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Trakya University Journal of Natural Sciences, 23(2): 167-176, 2022
ISSN 2528-9691
DOI: 10.23902/trkjnat.1131400

Research Article

THE SAGA COMPLEX IS ESSENTIAL FOR THE REGULATION OF GENES INVOLVED IN YEAST TREHALOSE METABOLISM

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Cite this article as:

Turgut Genç T. 2022. The SAGA complex is essential for the regulation of genes involved in yeast trehalose metabolism. *Trakya Univ J Nat Sci*, 23(2): 167-176, DOI: 10.23902/trkjnat.1131400

Received: 15 June 2022, Accepted: 05 September 2022, Online First: 20 September 2022, Published: 15 October 2022

Edited by:
Özkan Danış

Key words:
Msn2/4
Nitrogen starvation
Nucleosome
SAGA
Trehalose

Abstract: *Saccharomyces cerevisiae* accumulates trehalose as a stress metabolite in adverse environmental conditions. The trehalose synthesis and breakdown are important for the regulation of trehalose levels within the yeast cell. Therefore, *TPS1* and *NTH1* gene expressions are tightly regulated during transcription and also translation. Since both genes contain Stress Response Elements (STRE) in the promoter regions, they are co-activated under stress conditions. However, the presence of similar regulatory elements in the promoter of both genes shows that these genes undergo a different regulation at the transcriptional level. In our study, the role of the Spt-Ada-Gcn5 Acetyltransferase (SAGA) complex in the transcriptional regulation of *TPS1* and *NTH1* genes was determined in nutrient-poor environment. For that purpose, the wild type and *Δada1* mutant yeast cells, where Ada1p is a member of the SAGA complex, were grown in normal and nitrogen starvation conditions. In addition, trehalose level was detected enzymatically in both wild type and mutant yeast cells. *In silico* promoter analysis of *TPS1* and *NTH1* promoters revealed that the STRE sequences required for binding of Msn2/4 transcription factors are closed by nucleosomes at the *NTH1* promoter, but open at the *TPS1* promoter. In the absence of Ada1p, stress-induced promoter activation in the *TPS1* gene was observed, while *NTH1* gene expression was not activated. According to these results, the nucleosomes spanning the STRE sequences could not be mobilized in the absence of Ada1 protein, and therefore the Msn2/4 transcription factors cannot bind to the promoter and activate the *NTH1* gene expression under stress conditions. It was also observed that in the absence of Ada1p, trehalose accumulation was reduced regardless of stress conditions.

Özet: *Saccharomyces cerevisiae* olumsuz çevre koşullarında stres metaboliti olarak trehaloz biriktirir. Hücre içi trehaloz miktarının düzenlenmesinde trehalozun sentezi ve yıkımı önemlidir. Bu nedenle, *TPS1* ve *NTH1* gen ekspresyonları transkripsiyon ve translasyon sırasında sıkı bir şekilde düzenlenmektedir. Her iki genin promotor bölgesinde Stres Tepki Elementleri (STRE) bulunduğundan stres koşullarında birlikte aktive olurlar. Ancak her iki genin promotor bölgesinde benzer düzenleyici elemanların bulunması bu genlerin transkripsiyon seviyesinde farklı bir regülasyona uğradıklarını göstermektedir. Çalışmamızda, *TPS1* ve *NTH1* genlerinin transkripsiyonel düzenlenmesinde Spt-Ada-Gcn5 Asetiltransferaz (SAGA) kompleksinin rolü besin yönünden zayıf ortamda belirlendi. Bu amaçla, SAGA kompleksinin alt ünitesi olan Ada1p içeren yaban tip ve içermeyen *Δada1* mutant maya hücreleri azot açlığında ve normal büyüme koşullarında üretildi. Ayrıca yaban tip ve mutant maya hücrelerinde trehaloz seviyesi enzimatik olarak tespit edildi. *TPS1* ve *NTH1* genlerinin *in silico* promotor analizi sonucunda Msn2/4 transkripsiyon faktörlerinin bağlanması için gerekli olan STRE dizilerinin *NTH1* promotorunda nükleozomlar tarafından kapatıldığı *TPS1* promotorunda ise açıkta kaldıkları belirlendi. Ada1 proteininin yokluğunda, *TPS1* geninde stres kaynaklı promotor aktivasyonu gözlenirken, *NTH1* geninde promotor aktivasyonu gözlenmedi. Bu sonuçlara göre, Ada1 proteininin yokluğunda STRE dizilerini kaplayan nükleozomlar mobilize edilemediğinden Msn2/4 transkripsiyon faktörleri stres koşullarında promotora bağlanamayarak transkripsiyonu aktive edememiş olabilir. Ayrıca stres koşullarından bağımsız olarak Ada1 proteininin yokluğunda maya hücrelerindeki trehaloz birikiminin azaldığı gözlendi.

