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## Non-Histone Proteins and the Mediator Complex are Essential for the Transcription of *HXT2* and *HXT4* Genes in *Saccharomyces cerevisiae*

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**Abstract:** Transcriptional activation of *HXT2* and *HXT4* genes require rapid reorganization of the chromatin structure on the promoter regions of these genes. In this study, it was found that the transcription of *HXT2* and *HXT4* genes decreased 4- to 7-fold in the yeast strain that does not contain the non-histone proteins 6A and 6B (Nhp6A/B). In addition, results of this research indicated that Nhp6A/B are required for the rapid derepression and activation of *HXT2* and *HXT4* transcription upon glucose signaling. Furthermore, it was found that *Med2p*, which provides the connection between the transcriptional activators and the basal transcription factors, is also required for the activation of *HXT2* and *HXT4* transcription. Transcription of *HXT2* and *HXT4* decreased 2- to 13-fold in *Med2* mutant *S. cerevisiae* cells. These results indicated that Nhp6A/B and the mediator complex are essential for the regulation of *HXT2* and *HXT4* gene transcription in *S. cerevisiae*.

**Key Words:** *Saccharomyces cerevisiae*, glucose repression, hexose transporters, mediator, non-histone proteins

### Non-Histon Proteinler ve Mediator Kompleksi *Saccharomyces cerevisiae*'da *HXT2* ve *HXT4* Genleri Transkripsiyonu İçin Gereklidir

**Özet:** *HXT2* ve *HXT4* genlerinin transkripsiyonunun aktivasyonu bu genlerin promotor bölgelerindeki kromatin yapısının hızlı bir şekilde yeniden organizasyonunu gerektirir. Bu çalışmada non-histon proteinler olan 6A ve 6B (Nhp6A/B) içermeyen maya suşunda *HXT2* ve *HXT4* genleri transkripsiyonunun 4-7 kat azaldığı bulundu. Buna ek olarak bu çalışmanın sonuçları Nhp6A/B'nin glukoz sinyalinin alınmasıyla *HXT2* ve *HXT4* genleri transkripsiyonunun hızlı bir şekilde aktivasyonu için gerekli olduğunu göstermektedir. Ayrıca, bazal transkripsiyon faktörleri ile transkripsiyon aktivatörleri arasında bağlantıyı sağlayan *Med2p*'nin de *HXT2* ve *HXT4* genlerinin transkripsiyonlarının aktivasyonu için gerekli olduğu bulundu. *HXT2* ve *HXT4* transkripsiyonu *Med2* mutanlığı *S. cerevisiae* suşunda 2-13 kat azaldı. Bu sonuçlar *S. cerevisiae*'da Nhp6A/B ve mediatör kompleksinin *HXT2* ve *HXT4* genleri transkripsiyonunun düzenlenmesi için gerekli olduğunu gösterdi.

**Anahtar Sözcükler:** *Saccharomyces cerevisiae*, glukoz baskılaması, Heksoz taşıyıcılar, mediatör, Non-histon proteinler

### Introduction

Glucose uptake in the yeast *Saccharomyces cerevisiae* is carried out by multiple hexose transporter proteins that are encoded by *HXT* genes (1). Among these genes, *HXT2* and *HXT4* encode intermediate affinity hexose transporters (1). Transcriptions of *HXT2* and *HXT4* is repressed by high glucose and activated by low levels of glucose (2). Mig1p, Ssn6p-Tup1p complex, and Rgt1p are required for the glucose repression of *HXT2* and *HXT4* (3). Transcriptional activation of these two *HXT* genes requires a low glucose signal, which is exerted through Snf3p action (3). The transcription factors

Gcr1p-Gcr2p complex and Rgt1p are essential for the activated level transcription of *HXT2* and *HXT4* in low glucose medium (3, 4).

Ssn6p-Tup1p complex represses the transcription of various genes with different mechanisms. This co-repressor complex is tethered to *HXT2* and *HXT4* promoters mainly by Rgt1p (5). Recent results indicate that non-histone protein Nhp6B also interacts with Ssn6p-Tup1p complex (6). Nhp6A and Nhp6B (Nhp6A/B) are highly homologous proteins and are encoded by the non-essential genes *Nhp6A* and *Nhp6B* (7). Nhp6A/B are involved in the transcriptional regulation of several genes

by modulating the chromatin structure at promoter regions (8, 9).

