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The effects of the Pho85 signaling pathway on invertase biosynthesis and glucose uptake in *Saccharomyces cerevisiae*

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Abstract: Glucose signaling controls a wide range of metabolic events in the yeast *Saccharomyces cerevisiae*. Glucose sensing and signaling processes require a variety of membrane-bound and cytoplasmic sensor proteins. Pho85p is a cyclin-dependent protein kinase that controls different metabolic events upon binding to its cyclin partners in the cytoplasm of yeast cells. In this study, we investigated the roles of Pho85p in the glucose signaling pathways that control invertase biosynthesis and glucose uptake in yeast. The biosynthesis of invertase enzyme from the *SUC2* gene is controlled by glucose repression and derepression mechanisms in *S. cerevisiae*. However, the results of this research indicated that invertase biosynthesis occurs at low levels in a deregulated manner in the Δ*pho85* mutant yeast strain. Furthermore, our results showed that the biosynthesis of invertase is not repressed when the Δ*pho85* mutant is exposed to high levels of glucose. Moreover, we found that the glucose consumption rate of the Δ*pho85* mutant is at least 2-fold lower than that of the wild-type yeast strain. Our results indicated that Pho85p functions are essential for the regulated biosynthesis of the invertase enzyme from the *SUC2* gene and for the high levels of glucose uptake in *S. cerevisiae*.

Key words: Pho85, glucose sensing, glucose uptake, *Saccharomyces cerevisiae*, invertase

Saccharomyces cerevisiae'da Pho85 sinyal iletim yolağının invertaz biyosentezi ve glukoz alımına etkileri

Özet: Glukoz sinyali *Saccharomyces cerevisiae*'da çok çeşitli metabolik olayları kontrol eder. Glukozun algılanması ve sinyal iletimi işlemi için hücre zarı ve sitoplazmasında bulunan çeşitli sensor proteinler gereklidir. Pho85p, sikline bağlı bir protein kinaz olup ilgili siklin proteinlerine bağlanarak maya hücreleri sitoplazmasında farklı metabolic olayları kontrol eder. Bu çalışmada Pho85p'nin glukoz alımı ve invertaz enzimi biyosentezini kontrol eden glukoz sinyal iletimi yolağındaki işlevleri araştırıldı. *S. cerevisiae*'da invertaz enziminin *SUC2* geninden biyosentezi glukoz baskılanması ve baskının kaldırılması mekanizması ile kontrol edilir. Bununla birlikte, bu araştırmada elde edilen sonuçlar Δ*pho85* mutantı maya hücrelerinde invertaz biyosentezinin düşük seviyede ve düzensiz olarak meydana geldiğini göstermektedir. Buna ek olarak, elde ettiğimiz sonuçlar Δ*pho85* mutantına yüksek glukoz uygulandığında invertaz biyosentezinin baskılanmadığını göstermektedir. Ayrıca, Δ*pho85* mutantında glukoz tüketim hızının yaban tip mayaya göre en az 2 kat daha düşük olduğunu belirledik. Sonuçlarımız *S. cerevisiae*'da Pho85 işlevinin *SUC2* geninden invertaz biyosentezinin kontrol edilmesi ve yüksek oranda glukoz alımı için gerekli olduğunu göstermektedir.

Anahtar sözcükler: Pho85, glukoz algılama, glukoz alımı, *Saccharomyces cerevisiae*, invertaz

Introduction

Glucose is a preferred carbon and energy source for the yeast *Saccharomyces cerevisiae* (1). The presence of glucose in the growth medium results in the repression or activation of 30% of the genes in *S. cerevisiae* (2,3). Depending on the extracellular glucose concentrations, either high affinity or low affinity sensors on the yeast cell membranes are activated (4). Intracellular glucose sensors that interact with membrane-bound sensors transmit the glucose signals to the cytoplasmic regulatory proteins (4,5).

Pho85p is a cyclin-dependent kinase (CDK) that interacts with at least 10 different Pho85-dependent cyclins (Pcl) within *S. cerevisiae* cells (6). Pho85-Pcl complexes control various metabolic pathways, such as morphogenesis, and cell-cycle progression (6,7). Glycogen accumulation is one of the metabolic events that Pho85 and its cyclins, Pcl6 and Pcl7, control in the yeast *S. cerevisiae* (8).

