

1-1-2018

## Oxidative Stress Upregulates the Transcription of Genes Involved in ThiamineMetabolism

BURCU KARTAL

AHMET AKÇAY

BEDİA PALABIYIK

Follow this and additional works at: <https://journals.tubitak.gov.tr/biology>



Part of the [Biology Commons](#)

### Recommended Citation

KARTAL, B, AKÇAY, A, & PALABIYIK, B (2018). Oxidative Stress Upregulates the Transcription of Genes Involved in ThiamineMetabolism. *Turkish Journal of Biology* 42 (5): 447-452. <https://doi.org/10.3906/biy-1801-51>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Biology by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact [academic.publications@tubitak.gov.tr](mailto:academic.publications@tubitak.gov.tr)

## Oxidative Stress Upregulates the Transcription of Genes Involved in Thiamine Metabolism

Burcu KARTAL<sup>1,2</sup>, Ahmet AKÇAY<sup>1</sup>, Bedia PALABIYIK<sup>3,\*</sup>

<sup>1</sup>Department of Molecular Biology and Genetics, Institute of Graduate Studies in Science and Engineering, İstanbul University, İstanbul, Turkey

<sup>2</sup>Department of Genetics and Bioengineering, Faculty of Engineering, Alanya Alaaddin Keykubat University, Antalya, Turkey

<sup>3</sup>Department of Molecular Biology and Genetics, Faculty of Science, İstanbul University, İstanbul, Turkey

Received: 24.01.2018 • Accepted/Published Online: 06.08.2018 • Final Version: 25.10.2018

**Abstract:** Thiamine is a major vitamin that acts as a cofactor in energy metabolism in all organisms, as well as in lipid and amino acid metabolisms, and is associated with many diseases. It is known that glucose starvation decreases the intracellular thiamine pool while increasing oxidative stress tolerance. Earlier, in whole genome analysis, we detected major differences in the expression of genes related to thiamine pathway against oxidative stress in *Schizosaccharomyces pombe*. We investigated the effects of oxidative stress and glucose repression to thiamine pathway in *S. pombe* by comparing some genes encoding key enzymes of each related pathway at the transcription level. In the present study, we found that the expression of genes related to thiamine biosynthesis and transport (*thi2*, *thi3*, and *pho1*) increased in wild type and *ird11* cells grown in thiamine-rich media under oxidative stress induced by H<sub>2</sub>O<sub>2</sub>. Based on our findings, we suggested that there might be an important effect of oxidative stress on thiamine biosynthesis and transport.

**Key words:** Fission yeast, thiamine, oxidative stress, glucose metabolism

### 1. Introduction

Thiamine diphosphate, the biological active form of thiamine (vitamin B<sub>1</sub>), is a cofactor for many enzymes (pyruvate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase,  $\alpha$ -ketoacid dehydrogenase, transketolase, and pyruvate decarboxylase) in universal metabolic pathways such as glycolysis, pentose phosphate pathway, and tricarboxylic acid cycle (TCA) (Friedrich, 1987).

Yeast synthesizes 5-(2-hydroxyethyl)-4-methylthiazole phosphate (HET-P) and 4-amino-5-hydroxymethyl-2-methylpyrimidine diphosphate (HMP-PP) precursors separately in thiamine biosynthesis, and the subsequent HET-P and HMP-PP unite to form ThMP with thiamine phosphate synthase (Kowalska and Kozik, 2008). In eukaryotes, ThMP is first converted to free thiamine, and then the free thiamine is converted to ThDP (Murata, 1982). Dephosphorylation is essential for extracellular thiamine phosphates to be transported through the cell membrane (Kowalska and Kozik, 2008).

Various defense mechanisms (oxidative stress response) have been developed for eliminating oxidative stress that arises from the increase of reactive oxygen species that are stable within the cell. Sty1p, the stress-activat-

ed MAPK, is a major regulator in stress response (Shiozaki and Russell, 1996; Shieh et al., 1997) and triggers the global stress response via stimulating Atf1p and Pap1p transcription factors. Activated transcription factors stimulate the expression of genes that encode oxidative stress response proteins such as glutathione peroxidase (Gpx1), neutral trehalose (Ntp1), cytoplasmic catalase (Ctt1), thioredoxin reductase (Trr1), and superoxide dismutase (Sod1) (Toone et al., 1998; Mutoh et al., 2002).

