Histone H3 Lysine 4 Hypermethylation Prevents Aberrant Nucleosome Remodeling at the *PHO5* Promoter[▽]

Shan-Shan Wang, Bo O. Zhou,* and Jin-Qiu Zhou*

State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Shanghai 200031, China

Received 5 January 2011/Returned for modification 3 February 2011/Accepted 25 May 2011

Recent studies have highlighted the histone H3K4 methylation (H3K4me)-dependent transcriptional repression in *Saccharomyces cerevisiae*; however, the underlying mechanism remains inexplicit. Here, we report that H3K4me inhibits the basal *PHO5* transcription under high-phosphate conditions by suppressing nucleosome disassembly at the promoter. We found that derepression of the *PHO5* promoter by *SET1* deletion resulted in a labile chromatin structure, allowing more binding of RNA polymerase II (Pol II) but not the transactivators Pho2 and Pho4. We further showed that Pho23 and Cti6, two plant homeodomain (PHD)-containing proteins, cooperatively anchored the large Rpd3 (Rpd3L) complex to the H3K4-methylated *PHO5* promoter. The deacetylation activity of Rpd3 on histone H3 was required for the function of Set1 at the *PHO5* promoter. Taken together, our data suggest that Set1-mediated H3K4me suppresses nucleosome remodeling at the *PHO5* promoter so as to reduce basal transcription of *PHO5* under repressive conditions. We propose that the restriction of aberrant nucleosome remodeling contributes to strict control of gene transcription by the transactivators.

Chromatin, the physiological template of all eukaryotic genetic information, is made up of repeating nucleosomes, each of which consists of 147-bp DNA wrapping around a histone octamer, including two copies each of H2A, H2B, H3, and H4 (30). In the process of gene transcription, chromatin structure can be modulated at several levels, such as ATP-dependent chromatin remodeling (11), histone modifications (36), and nucleosome disassembly and reassembly (42). Over the past decade, cross talks between different chromatin modifications have been reported, although the blueprint for this network is still elusive (61).

Cumulative studies have demonstrated that nucleosome disassembly at gene promoters is a general characteristic of gene activation in eukaryotes (9, 35, 51, 53). The *Saccharomyces cerevisiae* *PHO5* gene is one of the best understood examples for nucleosome dynamics (1, 4, 5). The *PHO5* gene encodes an acid phosphatase that mediates periplasmic phosphate hydrolysis. The expression of *PHO5* is stringently controlled by intracellular P_i concentration. Under high-phosphate (P_i^+) conditions, the sequence-specific activator Pho4 is retained in the cytoplasm, preventing activation of *PHO5* (45). Under phosphate depletion (P_i^-) conditions, Pho4 becomes localized to the nucleus, where it interacts with another activator, Pho2, and together they bind the *PHO5* promoter (6, 45). Correspondingly, under P_i^+ conditions, four positioned nucleosomes, including UASp2 and the TATA box, reside over the

PHO5 promoter containing the Pho4 binding site (62). After the switch to P_i^- conditions, the four positioned nucleosomes are gradually disassembled from the *PHO5* promoter (1, 9, 51). Disassembly of the *PHO5* promoter nucleosomes is dispensable for the partial recruitment of activators but is indispensable for the recruitment of general transcriptional machinery and coactivators (1, 3, 18, 66).

Rpd3 is one of the major histone deacetylases in yeast and regulates the expression of a large number of genes (70). Two known Rpd3 complexes share a core of three subunits, i.e., Rpd3, Sin3, and Ume1 (14, 26, 27, 34, 40, 49, 71). The small Rpd3 (Rpd3S) complex is targeted primarily to the transcribed region and has been found to suppress spurious intragenic transcription during elongation and is implicated in controlling promoter fidelity (24, 27, 47), while the large Rpd3 (Rpd3L) complex occupies mainly gene promoters and functions to regulate transcription initiation (54, 55). The chromatin association of the Rpd3S complex requires Set2-dependent H3K36 methylation (H3K36me) simultaneously recognized by two of its subunits, the Chromo domain-containing protein Eaf3 and the plant homeodomain (PHD)-containing protein Rco1 (37). Interestingly, there are two PHD-containing subunits in the Rpd3L complex, Pho23 and Cti6, both of which specifically recognize methylated H3K4 peptide *in vitro* (55, 56). However, it is still unclear whether or how Pho23 and Cti6 direct the Rpd3L complex to chromatin *in vivo*.

Histone H3K4 trimethylation (H3K4me3), catalyzed by Set1, is associated predominantly with actively transcribed genes (32, 43). Nevertheless, a number of studies have highlighted the repressive role of histone H3K4 methylation in gene transcription, such as for *PHO5* and *GAL1* (15, 47), although the mechanism remains elusive. In this study, using the yeast *PHO5* gene as a model, we elucidated the molecular mechanism by which Set1-mediated H3K4me represses gene

* Corresponding author. Mailing address: State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Shanghai 200031, China. Phone: 86-21-54921076. Fax: 86-21-54921075. E-mail for Bo O. Zhou: zhoubo@sibs.ac.cn. E-mail for Jin-Qiu Zhou: jqzhou@sibs.ac.cn.

[▽] Published ahead of print on 6 June 2011.

TABLE 1. Yeast strains constructed in this work

Strain	Genotype
JQW118.....BY4742 <i>pho2Δ::KanMX set1Δ::URA3</i>	
JQW119.....BY4742 <i>pho4Δ::KanMX set1Δ::URA3</i>	
JQW120.....BY4742 <i>pho80Δ::KanMX set1Δ::URA3</i>	
JQW121.....BY4742 <i>rdp3Δ::KanMX set1Δ::URA3</i>	
JQW122.....BY4742 <i>pho23Δ::KanMX cti6Δ::URA3</i>	
JQW123.....BY4742 <i>PHO2-MYC::HIS3</i>	
JQW124.....BY4742 <i>PHO4-MYC::HIS3</i>	
JQW126.....BY4742 <i>SPT6-MYC::HIS3</i>	
JQW128.....BY4742 <i>RPB3-MYC::HIS3</i>	
JQW129.....BY4742 <i>RPD3-MYC::HIS3</i>	
JQW132.....BY4742 <i>set1Δ::URA3 PHO2-MYC::HIS3</i>	
JQW133.....BY4742 <i>set1Δ::URA3 PHO4-MYC::HIS3</i>	
JQW134.....BY4742 <i>set1Δ::URA3 SPT6-MYC::HIS3</i>	
JQW135.....BY4742 <i>set1Δ::URA3 RPB3-13MYC::HIS3</i>	
JQW136.....BY4742 <i>set1Δ::URA3 RPD3-MYC::HIS3</i>	
JQW137.....BY4742 <i>cti6Δ::KanMX RPD3-MYC::HIS3</i>	
JQW138.....BY4742 <i>pho23Δ::KanMX RPD3-MYC::HIS3</i>	
JQW139.....BY4742 <i>pho23Δ::KanMX cti6Δ::URA3 RPD3-MYC::HIS3</i>	
JQW142.....BY4742 pRS415[<i>LEU2 GAL1-10 HHT1-HA HHT1</i>]	
JQW143.....BY4742 <i>set1Δ::URA3 pRS415 [LEU2 GAL1-10 HHT1-HA HHT1]</i>	
JQW144.....BY4742 <i>rdp3Δ::KanMX pRR608 [CEN LEU2 RPD3]</i>	
JQW145.....BY4742 <i>rdp3Δ::KanMX pRR609 [CEN LEU2 rdp3(H150A)]</i>	
JQW146.....MATα <i>ura3-52 leu2-3,112 trp1-289 his3Δ1Δ (hlf1-hht1) (hlf2-hht2) pNS329 [CEN TRP1 HHT1^{K4A}-HHF1]</i>	

expression. We found that histone H3K4 was hypermethylated at the *PHO5* promoter independent of the activation state. Elimination of H3K4me did not affect the recruitment kinetics of Pho2 and Pho4 but led to a relatively open chromatin structure of the *PHO5* promoter, suggesting that Set1-mediated H3K4me inhibits *PHO5* transcription by a chromatin-based mechanism. We also showed that the Rpd3L complex was targeted to the *PHO5* promoter by H3K4 di- and trimethylation (H3K4me_{2/3}) and mediated the function of Set1. Although Rpd3 deacetylated both H3 and H4 at the *PHO5* promoter, Set1 specifically affected H3 deacetylation. Therefore, we conclude that the deacetylation activity on H3 is required for the regulation of *PHO5* transcription by Set1-mediated H3K4me.