Glucose is transported into yeast cells by the high affinity or low affinity hexose transporters (9). There are 20 hexose transporter-related genes (*HXT*) in *S. cerevisiae*. Transcriptions of certain *HXT* genes are also regulated by glucose signaling in yeast cells (9,10). Glucose sensing and signaling systems include multiple factors (4,5). The invertase enzyme (E.C. 3.2.1.26) is encoded by the *SUC2* gene (11). It is required for the utilization of sucrose and raffinose as carbon and energy sources for *S. cerevisiae* (11). Biosynthesis of the invertase is regulated by a glucose repression mechanism at the transcriptional level (1, 12). In the presence of high glucose levels (more than 0.1% w/v) in the growth medium, transcription of *SUC2* is repressed by the repressor proteins Mig1p, Ssn6p, Tup1p complex, and nucleosomes (13, 14). However, in the presence of low glucose (less than 0.1%) or sucrose, transcription of *SUC2* becomes derepressed and is activated by transcription factors (15,16). Invertase is synthesized from *SUC2* mRNA and exported to the periplasmic area as an active enzyme.

Previously, we have shown that the transcription of *SUC2* and certain *HXT* genes are regulated by the transcriptional activator protein Gcr1p (15,17). Transcription of the *HXT4* gene decreased about 35-fold in the *gcr1* mutant (17). A significant decrease in the low affinity glucose uptake system has been reported for the *gcr1* mutant yeast (18). In addition to the defect in glucose uptake, *SUC2* gene expression is also deregulated in *gcr1* mutant yeast cells. It was shown that the expression of the *SUC2* gene becomes insensitive to glucose repression in this mutant (15). Gcr1p specifically binds to several sites on the *SUC2*, *HXT2,* and *HXT4* genes' promoter regions (15,17). Gcr1p is a phosphoprotein and interacts with the Pho85p complex (19,20). Gcr1p requires Pho85p for its activation functions (20). However, the exact physiological role of Pho85p in the Gcr1p dependent metabolic events such as glucose uptake and invertase biosynthesis is not known. In this study, we analyzed the involvement of Pho85p in glucose uptake and invertase biosynthesis in *S. cerevisiae*.

Materials and Methods

Yeast strains and growth medium

The *S. cerevisiae* strains used in this research were BY4741 (MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0) and Y02747 (MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, YPL031c::kanMX4). Yeast strains BY4741 and Y02747 are isogenic, except for the Δ*pho85* mutation (21). They were purchased from the EUROSCARF yeast collection (University of Frankfurt. Germany). The yeast strains were grown in a YPD medium (1% yeast extract, 2% peptone, 2% dextrose) with constant shaking (130 rev/min) at 30 °C in an incubator shaker. Glucose derepressed cells were prepared as described previously (22).

Invertase assays

In order to compare the derepression rates of the invertase biosynthesis in the wild-type and mutant strains, yeast cells were first grown in 50 mL of YPD medium under the glucose repressed conditions until the early logarithmic stage (OD_{600}: 0.7–0.8). Yeast cells were then harvested and washed twice with an equal volume of sterile distilled water. They were resuspended in 50 mL of fresh YP medium that contained 0.05% glucose (w/v) for derepression of invertase synthesis. The yeast cells were further incubated on a shaker (130 rev/min) at 30 °C. The samples were removed from the cultures at specific time intervals as indicated in Figure 1.

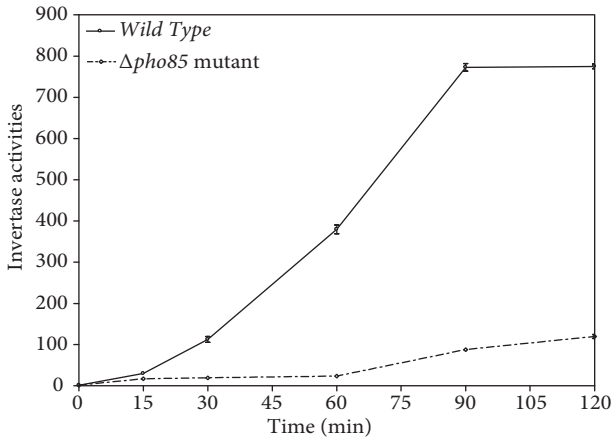

Figure 1. Derepression patterns of the invertase biosynthesis in the wild-type and Δ*pho85* mutant yeast strains.

