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Research Article

TRANSCRIPTOMIC ANALYSIS OF GLYCOGEN METABOLISM AFTER CAFFEINE TREATMENT IN *Saccharomyces cerevisiae*

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ABSTRACT. *Saccharomyces cerevisiae* is used as a model organism for investigating the metabolic and genetic regulations in higher organisms. Glycogen is a storage carbohydrate in yeast cells and is used as an energy source. Glycogen is accumulated at the onset of the stationary phase and the logarithmic phase during different environmental and intracellular stress conditions. Glycogen level in the cell is regulated by different sensory and signaling pathways. TOR signaling pathway is activated when the environmental conditions are suitable, but it is repressed after rapamycin/caffeine treatment or nutrient starvation. In this study, the effect of the TOR signaling pathway on glycogen metabolism was determined with the Next Generation Sequencing method in *S. cerevisiae*. The total RNA isolated from yeast cells grown in a medium containing caffeine was used for the Next Generation Sequencing analysis. The differentially expressed genes after caffeine treatment were determined by comparing the caffeine-treated cells to untreated cells. It was determined that the transcription of 44% of the genes was expressed differentially after caffeine treatment, and 20% of differentially expressed genes were found to be up-regulated. The transcription of genes involved in glycogen metabolism, except *PGM1*, was up-regulated after caffeine treatment. The inactivation of Tor1p caused to increase in *PGM2*, *UGP1*, *GLG1*, *GSY1*, *GSY2*, *GLC3*, *GPH1*, and *GDB1* transcription at least 2 fold. *In silico* analysis revealed that these genes include at least one STRE sequence in their promoter regions for binding of Msn2/4 transcription factors. It was observed that after caffeine treatment *MSN2* transcription was down-regulated while *MSN4* transcription was upregulated. This indicates that the Msn4 transcription factor was more effective than Msn2p in up-regulating genes in glycogen metabolism. In conclusion, repression of the TOR signaling pathway by caffeine causes Msn4p-dependent transcriptional activation of genes involved in glycogen metabolism.

Keywords: *Caffeine, Glycogen, TOR pathway, Saccharomyces cerevisiae*

INTRODUCTION

S. cerevisiae yeast species is the first unicellular eukaryotic organism whose genome was sequenced. The genome of *S. cerevisiae* consists of 16 chromosomes and is approximately 13,392 kb in size. Of the 6275 genes, 5800 are thought to be functional. Having its entire genome sequenced provides a great opportunity to study various cellular processes. Glycogen is a polysaccharide containing α-1,4- in a straight chain of glucose subunits and α-1,6-glycosidic bonds at branching points. In *S. cerevisiae* yeast cells, the amount of glycogen in the cell varies according to the physiological state of the cells, growth phases, and conditions. Reserve carbohydrates can constitute 1-25% of the dry weight of yeast cells, depending on environmental conditions and growth phases

[1]. Glycogen is accumulated in limited growth conditions (lack of nitrogen, phosphorus, sulfur, biotin, etc.) and at the end of the logarithmic stage [2]. Glycogen synthesis starts with the conversion of glucose 6-phosphate to glucose 1-phosphate catalyzed by phosphoglucomutase, encoded by *PGM1* and *PGM2*. UDP-glucose (UDPG) is synthesized from uridine triphosphate (UTP) and glucose-1-phosphate by the action of UDPG pyrophosphorylase encoded by *UGP1*. The glycogenin protein is a self-glycosylated initiator protein and has glycosyltransferase activity encoded by *GLG1* and *GLG2*. Glycosyltransferase enzyme transfers glucose from UDPG to the glycogenin with α-1,4-glycosidic bond, up to 8-10 glucose. The oligosaccharide including the glycogenin molecule continues to be synthesized by the activity of the glycogen synthase enzyme encoded by *GSY1* and *GSY2* and the branching enzyme encoded by the *GLC3* gene (Fig. 1) [3]. Degradation of glycogen into glucose 1-phosphate and free glucose occurs by the activity of glycogen phosphorylase (Gph1p) and debranching enzymes (Gdb1p), respectively. In addition, glycogen is reduced to non-phosphorylated glucose by the activity of the vacuolar glucoamylase enzyme encoded by *SGA1* [3].

