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Analysis of the Effects of Transcription Factors Gcr2p and Sgc1p on the Control of the *SUC2* Gene Expression in *Saccharomyces cerevisiae*

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Abstract: The transcription of the *SUC2* gene is regulated by glucose repression and the derepression mechanism in the yeast *Saccharomyces cerevisiae*. The Mig1p-Tup1p-Ssn6p repressor complex, hexokinase PII and nucleosomes are required for the repression of the *SUC2* gene. The Snf/Swi and SAGA complexes act on Mig1p and nucleosomes in low glucose media. *SUC2* transcription thus becomes activated. In this study, it is shown that the transcription factors Gcr2p and Sgc1p are also required for the high level transcription of the *SUC2* gene. *SUC2* transcription and the invertase synthesis decreased at significant levels in *gcr2* and *sgc1* mutant yeast cells. In addition, it is also shown that the derepression of the *SUC2* gene transcription is very slow in *gcr2* and *sgc1* mutant yeast strains. The glucose repression rate seems to be normal in *gcr2* and *sgc1* mutants, indicating that Gcr2p and Sgc1p are essential for the high level transcription of the *SUC2* gene.

Key Words: Invertase, Glucose repression, *GCR2, SUC2, SGC1*, *Saccharomyces cerevisiae*, Transcription.

Transkripsiyon Faktörleri Gcr2p ve Sgc1p'nin *Saccharomyces cerevisiae*'da *SUC2* Gen İfadesinin Kontrolüne Etkilerinin Analizi

Özet: *Saccharomyces cerevisiae*'da *SUC2* geninin transkripsiyonu glikoz baskılaması ve baskının kaldırılması mekanizması ile düzenlenmektedir. Mig1p-Tup1p-Ssn6p represör kompleksi, hekzokinaz II proteini ve nükleozomlar *SUC2* geninin baskılanması için gereklidirler. Düşük miktarda glikoz içeren üreme ortamında ise Snf/Swi ve SAGA kompleksleri Mig1p kompleksi ve nükleozomlara etki ederler ve daha sonra da *SUC2* geni transkripsiyonu aktive edilmiş olur. Bu çalışmada transkripsiyon faktörleri Gcr2p'nin ve Sgc1p'nin *SUC2* geni transkripsiyonunun fazla miktarda yapılabilmesi için gerekli olduğu gösterildi. *SUC2* transkripsiyonu ve invertaz sentezinin *gcr2* ve *sgc1* mutantı maya suşlarında önemli miktarda azaldığı bulundu. Ayrıca, *gcr2* ve *sgc1* mutant suşlarında *SUC2* transkripsiyonunda glikoz baskılamasının kaldırılmasının çok yavaş olduğu gösterildi. Glikoz baskılama hızının *gcr2* ve *sgc1* mutant suşlarında normal bulunması, Gcr2p ve Sgc1p'nin *SUC2* geni transkripsiyonunun yüksek seviyede yapılması için gerekli olduklarını göstermiştir.

Anahtar Sözcükler: İnvertaz, Glikoz baskılaması, *GCR2, SUC2, SGC1*, *Saccharomyces cerevisiae*, Transkripsiyon.

Introduction

Invertase (E.C. 3. 2. 1. 26) is encoded by the *SUC2* gene of the yeast *Saccharomyces cerevisiae* (1). Extracellular invertase is highly glycosylated in its secreted form. It is required for the hydrolysis of sucrose and raffinose in growth media. (2,3). The transcription of the *SUC2* gene is subject to glucose repression (4). The Mig1p-Ssn6p-Tup1p complex and Hxk2p bind directly to the promoter region and repress its transcription in the presence of high glucose (2% w/v) in the growth media of *S. cerevisiae* (5-8). Nucleosomes are required for the formation of a repressed chromatin structure on the *SUC2* promoter region (9-11). Other regulatory factors including Sko1p, Glc7p, Gal11p, Grr1p and Med8p are also required for the regulation of the *SUC2* transcription (12,13)