In order to analyze the effects of Pho85p on the repression of invertase biosynthesis, yeast cells were first pre-cultured in 10 mL of YPGL medium (1% yeast extract, 2% peptone, 2% glycerol, 2% sodium lactate) to the logarithmic stage. Glucose was added to the portion of the yeast cultures at repressing concentrations (2% w/v) and incubated for 2 h. At the end of the incubation period, yeast cells were harvested and the invertase activities of the yeast cultures were determined as described by the glucose oxidase peroxidase (GOD-POD) method (23,24). One unit of the invertase activity is equal to the amount of enzyme that catalyzes the deliberation of 1 μmol glucose per minute per 100 mg dry weight of yeast cells (22).

Analysis of glucose consumption rates

In order to determine the glucose consumption rates of the wild-type (BY4741) and the Δ*pho85* mutant (Y02797), first the yeast cells were cultivated in 25 mL of YPD medium until the mid-logarithmic stage (OD_{600}: 1.0). Yeast cells were harvested and washed twice with an equal volume of sterile distilled water. Then they were resuspended in 25 mL of a YP medium that contained 0.1% glucose. Yeast cultures were incubated on a shaker (130 rev/min) at 30 °C. Samples (1 mL) were removed from the cultures every 20 min. Yeast cells in the samples were pelleted rapidly by centrifugation. The amounts of glucose in the growth medium were determined with a glucose oxidase peroxidase assay, using 5-10 μL of samples as described (24). The glucose concentrations of the growth medium were plotted versus the time points to obtain the glucose consumption patterns of the wild-type and the Δ*pho85* mutant yeast cells.

All experiments were done in triplicate and repeated at least once under the same growth conditions. Hence, the numbers that are given in the tables and figures are the mean values of at least 6 independent experiments.

Results

Effects of Pho85p on the regulation of invertase biosynthesis

In order to determine whether Pho85p is involved in the regulated biosynthesis of invertase, invertase activities were measured in both the wild and the Δ*pho85* mutant yeast strains. When the yeast cultures were grown in a high glucose medium (2% w/v), invertase activities of the wild-type and the Δ*pho85* mutant yeast cells were at their basal levels (3–5 units) (Table 1). The invertase biosynthesis was activated and yielded 1106 units in the wild-type yeast cells once they were transferred to the derepressed growth medium. However, the invertase biosynthesis in the Δ*pho85* mutant was measured as 131 units in the derepressed growth medium (Table 1). Invertase biosynthesis in the Δ*pho85* mutant was approximately 8-fold lower than in the wild-type yeast cells, indicating that the cyclin dependent kinase Pho85p is essential for the high level biosynthesis of invertase enzyme in *S. cerevisiae*.

Moreover, we analyzed the involvement of Pho85p in the repression of invertase biosynthesis in

Table 1. Invertase synthesized at low levels in Δ*pho85* mutant yeast.

	Invertase activities[a] (±SD)	
Yeast Strains	Repressed	Derepressed
Wild Type	5 ± 2	1106 ± 110
Δ*pho85 mutant*	3 ± 2	131 ± 18

[a]Invertase activities are expressed as the amount of enzyme that catalyzes the deliberation of 1 μmol glucose per minute per 100 mg dry weight of yeast cells.
(±SD): Standard Deviations

derepressed yeast cells. Therefore, the wild-type and the Δ*pho85* mutant yeast strains were cultivated in a glycerol lactate medium, in which *SUC2* gene expression and invertase synthesis took place at a moderate level. When glucose was added to the aliquots of these yeast cultures, the invertase activities of the wild-type yeast cells were repressed 3-fold within the 2 h of the incubation period in the presence of high glucose levels in the growth medium. However, we found that the invertase activities remained approximately at the same levels in the presence of high glucose in the Δ*pho85* mutant yeast strain (Table 2). These results suggest that Pho85p activity is essential for the glucose signaling dependent regulation of invertase synthesis in *S. cerevisiae*.