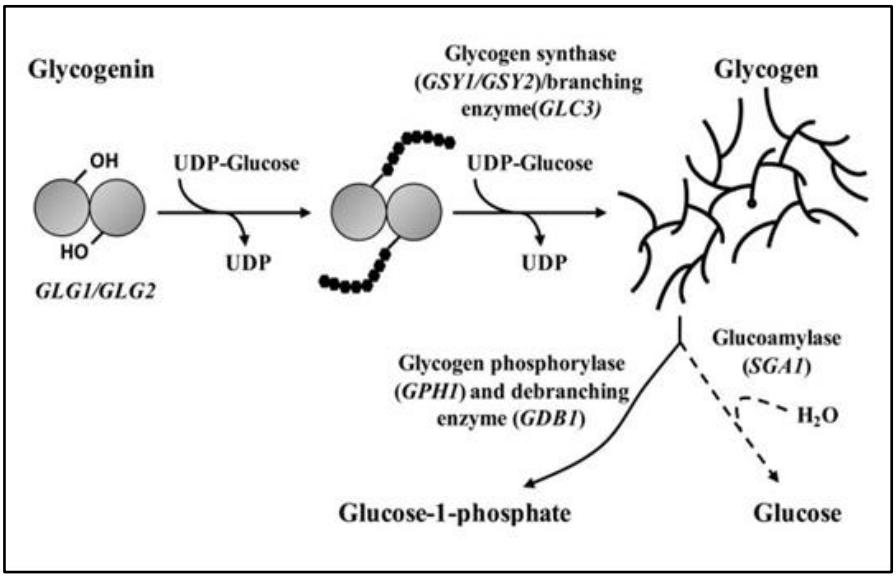

Fig. 1. *Glycogen metabolism in S. cerevisiae yeast cells*

The intracellular glycogen level of *S. cerevisiae* yeast cells is regulated cooperatively with different pathways such as cAMP-dependent protein kinase (PKA) and the Target of rapamycin (TOR) pathway depending on the growth conditions. TOR signaling pathway has a role in the regulation of cell growth according to nutrient availability. In *S. cerevisiae*, there are two different protein kinases, Tor1p and Tor2p. Tor1p and Tor2p control cell growth by participating in the formation of rapamycin-sensitive TOR Complex 1 (TORCI), while TORCII is not sensitive to rapamycin [4, 5, 6]. Incubation of cells with rapamycin causes important physiological changes such as the arrest of the G1 phase, inhibition of protein synthesis, increase in autophagy, and glycogen accumulation [7]. Rapamycin is an immunosuppressive and anti-proliferative antibiotic and its target protein is Tor kinase. Tor kinase is a complex protein belonging to the phosphatidylinositol 3-kinase-like-kinase family [4, 8, 9, 10]. Mutations in the TOR pathway alter the chronological life span (CLS) of yeast cells. Caffeine, which belongs to the methylxanthine family, is a pharmacological agent that targets the Tor protein and acts on the Tor signaling pathway, like rapamycin. As with rapamycin and caffeine,

nitrogen starvation represses the Tor protein. [11, 12]. Tor1p is activated in a strong nitrogen source and activates the Tap42 protein. Tap42p inactivates the Sit4 protein phosphatase enzyme and prevents the dephosphorylation of Msn2/Msn4 transcription factors. Thus, Tap42/Sit4p ensures that Msn2/Msn4p proteins phosphorylated by cAMP/PKA, remain in the cytoplasm. In a weak nitrogen source or in the presence of rapamycin/caffeine, Tor1p became inactive and Tap42 activation terminated. Sit4p phosphatase dephosphorylates Msn2/Msn4 proteins and allows them to pass into the nucleus [13]. Msn2 and Msn4 transcriptional activator proteins bind to STRE motifs which are present in the promoter region of genes involved in glycogen metabolism.

The stress responses of yeast cells are revealed in more detail with genomic and transcriptomic studies. The transcriptome is all of the transcripts that are formed as a result of the genetic response given by the cell at a certain developmental stage, during and/or after a certain physiological condition. The relative transcript levels of all genes in the yeast genome can be quickly determined using DNA sequences. By analyzing the obtained genomic expression data, important information about gene function, genetic regulation mechanisms, and the general physiological response of the cell can be determined. Therefore, this study was aimed to determine the effect of the TOR signaling pathway on glycogen metabolism by using the Next Generation Sequencing (NGS) method.

MATERIALS AND METHODS

Yeast Strain and Growth Conditions

BY4741 *S. cerevisiae* (MATa; *ura3Δ0; leu2Δ0; his3Δ1; met15Δ0*) yeast strain was used in the study. BY4741 is a standard haploid *S. cerevisiae* strain whose genome has been completely sequenced and does not contain any mutations in the genes related to metabolic pathways. BY4741 yeast strain was purchased from the EUROSCARF (European *Saccharomyces cerevisiae* Archive for Functional Analysis) collection. As a growth medium Sc-Ura$^-$ (minimal synthetic growth medium without uracil) supplemented with 2% glucose and 20% uracil was used. Yeast cells were grown in Sc-Ura liquid growth medium with constant temperature (30°C) and shaking (120rpm) up to the logarithmic phase. At this stage, half of the yeast culture was washed and transferred to a fresh Sc-Ura medium containing 8mM caffeine, and the other half to a fresh Sc-Ura medium without caffeine then incubated for 4 hours under the same conditions. At the end of this period, yeast cells were precipitated and washed with sterile distilled water and used for total RNA isolation. The amount of caffeine that inhibits the TOR pathway was determined as 8 mM in previous research [9].