In the presence of low glucose (0.05-0.1% w/v) or a non-fermentable carbon source in the growth media of *S. cerevisiae*, the transcription of *SUC2* becomes derepressed. Mig1p is phosphorylated by the Snf1p protein kinase complex (14). It dissociates from the *SUC2* promoter and is exported from nuclei (15). Hxk2p is

dephosphorylated by protein phosphatase Cid1p and dissociates from the *SUC2* promoter under derepressed growth conditions (7). Finally, nucleosomes are modified and partially displaced from the *SUC2* promoter and derepressed chromatin structure forms (9,10,16)

However, derepression of the *SUC2* promoter is not sufficient to maintain long-term and high levels of transcription. Transcriptional activator binding sites (UASs) required for the activation have been identified on the *SUC2* promoter by deletions and site directed mutations (11). Continuous presence of the Snf/Swi remodeling complex on the *SUC2* promoter is also necessary for the maintenance of the long-term derepressed and activated level of the *SUC2* transcription (16). Recently, our group has shown that the transcription factor Gcr1p (Glycolysis Regulatory protein-1) specifically binds to the promoter region of *SUC2*. (17). Gcr1p is a DNA binding transcription factor that specifically interacts with the 5'-CTTCC-3' (CT-box) sequence (18). Gcr1p is required for the high-level expression of glycolytic enzyme genes. Levels of glycolytic enzymes decrease to 1-10% of their wild type level in *gcr1* mutants (19).

In most cases, Gcr1p is present together with the transcription factors Gcr2p and Rap1p. It has been shown that Rap1p (Repressor/Activator Protein-1) facilitates Gcr1p binding to its binding sites on certain types of promoters (20,21). Nonetheless, Rap1p is dispensable for the functions of the Gcr1p-Gcr2p complex. Gcr2p is also required for high-level expressions of glycolytic enzyme genes (20,22). Gcr2p is not a DNA binding transcription factor, but genetic and biochemical evidence indicates that Gcr2p physically interacts with Gcr1p (22,23). Gcr2p is required for the transcriptional activation function and hyperphosphorylation of Gcr1p (22,24).

In addition to Rap1p, the Gcr1p-Gcr2p complex may also require the function of the transcription factor Sgc1p (Suppressor of GCR1), since certain types of mutation in the *SGC1* gene suppress the requirement for the Gcr1p-Gcr2p complex (25,26). The *SGC1* gene encodes a basic helix-loop-helix (bHLH) type transcription factor, which specifically interacts with the nucleotide sequence (5'-CANNTG-3') known as E-box (27). Sgc1p is also required for the high level expression of several glycolytic genes in *S. cerevisiae* (25).

Our previous results indicated that *SUC2* transcription and the invertase synthesis in *gcr1* mutant yeast cells are non-glucose repressible, suggesting that the Gcr1p function is required for the regulated expression of the *SUC2* gene (17,28). In this study, we analyzed the effect of Gcr2p and Sgc1p on the *SUC2* transcription and invertase synthesis in *S. cerevisiae*. In order to analyze if there are any differences in the rate and pattern of the repression and derepression of the *SUC2* gene expression among the wild type, *gcr2* and *sgc1* mutant strains, the invertase activities were also measured in actively growing cultures of these strains in a time-course dependent manner.

Materials and Methods

Yeast strains and plasmids

The genotypes of *S. cerevisiae* strains used in this study are: YST104 (MATα, ura3-52, his6, leu2-3,112), YST105 (MATα, ura3-52, his6, leu2-3,112, *gcr2*-1) and YST133 (MATα, ura3-52, his6, leu2-3,112, Δ*sgc1*::LEU2). They are all isogenic except for the indicated *gcr2* and *sgc1* mutations (20,23).

The structure and construction of *SUC2*-LacZ gene fusion on the yeast expression plasmid have been described previously (17). This is a 2μM-*URA3* based yeast expression vector that contains the *SUC2* promoter region extending from –384 to –900 bp (relative to the first ATG codon of the *SUC2* gene). As a control, *Cyc1*-LacZ reporter gene fusion on a 2μM-*URA3* based expression vector was used, since the expression of *CYC1* is also regulated by glucose repression (29). Yeast transformations were performed as described previously using lithium acetate (30).