MATERIALS AND METHODS

Yeast strains, growth conditions, and antibodies. Most yeast strains originated from Euroscarf. Strains constructed in this work are shown in Table 1. The induction of *PHO5* was achieved by immediately shifting yeast cells from high-phosphate medium (yeast extract-peptone-dextrose [YPD] plus 1 g/liter KH₂PO₄) to synthetic phosphate-free medium (yeast nitrogen base without phosphate was bought from MP Biomedicals). Histone modification antibodies were from Upstate. The histone H4 antibody was from Abcam. The anti-myc monoclonal antibody (clone 9E10) was from Sigma.

Chromatin immunoprecipitation (ChIP) assay. Cells were cross-linked with 1% formaldehyde for 15 min at room temperature and then resuspended in lysis buffer (50 mM HEPES, pH 7.9, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100 [vol/vol], 0.1% Na-deoxycholate [wt/vol], 1 mM phenylmethylsulfonyl fluoride [PMSF], protease inhibitor cocktail). Cells were lysed using glass beads and sonicated to shear the chromatin to fragment sizes of approximately 200 to 500 bp. Cross-linked chromatin fragments were immunoprecipitated with antibody for 4 h. Protein G/A-Sepharose beads were then added to the samples, and samples were incubated for an additional 2 h. The immunoprecipitated complexes were washed with lysis buffer, lysis buffer containing 500 mM NaCl, wash buffer (50 mM HEPES, pH 7.9, 300 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% NP-40 [vol/vol], 0.1% Na-deoxycholate), and TE (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). Next, the immunoprecipitated chromatin was eluted from

beads with elution buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1% SDS [wt/vol]). Formaldehyde cross-linking was reversed by incubating the eluates at 65°C overnight. DNA from the eluates was treated with 100 μg/ml proteinase K and purified with a QIAquick PCR purification kit (Qiagen). Immunoprecipitated fractions (IP) and whole-cell extracts (Input) containing DNA were analyzed by real-time PCR. Primers used in real-time PCR were as follows: for TELVIR (subtelomeric region of chromosome VI-R), CCATAATGCCTCCTA TATTTAGCCTTT/GACATATCCTTCACGAATATTGTTAGA; for *PHO5* UASp2, GAATAGGCAATCTCTAAATGAATCGA/GAAAACAGGGACCA GAATCATAAAATT; for the *PHO5* 5' open reading frame (ORF), GTTTAAAG TCTGTGTGTTTATTC/CCAATCTTGTGACATCGGCTA; for the *PHO5* 3' ORF, CGGACCATACTACTCTTTCCCT/CCAGACTGACAGTAGGGTA TCT; and for ACT1, TGTCTTGTACTCTTCCGGT/CCGGCCAAATCGAT TCTCAA.

Quantitative reverse transcription-PCR (qRT-PCR). Total RNA was isolated from cells grown to a concentration of approximately 1.0×10^7 cells/ml with an RNeasy minikit (Qiagen). cDNA was synthesized using a Moloney murine leukemia virus (MMLV) reverse transcriptase system and oligo(dT) (Promega). One microliter of the RT reaction was used in the subsequent real-time fluorescence quantitative PCR (Eppendorf).

Immunofluorescence of yeast cells. Immunostaining experiments were performed as previously described (72). Confocal microscopy was performed on a Leica TCS SP2 microscope with a 63× lambda blue oil objective. Image processing, including similar filtration and threshold levels, was standardized for all images.

Peptide pulldown assay. Biotinylated histone tail peptides were prebound to streptavidin beads (Invitrogen) at a ratio of 2 μg of peptide to 30 μl bead slurry in peptide binding buffer (PBB) (20 mM Tris-Cl at pH 8.0, 150 mM NaCl, 5% glycerol [vol/vol], 0.05% Tween 20 [vol/vol], 1 mM EDTA, 1 mM β-mercaptoethanol, protease inhibitors). Beads were washed 3 times with PBB and blocked with 0.5 mg/ml bovine serum albumin (BSA). Recombinant protein or yeast whole-cell extract was then added into the prebound bead slurry in equal volumes. Reaction samples were incubated for 1.5 h at 4°C and washed twice with PBB and twice with PBB containing 200 mM NaCl. Specific proteins were eluted by boiling in SDS sample buffer prior to Western blot analysis.

Nuclease digestion of yeast chromatin. Preparation and digestion of yeast nuclei were performed as described previously (4). DNA was prepared with phenol-chloroform extraction followed ethanol precipitation.

Southern blotting. Yeast genomic DNAs were digested with restriction enzymes as indicated in Fig. 4A, separated by 1.5% agarose gel electrophoresis, and transferred to a Hybond-N membrane (Amersham). The blot was hybridized with a [³²P]dCTP incorporated probe as indicated in Fig. 4A. The radioactive signal was detected and quantitated by phosphorimager.

Purification of recombinant proteins. The glutathione S-transferase (GST)-fused PHDs of Pho23 and Cti6 were overexpressed in *Escherichia coli* and purified using glutathione Sepharose 4B medium (GE Healthcare).

RESULTS

Histone H3K4 is hypermethylated at the *PHO5* promoter irrespective of its activation state. Since Set1-mediated H3K4me regulated *PHO5* transcription (15), we first investigated how H3K4me associated with *PHO5*. We performed chromatin immunoprecipitation (ChIP), combined with real-time fluorescent quantitative PCR, in a time course experiment of *PHO5* induction to detect the IP (% Input) of trimethylated H3K4 (H3K4me₃) at the *PHO5* promoter versus that at the subtelomeric region of chromosome VI-R (Fig. 1A). The induction of *PHO5* was achieved by immediately shifting yeast cells from high-phosphate (P_i⁺) to phosphate-free (P_i⁻) medium. The value of H3K4me₃ was corrected by the histone occupancy. Consistent with a previous report (15), we found that the H3K4me₃ marker was present at the repressed *PHO5* promoter when cells were cultured in P_i⁺ medium. Unexpectedly, the level of H3K4me₃ remained unchanged after P_i withdrawal (Fig. 1B). Therefore, H3K4me₃ is not coupled with active gene transcription in the case of *PHO5*.

FIG. 1. Set1-mediated H3K4 trimethylation associates with the *PHO5* promoter and represses *PHO5* transcription. (A) Schematic of the nucleosomal organization of the repressed promoter. The primer sets used for ChIP analysis are listed in the text. The light-gray ovals indicate nucleosomes that are removed during activation of *PHO5*, while the dark-gray ovals indicate nucleosomes that are not removed. (B) ChIP analysis of H3K4me3 at the promoter of *PHO5*. Yeast cells grown in P_i^+ medium were shifted to phosphate-depleted medium, and the cells were harvested for ChIP at timed intervals. The H3K4me3 was corrected by histone H4 occupancy. The IP (% Input) at the *PHO5* promoter and the subtelomeric region of chromosome VI-R are shown. (C and D) Analysis of *PHO5* induction in wild-type and *set1Δ* cells. (C) Relative mRNA level of *PHO5* at timed intervals after P_i withdrawal, normalized to *ACT1*. (D) The difference between the beginning and ending levels was set as 100%, and the values at the indicated times were determined. (E) Analysis of *PHO5* induction in wild-type and H3K4A mutant cells. mRNA analysis was performed as described for panel C. (F) ChIP analysis of myc-tagged Rpb3 at *PHO5*. The corresponding regions of the PCR products are shown in panel A. Error bars represent standard errors of the means from three trials.

