

Research Article

Amino Acid Starvation Enhances Programmed Ribosomal Frameshift in Metavirus Ty3 of *Saccharomyces cerevisiae*

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Ty3 is a retroviral-like element and propagates with a retroviral-like mechanism within the yeast cells. Ty3 mRNA contains two coding regions, which are GAG3 and POL3. The coding region POL3 is translated as a GAG3-POL3 fusion protein by a +1 programmed frameshift. In this study, it was shown that the Ty3 frameshift frequency is significantly increased by amino acid starvation in a Gcn2p complex dependent manner. When the yeast cells were subjected to amino acid starvation, the frameshift frequency of Ty3 increased more than 2-fold in the wild-type yeast cells, mostly independent of Gcn4p. However, Ty3 frameshift frequency remained at basal level in the *gcn1*, *gcn20*, or *gcn2* mutant yeast cells in amino acid starved yeasts. Gcn1p forms a complex with Gcn2p and Gcn20p and is involved in the sensing of uncharged tRNAs on the ribosomal A-site during translation. Increases in uncharged tRNA levels due to amino acid depletion lead to ribosomal pauses. These ribosomal pauses are significant actors in the regulation of Ty3 frameshift frequency. Results of this research revealed that frameshift frequency in Ty3 is regulated by the Gcn2p complex in response to amino acid starvation in yeast.

1. Introduction

Ty elements of the yeast *Saccharomyces cerevisiae* belong to the retrotransposon family of eukaryotic mobile genetic elements [1]. Ty3 is one of the five different retroviral-like elements that are present in the yeast *S. cerevisiae* genome [2]. Due to its genome organization, Ty3 has been classified as the member of the Metaviridae group of the retrovirales order [3]. Ty elements transpose via an RNA intermediate using a similar strategy to the vertebrate retroviruses [4].

Ty3 genome encodes a single mRNA that contains two overlapping coding regions [2]. These coding regions are named TY3A (GAG3) and TY3B (POL3), and they encode retroviral gag and pol analogs, respectively. POL3 is synthesized by +1 translational frameshifting as a fusion protein with GAG3 [5, 6]. Posttranslational cleavage of GAG3 generates nucleocapsid proteins that are required for the formation of TY virus like particles (Ty-VLP). Posttranslational proteolytic cleavage of POL3 gives three different proteins that show protease (PR), integrase (IN), and reverse transcriptase/RNase H (RT/RH) activities [7]. The programmed

ribosomal frameshifting (PRF) in Ty3 elements is caused by the slow decoding of AGU codon by tRNA-Ser-GCU followed by the out-of-frame binding of tRNA-Val-GUU at +1 direction in the frameshift site of Ty3 [5]. The rate of PRF shows variations among retroviruses and retroviral-like elements [6]. The PRF rate is approximately 4-5% in Ty3, while it is 25% in Ty1 under standard growth conditions in most of the laboratory strains of *S. cerevisiae* (in synthetic complete medium supplemented with 2% glucose in logarithmically grown cells at 30°C) [8, 9].

Several lines of evidence indicate that the PRF is not a constitutive event, and it is regulated by different cellular signals. Previously, we have shown that the programmed ribosomal frameshift (PRF) event in Ty3 is regulated by glucose signaling [9]. The PRF rate in Ty3 is activated more than 3-fold in the yeast cells that are grown in low glucose medium in a Snf1p dependent manner. cAMP dependent protein kinase-A is also involved in the regulation of frameshift efficiency in Ty3 [9]. In addition, we have analyzed the effects of glucose signaling on the PRF efficiency in Ty1 elements and shown that the Ty1 type +1 PRF frequency decreases several

fold in glycerol-lactate-grown *S. cerevisiae* cells [8]. Atkins and Björk [10] have also reported that mutations in certain ribosomal proteins and tRNAs also affect the PRF rate in both bacteria and yeast.