The SAGA complex (Spt, Ada, Gcn5, and Acetyltransferase) is another chromatin-modifying complex that controls a large set of genes in *S. cerevisiae*. It consists of 14 subunits in *S. cerevisiae* (10). Depending on the promoter context of the target genes, the SAGA complex can interact with activators and also with basal transcription factors (11,12). Functional cooperation between Nhp6A/B and the SAGA complex has also been reported. The mediator complex is also involved in the control of gene expression in *S. cerevisiae*. The mediator complex of *S. cerevisiae* is composed of 24 different subunits and it is essential for the connection of transcriptional activators to basal transcription factors on the promoter regions (13,14).

Derepression of *HXT2* and *HXT4* gene transcription requires rapid reorganization of the promoter region, which results in the redistribution of the transcription factors on the promoter regions of these genes. In this research, the functions of Nhp6A/B and the mediator complex in the regulation of *HXT2* and *HXT4* genes were investigated using the isogenic wild type and mutant *S. cerevisiae* strains.

## Materials and Methods

### Yeast strains and Plasmids

The genotypes of *S. cerevisiae* strains used in study are listed in Table 1. YST150 and YST151 are isogenic, except for the indicated  $\Delta nhp6A/B$  double mutation (6). YST 124 and YST 165 are also isogenic, except for the  $\Delta med2$  mutation (15).

The structures of the plasmids that contain *HXT2-lacZ* and *HXT4-lacZ* gene fusions were described previously.

Both plasmids are the derivatives of the 2  $\mu$ M -URA3-based expression vector YEp356R. The *HXT2-lacZ* gene fusion plasmid contains the 0.9 bp-long promoter region of the *HXT2* gene and the *HXT4-lacZ* gene fusion plasmid contains the 1605 bp-long UAS region of the *HXT4* gene (16,17). Plasmid that carries Suc2-LacZ gene fusion is also a 2  $\mu$ M-URA3-based expression vector, which has been previously defined (18,19). It was shown that the promoter regions of *HXT2*, *HXT4*, and *SUC2* genes used in the construction of LacZ gene fusions are able to exert glucose-dependent transcriptional control of heterologous genes (17,19). Suc2-lacZ gene fusion was used as a control plasmid since *SUC2* expression is controlled by glucose repression with chromatin factors (20). It is known that these 2  $\mu$ M-URA3-based yeast expression vectors that contain lacZ gene fusions are stably maintained in the yeast transformants under selective growth conditions (21). It was also shown that  $\beta$ -galactosidase activities expressed from these gene fusions correlate to mRNA levels of the same gene fusions (2).

### Growth conditions and $\beta$ -galactosidase assays

Yeast strains were cultivated in YPAD (1% yeast extract, 2% peptone, 20 mg/l adenine, and 2% glucose) medium for transformation. Yeast transformations were performed using the lithium acetate procedure (22). Yeast transformants were grown in synthetic complete medium without uracil (SC-Ura), supplemented with 4% glucose (2). For  $\beta$ -galactosidase assays, first the yeast transformants were cultivated in 10 ml of SC-Ura medium supplemented with 4% glucose, with constant shaking at 30 °C (130 rev per min) to the stationary stage, in triplicate. Then, 100  $\mu$ l of these saturated cultures were transferred to fresh media and grown to

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

Strain name (Original Name)	Genotype (relevant mutation)	Source
YST150 (JD52)	MATa, <i>Ura3-52</i> , <i>leu2-3</i> , <i>his3<math>\Delta</math>200</i> , <i>lys2-801</i> , <i>trp1<math>\Delta</math>63</i> , <i>ade2::hisG</i>	N. Lehming
YST151 (JD52)	MATa, <i>Ura3-52</i> , <i>leu2-3</i> , <i>his3<math>\Delta</math>200</i> , <i>lys2-801</i> , <i>trp1<math>\Delta</math>63</i> , <i>ade2::hisG</i> , $\Delta nhp6A$ , $\Delta nhp6B$ ( $\Delta nhp6A/B$ )	N. Lehming
YST124 (BY4741)	MATa, <i>his3<math>\Delta</math>1</i> ; <i>leu2<math>\Delta</math>0</i> ; <i>met15<math>\Delta</math>0</i> ; <i>ura3<math>\Delta</math>0</i>	EUROSCARF
YST165 (Y13701)	MAT $\alpha$ , <i>his3<math>\Delta</math>1</i> ; <i>leu2<math>\Delta</math>0</i> ; <i>met15<math>\Delta</math>0</i> ; <i>ura3<math>\Delta</math>0</i> , <i>YDL005c::kanMX4</i> ( $\Delta med2$ )	EUROSCARF

the logarithmic stage ( $A_{600} = 1.0$ ). However, 4 h prior to harvest, 5 ml of growing cultures were shifted to 5 ml of SC-Ura medium supplemented with 0.1% glucose, 2% sodium lactate, and 2% glycerol, for the derepression of *HXT2* and *HXT4* gene transcription (4). At the end of the growth periods, yeast transformants were harvested and washed once with ice-cold sterile distilled water, and then resuspended in 200  $\mu$ l of breaking buffer (23).  $\beta$ -galactosidase assays were performed in triplicate, as previously described (23).  $\beta$ -galactosidase activities were given as Miller Units (23).