Elimination of H3K4me derepresses *PHO5* transcription.

To investigate the effect of Set1 deletion on *PHO5* induction, we performed qRT-PCR to measure the mRNA level of *PHO5* at timed intervals upon P_i withdrawal. In the wild-type strain, *PHO5* transcription was strongly repressed in P_i^+ medium and was gradually increased upon the shift to P_i^- medium. After a 240-min induction, the level of *PHO5* mRNA was over 160-fold above the initial level (Fig. 1C). In the *set1Δ* strain, much more *PHO5* mRNA was produced even under P_i^+ conditions (Fig. 1C, 0 min). *SET1* deletion also increased the induction

rate of *PHO5* (Fig. 1C and D) but had a minor effect on *PHO5* transcription after growth under P_i^- conditions for hours (Fig. 1C). An essentially identical result was also observed in an H3 lysine 4-to-alanine mutation strain (H3K4A strain), where H3K4 methylation by Set1 was prevented (Fig. 1E). Altogether, these data indicate that Set1-dependent H3K4me acts on *PHO5* transcription both under P_i^+ conditions and at early times after withdrawal of P_i .

We further investigated the effect of *SET1* deletion on the action of RNA polymerase II (Pol II) at the *PHO5* gene. The level of Rpb3, one of the key subunits of RNA Pol II (28), at the promoter, 5' ORF, and 3' ORF of *PHO5* was measured in either P_i^+ or P_i^- medium. Consistent with the mRNA analysis results (Fig. 1C), the overall binding of Rpb3 at the *PHO5* promoter in *set1Δ* cells was higher than that in wild-type cells (Fig. 1F). Notably, in both wild-type and *set1Δ* cells, Rpb3 was equally distributed at *PHO5*, exhibiting no 5' or 3' ORF bias, suggesting that Set1 does not affect the elongation efficiency of RNA Pol II.

Set1-mediated H3K4me does not affect the recruitment of Pho2 and Pho4 onto the *PHO5* promoter. Transcription of *PHO5* is tightly controlled by the *PHO* gene regulatory cascade (Fig. 2A). Pho2 and Pho4 are essential transactivators for *PHO5* transcription. To determine whether the increase of *PHO5* transcription in *set1Δ* cells depends on Pho2 and Pho4, we examined the *PHO5* mRNA level under the wild-type, *pho2Δ*, or *pho4Δ* genetic background. Consistent with the general idea, no mRNA accumulation of *PHO5* was detected in *pho2Δ* and *pho4Δ* cells (Fig. 2B). Although *SET1* deletion elevated the *PHO5* mRNA level under the wild-type background (Fig. 1C and 2B), it did not change the *PHO5* mRNA level under the *pho2Δ* or *pho4Δ* background (Fig. 2B), suggesting that the function of H3K4me in *PHO5* transcription requires the presence of the activators. Interestingly, neither *PHO2* nor *PHO4* deletion affected H3K4me at the *PHO5* promoter (Fig. 2C), indicating that the establishment of H3K4me at the *PHO5* promoter does not require the activators.

Further, we investigated the effect of *SET1* deletion on *PHO5* transcription when the activators constitutively bound the *PHO5* promoter. We capitalized on the *pho80Δ* mutant, where Pho4 goes into the nucleus and constitutively activates *PHO5* transcription (45). We found that the mRNA level of *PHO5* in *set1Δ pho80Δ* cells was similar to that in *pho80Δ* cells (Fig. 2B), suggesting that H3K4me barely affects the *PHO5* transcription when transactivators saturate the *PHO5* promoter.

Since the function of Set1-mediated H3K4me in *PHO5* transcription relied on the regulatory cascade, we then wondered whether H3K4 methylation influenced *PHO5* transcription through affecting the recruitment of the activators. First, the cellular localizations of Pho4 were compared between wild-type and *set1Δ* cells. Under P_i^+ conditions, the Pho4 staining was dispersed in wild-type cells, while after the depletion of P_i , the Pho4 activator was relocalized from the cytoplasm to the nucleus (45) (Fig. 2D, left). *SET1* deletion did not affect the localization or the relocalization kinetics of Pho4 (Fig. 2D, right). Further, we compared the recruitments of Pho2 and Pho4 at the *PHO5* promoter between wild-type and *set1Δ* cells. ChIP experiments were performed using anti-myc antibody to detect the binding of myc-tagged Pho2 or Pho4 at the *PHO5*

FIG. 2. Set1-mediated H3K4me does not affect the recruitment of Pho2 and Pho4 onto the *PHO5* promoter. (A) Schematic of the regulatory circuit for phosphatase genes (46). (B) mRNA analysis of *PHO5* in cells from different isogenic strains. Yeast cells were cultured in either P_i^+ medium (top) or P_i^- medium (bottom). (C) ChIP analysis of H3K4me3 at the *PHO5* promoter. The conditions and the isogenic strains are indicated. ChIP experiments were performed as described for Fig. 1B. (D) Confocal images of the immunolocalization of myc-tagged Pho4 in wild-type and *set1Δ* cells. Pho4-myc was stained with mouse anti-Myc monoclonal antibody and detected with a Cy3-conjugated secondary antibody. DNA is stained with DAPI (4',6-diamidino-2-phenylindole). (E and F) ChIP analysis of myc-tagged Pho4 and Pho2 level at the *PHO5* promoter region in wild-type and *set1Δ* cells. Experiments were performed as described for Fig. 1B. Error bars represent standard errors of the means from three trials.

promoter during *PHO5* induction. Upon P_i withdrawal, the abundance of both Pho2 and Pho4 at the *PHO5* promoter was dramatically increased (Fig. 2E and F). Notably, the recruitment curves of Pho2 and Pho4 in *set1Δ* cells resembled those in wild-type cells (Fig. 2E and F), indicating that Set1-mediated H3K4me does not regulate the recruitment of the transactivators at the *PHO5* promoter.

Lack of H3K4 methylation promotes nucleosome remodeling at the *PHO5* promoter. Nucleosome disruption at the *PHO5* promoter, controlled by activator binding, is the key step before the loading and action of transcriptional machinery (1, 60). Since Set1-mediated H3K4me did not function through regulating activator recruitment, we hypothesized that H3K4me influences the chromatin structure of the *PHO5* gene. To test this idea, we detected the histone occupancy at the promoter or ORF region of the *PHO5* gene in wild-type and *set1Δ* cells (Fig. 1A). We used the commercially available version of anti-histone H4 antibody, which was used in a previous study establishing that nucleosomes lose contact with the activated *PHO5* promoter (51). As expected, nucleosome occu-

pancy at the *PHO5* promoter was high under P_i^+ conditions (Fig. 3A). After withdrawal of P_i , we observed a decrease ($\sim 21\%$ of original level at 240 min) in the H4 level at the promoter region of *PHO5* (Fig. 3A, wild type). Strikingly, in *set1Δ* cells, the H4 level at the *PHO5* promoter was only $\sim 65\%$ of that in wild-type cells under P_i^+ conditions (Fig. 3A, 0 min). *SET1* deletion also accelerated the rate of nucleosome disassembly at the *PHO5* promoter (Fig. 3A and data not shown). However, at 240 min after withdrawal of P_i , the histone occupancy at the *PHO5* promoter in *set1Δ* cells was comparable to that seen in wild-type cells (Fig. 3A). We also detected the histone occupancy at the 3' ORF region of *PHO5* and found that histone occupancy was not changed by P_i withdrawal in either wild-type or *set1Δ* cells (Fig. 3B). Taken together, these data suggest that *SET1* deletion reduces nucleosome occupancy of the *PHO5* promoter.

