ISTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE

Eur J Biol 2020; 79(1): 36-42

Apoptosis Signaling Pathway Regulates the Gene Expression in the Yeast Retrotransposons Ty1 and Ty2

Ceyda Colakoglu[1], Sezai Türkel[1]

[1]Bursa Uludag University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Bursa, Turkey

ORCID IDs of the authors: C.C. 0000-0002-7471-5071; S.T. 0000-0001-7128-6948

Please cite this article as: Colakoglu C, Türkel S. Apoptosis Signaling Pathway Regulates the Gene Expression in the Yeast Retrotransposons Ty1 and Ty2. Eur J Biol 2020; 79(1): 36-42. DOI: 10.26650/EurJBiol.2020.0016

ABSTRACT

Objective: Ty elements are retroviral–like entities present in the yeast *Saccharomyces cerevisiae*. Apoptosis is a programmed cell death mechanism, conserved in all eukaryotes. In this study, we aimed to analyze how apoptotic signals affect the transcriptions of Ty1 and Ty2 elements in *S. cerevisiae*.

Materials and Methods: To analyze the effects of apoptotic signals on the transcription of Ty element, Ty1-LacZ, and Ty2-LacZ gene fusions were used as reporter genes. These gene fusions were transformed into the wild type and certain yeast mutants that are defective in various signaling pathways. Acetic acid was added to the growth medium of logarithmically growing yeast transformants to induce apoptosis. Transcription levels of the Ty-lacZ gene fusions were analyzed by ß-Galactosidase assays.

Results: The results of this study show that transcription of Ty1 and Ty2 decreases approximately 3-fold in response to apoptosis in *S. cerevisiae*. It appears that apoptosis acts through the transcription factors Tec1p and Sgc1p that associate with the regulatory region of Ty1 and Ty2. Moreover, AMP-activated protein kinase Snf1p, and to a lesser extent Tor1p, seem to be required for the transcriptional repression of Ty1 and Ty2 in apoptosis-induced yeast cells.

Conclusion: Ty1 and Ty2 transcription is regulated in response to apoptosis signaling in a differential manner. It seems that protein kinases Tor1p and Snf1p and transcription factors Tec1p and Sgc1p are involved in the apoptosis dependent regulation of Ty transcription.

Keywords: Ty elements, Apoptosis, Transcription, Yeast, Protein kinases

INTRODUCTION

Saccharomyces cerevisiae contains 5 different classes of retrotransposons, called Ty1-Ty5, in its genome (1). Transposition of these retrotransposons takes place via an RNA intermediate (2). Due to their genome organization and their intracellular propagation pattern, Ty elements are classified within the pseudovirales class of virales order (3). Ty1, Ty2, and Ty4 have a similar genome structure and organization. Ty1 and Ty2 have 334 bp Long Terminal Repeat (LTR) sequences in their 5′ and 3′ ends, named as delta elements. Ty1 has 30 copies per genome while Ty2 has about 10 copies per genome in yeast (1). Apart from full length Ty elements, the yeast genome contains a large number of solo LTR sequences. Ty3 and Ty5 have a different genome organization (1). The genome organization of Ty3 is similar to the human immunodeficiency virus. It is present as 4 copies per genome. Its LTRs are called sigma elements. The genome sizes of Ty elements vary from 4 Kbp to 6 Kbp (1). Ty elements encode two overlapping peptides, named TYA and TYB. These coding regions show structural and functional homologies to retroviral gag and pol polypeptides, respectively (4,5). TYB is translated by programmed ribosomal frameshift mechanisms as a TYA-TYB fusion protein (6).

Transcription of Ty elements starts at 5′ Delta and ends at 3′ Delta. Promoter elements of Ty elements are located both upstream and downstream of the transcription

Corresponding Author: Sezai Türkel E-mail: sturkel@uludag.edu.tr
Submitted: 11.04.2020 • **Revision Requested:** 17.05.2020 • **Last Revision Received:** 22.05.2020 • **Accepted:** 26.05.2020 • **Published Online**: 12.06.2020

initiation site (7,8). The transcriptional regulatory region of Ty2 is located within the first 750 regions of these elements (8,9). Regulatory regions of Ty1 encompass much longer regions and extend up to the first 1800 bp region of Ty1 (10,11). In upstream, TATA and UAS sequences are located within the first 150 bp region of Ty1 and Ty2. Unexpectedly, most of the transcription regulatory sites, such as enhancer elements, downstream repression sites, and cell type-specific transcription factors' binding sequences are located within the coding region of Ty1 and Ty2 (7-11). Both cis-acting regulatory sequences and transcriptional activators that associate with regulatory sites of Ty 1 and Ty2 have been identified in previous molecular and biochemical studies (7-11). Ty1 transcription is 10-times lower in diploids than in haploid yeast cells (12). The transcription of Ty1 is controlled by the heterodimeric repressor factor in diploids (13).

Ty1 is under the control of at least nine different transcription factors. These are Ste12, Tye7, Tec1, Mcm1, Rap1, Tea1/Ibf1, Gcn4, Gcr1 and Mot3 (5,13-17). Besides transcription factors, chromatin-modifying complexes such as Swi/Snf, SAGA and ISWI are also involved in the regulation of Ty transcription (5). Transcription of Ty2 is controlled by glucose signaling (17).