Using microarray technology earlier, we found global changes in the gene expressions in response to oxidative stress induced by H<sub>2</sub>O<sub>2</sub> in wild type and *ird11* mutant (resistant to glucose repression and oxidative stress) cells grown in optimal conditions (Kig et al., 2005; Palabiyik et al., 2012). Because of the involvement of some of these genes in thiamine metabolism (Palabiyik et al., 2014), we aimed to investigate the relationship between oxidative stress, glucose metabolism, and thiamine metabolism. Expression levels of thiamine metabolism, glucose metabolism, and stress response pathways genes were measured with quantitative real-time PCR (qRT-PCR) at a level of transcription, in both normal and hydrogen peroxide-induced oxidative stress conditions with/without thiamine.

\* Correspondence: bediag@istanbul.edu.tr

## 2. Materials and methods

### 2.1. Growth conditions and *S. pombe* strains

*S. pombe* Lindner liquefaciens (wild type, 972*h*) strain and *ird11* mutant (Kig et al., 2005) were cultivated till midlogarithmic phase in minimal media (MML) with/without thiamine (Gutz et al., 1974). 2 mM H<sub>2</sub>O<sub>2</sub> was applied as an oxidative stress agent for 1 h at 30 °C (Bayram et al., 2008).

### 2.2. Total RNA isolation

Total RNA from control and experimental groups were acquired using a commercial kit (High Pure RNA Isolation Kit, Roche) according to the manufacturer's recommendations. Spectrophotometrical measurements of RNA samples at 260 nm were calculated via  $\mu\text{g/mL} = \text{A}_{260} \times \text{dilution factor} \times 40$  formulae (Sambrook, 1989). RNA samples were separated into amounts of 20  $\mu\text{L}$  and stored at  $-70$  °C.

### 2.3. cDNA synthesis

cDNA synthesis from RNA molecules was done with a commercial kit (Transcriptor High Fidelity cDNA Synthesis Kit, Roche) according to the manufacturer's instructions; cDNA samples were stored at  $-20$  °C.

### 2.4. Real-time PCR

Variation in expression profiles of the genes of interest under different conditions was determined with quantitative RT-PCR that is based on "SYBR green". Fast Start SYBR Green Master Kit (Roche) and "Roche, 480" were used according to the manufacturer's instructions. Gene-specific primers were designed at Primer 3 program and are listed in Table. *S. pombe* actin gene (*act1*) was used as a reference gene for normalization of the results (Xue-Franzen et al., 2006).

PCR was carried out under the following conditions: 95 °C for 10 min (preincubation), followed by 45 cycles of 95 °C for 10 s, 53 °C for 7 s, and 72 °C for 5 s.

### 2.5. Statistical analysis

The variation in gene expression level (R) was calculated according to Pfaffl equation (Pfaffl, 2001), which is based on the proportion of crossing threshold (Ct) measured for target and reference genes depending on the yield at each reaction (E). Statistical analysis of experiment results was done with "Graphpad Ver.5" software.

$$R = \left( E_{\text{target gene}} \right)^{\Delta C_{\text{t target gene (control-test)}}} / \left( E_{\text{reference gene}} \right)^{\Delta C_{\text{t reference gene}}}$$

## 3. Results

### 3.1. The effect of thiamine on the expression of genes related to thiamine metabolism

Expression profiles of genes that are included in thiamine intake and biosynthesis pathway were examined in *S. pombe* 972*h* wild type and *ird11* mutant strains grown in thiamine-rich media (Figure 1). The expression of *thi2* and *thi3* genes was downregulated (87.29', 78.06', respectively) in the wild type, as expected (Figure 1A), while in *ird11*

strain the expression of *thi3* did not change, but *thi2* and *pho1* genes were upregulated (1.69', 2.55', respectively) (Figure 1B).

