

The *GCR2* Gene Is Required for the Transcriptional Activation of Retrotransposon Ty2-917 in *Saccharomyces cerevisiae*

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Ty2 retrotransposons are the mobile genetic elements of the yeast *Saccharomyces cerevisiae*. Transcriptional regulation of Ty2-917 requires a complex set of cis acting elements which are located both upstream and downstream of the transcription initiation site. Previously, the glycolysis regulatory protein Gcr1p has been identified as the major transcriptional regulator of Ty2-917. Gcr1p is a DNA binding transcription factor that requires Gcr2p for its functions. In this study, the effect of Gcr2p on the transcriptional regulation of Ty2-917 was analyzed. The result of this study indicates that Ty2-917 transcription decreases 24-fold in *gcr2* mutant yeast cells. In addition, Ty2 enhancer element dependent transcriptional activation of a heterolog promoter also decreases at a significant level. These results showed that Gcr2p is essential for the high level transcription of Ty2-917.

Key words retrotransposon; transcription; Ty2; *GCR2*; *Saccharomyces cerevisiae*

Ty elements of the yeast *Saccharomyces cerevisiae* are similar to vertebrate retroviruses.^{1,2)} Ty2 elements are present as 5—15 copies per haploid genome in the yeast.³⁾ Transcription of Ty2-917 is controlled by multiple regulatory regions that are located both in the upstream activation sequence (UAS) and also in the transcribed region.⁴⁾ Ty2-917 UAS is a weak transcriptional activator region.⁵⁾ Previous studies revealed that Ty2-917 enhancer, which is located downstream of the transcription initiation site, and UAS collaborate in the transcriptional activation of Ty2.⁴⁾ Ty2 enhancer element can also activate transcription at very high levels when it is cloned upstream of a heterologous promoter.

A large set of transcription factors associated with the regulatory regions of Ty2-917 have been identified in previous studies. Among these trans-acting factors, SAGA complex (Spt/Ada/Gcn5/Acetylase) and Gcr1p (Glycolysis regulation 1) seem to be the major regulators of Ty2 transcription.^{6,7)} Gcr1p specifically interacts with Gcr2p.⁸⁾ The role of Gcr1p in the Ty2 transcription is partly structural because the effects of Gcr1p on the Ty2 enhancer disappears when the enhancer element is placed upstream of a heterologous promoter.⁶⁾

In this study, the effect of Gcr2p on Ty2-917 transcription was analyzed. It was shown that the transcription of Ty2-917 decreased 24-fold in the *gcr2* mutant *S. cerevisiae* strain. In addition, Ty2-917 enhancer element-dependent transcription of a heterologous promoter also decreased at moderate levels in the *gcr2* mutant yeast cells. These results indicate that Gcr2p is essential for the transcriptional activation of Ty2-917.

MATERIALS AND METHODS

Yeast Strains and Growth Conditions The genotypes of the yeast strains used in this study are: YST104 (MAT α *ura3-52 his6 leu2-3,112*) and YST105 (MAT α *ura3-52 his6 leu2-3,112 gcr2-1*). They are isogenic other than the *gcr2* mutation.⁹⁾ Yeast cells were grown in YPD (1% yeast extract, 2% peptone, supplemented with 2% dextrose) media for transformation. Plasmids were transformed into the yeast cells as described previously.¹⁰⁾ Transformants were grown in YNB media supplemented with auxotrophic requirements and 2% dextrose. Uracil was omitted from the growth media

for maintenance of the plasmids.

Plasmids and β -Galactosidase Assays The structure and construction of the plasmids used in this study have been described previously.⁶⁾ Briefly, YEp917-555 contains the first 555 bp region of Ty2-917 element fused to *Escherichia coli* lacZ gene on the 2 μ m-URA3 based yeast expression vector. pST1-Enh contains an enhancer region of Ty2-917 in upstream of the UAS-less His4-lacZ fusion gene on the 2 μ m-URA3 based yeast expression vector. pFN8X-n is used as a control plasmid, which contains the His4-lacZ fusion gene.¹¹⁾

Yeast transformants were grown in YNB media (supplemented with auxotrophic requirements and 2% dextrose) until the logarithmic stage, in triplicate. β -Galactosidase assays were done using permeabilized yeast cells as described previously.¹²⁾ β -Galactosidase activities were expressed as μ mol ONPG cleaved per mg of protein per minute.