We next monitored binding of the histone chaperone Spt6, which is required for chromatin reassembly at *PHO5* (2). Unexpectedly, more binding of Spt6 was detected at both the promoter and the 3' ORF of *PHO5* in *set1Δ* cells than in

FIG. 3. H3K4 methylation suppresses nucleosome remodeling at the *PHO5* promoter. (A and B) ChIP analysis of histone H4 level at the promoter (A) and the 3' ORF (B) of *PHO5* in wild-type and *set1Δ* cells upon phosphate starvation. ChIP assays were performed as described for Fig. 1B. (C and D) ChIP analysis of myc-tagged Spt6 at the promoter (C) and the 3' ORF (D) of *PHO5* in wild-type and *set1Δ* cells upon phosphate starvation. ChIP assays were performed as described for Fig. 1B. (E) Schematic of the extrachromosomal copy of *HHT1-HHF1* expressing H3 and HA-tagged H4 (H4-HA) under the control of the *GAL1* promoter. (F) Schematic of the experimental procedures used to obtain results shown in panels G through I. (G to I) ChIP of H4-HA at the *PHO5* promoter (G), the 3' ORF (H), and the *ACT1* ORF (I) after galactose induction and P_i withdrawal. Error bars represent standard errors of the means from three trials.

wild-type cells (Fig. 3C and D), implying that *SET1* deletion activates chromatin reassembly events at *PHO5*. To validate that chromatin reassembly events occurred in *set1Δ* cells, we constructed a strain into which an extra copy of *HHT1-HHF1* was introduced. *HHF1* was tagged with hemagglutinin (HA) epitope at its C terminus, and its promoter was replaced with a *GAL1* promoter (Fig. 3E). The expression of H4-HA was induced by galactose 1 h before P_i withdrawal (Fig. 3F). By measuring the abundance of H4-HA at *PHO5*, we could monitor the nucleosome reassembly under P_i^+ conditions and at early times after withdrawal of P_i . After long-term H4-HA expression (>2 h), chromatin in most cells was reassembled, and accordingly the abundance of H4-HA at *PHO5* was reduced. The results showed that, in wild-type cells, the H4-HA was not incorporated into the *PHO5* promoter throughout the process (Fig. 3G). In striking contrast, in *set1Δ* cells, H4-HA was incorporated into the *PHO5* promoter right after galactose addition (Fig. 3G), supporting the idea that, under physiological conditions, H3K4me3 suppresses chromatin reassembly events at *PHO5*. In addition, we found that H4-HA was not detected at the 3'ORF of *PHO5* in either wild-type or *set1Δ* cells (Fig. 3H). These results were expected because nucleosomes at the *PHO5* ORF were well packaged even after *PHO5* activation (Fig. 3B). At the control locus of *ACT1*, *SET1* deletion did not affect nucleosome reassembly (Fig. 3I). Altogether, these data suggest that nucleosomes at the *PHO5* promoter in *set1Δ* cells are dynamically disassembled and reassembled even under P_i^+ conditions.

Derepression of the *PHO5* promoter by *SET1* deletion leads to a relatively open chromatin structure. To compare the chromatin structures of the *PHO5* promoter between wild-type and *set1Δ* cells, we performed restriction endonuclease digestion

experiments. As shown in the schematic in Fig. 4A, the *Bam*HI site falls within the linker regions between the positioned nucleosomes of the repressed promoter, whereas the *Cla*I and *Hha*I restriction enzymes have intranucleosomal sites located in the nucleosomes -2 and -3 , respectively. Analyzing the accessibility of the *Cla*I and *Hha*I restriction sites at the *PHO5* promoter is a reliable and quantitative way to access chromatin structure (5, 9). We isolated nuclei from wild-type and *set1Δ* strains grown in either P_i^+ or P_i^- medium, treated them with *Cla*I or *Hha*I, and then performed Southern blotting. Interestingly, in P_i^+ medium, we observed a clear increase of restriction nuclease accessibility in *set1Δ* cells compared with that in wild-type cells (Fig. 4B and C). In P_i^- medium, however, the restriction sites became equally accessible in wild-type and *set1Δ* strains (Fig. 4B and C). These data indicate that derepression of the *PHO5* promoter by *SET1* deletion increases the accessibility of nucleosomes -2 and -3 .

We next performed micrococcal nuclease (MNase) digestion to address whether nucleosome positioning at the *PHO5* promoter was changed in the *set1Δ* strain. Nuclei were isolated from strains in P_i^+ medium and digested with MNase. Southern blotting showed that the overall chromatin organizations in wild-type and *set1Δ* cells were essentially the same (Fig. 4D). In addition, no detectable increase in MNase accessibility was detected in *set1Δ* cells (Fig. 4D). We reasoned that the modest increase of chromatin accessibility to DNA binding proteins detected by restriction endonuclease accessibility was not detectable by MNase accessibility.

The Rpd3L complex is recruited by Set1-mediated H3K4me and represses *PHO5* transcription. It has been shown that the Rpd3 complex represses *PHO5* transcription and that the Rpd3S complex is involved in the Set1-mediated repression of

FIG. 4. *SET1* deletion leads to a more accessible chromatin structure at the *PHO5* promoter. (A) Schematic of the recognition sites of the restriction enzymes used in nuclease assays and Southern blotting. (B and C) Restriction enzyme digestion of yeast nuclei. Nuclei were digested with 5 U or 50 U *Cla*I (B) or *Hha*I (C) for 120 min. To monitor the extent of cleavage, DNA was isolated, cleaved with *Hae*III, separated by a 1.5% agarose gel, blotted, and hybridized with the probe indicated in panel A. Histograms represent the percentages of enzyme cleavage, quantified by phosphorimager. (D) MNase digestion of yeast nuclei. Nuclei were digested with increasing amounts of MNase for 15 min. Southern blotting was performed as described for panel B, except that the DNA was cleaved with *Apa*I. Black arrows denote sensitive sites representing the internucleosomal regions. Positioned nucleosomes at the *PHO5* promoter are represented (ovals) on the right.

GAL1 induction (15, 47). We proposed that Rpd3 was the effector of Set1-mediated H3K4me in the regulation of *PHO5* transcription. To test this hypothesis, we first performed ChIP to investigate the dependence of Rpd3 binding at the *PHO5* promoter on Set1. As a result, the Rpd3 binding level in *set1*Δ cells was only 13% of the wild-type level (Fig. 5A), indicating that Set1 is required for Rpd3 recruitment at the *PHO5* promoter. To validate that Rpd3 and Set1 functioned in the same genetic pathway, we compared the *PHO5* mRNA levels during induction between *rpd3*Δ and *rpd3*Δ *set1*Δ strains. As shown in Fig. 5B, *RPD3* deletion also increased the basal transcription of *PHO5*. Notably, the *PHO5* induction curve in *rpd3*Δ *set1*Δ cells resembled that in *rpd3*Δ cells, suggesting that Rpd3 is responsible for the Set1-dependent transcriptional repression of *PHO5*.

Rpd3 exists in two separate complexes: Rpd3S and Rpd3L (14, 26, 27, 34, 40, 49, 71). We wondered which complex participated in *PHO5* transcriptional regulation. Therefore, we compared the *PHO5* mRNA levels in Rpd3S and Rpd3L complex mutant strains with that in the wild-type strain. All strains were cultured in *P*_i⁺ medium. The relative *PHO5* mRNA level of the wild-type strain was set as 1. The result showed that deletions of each of the shared subunits, *RPD3*, *SIN3*, and *UME1*, caused a dramatic increase in *PHO5* transcription (Fig. 5C). Deletion of Rpd3S complex-specific genes *EAF3* and *RCO1* barely affected *PHO5* transcription, while deletion of Rpd3L complex-specific genes, such as *SAP30*, *SDS3*, *DEP1*, *RXT2*, *PHO23*, and *CTI6*, increased *PHO5* transcription (Fig. 5C). Therefore, we conclude that the Rpd3L complex participates in the regulation of *PHO5* transcription.