RNA Extraction, cDNA Preparation, and RNA Sequencing

Yeast cells harvested from the medium with and without caffeine were used for RNA-Seq analysis. Total RNA extraction of yeast strains was carried out by using Invitrogen™ RiboPure™ RNA Purification Kit for yeast. RNA concentration, purity, and integrity were approved as qualitative and quantitative using Qubit® RNA HS Assay Kit in Qubit 3.0 Fluorometer, agarose gel electrophoresis, and Agilent™ Bioanalyzer tools. mRNA library was constructed with Dynabeads® mRNA DIRECT™ Micro and Ion Total RNA-Seq Kit v2. All the processes were performed according to the manufacturer's instructors, and the RNA samples with RIN values more than 7 and 280:260 rates more than 2 were utilized for RNA sequencing. Adaptor

ligations, construction cDNA library, cleaning and amplification process was performed using Ion Total RNA-Seq Kit v2 Kit and Ion Torrent™ Ion Xpress™ RNA-seq Barcode 1-16 kit. Clonal amplification was studied with the Emulsion PCR method using Iontorrent Ion One Touch System and Ion 540 Kit-OT2. Single-end reads were sequenced with Ion-Torrent Platform Ion S5 XL (Thermo-IonTorrent, A27214).

Data Analysis

All reads were attained as UBAM (unmapped BAM) with adapter regions trimmed, low quality reads eliminated. The UBAM files were converted ".fastq" format using the GALAXY platform (https://usegalaxy.org). The quality control of all data files was analyzed and the mapping of RNA-seq reads was carried out with the TopHat2 program and obtained .bam files [14]. Using .bam files, RNA seq reads were counted with the HTSeq-count tool, and statistical analysis of differential expression was studied with the DeSeq2 program [15, 16].

RESULTS AND DISCUSSION

The effect of Tor1 protein on transcription in *S. cerevisiae* was evaluated by comparing the transcriptome of yeast cells grown under minimal growth conditions supplemented with and without caffeine. *S. cerevisiae* genome (SacCer3) was used as the reference genome in transcriptomic analyses. The expressed genes differentially after caffeine treatment were determined by comparing the caffeine-treated cells to untreated cells. The data revealed that 43,5% of genes were differentially expressed in response to the caffeine treatment. While 46% of differentially expressed genes were upregulated, 54% were downregulated (Fig. 2a). When all differentially expressed genes (DEG) ($p<0.05$) were mapped to terms of KEGG (Kyoto Encyclopedia of Genes and Genomes) database (http:// www.kegg.jp/), the up-regulated genes were mainly associated with metabolic pathways while the down-regulated genes were associated with ribosome biosynthesis. Gene ontology (GO) annotation of DEG genes showed that 65.7% of genes were up-regulated and 34.3% of genes down-regulated in terms of molecular function, 33.7% of genes up-regulated and 66.3% of genes down-regulated in terms of biological process, and 23.8% of genes up-regulated and 76.2% of genes down-regulated in terms of cellular component (Fig. 2b).

In *S. cerevisiae* yeast cells *PGM1, PGM2, UGP1, YHL012W, GLG1, GLG2, GSY1, GSY2,* and *GLC3* genes are involved in the synthesis of glycogen. The transcription rates of these genes after caffeine application (Log_2 Fold Change, LFC) are given in Fig. 3. The genes in the glycogen synthesis were up-regulated except *PGM1*. Pgm1p and Pgm2p are the minor and major isoforms of phosphoglucomutase, respectively. While the *PGM1* gene is constitutively expressed up to a certain level, the *PGM2* gene is expressed continuously in a constitutive manner [17]. Expression of the *PGM2* gene is increased in glucose repression, in the absence of glucose, in the presence of galactose, at the diauxic shift, and in different stress conditions such as heat, ethanol, salt, cold, and lithium stress [17, 18, 19, 20, 21, 22, 23, 24, 25]. The increase in *PGM2* expression in diauxic shift, heat shock, and salt stress is mediated by the Msn2/Msn4 transcription factors [21, 24]. It has been demonstrated by mRNA measurements that *PGM2* transcription is completely repressed in *Δmsn2msn4* mutant yeast cells [24]. In our promoter analysis using the YEASTRACT (http://www.yeastract.com) and SCPD (*Saccharomyces cerevisiae* Promoter Database) (http://rulai.cshl.edu/SCPD) databases,

we determined two and five STRE sequences in *PGM1* and *PGM2* promoters, respectively. Inactivation of Tor1 protein by caffeine treatment may have caused Msn2/4p to transport from the cytoplasm to the nucleus and bind to the *PGM2* promotor for upregulating the transcription. However, even if the presence of Msn2/4p binding site on the *PGM1* promoter, it was observed that *PGM1* transcription was downregulated. After caffeine treatment, *MSN2* transcription was downregulated (LFC<0) and *MSN4* transcription was upregulated (LFC≥6). This suggests that the *PGM1* gene may be regulated via an Msn2p-dependent manner, while the *PGM2* gene may be regulated in an Msn4p-dependent way.