Apoptosis is an evolutionary conserved programmed cell death mechanism that takes place in all eukaryotes (18-20). Different external and internal signals trigger apoptotic signaling in eukaryotes (21,22). Upon activation of apoptosis by one of the signaling pathways, initiator caspases trigger a caspase cascade, which ends up with the complete destruction of targeted cells (21,22).

Apoptosis is also defined in the yeast cells. The first time, apoptosis-like cellular processes are defined in cdc48 mutants of *S. cerevisiae* (23,24). Madeo et al, (1997) found that when starved for nutrients, cdc48 mutants of *S. cerevisiae* go through cellular destruction, similar to apoptotic events in human cells. Later, it was shown that over-expression of the human Bax gene, a proapoptotic factor for human cells, also activated apoptosis-like cell death in *S. pombe* (25).

In search of molecular components of apoptotic signaling pathways in *S. cerevisiae*, the *YCA1* gene (also known as Metacaspase-1, *MCA1*) has been identified and its function in *S. cerevisiae* apoptosis has been confirmed by genetic analyses (26,27). Unlike human caspases, yeast caspase Mca1p has a calcium-dependent cysteine-type endopeptidase activity on the targeted proteins (28,29). Excess H_2O_2, acetic acid, osmotic stress, and certain metal ions are the external activator of apoptosis in yeast. Aging, DNA-damaging drugs, expression of heterologous genes such as human Bax, and α-synuclein are known intracellular effectors that activate apoptosis in *S. cerevisiae* (30). Acetic acid is the well-defined activator of the apoptosis in *S. cerevisiae* (31,32).

Apoptosis has global effects on the metabolism of eukaryotic cells. Once the apoptosis is triggered by internal or external signaling, different kinases are activated or repressed. It is known that protein kinases Tor1p, Snf1p, and Gcn2p have significant and multiple functions in the nutrient signaling in yeast (33-35). These protein kinases are also conserved in human cells. Recent evidence indicates that Tor1p and Snf1p also have a function in apoptosis (33-35).

Unlike retroviruses, Ty elements cannot leave the yeast cells. Therefore Ty elements can be considered as mandatory genetic components of the yeast cells. Ty genomes do not encode any known transcription or translation regulatory factors. Hence, Ty viruses are completely dependent on the yeast encoded transcription and translation factor for their genomic propagation. In this study, we investigated how apoptotic signals affect the transcriptional regulations of Ty1 and Ty2 elements in *S. cerevisiae*. Our results indicate that activation of apoptosis in yeast cells result in significant decreases in Ty1 and Ty2 transcription. We also show that protein kinases Tor1p and Snf1p were involved in the apoptosis-dependent repression of Ty1 and Ty2 transcription, albeit at the differential level. Effects of Tec1p and Sgc1p were also analyzed to test if apoptosis operates through these factors to regulate Ty transcription.

MATERIALS AND METHODS

Yeast Strains and Ty Expression Vectors

S. cerevisiae strains used in this research were purchased from the EUROSCARF (University of Frankfurt, Germany). Their genotypes and accession numbers for EUROSCARF collection are given in Table 1. The construction of kanMX deletion mutants of these strains was explained previously (36). It is known that these yeast strains do not contain any other mutations in their genomes other than the indicated ones.

The plasmids Ty1-144-lacZ, Ty2-754-lacZ, p-ENC and Ty2-555-lacZ are 2 micron-*URA3* based, yeast episomal (YEp) type expression vectors. The Ty2-754-lacZ plasmid contains the first 754 nucleotides of the Ty2 genome fused to *E. coli* lacZ gene. It contains all of the regulatory sequences required for the regulated transcription of Ty2. The Ty2-555-lacZ reporter plasmid has the same general features except that it does not contain downstream repression sites for Ty2. p-ENC is also a 2 µm-*URA3* based expression vector that contains an enhancer region of Ty2, upstream of the TATA box in the UAS-less His4-lacZ gene fusion. Structure and constructions of Ty2-lacZ gene fusions have been published previously (7-9).

Ty1-144-lacZ plasmid is a 2 µm-URA3 based, YEp vector and contains the first 1571 bp region of Ty1 element fused to LacZ gene. Its whole structure and construction have been explained previously (10,11). It is known that these yeast expression vectors are stably maintained in the selective growth medium, and their copy number does not vary in yeast transformants (7).

Growth Conditions and Transformation

Yeast strains were cultured in YPD (yeast extract, peptone, dextrose) media for transformations. Transformation of Ty expression vectors to competent *S. cerevisiae* strains was done using the Polyethylene glycol-lithium acetate method as described previously (37). Yeast transformants were grown in 10 ml of

Table 1. *S. cerevisiae* strains and their relevant genotypes used in this research