Growth conditions and enzyme assays

β-Galactosidase activities of yeast transformants were determined in permeabilized yeast cells. First, yeast transformants were grown to the logarithmic stage in 10 ml of uracil minus synthetic complete media (-Ura, SC) supplemented with 2% glycerol and 2% lactate in triplicates at 30 ºC (31). Uracil was omitted for the maintenance of plasmids in the yeast cells. Thereafter, yeast cultures were harvested, washed twice with 10 ml of sterile distilled water, and resuspended in 10 ml of –Ura SC medium. Then the aliquots of the yeast cultures

(5 ml each) were grown as glucose repressed (2% glucose) or glucose derepressed (0.05% glucose) for 4 h and then harvested for β-Galactosidase assays. β-Galactosidase activities were determined in triplicates as described previously (32). β-Galactosidase activities were given as μM O-Nitrophenyl β-D-Galactopyranoside (ONPG) cleaved per minute per mg of protein, and represent the mean values of 18 independent assays of 6 independent transformants. Standard deviation in these assays was less than 10%.

In order to determine the derepression rates and the pattern of invertase synthesis of the wild type, *gcr2* and *sgc1* mutant yeast strains, these yeast strains were first pre-cultured in 10 ml of YPD medium (1% yeast extract, 2% peptone, 2% dextrose) to the stationary stage (31). Then yeast cells were harvested and washed twice with 10 ml of sterile distilled water. These yeast stocks were used to inoculate 50 ml of YPG/L medium (1% yeast extract, 2% peptone, 2% glycerol and 2% lactate) supplemented with 0.05% glucose for derepression. The initial cell densities of each yeast culture were adjusted to OD_{600}: 0.3. The yeast cultures were transferred to an incubator shaker. Samples were removed at time intervals until the late logarithmic stage (OD_{600}: 1.5) as indicated in Figure 1. The secreted invertase activities of the yeast strains were determined using whole cells by the glucose oxidase-peroxidase method as described previously (33). One unit of invertase activity was expressed as the amount of extracellular invertase that catalyses the release of 1 μM of glucose per minute per 100 mg of dry weight. To analyze the glucose repression rate, yeast strains were grown to the logarithmic stage in YPD medium and then derepressed for 2 h. Following complete derepression, glucose was added to the yeast cultures at 2% and the yeast samples were removed at time intervals as indicated in Figure 2. Invertase activities were determined as described previously (33).

Results

GCR2 and *SGC1* are required for the high level expression of *SUC2* gene

Transcription of the *SUC2* gene is regulated by the glucose repression and derepression mechanism in *S. cerevisiae*. Regulated transcription of the *SUC2* gene requires the activities of chromatin modifying complexes and transcriptional activators that specifically interact with the *SUC2* promoter region (34). Gcr1p is one of the activators that specifically bind to the *SUC2* promoter (17,28). In most cases, Gcr1p forms a heterodimer with Gcr2p and associates with Sgc1p. In order to further investigate the role of Gcr2p and Sgc1p in the regulation of the *SUC2* gene the *SUC2* transcription in the *gcr2* and *sgc1* mutant yeast strains was analyzed.

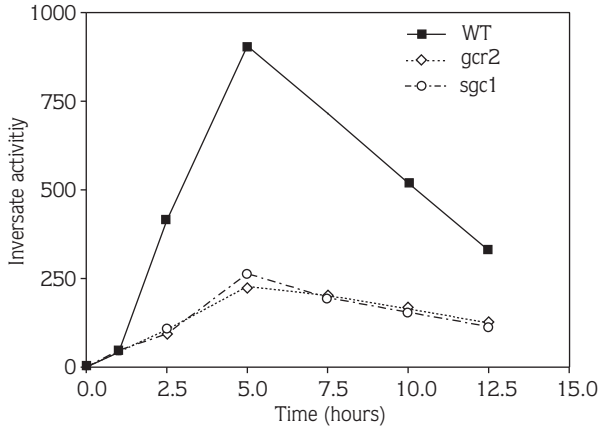

Figure 1. Long-term analysis of the derepression of invertase synthesis in wild type *gcr2* and *sgc1* mutant yeast strains. There is a rapid derepression of invertase synthesis in the wild type yeast strain. However, derepression is very slow in the *gcr2* and *sgc1* mutant yeast strains. WT: wild type yeast strain (YST104), *gcr2*: (YST105) and *sgc1*: (YST133). Invertase activities are expressed as micromoles of glucose deliberated/min/100 mg of dry weight.

