phosphate dipyrophosphorylase/ hydroxyethylthiazole kinase	1.172	0.602	2.301

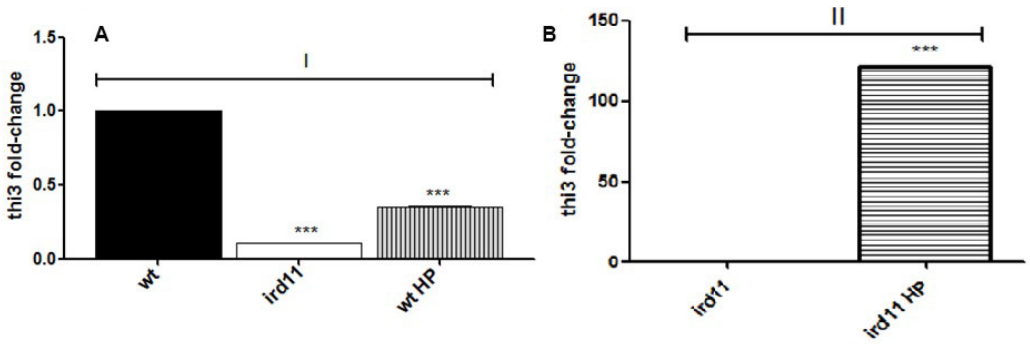

Figure 3. Expression levels of the *thi3* gene relative to the *act1* gene. Expression levels of the *thi3* gene in wild type and *ird11* mutant cells treated with/without H_2O_2 after grown in rich media containing 2% glucose (repressed media), were determined according to Pfaffl method. Significant differences between: **A.** untreated wild type and untreated *ird11* and treated wild type cells, **B.** untreated and treated *ird11* cells were evaluated by the Student *t*-test. **P < 0.001. wt = wild type; HP = treated with H_2O_2.

DISCUSSION

In yeast, the external glucose level controls the switch between respiration and fermentation. During aerobic conditions and high glucose concentration, *S. pombe* temporarily (during the exponential growth phase) produces CO_2 and ethanol. By contrast, in conditions

of low glucose concentration, respiration is much more highly activated, resulting in a sharp elevation in the level of ROS generation. The purpose of this study was to determine how *ird11* is resistant to glucose repression and oxidative stress. Our microarray results suggested that increased g*ht3* gene expression might play a role in the protection of the *S. pombe ird11* mutant from the deleterious effects of oxidative stress, as has been inferred in previous studies (Heiland et al., 2000; Madrid et al., 2004; Roux et al., 2010). This might clarify the lower glucose consumption rate (63%) in *ird11* compared to the wild type (Kig et al., 2005). We propose that in spite of the surplus glucose availability in the medium, the upregulation of *ght3*, which encodes the high affinity hexose transporter, may be due to erroneous starvation signaling. With the exception of *ght8* and a few of the genes listed in Table 1, no changes in the expression of other glucose-sensing signaling-related genes were observed in the microarray results.

In *S. pombe*, thiamine (vitamin B1) represses the mRNA synthesis of many genes involved in thiamine metabolism, including *thi2*, *thi3*, *thi4*, *pho4*, and *car1*. In addition, glucose starvation in the wild type drastically decreases the intracellular thiamine pool (Schweingruber et al., 1991). Considering the fact that thiamine acts as a vital cofactor at different stages of glucose metabolism, downregulation of the 7 genes associated with its transport and metabolism, such as *thi2*, *thi3*, *thi4*, and *car1/bsu1*, SPBP8B7.18c, and SPCC18B5.05c, may be partially explained by its potential role in the regulation of carbohydrate metabolism, growth rate, and ROS production.

Intriguingly, the microarray results showed the downregulation of 10 cDNA elements and the upregulation of 3 cDNA elements in *ird11* relative to the wild type, all of which are related to carbohydrate metabolism pathways (Table 1).