Yeast Strains' Lab Code, (relevant mutations) and EUROSCARF Accession numbers	Genotypes
YST124 (wild type) Y00000 (BY4741)	MATα *his*Δ1; *leu2*Δ0;*met15*Δ0;*ura3*Δ0
YST155 (*tor1*Δ) Y06864	MATα *his*Δ1; *leu2*Δ0;*met15*Δ0;*ura3*Δ0; YJR66w::kanMX4
YST159 (*snf1*Δ) Y14311	MATα *his*Δ1; *leu2*Δ0;*met15*Δ0;*ura3*Δ0; YDR477w::kanMX4
YST230 (*gcn2*Δ) Y03642	MATα *his*Δ1; *leu2*Δ0;*met15*Δ0;*ura3*Δ0; YDR283c::kanMX4
YST265 (*tec1*Δ) Y07155	MATα *his*Δ1; *leu2*Δ0;*met15*Δ0;*ura3*Δ0; YBR083w::kanMX4
YST289 (*sgc1*Δ) Y01641	MATα *his*Δ1; *leu2*Δ0;*met15*Δ0;*ura3*Δ0; YOR344c::kanMX4

synthetic complete medium without uracil that contains 2% glucose (Sc-Ura, Dext) in triplicates as described (38). At logarithmic stages, yeast cultures were divided into two aliquots (5 ml each). To induce apoptosis, acetic acid was added to yield 60 mM (pH 3) final concentration to the growth medium of the one portion (5 ml) of the yeast transformants. Yeast cultures (control group and apoptosis induced ones) further grown for five hours at 30°C in an incubator shaker at 150 revolutions per minute. At the end of growth periods, yeast transformants were harvested and processed for ß-galactosidase assays.

ß-Galactosidase Enzyme Assays

The yeast transformants that were grown with or without acetic acid, harvested at the end of 5 hours of incubation periods, were spun down with centrifugation at 1000 g for 5 minutes. Harvested yeast cells washed with 1 ml of sterile distilled water and transferred to microfuge tubes and harvested again by centrifugation at 15,000 g for 1 minute. Yeast pellets were resuspended in 200 µl of break buffer and stored at -80°C till enzyme assays. ß-galactosidase assays were done essentially as described by Guarantee (39). In brief, yeast cells, suspended in break buffer, were permeabilized with 20 µl of chloroform and 20 µl of 0.1% SDS by vortexing at top speed for 1 minute to get permeabilized yeast cell suspensions (39). ß-galactosidase assays were done in triplicates using permeabilized yeast cell suspensions and ONPG as substrate as described (39). Protein concentrations in permeabilized yeast cells were determined by the Lowry assay (40). ß-galactosidase units are expressed in nm ONPG cleaved per minute per mg of protein in the permeabilized yeast cell suspensions. All assays were repeated at least twice in triplicates. The number representing β-galactosidase units in figures are the averages of 9 independent ß-galactosidase assays. Standard deviations were less than 10% in all of the ß-galactosidase units given in figures. We have used the T-test to compare two groups of data (such as wild type versus mutant, normal growth versus apoptosis conditions). Transcription units were significantly different ($p<0.05$).

RESULTS

Effects of Apoptosis Signaling on the Transcription of Ty Elements

We analyzed the effects of apoptosis on two different Ty elements, Ty1 and Ty2, respectively. Under normal growth conditions, transcription from Ty1-144-LacZ gene fusion yields a high level of expression (3074 Units), as expected. However, induction of apoptosis by acetic acid for 5 hours resulted in more than a 3-fold decrease in Ty1 transcription (Figure 1). Similarly, transcription from the Ty2-754-LacZ reporter gene is also repressed upon the activation of apoptosis. Transcription from Ty2-754-LacZ gene fusion is determined as 185 units under normal growth conditions. Induction of apoptosis by acetic acid result-

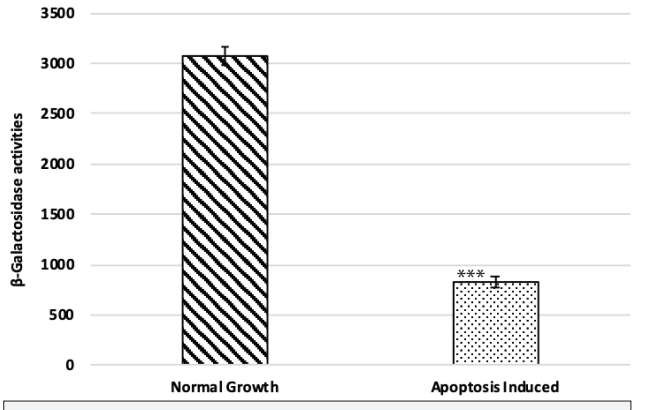

Figure 1. Effects of apoptosis induction on Ty1-144 transcription in wild type yeast. Error bars indicate the standard deviations (***$p<0.001$ compared with untreated control).

ed in a nearly 3-fold decrease in Ty2 transcription too (Figure 2). It is known that Ty1 is transcribed at much higher rates than Ty2 (12). It was previously reported that Ty1 mRNA constitutes about 0.8% of total cellular RNA in yeast (12). Hence, there is a clear difference between Ty1 and Ty2 transcriptions in normal growth medium, as seen in Figures 1 and 2. The transcription of both Ty elements decreases approximately 3-fold by the apoptosis signaling. A *p*-value which is less than 0.001 ($p \leq 0.001$) indicates that there are statistically significant differences in expression levels of Ty1 and Ty2 elements compared to untreated control.