Analysis of the derepression rate of invertase biosynthesis

The biosynthesis of invertase is regulated by glucose signaling. In order to test whether there was any change or fluctuations during the derepression stage, invertase activities in the Δ*pho85* mutant and in the wild-type yeast cells were determined in a time-dependent manner. Invertase biosynthesis was derepressed rapidly in the wild-type yeast strains and reached its maximum levels (775 units) 90 min after derepression (Figure 1). In contrast to the wild-type yeast strain, derepression of invertase biosynthesis in the Δ*pho85* mutant yeast strain occurred very slowly. Its invertase activity was 7-fold lower than that of the wild-type yeast cells at the end of the incubation period. This result clearly shows that although the invertase biosynthesis was derepressed in the Δ*pho85* mutant yeast strain in response to low glucose signals it took place several times more slowly than that of the wild-type *S. cerevisiae* strain (Figure 1).

Effects of Pho85p on the glucose consumption of *S. cerevisiae*

Glucose uptake and consumption is dependent on the presence of an efficient and active glucose signaling pathway in *S. cerevisiae*. After showing that the glucose signaling for the regulated biosynthesis of the invertase enzyme is defective in the Δ*pho85* mutant yeast cells, we decided to analyze whether the Pho85p function is also essential for the normal level of glucose consumption in the yeast cells. Glucose consumption was measured as the time-dependent decrease in the glucose amount in the growth medium of the yeast cells. The wild-type yeast cells consumed all of the glucose in the growth medium within 90 min (Figure 2). The rate of glucose consumption was approximately 0.2–0.3 mM/min/OD_{600} in the wild-type yeast cells under these growth conditions. However, the glucose consumption rate in the Δ*pho85* mutant strain was 2- to 3-fold lower than that of the wild-type yeast. This consumption rate was determined to be 0.1 to 0.15 mM/min/OD_{600} in the Δ*pho85* mutant yeast strain (Figure 2). These results indicated that the cyclin dependent protein kinase Pho85p is required for the normal level of glucose uptake in *S. cerevisiae*.

Table 2. Invertase synthesis is not repressed after long-term derepression in the Δ*pho85* mutant.

Yeast Strains	Invertase activities[a] (±SD)	
	Derepressed (YPGL)	Repressed (YPGL to 2% Glucose)
Wild Type	256 ± 24	94 ± 3
Δ*pho85 mutant*	123 ± 8	124 ± 1

[a]Invertase activities are expressed as the amount of enzyme that catalyzes the deliberation of 1 μmol glucose per minute per 100 mg dry weight of yeast cells.
(±SD): Standard Deviations

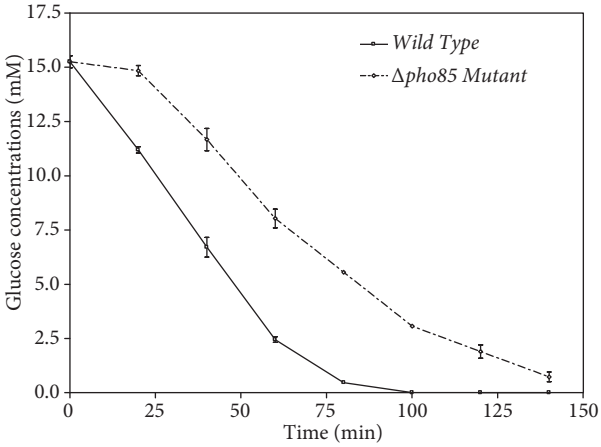

Figure 2. Glucose consumption in the in the wild-type and Δ*pho85* mutant yeast strains.