Amino acid starvation causes the transcriptional activation of more than 30 genes of 10 different pathways required for amino acid biosynthesis by the transcriptional activator Gcn4p [11]. The key molecular event that activates Gcn4p synthesis is the accumulation of the uncharged tRNAs under amino acid starvation conditions. The protein kinase Gcn2p interacts with the uncharged tRNAs and initiates a cascade of events which results in the derepression of *GCN4* expression [12]. In addition to amino acid starvation, several metabolic signals also activate Gcn2p [13]. Gcn2p is associated with two different effector proteins, namely, Gcn1p and Gcn20p [14]. These effector proteins are required for the kinase function of Gcn2p during amino acid starvation conditions [14, 15]. It was shown that the Gcn2p, associated with Gcn1p and Gcn20p, is also present in elongating ribosomes during translation [15, 16]. Gcn1p has three different functional regions. These interaction sites are required for ribosome binding, for Gcn2p interactions, and for Gcn20p interactions in amino acid starved yeast cells [17]. There is additional evidence indicating that Gcn2p interacts with translation elongation factors eEF1A and eEF3 in different growth conditions. It was shown that eEF1A and eEF3 deactivate Gcn2p function in nutrient replete conditions [16, 18].

In this study, I have analyzed the effects of the amino acid starvation on the programmed ribosomal frameshift rate in Ty3. Results of this research indicate that programmed ribosomal frameshift efficiency is regulated by amino acid starvation in a Gcn1p-Gcn20p-Gcn2p complex dependent manner in *S. cerevisiae*.

2. Materials and Methods

2.1. Yeast Strains and Plasmids. The genotypes of the yeast strains used in this study are WY281 (*MATa ura3-52 ino1*), F212 (*MATa ura3-52 ino1 gcn4-Δ1*), WY282 (*MATa ura3-52; ino1; Δgcn1*), WY283 (*MATa ura3-52; ino1; Δgcn20*), WY284 (*MATa ura3-52; ino1; Δgcn1; Δgcn20*), and H1538 (*MATa ura3-52, leu2-3,112, trp1-Δ63, gcn2Δ*). *S. cerevisiae* strain WY281 is used as standard wild-type yeast in this research since these yeasts have functional Gcn1p, Gcn2p, Gcn20p, and Gcn4p [15].

Structures and constructions of Ty3 frameshift (Ty3-FS) and Ty3 frame fusion (Ty3-FF) reporter plasmids were explained previously [5]. These plasmids are 2 μ m *URA3*-based shuttle vectors and based on the previously described expression vector pMB38 [19]. Ty3 frameshift site (GCG-AGT-T) is fused to the *E. coli* lacZ gene in the +1 reading frame in Ty3-frameshift reporter plasmid (TY3-FS). In this vector, translation of the lacZ fusion protein depends on the occurrence of frameshift event in the +1 direction at the 5'-GCG-AGU-U-3' sequence of mRNA. The Ty3-frame fusion plasmid does not contain the frameshift site; hence, translation of the lacZ fusion protein does not require a frameshift event (Ty3-FF) [5]. Plasmids were transformed

into the yeast cells as described previously using the lithium acetate, PEG method [20]. These 2 μ m *URA3*-based plasmids can be stably maintained with no drastic change in copy numbers in different yeast transformants under selective growth conditions [21].

2.2. Growth Conditions. Yeast cells were grown in liquid Yeast Nitrogen Base (YNB) medium supplemented with 2% glucose and necessary amino acids as described previously [22]. For amino acid starvation experiments, yeast transformants were first grown to saturation in 5 mL of liquid YNB media supplemented with 2% glucose and necessary amino acids and then diluted to OD₆₀₀: 0.1–0.15 in 10 mL of fresh YNB liquid media. Once yeast cell density reached OD₆₀₀: 0.5–0.6, yeast cultures were divided into two aliquots (5 mL each). Filter sterilized 3-amino 1,2,4-triazole (3-AT) was added to 10 mM final concentration to the one set of (5 mL) yeast cultures, as described previously [23]. 3-AT is a competitive inhibitor of *HIS3* gene product and triggers amino acid starvation in the yeast cells [24]. 3-AT treated and untreated yeast transformants were grown for additional 5 hours and harvested for β -galactosidase assays.

2.3. Enzyme Assays. β -Galactosidase assays were done in triplicate as described previously [25]. β -Galactosidase units are given in nmol of ONPG (2-nitrophenyl β -D-galactopyranoside) cleaved per minute per mg of protein in permeabilized yeast cells. Protein concentrations in the permeabilized yeast lysates were determined by the Lowry assay as described [26]. Frameshift rates were calculated as the percent of the ratio of β -galactosidase activities expressed from Ty3-FS plasmid to the β -galactosidase activities expressed from Ty-FF plasmid in the relevant yeast transformants. Yeast transformants were grown in triplicate and all experiments were repeated at least once under the same growth concentrations. Hence, the Ty3 FS rate values given in the tables are the mean values of at least 18 independent assays. Standard deviations for β -galactosidase units were below 10% in triplicate assays.