Introduction

Saccharomyces cerevisiae accumulates trehalose sugar to withstand or adapt to unfavorable environmental conditions. When the stress conditions are terminated, yeast cells break down trehalose into glucose and use it as a carbon source. Similarly, the yeast cells accumulate trehalose when entering the quiescence (G_0) phase and use

it again as an energy source when exiting. The Trehalose Phosphate Synthase (TPS) enzyme complex responsible for the trehalose synthesis consists of four subunits: Tps1p, Tps2p, Tps3p and Tsl1p (Trevisol *et al.* 2014). Trehalose biosynthesis is carried out in two steps: in the first step, the Tps1 enzyme synthesizes trehalose-6-phosphate from UDP-glucose and glucose-6-phosphate, and in the second step, trehalose-6-phosphate is dephosphorylated by the Tps2 subunit. Other regulatory subunits, Tps3p and Tsl1p, ensure the complex remains stable (Bell *et al.* 1998, Trevisol *et al.* 2014). When the stress conditions are terminated, the accumulated trehalose is rapidly converted to glucose by the neutral trehalase enzyme encoded by the *NTH1* and *NTH2* genes (Nwaka & Holzer 1997). Nth1p is phosphorylated by Protein Kinase A (PKA) and the enzymatic activity of the protein is controlled by phosphorylation and dephosphorylation mechanisms (App & Holzer 1989, Schepers *et al.* 2012). Transcription of both *TPS1* and *NTH1* genes is activated under different stress conditions such as heat shock, oxidative stress and metal stress (Zähringer *et al.* 1997, Parrou *et al*. 1999, Estruch 2000, Asada *et al*. 2022).

The promoters of *TPS1* and *NTH1* include Stress Response Elements (STRE) (5'–CCCCT–3') where the general stress factors, Msn2/4p, can bind and activate transcription (Zähringer *et al.* 2000, Stewart-Ornstein *et al.* 2013). Msn2/4 zinc finger transcription factors bind to STRE sequences and activate the transcription of many genes in response to environmental stresses (Gasch *et al.* 2000, Huebert *et al.* 2012, Stewart-Ornstein *et al.* 2013, Rajvanshi *et al.* 2017). Msn2/4 proteins translocate from the cytoplasm to the nucleus when the glucose is depleted or the cells encounter stress such as nutrient starvation (Görner *et al.* 2002). Cellular localization of Msn2/4 is regulated mainly by cAMP-dependent PKA and TOR pathways depending on the environmental conditions (Beck & Hall 1999, Görner *et al.* 2002).

The mobilization of nucleosomes localized in the promoter region is important for the binding of basic transcription initiation factors and also regulatory proteins such as activators or repressors. The chromatin remodeling factors (CRFs) are multi-protein complexes that relocalized the nucleosomes through nucleosome sliding or nucleosome eviction mechanisms (Fazzio & Tsukiyama 2003, Boeger *et al.* 2004). Spt-Ada-Gcn5 Acetyltransferase (SAGA) complex is a chromatin re-modulator that is involved in the acetylation and deubiquitination of histone and non-histone proteins (Nagy & Tora 2007, Rodriguez-Navarro 2009). The SAGA complex consists of functional modules containing different numbers of subunits. The architecture unit or core unit composed of Spt7, Spt20 and Ada1 proteins is essential for the integrity of the SAGA complex. The formation of an active SAGA complex does not occur in *Δspt7*, *Δspt20* and *Δada1* mutants (Sterner *et al.* 1999, Wu & Winston 2002). The SAGA complex is responsible for the recruitment of the basal transcription machinery to the promoters and also activates transcription by interacting with different coactivator proteins or repressing transcription in some promoters (Lee *et al.* 2000, Belotserkovskaya *et al.* 2000, Narlikar *et al.* 2002, Antonazzi *et al.* 2021). The osmotic stress and ethanol increase the expression of *SPT3* and *SPT15* genes which are the subunits of the SAGA complex (Yo *et al.* 2012). The genotoxic stress causes the SAGA complex to bind the promoters of TPS genes and induce gene expression for trehalose synthesis (Yu *et al.* 2021). These studies indicate that the SAGA complex can have an important role in trehalose metabolism.

Therefore, in our study, we aimed to determine the role of the SAGA complex in the transcriptional regulation of *TPS1* and *NTH1* genes using *Δada1* mutant yeast cells. Our results showed that the stress-induced *TPS1* activation was observed in the absence of Ada1p, but the *NTH1* promoter could not be activated and transcription remained at the basal level. *NTH1* promoter activation may not have been observed in stress conditions, since the nucleosomes localized on the STRE sequences cannot be mobilized and Msn2/4 transcription factors cannot bind to these sequences in the *Δada1* mutant yeast cells. It was also observed that in the absence of Ada1p, trehalose accumulation decreased regardless of stress conditions.

Materials and Methods

Strains and plasmids

Saccharomyces. cerevisiae BY4741 (MATa; *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*) and mutant derivative Y01038 (MATa; *ura3Δ0; leu2Δ0; his3Δ1; met15Δ0;* YPL254w::kanMX4) were used in this study (Brachmann *et al.* 1998). All yeast strains were obtained from EUROSCARF (Frankfurt, Germany). *ADA1* gene was completely replaced with the geneticin resistance-coding KanMX4 module in the Y01038 (*Δada1*) strain. BY4741 strain has no known mutations relevant to trehalose metabolism. *Escherichia coli* XL1 Blue bacterial cells were used for plasmid amplification. The plasmids used in this research, pNL1 expression vectors including *TPS1*- and *NTH1*-lacZ gene fusions were used to determine the promoter activities. In these expression vectors, *TPS1* (1000 bp) and *NTH1* (770 bp) promoter regions fused in-frame to the lacZ gene. The promoter regions contain all of the regulatory sites required for *TPS1* and *NTH1* gene expressions (Parrou *et al.* 1997). The other plasmid contains *SUC2*-lacZ gene fusion which is independent of the stress regulation, so it was used as a control. In this expression vector, the 616 bp promoter region of the *SUC2* gene (extending from-384 to -900 bp) was replaced with the *CYC1* UAS in 2 μm-URA3-based expression vector pLGΔ31225 (Türkel *et al.* 2003a).

