Journal of Applied Biological Sciences 9 (1): 01-05, 2015
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Glucose Signaling Pathway Regulates the Transcription of Pseudovirus Ty1 in the Cell Type Dependent Manner in *Saccharomyces cerevisiae*

Sezai TÜRKEL*[1] Elif ARIK[2]
[1]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Uludag University, Bursa, Turkey
[2]Department of Biology, Faculty of Arts and Sciences, Uludag University, Bursa, Turkey

*Corresponding author
E-mail: sturkel@uludag.edu.tr

Received: November 01, 2014
Accepted: December 12, 2014

Abstract

Transcription of the yeast retrotransposon Ty1 is regulated by a large set of transcription factors and chromatin modifying complexes in a cell type dependent manner. In this study, we have shown that the transcription of Ty1 is activated by low glucose signaling in diploid *S. cerevisiae* cells. Growth of diploid yeast cells in low glucose medium lead to at least 3-fold activation of Ty1 transcription. Our results indicated that this activation largely depends on the transcription factor Tup1p. In addition to Tup1p, Snf2p, one of the subunits of chromatin modifying complex Snf/Swi, is also essential for the regulation of Ty1 transcription. Moreover, our results indicated that the transcription of Ty1 in haploid *S. cerevisiae* cells were not affected by glucose signaling and remained at same levels in high glucose or in low glucose medium. In *tup1* mutant diploid yeast cells, transcription of Ty1 remained at the basal level and it is not regulated by glucose signaling. These results indicated that transcription of Ty1 is regulated differentially by glucose signaling in a cell type, Tup1p and Snf2p dependent manner.

Keywords: Ty elements, Glucose signaling, Transcription, Yeast, Chromatin factors

INTRODUCTION

Ty1 is one of the retroviral-like elements of the yeast *Saccharomyces cerevisisae* [1]. It is present as high copy element that can be found up to 30 copies per haploid yeast genome [2]. Ty1 propagates via an RNA intermediate using a similar strategy as vertebrate retroviruses [3]. Ty elements are re-classified based on their genomic structures. In the new classification system, Ty1, Ty2, Ty4 and Ty5 are named as "Pseudoviruses" and classified within the Pseudoviridae family of the Retrovirales order [4].

Pseudovirus Ty1 is 5.9 Kbp long and contains 330 bp terminal repeats on its 5′ and 3′ ends known as delta elements [5, 6]. Ty1 genome encodes 5.7 Kb long single mRNA molecules with poly-A tail [5, 6]. Transcriptional regulatory regions of Ty1 locate within 5' LTR and also within the coding region encompassing the first 1500 bp region of this pseudovirus [7-9]. Ty1 genome does not encode any known transcriptional regulatory factors for their gene expression. Therefore, transcription of pseudovirus Ty1 is totally dependent on the yeast encoded transcription factors. Some of these transcription factors are Gcn4p, Tec1p, Ste12p, Sgc1p and Mot3p [8, 10-13]. In addition to transcriptional activators, chromatin modifying complexes such as SAGA (Spt-Ada-Gcn5-Acetylase) and Snf/Swi are also involved in the regulation of Ty1 transcription [10-12]. In addition, transcription of Ty1 is also regulated by a cell type dependent manner. In diploid *S. cerevisiae* cells, transcription of Ty1 is regulated by a1-α2 heterodimers [7, 13].

Glucose signaling affects a large set of genes in *S. cerevisiae* [14]. It is known that approximately 30% of yeast genes either activated or repressed by glucose [15, 16]. Previously, it was shown that the transcription of Ty2 is activated by high glucose [17, 18]. In the present study, we have analyzed the effects of glucose signaling on the transcriptional regulation of Ty1 in haploid and diploid yeasts. Our results indicated that the transcription of Ty1 is differently regulated in haploid and diploid yeasts in response to glucose signaling by Tup1p and Snf2 dependent manner.

MATERIALS AND METHODS

Yeast strains and plasmids

The *S. cerevisiae* strains used in this research are based on BY4741 (*MATa, his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) genetic back ground. The yeast strain used in this study was listed in Table 1. Yeast strains was purchased from the EUROSCARF (University of Frankfurt, Germany) and used in our research as a standard *S. cerevisae* strain [19].