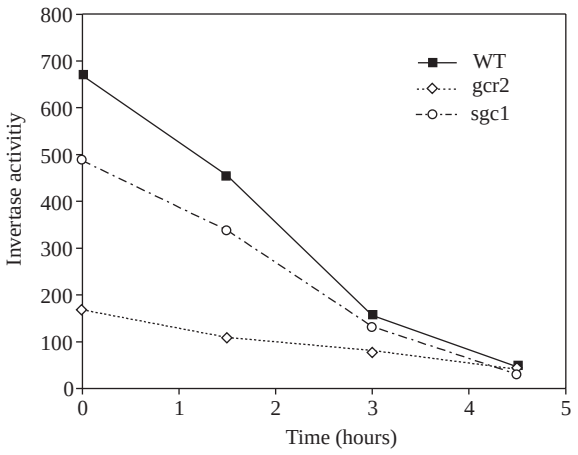

Figure 2. Analysis of the glucose repression patterns of invertase synthesis in wild type (YST104), *gcr2* (YST105) and *sgc1* (YST133) mutant yeast strains. Glucose repression takes place at a normal level in all strains. Invertase activities are expressed as micromoles of glucose deliberated/min/100 mg of dry weight.

SUC2-LacZ reporter gene fusion was used to monitor the transcription pattern of the *SUC2* gene under repressed or derepressed growth conditions. The *SUC2*-LacZ gene fusion yielded 1091 units of β-Galactosidase activity in the *GCR2*$^+$ wild-type strain under derepressed growth conditions. As expected, its expression dramatically decreased to 183 units in glucose repressed yeast cells (Table). However, the expression of the *SUC2*-LacZ gene fusion in the *gcr2* mutant yeast strain yielded approximately 2-fold lower (495 units) β-Galactosidase activity under derepressing conditions, indicating that Gcr2p is involved in the high level expression of the *SUC2* gene in derepressed yeast cells. β-Galactosidase activity expressed from the *SUC2*-LacZ gene fusion was still under the control of glucose repression in the *gcr2* mutant. Four hours after the repression, β-Galactosidase activity expressed from the *SUC2*-LacZ gene fusion in the *gcr2* mutant decreased to 199 units of activity (Table 1). Similarly, the expression of the *SUC2*-LacZ gene fusion decreased also approximately 4-fold in the *sgc1* mutant yeast strain. In derepressed *sgc1* mutant yeast cells, β-Galactosidase activity expressed from the *SUC2*-LacZ gene was 277 units. The transcription of the *SUC2*-LacZ was repressed in the presence of 2% glucose in the growth medium and gave only 163 units of β-Galactosidase activity. These results indicate that Gcr2p and Sgc1p functions are necessary for the derepressed level expression of the *SUC2* gene.

Table 1. Gcr2p and Sgc1p required for the regulated transcription of the *SUC2* gene.

	β-Galactosidase Activities[a]					
	WT[b]		*gcr2-1*		*sgc1*	
Plasmids	R[c]	Dr	R	Dr	R	Dr
Suc2-LacZ	183	1091	199	495	163	277
Cyc1-LacZ	4956	9800	6086	10596	7915	14470

[a]β-Galactosidase activities are given in nanomoles of ONPG cleaved/min/mg of protein. [b]*WT*, *gcr2-1* and *sgc1* represent the yeast strains YST104, YST105 and YST133. [c]DR: derepressed (0.05% glucose), R: repressed (2% Glucose) growth conditions.