Cooperative action of Pho23 and Cti6 mediates the binding of Rpd3 at the *PHO5* promoter. The binding of proteins with methylated H3K4 involves recognition of H3K4me by specific domains, such as the plant homeodomain (PHD) (55). Interestingly, there are two PHD-containing subunits, Pho23 and Cti6, in the Rpd3L complex, both of which recognize trimethylated H3K4 *in vitro* (55, 56). To investigate which subunit might be responsible for the Rpd3L complex recruitment to the methylated *PHO5* promoter, we detected Rpd3 binding in wild-type, *pho23*Δ, and *cti6*Δ strains. The ChIP results showed that mutation of either *PHO23* or *CTI6* caused a modest decrease in Rpd3 binding at the *PHO5* promoter (Fig. 5D), suggesting that both subunits contribute to the recruitment of the Rpd3L complex to the *PHO5* promoter. Notably, when we deleted both *PHO23* and *CTI6*, the binding of Rpd3 at *PHO5* was dramatically reduced (Fig. 5D), suggesting a synthetic effect of the two PHD-containing proteins on the Rpd3L complex association with the H3K4-methylated *PHO5* promoter. Consistently, double deletion of *PHO23* and *CTI6* led to a higher mRNA level of *PHO5* than single deletion did (Fig. 5C). Taken together, these data suggest that Pho23 and Cti6 cooperatively mediate the association and function of the Rpd3L complex on the *PHO5* gene.

To directly assess the roles of Pho23 and Cti6 in the Rpd3L complex recognizing H3K4me, *in vitro* binding assays with biotinylated histone peptides were employed. First, we examined the binding affinity of the PHDs of Pho23 and Cti6 with unmodified or K4-monomethylated, -dimethylated, or -trimethylated histone H3 peptides (amino acids 1 to 27). The GST-fused recombinant PHDs were overexpressed in bacteria and puri-

FIG. 5. H3K4me recruits the Rpd3L complex for *PHO5* repression. (A) ChIP analysis of myc-tagged Rpd3 at the *PHO5* promoter in wild-type and *set1Δ* cells. Yeast cells were grown in P_i^+ medium. (B) Analysis of *PHO5* induction in wild-type, *rpd3Δ*, and *rpd3Δ set1Δ* cells. *PHO5* mRNA was analyzed as described for Fig. 1C. (C) mRNA analysis of *PHO5* in wild-type and Rpd3 complex mutant cells. Yeast was grown in P_i^+ medium. The wild-type level of *PHO5* was set as 1. The log₂ values above 0 indicate increased transcription of *PHO5*. (D) ChIP analysis of myc-tagged Rpd3 at the *PHO5* promoter in wild-type and mutant cells. ChIP was performed as described for panel A. Error bars represent standard errors of the means from three trials. (E) Pulldown assay of the PHD of either Cti6 or Pho23 with H3 N-terminal peptides. Agarose beads conjugated by peptides un-, mono-, di-, or trimethylated at H3K4 were incubated with equal amounts of recombinant proteins as indicated on the right. The peptide-bound proteins were detected by Western blotting with anti-GST antibody. "Mock" indicates a no-peptide control. Coomassie blue-stained peptides are shown at the bottom. (F) Pulldown assay of whole-cell extracts with H3 N-terminal peptides. The whole-cell extracts were derived from yeasts with the genetic backgrounds indicated on the left. The bound Rpd3-myc was detected with anti-myc antibody.

fied to homogeneity (data not shown). Then, equal amounts of the recombinant proteins were incubated with peptide-conjugated agarose beads. The peptide-bound proteins were washed several times and subjected to Western blotting with anti-GST antibody. The results showed that both PHDs bound most strongly to H3K4me3 peptide, less to H3K4me2 peptide, even less to H3K4me1 peptide, and not at all to unmodified peptides (Fig. 5E), supporting the conclusion that Pho23 and Cti6 directly recognize hypermethylated H3K4. Then, we tagged Rpd3 with myc epitope in wild-type, *cti6Δ*, *pho23Δ*, and *cti6Δ pho23Δ* cells and examined the binding affinity of Rpd3-myc with the H3 peptides. As expected, the myc-tagged Rpd3 had a higher affinity to hypermethylated H3K4 peptides in the wild-type strain than in the mutant strains (Fig. 5F). Surprisingly, single mutation of either *PHO23* or *CTI6* did not affect the preference, while double deletion of *PHO23* and *CTI6* almost eliminated the binding of Rpd3 with methylated H3K4 (Fig. 5F). Taken together, these data support the conclusion that Pho23 and Cti6 cooperatively recruit the Rpd3L complex to H3K4-methylated chromatin.

The deacetylation activity of Rpd3 is essential for Set1-dependent suppression of nucleosome remodeling at the *PHO5* promoter. We then asked whether the deacetylation activity of Rpd3 was involved in the regulation of nucleosome remodeling by Set1. To this end, we inactivated the histone deacetylation activity of Rpd3 by mutating the histidine at amino acid 150 to alanine (25) and examined the effect on *PHO5* promoter structure. The ChIP results showed that the initial nucleosome occupancy in the *rpd3*(H150A) mutant strain was only 68% of the wild-type level, which was similar to that in the *rpd3Δ* strain

and the *set1Δ* strain (Fig. 6A and 3A). Thus, histone deacetylation by Rpd3 mostly likely mediates the Set1-dependent regulation of nucleosome disassembly.

Set1-mediated H3K4 methylation specifically inhibits histone H3 acetylation at the *PHO5* promoter. According to the literature, the major substrates of Rpd3 on nucleosomes are the lysine residues at the N termini of histones H3 and H4 (13). To investigate how Set1 and Rpd3 affected the acetylation status of the *PHO5* promoter, we used anti-acetyl-H3K9/14 (α -AcH3) antibody and anti-acetyl-H4K5/8/12/16 (α -AcH4) antibody in ChIP experiments to analyze the relative histone acetylation levels at the promoter of *PHO5*. Although the ChIP signals for AcH3 and AcH4 decreased upon P_i withdrawal (data not shown), the relative acetylation levels, achieved by normalizing the acetylation signals to the H4 signal, were essentially stable during the induction process (Fig. 6B and C). Consistent with a previous report (52), in *rpd3Δ* cells, we observed a marked increase of both AcH3 and AcH4 levels (Fig. 6B and C). However, in *set1Δ* cells, we found that the AcH3 level was greatly elevated (Fig. 6B) while the AcH4 level was slightly reduced (Fig. 6C). Combined with the above-described ChIP results (Fig. 6A), these data suggest that Rpd3-mediated H3 deacetylation is responsible for the restriction of nucleosome remodeling by Set1-mediated H3K4me.

DISCUSSION

In this study, we have characterized the mechanism for Set1-dependent *PHO5* transcriptional repression. We found that

FIG. 6. Set1 suppresses nucleosome disassembly by Rpd3-dependent H3 deacetylation. (A) ChIP analysis of histone H4 level at the promoter region of *PHO5* in wild-type and *rpd3* mutant cells upon phosphate starvation. ChIP assays were performed as described for Fig. 3A. (B and C) ChIP analysis of AcH3 (B) and AcH4 (C) at the promoter region of *PHO5* in wild-type and mutant cells upon phosphate starvation. ChIP assays were performed as described for Fig. 1B. The IP (% Input) values of histone acetylation were then corrected by histone H4 density. Error bars represent standard errors of the means from three trials.