In order to analyze the derepression patterns of the *HXT2* and *HXT4* genes in the wild type and  $\Delta nhp6A/B$  double mutant, yeast transformants were grown to the logarithmic stage in SC-Ura medium supplemented with 20 mg/l adenine, and 4% glucose, for glucose-repressed conditions. Then, cells were harvested and washed twice with sterile distilled water. After that, cells were inoculated into SC-Ura medium supplemented with 20 mg/l adenine, 2% glycerol, 2% sodium lactate, and 0.1% glucose, for derepression. Cultures were incubated on a shaker (30 °C, 130 rev per min) and 5 ml yeast samples were removed for  $\beta$ -galactosidase assays at specified time intervals.

## Results

### Nhp6A/B are essential for the regulated expression of *HXT2* and *HXT4* genes

*HXT2* transcription was decreased 4- to 5-fold in the wild type and  $\Delta nhp6A/B$  double mutant yeast cells when the yeast cells were transferred to low glucose medium, as expected (Table 2). However, the overall level of *HXT2* transcription was approximately 5-fold lower in

$\Delta nhp6A/B$  double mutants than in the wild type yeast cells. Although the repression and derepression of *HXT2* takes place in the  $\Delta nhp6A/B$  mutants, both repressed and derepressed level transcription were much lower in  $\Delta nhp6A/B$  mutants than the wild type yeast (Table 2).

Transcription of *HXT4* is also regulated by glucose repression and derepression mechanisms; hence, the expression of *HXT4* in the wild type yeast strain grown in low glucose medium resulted in a 73-fold derepression of the transcription of this gene fusion (Table 2). Derepression of *HXT4* also occurred in the  $\Delta nhp6A/B$  mutant yeast cells; but, its level was 7.5-fold lower than the wild type yeast cells.

Transcription of the *SUC2* gene is regulated by glucose repression (20). It is known that *Nhp6A/B* are also required for the regulated expression of the *SUC2* gene (24); hence, the transcription of *SUC2* partially derepressed in the  $\Delta nhp6A/B$  mutant yeasts when compared to its expression level in the wild type yeast strain (Table 2).

### Derepression of *HXT2* and *HXT4* are very slow in $\Delta nhp6A/B$ mutant yeast cells

In order to see if there are any fluctuations in the transcription of *HXT2* and *HXT4* genes during the derepression process in the  $\Delta nhp6A/B$  double mutant, the derepression patterns of *HXT2* and *HXT4* were analyzed in a time course-dependent manner in this mutant. Transcriptions of *HXT2* and *HXT4* rapidly increased to their maximum levels after 3 h of incubation in the wild type yeast strain (Figure 1). However, contrary to the wild type, low-level derepression of *HXT2* and *HXT4* gene transcription took place very slowly in the  $\Delta nhp6A/B$

Table 2. Effects of Nhp6A and Nhp6B proteins on the expression of *HXT2* and *HXT4* genes

Gene Fusions	Mean $\beta$ -Galactosidase activity $\pm$ SD			
	Wild type		$\Delta nhp6A/B$	
	R <sup>a</sup>	DR	R	DR
Hxt2-LacZ	287 $\pm$ 9	1076 $\pm$ 94	42 $\pm$ 5	236 $\pm$ 23
Hxt4-LacZ	18 $\pm$ 2	1321 $\pm$ 124	16 $\pm$ 2	175 $\pm$ 12
Suc2-LacZ	3 $\pm$ 1	831 $\pm$ 75	2 $\pm$ 1	56 $\pm$ 3

<sup>a</sup>R: Repressing (4% glucose); DR, derepressing (0.1% glucose) growth conditions.  
 $\pm$  SD: Standard Deviations.