The expression of the *Cyc1*-LacZ gene fusion was glucose repressed as expected. However, the molecular mechanism of the glucose repression of *CYC1* is different from that of *SUC2*. Hence, the expression of the *Cyc1*-LacZ gene was not affected by the *gcr2* or *sgc1* mutations as expected. The transcription of the *Cyc1*-LacZ gene fusion was glucose repressible by approximately 50% in all 3 strains (Table).

Derepression rate of invertase activities are very slow in *gcr2* and *sgc1* mutants

In order to determine the derepression pattern of invertase synthesis in *gcr2* and *Sgc1* mutants during prolonged incubation times under derepressed growth conditions, invertase activities were determined in a time-course dependent manner. First, the wild type, *gcr2* and *sgc1* mutant strains were pre-cultured in YPD media until the stationary stage. Then yeast strains were inoculated into YPG/L medium supplemented with 0.05% glucose for derepression of invertase synthesis. Starting from the pre-logarithmic stage (OD_{600}: 0.3), yeast samples were taken at time intervals up to the late logarithmic stage (OD_{600}: 1.5), as indicated in Figure 1, and the invertase activities were determined as described previously (33). Invertase synthesis in the wild type strain increased rapidly and reached its maximum level (900-1000 units) after 5 h of incubation. However, the derepression of the invertase synthesis in the *gcr2* and *sgc1* mutants was very slow. Full level derepression of invertase synthesis, which gave approximately 250 units of invertase activity, was completed in 5 h in *gcr2* and *sgc1* mutants. As expected, invertase synthesis in all 3 strains rapidly decreased at the end of the logarithmic stage. These results indicate that Gcr2p and Sgc1p are involved in rapid derepression of the *SUC2* gene and invertase synthesis in *S. cerevisiae*.

Previously, it was shown that the invertase synthesis is not glucose repressible in the *gcr1* mutant strain (17). To further elucidate the mechanisms of Gcr2p and Sgc1p requirement in the *SUC2* gene expression, the glucose repression rate of invertase synthesis was measured. First, invertase synthesis was derepressed by growing yeast cells in low glucose medium. After reaching a full level of derepression, glucose was added to the growth medium at repressing concentrations (2%). Thereafter, yeast samples were removed at the time intervals and invertase activities determined, as indicated in Figure 2. From these results, it is clear that the glucose repression takes place in both the *gcr2* and *sgc1* mutant yeast strains. Invertase activities reached their lowest levels (30-40 units) in the wild type, *gcr2* and *sgc1* mutant

yeast strains at the end of the incubation period (Figure 2). Due to the glucose repression, invertase activity decreased 14.5-fold (from 669 to 46 units) in the wild type strain (YST104). Similarly, it decreased about 16-fold (from 487 to 30 units) in the *sgc1* mutant (YST133) and 4-fold (from 168 to 40 units) in the *gcr2* mutant (YST105) yeast strains. These results confirmed that, unlike Gcr1p, Gcr2p and Sgc1p are not required for the glucose repression of the *SUC2* gene transcription.

Discussion

The transcription of the *SUC2* gene is regulated by glucose repression and the derepression mechanism and requires multiple sets of trans-acting factors.

It has been shown that 4 well-positioned nucleosomes cover the upstream regulatory region of the *SUC2* promoter, including TATA box, under glucose repressed conditions (9,10). The Mig1-Tup1-Ssn6 complex is required for the establishment of the repressed state of the *SUC2* promoter. Tup1p interacts with histones H3 and H4, and possibly recruits nucleosomes on target promoters (35). Recently, it was also shown that the Ssn6-Tup1 complex interacts with histone deacetylases once the nucleosomes are recruited to the *SUC2* promoter, and then deacetylated nucleosomes form a more stable repressed chromatin structure (36).