Growth conditions

Yeast strains were cultured in a YPD medium (10 g/L Yeast extract, 20 g/L Bacto-peptone and 20 g/L Glucose) for plasmid transformation. The plasmids were transformed into wild type and *Δada1* mutant yeast strains

using the Lithium Acetate-Polyethylene Glycol procedure as described previously (Ausubel *et al.* 1993). Transformants were plated on Yeast Synthetic Minimal (YSM) medium without uracil (1.7 g/L yeast nitrogen base (w/o amino acids and ammonium sulfate), 5 g/L ammonium sulfate, 20 mg/L histidine, 60 mg/L leucine, 20 mg/L methionine) supplemented with 2% glucose and grown at 30°C. Transformant yeast colonies were used in liquid culture inoculations. For nitrogen starvation stress 0.1% proline was added to YSM culture instead of ammonium sulfate (Park *et al.* 1996).

β–galactosidase activity assay

After completion of incubation, the harvested yeast cells were washed and used for determining β–galactosidase activities as described previously with some modifications (Guarente 1983, Türkel *et al.* 2003b). Beta-galactosidase units were given in nanomoles of ONPG (o-Nitrophenyl β-D-Galactopyranoside) cleaved per minute per milligram of protein in permeabilized yeast cells. Protein concentrations were determined by the Lowry assay (Lowry *et al.* 1951).

Trehalose assay

Trehalose assay of yeast cells was conducted as described previously (Chen & Futcher 2017). Yeast cells were removed and washed with ice-cold water and then resuspended in 125 µl of 0.25 M Na_2CO_3 and incubated at 95°C for 3 hours. The cell mixture was incubated at 37°C for 18 hr in the presence of 3 mU trehalase enzyme (Sigma, T8778, 0.25 U/mL) for trehalose assay. The amount of the liberated glucose was determined enzymatically via the glucose oxidase-peroxidase system using a commercial kit (Fluitest®- GLU, Biocon, Germany). The determined trehalose content of yeast cells was given as micrograms of glucose equivalent per milligram of wet mass (µg/mg) of the yeast cells.

In silico analysis

TPS1 and *NTH1* promoter sequences (1 kb) were obtained from the SGD (*Saccharomyces* Genome Database) database (Engel *et al.* 2014). Gcr1, Gcn4 and Msn2/4 binding sites in the *TPS1* and *NTH1* promoter regions were determined using YEASTRACT and EPD (Eukaryotic Promoter Database) databases (Monteiro *et al.* 2020, Meylan *et al.* 2020). The probable nucleosome positions in *TPS1* and *NTH1* promoter regions were determined with the ICM (Interactive Chromatin Modeling) program (Stolz & Bishop 2010). The energy calculations ($E_{nuc} = ½\sum K (X_{nuc}-X_{DNA})^2$) suggested by the program were used to determine the nucleosome positions.

Statistical analysis

The results are reported as means ± standard deviation (SD) of at least three independent transformants and three independent experiments. Data analysis was performed using the Graph Pad Prism software 5 (Graph Pad Software Inc., La Jolla, USA). The unpaired, parametric, two-tailed student's t-test was used with a 95% confidence level. Differences were considered statistically significant when a *p* value was less than 0.05.

Results

In silico analysis of promoters

The number of nucleosomes and their positions at the *TPS1* and *NTH1* promoter regions were determined using the ICM program. For analysis, 1000 bp regions of *TPS1* and *NTH1* promoters were used. The analysis showed that there are four possible nucleosomes localized at the *TPS1* and *NTH1* promoters. The chromatin modeling of *NTH1* and *TPS1* promoters are given in Fig. 1. The positions of nucleosomes at the *NTH1* promoter were determined as follows: the first histone extends from -96 to -242 bp, the second histone extends from -306 to -452 bp, the third histone extends from -605 to -751 bp and the fourth histone extends from -780 to -926 bp. Similarly, the positions of nucleosomes at the *TPS1* promoter were as follows: the first histone extends from -40 to -186 bp, the second histone extends from -471 to -617 bp, the third histone extends from -687 to -833 bp and the fourth histone extends from -852 to -998 bp. The binding sequences of Gcr1, Gcn4 and Msn2/4 proteins in the *NTH1* and *TPS1* promoter regions are shown in Fig. 2 and Fig. 3, respectively. The possible nucleosome positions obtained from ICM were marked on the *NTH1* and *TPS1* promoters. In the *NTH1* promoter region, three Msn2/4 binding sites which were closed by first and second nucleosomes, were determined (Fig. 2). In the *TPS1* promoter region six Msn2/4 binding sites were determined (Fig. 3). In contrast to the *NTH1* promoter, all Msn2/4 binding sites in *TPS1* promoter were not closed with the nucleosomes.