Set1 affected *PHO5* transcription through regulating chromatin structure but not the recruitment of transactivators. Notably, in *set1Δ* cells, the four positioned nucleosomes at the *PHO5* promoter were dynamically disassembled and reassembled under P_i^+ conditions. We further revealed the Rpd3L complex, rather than the Rpd3S complex, as the effector of H3K4me in inhibiting *PHO5* transcription. The deacetylation activity of Rpd3 on histone H3 is essential for the transcriptional repression of *PHO5*. Given that H3 acetylation is required for nucleosome remodeling at the *PHO* gene promoter (23, 50), the erasure of H3 acetylation by Rpd3 suppressed nucleosome remodeling at the *PHO5* promoter. Therefore, our data suggest that the Set1-mediated H3K4me sets a nucleosomal barrier for the basal transcription of *PHO5* under repressed conditions.

Repressive role of H3K4me in gene transcription. When analyzing H3K4me distribution patterns genome-wide, one notices that H3K4me2/3 are occasionally present at transcriptionally inactive genes (48). Clearly, then, an important issue to address is how these epigenetic marks occur at inactive loci and what their functional outcomes are. One possibility is that H3K4me2/3 originate from weak cryptic noncoding RNAs (ncRNAs). Indeed, antisense RNAs were detected at *PHO5* as well as the *PHO84* gene (12, 65). Other supportive evidence came from studies of the *GAL1-10* gene cluster. In glucose, the 3' ORF of *GAL10* was trimethylated at H3K4 and generated a long ncRNA that was reciprocal to the *GAL1* gene (24). The generation of ncRNA required the Reb1 transcriptional factor, whose depletion led to a decrease of H3K4me3 at the

GAL1-10 locus (47). However, in the case of the *PHO5* gene, the establishment of H3K4me did not require ncRNAs (65).

Despite the general idea that Set1 is associated with active transcription (31, 57, 59), an increasing number of arguments suggest that Set1-mediated H3K4me2/3 are also involved in gene repression. It was shown that Set1 was required for silencing of Ty1 retrotransposons *GAL1* and *PHO84* (10, 15), subsequently found to be ncRNA mediated (8, 12, 24, 47). Recently, Morillon's team provided evidence that H3K4me2/3 recruit the Rpd3S complex to *GAL1* as well as many other inducible genes that showed cryptic transcription (47, 69). Paradoxically, according to the proposed model, the Rpd3S complex preferentially recognizes Set2-mediated H3K36me3 at the 3' end of actively transcribed genes and represses promoters hidden within the coding region (37–39). It is still elusive how the Rpd3S complex is associated with H3K4me at gene promoters. In the case of *PHO5*, the Rpd3S complex-specific subunits had no effect on the *PHO5* mRNA level (Fig. 5C). In contrast, many Rpd3L complex-specific subunits negatively regulate *PHO5* transcription (16, 33, 40) (Fig. 5C). Our observations that both the Pho23 and the Cti6 subunit cooperatively recruited Rpd3 enzyme to the H3K4-methylated *PHO5* promoter (Fig. 5D to F) provide a conceivable explanation for how H3K4me and the Rpd3L complex interact to repress *PHO5* transcription.

Regulation of chromatin remodeling at the *PHO5* promoter by histone acetylation and deacetylation. It is generally considered that neutralization of the positive charges on lysine residues in the histone tails by acetylation disrupts electrostatic interactions between the histones and the phosphate groups in the DNA, leading to a looser configuration (58, 64). The fundamental role of histone acetylation in disruption of the promoter nucleosome structure during *PHO5* induction has been revealed (7, 22, 44, 50, 51, 68). Under repressed conditions, the NuA4 acetyltransferase complex, recruited by Pho2, maintains a hyperacetylated state of the nucleosomes so as to poise the promoter for chromatin remodeling (44). The SAGA complex is recruited to the *PHO5* promoter upon induction and directly regulates the opening of the nucleosome structure (7, 17, 22). However, hyperacetylation of histone H3 is detected in the absence of Swi2 only when the remodeling process is frozen subsequent to activator binding (50). If remodeling is permitted to go to completion, hyperacetylation is lost (50, 51). Thus, we propose that in the process of *PHO5* activation, the promoter nucleosomes acetylated at histone H3 are quickly removed during induction. The unremodeled nucleosomes restore their initial acetylation level, probably by Rpd3-dependent histone deacetylation. In *set1Δ* or *rpd3Δ* cells, we found that histone H3 was hyperacetylated and prone to be removed from the *PHO5* promoter without induction (Fig. 6A and B). This looser nucleosome confirmation potentially increased the binding of chromatin remodelers and thereby further facilitated the chromatin remodeling process (Fig. 4 and 5). Therefore, our study suggests that histone hyperacetylation at both H3 and H4 tends to be removed from the *PHO5* promoter.

Interestingly, both NuA4 and the Rpd3 complex are able to recognize H3K4me (41, 55, 56). In the case of *PHO5*, we found that H3K4me was critical for the recruitment of Rpd3 and contributed to the recruitment of NuA4 at the *PHO5* promoter

FIG. 7. Predicted model for the regulation of nucleosome remodeling at the *PHO5* promoter. (A) Under P_i^+ conditions, the four nucleosomes at the *PHO5* promoter are well positioned, inaccessible to chromatin remodelers or coactivators. Histone H4 (shown as red sector) N-terminal lysines are acetylated by Pho2-recruited NuA4. H4 acetylation is required but not sufficient for initiation of promoter chromatin remodeling. Histone H3 (shown as blue sector) is hypermethylated at K4, which recruits the Rpd3L complex to maintain a hypoacetylated state of H3 lysines. At this stage, there is only basal *PHO5* transcription. (B) Upon P_i withdrawal, histone modifiers and chromatin remodelers are targeted to the *PHO5* promoter by the relocalized Pho4. Remodeling of the promoter nucleosomes requires acetylation of histone H3. Once acetylated, histone H3 is rapidly removed from the *PHO5* promoter; otherwise, it will be deacetylated by the Rpd3L complex. Therefore, the relative acetylation level at the *PHO5* promoter is essentially stable during the induction process. At this intermediate stage, the *PHO5* transcript is being accumulated but still at a low level. (C) After growth in P_i^- medium, nucleosomes at the *PHO5* promoter are completely unfolded, allowing efficient action of the transcriptional machinery. At this stage, Set1-mediated H3K4me is dispensable. (D) When *SET1* is deleted, the promoter becomes partially accessible to chromatin remodelers even under P_i^+ conditions. Loss of Rpd3 increases histone H3 acetylation and thereby elevates basal chromatin remodeling. The four nucleosomes are dynamically disassembled and reassembled (shown as dashed circles). Such a labile promoter structure results in high basal transcription of *PHO5* even under repressed conditions.

(Fig. 5A and data not shown). These observations are consistent with the predicted model where both histone acetyltransferases and deacetylases are associated with inactive genes primed by H3K4 methylation (67). A previous study has indicated that Rpd3 can compete with NuA4 for chromatin binding by deacetylating and destabilizing the Yng2 subunit of NuA4 (38). Indeed, we observed an increase of Esa1 binding at the *PHO5* promoter in *rp3Δ* cells (data not shown). Therefore, elimination of H3K4me results in a reduced binding of Esa1 as well as Rpd3, the competitor of Esa1. This could explain the discrepancy between Set1 and Rpd3 in regulating H4 acetylation at the *PHO5* promoter (Fig. 6C). Altogether, our work reveals a complicated cross talk among histone methylation, acetylation, and deacetylation at the *PHO5* promoter that orchestrates the chromatin structure.