3. Results

3.1. Effects of the Amino Acid Starvation on the Ty3 Frameshift Rate. Ty elements do not encode any known regulatory protein for their own gene expression. In this regard, they completely depend on the host-encoded transcription and translation factors for their own replication. Ty elements of *S. cerevisiae* cannot transpose intercellularly. Being an obligatory resident in yeast cells, genes expressed in Ty elements are also subjected to physiological changes that modulate various metabolic events in yeast cells.

In order to investigate if the frameshift event in Ty3 will be affected from physiological stresses such as amino acid starvation, yeast plasmids that contain Ty3 frameshift (Ty3-FS) and Ty3 frame fusion (Ty3-FF) expression cassettes were transformed into wild-type yeast cells. Yeast transformants were grown in minimal medium supplemented with 2% glucose and auxotrophic requirements. The programmed

TABLE 1: Amino acid starvation increases PRF efficiency in a partially Gcn4p dependent manner in Ty3.

Yeast strains and relevant mutations	% frameshift rates* \pm SD	
	Normal growth	3-AT treated
Wild type	4.0 \pm 0.2	9.2 \pm 0.5
$\Delta gcn4$	4.0 \pm 0.3	7.0 \pm 0.5

*% frameshift rates were calculated as the percent of the ratio of β -galactosidase activities expressed from the Ty3-frameshift plasmid to the β -galactosidase activities expressed from the Ty3-frame fusion plasmid in the relevant yeast transformants.

\pm SD: standard deviations.

ribosomal frameshift frequencies for Ty3 in yeast cells grown in normal conditions were measured as 4% \pm 0.2 (Table 1). To test the effects of amino acid starvation on frameshift frequency in Ty3, aliquots of the yeast cells were subjected to amino acid starvation by adding 3-amino triazole, a competitive inhibitor for the *HIS3* gene product that triggers amino acid starvation in yeast cells. The PRF frequency of Ty3 in the amino acid starved yeast strain increased more than 2-fold and was measured as 9.2% \pm 0.5 (Table 1).

Amino acid starvation leads to the activation of the protein kinase Gcn2p and transcriptional activator Gcn4p. Transcription factor Gcn4p is a multifunctional transcriptional activator and coordinately activates the transcription of genes involved in amino acid biosynthesis [11, 27]. In order to test if Gcn4p has any function in the activation of Ty3 frameshift frequency in amino acid starved yeast cells, plasmids containing Ty3-FS and Ty3-FF expression cassettes were transformed into $\Delta gcn4$ mutant and its isogenic wild-type strains. Frameshift frequency of Ty3 was measured as 4% \pm 0.3 in *gcn4* mutant yeast cells in normal growth conditions (Table 1). Frameshift frequency in amino acid starving $\Delta gcn4$ mutant yeast cells also increased as in the wild-type strains and resulted in 7% \pm 0.5 frameshift frequency (Table 1). These results indicated that Gcn4p is partially involved in the regulation of frameshift frequency in Ty3.

3.2. Gcn2p Is Essential for the Regulation of Ty3 Frameshift Frequency. Programmed ribosomal frameshift events take place during the translational elongation stage. Gcn2p is a protein kinase that has multiple functions in the sensing and signaling processes of amino acid starvation in yeast. Recent evidence also indicates that Gcn2p associates with ribosomes and may also be involved in translational elongation [16, 18]. Hence, the effects of Gcn2p on frameshift frequency in Ty3 were analyzed in the wild-type and *gcn2* mutant yeast cells. Frameshift frequencies of Ty3 in the wild-type yeast cells were measured as 4% \pm 0.2 and 9.2% \pm 0.5 in normal and in amino acid starved yeast cells, respectively (Table 2). Frameshift frequency of Ty3 was measured as 4% \pm 0.4 in *gcn2* mutant cells that were grown under normal growth conditions (Table 2). When the *gcn2* mutant yeast cells were subjected to amino acid starvation, frameshift frequency remained approximately at the same levels (5% \pm 0.5). These results indicated that functional Gcn2p is essential for the regulation of the frameshift event in amino acid starved yeast cells.