The effect of Ada1 protein in NTH1 and TPS1 gene expressions

The SAGA complex acts as a transcriptional regulator depending on the subunits and also growth conditions. Ada1 subunit of SAGA is essential for the integrity of the complex. The role of the Ada1 protein and thus the SAGA complex in *NTH1* and *TPS1* gene activations were determined under normal growth conditions and nitrogen starvation. First, transformant wild type and mutant yeast cells were grown in the medium containing ammonium sulfate up to the logarithmic stage and β-galactosidase enzyme activities were determined. The measurement of beta-galactosidase serves as a rapid and semi-quantitative assay for gene activation. Beta-galactosidase activities of wild-type and mutant yeast cells were measured as 102.3±16.6 units and 124.5±39.2 units in *NTH1*-LacZ gene fusion, respectively. And, the promoter activation of wild-type and mutant yeast cells were measured as 2323.4±153.2 units and 2775.2±157.4 units in *TPS1*-LacZ gene fusion, respectively (Fig. 4). There was no significant change in the *NTH1* and *TPS1* promoter activities of wild-type and mutant yeast cells under normal growth conditions (P=0.9912).

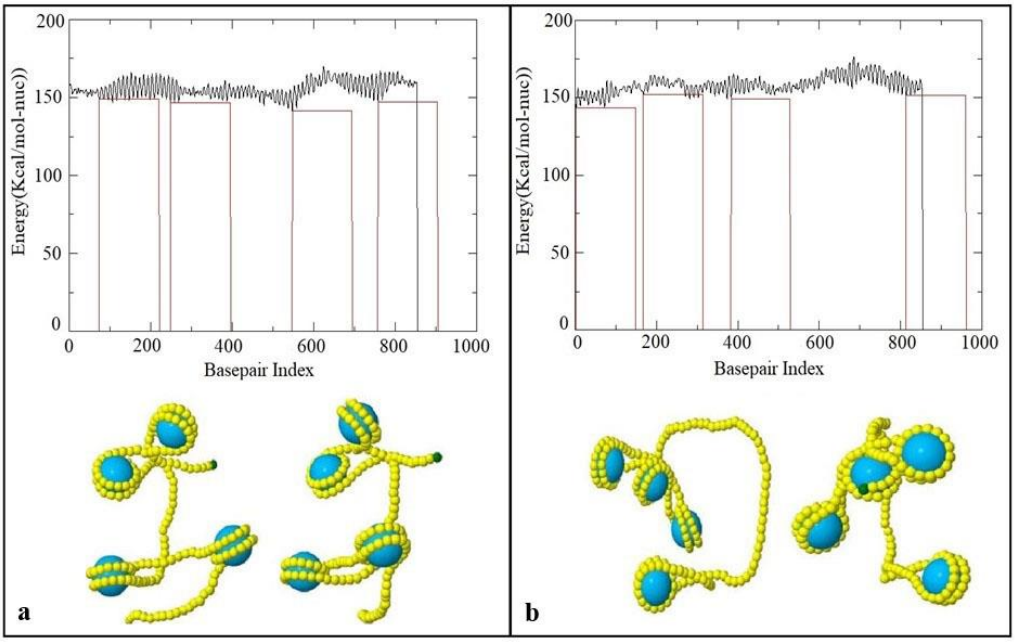

Fig. 1. Chromatin modeling of **a.** *NTH1* promoter and **b.** *TPS1* promoter created by ICM program. Blue-colored structures indicate histone cores; yellow-colored structures show the DNA sequence containing 5 nucleotides.

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TATTCTGGAG CAATGATGTG GTTAGCACAA ATCTTTAAGG TCTTGTCTCT TCTCATCAAT  60
ATTCTAACCT TGTTAGTCTT TTTGTTCTTC AAGAACTTAC AGTCACCAGT ACCTCTTTCT 120
TTCCATTCCT TGGCATCGGC ATCGAATCTG AAAAGCTTGG CTCTGACCTT GTAAAGAACT 180
TCTTCGTCTT CTTCCATTGT CTTAACATCT ACCTTTTCCA GGTGAACCAC TGGTTCAAAA 240
TGGATATCTG GTGATTCTGG AGCATCATCA CCTTCCTTCT TGGTTTCCTC CTTGGTATCT 300
TCTTCGTCTT TCTTGGTTTC TGGCTTTTCG GCCTTCTTAC CACCAAACAT GGAGAAGACA 360
GCAGAGGATG GTGGCTTTGG AGCAGCCTCT TCCTTCTTGT CGACGACAGG TTTCTTATCT 420
TCGCTAGACA TTCTTTTTCT ATAGTTGTGT TTATCTTCTT GCTTTTATTT CAAATTAAAC 480
AAGATCTTTC TTCTTTCTCC AATTGAATAT CAATTTCATC ATCAGATTTT AGTTTCTGTT 540
TTATTTTTTA TTTTTTTATT TTTTTTTTGT TTCTTGTTTT CCGCGTACTT CCCGCTGGGC 600
GAAAAAAGAA ATGAAAAAAA GAAACGACAG GAGCATCGTG TAGGACGAAG CCCCTTATCC 660
CCTAGTTACC GAAGAAGGCC ACCAATCTTA AGTTTGATAG AGCAGTACTT ATATAAGGCT 720
ATATATAGAC TGGTTCACAA GGTTATCAAT ATGAAACTTG CGCGATCACC GATTTACGGG 780
ATTTTTCAGG AGCGAGGTAC AAGATTTGTT GGCCTGAAAA GATCGCAAAA CATTAGCTAG 840
AAATTTTCCC CCTATCGTTT TCCGTAGAGT AAATATAATA TCAAGAAGAT AGTTTTATAT 900
TGACTGATTT CACAACCAAC TGCATAGATA TAAGGAGATT ACTAGATACA AGAACGCCTG 960
ATAAACAAAA AAAGAAAAAT TAACAAAAAA AATCAGTAGA                      1000
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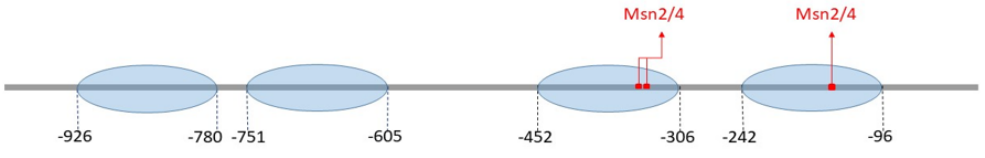