The plasmid pTY1A-lacZ is a 2 μm-*URA3* based shuttle vector and contains a fusion of the first 1571 bp region of Ty1 element to the *E. coli* lacZ gene [8, 20]. It is used as reporter gene fusion to determine the transcriptional activity of Ty1 in different growth conditions as described [8]. Ty1A-lacZ gene fusion plasmid was transformed in to the competent yeast cells as described previously using lithium acetate method [21]. Yeast transformants were plated on synthetic complete dextrose medium without Uracil (Sc–Ura, + 2% glucose). To use in liquid culture inoculations for β-Galactosidase assay, 9-12 transformant colonies were randomly selected for pTy1A-lacZ plasmid for each yeast strains and patched on Sc – uracil dextrose plates. Previously, it was shown that these types of 2 μm-*URA3* based Ty-lacZ expression vectors are stably maintained in yeast transformants under selective growth conditions [22, 23]. Moreover, it is also known that the β-Galactosidase activities expressed from Ty-lacZ gene fusions are correlated to native Ty mRNA levels [8, 20, 22, 23].

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

Euroscarf Access No	Genotype	Relevant mutations
Y00000	MATa,his3Δ1; leu2Δ0; met15Δ0; ura3Δ0.	Haploid, wild type
Y20000	MATa/α,his3Δ1/his3Δ1; leu2Δ0/leu2Δ0; lys2Δ0/LYS2; MET15/met15Δ0; ura3Δ0/ura3Δ0.	Diploid, wild type
Y07198	MATa,his3Δ1; leu2Δ0; met15Δ0; ura3Δ0, YCR084c::kanMX4	Δ*tup1*
Y37198	MATa/α,his3Δ1/his3Δ1; leu2Δ0/leu2Δ0; lys2Δ0/LYS2; MET15/met15Δ0; ura3Δ0/ura3Δ0, YCR084c::kanMX4/ YCR084c::kanMX4	Δ*tup1*/Δ*tup1*
Y01586	MATa,his3Δ1; leu2Δ0; met15Δ0; ura3Δ0, YOR290c::kanMX4	Δ*snf2*
Y31586	MATa/α,his3Δ1/his3Δ1; leu2Δ0/leu2Δ0; lys2Δ0/LYS2; MET15/met15Δ0; ura3Δ0/ura3Δ0, YOR290c::kanMX4/ YOR290c::kanMX4	Δ*snf2*/Δ*snf2*

Growth Conditions

S. cerevisiae cells were grown in YPD (1% Yeast Extract, 2% Bacto Peptone, 2% Glucose) medium for transformation [24]. pTy1A-lacZ expression vector was transformed into wild type and mutant strains that lack relevant factors to test the effects of various regulatory factors that involve in the transcriptional regulation of Ty1. Since the gene expression in Ty1 is regulated in a cell type dependent manner, both haploid (BY4741) and diploid strains (BY4743) were used in this study [19]. Mutations in the yeast strains relevant to this study were indicated in Table 1 and also in table legends.

In order to test the effects of glucose signaling on the gene expression in Ty1, yeast transformants were pre-cultured in 10 mL of Sc-Ura medium supplemented with 2% glucose up to logarithmic stage at 30 C, in an incubator shaker. Then, 5 mL of yeast culture was harvested and washed twice with 5 mL of sterile, ice-cold distilled water. After that, these yeast samples were resuspended in 5 ml Sc-Ura medium supplemented with 0.05% glucose or with 2% glycerol and 2% lactate, and further incubated for 4 hours in incubator shaker.

Enzyme assays

Yeast cells were harvested at the end of incubation periods and washed once with 1 mL of sterile distilled water and then resusupended in 200 μL of yeast cell breaking buffer [25]. Yeast stocks were permeabilized with 20 μL of 0.1% SDS and 20 μL of chloroform to obtain lysates. β-Galactosidase assays were done in triplicates and repeated at least once under same experimental conditions. β-Galactosidase units are given in nanomoles of ONPG cleaved per minute per mg of protein in permeabilized yeast cells. Protein concentrations in the permeabilized yeast cell lysates were determined by the Lowry assay as described [26].