TABLE 2: Gcn2p is required for the increase of PRF efficiency in response to amino acid starvation.

Yeast strains and relevant mutations	% frameshift rates* \pm SD	
	Normal growth	3-AT treated
Wild type	4.0 \pm 0.2	9.2 \pm 0.5
($\Delta gcn2$)	4.0 \pm 0.4	5.0 \pm 0.5

*% frameshift rates were calculated as the percent of the ratio of β -galactosidase activities expressed from the Ty3-frameshift plasmid to the β -galactosidase activities expressed from the Ty3-frame fusion plasmid in the relevant yeast transformants.

\pm SD: standard deviations.

TABLE 3: Gcn1p and Gcn20p are required for the increase of PRF efficiency in response to amino acid starvation.

Yeast strains and relevant mutations	% frameshift rates* \pm SD	
	Normal growth	3-AT treated
Wild type	4.0 \pm 0.2	9.2 \pm 0.5
($\Delta gcn1$)	4.3 \pm 0.1	4.4 \pm 0.2
($\Delta gcn20$)	4.4 \pm 0.2	4.3 \pm 0.4
($\Delta gcn1/\Delta gcn20$)	4.7 \pm 0.2	4.3 \pm 0.5

*% frameshift rates were calculated as the percent of the ratio of β -galactosidase activities expressed from the Ty3-frameshift plasmid to the β -galactosidase activities expressed from the Ty3-frame fusion plasmid in the relevant yeast transformants.

\pm SD: standard deviations.

3.3. Gcn1p and Gcn20p are Involved in the Regulation of Ty3 Frameshift Frequency. Previous results suggest a model in which Gcn1p and Gcn20p associate with Gcn2p in elongating ribosomes [16, 18]. After showing that Gcn2p is involved in the activation of frameshift during amino acid starvation, the effects of Gcn1p and Gcn20p on the frameshift frequency of Ty3 were also analyzed in the wild-type and isogenic *gcn1*, *gcn20*, and *gcn1/gcn20* double mutant yeast strains. Yeast plasmids that have Ty3-FS and Ty3-FF expression cassettes were transformed into wild-type and isogenic *gcn1*, *gcn20*, and *gcn1/gcn20* double mutant yeasts. Transformants were grown under normal growth conditions and were subjected to amino acid starvation by adding 3-AT to the growth mediums.

Frameshift frequency in the wild-type *gcn1*, *gcn20*, and *gcn1/gcn20* double mutants remained at approximately the same basal levels (approximately 4% \pm 0.5) as in the yeast cells under standard growth conditions (Table 3). As expected, frameshift frequency of Ty3 in the wild-type yeast strain increased more than 2-fold (from 4% to 9%) in amino acid starved yeast cells (Table 3). However, when the *gcn1*, *gcn20*, and *gcn1/gcn20* mutants were subjected to amino acid starvation to activate the Gcn2p pathway, frameshift frequency of Ty3 did not increase in these *gcn* mutants and remained essentially at the same basal levels (approximately 4% \pm 0.5) (Table 3). These results suggest that the functional Gcn2p complex which also contains Gcn1p and Gcn20p is involved in the regulation of frameshift frequency of Ty3 in amino acid starved yeast cells. Moreover, it is clear from the *gcn1/gcn20* double mutant that Gcn1p and Gcn2p do not have

any cumulative effects on the frameshift efficiency in Ty3 during amino acid starvation.

4. Discussion

Programmed ribosomal frameshift is one of the regulatory mechanisms in retroviral gene expression [6]. It occurs during the translational elongation stage. The molecular mechanisms of the PRF in Ty3 have been uncovered previously [5, 28]. The core nucleotide sequence of the frameshift site on Ty3 is GCG-AGU-U. Frameshift is stimulated by the ribosomal pause resulting from the slow decoding of the AGU codon when it arrives in the A-site of elongating ribosomes. Peptidyl-tRNA^{Ala} which decodes the GCG codon associates with the P-site of the ribosome. Frameshift occurs at +1 direction without tRNA slippage by out-of-frame binding of valyl-tRNA to the GUU codon at the A-site of the ribosome [28]. Previously, it was reported that the frameshift frequency in Ty3 can be modulated by growth conditions [9]. It is activated by nonfermentable carbon sources up to 3-fold in a Snf1p dependent manner.