### 3.2. Thiamine-mediated glucose metabolism and stress response pathway

The expression profiles of genes involved in glucose metabolism and stress response pathways were examined in *S. pombe* 972*h* wild type and *ird11* mutant strains grown in thiamine-rich media, using qRT-PCR (Figure 2). It was determined that the *fbp1* gene encoding phosphofructokinase 1 enzyme, which is a gluconeogenic enzyme, was downregulated, although not statistically significantly in both of the strains at the presence of thiamine. No significant variation was observed in the expression of the *hck2* gene encoding hexokinase 2 enzyme, which starts glycolytic flow. It is suggested that there is an optimal glycolytic flow in both of the strains in all growth media. The transcription of stress response genes (*sty1*, *sod1*, and *ctt1*) did not change in both strains in the absence and presence of thiamine (Figures 2A and 2B). Only the expression of the *sod1* gene, which encodes the superoxide dismutase 1 enzyme in *ird11*, was increased (2.48') in thiamine-rich media relative to thiamine-free media (Figure 2B).

### 3.3. Activation of thiamine metabolism related genes under oxidative stress

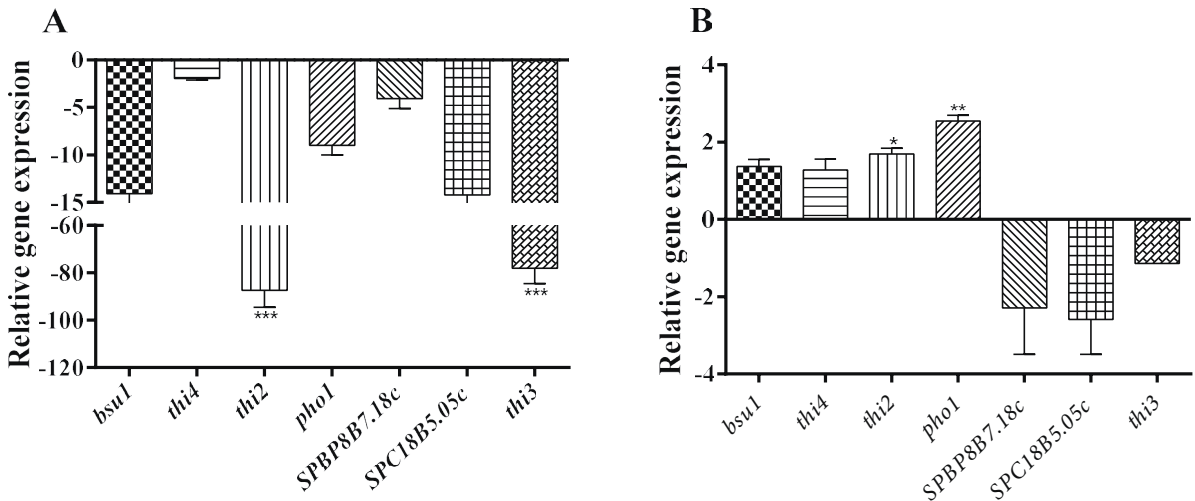
Expression levels of genes encoding main enzymes involved in oxidative stress response pathways, thiamine metabolism, and glucose metabolism were determined at the level of transcription after exposing *S. pombe* wild type and *ird11* cells to oxidative stress (2 mM H<sub>2</sub>O<sub>2</sub>, 1 h) in thiamine-rich media (Figure 3). No significance was observed in the expression of genes related to glucose metabolism and oxidative stress response pathway in both strains. In wild type, a significant increase was observed only in the expression of the *pho1* gene encoding acid phosphatase, which allows thiamine to be introduced into the cell (Figure 3A). On the other hand, the expression of the *thi2* and *thi3* genes, which encode thiazole biosynthetic enzyme (participates in the synthesis of the thiazole ring) and 4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate synthase enzyme (participates in the synthesis of the pyrimidine ring), respectively, and also the expression of *pho1* gene were upregulated in *ird11* as if thiamine was absent in the media (Figure 3B).