Previously, it was reported that Gcr1p is involved in the nucleosome displacement under glucose derepressed conditions from the *TDH3* gene (Triosephosphate Dehydrogenase-3) promoter (37). Hence, it is conceivable that Gcr1p plays a similar role in the remodeling of the *SUC2* promoter under glucose repressed and derepressed conditions. Gcr1p may act as a remodeling factor to displace the positioned nucleosomes from *SUC2*'s upstream region. Gcr1p may also play a significant role in the recruitment of the Snf/Swi and SAGA complexes to the *SUC2* promoter. According to this model, Gcr1p binding to the *SUC2* promoter facilitates the binding of another transcriptional activator(s) to the opened *SUC2* promoter region. It is also known that the components of the glucose repression pathway are intact in *gcr1* mutant yeast cells (17,28). Hence, it seems that the Mig1p-Tup1p-Ssn6p complex and other components of the glucose repression are present within the *gcr1* mutant yeast cells, but they cannot form a repressed chromatin structure efficiently on the *SUC2* promoter.

Gcr1p is not a strong transcriptional activator, and in general the Gcr1p dependent promoters require additional transcriptional activators such as Gcr2p, Sgc1p and Rap1p (20). The presence of transcriptional activators in the *SUC2* promoter has been verified by deletion analysis of this promoter (11). Gcr1p can also form a homodimer or heterodimer with Gcr2p once the derepressed chromatin structure forms on the *SUC2* promoter.

The absence of Gcr2p or Sgc1p results in an approximately 3-4-fold decrease in the derepressed level invertase activity of the yeast cells, indicating that the functions of these transcription factors are required for the derepression, but not for the repression, of the *SUC2* gene. It seems that Gcr1p alone is not sufficient to restore high levels of *SUC2* expression but, contrary to the Gcr2p and Sgc1p functions, Gcr1p is required for the complete repression of *SUC2* expression. Stanway et al. (38) showed that Gal11p is required for the Gcr1p dependent transcriptional activation of the Phosphoglycerate Kinase (*PGK*) gene in *S. cerevisiae*. Gal11p is a transcriptional mediator that directly interacts with the RNA Polymerase–II complex. It is also known that Gal11p is required for the regulated expression of *SUC2* (12).

Med8p is a recently characterized transcriptional mediator that is required for the derepression of the *SUC2* promoter, and it also interacts with Gal11p (13, 39). Hence, the Gcr1p-Gcr2p complex and Sgc1p may facilitate the formation of an open chromatin structure over the promoter region of *SUC2* by recruiting the Snf/Swi and SAGA complexes. Thereafter, Gcr2p may couple with the effects of Gcr1p to basal transcription factors through Gal11p and Med8p. Unlike Gcr1p, our results indicated that Gcr2p and Sgc1p are not necessary for the glucose repression of the *SUC2* gene. Gcr1p-Gcr2p may have a dual function in the regulation of *SUC2* transcription. It may function as a nucleosome displacement factor on *SUC2* promoter as in the *TDH3* promoter. Hence, *SUC2* expression is still glucose repressible in *GCR1*$^{+}$ *gcr2* or *GCR1*$^{+}$ *sgc1* yeast cells. Therefore, it is likely that Gcr2p acts as a co-activator of Gcr1p and is required for the high level expression of the *SUC2* gene in *S. cerevisiae*.

Sgc1p is a bHLH motif containing transcription factor. Transcription factors in this family specifically bind to the nucleotide sequence 5'-CANNTG-3' or are related once in the promoter regions of genes that they regulate (27).

The involvement of Sgc1p in the regulation of several biological processes has been reported previously (27). However, consensus binding sites for Sgc1p have not yet been identified in detail. Nonetheless, analysis of the promoter region of the *SUC2* gene reveals that sequences similar to bHLH factors binding sites are also present in the regulatory region of *SUC2*. Hence it is conceivable that Sgc1p and the Gcr1p-Gcr2p complex function together in the regulated expression of the *SUC2* gene in *S. cerevisiae*.