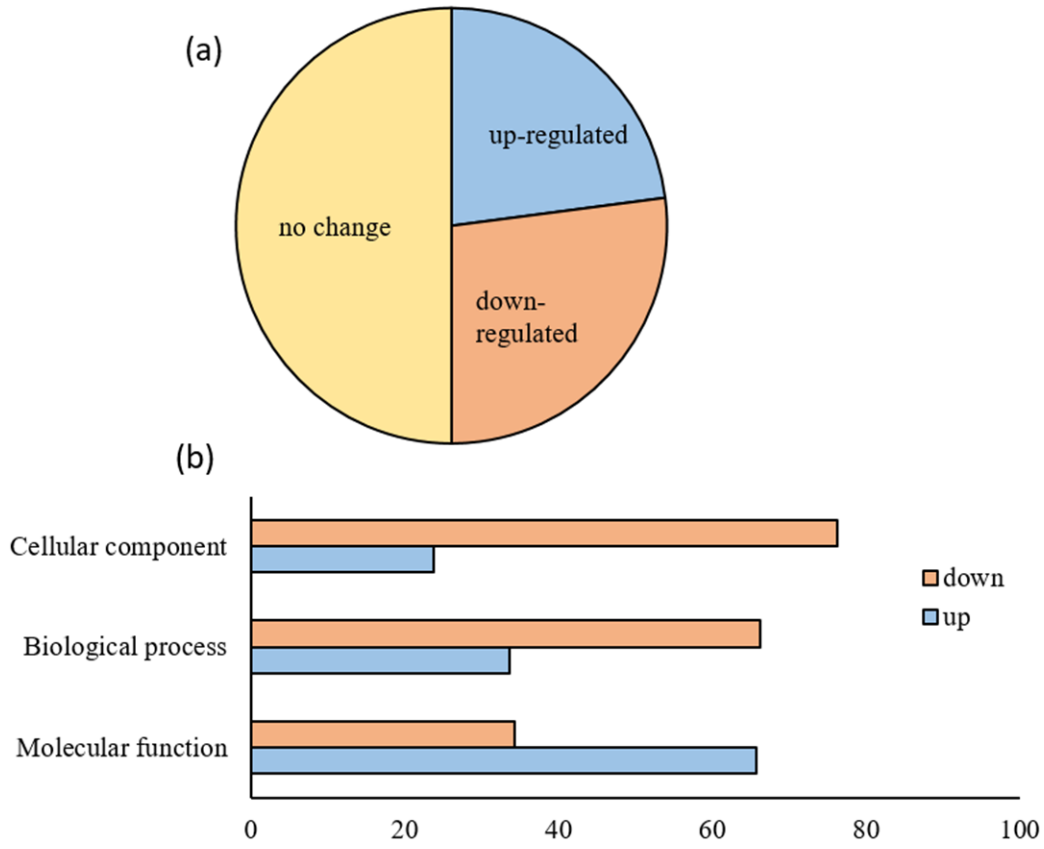

Fig. 2. *(a) Differentially expressed genes in response to the caffeine treatment (b) Gene ontology annotation of differentially expressed genes*

Although the YHL012W and *UGP1* genes encoded for the uridyltranferase enzyme are paralogous, it was observed that *Δyhl012w* yeast cells are viable whereas *Δugp1* yeast cells cannot [26]. In our promoter analysis, there is no STRE element in the YHL012W promoter region, while there are 3 STRE sequences in the *UGP1* promoter region. It has been determined that Msn2/4 transcription factors regulate *UGP1* transcription by binding to these sequences in the *UGP1* promoter, and the level of *UGP1* mRNA increases under some stress conditions [27]. Therefore, the increase in *UGP1* transcription after caffeine treatment may have been Msn4p-dependent.