Taken together, our observations, such as the increase in *ght3* with the concurrent decrease in glycolytic enzyme enolase and thiamine synthesis/transport-related gene expression levels, may have resulted from the flux redirection toward mitochondrial respiration. Therefore, this might confer the ability to enhance NADPH generation in order to prevent the high ROS accumulation resulting from respiration. Since NADPH plays a key role as a cofactor in fatty acid synthesis, recycling steps through glutathione, thioredoxin, and peroxiredoxin pathways, its redox status is very important (Pollak et al., 2007). The reduced form of NADPH is supplied by the pentose phosphate pathway (PPP) (Slekar et al., 1996; Wamelink et al., 2008), and during stress conditions, it acts as a regulator of the expression level of some genes (Krüger et al., 2011). Ralser et al. (2007) confirmed that inactivation of GAPDH by ROS leads to redirection of the glucose flux to the PPP in order to generate more NADPH. Grüning and Ralser (2011) reported that low pyruvate kinase activity activates respiration without increasing intracellular ROS, and this adaptation is gained by stimulation of the PPP. Furthermore, our previous study showed that the reduced form of glutathione (GSH) significantly increased both in *ird11* compared to wild type cells grown in normal conditions and in *ird11* after exposure to H_2O_2 (Palabiyik et al., 2012).

The microarray results showed elevated levels of some purine and pyrimidine synthesis pathway-related genes. This result suggests that indeed, excessive glucose uptake by the *ird11* mutant is mainly redirected to the PPP, thereby allowing the generation of NADPH to sustain the antioxidant response, which is indispensable for cell survival. A strong effect (about 8-fold elevation) in the *fbp1* gene expression level found in *ird11* (Palabiyik et al., 2012) could be explained by activation of the gluconeogenetic pathway, which is induced by low glucose concentration sensing. In addition, the expression profiles of genes identified as *rpl302* and *mpg1* in the *ird11* mutant by using the differential display technique provided additional evidence of this effect, suggesting that the *ird11* mutant may be used as a convenient

model cell for studies on glucose sensing/signaling and the oxidative stress response pathways (Suslu et al., 2011).

On the other hand, although the expression level of *sty1*, which is involved in the MAPK pathway and is elicited by low glucose concentration sensing-signaling, was not altered in the *ird11* mutant compared to the wild type in normalgrowth conditions, it decreased after exposure to H_2O_2.

However, the immunoblotting results clearly showed that phosphorylated Sty1 MAPK, which is much more stable, is enhanced in the *ird11* mutant, and in line with this, our microarray results indicated an approximately 2-fold decrease in the *win1* gene expression level, which encodes Win1 MAPKKK and is located upstream of Sty1 MAPK.

Despite the low glucose concentration sensing-signaling, several lines of evidence suggested that there is a flux shift toward fermentation instead of respiration, including: 1) *ird11* grows at a similar rate to the wild type, 2) no significant change in *hxk2* gene expression compared to wild type (Palabiyik et al., 2012), 3) the downregulation of the *pdx1* gene encoding pyruvate dehydrogenase protein X component (Pdx1) and upregulation of the gene encoding pyruvate decarboxylase alongside the decreased expression of some mitochondrial genes, such as the mitochondrial glycerol dehydrogenase (Gld1) gene, *gld1* and the alcohol dehydrogenase (Adh4) gene, *adh4*, in *ird11* compared to the wild type.

The expression of *gld1* is regulated by glucose repression (Matsuzawa et al., 2010). Muller et al. (1999) reported that both of the pyruvate decarboxylase genes, PDC1 and PDC5, that are conserved among yeast, bacteria, and plants, were expressed in *S. cerevisiae* under thiamine limitation. The PDC5 (minor form) gene was particularly repressed by thiamine. The mammalian multienzyme pyruvate dehydrogenase complex is located in the mitochondria matrix and is regulated in numerous ways such as by thiamine, ATP, NADH, and acetyl-CoA (Strumiło, 2005), and by the concerted activity of two kinases and two phosphatases (Gey et al., 2008).

Shifting to fermentation leads to a decrease in Krebs cycle activity. Consequently, the thiamine level necessary for its activation decreases, resulting in decreased thiamine transport and biosynthesis-related gene expression. Nevertheless, because of the presence of a thiamine pool in the cytosol, the PPP would be enhanced. Together, these lines of evidence indicate that *ird11* cells behave as a double-edged sword.

ATP produced at a moderately high rate, alongside low glucose concentration sensing-signaling induced impairment of the respiratory chain and fermentation balance leading to behavior in opposite directions.

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