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Recruitment of the NuA4 complex poises the *PHO5* promoter for chromatin remodeling and activation

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The remodeling of the promoter chromatin structure is a key event for the induction of the *PHO5* gene. Two DNA-binding proteins Pho2 and Pho4 are critical for this step. We found that the NuA4 histone acetyltransferase complex is essential for *PHO5* transcriptional induction without affecting Pho4 translocation upon phosphate starvation. Our data also indicate that NuA4 is critical for the chromatin remodeling event that occurs over the *PHO5* promoter prior to activation. Using Chromatin IP analysis, we found that Esa1-dependent histone H4 acetylation at the *PHO5* promoter correlates with specific recruitment of the NuA4 complex to this locus under repressing conditions. We demonstrate that the homeodomain transcriptional activator Pho2 is responsible for this recruitment *in vivo* and interacts directly with the NuA4 complex. Finally, we show that Pho4 is unable to bind the *PHO5* promoter without prior action of NuA4. These results indicate that, before induction, NuA4 complex recruitment by Pho2 is an essential event that presets the *PHO5* promoter for subsequent binding by Pho4, chromatin remodeling and transcription.

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Introduction

In *Saccharomyces cerevisiae*, phosphate metabolism is under tight control of the PHO system, which helps to adapt the cell to the consequence of external changes in inorganic phosphate availability. Phosphate deprivation from the culture medium results in an important induction of secreted acid phosphatase enzymes. The most abundant enzyme is the tightly regulated *PHO5* gene product whose expression is repressed at the transcriptional level by a particular promoter chromatin structure, making the regulation of this gene one of the most popular models to study the relation between chromatin structure and transcription (Svaren and Horz, 1997). Four stably positioned nucleosomes are present on the *PHO5* promoter and the induction of this gene correlates with the alteration of the structure of these nucleosomes. Two positive regulators, homeodomain protein Pho2 (Bas2) and basic helix–loop–helix factor Pho4, are essential for induction and for remodeling of the promoter chromatin structure. Pho4 binding to both of the UAS(s) is essential for chromatin remodeling and is observed only after phosphate starvation (Svaren and Horz, 1997). The critical binding of Pho4 to UAS2 is prevented by nucleosome −2, whose remodeling is essential to allow this interaction and subsequent transcriptional induction. The Pho4 protein is subjected to post-translational regulation by the Pho80–85 Cyclin–CDK complex that phosphorylates it in phosphate-rich media and prevents its nuclear localization (Svaren and Horz, 1997). The exact role of Pho2 in the *PHO5* transition is less clear. Pho2 was shown to interact and cooperate with Pho4 for binding at UAS1 and for an efficient transactivation at UAS2 (Barbaric *et al*, 1998).

In addition to Pho2 and 4, other activities play an important role in the chromatin remodeling at the *PHO5* promoter, which is the key event in the transition from repressed to activated state. The INO80 ATP-dependent chromatin remodeling complex is required for full activation, and the SWI/SNF complex has also been implicated by itself or in association with the histone variant Htz1 (Santisteban *et al*, 2000; Steger *et al*, 2003). Recently, two studies demonstrated that specific nucleosomes are not only remodeled during activation but in fact also displaced from the promoter DNA (Boeger *et al*, 2003; Reinke and Horz, 2003).

Studies have also pointed out the importance of histone acetylation in *PHO5* regulation; however, none demonstrated an absolute requirement for a specific histone acetyltransferase (HAT) in the transition from repressed to derepressed state. It was shown that the histone H3-specific Gcn5 HAT is not essential for derepression of the *PHO5* gene, but could affect the chromatin structure in the constitutively derepressed *Pho80* mutant (Gregory *et al*, 1998). Although Gcn5 does not affect the final *PHO5*-activated steady-state level, it seems to increase the rate of gene induction by accelerating *PHO5* chromatin remodeling (Barbaric *et al*, 2001). Deletion of the Rpd3 histone deacetylase loosens the repression by increasing *PHO5* expression in phosphate-rich media, and delays the inactivation after shifting from inducing to non-inducing medium (Svaren and Horz, 1997; Vogelauer *et al*, 2000). Importantly, direct evidence of histone tail involvement in the control of *PHO5* expression was obtained by genetic analysis. The activation of the *PHO5* promoter is significantly and specifically reduced after deletion of the histone H4 tail or mutation of the acetylatable lysines (Durrin *et al*, 1991). It was also shown that deletion of histone H4 tail, like deletion of Gcn5, delays the transition from repressed to activated *PHO5* (Barbaric *et al*, 2001). In contrast to *PHO5*, the coregulated *PHO8* gene is fully dependent on both Gcn5 and SWI/SNF for chromatin remodeling over its promoter upon induction (Gregory *et al*, 1999). Thus, chromatin

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acetylation by Gcn5 plays different roles in the induction of the coregulated *PHO5* and *PHO8* genes.