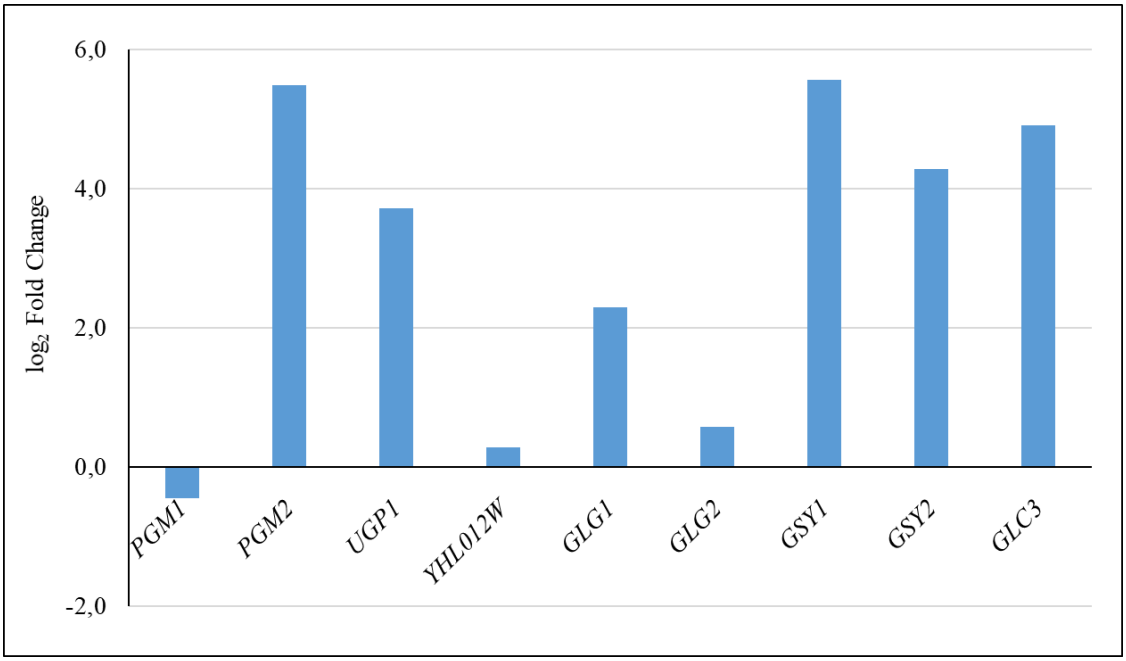

Fig. 3. *Transcriptional changes of genes involved in glycogen synthesis after caffeine treatment*

Glg1p and Glg2p use UDP-glucose to form a short α-1,4-glucosyl chain to the amino acid tyrosine in their structure. The amount of *GLG1* and *GLG2* mRNA starts to increase when half of the glucose in the environment is consumed and reaches its maximum level when it is depleted [28]. The glycogen accumulation in *Δglg1* and *Δglg2* yeast cells is not affected while the accumulation stops in *Δglg1glg2* yeast cells [29]. As in the case of YHL012W and *UGP1*, the *GLG2* promoter has no STRE sequence while the *GLG1* promoter has 2 STRE sequences. Therefore, the *GLG1* transcription may be upregulated because of the Msn4-dependent way, as in *PGM2* and *UGP1* genes. Tor1p-dependent regulation of the *GLG1* gene was demonstrated for the first time in our study.

The paralogous genes, *GSY1* and *GSY2*, encode the glycogen synthase enzyme that catalyzes the formation of α-1,4-glycosidic bonds. In addition, the *GLC3* gene encodes a glycogen branching enzyme that catalyzes α-1,6-glycosidic bond formation using UDP-glucose. As with other genes involved in glycogen metabolism, the amount of *GSY1*, *GSY2*, and *GLC3* mRNA begins to increase when half of the glucose in the growth medium is consumed and reaches its maximum level when the glucose is completely depleted [28]. After the deletion of the *GSY1*, *GSY2*, and *GLC3* genes yeast cells can survive but could not accumulate glycogen [30]. It is known that Msn2/4 transcription factors regulate transcription by binding to the promoter region of the *GSY1*, *GSY2*, and *GLC3* genes [31, 32, 33, 34, 35]. Gsy2p and Gsy1p have different enzyme activities under variable stress conditions (nutrient starvation, nitrogen starvation and environmental stress) and Gsy2p activity is more dominant than Gsy1p [31, 33, 36]. In addition to STRE elements, the *GSY1* promoter region includes binding sites for negative transcription regulators (Mig1p and Rox1p) and its transcription is repressed by Nrg1 and Nrg2 repressor proteins [31, 37, 38]. Similarly, the *GSY2* gene expression is regulated by STRE-dependent or -independent ways. The *GSY2* gene is positively controlled by Msn2/Msn4 transcription factors and Snf1 kinase, and it is negatively

controlled by Pho85 and cAMP-dependent protein kinase activities [39]. In our promoter analysis, two and three STRE sequences were determined in the *GSY1*/*GSY2* and *GLC3* genes, respectively. The transcription levels of *GSY1*, *GSY2*, and *GLC3* genes increased significantly after caffeine treatment in our study. This indicates that *GSY1*, *GSY2*, and *GLC3* genes are also controlled by Tor1 kinase activity.

GPH1, *GDB1*, and *SGA1* genes are involved in the breakdown of glycogen in *S. cerevisiae*. The transcription rates of these catabolic genes after caffeine treatment are given in Fig. 4. Glycogen is breakdown into glucose-1-phosphate and glucose by the glycogen phosphorylase (Gph1) and the debranching enzyme (Gdb1), respectively [40, 41]. Gph1p activity is controlled via cAMP-dependent phosphorylation and Hog1p-MAP kinase-dependent pathway [42]. *GPH1* and *GDB1* transcription undergo STRE-dependent regulation [41, 42]. It has been observed that yeast cells can survive and accumulate high glycogen after the deletion of *GPH1* and *GDB1* genes [2, 41]. In our promoter analysis, it was determined that there were 3 STRE elements in the *GPH1* promoter and one STRE element in the *GDB1* promoter. Msn4 transcription factor may have increased the *GPH1* and *GDB1* transcriptions by binding to STRE sequences after caffeine treatment.