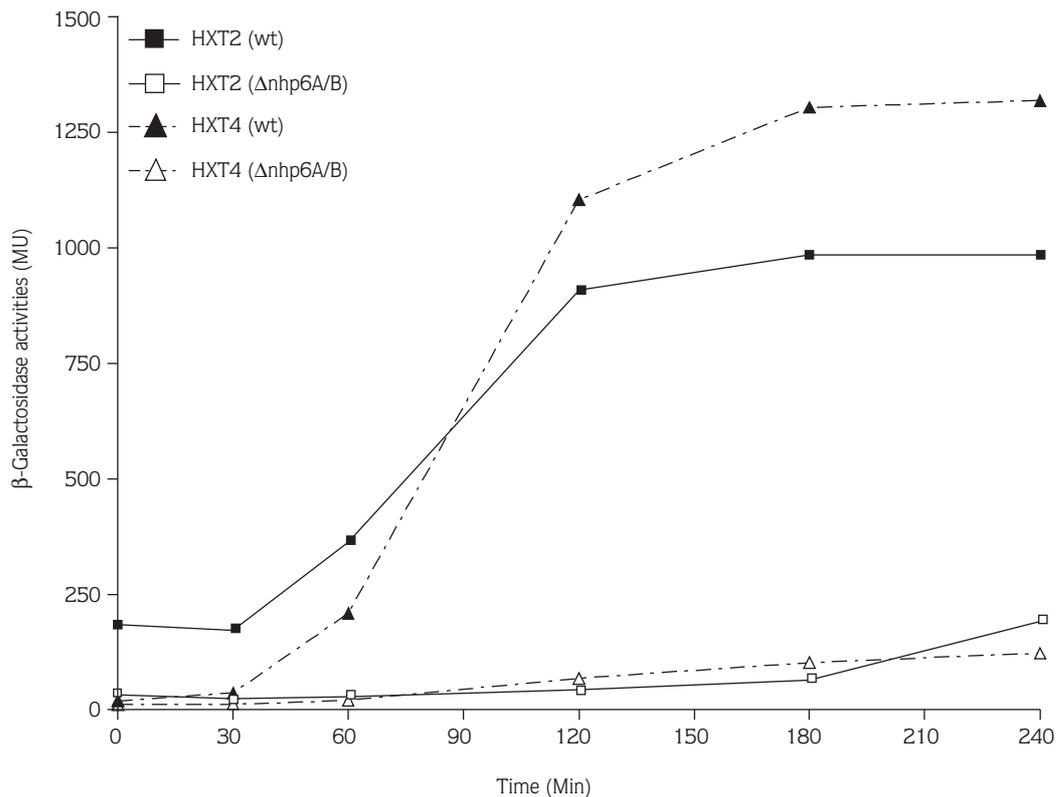


Figure 1. Time course analysis of the derepression of *HXT2* and *HXT4* expression in the wild type (YST150) and  $\Delta nhp6A/B$  double mutant (YST151) yeast strains. Yeast cells pregrown on synthetic complete medium without uracil, supplemented with 4% glucose to log phase. At time zero, yeast transformants were transferred to glucose derepressed conditions as described in the materials and methods section. Samples were removed from the cultures at specified time intervals and  $\beta$ -galactosidase activities were determined.  $\beta$ -galactosidase activities were expressed in Miller Units.

mutants. Clearly, there was no fluctuation during the derepression of *HXT2* and *HXT4*, either in the wild type or in the  $\Delta nhp6A/B$  mutant yeasts.

#### Med2p Function Is Required for *HXT2* and *HXT4* Transcription

The *S. cerevisiae* mediator complex provides the connection between the transcription activators and the basal transcription factors. We have tested whether *HXT2* and *HXT4* requires a transcriptional mediator for their transcription. Expression of *HXT2* decreased approximately 2-fold in glucose repressed conditions in  $\Delta med2$  mutant yeast cells. Transcription of *HXT4* also decreased 7-fold in the  $\Delta med2$  mutant, in glucose repressed growth conditions (Table 3).

When the yeast transformants shifted to glucose derepressed conditions, transcription of *HXT2* was not fully derepressed in the  $\Delta med2$  mutant and it was 2.6-fold lower than the wild type yeast cells. Likewise, transcription of the *HXT4* gene was also 13-fold lower than the wild type yeast cells when they were grown in derepressed conditions (Table 3). This result suggests that the function of the yeast mediator complex is essential for both basal and activated level transcription of *HXT2* and *HXT4* genes in *S. cerevisiae*.

Transcription of *SUC2* also decreased 4-fold in  $\Delta med2$  mutant yeast cells, in derepressed growth conditions. It is known that the mediator complex is essential for the regulated transcription of the *SUC2* gene (25).