Fig. 2. *In silico* promoter analysis of *NTH1*. Positions of nucleosomes were determined using an energy diagram obtained from the ICM program. The positions of the nucleosomes were marked according to the first nucleotide (T) in the 1000 bp promoter region (not the transcription start nucleotide, A^{+1}). The nucleotides covered by the nucleosomes are underlined, the transcription start nucleotide is indicated in bold, and the Msn2/4p binding site is highlighted in yellow and bold.

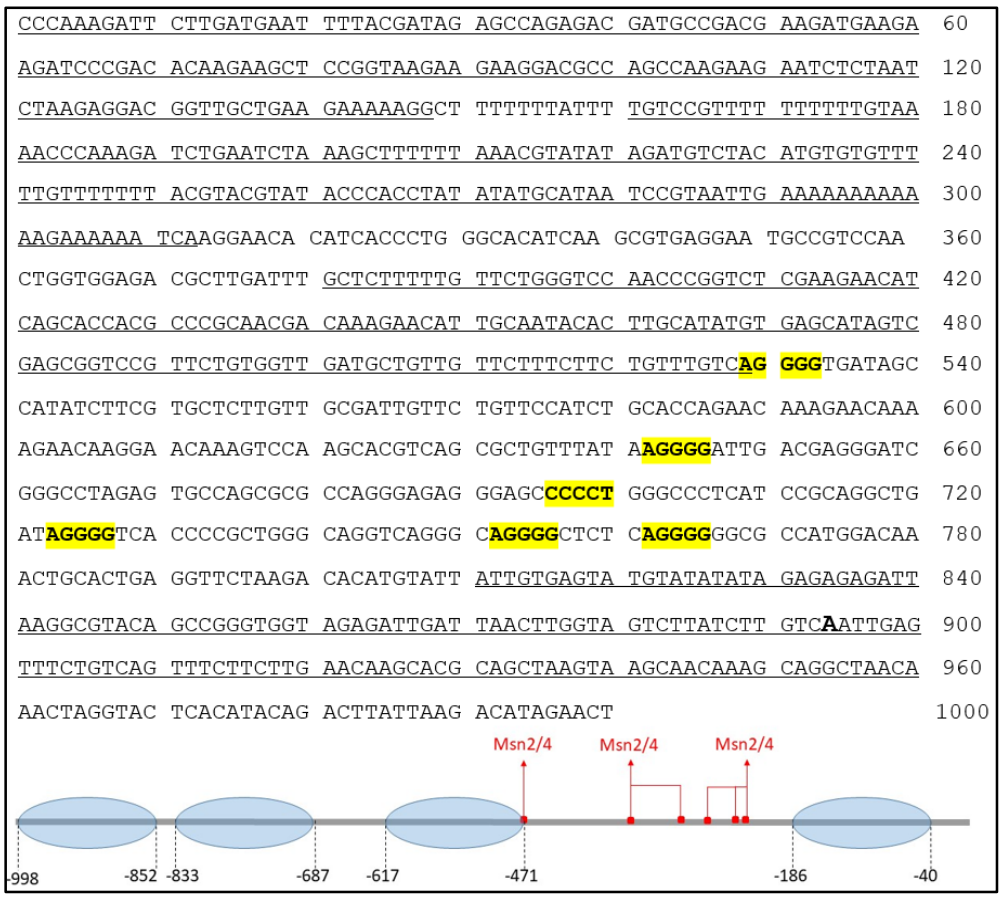

Fig. 3. In *silico* promoter analysis of *TPS1*. Positions of nucleosomes were determined using an energy diagram obtained from the ICM program. The positions of the nucleosomes were marked according to the first nucleotide (C) in the 1000 bp promoter region (not the transcription start nucleotide, A^{+1}). The nucleotides covered by the nucleosomes are underlined, the transcription start nucleotide is indicated in bold, and the Msn2/4p binding site is highlighted in yellow and bold.

However, the *NTH1* gene activation was 22-23 times lower than *TPS1* both in wild-type and mutant yeast cells (P<0.0001). The expression of *SUC2*-LacZ gene fusion as the control was not affected in the absence of Ada1 protein and yielded very low levels of beta-galactosidase activity (for wild type 1.48±0.02 units and *Δada1* mutant yeast 1.88±0.02 units) under normal growth conditions.