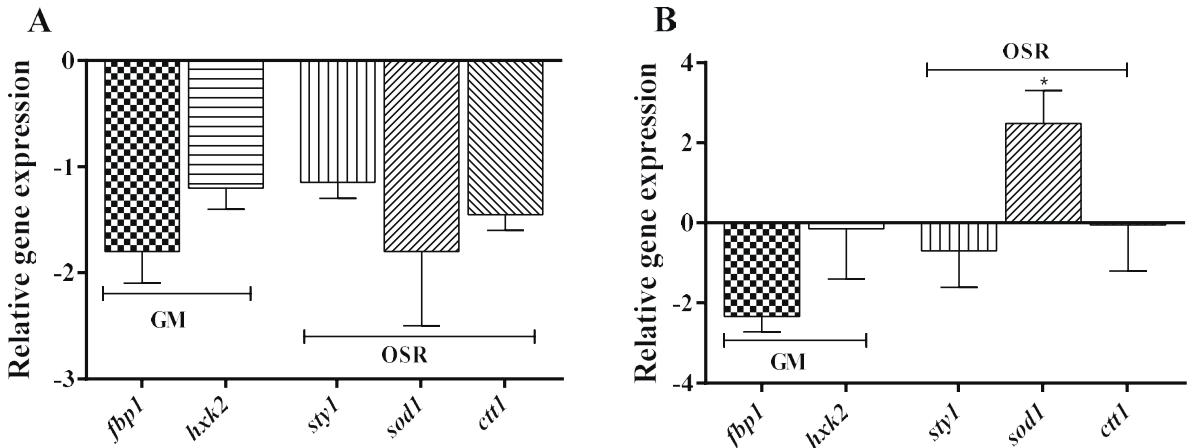
## 4. Discussion

There are many critical diseases occurring due to thiamine deficiency with little progress in diagnosis and treatment. Thiamine deficiency increases oxidative stress in neurodegenerative diseases such as Alzheimer, Parkinson, Huntington, and Wernicke-Korsakoff syndrome; furthermore, thiamine-dependent enzymes are more sensitive to oxidative stress (Gibson et al., 1999; Lin and Beal, 2006; Hazell

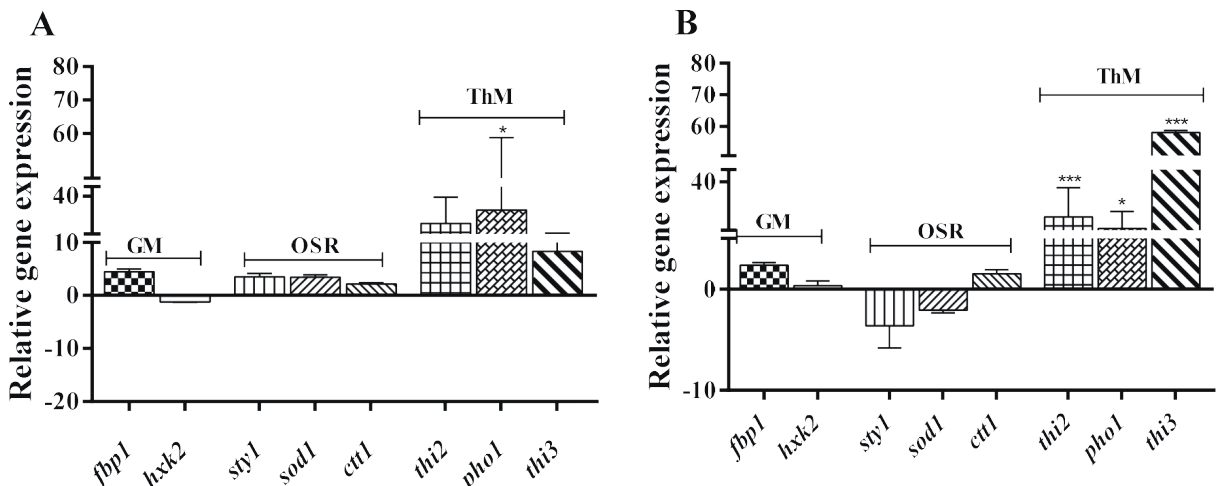
**Table.** Primers used throughout the study. (<sup>1</sup>: Forward sequence; <sup>2</sup>: Reverse sequence).