RESULT AND DISCUSSION

Effects of Glucose signaling on the Transcription of Ty1

Transcription of pseudovirus Ty1 is regulated by different metabolic signals. Previously, it was shown that the transcription of Ty1 is regulated by adenine starvation [20]. We have previously reported that transcription of Ty2, closely related to Ty1, is regulated by glucose signaling [17, 18]. Recently, we have also reported that the ribosomal frameshift efficiency, which involves for the translation of Gag-Pol fusion protein of Ty1 and Ty3, is also regulated by glucose signaling [17, 27]. Hence we wanted to see if glucose signaling also affects the transcription of Ty1 elements. Ty1 and Ty2 have a similar genetic organization. Transcription of Ty2 does not depend on the cell types in *S. cerevisiae*. Unlike Ty2, transcription of Ty1 also depends on the cell type [7, 18]. It was shown that the transcription of Ty1 is regulated by diploid specific transcription factor a1-α2 heterodimer. Hence the effect of glucose signaling on Ty1 transcription was investigated both in haploid and also in diploid yeast strains.

Growth of haploid yeast cells in high glucose (2%) medium resulted in approximately 3000 units of transcriptional activity in Ty1 gene fusion. Shifting of yeast cells from high glucose to low glucose medium or to the growth medium that contains glycerol and lactate as sole carbon sources did not changed the transcription level in Ty1 at significant level, and also resulted in approximately 3000 units of transcriptional activity (Table 2). From these results, it is clear that glucose signaling does not affect the transcriptional activity of Ty1 in haploid yeast strains. To see the effects of glucose signaling on Ty1, its

transcriptional activity was also determined in diploid yeast cells. Surprisingly, we have shown that the transcription of Ty1 is activated more then 3-fold (from 272 units to 998 units) in diploid yeast cells in low glucose medium (Table 2). It is known that the transcriptional activity of Ty1 is a lot lower in the diploid yeast cells than in the haploids. As expected, Ty1 transcription was measured approximately 300 units in diploid yeast strains. However, transcription of Ty1 activated 2-3 folds in the yeast cells grown in low glucose or in non-fermentable carbon sources (Table 2).

It is also known that Ty1 transcription is regulated by a large number of transcription factors and chromatin modifying complexes. These transcription factors are Gcn4p, Sgc1p (Tye7), Tec1p, Mot3p, Ste12p and the chromatin modifying complexes Snf/Swi and SAGA complexes [8, 10-12, 28-30]. To see if any one of these factors involved in the glucose mediated regulation of Ty1 transcription we have analyzed the expression level of Ty1A-lacZ gene fusion in mutant strains that lack one of these factors. We did not see any significant effects from the lack of Gcn4p, Tec1p, Sgc1p, Ste12p, Mot3p and Spt11p in glucose signaling dependent regulation of Ty1 transcription (data not shown) either in haploid or in diploid yeast strains that have deletions for the above mentioned genes.

Table 2. The Effects of glucose signaling on the transcription the retroviral-like element Ty1 in haploid and diploid wild type *S. cerevisiae* cells.

Yeast Cell Types	β-Galactosidase activities ±SD*		
	Repressed	Derepressed	2% Glycerol 2% Lactate
Haploid wild type	2877±102	3155±214	3307±306
Diploid wild type	272±14	405±5	998±42

∗β-Galactosidase activities are expressed as nmol ONPG/min/mg of protein
SD: Standard deviations

Tup1p involved in the glucose signaling dependent regulation of Ty1 transcription

It is known that the transcription of Ty1 is regulated by diploid specific regulatory factors a1-α2 heterodimers in diploid yeast cells [7, 13]. a1-α2 interacts with Tup1p [31, 32]. It is known that Tup1p also interacts with MCM1 and Rap1p [17, 26]. Hence, we wanted to test the effects of Tup1p on the glucose dependent regulation of Ty1 transcription in haploid and diploid yeasts. Ty1A-lacZ gene fusion was transformed into haploid and diploid isogenic *tup1* mutant yeast strains. As shown in Table 2, transcription of Ty1 gene fusion yielded 3000 units of β-Galactosidase activity in standard growth conditions. However, while transcription of Ty1 is activated by low glucose signaling in the diploid wild type, its remained essentially at the same levels (240 units) in low glucose medium in diploid *tup1* mutant yeast (Table 3). A similar pattern of regulation was also obtained from the *tup1* mutant haploid yeast strain. Even thought glucose signaling does not affect Ty1 transcription in haploid yeast strains, Ty1 transcription decreased approximately 3-fold (from 3000 units to 900 units) and remained at the same levels both in high or low glucose medium in haploid *tup1* mutants (Table 3). It appears that Tup1p is essential component for the basal level transcription in haploid yeast cells. From these results, it is clear that the Tup1p is the major regulator of Ty1 for the glucose signaling dependent transcriptional regulation in diploid yeast cells.