Both *NTH1* and *TPS1* gene expressions increase under different physiological and environmental stress conditions. Therefore, *NTH1* and *TPS1* promoter activations were determined in nitrogen starvation conditions. For this purpose, the yeast cells growing exponentially were shifted to a nitrogen-poor environment and enzyme assays were done after 4 hr incubation. When wild type yeast cells were shifted into the fresh medium containing a stress-inducing agent, the promoter activity increased from 102.3±16.6 units to 802.4±74.3 units for the *NTH1*, and from 2323.4±153.2 units to 7982.3±234.2 units for the *TPS1* gene (Fig. 4). *NTH1* and *TPS1* gene expressions of wild type yeast cells elevated nearly 8-fold and 3-fold in a nitrogen-poor environment, respectively (P=0.0023 for *NTH1* and P=0.0018 for *TPS1*). Similarly, when *Δada1* yeast cells were transferred into the poor nitrogen source, no change was observed in the *NTH1* gene expression level (P<0.0001), whereas the *TPS1* gene expression increased approximately 3-fold (from 2775.2±157.4 units to 9267.2±315.2 units).

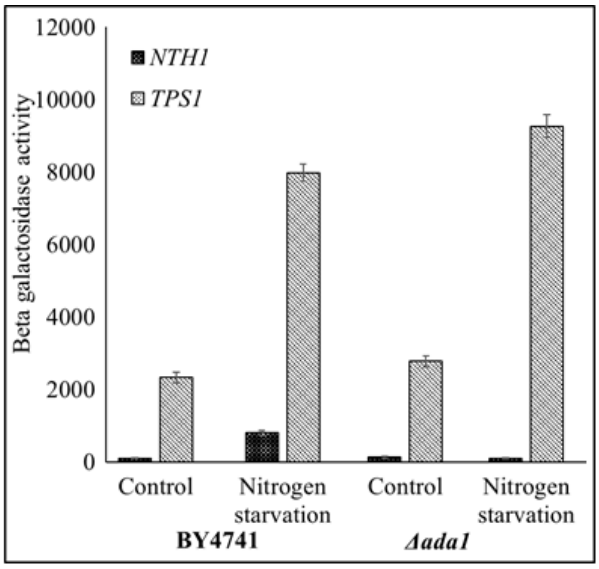

Fig. 4. *NTH1* and *TPS1* promoter activities of wild-type (BY4741) and mutant (*Δada1*) yeast cells under normal and nitrogen starvation conditions. The beta-galactosidase activity was given as hydrolyzed nmol ONPG/mg protein/min.

While the promoter activity of the *TPS1* gene in *Δada1* yeast cells was significant (*P*=0.0021) after starvation shift, *NTH1* promoter activity was not significant. *NTH1* gene expression of mutant yeast cells under nitrogen starvation condition was 8-fold lower than the wild type yeast cells (*P*=0.0025), but the *TPS1* gene expression in wild type and mutant yeast cells were similar. The promoter activity of the *SUC2* gene in a nitrogen-poor environment was measured as 11.12±3.2 unit and 14.18±3.7 unit for wild type and *Δada1* mutant yeast cells, respectively.

Effects of Ada1 protein on trehalose accumulation

The intracellular trehalose accumulations of wild type and mutant yeast cells were determined in normal growth conditions and nitrogen starvation. First, wild type and mutant yeast cells were grown in a nitrogen-rich environment up to the logarithmic stage and trehalose accumulations were determined. Then the yeast cells were transferred to a nitrogen-poor environment and trehalose accumulations were determined after 4 hr incubation. In normal growth conditions, the trehalose accumulations of wild type and mutant yeast cells were measured as 179.9±33.2 and 56.3±17.2 µg glucose/mg wet weight of cells, respectively. The level of trehalose in mutant yeast cells was 3-fold less than the wild type yeast cells (*P*=0.0031) (Fig. 5). When the wild type and mutant yeast cells were shifted into the nitrogen-starved environment, trehalose accumulations were determined as 1578.4±189.3 and 438.1±73.4 µg glucose/mg wet weight of cells, respectively. The levels of trehalose both in wild type and mutant yeast cells were increased 8-9 fold in nitrogen starvation conditions (*P*=0.0021 for wild type and *P*=0.0022 for *Δada1*). However, the accumulation of trehalose in mutant yeast cells was nearly 3- to 4-fold less than the wild type yeast cells regardless of the growth environment (*P*<0.0001).

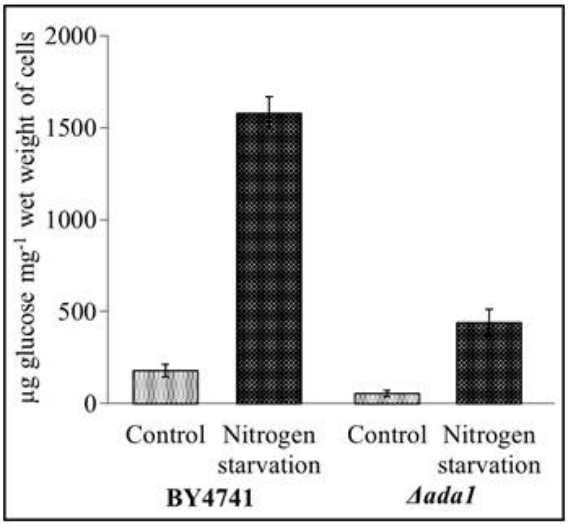

Fig. 5. Trehalose accumulation of wild type (BY4741) and mutant (*Δada1*) yeast cells under normal and nutrient starvation conditions. Trehalose content of yeast cells was given as micrograms of glucose equivalent per milligram of wet weight of the yeast cells.