Discussion and conclusions

Pho85p controls diverse metabolic events in *S. cerevisiae* (25). Our results indicated that the Pho85p functions are also essential for the glucose signaling that results in the regulation of invertase biosynthesis from the *SUC2* gene. Regulation of *SUC2* transcription by glucose signaling requires both repressors and activators (15,16). At high glucose levels, the Mig1p complex, nucleosomes, and non-histone proteins Nhp6A/B repress the transcription of the *SUC2* gene, which results in a very low level of invertase activity in *S. cerevisiae* (13,26). *SUC2* gene expression is not fully repressed in Δ*pho85* mutants at high glucose levels in the short term. It is known that the nucleosome positioning and the repressor protein complex Mig1p-Ssn6p-Tup1p's complex binding is required for the rapid and complete repression of *SUC2* expression (16,27). Our results on the effects of Pho85p on the biosynthesis of invertase indicate that Pho85p targets at least one of the factors that participate in the repression of the *SUC2* gene. One of the possibilities is that Pho85p may act on the chromatin binding and/or modifying complexes for rapid formation of the repressed chromatin structure over the *SUC2* promoter. Asf1p, which interacts with Pho85p, functions as a nucleosome assembly factor in *S. cerevisiae* (28-30). Hence, it is conceivable that the low level activity of Asf1p in the Δ*pho85* mutant might lead to the inefficient or slow formation of a repressed chromatin structure over the *SUC2* promoter.

Lenburg and O'Shea (20) reported that Pho85p is required for the function of Gcr1p and Spt7p in *S. cerevisiae*. Physical interactions between Pho85p and Spt7p were also shown (31). It is known that the transcriptional regulation of *SUC2* requires Gcr1p and SAGA histone acetyl transferase (HAT) complex (15,32). Furthermore, it was shown that the lack of histone acetyltransferase activity decreases the rate of chromatin remodeling on certain promoters, which then leads to the significant drop in the rate of transcriptional activation of targeted genes (33). Consistent with this observation, the lack of Pho85p activity may lead to the slow remodeling of the *SUC2* promoter, resulting in the rather slow derepression of invertase biosynthesis in the Δ*pho85* mutant.

We propose that the Pho85 dependent phosphorylation of Gcr1p in response to low glucose signaling results in the rapid activation of invertase biosynthesis from *SUC2* in the wild-type yeast cells. The same events may also occur in the transcriptional regulation of *HXT* genes. The expression of certain *HXT* genes also depends on the Gcr1p and SAGA complex (17,34). Lack of Pho85p functions in Δ*pho85* mutant yeast might result in a less active Gcr1p complex that in turn leads to a low level of expression of *HXT* genes, which might result in the low level of glucose consumption.

Our results indicate that the Pho85p functions are essential for the intracellular glucose signaling that results in the regulated biosynthesis of invertase and the high level of glucose uptake in *S. cerevisiae*. Therefore, it appears that the slow growth phenotype results from the inefficient glucose uptake and inefficient glucose signaling in the Δ*pho85* mutant yeast strain.

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References

1. Carlson M. Regulation of glucose utilization in yeast. Curr Opin Genet Dev 8: 560-564, 1998.

2. Rolland F, Winderickx J, Thevelein JM. Glucose-sensing and -signaling mechanisms in yeast. FEMS Yeast Res 2: 183-201, 2002.