Cross talk between the transcriptional activators and chromatin remodeling at the *PHO5* gene. The transcription of *PHO5* is tightly controlled by the *PHO* gene regulatory cascade. The absence of transcriptional activator Pho2 or Pho4 from the *PHO5* promoter causes a complete loss of *PHO5* transcription (6, 45). Recruitment of transcriptional activators is required for remodeling of the four positioned nucleosomes at *PHO5* (19–21, 63). Therefore, it is not surprising to find that the regulation of *PHO5* transcription by Set1 also requires the presence of Pho2 and Pho4 (Fig. 2B). Since chromatin is com-

pletely unfolded at the active *PHO5* promoter (9), Set1 no longer has an effect on the transcription of activated *PHO5* (Fig. 2B). On the other hand, it has been shown that nucleosome disassembly from the *PHO5* promoter is a prerequisite for action of the general transcription machinery, activators, and coactivators (1, 3, 18, 29). In our study, we found that *SET1* deletion altered the chromatin structure at the *PHO5* promoter (Fig. 2 and 3) but did not affect the recruitment of transactivators. We reason that the chromatin structure at the *PHO5* promoter, although partially relaxed in *set1Δ* cells, is still restrictive to activator binding. Given the fact that the activators are already present at the *PHO5* promoter even under repressed conditions (44) (Fig. 2E and F), the elimination of H3K4me at the *PHO5* promoter may exaggerate the activity of activators in nucleosome remodeling.

In summary, we have presented a Set1-dependent chromatin-based mechanism that prevents basal transcription of *PHO5* under repressed conditions (Fig. 7). The nucleosomes at the *PHO5* promoter become more dynamic and more accessible to the transcription machinery even under repressive conditions when H3K4 is hypomethylated. We propose that the stringent regulation of *PHO5* transcription is achieved not only by the regulatory cascade but also by the well-controlled chromatin structure at their promoters.

ACKNOWLEDGMENTS

This work is supported by grants from the National Natural Science Foundation of China (90919027) and the Ministry of Science and Technology of China (2011CB966300).

REFERENCES

- Adkins, M. W., S. R. Howar, and J. K. Tyler. 2004. Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast *PHO5* and *PHO8* genes. *Mol. Cell* **14**:657–666.
- Adkins, M. W., and J. K. Tyler. 2006. Transcriptional activators are dispensable for transcription in the absence of Spt6-mediated chromatin reassembly of promoter regions. *Mol. Cell* **21**:405–416.
- Adkins, M. W., S. K. Williams, J. Linger, and J. K. Tyler. 2007. Chromatin disassembly from the *PHO5* promoter is essential for the recruitment of the general transcription machinery and coactivators. *Mol. Cell. Biol.* **27**:6372–6382.
- Almer, A., and W. Hörz. 1986. Nuclease hypersensitive regions with adjacent positioned nucleosomes mark the gene boundaries of the *PHO5/PHO3* locus in yeast. *EMBO J.* **5**:2681–2687.
- Almer, A., H. Rudolph, A. Hinnen, and W. Hörz. 1986. Removal of positioned nucleosomes from the yeast *PHO5* promoter upon *PHO5* induction releases additional upstream activating DNA elements. *EMBO J.* **5**:2689–2696.
- Barbaric, S., M. Munsterkotter, J. Svaren, and W. Hörz. 1996. The homeodomain protein Pho2 and the basic-helix-loop-helix protein Pho4 bind DNA cooperatively at the yeast *PHO5* promoter. *Nucleic Acids Res.* **24**:4479–4486.
- Barbaric, S., H. Reinke, and W. Hörz. 2003. Multiple mechanistically distinct functions of SAGA at the *PHO5* promoter. *Mol. Cell. Biol.* **23**:3468–3476.
- Berretta, J., M. Pinskaya, and A. Morillon. 2008. A cryptic unstable transcript mediates transcriptional trans-silencing of the Ty1 retrotransposon in *S. cerevisiae*. *Genes Dev.* **22**:615–626.
- Boeger, H., J. Griesenbeck, J. S. Strattan, and R. D. Kornberg. 2003. Nucleosomes unfold completely at a transcriptionally active promoter. *Mol. Cell* **11**:1587–1598.
- Briggs, S. D., et al. 2001. Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in *Saccharomyces cerevisiae*. *Genes Dev.* **15**:3286–3295.
- Cairns, B. R. 2005. Chromatin remodeling complexes: strength in diversity, precision through specialization. *Curr. Opin. Genet. Dev.* **15**:185–190.
- Cambong, J., et al. 2009. Trans-acting antisense RNAs mediate transcriptional gene co-suppression in *S. cerevisiae*. *Genes Dev.* **23**:1534–1545.
- Carmen, A. A., S. E. Rundlett, and M. Grunstein. 1996. HDA1 and HDA3 are components of a yeast histone deacetylase (HDA) complex. *J. Biol. Chem.* **271**:15837–15844.
- Carrozza, M. J., et al. 2005. Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. *Cell* **123**:581–592.
- Carvin, C. D., and M. P. Kladde. 2004. Effectors of lysine 4 methylation of histone H3 in *Saccharomyces cerevisiae* are negative regulators of *PHO5* and *GALI-10*. *J. Biol. Chem.* **279**:33057–33062.
- Colina, A. R., and D. Young. 2005. Raf60, a novel component of the Rpd3 histone deacetylase complex required for Rpd3 activity in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **280**:42552–42556.
- Dhasarathy, A., and M. P. Kladde. 2005. Promoter occupancy is a major determinant of chromatin remodeling enzyme requirements. *Mol. Cell. Biol.* **25**:2698–2707.
- Ertel, F., et al. 2010. *In vitro* reconstitution of *PHO5* promoter chromatin remodeling points to a role for activator-nucleosome competition in vivo. *Mol. Cell. Biol.* **30**:4060–4076.
- Fascher, K. D., J. Schmitz, and W. Hörz. 1990. Role of trans-activating proteins in the generation of active chromatin at the *PHO5* promoter in *S. cerevisiae*. *EMBO J.* **9**:2523–2528.
- Fascher, K. D., J. Schmitz, and W. Hörz. 1993. Structural and functional requirements for the chromatin transition at the *PHO5* promoter in *Saccharomyces cerevisiae* upon *PHO5* activation. *J. Mol. Biol.* **231**:658–667.
- Gaudreau, L., A. Schmid, D. Blaschke, M. Ptashne, and W. Hörz. 1997. RNA polymerase II holoenzyme recruitment is sufficient to remodel chromatin at the yeast *PHO5* promoter. *Cell* **89**:55–62.
- Gregory, P. D., et al. 1998. Absence of Gcn5 HAT activity defines a novel state in the opening of chromatin at the *PHO5* promoter in yeast. *Mol. Cell* **1**:495–505.
- Gregory, P. D., A. Schmid, M. Zavari, M. Munsterkotter, and W. Hörz. 1999. Chromatin remodeling at the *PHO8* promoter requires SWI-SNF and SAGA at a step subsequent to activator binding. *EMBO J.* **18**:6407–6414.
- Houseley, J., L. Rubbi, M. Grunstein, D. Tollervey, and M. Vogelauer. 2008. A ncRNA modulates histone modification and mRNA induction in the yeast GAL gene cluster. *Mol. Cell* **32**:685–695.
- Kadosh, D., and K. Struhl. 1998. Histone deacetylase activity of Rpd3 is important for transcriptional repression in vivo. *Genes Dev.* **12**:797–805.
- Kasten, M. M., S. Dorland, and D. J. Stillman. 1997. A large protein complex containing the yeast Sin3p and Rpd3p transcriptional regulators. *Mol. Cell. Biol.* **17**:4852–4858.
- Keogh, M. C., et al. 2005. Cotranscriptional set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. *Cell* **123**:593–605.
- Kolodziej, P., and R. A. Young. 1989. RNA polymerase II subunit RPB3 is an essential component of the mRNA transcription apparatus. *Mol. Cell. Biol.* **9**:5387–5394.
- Korber, P., et al. 2006. The histone chaperone Asf1 increases the rate of histone eviction at the yeast *PHO5* and *PHO8* promoters. *J. Biol. Chem.* **281**:5539–5545.
- Kornberg, R. D. 1974. Chromatin structure: a repeating unit of histones and DNA. *Science* **184**:868–871.
- Kouzarides, T. 2007. Chromatin modifications and their function. *Cell* **128**:693–705.
- Krogan, N. J., et al. 2003. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol. Cell* **11**:721–729.
- Lau, W. W., K. R. Schneider, and E. K. O'Shea. 1998. A genetic study of signaling processes for repression of *PHO5* transcription in *Saccharomyces cerevisiae*. *Genetics* **150**:1349–1359.
- Lechner, T., et al. 2000. Sds3 (suppressor of defective silencing 3) is an integral component of the yeast Sin3[middle dot]Rpd3 histone deacetylase complex and is required for histone deacetylase activity. *J. Biol. Chem.* **275**:40961–40966.
- Lee, C. K., Y. Shibata, B. Rao, B. D. Strahl, and J. D. Lieb. 2004. Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat. Genet.* **36**:900–905.
- Li, B., M. Carey, and J. L. Workman. 2007. The role of chromatin during transcription. *Cell* **128**:707–719.
- Li, B., et al. 2007. Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. *Science* **316**:1050–1054.
- Li, B., et al. 2007. Infrequently transcribed long genes depend on the Set2/Rpd3S pathway for accurate transcription. *Genes Dev.* **21**:1422–1430.
- Li, B., et al. 2009. Histone H3 lysine 36 dimethylation (H3K36me2) is sufficient to recruit the Rpd3s histone deacetylase complex and to repress spurious transcription. *J. Biol. Chem.* **284**:7970–7976.
- Loewith, R., et al. 2001. Pho23 is associated with the Rpd3 histone deacetylase and is required for its normal function in regulation of gene expression and silencing in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **276**:24068–24074.
- McManus, K. J., V. L. Biron, R. Heit, D. A. Underhill, and M. J. Hendzel. 2006. Dynamic changes in histone H3 lysine 9 methylations: identification of a mitosis-specific function for dynamic methylation in chromosome congression and segregation. *J. Biol. Chem.* **281**:8888–8897.
- Mellor, J. 2006. Dynamic nucleosomes and gene transcription. *Trends Genet.* **22**:320–329.
- Ng, H. H., F. Robert, R. A. Young, and K. Struhl. 2003. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol. Cell* **11**:709–719.
- Nourani, A., R. T. Utley, S. Allard, and J. Côté. 2004. Recruitment of the NuA4 complex poises the *PHO5* promoter for chromatin remodeling and activation. *EMBO J.* **23**:2597–2607.
- O'Neill, E. M., A. Kaffman, E. R. Jolly, and E. K. O'Shea. 1996. Regulation of PHO4 nuclear localization by the PHO8-*PHO85* cyclin-CDK complex. *Science* **271**:209–212.
- Oshima, Y., N. Ogawa, and S. Harashima. 1996. Regulation of phosphatase synthesis in *Saccharomyces cerevisiae*—a review. *Gene* **179**:171–177.
- Pinskaya, M., S. Gourvennec, and A. Morillon. 2009. H3 lysine 4 di- and tri-methylation deposited by cryptic transcription attenuates promoter activation. *EMBO J.* **28**:1697–1707.
- Pokholok, D. K., et al. 2005. Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* **122**:517–527.
- Puig, S., M. Lau, and D. J. Thiele. 2004. Cti6 is an Rpd3-Sin3 histone deacetylase-associated protein required for growth under iron-limiting conditions in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **279**:30298–30306.
- Reinke, H., P. D. Gregory, and W. Hörz. 2001. A transient histone hyperacetylation signal marks nucleosomes for remodeling at the *PHO8* promoter in vivo. *Mol. Cell* **7**:529–538.
- Reinke, H., and W. Hörz. 2003. Histones are first hyperacetylated and then lose contact with the activated *PHO5* promoter. *Mol. Cell* **11**:1599–1607.
- Schultz, J. 1936. Variegation in *Drosophila* and the inert chromosome regions. *Proc. Natl. Acad. Sci. U. S. A.* **22**:27–33.
- Schwabish, M. A., and K. Struhl. 2004. Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. *Mol. Cell. Biol.* **24**:10111–10117.
- Sharma, V. M., R. S. Tomar, A. E. Dempsey, and J. C. Reese. 2007. Histone deacetylases RPD3 and HOS2 regulate the transcriptional activation of DNA damage-inducible genes. *Mol. Cell. Biol.* **27**:3199–3210.
- Shi, X., et al. 2006. ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. *Nature* **442**:96–99.
- Shi, X., et al. 2007. Proteome-wide analysis in *Saccharomyces cerevisiae* identifies several PHD fingers as novel direct and selective binding modules