In the present work, we demonstrate that the NuA4 HAT complex is essential for *PHO5* transition from transcriptionally repressed to activated state and for the chromatin remodeling step over the promoter region. NuA4 becomes dispensable once *PHO5* is induced, arguing for an early role of presetting the promoter for activation. We demonstrate that the NuA4 complex is present at the *PHO5* promoter under repressive conditions, which parallels the reported presence of Esa1-dependent acetylated histone H4 isoforms under the same conditions (Vogelauer *et al*, 2000). We also found that the Pho2 homeodomain protein interacts specifically with the NuA4 complex and is responsible for specific recruitment of NuA4 to the *PHO5* promoter under uninduced conditions. Moreover, we show that, in the absence of NuA4, Pho4 is unable to bind the *PHO5* promoter *in vivo*. Altogether, these results suggest a mechanism in which NuA4 recruitment by Pho2 leads to local histone hyperacetylation, which in turn poises the promoter for rapid activation upon Pho4 nuclear translocation. Thus, the NuA4 complex plays an essential role in *PHO5* induction before the chromatin remodeling step by presetting the promoter for activation.

Results

The NuA4 complex is essential for transcriptional induction of the PHO5 gene

We have previously shown that the level of *PHO5* mRNA is reduced in several NuA4 mutants compared to wild-type strain (Boudreault *et al* (2003) and references therein). However, these observations were made only on basal non-induced levels of *PHO5* transcription. In order to assess the role of NuA4 in the activation of the *PHO5* gene, we analyzed by Northern blotting the rate of *PHO5* mRNA accumulation after shift to low phosphate (−Pi) media. In this experiment, we used a temperature-sensitive (ts) mutant for Esa1, NuA4 catalytic HAT subunit, the isogenic wild-type strain, and compared the induction of *PHO5* at permissive (RT) and restrictive temperature (37°C). We have previously demonstrated that this *esa1* ts mutant (*esa1-Δ414* (Clarke *et al*, 1999)) is deficient in NuA4 HAT activity at permissive temperature and that shifting to restrictive conditions completely abolishes the activity and disrupts the complex (Allard *et al*, 1999). In the wild-type strain, *PHO5* mRNA levels are significantly induced after 2 h in low Pi medium and reached the maximum level after 5 h at the permissive temperature or 4 h at 37°C (Figure 1A and B). In the mutant strain no induction is observed at the restrictive temperature, indicating that Esa1 and/or the NuA4 complex is essential for induction of the *PHO5* gene. Moreover, under permissive conditions the level of *PHO5* mRNA is significantly reduced in the *esa1* mutant compared to the wild type. No further induction was observed after longer incubation in low Pi media (data not shown). Almost identical results were also obtained with a different *esa1* ts mutant (*esa1-L254P* (Clarke *et al*, 1999)) (data not shown). These observations demonstrate that wild-type Esa1 activity is required for induction of the *PHO5* gene, and identify NuA4 as the first coactivator HAT complex essential for the transition from inactivated to activated state.

To investigate whether this effect is specific to *PHO5*, we analyzed the induction of the coregulated *PHO8* gene. After shifting the cell to low phosphate media, we observed an induction of the *PHO8* gene in the wild-type and *esa1* mutant