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References

1. Carlson M, Botstein D. Two differentially regulated mRNAs with different 5` ends encode secreted and intracellular forms of yeast invertase. Cell 28: 145-154, 1982.
2. Perlman D, Halvorson HO, Cannon LE. Presecretory and cytoplasmic invertase polypeptides encoded by distinct mRNAs derived from the same structural gene differ by a signal sequence. Proc Natl Acad Sci USA 79: 781-785, 1982.
3. Perlman D, Raney P, Halvorson HO. Cytoplasmic and secreted *Saccharomyces cerevisiae* invertase mRNAs encoded by one gene can be differentially or coordinately regulated. Mol Cell Biol 9: 1682-1688, 1984.
4. Sarokin L, Carlson M. Upstream region of the *SUC2* gene confers regulated expression to a heterologous gene in *Saccharomyces cerevisiae*. Mol Cell Biol 5: 2521-2526, 1985.
5. Trumbly RJ. Glucose repression in the yeast *Saccharomyces cerevisiae*. Molecular Microbiol 6: 15-21, 1992.
6. Lutfiyya LL, Johnston M. Two zinc-finger-containing repressors are responsible for glucose repression of *SUC2* expression. Mol Cell Biol 16: 4790-4797, 1996.
7. Randez-Gil F, Sanz P, Entien K.-D, et al. Carbon source-dependent phosphorylation of hexokinase PII and its role in the glucose-signaling response in yeast. Mol Cell Biol 18: 2940-2948, 1998.
8. Herrero P, Martinez-Campa C, Moreno F. The hexokinase 2 protein participates in regulatory DNA-protein complexes necessary for glucose repression of the *SUC2* gene in *Saccharomyces cerevisiae*. FEBS Letters 434: 71-76, 1998.
9. Gavin IM, Simpson RT. Interplay of yeast global transcriptional regulators Ssn6p-Tup1p and Swi-Snf and their effect on chromatin structure. EMBO J 16: 6263-6271, 1997.
10. Wu L, Winston F. Evidence that Snf-Swi controls chromatin structure over both the TATA and UAS regions of the *SUC2* promoter in *Saccharomyces cerevisiae*. Nucleic Acids Res 25: 4230-4234, 1997.
11. Bu Y, Schmidt MC. Identification of cis-acting elements in the *SUC2* promoter of *Saccharomyces cerevisiae* required for activation of transcription. Nucleic Acids Res 26: 1002-1009, 1998.
12. Vallier LG, Carlson M. New *SNF* genes *GAL11* and *GRR1* affect *SUC2* expression in *Saccharomyces cerevisiae*. Genetics 129: 675-684, 1991.
13. Moreno-Herrero F, Herrero P, Colchero J, et al. Analysis by atomic force microscopy of Med8 binding to cis-acting regulatory elements of the *SUC2* and *HXK2* genes of *Saccharomyces cerevisiae*. FEBS Letters 459: 427-432, 1999.
14. Treitel MA, Kuchin S, Carlson M. Snf1 protein kinase regulates phosphorylation of the Mig1 repressor in *Saccharomyces cerevisiae*. Mol Cell Biol 18: 6273-6280, 1998.
15. De Vit MJ, Johnson M. The nuclear exportin Msn5 is required for nuclear export of the Mig1 glucose repressor of *Saccharomyces cerevisiae*. Current Biology 9: 1231-1241, 1999.
16. Sundarsanam P, Cao Y, Wu L, et al. The nucleosome remodeling complex, Snf/Swi, is required for the maintenance of transcription in vivo and is partially redundant with the histone acetyltransferase, Gcn5. EMBO J 18: 3101-3106, 1999.
17. Türkel S, Turgut T, Uemura H, et al. Mutations in *gcr1* derepress *SUC2* gene expression in *Saccharomyces cerevisiae*. Mol Genet Genomics 268: 825-831, 2003.
18. Baker HV. GCR1 of *Saccharomyces cerevisiae* encodes a DNA binding protein whose binding is abolished by mutations in the CTTCC sequence motif. Proc Natl Acad Sci USA 88: 9443-9447, 1991.
19. Clifton D, Fraenkel DG. The gcr (glycolysis regulation) mutation of *Saccharomyces cerevisiae*. J Biol Chem 256: 13074-13078, 1981.
20. Uemura H, Koshio M, Inoue Y, et al. The role of Gcr1p in the transcriptional activation of glycolytic genes in yeast *Saccharomyces cerevisiae*. Genetics 147: 521-532, 1997.
21. Lopez MC, Smerage JB, Baker HV. Multiple domains of repressor activator protein 1 contribute to facilitated binding of glycolysis regulatory protein 1. Proc Natl Acad Sci USA 95: 14112-14117, 1998.