Table 3. Effects of Med2p on the expression of *HXT2* and *HXT4* genes

Gene Fusions	Mean $\beta$ -Galactosidase activity $\pm$ SD			
	Wild type		$\Delta med2$	
	R <sup>a</sup>	DR	R	DR
Hxt2-LacZ	606 $\pm$ 32	1809 $\pm$ 60	324 $\pm$ 26	676 $\pm$ 14
Hxt4-LacZ	234 $\pm$ 37	2991 $\pm$ 81	32 $\pm$ 8	222 $\pm$ 23
Suc2-LacZ	3 $\pm$ 1	840 $\pm$ 83	5 $\pm$ 2	240 $\pm$ 7

<sup>a</sup>R: Repressing (4% glucose); DR, derepressing (0.1% glucose) growth conditions.

$\pm$  SD: Standard Deviations.

## Discussion and Conclusion

Chromatin factors and chromatin-modifying complexes have a large impact on the regulation of gene expression in *S. cerevisiae* (26). A transcription profile of the  $\Delta nhp6A/B$  double mutant yeast cells shows that Nhp6A/B also control the transcription of a large number of genes in *S. cerevisiae* (9).

Rgt1p and Gcr1p complex are essential for the transcriptional activation of *HXT2* and *HXT4* genes (2,4); however, results presented in this study suggest that Rgt1p and Gcr1p complexes cannot activate the transcription of *HXT2* and *HXT4* in the absence of Nhp6A/B. Since Nhp6A/B bend the DNA molecule, these architectural factors might be required for the rapid association of the Rgt1p and Gcr1p complexes on the *HXT2* and *HXT4* promoters (8,9). In addition, Nhp6A/B may also be involved in the stable binding of Rgt1p and Gcr1p complexes to their cognate binding sites on the *HXT2* and *HXT4* promoters. Hence, a lack of Nhp6A/B function in the  $\Delta nhp6A/B$  double mutant yeast cells may result in the destabilization of the Rgt1p and Gcr1p complexes binding to the promoter regions of *HXT2* and *HXT4*. This may lead to low levels of transcriptional activation of these genes, even under derepressed growth conditions (27).

Transcription of *HXT2* and *HXT4* is repressed in the  $\Delta nhp6A/B$  double mutant strains in a high glucose medium; therefore, it appears that Nhp6A/B are not required for the formation of the repressed chromatin structure on the *HXT2* and *HXT4* promoter regions. This result indicates that the Rgt1p-Ssn6-Tup1p complex and Mig1p function properly for the repression of the *HXT2* and *HXT4* genes in  $\Delta nhp6A/B$  mutant cells. It seems that Nhp6A/B are essential for the rapid derepression and

high-level expression of *HXT2* and *HXT4* since the activated level transcriptions of both *HXT2* and *HXT4* were very low in the  $\Delta nhp6A/B$  mutant strains. Activation of *HXT2* and *HXT4* transcription requires Rgt1p and Gcr1p complexes. Recently, it was also shown that Nhp6A/B have a similar function in the regulated expression of the *SUC2* gene in *S. cerevisiae* (24). We suggest that Nhp6A/B act together with Rgt1p and Gcr1p complexes for the activation and high-level expression of *HXT2* and *HXT4* genes.

It is known that the SAGA complex interacts with chromatin factors and transcriptional activators to unfold the chromatin structures on the target promoter regions (28-30). The SAGA complex and non-histone proteins 6A/B may function as architectural factors, and together they may participate in the formation of transcriptional activation complex on the promoter regions of *HXT2* and *HXT4* genes. It was recently shown that the SAGA complex is involved in the regulation of *HXT2* and *HXT4* transcription (31). Components of the SAGA complex may function in activator recruitment to *HXT2* and, especially, to the *HXT4* promoter. Since the SAGA complex and Gcr1p interact with each other, it is conceivable that the SAGA complex recruits the Gcr1p complex to *HXT2* and *HXT4* promoters for the rapid formation of a stable activation complex, as suggested for different promoters (30,32). It is also known that the SAGA complex and Nhp6A/B function in parallel pathways for the reorganization of the chromatin structures on certain promoters (12).

Our results also show that the yeast mediator complex is involved in the regulation of both *HXT2* and *HXT4* transcription. It was previously shown that the deletion of the *Med2* gene results in significant decreases in the

expression levels of several genes in *S. cerevisiae* (33). In that study, a 2-fold decrease was reported in the transcription of the *HXT6* gene in galactose grown *Med2* mutant yeast cells. Our results show that the *Med2p* subunit of the yeast mediator complex is also essential for the regulation of *HXT2* and *HXT4* genes. The results obtained from this research suggest that Nhp6A/B and the yeast mediator complex are essential for the rapid derepression of *HXT2* and *HXT4* genes in *S. cerevisiae*.

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