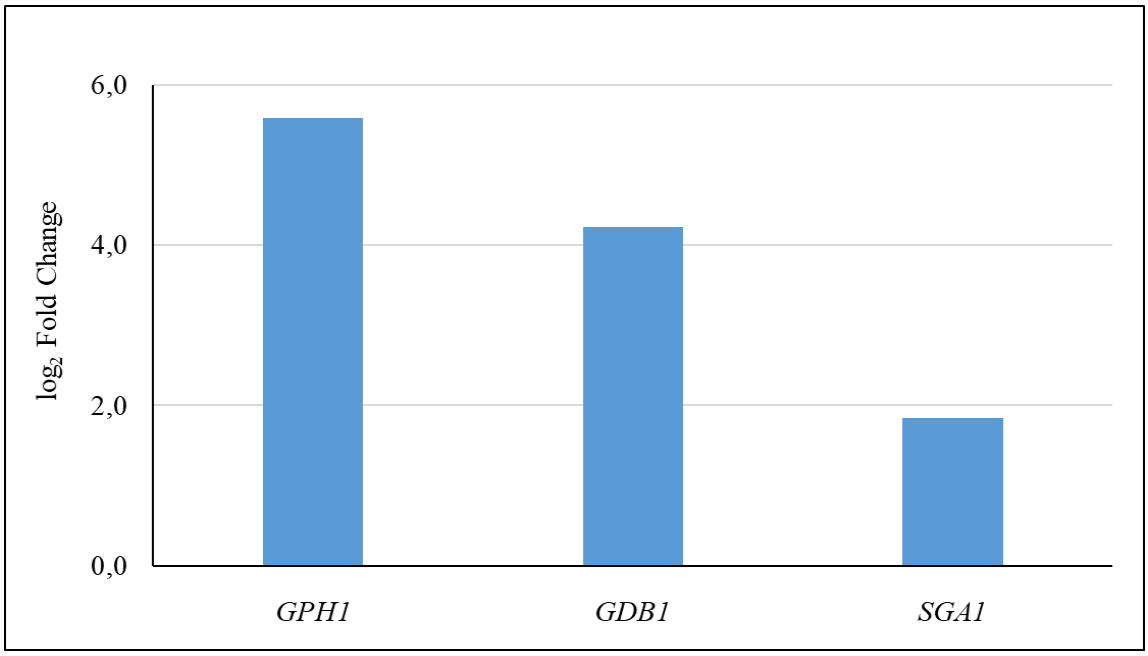

Fig. 4. *Transcriptional changes of genes involved in glycogen catabolism after caffeine treatment*

Yeast cells go to sporulation when grown on nonfermentable carbon sources and starved for nitrogen. *SGA1* encodes sporulation-specific glucoamylase bearing α-1,4 and α-1,6 glucosidase activities [2]. During sporulation, the *SGA1* gene product hydrolyses glycogen to produce glucose [2]. *SGA1* expression is controlled by Ime1p and Rme1p [43]. In addition, *SGA1* expression is repressed by Nrg1p and Nrg2p [38]. Since the target genes of Nrg1 and Nrg2 transcriptional repressors contain STRE or STRE-like elements, they are also regulated by Msn2/4 transcription factors [38]. The determination of 6 STRE sequences in the *SGA1* promoter region in promoter analysis indicates that *SGA1* gene regulation can be also Msn4-dependent way.

CONCLUSION

In this study, the effect of the TOR signaling pathway on glycogen metabolism was determined with the Next Generation Sequencing method in *S. cerevisiae*. It was determined that the transcription of genes involved in glycogen metabolism was increased after caffeine treatment, except *PGM1*. Particularly, the inactivation of Tor1p caused to increase in *PGM2*, *UGP1*, *GLG1*, *GSY1*, *GSY2*, *GLC3*, *GPH1*, and *GDB1* transcription at least 2 fold (LFC>2). *In silico* promoter analysis revealed that all genes in glycogen metabolism, except YHL012W and *GLG2*, include at least one STRE sequence for binding of Msn2/4 transcription factors. Inactivation of Tor1 protein with caffeine may have caused Msn2/4p to transport from cytoplasm to the nucleus and bind to the related gene promotor for activating the transcription. To support this result, it would be appropriate to quantify the related gene transcripts by RT-qPCR in *Δmsn2, Δmsn4*, and *Δmsn2msn4* yeast cells, or to determine LacZ gene expressions in these mutants by constructing the LacZ gene fusions including related gene promoter regions.

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REFERENCES

[1] Lillie, S. H., Pringle, J. R. (1980): Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. Journal of Bacteriology, 143: 1384-1394.

[2] François, J., Parrou, J. L. (2001): Reserve carbohydrates metabolism in the yeast *Saccharomyces cerevisiae*. FEMS Microbiology Reviews, 25(1): 125-145.

[3] Wilson, W. A., Roach, P. J., Montero, M., Baroja-Fernández, E., Muñoz, F. J., Eydallin, G., Viale, A. M. Pozueta-Romer, J. (2010): Regulation of glycogen metabolism in yeast and bacteria. FEMS Microbiology Reviews, 34(6): 952-985.

[4] Loewith, R., Jacinto, E., Wullschleger, S., Lorberg, A., Crespo, J.L., Bonenfant, D., Oppliger, W., Jenoe, P., Hall, M. N. (2002): Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Molecular Cell, 10: 457-468.

[5] Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Ruegg, M. A., Hall, A., Hall, M. N. (2004): Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nature Cell Biology, 6: 1122-1128.