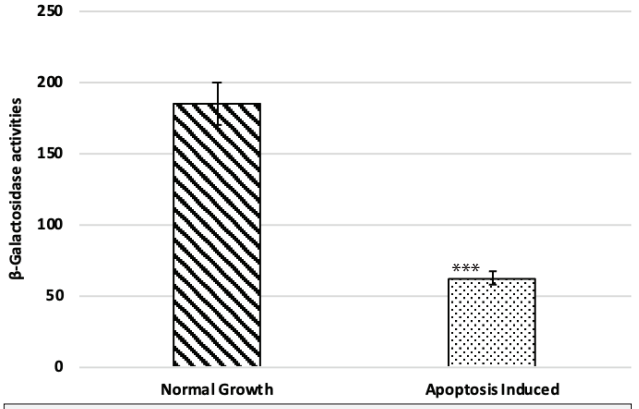

Figure 2. Effects of apoptosis induction on Ty2-754 transcription in wild type yeast. Error bars indicate the standard deviations (***p<0.001 compared with untreated control).

Effects of Different Protein Kinases on Ty Transcription in Apoptosis Induced Cells

Transcription of Ty1 decreased 20-40% in these kinase mutants in normal growth conditions (Figure 3). But the highest decrease in Ty1 transcription was seen in *snf1* deletion mutant yeast cells, indicating that functional Snf1p kinase is essential for optimal level transcription of Ty1. It has already been shown that Snf1p is required for Ty transcription (41). Activation of apoptosis by acetic acid leads to a further decrease in the transcription of Ty1-144-LacZ gene fusion in this kinase mutant. It seems that

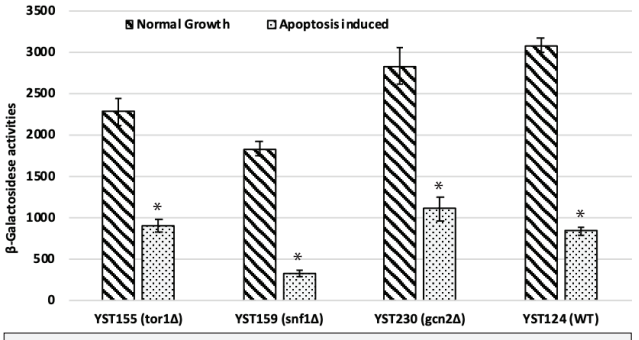

Figure 3. Effects of different protein kinases on the Ty1-144-LacZ transcription in apoptosis induced conditions. Error bars indicate the standard deviations (*p<0.05 compared with untreated control).

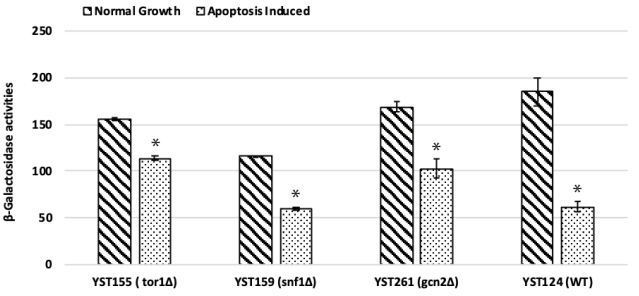

Figure 4. Effects of different protein kinases on the Ty2-754-LacZ transcription in apoptosis induced conditions. Error bars indicate the standard deviations (*p<0.05 compared with untreated control).

inactivation or degradation of Snf1p in apoptosis-induced cells results in a nearly 6-fold decrease (from 1824 units to 323 units) in Ty1 transcription (Figure 4). Ty1 transcription also decreased approximately 2-fold in apoptosis induced Δ*tor1* and Δ*gcn2* mutants and was reduced down to wild type level expression. These results indicate that Tor1p and Gcn2p are not involved in the Ty1 transcription when apoptosis-induced, although they are required for transcription in normal growth conditions. A *p*-value which is less than 0.05 ($p \leq 0.05$) indicates that there are statistically significant differences in expression levels of Ty1 compared to untreated control (Figure 3).

We also tested the effects of the above-mentioned kinases if they were involved in the Ty2 transcription when yeast cells shifted to apoptotic growth conditions. Interestingly, lack of Tor1p, Gcn2p or Snf1p in normal growth conditions did not influence Ty2 transcription at significant levels. (Figure 4). However, once apoptosis was induced by adding acetic acid to the growth medium of these kinase mutants, transcription of Ty2-754-lacZ gene fusion differentially affected by each mutant. Transcription of Ty2-754-LacZ in Δ*snf1* decreased to wild type level expression in the apoptosis induced yeast cells. It is clear that transcription of Ty2 in apoptosis-induced Δ*tor1* and Δ*gcn2* mutants are not affected by these mutations as much as in Ty1-LacZ transcription (Figure 4). A *p*-value which is less than 0.05 ($p \leq 0.05$) indicates that there are statistically significant differences in expression levels of Ty2 compared to untreated control (Figure 3).

