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Analysis of the Effects of Chromatin Modifying Complexes on the Transcription of Retrotransposon Ty2-917 in *Saccharomyces cerevisiae*

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Abstract: Transcription of the yeast retrotransposon Ty2-917 is regulated by a complex set of regulatory regions located both upstream and downstream of the transcription initiation site. In this study, it was found that certain chromatin modifying factors are essential for the transcriptional regulation of Ty2-917. Among the chromatin binding factors tested in this research, non-histone proteins Nhp6A/B have the largest effect on the transcription of Ty2-917. In *Δnhp6A/B* double mutant yeast strain transcription of Ty2 decreased 20-fold, indicating that these non-histone proteins are essential for the transcription of Ty2-917. Furthermore, it was also shown that the transcription of Ty2-917 decreased by 2-fold in the in *Δisw2* mutant, which shows that Isw2p is also involved in the activation of Ty2-917 transcription. In addition, results of this research show that the Spt7p, which is the critical subunit of SAGA complex, is essential for the maximal level transcription of Ty2-917.

Key Words: Ty elements, Yeast, Retrotransposon, Chromatin factors, Transcription

Kromatin Modifiye Eden Komplekslerin *Saccharomyces cerevisiae* Retrotranspozonu Ty2-917 Transkripsiyonuna Etkilerinin Analizi

Özet: Maya retrotranspozonu Ty2-917'nin transkripsiyonu transkripsiyon başlama yerinin alt ve üst bölgesinde yer alan kompleks yapıdaki düzenleyici bölgeler tarafından kontrol edilir. Bu çalışmada bazı kromatin modifiye edici faktörlerin Ty2-917 transkripsiyonunun düzenlenmesi için gerekli olduğu bulundu. Bu araştırmada test edilen kromatin bağlanma faktörlerinden Ty2-917 transkripsiyonuna en büyük etkisi olan faktör non-histon proteinleri olan Nhp6A/B olarak belirlendi. *Δnhp6A/B* çifte mutantı maya suşunda Ty2 transkripsiyonunun 20 kat azalması bu non-histon proteinlerinin Ty2-917 transkripsiyonu için gerekli olduğunu göstermektedir. Buna ek olarak, Ty2-917 transkripsiyonunun *Δisw2* mutantında 2 kat azaldığı gösterildi. Bu sonuç Isw2p'nin de Ty2-917 transkripsiyonunun aktivasyonunda yer aldığını göstermektedir. Ayrıca, bu araştırma sonuçları SAGA kompleksinin önemli bir alt birimi olan Spt7p'nin de maksimum seviyede Ty2-917 transkripsiyonu için gerekli olduğunu göstermektedir.

Anahtar Sözcükler: Ty elementleri, Maya, Retrotranspozon, Kromatin faktörleri, Transkripsiyon

Introduction

Ty elements of the yeast *Saccharomyces cerevisiae* show structural and functional similarities to eukaryotic retroviruses (1). Ty elements transpose via an RNA intermediate using a similar strategy as in vertebrate retroviruses (2). Five different families of Ty elements, Ty1 through Ty5, have been characterized in yeast cells so far (3). Ty1, Ty2, Ty4, and Ty5 belong to the family pseudoviridae of the order retrovirales. Ty3 has a different genomic organization, and hence it has been classified as the member of the genus metaviridae (4).

Yeast retrotransposon Ty2-917 has a very compact genomic structure (5). Transcriptional regulatory regions of Ty2-917 are located within the first 754 bp of this element. An enhancer element located downstream of the transcription initiation site and the UAS (Upstream Activating Site) are required for the activation of Ty2 transcription (6). In addition, the negative regulatory region, also located downstream of transcription initiation site, represses Ty2 transcription 2-4 fold (7). Hence, interactions between the basal transcription factors and the transcriptional activators, which bind to the downstream regulatory elements, are essential for

the formation of productive transcription initiation and activation complex on Ty2-917 promoter region. However, only a few of the transcription factors required for the activation of Ty2-917 have been identified so far. The major transcriptional regulator of Ty2-917 is the Gcr1p complex (8,9). Transcription of Ty2-917 diminishes completely in the *gcr1* mutant yeast cells. In addition to its activation function, Gcr1p also introduces sharp bends on DNA and may be involved in the nucleosome positioning on certain promoters (10,11).