RESULTS

Transcription of the yeast retrotransposon Ty2-917 is regulated by a large set of transcription factors. Transcriptional regulatory regions of the yeast retrotransposon Ty2-917 are located both upstream and downstream of the transcription initiation site and contain activator and repressor binding regions.^{4—6,13,14)} Previously, it was shown that Gcr1p is the main transcriptional regulator of Ty2-917, and it specifically binds to several regions within the Ty2-917 enhancer element and UAS region. In the *gcr1* mutant yeast strain, transcription of Ty2-917 decreased about 100-fold.⁶⁾

In most of the cases, Gcr1p is present together with Rap1p and Gcr2p on different promoters.^{15,16)} Gcr2p specifically interacts with Gcr1p and may act as the co-activator of Gcr1p.⁸⁾ In this study, the effects of Gcr2p on Ty2-917 transcription were analyzed using Ty2-lacZ gene fusion. Transcriptional activator regions of Ty2-917 are located within the first 555 bp region of this element. Hence, plasmid carrying the first 555 bp region of Ty2-917 which fused to the *E. coli* lacZ gene was transformed into wild type (YST104) and *gcr2* mutant (YST105) yeast cells. β -galactosidase activities expressed from this gene fusion were determined in yeast transformants, as described previously. The expression of Ty2-lacZ gene fusion in wild type yeast cells yielded 1928 units

Table 1. The *GCR2* Gene is Required for Ty2-917 Transcription

Plasmids	β -Galactosidase activities ^{a)}	
	Wild type ^{b)}	<i>gcr2-1</i>
YEp917-555	1928	80
pST1-Enh	478	182
pFN8X-n	382	319

a) β -Galactosidase activities are expressed as μ M ONPG cleaved per minute per mg of protein. b) Wild type and *gcr2-1* represents the yeast strains YST104 and YST105, respectively.

of β -galactosidase activity, as expected. But the expression level of Ty2-lacZ gene fusion in the *gcr2* mutant yeast strain resulted in a 24-fold decrease, and gave only 80 units of β -galactosidase activity (Table 1). These results indicated that Gcr2p is absolutely essential for the transcriptional activation of the Ty2-917 element in the yeast cells.

An enhancer element, which is located within the transcribed portion of Ty2-917, is also required for its transcriptional activation. Like other enhancer elements, this region can activate the transcription of heterologous promoters when it is inserted upstream of the transcription initiation site.⁴⁾ In order to further investigate the role of Gcr2p on Ty2-917 transcription, transcription driven by this enhancer element from the UAS-less His4-lacZ gene fusion was analyzed. Transcription activated by the Ty2 enhancer element from this gene fusion gave 478 units of β -galactosidase activity in the wild type yeast strain. However, transcription of the same gene fusion in the *gcr2* mutant yeast cells gave only 182 units of β -galactosidase activity, indicating that the enhancer element of Ty2-917 largely depends on the presence of functional Gcr2p. Transcription of His4-lacZ gene fusion on plasmid pFN8X-n was not affected by *gcr2* mutation at significant levels. Its transcription resulted with high levels of β -galactosidase activity, both in the wild type and in the *gcr2* mutant yeast strains, as expected.

DISCUSSION

S. cerevisiae contains five different classes of retroviral-like mobile genetic elements known as Ty elements. Like eukaryotic retroviruses, yeast retrotransposons also replicate with an RNA intermediates, and they can form virus-like particles in yeast cells.^{2,17)} Gene expression in the Ty elements of the yeast *S. cerevisiae* is regulated at transcriptional and translational levels.¹⁸⁾ Ty elements have a very small genome size. Their promoter regions are saturated with the binding sites of various transcription factors. Ty elements do not encode any known transcription factors. Therefore, their transcription totally depends on the yeast encoded transcription factors. Some of these transcription factors bind to the transcribed portion of Ty2-917. Hence, the formation of a productive transcriptional activation complex on Ty2-917 promoter may require extensive chromatin folding and nucleosome displacement.

Previous studies indicated that Gcr1p specifically binds to several sites on the UAS and enhancer region of Ty2-917.⁶⁾ In the *gcr1* mutant yeast strain, transcription of Ty2-917 decreased about 100-fold. The results presented in this study

suggest that Gcr2p is also required for Ty2 transcription, since deletion of the *GCR2* gene results in a 24-fold decrease in Ty2-917 transcription.

It has been proposed that the role of Gcr1p in the regulation of Ty2-917 transcription is structural, because, when the enhancer element is located within the transcribed portion of the Ty2-917 element, the lack of Gcr1p results in a 100-fold decrease in the transcription of Ty2-917 element. However, when the enhancer element is cloned upstream of the transcription initiation site, it becomes independent of Gcr1p. It is known that Gcr1p is involved in DNA bending and chromatin organization.^{19,20)} It was clearly shown that Gcr1p associates with the chromatin modifying SAGA complex.⁷⁾ In addition to Gcr1p and SAGA complex, *SNF/SWI* and *SPT* families of transcription factors, which are involved in chromatin folding on various promoters, are also required for the regulation of Ty2 transcription.^{21–23)}

Nonetheless, it seems that Gcr1p, SAGA complex and other chromatin modifying factors are not sufficient for the regulation of Ty2-917 transcription. Gcr2p is also essential for Gcr1p function in the activation of Ty2 transcription. Gcr1p is a phosphoprotein. The formation of a Gcr1p-Gcr2p complex is essential for the hyperphosphorylation of Gcr1p.⁸⁾ Hence, it can be suggested that unphosphorylated Gcr1p in *gcr2* mutant yeast cells cannot function properly in the formation of an efficient transcription initiation complex on the Ty2-917 promoter region.

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