In this study, the effects of amino acid starvation caused by 3-AT on the frameshift frequency in Ty3 were analyzed. 3-AT inhibits the histidine biosynthesis by acting on the product of the HIS3 gene, imidazole glycerol-phosphate dehydratase [23, 24]. Presence of 3-AT also increases the amount of uncharged tRNA within cytoplasm, which results in the activation of Gcn2p kinase activity [11, 12].

It is found that amino acid starvation increases frameshift frequency more than 2-fold in a Gcn1p dependent manner. Gcn1p, which also interacts with Gcn20p, is the effector protein for the activation of the kinase function of Gcn2p [29]. Gcn1p-Gcn20p-Gcn2p complex associates with ribosomal A-site in amino acid starved yeast cells [17, 29]. It can be proposed that the interaction of this complex with ribosomal A-site is crucial for the induction of the Gcn2p kinase activity [29]. Ribosomal pause is one of the critical reasons for the frameshift in Ty3. It was suggested that the Gcn1p-Gcn2p complex delivers uncharged tRNAs to ribosomal A-site during amino acid starvation. Hence, it is reasonable to propose that when the A-site is occupied by uncharged tRNAs, it leads to an increase in ribosomal pause in amino acid starved yeast cells. This prolonged pause further increases the frameshift frequency in Ty3 in wild-type *GCN⁺* yeast cells. Longer pause durations increase the likelihood of out-of-frame binding of valyl-tRNA to the GUU codon at the A-site of the ribosome. Frameshift frequency in any one of the *gcn1*, *gcn20*, or in *gcn2* mutant strains did not change in amino acid starvation conditions and remained at amino acid replete levels. It is also clear that the lack of both Gcn1p and Gcn20p in the double mutant strain did not have any cumulative effect on the frameshift frequency, which indicates that all of these factors are involved in the increase of frameshift frequency in Ty3 in amino acid starved yeast cells.

Gcn4p is a multifunctional transcriptional activator and its translation is also activated by amino acid starvation in a Gcn2p dependent manner [30]. Gcn4p activates the expression of purine biosynthetic genes [31]. Gcn4p is also involved

in the regulation of the genes encoding ribosomal proteins [32]. To test if Gcn4p affects frameshift frequency indirectly, frameshift frequency of Ty3 was also analyzed in the *gcn4* mutant strain. Results of this study showed that Gcn4p has a partial effect on the regulation of frameshift frequency in the amino acid starved yeast cells.

Programmed ribosomal frameshift is a good model to use in the analysis of the regulation of translation elongation stage. PRF in Ty3 can be used to analyze the regulatory events during the translation elongation of protein synthesis. Another question is how this change will affect the replicative cycle of Ty3 within the yeast. It was suggested that yeast Ty elements are under continuous selective pressure for the repression of transposition and for elimination from the yeast cell. Ty elements must overcome host pressure and elimination and still coexist with the yeast genome [33]. It has already been shown that the alterations in frameshift rate dramatically affect the transposition events in the yeast Ty1 elements [34]. Reduction of TYB protein levels, which are similar to retroviral *pol* products, resulted in a decreased rate of transposition in the yeast [35]. Kirchner et al. [35] also found that the perturbation of GAG3 and POL3 ratios also affects the transposition rate of Ty3 in *S. cerevisiae*.

5. Conclusion

It is known that environmental factors such as various stresses can increase transposable elements' mobility to generate genetic diversity in the host organisms. Amino acid starvation and the glucose limitations are metabolic stress conditions that affect many cellular processes and the pathways in the yeast cells. Results presented in this study indicate that the programmed frameshift level in the metavirus Ty3 can be regulated by nutrient limiting conditions through the protein kinase Gcn2p complex. Increases in the frameshift rate in response to these stresses also lead to the biosynthesis of more TY3B polypeptide which might lead to increases in the Ty3 propagation in the host cells.

Competing Interests

The author declares that there are no competing interests regarding the publication of this paper.