Chromatin modifying complexes has a significant effect on the control of gene expression in yeast (12). Previously, it was shown that Spt7p, core subunit of the SAGA protein complex, is required for the Ty1 transcription. Involvements of SNF/SWI and SPT family of transcription factors in Ty1 elements transcription have been studied extensively (13,14). The effects of SAGA complex on the Ty delta element mediated transcription of *HIS4* gene was analyzed. It was shown that Gcr1p and Gcn4p specifically interact with the SAGA complex when they bind to delta element (15). Recently Morillon et al. (2002) have shown that the transcription of Ty1 varies up to 50-fold, depending on the genomic locations within the yeast *S. cerevisiae* (16). Ty1 and Ty2 are highly homologous elements but they show sequence diversity within their regulatory sites (3).

In addition to SAGA complex, non-histone proteins 6A and 6B (Nhp6A/B), and Isw2p are also involved in the regulation of gene expression by altering chromatin structures (17,18). Nhp6A/B, which belongs to high mobility group protein family, has DNA bending and DNA looping activity (19). Functional cooperation between the SAGA complex and Nhp6A/B for the regulation of certain promoters was shown in yeast (20). Nhp6A/B also required for the formation of transcription pre-initiation complex in certain genes (21). Isw2p is a multi-functional protein that has an ATP dependent chromatin folding ability and present as different protein complexes in yeast (18). Interestingly, it was recently found that Isw2p is involved in the integration of Ty1 on the targeted regions on yeast chromosomes (22).

The effects of chromatin modifying complexes on the transcription of Ty2-917 have not been reported yet. In this study, we analyzed the effects of non-histone proteins Nhp6A/B, Isw2p and Spt7p on the Ty2-917 transcription.

Materials and Methods

Yeast Strains and Plasmids

S. cerevisiae strains used in this study and their genotypes are listed in Table 1. The yeast strains YST150 and YST151 are isogenic except for the Δ*nhp6A* and Δ*nhp6B* mutations (23). The yeast strains YST124, YST164, and YST167 are also isogenic strains except for the Δ*isw2*, and Δ*spt7* mutations (Table 1).

Structures and construction of plasmids that contain various Ty2-lacZ gene fusions were described previously (8). Briefly, plasmid pST1 is 2μ-*URA3* based expression vector that carries His4-LacZ fusion to codon 32 of *HIS4*. In this plasmid, transcriptional activator binding sites on *HIS4* promoter are replaced by a polylinker, which contains restriction sites for Xho1, Not1, Kpn1 and BamH1 enzymes (24). Then, the enhancer element of Ty2-917, which extents from 240 to 559 bp in Ty2-917, was cloned into the polylinker site giving Ty2 enhancer and *E. coli* lacZ (Enh-lacZ) gene fusion plasmid. YEp917-555 and YEp917-754 are also 2μ-*URA3* based expression vectors, which carries Ty2-917 LacZ fusion at position 555 (Ty2-555-lacZ) and at position 754 (Ty2-

Table 1. List of *S. cerevisiae* strains used in this study.

Strain name (Original Name)	Genotype (relevant mutation)	Source/reference
YST150 (JD52)	MATa, *Ura3-52, leu2-3, his3Δ200, lys2-801, trp1Δ63, ade2::hisG*	N. Lehming (23)
YST151 (JD52)	MATa, *Ura3-52, leu2-3, his3Δ200, lys2-801, trp1Δ63, ade2::hisG, Δnhp6A, Δnhp6B*	N. Lehming (23)
YST124 (BY4741)	MATa, *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	EUROSCARF (35)
YST164 (Y01601)	MATa, *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0, YOR304w::kanMX4 (Δisw2)*	EUROSCARF (35)
YST167 (Y03218)	MATa, *his3Δ1; leu2Δ0; met15Δ0; ura3Δ0, YBR081c::kanMX4 (Δspt7)*	EUROSCARF (35)

754-LacZ) of Ty2-917 to *E. coli* LacZ gene, respectively. Structure and construction of Suc2-LacZ gene fusion was also described previously. In this plasmid, UAS region of the *SUC2* gene extending from –384 to –900 was replaced with the *CYC1* UAS in 2µm–*URA3*-based expression vector pLGΔ312 (25). pLGΔ312 is a 2µm-*URA3* based plasmid bearing a fusion of the yeast wild type *CYC1* gene to the *E. coli* lacZ gene (26).

Plasmids were transformed to the given yeast strains using lithium acetate and PEG-4000 as described (27). It is known that these plasmids stably maintained in selective growth medium and their copy numbers do not vary in various yeast transformants (5,28).