Discussion

In this study, the role of the SAGA complex in the transcriptional regulation of *TPS1* and *NTH1* genes was determined using *Δada1* mutant yeast cells under normal and nitrogen starvation conditions. Stress-induced gene expression was detected in both *TPS1* and *NTH1* genes in the presence of Ada1p. However, in the absence of Ada1p, it was determined that *TPS1* gene expression was activated and *NTH1* gene expression did not change. In addition, a significant reduction in trehalose accumulation was observed in *Δada1* yeast cells under both normal and nitrogen starvation conditions.

The promoter regions of the *TPS1* and *NTH1* genes include STRE sequences for binding transcription factors and other regulatory proteins (Nwaka *et al.* 1995, Winderickx *et al.* 1996). Msn2/4 transcription factors bind to STRE sequences and regulate the transcription in stress conditions (Martinez-Pastor *et al.* 1996, Stewart-Ornstein *et al.* 2013, Rajvanshi *et al.* 2017). *In silico* analysis revealed four possible nucleosome localizations at the *TPS1* and *NTH1* promoter regions. In addition, all Msn2/4 binding sites on the *NTH1* promoter were closed by nucleosomes while they were open in the *TPS1* promoter. To validate these results, the positions of nucleosomes need to be confirmed using different experimental approaches, such as Electrophoretic Mobility Shift Assay (EMSA). *In silico* analysis suggested that, although both promoters are activated simultaneously under stress conditions, the transcriptional activation mechanisms and the activators involved in the activation mechanism are highly likely to be different. In other words, while nucleosome mobilization is required for the transcriptional activation of the *NTH1* gene under stress conditions, it may not be necessary for the *TPS1* promoter. This may cause *TPS1* gene expression to be higher than *NTH1* gene expression under the same conditions. Indeed, in a previous study, the amount of *NTH1* and *TPS1* mRNA levels were determined by RT-qPCR and they found that the level of *TPS1* mRNA was higher than the *NTH1* mRNA level (Flores *et al.* 2011).

The SAGA complex, known as transcriptional co-activator, has Histone Acetyl Transferase (HAT) activity and plays a role in the mobilization of nucleosomes. ADA proteins (Ada1-5) are generally associated with transcriptional repression. The SAGA complex acts as a transcriptional activator or repressor depending on the subunits and also growth conditions (Ricci *et al.* 2002, Chen & Dent 2021). Therefore, in this study, the role of the SAGA complex in the transcriptional regulation of *NTH1* and *TPS1* genes was determined under normal growth conditions and nitrogen starvation in the presence and absence of Ada1p. It was determined that there was no significant change in *NTH1* and *TPS1* promoter activities in wild type and mutant yeast cells under normal growth conditions. This indicates that the Ada1 protein has no role in the regulation of *NTH1* and *TPS1* gene expressions under standard growth conditions.

In yeast cells, *NTH1* and *TPS1* transcription starts at the exponential stage and reaches its maximum level in the diauxic stage. In our study, *NTH1* and *TPS1* promoter activation was determined at the exponential stage so the promoter activities of both genes were at the basal transcription level. *TPS1* gene activation was observed nearly 23-fold higher than *NTH1* both in the presence and absence of Ada1 protein. This indicates that the basal transcription of *TPS1* gene is higher than *NTH1*. The only known function of the Nth1 protein to date is trehalase activity. However, Tps1 protein is a multifunctional protein that plays a role in the regulation of the glycolytic pathway and glucose-dependent suppression of gluconeogenic genes in addition to trehalose 6-phosphate synthase enzyme activity (Bonini *et al.* 2003, Deroover *et al.* 2016, Gancedo *et al.* 2016). Therefore, the basal level of *TPS1* gene expression in yeast cells may be higher than the basal level of *NTH1* gene expression.

Gene expression in both *NTH1* and *TPS1* increases under variable stress conditions. In nutrient starvation (eg. glucose and nitrogen), the accumulation of trehalose increases independently from the growth phase (François & Parrou 2001). Therefore, *NTH1* and *TPS1* gene expressions were determined in nitrogen starvation conditions. In a nitrogen-poor environment, *TPS1* gene expression was observed to be 10-fold and 96-fold higher than *NTH1* gene expression in the presence and absence of Ada1p, respectively. In addition, *TPS1* gene expression increased approximately 3-fold under stress conditions in the presence and absence of Ada1p. This indicates that the Ada1p has no role in *TPS1* gene expression. The low *NTH1* gene expression in the absence of Ada1p indicates that the Ada1 protein has a regulatory role in *NTH1* gene expression under stress conditions. Based on the *in silico* promoter analysis, *NTH1* transcription may not have been activated due to the inability to mobilize nucleosomes that close the Msn2/4 binding sites required for transcriptional activation. In addition, *TPS1* transcription activation may have been observed since the promoter elements required for activation are not blocked by nucleosomes in the *TPS1* promoter.

In normal growth conditions, the amount of trehalose accumulated in wild-type yeast cells was 3 times greater than in mutant yeast cells. Similarly, in nitrogen starvation conditions, the level of trehalose accumulated by wild-type yeast cells was observed to be 4-fold higher than that of mutant yeast cells. Although nitrogen starvation-induced trehalose accumulation approximately 8-9-fold in the presence or absence of Ada1p, the trehalose levels were reduced 3-fold in the absence of Ada1p in both normal and nitrogen-starved conditions. Under normal growth conditions, yeast cells begin to synthesize trehalose at the end of the exponential phase and continue throughout the stationary phase. However, under stress conditions, trehalose is synthesized independently of the growth phase and continues until the end of stress conditions (Lillie & Pringle 1980, Thevelein 1984). In our study, the trehalose level in wild-type yeast cells under normal growth conditions was much lower than the amount of trehalose measured under stress conditions. This is an expected result since nitrogen starvation is known to cause trehalose accumulation in yeast. (Lillie and Pringle 1980).