[6] Fadri, M., Daquinag, A., Wang, S., Xue, T., Kunz J. (2005): The pleckstrin homology domain proteins Slm1 and Slm2 are required for actin cytoskeleton organization in yeast and bind phosphatidylinositol-4,5-bisphosphate and TORC2. Molecular Biology of Cell, 16(4): 1883-1900.

[7] Rohde, J. R., Bastidas, R., Puria, R., Cardenas, M. E. (2008): Nutritional control via Tor signaling in *Saccharomyces cerevisiae*. Current Opinion in Microbiology, 11: 153-160.

[8] Reinke, A., Anderson, S., McCaffery, J. M., Yates, J., Aronova, S., Chu, S., Fairclough, S., Iverson, C., Wedaman, K. P., Powers, T. (2004): TOR complex 1 includes a novel component, Tco89p (YPL180w), and cooperates with Ssd1p to maintain cellular integrity in *Saccharomyces cerevisiae*. Journal of Biological Chemistry 279: 14752-14762.

[9] Reinke, A., Chen, J. C. Y., Aronova, S., Powers, T. (2006): Caffeine targets TOR complex I and provides evidence for a regulatory link between the FRB and kinase domains of Tor1p. Journal of Biological Chemistry 281: 31616-31626.

[10] Wullschleger, S., Loewith, R. Hall, M. N. (2006): TOR signaling in growth and metabolism. Cell 124(3): 471-484.
[11] Sarkaria, J. N., Busby, E. C., Tibbetts, R. S., Roos, P., Taya, Y., Karnitz, L. M., Abraham, R. T. (1999): Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine. Cancer Research 59: 4375-4382.
[12] McMahon, L. P., Yue, W., Santen, R. J., Lawrence, J. C. Jr. (2005): Farnesylthiosalicylic acid inhibits mammalian target of rapamycin (mTOR) activity both in cells and *in vitro* by promoting dissociation of the mTOR-raptor complex. Molecular Endocrinology 19: 175-183.
[13] Inoki, K., Ouyang, H., Li, Y., Guan K. L. (2005): Signaling by target of rapamycin proteins in cell growth control. Microbiology and Molecular Biology Reviews 69(1): 79-100.
[14] Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., Salzberg, S. L. (2013): TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biology 14(R36): 1-13.
[15] Anders, S., Pyl, P. T., Huber, W. (2014): HTSeq–a Python framework to work with high-throughput sequencing data. Bioinformatics 31(2): 166-169.
[16] Love, M. I., Huber, W., Anders, S. (2014): Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15(550): 1-22.
[17] Fu. L., Miseta, A., Hunton, D., Marchase, R. B., Bedwell, M. D. (2000): Loss of the major isoform of phosphoglucomutase results in altered calcium homeostasis in *Saccharomyces cerevisiae*. Journal of Biochemical Chemistry 275(8): 5431-5440.
[18] Oh, D., Hopper, J. E. (1990): Transcription of a yeast phosphoglucomutase isozyme gene is galactose inducible and glucose repressible. Molecular Biology of the Cell 10(4): 1415-1422.
[19] Dey., N. B., Bounelis, P., Fritz, T. A., Bedwell, D. M., Marchase, R. B. (1994): The glycosylation of phosphoglucomutase is modulated by carbon source and heat shock in *Saccharomyces cerevisiae*. The Journal of Biological Chemistry 269(43): 27143-27148.
[20] Fu, L., Bounelis, P., Dey, N., Browne, B. L., Marchase, R. B., Bedwell, D. M. (1995): The posttranslational modification of phosphoglucomutase is regulated by galactose induction and glucose repression in *Saccharomyces cerevisiae*. Journal of Bacteriology 177(11): 3087-3094.
[21] Boy-Marcotte, E., Perrot, M., Bussereau, F., Boucherie, H., Jacquet, M. (1998): Msn2p and Msn4p control a large number of genes induced at the diauxic transition which are repressed by cyclic AMP in *Saccharomyces cerevisiae*. Journal of Bacteriology 180(5): 1044-1052.
[22] Alexandre, H., Ansanay-Galeote, V. Blondin D. B. (2001): Global gene expression during short-term ethanol stress in *Saccharomyces cerevisiae*. FEBS Letter 498(1): 98-103.
[23] Masuda, C. A., Xavier, M. A., Mattos, K. A., Galina, A. (2001): Phosphoglucomutase is an in vivo lithium target in yeast. Journal of Biological Chemistry 276(41): 37794-37801.
[24] Hirata, Y., Andoh, T., Asahara, T., Kikuchi, A. (2003): Yeast glycogen synthase kinase-3 activates Msn2p-dependent transcription of stress responsive genes. Molecular Biology of the Cell 14(1): 302-312.
[25] Schade, B., Jansen, G., Whiteway, M., Entia, K. D., Thomas, D. Y. (2004): Cold adaptation in budding yeast. Molecular Biology of the Cell 15(12): 5492-5502.
[26] Daran, J. M., Daran, M., Dallies, N., Thines-Sempoux, D., Paquet, V., François, J. (1995): Genetic and biochemical characterization of the *UGP1* gene encoding the UDP-glucose pyrophosphorylase from *Saccharomyces cerevisiae*. European Journal of Biochemistry 233(2): 520-530.
[27] Yi, D. G., Huh, W. K. (2015): UDP-glucose pyrophosphorylase Ugp1 is involved in oxidative stress response and long-term survival during stationary phase in

Saccharomyces cerevisiae. Biochemical and Biophysical Research Communications 467(4): 657-663.