- of histone H3 methylated at either lysine 4 or lysine 36. *J. Biol. Chem.* **282**:2450–2455.
57. **Shilatifard, A.** 2006. Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. *Annu. Rev. Biochem.* **75**:243–269.
 58. **Shogren-Knaak, M., et al.** 2006. Histone H4–K16 acetylation controls chromatin structure and protein interactions. *Science* **311**:844–847.
 59. **Sims, R. J., III, and D. Reinberg.** 2006. Histone H3 Lys 4 methylation: caught in a bind? *Genes Dev.* **20**:2779–2786.
 60. **Straka, C., and W. Hörz.** 1991. A functional role for nucleosomes in the repression of a yeast promoter. *EMBO J.* **10**:361–368.
 61. **Suganuma, T., and J. L. Workman.** 2008. Crosstalk among histone modifications. *Cell* **135**:604–607.
 62. **Svaren, J., and W. Hörz.** 1995. Interplay between nucleosomes and transcription factors at the yeast *PHO5* promoter. *Semin. Cell Biol.* **6**:177–183.
 63. **Svaren, J., J. Schmitz, and W. Hörz.** 1994. The transactivation domain of Pho4 is required for nucleosome disruption at the *PHO5* promoter. *EMBO J.* **13**:4856–4862.
 64. **Szerlong, H. J., J. E. Prenni, J. K. Nyborg, and J. C. Hansen.** 2010. Activator-dependent p300 acetylation of chromatin in vitro: enhancement of transcription by disruption of repressive nucleosome-nucleosome interactions. *J. Biol. Chem.* **285**:31954–31964.
 65. **Uhler, J. P., C. Hertel, and J. Q. Svejstrup.** 2007. A role for noncoding transcription in activation of the yeast *PHO5* gene. *Proc. Natl. Acad. Sci. U. S. A.* **104**:8011–8016.
 66. **Venter, U., J. Svaren, J. Schmitz, A. Schmid, and W. Hörz.** 1994. A nucleosome precludes binding of the transcription factor Pho4 in vivo to a critical target site in the *PHO5* promoter. *EMBO J.* **13**:4848–4855.
 67. **Wang, Z., et al.** 2009. Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* **138**:1019–1031.
 68. **Williams, S. K., D. Truong, and J. K. Tyler.** 2008. Acetylation in the globular core of histone H3 on lysine-56 promotes chromatin disassembly during transcriptional activation. *Proc. Natl. Acad. Sci. U. S. A.* **105**:9000–9005.
 69. **Xu, Z., et al.** 2009. Bidirectional promoters generate pervasive transcription in yeast. *Nature* **457**:1033–1037.
 70. **Yang, X. J., and E. Seto.** 2008. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. *Nat. Rev. Mol. Cell Biol.* **9**:206–218.
 71. **Zhang, Y., et al.** 1998. SAP30, a novel protein conserved between human and yeast, is a component of a histone deacetylase complex. *Mol. Cell* **1**:1021–1031.
 72. **Zhou, J., B. O. Zhou, B. A. Lenzmeier, and J. Q. Zhou.** 2009. Histone deacetylase Rpd3 antagonizes Sir2-dependent silent chromatin propagation. *Nucleic Acids Res.* **37**:3699–3713.