To see if the apoptosis signaling acts on the transcriptional activators that associate with the Ty2 enhancer region, we also tested the effects of apoptosis signaling on Ty2 enhancer element-dependent transcription independent of Ty2 promoter context. Enhancer element-dependent transcription of a heterologous promoter from p-ENC-LacZ expression vector was analyzed under normal and apoptosis induced growth conditions (Figure 5). In addition, we also tested the effects of apoptosis on the Ty2 transcription that contains upstream activation sequence (UAS) and enhancer elements only as the activator regions in the native Ty2 promoter context. Ty2-555-LacZ expression vector is the truncated version of Ty2-754-LacZ vec-

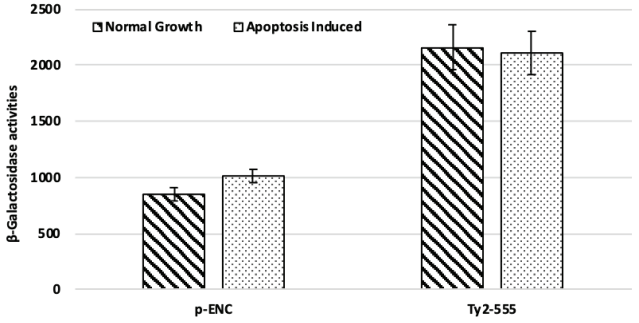

Figure 5. Analysis of the effects of apoptosis on the Ty2-555 and Ty2-Enhancer element dependent transcription in the wild type yeast. Error bars indicate the standard deviations.

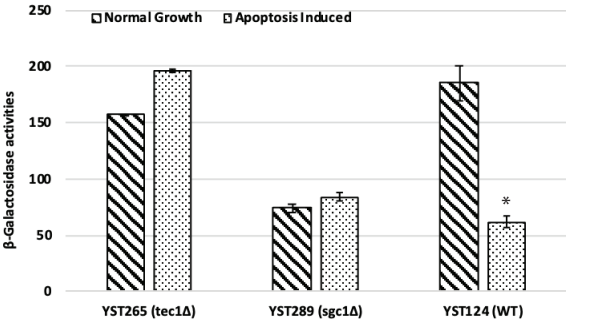

Figure 7. Effects of Tec1p and Sgc1p on Ty2-754-LacZ transcription in apoptosis induced yeast cells. Error bars indicate the standard deviations (*p<0.05 compared with untreated control).

tor that does not contain a negative regulatory region of Ty2 (7-9). Hence, transcription from Ty2-555-LacZ gene fusion was much higher than the Ty2-754-LacZ gene fusion that contained negative regulatory sites for Ty2. As seen in Figure 5, apoptosis signaling did not affect transcription from Ty2-555-LacZ nor p-ENC-LacZ dependent transcription (Figure 5). These results suggest that apoptosis signaling targets regulatory factors that associate with the full-length promoter region of Ty2 in its native context (Figure 5).

Effects of Tec1p and Sgc1p on the Ty1 and Ty2 Transcription in Apoptosis Induced Cells.

To analyze if Tec1p and Sgc1p were involved in the apoptosis signaling-dependent repression of Ty1 and Ty2 transcription, we analyzed the transcription levels of Ty1-144-LacZ and Ty2-754-LacZ gene fusion in Δ*tec1* and Δ*sgc1* mutant strains of *S. cerevisiae* (Figures 6 and 7).

When compared to the expression levels of Ty1-144-LacZ gene fusions in the wild type yeast strains, Ty1 transcription was not affected by the apoptosis signaling in Δ*tec1* and Δ*sgc1* mutants. In other words, it seems that functional Tec1p and Sgc1p were the targets of apoptosis signaling for repression of Ty1 transcription. Similarly, transcription from Ty2-754-LacZ gene fusion also was not affected by apoptosis signaling in Δ*tec1* and Δ*sgc1* mutants. We saw slight increases in Ty2-754-LacZ transcription in these mutants when apoptosis was induced by acetic acid (Figure 7).

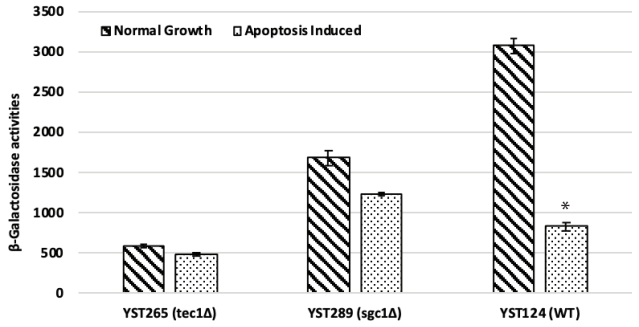

Figure 6. Effects of Tec1p and Sgc1p on Ty1-144-LacZ transcription in apoptosis induced yeast cells. Error bars indicate the standard deviations (*p<0.05 compared with untreated control).

DISCUSSION

Ty elements have been identified as intracellular mobile genetic elements of the yeast *S. cerevisiae* (1). Their genome structures and intracellular propagation mechanisms resemble retroviruses (2,5). Ty elements do not encode any regulatory factors for their transcription and translation from their genomes. Hence, transcription of Ty elements and translation of their mRNA completely depends on yeast encoded regulatory factors. Regulator factors that are involved in the transcription of Ty have been identified previously (5,13-17). It can be expected that the cellular metabolic events that affect gene expression in the yeast genome should also affect the gene expression in Ty. In accord with this, it has been shown that glucose signaling, amino acid starvation, and adenine starvation affects the transcription of Ty1 and Ty2 (10,17). It is known that the Ty1 transcription is regulated by a cell type-dependent manner, and repressed several-fold by repressor proteins in diploid *S. cerevisiae* cells (13). The expression of Ty2 is not affected by a cell type. Nonetheless, even though the transcription of Ty1 and Ty2 is activated or repressed by cellular signals, it is known that the genomic copy numbers of Ty elements within the yeast genome do not change drastically during consecutive cell divisions. Several mechanisms have been proposed for the copy number maintenance for the Ty element (5,42).