[28] Parrou, J. L., Enjalbert, B., Plourde, L., Bauche, A., Gonzalez, B., François, J. M. (1999): Dynamic responses of reserve carbohydrate metabolism under carbon and nitrogen limitations in *Saccharomyces cerevisiae*. Yeast 15(3): 191-203.

[29] Cheng, C., Mu, J., Farkas, I., Huang, D., Goebl, M. G., Roach, P. J. (1995): Requirement of the self-glucosylating initiator proteins Glg1p and Glg2p for glycogen accumulation in *Saccharomyces cerevisiae*. Molecular and Cellular Biology 15(12): 6632-6640.

[30] Thon, V. J., Vigneron-Lesens, C., Marianne-Pepin, T., Montreuil, J., Decq, A., Rachez, C., Ball, S. G., Cannon, J. F. (1992): Coordinate regulation of glycogen metabolism in the yeast *Saccharomyces cerevisiae*. Induction of glycogen branching enzyme. Journal of Biological Chemistry 267(21):15224-15228.

[31] Estruch, F. (2000): Stress-controlled transcription factors, stress-induced genes and stress tolerance in budding yeast. FEMS Microbiology Reviews 24(4): 469-486.

[32] Causton, H. C., Ren, B., Koh, S. S., Harbison, C. T., Kanin, E., Jennings, E. G., Lee, T. I., True, H. L., Lander E. S., Young, Y. R. (2001): Remodeling of yeast genome expression in response to environmental changes. Molecular Biology of the Cell 12(2): 323-337.

[33] Gasch, A. P. (2003): The environmental stress response: a common yeast response to diverse environmental stresses. In S. Hohmann & W. H. Mager (eds.). Yeast Stress Response. Berlin: Springer-Verlag, pp. 11-70.

[34] Sadeh, A., Movshovich, N., Volokh, M., Gheber, L., Aharoni, A. (2011): Fine-tuning of the Msn2/4-mediated yeast stress responses as revealed by systematic deletion of Msn2/4 partners. Molecular Biology of the Cell, 22: 3127-3138.

[35] Nanyan, N., Takagi, H. (2020): Proline homeostasis in *Saccharomyces cerevisiae*: how does the stress-responsive transcription factor Msn2 play a role. Frontiers in Genetics 11(438): 1-9.

[36] Farkas, I., Hardy, T. A., Goebl, M. G., Roach, P. J. (1991): Two glycogen synthase isoforms in *Saccharomyces cerevisiae* are coded by distinct genes that are differentially controlled. The Journal of Biological Chemistry 266(24): 15602-15607.

[37] Unnikrishnan, I., Miller, S., Meinke, M., LaPorte, D. C. (2003): Multiple positive and negative elements involved in the regulation of expression of *GSY1* in *Saccharomyces cerevisiae*. Journal of Biological Chemistry 278(29): 26450-26457.

[38] Vyas V. K., Berkey, C. D., Miyao, T. Carlson, M. (2005): Repressors Nrg1 and Nrg2 regulate a set of stress-responsive genes in *Saccharomyces cerevisiae*. Eukaryotic Cell 4(11): 1882-1891.

[39] Enjalbert. B., Parrou, J. L., Taste, M. A., François, J. (2004): Combinatorial control by the protein kinases PKA, PHO85 and SNF1 of transcriptional induction of the *Saccharomyces cerevisiae GSY2* gene at the diauxic shift. Molecular Genetics and Genomics 271(6): 697-708.

[40] Hwang, P. K., Tugendreich, S., Fletterick, R. J. (1989): Molecular analysis of *GPH1*, the gene encoding glycogen phosphorylase in *Saccharomyces cerevisiae*. Molecular and Cellular Biology 9(4): 1659-1666.

[41] Teste, M. A., Enjalbert, B., Parrou, J., François, J. M. (2000): The Saccharomyces cerevisiae YPR184W gene encodes the glycogen debranching enzyme. FEMS Microbiology Letters 193(1): 105-110.

[42] Sunnarborg, S. W., Miller, S. P., Unnikrishnan, I., LaPorte, D. C. (2001): Expression of the yeast glycogen phosphorylase gene is regulated by stress-response elements and by the HOG MAP kinase pathway. Yeast 18(16):1505-1514.

[43] Kihara, K., Nakamura, M., Akada, R., Yamashita, I. (1991): Positive and negative elements upstream of the meiosis-specific glucoamylase gene in *Saccharomyces cerevisiae*. Molecular Genetics and Genomics 226(3): 383-392.