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References

- [1] J. R. Cameron, E. Y. Loh, and R. W. Davis, "Evidence for transposition of dispersed repetitive DNA families in yeast," *Cell*, vol. 16, no. 4, pp. 739–751, 1979.
- [2] D. J. Clark, V. W. Bilanchone, L. J. Haywood, S. L. Dildine, and S. B. Sandmeyer, "A yeast sigma composite element, TY3, has properties of a retrotransposon," *The Journal of Biological Chemistry*, vol. 263, no. 3, pp. 1413–1423, 1988.

- [3] P. Capy, "Classification and nomenclature of retrotransposable elements," *Cytogenetic and Genome Research*, vol. 110, no. 1–4, pp. 457–461, 2005.
- [4] J. D. Boeke, D. J. Garfinkel, C. A. Styles, and G. R. Fink, "Ty elements transpose through an RNA intermediate," *Cell*, vol. 40, no. 3, pp. 491–500, 1985.
- [5] P. J. Farabaugh, H. Zhao, and A. Vimaladithan, "A novel programmed frameshift expresses the *POL3* gene of retrotransposon Ty3 of yeast: frameshifting without tRNA slippage," *Cell*, vol. 74, no. 1, pp. 93–103, 1993.
- [6] P. J. Farabaugh, "Programmed translational frameshifting," *Microbiological Reviews*, vol. 60, no. 1, pp. 103–134, 1996.
- [7] J.-F. Roth, "The yeast Ty virus-like particles," *Yeast*, vol. 16, no. 9, pp. 785–795, 2000.
- [8] S. Türkel, Ö. Bayram, and E. Arik, "Glucose signaling pathway and growth conditions regulate gene expression in retrotransposon Ty2," *Zeitschrift für Naturforschung—Section C*, vol. 64, no. 7–8, pp. 526–532, 2009.
- [9] S. Türkel, G. Kaplan, and P. J. Farabaugh, "Glucose signalling pathway controls the programmed ribosomal frameshift efficiency in retroviral-like element Ty3 in *Saccharomyces cerevisiae*," *Yeast*, vol. 28, no. 11, pp. 799–808, 2011.
- [10] J. F. Atkins and G. R. Björk, "A gripping tale of ribosomal frameshifting: extragenic suppressors of frameshift mutations spotlight P-site realignment," *Microbiology and Molecular Biology Reviews*, vol. 73, no. 1, pp. 178–210, 2009.
- [11] A. G. Hinnebusch, "Mechanisms of gene regulation in the general control of amino acid biosynthesis in *Saccharomyces cerevisiae*," *Microbiological Reviews*, vol. 52, no. 2, pp. 248–273, 1988.
- [12] R. C. Wek, M. Ramirez, B. M. Jackson, and A. G. Hinnebusch, "Identification of positive-acting domains in GCN2 protein kinase required for translational activation of GCN4 expression," *Molecular and Cellular Biology*, vol. 10, no. 6, pp. 2820–2831, 1990.
- [13] B. A. Castilho, R. Shanmugam, R. C. Silva, R. Ramesh, B. M. Himme, and E. Sattlegger, "Keeping the eIF2 alpha kinase Gcn2 in check," *Biochimica et Biophysica Acta (BBA)—Molecular Cell Research*, vol. 1843, no. 9, pp. 1948–1968, 2014.
- [14] C. R. Vazquez de Aldana, M. J. Marton, and A. G. Hinnebusch, "GCN20, a novel ATP binding cassette protein, and GCN1 reside in a complex that mediates activation of the eIF-2 α kinase GCN2 in amino acid-starved cells," *The EMBO Journal*, vol. 14, no. 13, pp. 3184–3199, 1995.
- [15] M. J. Marton, C. R. Vazquez De Aldana, H. Qiu, K. Chakraborty, and A. G. Hinnebusch, "Evidence that GCN1 and GCN20, translational regulators of GCN4, function on elongating ribosomes in activation of eIF2 α kinase GCN2," *Molecular and Cellular Biology*, vol. 17, no. 8, pp. 4474–4489, 1997.
- [16] J. Visweswaraiah, S. Lageix, B. A. Castilho et al., "Evidence that eukaryotic translation elongation factor 1A (eEF1A) binds the Gcn2 protein C terminus and inhibits Gcn2 activity," *The Journal of Biological Chemistry*, vol. 286, no. 42, pp. 36568–36579, 2011.
- [17] E. Sattlegger and A. G. Hinnebusch, "Separate domains in GCN1 for binding protein kinase GCN2 and ribosomes are required for GCN2 activation in amino acid-starved cells," *The EMBO Journal*, vol. 19, no. 23, pp. 6622–6633, 2000.