22. Uemura H, Fraenkel DG. *Gcr2*, a new mutation affecting glycolytic gene expression in *Saccharomyces cerevisiae*. Mol Cell Biol 10: 6389-6396, 1990.

23. Uemura H, Jigami Y. Mutations in *GCR1*, a transcriptional activator of *Saccharomyces cerevisiae* glycolytic genes, function as suppressors of *gcr2* mutations. Genetics 139: 511-521, 1995.

24. Zeng X, Deminoff SJ, Santangelo GM. Specialized Rap1p/Gcr1p transcriptional activation through Gcr1p DNA contacts requires Gcr2p, as does hyperphosphorylation of Gcr1p. Genetics 147: 493-505, 1997.

25. Nishi K, Park CS, Pepper AE, et al. The *GCR1* requirement for yeast glycolytic gene expression is suppressed by dominant mutations in the *SGC1* gene, which encodes a novel basic-helix-loop-helix protein. Mol Cell Biol 15: 2646-2653, 1995.

26. Sato T, Lopez MC, Sugioka S et al. The E-Box DNA binding protein Sgc1p suppresses the *gcr2* mutation, which is involved in transcriptional activation of glycolytic genes in *Saccharomyces cerevisiae*. FEBS Letters 463: 307-311, 1999.

27. Robinson KA, Lopes JM. *Saccharomyces cerevisiae* basic helix-loop-helix proteins regulate diverse biological processes. Nucleic Acids Res 28: 1499-1505, 2000.

28. Lopez MC, Baker HV. Understanding the growth phenotype of the yeast *gcr1* mutant in terms of global genomic expression patterns. J Bacteriol 182: 4970-4978, 2000.

29. Guarente L, Ptashne M. Fusion of E. coli *LacZ* to Cytochrome C gene of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 78: 2199-2203, 1981.

30. Ito H, Fukuda Y, Murata K, et al. Transformation of intact yeast cells treated with alkali cations. J Bacteriol 153: 163-168, 1983.

31. Guthrie C, Fink GR. Guide to yeast genetics and molecular biology. Methods in Enzymol 194: 1-932, 1991.

32. Guarente L. Yeast promoters and lacZ fusions designed to study expression of cloned genes in yeast. Methods in Enzymol 101: 181-191, 1983.

33. Rothe C, Lehle L. Sorting of invertase signal peptide mutants in yeast dependent and independent on the signal recognition particle. Eur J Biochem 252: 16-24, 1998.

34. Pollard KJ, Peterson CL. Role for *ADA/GCN5* products in antagonizing chromatin mediated transcriptional repression. Mol Cell Biol 17: 6212-6222, 1997.

35. Edmondson DG, Smith MM, Roth SY. Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. Genes and Dev 10: 1247-1259, 1996.

36. Watson AD, Edmondson DG, Bone JR, et al. Ssn6-Tup1 interacts with class I histone deacetylases required for repression. Genes and Dev 14: 2737-2744, 2000.

37. Pavlovic B, Horz W. The chromatin structure at the promoter of a glyceraldehyde phosphate dehydrogenase gene from *Saccharomyces cerevisiae* reflects its functional state. Mol Cell Biol 8: 5513-5520, 1988.

38. Stanway CA, Gibbs JM, Kearsey SE, et al. The yeast co-activator GAL11 positively influences transcription of the phosphoglycerate kinase gene, but only when RAP1 is bound to its upstream activation sequence. Mol Gen Genet 243: 207-214, 1994.

39. Malik S, Roeder RG. Transcriptional regulation through mediator-like coactivators in yeast and metazoan cells. Trends in Biochem Sci 25: 277-283, 2000.