Growth Media and β-Galactosidase Assays

Yeast transformants were grown in synthetic complete medium without uracil (Sc –Ura) supplemented with 2% dextrose at 30 ºC with constant shaking (130 rev/min) to stationary stage first (29). Then, 50 µl of these saturated cultures were inoculated into 5 ml of Sc –Ura medium supplemented with 2% dextrose and grown to the logarithmic stage. Yeast cells were harvested and β-galactosidase assays were done in triplicates as described. β-galactosidase activities were given in Miller Units (µmol ONPG hydrolyzed to O-nitrophenol and D-galactose per minute per OD_{600} of yeast cultures) (30). Yeast transformants were grown in duplicates and β-galactosidase assays repeated twice. Hence, enzyme units given tables are the mean values of at least 12 independent assays. Standard deviations for β-galactosidase units were approximately 10% in triplicate assays.

Results

Non-histone proteins are required for the activation of Ty2-917 transcription

Nhp6A/B has significant effects on the transcriptional regulation of a large set of genes in *S. cerevisiae* (17). We wanted to investigate if the Nhp6A/B is involved in the regulation of Ty2-917 transcription. We have used 3 different Ty2–lacZ gene fusion in our analyses. It is known that the steady-state Ty2-lacZ mRNA levels are proportional to β-galactosidase activities (7). Transcription of Ty2-555-lacZ gene fusion decreased to basal level (20-fold) in Δ*nhp6A /B* mutant indicating that non-histone proteins 6A and 6B participate in the formation of transcriptional activation complex. Likewise, transcription from Ty2-754-lacZ gene fusion also decreased 7-fold in the Δ*nhp6A/*B mutant (Table 2). Since Ty2-754-lacZ gene fusion contains the negative regulatory region, transcription from this gene fusion is approximately 9- to 10-fold lower than the Ty2-555-lacZ gene fusion in the wild type yeast strains (Tables 2 and 3). Ty2 enhancer element activates transcription when it is present upstream of a heterologous promoter. Ty2 enhancer element activated transcription of a heterologous promoter is also decreased approximately 32-fold in Δ*nhp6A/B* mutant yeast cells (Table 2). Transcription of the control gene fusions Cyc1-lacZ decreased to 5 units in the Δ*nhp6A/B* mutant strain. It is already known that *CYC1* transcription is regulated by Nhp6A/B (21). Basal level transcription from the Suc2-lacZ gene fusion is not affected by the Δ*nhp6A/B* mutation (Table 2). From these results we have concluded that non-histone proteins 6A/B are essential for the transcription of Ty2-917 and the effects of Nhp6A/B is exerted through the enhancer element on Ty2-917.

Isw2p and Spt7p are required for the activation of Ty2-917 transcription

We have shown that transcription of Ty2 and Ty2 enhancer element dependent transcription of a heterologous promoter was completely dependent on the Nhp6A/B. Then we wanted to test the effect of Isw2p on the Ty2-917 transcription. We have measured the effect of Isw2p on the Ty2 transcription in Δ*isw2* mutant and isogenic wild type yeast strain using Ty2-lacZ gene fusions. We found that transcription of Ty2-555-lacZ gene fusion decreased 2.5-fold in the Δ*isw2* mutant yeast cells (Table 3). Transcription of Ty2-754-lacZ and the Ty2 enhancer activated transcription of a heterologous

Table 2. Effects of Nhp6A/B on the Ty2-917 transcription.

Gene Fusions	β-Galactosidase Activities*	
	YST150 (Wild type)	YST151 (*Δnhp6A/B*)
Ty2-555-lacZ	184	9
Ty2-754-lacZ	21	3
Enh-lacZ	97	3
Suc2-lacZ	1	2
Cyc1-lacZ	30	5

*β-Galactosidase units are expressed in Miller Units

Table 3. Effects of Isw2p and Spt7p on the Ty2-917 transcription.

Gene Fusions	β-Galactosidase Activities*		
	YST124 (Wild type)	YST164 (*Δisw2*)	YST167 (*Δspt7*)
Ty2-555-lacZ	568	226	49
Ty2-754-lacZ	51	25	24
Enh-lacZ	230	95	44
Suc2-lacZ	3	3	5
Cyc1-lacZ	39	44	35

*β-Galactosidase units are expressed in Miller Units

promoter was also decreased approximately 2-fold in Δ*isw2* mutant yeast strain, indicating that functional Isw2p is required for the maximal level transcription from Ty2-917 promoter (Table 3). In addition, 2.4-fold decrease in the enhancer element dependent transcription of a heterologous promoter suggests that Isw2p also targets the enhancer element of Ty2-917. Basal level transcription from the control gene fusions Suc2-LacZ and also Cyc1-lacZ is not affected by Δ*isw2* mutation at significant level.