Programmed cell death is a global cellular response to various extracellular and intracellular signals for cell destruction without any cellular artifact (21,22). Molecular mechanisms and biological functions of apoptosis in multi-cellular organisms have been studied extensively (21,22). Apoptosis and its function have also been explained in the yeast *S. cerevisiae* (23,26,27). It occurs in response to external chemical stressors, like acetic acid and hydrogen peroxide in *S. cerevisiae* (30-33). Apoptosis is also activated in aging yeast cells (30-33).

It appears that transcription of Ty1 and Ty2 is also affected by external apoptosis signaling generated by acetic acid. Our results indicated that while the levels of transcription show large differences between Ty1 and Ty2, their transcription was repressed at about the same levels, by 3-fold, in response to apoptosis signaling in the wild type *S. cerevisiae* cells. This result implies that common regulator factors act on Ty1 and Ty2 promoter for repression of Ty transcription when apoptosis-induced. Several transcription factors are involved in the transcriptional activation of both Ty1 and Ty2. Some of these well-known transcriptional activators are Gcn4p, Gcr1p, Tec1p and Sgc1p (Tye7p). The deletion of *GCR1* gene has a severe defect on *S. cerevisiae*, and its deletion has lethal effects on some yeast strains (43). Therefore, we could not analyze the effects of Gcr1p on Ty transcription in apoptosis-induced Δ*gcr1* mutant strain.

Transcription factor Gcn4p is also involved in transcriptional activation of Ty1 when amino acid or adenine starvation-induced (10,11). But, Gcn4p is not actively expressed in normal growth conditions, and its activation requires amino acid or purine starvation (10,11). To test the effects of Gcn4p on the Ty1 and Ty2 transcription in apoptosis induced cells, expression of *GCN4* gene must be activated by amino acid or purine starvation. This type of experimental set up will lead to the application of two independent stress conditions, apoptosis and amino acid starvation, on the same yeast cells, which might result in misleading evaluations for the effects of Gcn4p involvement in apoptosis-induced cells. Therefore, we did not test the effects of Gcn4p on Ty1 and Ty2 transcription in apoptosis induced Δ*gcn4* mutants.

Instead, we analyzed the effects of Tec1p and Sgc1p on the transcription of Ty1 and Ty2 in the wild type and mutant yeast that is viable under normal growth conditions. Recent evidence indicates that Tec1p also involves the TOR and other MAPK signaling pathways (44,45). Ty1 transcription largely depends on Tec1p (14). Therefore, transcription of Ty1 decreased nearly 6-fold in Δ*tec1* mutants strain grown in normal conditions. The transcript of Ty2 is not affected by Δ*tec1*. It was already shown that Tec1p has no drastic effects on Ty2 transcription (14). But, when apoptosis-induced by acetic acid, transcription Ty2 also remained at the same levels. In other words, apoptosis signaling did not affect Ty2 transcription in Δ*tec1* mutants (Figure 7). These results indicated functional Tec1p is one of the targeted regulatory factors for the Ty1 repression in apoptosis induced conditions. The effects of Tec1p in apoptosis-induced and uninduced cells for Ty2 seems to be more complex than Ty1. First of all, a lack of functional Tec1p in Δ*tec1* mutant did not have any effects on Ty2 transcription. But, the induction of apoptosis by acetic acid did not repress Ty2 transcription in this mutant. This result suggests that although functional Tec1p is not essential for Ty2 transcription in normal conditions, it is essential for the repression or downregulation of Ty2 transcription by apoptosis.

Ty promoters contain activator and repressor binding sites in both upstream and also in downstream of transcription initiation sites (7-9). We analyzed the effect of apoptosis signaling on the truncated Ty2 promoter (Ty2-555-LacZ reporter) that contains only the enhancer region (7,8). Interestingly, our results indicated that activation of apoptosis signaling did not have any effects on Ty2 transcription when it contains only the enhancer region. We also tested the effects of apoptosis signaling on the Ty2 enhancer element-dependent transcription when present outside of the Ty2 promoter. As in the truncated Ty2 promoter, enhancer element-dependent transcription of a heterologous promoter is not affected by apoptosis signaling (Figure 5). These results may indicate that apoptosis signaling targets the regulatory factors that associate with the negative regulatory region of Ty2 element that is located within the 555-754 bp region of this element (7,8).