It was previously reported that Tup1p can function as the transcriptional activator for different promoters in yeast [31-33]. It appears that Tup1p is the activator of Ty1 when diploid yeast cells are grown in low glucose medium. The effect of Tup1p is more drastic in diploids then haploid yeasts. It might be possible that, in haploid yeasts, transcription of Ty1 largely depends on the Gcn4p, Mot3p, Sgc1p, Rap1p, Tec1p and other transcription factors. Transcription of Ty1 is 8-10 folds higher in haploids then in diploids. On the other hand, when we analyzed the transcriptional status of Ty1 in diploids, it becomes Tup1p dependent by the effects of a1-α2-Tup1p complex. Hence the lack of Tup1p in diploids yields more pronounced decrease in Ty1 transcription. Tup1p is also involved in the overall organization of yeast promoters and stabilization of transcription factors on the promoter regions [32, 33]. It seems that Tup1p also has a structural role in the Ty1 promoter organization, since Ty1 transcription significantly decreases both in haploid and diploid yeasts and becomes constitutive with respect to glucose signaling.

Table 3. Tup1p is involved in the cell type dependent regulation of Ty1 transcription in response to low glucose signaling.

Yeast Cell Types	β-Galactosidase activities ±SD*		
	Repressed	Derepressed	2% Glycerol 2% Lactate
Haploid Δ*tup1* mutant	952±36	873±42	874±27
Diploid Δ*tup1*/Δ*tup1* mutant	292±18	248±10	221±18

∗β-Galactosidase activities are expressed as nmol ONPG/min/mg of protein
SD: Standard deviations

The effects of Snf2p on the glucose signaling dependent regulation of Ty1 transcription

It is known that the chromatin modifying complexes SAGA and Snf/Swi regulate the transcription of Ty1 [10, 28, 29]. Hence, we wanted to see if these complexes are involved in the glucose dependent activation of Ty1 transcription. Both the SAGA and Snf/Swi of these complexes are composed of different subunits [29, 30]. We have tested the effects of SAGA complex on Ty1 by using the *Spt11* deletion strain. Ty1A-lacZ gene fusion was transformed in to Δ*spt11* mutant strain. We did not see any effects of Spt11p on the low glucose signaling dependent activation of Ty1 transcription (data not shown).

We have tested the effects of Snf/Swi complex on Ty1 transcription using *snf2* mutant yeast strain. Snf2p is the catalytic subunit of the Snf/Swi remodeling complex [28-30]. Previous reports also indicated that Snf2p involves in Ty1 regulation [28]. Ty1A-lacZ gene fusion was transformed into haploid and diploid Δ*snf2* mutant yeast strains. As shown in Table 4, transcription from Ty1 gene fusion yielded 1475 units of β-Galactosidase activity in the haploid Δ*snf2* mutant yeast. This was approximately 2-fold lower than the wild type yeast strain, indicating that Snf2p is essential for the maximal level transcription of Ty1. Growth of Δ*snf2* in low glucose medium resulted in a slight increase in Ty1 transcription levels (Table 4).

However, transcription of Ty1 gene fusion in diploid Δ*snf2*/Δ*snf2* mutant strain resulted in constitutive, low level transcription of Ty1 regardless of glucose concentrations. Transcription of Ty1 decreased more than 2-fold (from 272 units to 122 units) in this mutant strain when the yeast cells were grown in high glucose medium (Table 4). Growth of this mutant in low glucose also resulted in approximately 3-fold lower transcriptional activity (from 405 units to 142 units) in Ty1. These results indicated that chromatin modifying complex Snf/Swi, but not the SAGA complex, is involved in the low glucose signaling dependent activation of Ty1 transcription.