3. Schneper L, Düvel K, Broach JR. Sense and sensibility: nutritional response and signal integration in yeast. Curr Opin Microbiol 7: 624-630, 2004.
4. Gancedo JM. The early steps of glucose signaling in yeast. FEMS Microbiol Rev 32: 673-704, 2008.
5. Santangelo GM. Glucose signaling in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 70: 253-282, 2006.
6. Carroll AS, O'Shea EK. *pho85* and signaling environmental conditions. Trends Biochem Sci 27: 87-93, 2002.
7. Measday V, Moore L, Retnakaran R et al. A family of cyclin-like proteins that interact with the *Pho85* cyclin-dependent kinase. Mol Cell Biol 17: 1212-1223, 1997.
8. Wang Z, Wilson WA, Fujino MA et al. The yeast cyclins Pcl6p and Pcl7p are involved in the control of glycogen storage by the cyclin-dependent protein kinase *pho85*p. FEBS Lett 506: 277-280, 2001.
9. Boles E, Hollenberg CP. The molecular genetics of hexose transport in yeasts. FEMS Microbiol Rev 21: 85-111, 1997.
10. Klockow C, Stahl F, Scheper T et al. In vivo regulation of glucose transporter genes at glucose concentrations between 0 and 500 mg/L in a wild type of *Saccharomyces cerevisiae*. J Biotechnol 135: 161-167, 2008.
11. 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.
12. Gancedo JM. Yeast carbon catabolite repression. Microbiol Mol Biol Rev 62: 334-361, 1998.
13. DeVit MJ, Waddle JA, Johnston M. Regulated nuclear translocation of the Mig1 glucose repressor. Mol Biol Cell 8: 1603-1618, 1997.
14. 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.
15. Türkel S, Turgut T, Lopez MC et al. Mutations in gcr1 affect *SUC2* gene expression in Sacchaomyces cerevisiae. Mol Genet Genomics 268, 825-831, 2003.
16. 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.
17. Türkel S, Bisson LF. Transcription of the HXT4 gene is regulated by Gcr1p and Gcr2p in the yeast *S. cerevisiae*. Yeast 15: 1045-1057, 1999.
18. Bisson LF, Coons DM, Kruckeberg AL, Lewis DA. Yeast sugar transporters. Crit Rev Biochem Mol Biol 28: 259-308, 1993.
19. 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.
20. Lenburg ME, O'Shea EK. Genetic evidence for a morphogenetic function of the *Saccharomyces cerevisiae* Pho85 cyclin-dependent kinase. Genetics 157: 39-51, 2001.
21. Brachmann CB, Davies A, Cost GJ et al. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14: 115-132, 1998.
22. Celenza JL, Carlson M. Cloning and genetic mapping of *SNF1*, a gene required for expression of glucose repressible genes in *Saccharomyces cerevisiae*. Mol Cell Biol 4: 49-53, 1984.
23. 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.
24. Goldstein A, Lampen JO. β-D-Fructofuranoside Fructohydrolase from yeast. Methods in Enzymol., 42c: 504-511, 1975.
25. Huang D, Friesen H, Andrews B. *Pho85*, a multifunctional cyclin-dependent protein kinase in budding yeast. Mol Microbiol 66: 303-314, 2007.
26. Türkel S. Non-histone proteins Nhp6A and Nhp6B are required for the regulated expression of *SUC2* gene of *Saccharomyces cerevisiae*. J Biosci Bioeng 98: 9-13, 2004.
27. Trumbly RJ. Glucose repression in the yeast *Saccharomyces cerevisiae*. Mol Microbiol 6: 15-21, 1992.
28. Pan X, Ye P, Yuan DS et al. A DNA integrity network in the yeast *Saccharomyces cerevisiae*. Cell 124: 1069-81, 2006.
29. English CM, Maluf NK, Tripet B. ASF1 binds to a heterodimer of histones H3 and H4: a two-step mechanism for the assembly of the H3-H4 heterotetramer on DNA. Biochemistry 44: 13673-13682, 2005.
30. Daganzo SM, Erzberger JP, Lam WM et al. Structure and function of the conserved core of histone deposition protein Asf1. Curr Biol 13: 2148-2158, 2003.
31. Huang D, Moffat J, Andrews B. Dissection of a complex phenotype by functional genomics reveals roles for the yeast cyclin-dependent protein kinase *pho85* in stress adaptation and cell integrity. Mol Cell Biol 22: 5076-5088, 2002.
32. Yu YX, Eriksson P, Stillman DJ. Architectural transcription factors and the SAGA complex function in parallel pathways to activate transcription. Mol Cell Biol 20: 2350-2357, 2000.
33. Barbaric S, Walker J, Schmid A et al. Increasing the rate of chromatin remodeling and gene activation - a novel role for the histone acetyltransferase Gcn5. EMBO J 20: 4944-4951, 2001.
34. Van Oevelen CJC, Van Teeffelen HAAM, Timmers HTM. Differential requirement of SAGA subunits for Mot1p and Taf1p recruitment in gene activation. Mol Cell Biol 25: 4863-4872, 2005.