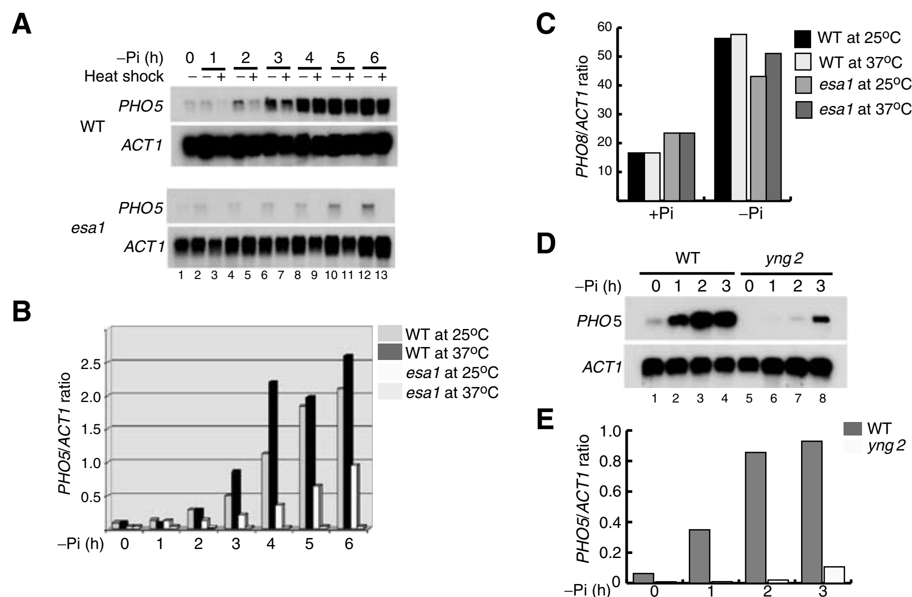

Figure 1 Esa1/NuA4 is essential for the transcriptional induction of the *PHO5* gene. (**A**) Transcriptional induction of the *PHO5* gene is severely impaired in the *esa1* ts mutant at restrictive temperature. WT or *esa1* ts mutant (*esa1*) were grown to mid-log phase in YPD at room temperature (25°C) and then transferred to phosphate-free medium (−Pi) for various times at (25°C) or heat shocked at (37°C). Extracted RNA was analyzed by Northern blots with *PHO5* and *ACT1* probes. (**B**) The histogram represents the *PHO5*/*ACT1* ratio for each condition shown in (A) (quantified by phosphorimager). (**C**) Transcriptional induction of the *PHO8* gene is NuA4 independent. As in (A), the WT or *esa1* mutant strains were grown in YPD (+Pi) and shifted to (−Pi) medium for 1 h at 25 or 37°C. The Northern blot was hybridized with *PHO8* and *ACT1* probes and the histogram represents the *PHO8*/*ACT1* ratio value ($\times 10^2$). (**D**) Deletion of the *YNG2* gene affects *PHO5* induction. Strains QY202 (WT) and QY203 (*yng2*) were grown in YPD and transferred to phosphate-free medium (−Pi) for 1, 2 and 3 h. RNA samples were analyzed as in (A). (**E**) Northern blot signals from (D) were quantified as in (B).

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strains at both permissive and restrictive temperatures (Figure 1C). These data demonstrate that Esa1 is not required for the induction of *PHO8*, indicating that its role is specific to *PHO5* gene activation.

We have previously demonstrated that cells carrying a deletion of *YNG2*, which encodes another NuA4 subunit, contains a complex with reduced nucleosomal HAT activity and lowered basal expression of *PHO5* (Nourani *et al*, 2001). We have also shown that Yng2 association with Esa1 greatly stimulates its activity towards chromatin substrates (Boudreault *et al*, 2003). In order to confirm that Esa1's role in *PHO5* induction is through NuA4 complex HAT activity, we analyzed *PHO5* transcript levels after phosphate starvation in *yng2* mutant and isogenic wild-type strains (Figure 1C and D). *PHO5* induction was detected after 1 h in the wild-type strain and reached a maximum level after 2 h. In contrast, the *yng2* mutant strain showed weak induction only after 3 h of incubation in low Pi media. However, in contrast to the *esa1* mutant, longer incubation under inducing conditions allows *PHO5* mRNA to accumulate (likely due to the reduced but still present NuA4 HAT activity, data not shown). Nevertheless, this result indicates that nucleosomal acetylation by the NuA4 complex is the key to *PHO5* transcription activation.

To address the possibility that NuA4 could indirectly affect *PHO5* induction by disrupting signaling to the Pho4 activator, we analyzed the localization of a Pho4–GFP fusion protein in the *esa1* and *yng2* mutants used above. In phosphate-rich media, there is no clear localization of Pho4–GFP in either wild-type or mutant strains (Figure 2). After 3 h of phosphate starvation, the majority of cells show nuclear accumulation of the fusion protein in both the wild-type and mutant strains. These results demonstrate that NuA4 does not affect Pho4 nuclear translocation upon phosphate starvation, suggesting that the complex is required at subsequent steps in the *PHO5* activation process.