Group	SEQ_ID	Name	Primer sequence (5'-3')
Reference gene	SPBC32H8.12c	Actin / <i>act1</i>	AGATTCTCATGGAGCGTGGT <sup>1</sup> TCAAAGTCCAAAGCGACGTA <sup>2</sup>
Thiamine metabolism	SPAC17A2.01	High-affinity import carrier for pyridoxine, pyridoxal, and pyridoxamine / <i>bsu1</i>	GCCCCGTTTACTTTTGTTC <sup>1</sup> GCAACACCGATGATGAAATG <sup>2</sup>
	SPAC23H4.10c	Bifunctional thiamine-phosphate diphosphorylase/hydroxyethylthiazole kinase / <i>thi4</i>	GTGATGGGTGTAACGGCTTC <sup>1</sup> GAGTTTTTCGCTTCCACTGC <sup>2</sup>
	SPBC26H8.01	Thiazole biosynthetic enzyme / <i>thi2</i>	CCCATTGTTGTTTCTGCT <sup>1</sup> CGCATGTCGTGAAGTTAGA <sup>2</sup>
	SPBP4G3.02	Acid phosphatase / <i>pho1</i>	AGCATTGACTTTCCACCAC <sup>1</sup> ATTCCAACAGCATCGAAAGC <sup>2</sup>
	SPBP8B7.18c	Phosphomethylpyrimidine kinase (predicted)	GCAGCCCTGAAATCGTTAAG <sup>1</sup> CGAGAGAATCCCCAGAAGT <sup>2</sup>
	SPCC18B5.05	Phosphomethylpyrimidine kinase (predicted)	GACGGCCGATCTGATTTATG <sup>1</sup> TGGCAGCTGTAAGAGAGCAA <sup>2</sup>
	SPCC1223.02	4-amino-5-hydroxymethyl-2-methylpyrimidine phosphate synthase / <i>nmt1</i>	TCCCCAGAGATTGGAACAAG <sup>1</sup> GGTCAAGTTCCCAGGTCAAG <sup>2</sup>
Glucose metabolism	SPBC1198.14c	Fructose-1,6-bisphosphatase / <i>fbp1</i>	GTATGGTGCTTCGGCTCATT <sup>1</sup> TTCATGTTTCGATGGGTCAA <sup>2</sup>
	SPAC4F8.07c	Hexokinase 2 / <i>hvk2</i>	CAACAAGGACTTTGCCCAAT <sup>1</sup> AAGGTGTCGCTCTCCTTTGA <sup>2</sup>
Stress response	SPAC24B11.06c	MAP kinase / <i>sty1</i>	TGTTTCATTCTGCCGGTGTTA <sup>1</sup> GAATACGAGCCAAACCGAAA <sup>2</sup>
	SPAC821.10c	Superoxide dismutase / <i>sod1</i>	ATTGGCCGTACCATTGTCAT <sup>1</sup> GACACCACAAGCGTTACGTG <sup>2</sup>
	SPCC757.07c	Catalase / <i>ctt1</i>	ATCCTCAATCCGACCACTG <sup>1</sup> AACGTCGGTAATTTCTGTTCA <sup>2</sup>

**Figure 1.** Relative expression profiles of genes related to thiamine metabolism. The expression of genes related to thiamine pathway in *S. pombe* cells growing in thiamine-rich media calculated relative to thiamine-free media. **A.** *S. pombe* wild type; **B.** *S. pombe ird11* mutant strain. Statistical analysis of experiment results was done with "Graphpad Ver.5" software. Error bars represent standard deviation of three experimental replicates. (Dunnet's test,  $P < 0.05^*$ ,  $P < 0.01^{**}$ ,  $P < 0.001^{***}$ ).



**Figure 2.** Relative expression profiles of genes related to glucose metabolism and oxidative stress response pathways. Expression of genes of interest in *S. pombe* cells growing in thiamine-rich media was calculated relative to thiamine-free media. **A.** *S. pombe* wild type; **B.** *S. pombe ird11* mutant strain. Statistical analysis of experiment results was done with “Graphpad Ver.5” software. Error bars represent standard deviation of at least two experimental replicates. (Dunnet’s test,  $P < 0.05^*$ ). GM: glucose metabolism; OSR: oxidative stress response.