- [18] J. Visweswaraiah, S. J. Lee, A. G. Hinnebusch, and E. Sattlegger, "Overexpression of eukaryotic translation elongation factor 3 impairs Gcn2 protein activation," *The Journal of Biological Chemistry*, vol. 287, pp. 37757–37768, 2012.
- [19] M. F. Belcourt and P. J. Farabaugh, "Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site," *Cell*, vol. 62, no. 2, pp. 339–352, 1990.
- [20] R. D. Gietz and R. H. Schiestl, "Transforming yeast with DNA," *Methods in Molecular and Cellular Biology*, vol. 5, no. 5, pp. 255–269, 1994.
- [21] X.-B. Liao, J. J. Clare, and P. J. Farabaugh, "The upstream activation site of a Ty2 element of yeast is necessary but not sufficient to promote maximal transcription of the element," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 23, pp. 8520–8524, 1987.
- [22] M. D. Rose, F. Winston, and P. Hieter, *Methods in Yeast Genetics. A Laboratory Course Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 1990.
- [23] C. M. Moehle and A. G. Hinnebusch, "Association of RAPI binding sites with stringent control of ribosomal protein gene transcription in *Saccharomyces cerevisiae*," *Molecular and Cellular Biology*, vol. 11, no. 5, pp. 2723–2735, 1991.
- [24] T. Klotowski and A. Wiater, "Synergism of aminotriazole and phosphate on the inhibition of yeast imidazole glycerol phosphate dehydratase," *Archives of Biochemistry and Biophysics*, vol. 112, no. 3, pp. 562–566, 1965.
- [25] L. Guarente, "Yeast promoters and lacZ fusions designed to study expression of cloned genes in yeast," *Methods in Enzymology*, vol. 101, pp. 181–191, 1983.
- [26] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement with the folin phenol reagent," *The Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.
- [27] K. Arndt and G. R. Fink, "GCN4 protein, a positive transcription factor in yeast, binds general control promoters at all 5' TGACTC 3' sequences," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 83, no. 22, pp. 8516–8520, 1986.
- [28] A. Vimaladithan and P. J. Farabaugh, "Special peptidyl-tRNA molecules can promote translational frameshifting without slippage," *Molecular and Cellular Biology*, vol. 14, no. 12, pp. 8107–8116, 1994.
- [29] E. Sattlegger and A. G. Hinnebusch, "Polyribosome binding by GCN1 is required for full activation of eukaryotic translation initiation factor 2 α kinase GCN2 during amino acid starvation," *The Journal of Biological Chemistry*, vol. 280, no. 16, pp. 16514–16521, 2005.
- [30] A. G. Hinnebusch and K. Natarajan, "Gcn4p, a master regulator of gene expression, is controlled at multiple levels by diverse signals of starvation and stress," *Eukaryotic Cell*, vol. 1, no. 1, pp. 22–32, 2002.
- [31] R. J. Rolfes and A. G. Hinnebusch, "Translation of the yeast transcriptional activator GCN4 is stimulated by purine limitation: implications for activation of the protein kinase GCN2," *Molecular and Cellular Biology*, vol. 13, no. 8, pp. 5099–5111, 1993.
- [32] Y. J. Joo, J.-H. Kim, U.-B. Kang, M.-H. Yu, and J. Kim, "Gcn4p-mediated transcriptional repression of ribosomal protein genes under amino-acid starvation," *The EMBO Journal*, vol. 30, no. 5, pp. 859–872, 2011.
- [33] I. K. Jordan and J. F. McDonald, "Tempo and mode of Ty element evolution in *Saccharomyces cerevisiae*," *Genetics*, vol. 151, no. 4, pp. 1341–1351, 1999.

- [34] H. Xu and J. D. Boeke, "Host genes that influence transposition in yeast: the abundance of a rare tRNA regulates Ty1 transposition frequency," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 21, pp. 8360–8364, 1990.
- [35] J. Kirchner, S. B. Sandmeyer, and D. B. Forrest, "Transposition of a Ty3 GAG3-POL3 fusion mutant is limited by availability of capsid protein," *Journal of Virology*, vol. 66, no. 10, pp. 6081–6092, 1992.



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