Our results clearly indicated that the transcription of the pseudovirus Ty1 is activated by low glucose signaling in a cell type dependent manner. It is also clear that the transcription factors Tup1p and Snf2p are essential for basal level transcription of Ty1 in both haploid and also in diploid yeast cells. Both Tup1p and Snf2p involves in the regulation of Ty1 transcription in response to low glucose signaling in diploid yeast cells.

Table 4. Snf2p is required for the basal level expression and also for the glucose dependent regulation of Ty1 transcription.

Yeast Cell Types	β-Galactosidase activities ±SD*	
	Repressed	**Derepressed**
Haploid Δ*snf2* mutant	1475±25	1812±74
Diploid Δ*snf2*/Δ*snf2*mutant	122±5	142±1

∗β-Galactosidase activities are expressed as nmol ONPG/min/mg of protein
SD: Standard deviations

Acknowledgements

We thank to Dr. Pascale Lesage (Universite Paris Diderot, Paris France) for kindly providing Ty1A-LacZ plasmid. This study is a part of PhD thesis of Elif Arik submitted to the Graduate School of Natural and Applied Sciences of Uludag University.

REFERENCES

[1] Cameron, J.R., Loh, E.Y., Davis, R.W. 1979. Evidence for transposition of dispersed repetitive DNA families in yeast. *Cell*, 16: 739-751.

[2] Kim, J.M., Vanguri, S., Boeke, J.D., Gabriel, A., Voytas, D.F. 1998. Transposable elements and genome organization: a comprehensive survey of retrotransposons revealed by the complete *Saccharomyces cerevisiae* genome sequence. *Genome Res*., 8: 464-478.

[3] Boeke, J.D., Garfinkel, D.J., Styles, C.A., Fink, G.R. 1985. Ty elements transpose through an RNA intermediate. *Cell*, 40: 491-500.

[4] Capy, P. 2005. Classification and nomenclature of retrotransposable elements. *Cytogenet. Genome Res.*, 110: 457-461.

[5] Elder, R.T., St John, T.P., Stinchcomb, D.T. Davis, R.W., 1981. Studies on the transposable element Ty1 of Yeast. I. RNA homologous to Ty1. *Cold Spring Harbor Symp. Quant. Biol.*, 45: 581-584.

[6] Elder, R.T., Loh, E.Y., Davis, R.W. 1983. RNA from the yeast transposable element Ty1 has both ends in th direct repeats, a structure similar to retrovirus RNA. *Proc. Natl. Acad. Sci. USA*, 80: 2432-2436.

[7] Errede, B. 1993. MCM1 binds to a transcriptional control element in Ty1. *Mol. Cell. Biol*., 13: 57-62.

[8] Morillon, A., Benard, L., Springer, M., Lesage, P. 2002. Differential effects of chromatin and Gcn4 on the 50-fold range of expression among individual yeast Ty1 retrotransposons. *Mol. Cell. Biol*., 22: 2078-2088.

[9] Roeder, S.G., Rose, A.B., Perlman, R.E. 1985. Transposable element sequences involved in the enhancement of yeast gene expression. *Proc. Natl. Acad. Sci. USA*, 82: 5428-5432.

[10] Dudley, A.M., Gansheroff, L.J., Winston, F. 1999. Specific components of the SAGA complex are required for Gcn4- and Gcr1-mediated activation of the his4-912δ promoter in *Saccharomyces cerevisiae*. *Genetics*, 151: 1365-1378.

[11] Gray W.M., Fassler, J.S. 1993. Role of *Saccharomyces cerevisiae* Rap1 protein in Ty1 and Ty-mediated transcription. *Gene Expression*, 3: 237-251.

[12] Laloux, I., Jacobs, E., Dubois, E. 1994. Involvement of SRE element of Ty1 transposon in TEC1-dependent transcriptional activation. *Nucleic Acids Res*., 22: 999-1005.