The effects of SAGA complex on the transcription of Ty2-917 were investigated using the Δ*spt7* mutant and the isogenic wild type yeast strains. Spt7p is an essential, core subunit of the SAGA complex (31). Transcription from Ty2-555-lacZ gene fusion decreased 11.6-fold in Δ*spt7* mutant yeast when compared to the expression level in the wild type strain (Table 3). Transcription of Ty2-754-LacZ and Ty2 enhancer element dependent transcription of a heterologous promoter was also decreased approximately 2-fold and 5.2-fold, in the Δ*spt7* mutant yeast cells, respectively (Table 3). These results are consistent with the previous observations on the involvement of the SAGA complex in the Ty1 and Ty delta element dependent transcription of target genes. But our results suggest that SAGA complex, like Nhp6A/B and Iswi2p, also acts on Ty2-917 enhancer element.

Transcription of Cyc1-lacZ gene fusion in *pLGΔ312* expression vector is not affected by Δ*spt7* mutation at significant level, indicating transcription of *CYC1* is not modulated by SAGA complex. Basal level transcription of the Suc2-lacZ gene fusion is not affected by the Δ*spt7* mutation (Table 3).

Discussion and Conclusion

Yeast retrotransposon Ty2-917 has a very compact genomic structure. All regulatory regions are located within the first 754 bp region of this element (5). Furthermore, significant portions of these regulatory sites are located within the downstream of transcription initiation site of Ty2-917. This type of promoter organization leads to the structural obstacle for the formation of transcriptional activation complex. First, interactions of transcriptional activators with basal transcription factors might require DNA looping or DNA folding, which is achieved mostly by chromatin modifying complexes. Second, transcriptional activators within the transcribed portion of Ty2 might be completely dissociated by each pass of transcription elongation complex from the region. Hence, rapid re-association of transcriptional activators to their binding sites within the transcribed part of Ty2-917 is also crucial for the continuous transcription initiation from the Ty2 promoter. Since the Ty elements are highly expressed and Ty RNA constitutes up to 5%-10% of total mRNA within the yeast cells, they seem to overcome these restrictions on their transcription (32).

Our results show that certain chromatin modifying complexes, Nhp6A/B being the most significant one, are involved in the organization of Ty2-917 transcription initiation complex. Transcription of Ty2-917 decreases to basal level in Δ*nhp6A/B* mutant yeast cells. This result clearly shows that Nhp6A/B acts through the transcriptional activators that bind to Ty2 enhancer elements. Like mammalian HMG proteins, Nhp6A/B is a multi-functional chromatin binding factor that bends DNA and participates in the formation of transcription initiation complex (21). We think that the Nhp6A/B has an architectural role in the Ty2-917 element. Nhp6A/B may have a function in the DNA looping that bridges Ty2 enhancer and Ty2 promoter region in their native form. Hence, Nhp6A/B may actively participate in the formation of transcription activation complex on Ty2-917 enhancer element. It is known that the HMG proteins function in the formation of enhanceosome complex in Epstein Barr Virus promoter (33).

Our result suggests that Spt7p, a core subunit of the SAGA complex, is also involved in the high level activation of Ty2-917 transcription. It is known that SAGA and Nhp6A/B has a functional cooperation for the regulation of a subset of genes. Triple mutant yeast cells that do not

contain functional Gcn5p, Nhp6A, and Nhp6B have a severe growth defect, indicating that these factors function in parallel pathways for the transcriptional activation of yeast genes (20). Isw2p is required for the target site selection for Ty1 but it does not have any effect on Ty1 transcription (22). However, our result suggests that Isw2p is also involved in the transcriptional activation of Ty2-917 and exerts its activity through an enhancer element.

We have also tested the effects of Rpd3p and Hda1p, which are involved in histone deacetylation, resulting in the repression of targeted genes. However, we found that neither Rpd3p nor Hda1p has significant effects on the Ty2-917 transcription (data not shown). This result suggests that nucleosome positioning is not involved in the transcriptional regulation of Ty2-917. Hence, the functions of the SAGA complex and the Isw2p is not the nucleosome displacement on Ty2-917 promoter.

It is already known that the SAGA complex interacts with Gcr1p, which is the main transcriptional activator of Ty2-917 (8,15). SAGA also functions as coactivator in certain promoters (34). Hence we suggest that the SAGA complex and Isw2p may function in the formation of transcription activation complex on Ty2-917 promoter together with Nhp6A/B. In conclusion, chromatin modifying complexes may involve in the DNA folding or looping for the formation of transcriptional activation complexes on the promoter regions of retroviruses that have a dispersed promoter organization.

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