**Figure 3.** Relative expression profiles of genes related to thiamine metabolism, glucose metabolism, and oxidative stress response pathways under oxidative stress. After *S. pombe* cells grown until midlogarithmic phase were exposed to 2 mM hydrogen peroxide, inducing oxidative stress in presence of thiamine, expression profiles of the genes were calculated relative to stress-free condition. **A.** *S. pombe* wild type; **B.** *S. pombe ird11* mutant strain. Statistical analysis of experiment results was done with “Graphpad Ver.5” software. Error bars represent standard deviation of at least three experimental replicates. (Dunnet’s test,  $P < 0.05^*$ ,  $P < 0.001^{***}$ ). GM: glucose metabolism; OSR: oxidative stress response; ThM: thiamine metabolism.

et al., 2010; Jhala and Hazell, 2011). It is also known that thiamine has an important role in signal transduction, immune system activation, and signaling in animal cells (Manzetti et al., 2014). It is believed that determining the expression profiles of genes responsible for thiamine biosynthesis and transport might lead to research into the diagnosis and treatment of the diseases. In this study, we in-

vestigated the relationship between thiamine metabolism, oxidative stress response, and glucose metabolism in both *S. pombe* wild type and the *ird11* mutant (Kig et al., 2005; Palabiyik et al., 2012).

The expression of genes related to biosynthesis and transport of thiamine is completely suppressed by thiamine in the media, whereas in the absence of thiamine

it is known that their expression increases at a high level (Maundrell, 1990; Praekelt et al., 1994; Nosaka, 2006). Besides, it has been reported that in *S. pombe*, thiamine represses the mRNA synthesis of many genes that are involved in their own metabolism, such as *thi2*, *thi3*, *thi4*, *pho4*, and *car1* (Schweingruber et al., 1991). It has been shown that with microarray analysis, in the absence of thiamine in *S. cerevisiae*, the expression of THI5, THI12 (ortholog of *S. pombe thi3* gene), and THI4 (ortholog of *S. pombe thi2* gene) genes were significantly increased (Nosaka et al., 2005). It was confirmed in the present study by the remarkable decrease (approximately 80-fold) in the expression of *thi3* and *thi2* genes in *S. pombe* wild type cells growing in the presence of thiamine, compared to the expression of other genes associated with thiamine biosynthesis and transport (Figure 1A).

In the presence of thiamine, the fact that the *fbp1* gene in both strains was downregulated suggested continuing glucose repression (Figures 2A and 2B). Likewise, the fact that no significant decrease was observed in stress response genes expression (*styl1*, *sod1*, and *ctt1*) in the wild type under thiamine-rich conditions (Figure 2A) suggests that the stress response is not affected by environmental thiamine. However, differences in the expression profiles of genes related to thiamine and oxidative stress response pathways between *ird11* and wild type cells might result from a lack of glucose repression in *ird11* (Figures 1B and 2B).

Palabiyik et al. (2014) have shown the relationship between oxidative stress and thiamine metabolism in microarray analyses, indicating that the expression of the *thi3* gene is 33.5-fold less than wild type in *ird11*, but 31-fold higher in *ird11* exposed to H<sub>2</sub>O<sub>2</sub>. We determined an increased expression of genes involved in thiamine biosyn-

thesis and transport (*thi2*, *thi3*, and *pho1*) when *ird11* and wild type cells were exposed to H<sub>2</sub>O<sub>2</sub> (Figure 3). Therefore, it is suggested that *S. pombe* cells need thiamine in defense mechanisms developed against oxidative stress. Recently, it has been reported that osmotic and oxidative stresses elevate the transcription of thiamine biosynthesis genes in oil palm (*Elaeis guineensis*) (Yee et al., 2016; Idris et al., 2018).