[13] Madison, J.M., Dudley, A.M., Winston, F. 1998. Identification and analysis of Mot3, a zinc finger protein that binds to the retrotransposon Ty long terminal repeat (δ) in *Saccharomyces cerevisiae*, *Mol. Cell. Biol*., 18: 1879-1890.

[14] Belinchon, M.M., J.M. Gancedo, J.M. 2007. Glucose controls multiple processes in *Saccharomyces cerevisiae* through diverse combinations of signaling pathways. *FEMS Yeast Res.*, 7: 808-818.

[15] DeRisi, J.L., Lyer, V.R., Brown, P.O. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science*, 278: 680–686.

[16] Schneper, L., Duvel, K., Broach, J.R. 2004. Sense and sensibility: nutritional response and signal integration in yeast. *Curr. Opin. Microbiol*., 7: 624-630.

[17] Türkel, S., Bayram, Ö., Arik, E. 2009. Glucose signaling pathway and growth conditions regulate gene expresion in retrotransposon Ty2. *Z Naturforsch*., 64c: 526–532.

[18] Türkel, S., Arik, E. 2007. Glucose signaling controls the transcription of retrotransposon Ty2-917 in *Saccharomyces cerevisiae*. *Virus Genes*, 35: 713-717.

[19] Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., Boeke, J.D. 1998. 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.

[20] Todeschini, A.-L., Morillon, A., Springer, M., Lesage, P. 2005. Severe Adenine Starvation Activates Ty1 Transcription and Retrotransposition in *Saccharomyces cerevisiae*. *Mol. Cell. Biol*., 25: 7459–7472.

[21] Gietz, R.D., Schiestl, R.H. 1995. Transforming yeast with DNA. *Methods Mol. Cell. Biol., 5:* 255-269.

[22] Farabaugh, P.J., Vimaladithan, A., Türkel, S., Johnson, R., Zhao, H. 1993. Three downstream sites repress transcription of a Ty2 retrotransposon in *Saccharomyces cerevisiae*, *Mol. Cell. Biol.,* 13: 2081-2090.

[23] Liao, X.-B., Clare, J.J., Farabaugh, P.J. 1987. The upstream activation site of a Ty2 element of yeast is necessary but not sufficient to promote maximal

transcription of the element. *Proc. Natl. Acad. Sci. USA*, 84: 8520-8524.

[24] Rose, M.D., Winston, F., Hieter, P. 1990. *Methods in Yeast Genetics. A Laboratory Course Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA.

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

[26] Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randal, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

[27] Türkel, S., Kaplan, G., Farabaugh, P.J. 2011. Glucose signaling pathway controls the programmed ribosomal frameshift efficiency in retroviral-like element Ty3 in *Saccharomyces cerevisiae*. *Yeast*, 28: 799-808.

[28] Happel, A.M., Swanson, M.S., Winston, F. 1991. The *SNF2*, *SNF5*, and *SNF6* genes are required for Ty transcription in *Saccharomyces cerevisiae*. *Genetics*, 128: 69-77.

[29] Peterson, C.L., Tamkun, J.W. 1995. The SWI/SNF complex: a chromatin remodeling machine? *Trends Biochem. Sci*., 20: 143-146.

[30] Tang, L., Nogales, E., Ciferri, C. 2010. Structure and function of SWI/SNF chromatin remodeling complexes and mechanistic implications for transcription. *Prog. Biophysics Mol. Biol*., 102: 122-128.

[31] Conlan, R.S., Gounalaki, N., Hatzis, P., Tzamarias, D. 1999. The Tup1-Cyc8 Protein Complex Can Shift from a Transcriptional Co-repressor to a Transcriptional Co-activator. *J. Biol. Chem*., 274: 205-210.

[32] Hanlon, S.E., Rizzo, J.M., Tatomer, D.C., Lieb, J.D., Buck, M.J. 2011. The Stress Response Factors Yap6, Cin5, Phd1, and Skn7 Direct Targeting of the Conserved Co-Repressor Tup1-Ssn6 in *S. cerevisiae*. *PLoS ONE*, 6(4): e19060.

[33] Rizzo, J.M., Mieczkowski, P.A., Buck, M.J. 2011. Tup1 stabilizes promoter nucleosome positioning and occupancy at transcriptionally plastic genes. *Nucleic Acids Res*., 39: 8803–8819.