The NuA4 complex is required for chromatin remodeling at the PHO5 promoter

The effect of *esa1* or *yng2* mutations on *PHO5* transcriptional induction could be due to a defect at the subsequent chromatin remodeling step. To this end, we compared chromatin remodeling at the *PHO5* promoter in both *esa1* and *yng2* mutants (Figure 3). Analyzing the accessibility of the *Cla*I restriction site located in the nucleosome −2 of the *PHO5* promoter is a reliable and quantitative way to assess the remodeling event (Svaren *et al*, 1995). We isolated nuclei from *esa1* mutant and wild-type (WT) strains after incubation in low Pi medium at the permissive and the restrictive temperature. In phosphate-rich medium, the *Cla*I site is poorly accessible in both wt and *esa1* mutant strains (Figure 3A, lanes 1, 2, 5 and 6). At 3 h after shifting to low Pi medium at the restrictive temperature, we observed a clear three-fold increase of the *Cla*I accessibility in wild type, while the level of digestion remained low in the *esa1* mutant (compare lanes 3, 4 with 7, 8). This indicates that Esa1 is required for the remodeling of the UAS2-containing nucleosome at the *PHO5* promoter upon induction. Even with longer incubation under inducing conditions at the permissive temperature (where some *PHO5* induction is detected in the *esa1* mutant, Figure 1A), *Cla*I digestion remained significantly lower in the mutant strain (compare lanes 10 and 12).

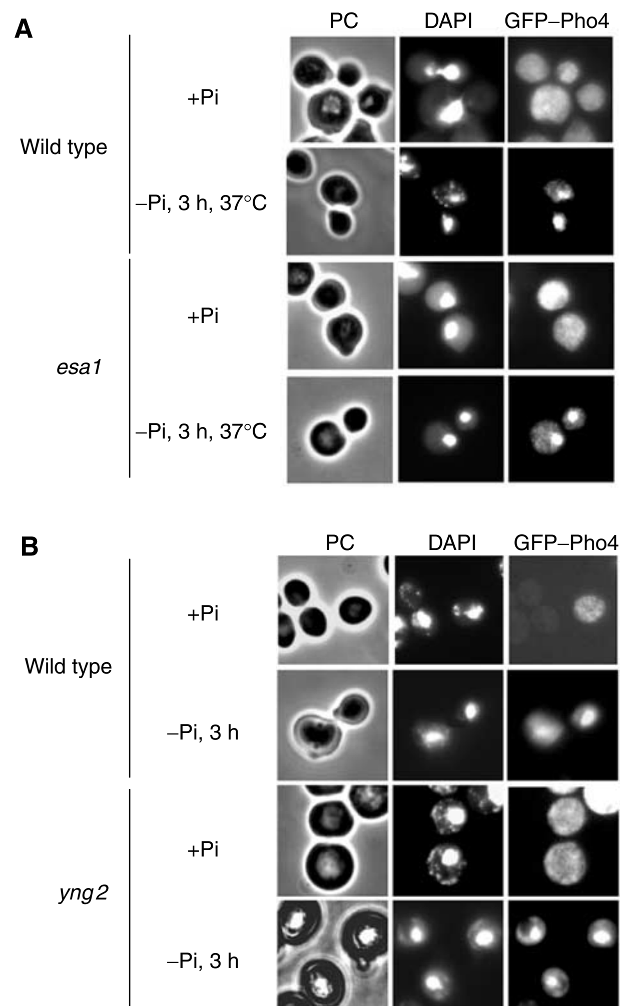

Figure 2 The Pho4 nuclear accumulation after phosphate starvation is not NuA4 dependent. (**A**) Esa1 does not affect Pho4 localization. The wild-type (WT) and *esa1* ts mutant (*esa1*) expressing a GFP–Pho4 fusion protein were cultured to mid log phase in YPD (+Pi) and then shifted to phosphate-deprived medium (−Pi) for 3 h at the restrictive temperature (37°C). The GFP–Pho4 fusion protein was observed by fluorescence microcopy (GFP–Pho4 panel). DAPI-stained nuclei are shown in the central panel, and cells visualized by phase contrast (PC) in the left panel. (**B**) Yng2 is not involved in Pho4 nuclear accumulation. The WT and *yng2* mutant expressing a GFP–Pho4 fusion protein were analyzed as in (A), but without heat shock.