S. cerevisiae has many different protein kinases that share functional and structural homologies to their human counterparts. Some of these kinases are Tor1p, Snf1p, and Gcn2p. These kinases are essential for nutrient signaling in yeasts. They also have functional roles in the signaling and progression of apoptosis and autophagy in yeast (34,35,45). The functional involvement of these kinases in the Ty1 and Ty2 transcription under apoptosis induced conditions were analyzed in the mutant yeast strains that do not have one of these kinases. Our results indicated that Snf1p has a significant function in the basal level transcription of Ty1 and Ty2 in normal growth conditions since the transcription of Ty1 and Ty2 decreased significantly in Δ*snf1* mutants. On the other hand, in apoptosis induced conditions, only transcription Ty1 further decreased in Δ*snf1* mutant yeast, showing the functional involvement of Snf1p kinase in Ty1 transcription in apoptosis conditions.

Ty1 and Ty2 transcription affected at the differential manner in the yeast mutants that do not contain functional Tor1p kinase. Transcription of Ty2 is not affected by apoptosis signaling in Δ*tor1* mutation, indicating that Tor1p kinase is involved in the repression of Ty2 transcription in apoptosis-induced cells. Gcn2p kinase seems to have a moderate level effect on Ty2 transcription both in normal and also in apoptosis induced conditions.

CONCLUSION

In this study, we have shown that the transcription of retroviral-like elements Ty1 and Ty2 was regulated in differential manners when the apoptosis signal was induced by acetic acid. It seems that protein kinases Tor1p and Snf1p and transcriptional activators Tec1p and Sgc1p are involved in the repression of Ty1 and Ty2 transcription in apoptosis induced yeast cells.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of study: S.T., C.C.; Data Acquisition: C.C., S.T..; Data Analysis/Interpretation: S.T., C.C.; Drafting Manuscript: S.T., C.C.; Critical Revision of Manuscript: S.T.; Final Approval and Accountability: S.T.; Technical or Material Support: S.T., C.C.; Supervision: S.T.

Conflict of Interest: The authors declare that they have no conflicts of interest to disclose.

Financial Disclosure: There are no funders to report for this submission.