Consequently, the fact that expression of genes related to thiamine biosynthesis and transport (*thi2*, *thi3*, and *pho1*) increased when wild type cells were exposed to H<sub>2</sub>O<sub>2</sub> (Figure 3A), while the expression of these genes decreased under thiamine-rich conditions (Figure 1A) in this study, suggests that oxidative stress upregulates thiamine metabolism to protect the cellular balance in fission yeast. This conclusion is supported by the findings obtained from *ird11*, which is resistant to glucose repression and oxidative stress (Figures 1B and 3B). Also, it has been reported that thiamine is involved in the stabilization of the redox levels of cells throughout the production of NADPH and glutathione during oxidative stress response and protects the tissues against oxidative damage via reduced NADP<sup>+</sup> (Depeint et al., 2006; Gioda et al., 2010). Besides, it has been put forward that in *S. cerevisiae* cells, free radical levels and protein oxidation have decreased under different stress conditions in the presence of thiamine (Wolak et al., 2014). Based on our findings, we hypothesize that thiamine pathway is affected by oxidative stress.

### Acknowledgments

This research was funded by the Scientific Research Project Coordination Unit of İstanbul University, project ID: 28781, and by The Scientific and Technological Research Council of Turkey, project number: 1919B011403361.

### References

- Bayram T, Pekmez M, Arda N, Yalçın AS (2008). Antioxidant activity of whey protein fractions isolated by gel exclusion chromatography and protease treatment. *Talanta* 75: 705-709. doi: 10.1016/j.talanta.2007.12.007.
- Depeint F, Bruce WR, Shangari N, Mehta R, O'Brien PJ (2006). Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism. *Chem-Biol Interact* 163: 94-112. doi: 10.1016/j.cbi.2006.04.014.
- Friedrich W (1987). *Hanbuch der Vitamine*. Munich, Germany: Urban & Schwarzenberg (in German).
- Gibson GE, Park LC, Zhang H, Sorbi S, Calingasan NY (1999). Oxidative stress and a key metabolic enzyme in Alzheimer brains, cultured cells, and an animal model of chronic oxidative deficits. *Ann NY Acad Sci* 893: 79-94.
- Gioda CR, de Oliveira Barreto T, Primola-Gomes TN, de Lima DC, Campos PP, Capettini Ldos S, Louton-Santos S, Vasconcelos AC, Coimbra CC, Lemos VS et al. (2010). Cardiac oxidative stress is involved in heart failure induced by thiamine deprivation in rats. *Am J Physiol-Heart C* 298: 2039-2045. doi:10.1152/ajpheart.00820.2009.
- GutzH, HeslotH, LeupoldU, LoprienoN (1974). *Schizosaccharomyces pombe*. In: King RC, editor. *Handbook of Genetics*. New York, NY, USA: Plenum Press, pp. 395-446.
- Hazell AS, Sheedy D, Oanea R, Aghourian M, Sun S, Jung JY, Wang D, Wang C (2010). Loss of astrocytic glutamate transporters in Wernicke encephalopathy. *Glia* 58: 148-156. doi:10.1002/glia.20908.