Interestingly, although *TPS1* gene expression of *Δada1* yeast cells was elevated under stress conditions, there was a 3-fold reduction in trehalose accumulation of mutant cells compared to wild type. Different physiological and genetic mechanisms may have caused the low level of trehalose in *Δada1* yeast cells. Independent of its enzymatic activity, Tps1p regulates glycolysis in response to stress resistance (Gibney *et al.* 2015, Gancedo *et al.* 2016). *Δtps1* cells have unrestricted hexokinase activity and cause the uncontrolled influx of glucose into glycolysis (Peeters *et al.* 2017). Therefore, although the level of *TPS1* gene expression is high, only some of the synthesized proteins may act as active enzymes and the others work as regulators. This may be the first possible reason for high gene expression and low trehalose level in *Δada1* yeast cells. However, it is necessary to determine the intracellular Tps1 protein level to support this proposition. Another possible reason is that the gene expression of *TPS2*, *TPS3* and *TSL1*, other subunits of the TPS complex, may be regulated in a SAGA-dependent way under stress conditions. Therefore, it will be appropriate to determine the promoter activations of other subunits in *Δada1* yeast cells. In the third possibility, the trehalose may have been synthesized in *Δada1* yeast cells as much as in wild type yeast cells, but the synthesized trehalose may have been hydrolyzed by other neutral trehalase (Nth2p) and/or acid trehalase (Ath1p) enzymes. In previous studies, it has been reported that the *NTH2* gene is 77% paralog with the *NTH1* gene but it does not have a functional trehalase activity (Nwaka *et al.* 1995). In recent studies, it has been determined that Nth2 protein has low trehalase activity (residual-neutral trehalase activity) depending on the growth conditions, and its activity increases in the stationary phase where the glucose is depleted (Jules *et al.* 2008). Because of the sensitivity of *Δnth2* yeast cells to heat, it may be involved in thermotolerance and may mediate resistance to other cellular stresses (Zaim *et al.* 2005). Therefore, Nth2p may hydrolyze the intracellular trehalose during stress. This may explain the low accumulation of trehalose in nitrogen starvation, but not the cause of low trehalose accumulation under normal growth conditions.

It is known that the acid trehalase enzyme encoded by the *ATH1* gene is localized to a small extent in the cell vacuole, predominantly on the cell surface (periplasmic space) and a small amount in the cell wall (Jules *et al.* 2004, Eleutherio *et al.* 2015). In a recent study, it has been reported that in the absence of Nth1 and Nth2 proteins, intracellular trehalose is transported to the cell surface where it is degraded to glucose via the periplasmic Ath1 protein. Although the transport systems transporting trehalose out of the cells are not yet known, it has been shown that this transport is Ath1p dependent (Jules *et al.*

2008, Magalhães *et al.* 2018). Because of the detection of active enzymes only in glucose-grown cells and the lack of STRE sequences at the *ATH1* gene promoter, *ATH1* gene expression is thought to be not activated by stress (Garre *et al.* 2009, Eleutherio *et al.* 2015). However, in salt stress, *ATH1* gene expression and acid trehalase activity increased more than *NTH1* gene expression and neutral trehalase activity in a Hog1p-dependent manner. In addition, the loss of Ath1 protein causes an increase in intracellular trehalose level (Garre *et al.* 2009). Similarly, *ATH1* and *NTH1* gene expressions were observed to be at least two times higher in the stress-resistant (SRM11, M8FE and H7) and caffeine-hyper resistant (Caf905-2) *S. cerevisiae* strains compared to the control yeast strain (Arslan *et al.* 2018, Balaban *et al.* 2019, Sürmeli *et al.* 2019, Kocaefe-Özşen *et al.* 2022). Based on the results of these studies, since *NHT1* gene expression cannot be activated in *Δada1* yeast cells, Ath1p-dependent cleavage of the accumulated trehalose in the cell is strongly probable. Determining trehalose accumulations of *Δnth2* and *Δath1* yeast cells under different stress conditions would be appropriate to confirm Nth2p- or Ath1p-dependent degradation of trehalose.

The core unit of the SAGA complex, consisting of Spt7, Spt20 and Ada1 proteins, is essential for the integrity and active form of the SAGA complex. For this reason, it would be appropriate to repeat this work with *Δspt7* and *Δspt20* yeast cells in future studies to support that the results obtained in *Δada1* yeast cells are SAGA-dependent. As a result of our study, we can say that the genes controlling trehalose metabolism are undergoing some kind of epigenetic regulation. This provides a new perspective on the regulation of genes that control the synthesis-breakdown mechanisms of intracellular metabolites.

Acknowledgement

The author is grateful to Professor Jean Marie François (Institut National Des Sciences Appliquées, Toulouse, France) for the gift of LacZ fusion systems.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Conflict of Interest: The author has no conflicts of interest to declare.

Funding: The author declared that this study has received no financial support.

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