REFERENCES

1. Kim JM, Vanguri S, Boeke JD, Gabriel A, Voytas DF. Transposable elements and genome organization: A comprehensive survey of retrotransposons revealed by the complete *Saccharomyces cerevisiae* genome sequence. Genome Res 1998; 8: 464-78.
2. Boeke J, Garfinkel DJ, Styles CA, Fink G. Ty elements transpose through an RNA intermediate. Cell 1985; 40: 491-500.
3. Capy P. Classification and nomenclature of retrotransposable elements. Cytogenet Genome Res 2005; 110: 457-61.
4. Farabaugh PJ. Translational frameshifting: implications for the mechanism of translational frame maintenance. Prog Nucleic Acid Res Mol Biol 2000; 64: 131-70.
5. Curcio MJ, Lutz S, Lesage P. The Ty1 LTR-retrotransposon of budding yeast, *Saccharomyces cerevisiae*. Microbiol Spectr 2015; 3: 1-35.
6. Belcourt MF, Farabaugh PJ. Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. Cell 1990; 62: 339-52.
7. Farabaugh PJ, XB. Liao, Belcourt M, Zhao H, Kapakos J, Clare J. Enhancer and silencer-like sites within the transcribed portion of a Ty2 transposable element of *S. cerevisiae*. Mol Cell Biol 1989; 9: 4824-34.
8. Farabaugh PJ, Vimaladithan A, Türkel S, Johnson R, Zhao H. Three downstream sites repress transcription of a Ty2 retrotransposon in *Saccharomyces cerevisiae*. Mol Cell Biol 1993; 13: 2081-90.
9. Türkel S, Farabaugh PJ. Interspersion of an unusual GCN4 activation site with a complex transcriptional repression site in Ty elements of *Saccharomyces cerevisiae*. Mol Cell Biol 1993; 13: 2091-103.
10. Morillon A, Bénard L, Springer M, Lesage P. Differential effects of chromatin and Gcn4 on the 50-fold range of expression among individual yeast Ty1 retrotransposons. Mol Cell Biol 2002; 22: 2078-88.
11. Todeschini AL, Morillon A, Springer M, Lesage P. Severe adenine starvation activates Ty1 transcription and retrotransposition in *Saccharomyces cerevisiae*. Mol Cell Biol 2005; 25: 7459-72.
12. Curcio MJ, Hedge AM, Boeke JD, Garfinkel DJ. Ty RNA levels determine the spectrum of retrotransposition events that activate gene expression in Saccharomyces cerevisiae. Mol Gen Genet 1990; 220: 213-21.
13. Company M, Errede BA. Ty1 cell-type-specific regulatory sequence is a recognition element for a constitutive binding factor. Mol Cell Biol 1988; 8: 5299-309.
14. Laloux I, Dubois E ,Dewerchin M, Jacobs E. *TEC1*, a gene involved in the activation of Tyl and Tyl-mediated gene expression in *Saccharomyces cerevisiae*: Cloning and molecular analysis. Mol Cell Biol 1990; 10: 3541-50.
15. Löhning C, Ciriacy M. The TYE7 gene of *Saccharomyces cerevisiae* encodes a putative bHLH-LZ transcription factor required for Ty1-mediated gene expression. Yeast. 1994; 10: 1329-39.
16. Türkel S, Yenice B. Analysis of the Effects of Chromatin Modifying Complexes on the Transcription of Retrotransposon Ty2-917 in Saccharomyces cerevisiae. Turk J Biol 2006; 30: 101-6.
17. Türkel S, Liao XB, Farabaugh PJ. Gcr1-dependent transcriptional activation of yeast retrotransposon Ty2-917. Yeast 1997; 13: 917-30.
18. Kerr JFR, Wyllie AH, Currie R. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-57.
19. Ellis HM, Horvitz HR. Genetic control of programmed cell death in the nematode *C. elegans*. Cell 1986; 44: 817-29.
20. Nunez G, Benedict, MA, Hu Y, Inohara N. Caspases: the proteases of the apoptotic pathway. Oncogene 1998; 17: 3237-45.
21. Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol 2007; 35: 495-516.
22. D'Arcy MS. Cell death: A review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Int 2019; 43: 582-92.
23. Madeo F, Herker E, Wissing S, Jungwirth H, Eisenberg T, Fröhlich KU. Apoptosis in yeast. Curr Opin Microbiol 2004; 7: 655-60.
24. Stolz A, Hilt W, Buchberger A, Wolf DH. Cdc48: a power machine in protein degradation. Trends Biochem Sci 2011; 36: 515-23.
25. Jürgensmeier JM, Krajewski S, Armstrong RC, Wilson GM, et al. Bax- and Bak-induced cell death in the fission yeast *Schizosaccharomyces pombe*. Mol Biol Cell 1997; 8: 325-39.
26. Mazzoni C, Falcone C. Caspase-dependent apoptosis in yeast. Biochim Biophys Acta (BBA). 2008; 1783: 1320-7.
27. Madeo F, Herker E, Maldener C, Wissing S. et al. A caspase-related protease regulates apoptosis in yeast. Mol Cell 2002; 9: 911-7.
28. Wilkinson D, Ramsdale M. Proteases and caspase-like activity in the yeast *Saccharomyces cerevisiae*. Biochem Soc Trans 2011; 39: 1502-8.
29. Shrestha A, Brunette S, Lloyd W, Lynn S, Megeney A. The metacaspase Yca1 maintains proteostasis through multiple interactions with the ubiquitin system. Cell Discovery 2019; 5: Article: 6.
30. Falcone C, Mazzoni C. External and internal triggers of cell death in yeast. Cell Mol Life Sci 2016; 73(11-12): 2237-50.
31. Giannattasio S, Guaragnella N, Zdralevic M, Marra E. Molecular mechanisms of *Saccharomyces cerevisiae* stress adaptation and programmed cell death in response to acetic acid. Front Microbiol 2013; 4, article 33.
32. Corte-Real M, Sousa MJ. Genome-wide identification of genes involved in the positive and negative regulation of acetic acid-induced programmed cell death in Saccharomyces cerevisiae. BMC Genomics, 2013; 14: 838.
33. Almeida B, Ohlmeier S, Almeida AJ, Madeo F, Leão C, Rodrigues F, Ludovico P. Yeast protein expression profile during acetic acid-induced apoptosis indicates causal involvement of the TOR pathway. Proteomics 2009; 9: 720-32.
34. Bonomelli B, Martegani E, Colombo S. Lack of SNF1 induces localization of active Ras in mitochondria and triggers apoptosis in the yeast *Saccharomyces cerevisiae*. Biochem Biophys Res Commun 2020; 523:130-4.
35. Xia X, Lei L, Qin W, Wang L, Zhang G, Hu J. GCN2 controls the cellular checkpoint: potential target for regulating inflammation. Cell Death Discovery 2018; 4: 20.
36. Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, Boeke JD. Designer Deletion Strains derived from Saccharomyces cerevisiae S288C: a Useful set of Strains and Plasmids for PCR-mediated Gene Disruption and Other Applications. Yeast 1998; 14: 115-32.
37. Gietz RD, Schiestl RH, Willems AR, Woods RA. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast 1995; 11: 355-60.
38. Rose MD, Winston F, Heiter P. Methods in Yeast Genetics. A Laboratory Course Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA. 1990.
39. Guarente L. Yeast promoters and lacZ fusions designed to study expression of cloned genes in yeast. Methods Enzymol 1983; 101: 181-91.
40. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-75.
41. Risler JK, Kenny AE, Palumbo RJ, Eric R, Gamache ER, Curcio MJ. Host co-factors of the retrovirus-like transposon Ty1. 2012; Mobile DNA 2012; 3: 12.
42. Garfinkel DJ, Nyswaner KM, Stefanisko KM, Chang C, Moore SP. Ty1 Copy number dynamics in *Saccharomyces*. Genetics 2005; 169: 1845-57.
43. Lopez MC, Baker HV. Understanding the growth phenotype of the yeast gcr1 mutant in terms of global genomic expression patterns. J Bacteriol 2000; 182: 4970-8.
44. Brückner S, Kern S, Birke R, Saugar I, Ulrich HD, Mösch HU. The TEA transcription factor Tec1 links TOR and MAPK pathways to coordinate yeast development. Genetics 2011: 189; 479–94
45. Carmona-Gutierrez D, Eisenberg T, Büttner S, Meisinger C, Kroemer G, Madeo F. Apoptosis in yeast: triggers, pathways, subroutines. Cell Death Differ 2010; 17: 763–73.