- Idris ZHC, Abidin AAZ, Subki A, Yusof ZNB (2018). The effect of oxidative stress towards the expression of thiamine biosynthesis genes (THIC and THI1/THI4) in oil palm (*Elaeis guineensis*). *Tropical Life Sciences Research* 29: 71-85. doi:10.21315/tlsr2018.29.1.5.
- Jhala SS, Hazell AS (2011). Modeling neurodegenerative disease pathophysiology in thiamine deficiency: consequences of impaired oxidative metabolism. *Neurochem Int* 58: 248-260. doi: 10.1016/j.neuint.2010.11.019.
- Kig C, Turkel S, Temizkan G (2005). Isolation and characterization of glucose derepressed invertase mutants from *Schizosaccharomyces pombe*. *Bioscience, Biotechnology, and Biochemistry* 69: 2475-2478. doi:10.1271/bbb.69.2475.
- Kowalska E, Kozik A (2008). The genes and enzymes involved in the biosynthesis of thiamin and thiamin diphosphate in yeasts. *Cell Mol Biol Lett* 13: 271-282. doi:10.2478/s11658-007-0055-5.
- Lin MT, Beal MF (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 443: 787-795. doi:10.1038/nature05292.
- Manzetti S, Zhang J, van der Spoel D (2014). Thiamin function, metabolism, uptake, and transport. *Biochemistry-US* 53: 821-835. doi:10.1021/bi401618y.
- Maundrell K (1990). nmt1 of fission yeast. A highly transcribed gene completely repressed by thiamine. *J Biol Chem* 265: 10857-10864.
- Murata K (1982). Actions of two types of thiaminase on thiamin and its analogues. *Ann NY Acad Sci* 378: 146-156.
- Mutoh N, Nakagawa CW, Yamada K (2002). Characterization of Cu, Zn-superoxide dismutase-deficient mutant of fission yeast *Schizosaccharomyces pombe*. *Curr Genet* 41: 82-88. doi:10.1007/s00294-002-0288-9.
- Nosaka K (2006). Recent progress in understanding thiamin biosynthesis and its genetic regulation in *Saccharomyces cerevisiae*. *Appl Microbiol Biot* 72: 30-40. doi:10.1007/s00253-006-0464-9.
- Nosaka K, Onozuka M, Konno H, Kawasaki Y, Nishimura H, Sano M, Akaji K (2005). Genetic regulation mediated by thiamin pyrophosphate-binding motif in *Saccharomyces cerevisiae*. *Mol Microbiol* 58: 467-479. doi:10.1111/j.1365-2958.2005.04835.x.
- Palabiyik B, Ghods FJ, Ucar EO (2014). A potential protective role for thiamine in glucose-driven oxidative stress. *Gen Mol Res* 13: 5582-5593. doi: 10.4238/2014.July.25.13.
- Palabiyik B, Kig C, Pekmez M, Dalyan L, Arda N, Temizkan G (2012). Investigation of the relationship between oxidative stress and glucose signaling in *Schizosaccharomyces pombe*. *Biochem Genet* 50: 336-349. doi:10.1007/s10528-011-9477-x.
- Pfaffl MW (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29: e45.
- Praekelt UM, Byrne KL, Meacock PA (1994). Regulation of THI4 (MOL1), a thiamine-biosynthetic gene of *Saccharomyces cerevisiae*. *Yeast* 10: 481-490. doi:10.1002/yea.320100407.
- Sambrook J, Fritsch EF, Maniatis T (1989). *Molecular Cloning: A Laboratory Manual*. 2nd ed. New York, NY, USA: Cold Spring Harbor Laboratory Press.
- Schweingruber AM, Dlugonski J, Edenharter E, Schweingruber ME (1991). Thiamine in *Schizosaccharomyces pombe*: dephosphorylation, intracellular pool, biosynthesis and transport. *Curr Genet* 19: 249-254.
- Shieh JC, Wilkinson MG, Buck V, Morgan BA, Makino K, Millar JB (1997). The Mcs4 response regulator coordinately controls the stress-activated Wak1-Wis1-Sty1 MAP kinase pathway and fission yeast cell cycle. *Gene Dev* 11: 1008-1022.
- Shiozaki K, Russell P (1996). Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast. *Gene Dev* 10: 2276-2288.
- Toone WM, Kuge S, Samuels M, Morgan BA, Toda T, Jones N (1998). Regulation of the fission yeast transcription factor Pap1 by oxidative stress: requirement for the nuclear export factor Crm1 (Exportin) and the stress-activated MAP kinase Sty1/Spc1. *Gene Dev* 12: 1453-1463.
- Wolak N, Kowalska E, Kozik A, Rapala-Kozik M (2014). Thiamine increases the resistance of baker's yeast *Saccharomyces cerevisiae* against oxidative, osmotic and thermal stress, through mechanisms partly independent of thiamine diphosphate-bound enzymes. *FEMS Yeast Res* 14: 1249-1262. doi:10.1111/1567-1364.12218.
- Xue-Franzen Y, Kjaerulff S, Holmberg C, Wright A, Nielsen O (2006). Genome wide identification of pheromone-targeted transcription in fission yeast. *BMC Genomics* 7: 303. doi:10.1186/1471-2164-7-303.
- Yee WS, Aziz SDA, Yusof ZNB (2016). Osmotic stress upregulates the transcription of thiamine (vitamin B1) biosynthesis genes (THIC and THI4) in oil palm (*Elaeis guineensis*). *Afr J Biotechnol* 15: 1566-1574. doi:10.5897/AJB2016.15222.