These results demonstrate a perfect correlation between the defect in transcription activation and inefficient chromatin remodeling at the *PHO5* promoter in the *esa1* mutant, indicating that normal levels of Esa1 activity are required for normal opening of this promoter. Similar results were obtained when *yng2* mutants were tested for *Cla*I accessibility after 3 h of phosphate starvation (Figure 3B). Again, while the wild-type cells showed an increase in digestion under inducing conditions, accessibility remained unchanged in *yng2* cells (compare lanes 1–4 and 5–8). This indicates that NuA4 complex activity is critical for remodeling of this nucleosome at the *PHO5* promoter.

To further characterize the role of the NuA4 complex in the changes of nucleosomal organization at the *PHO5* promoter upon activation, *in vivo* DNaseI protection assays were performed and DNA was analyzed by Southern blotting

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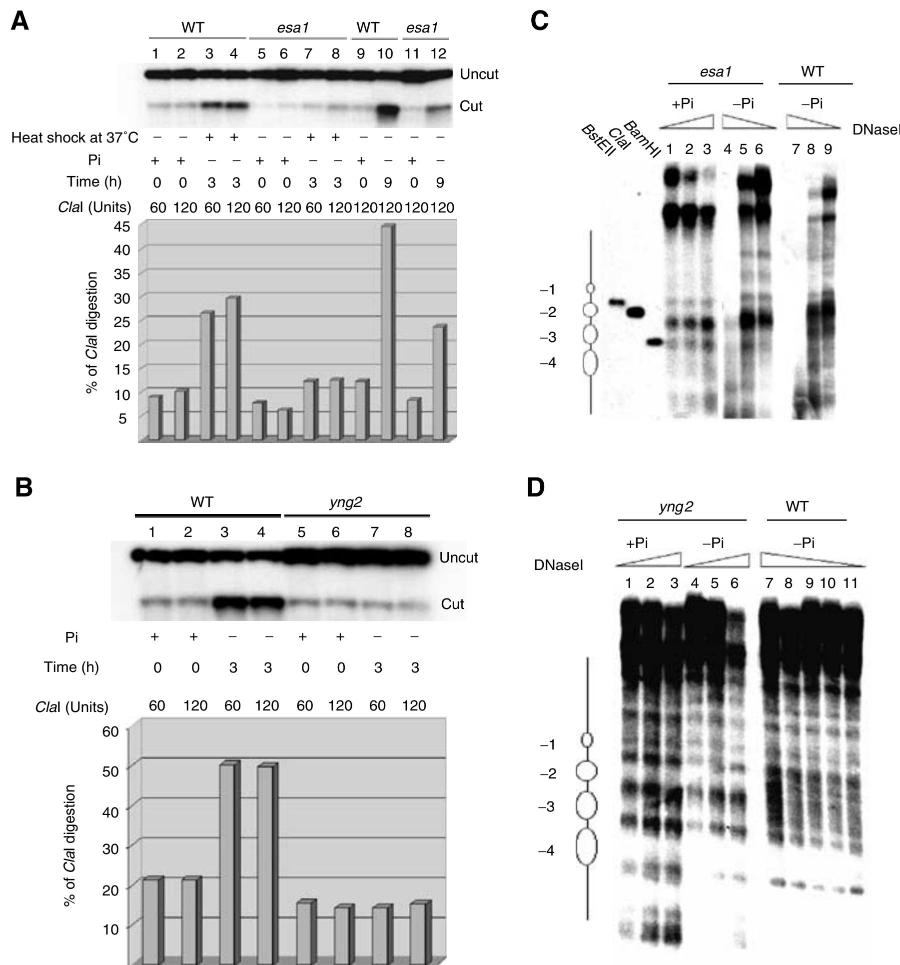

Figure 3 The NuA4 complex controls *PHO5* promoter remodeling during phosphate starvation. (**A**) Esa1 is required for the *PHO5* promoter transition upon phosphate deprivation. Nuclei were isolated from wild-type (WT) or *esa1* ts mutant (*esa1*) grown in YPD (+Pi) and shifted to phosphate-poor medium (−Pi) for 3 h at 37°C or 9 h at 25°C. The nuclei were treated with 60 or 120 Units of *Cla*I for 30 min, DNA was then isolated and analyzed by Southern blot. The histogram represents the percentage of *Cla*I cleavage (quantified by phosphorimager). (**B**) Yng2 has a major impact on *PHO5* promoter remodeling. Nuclei were isolated from wild type (WT) or *YNG2* deleted strain (*yng2*) grown in YPD (+Pi) and shifted to phosphate-poor medium (−Pi) for 3 h. The *Cla*I cleavage assay was performed as in (A). (**C**) *PHO5* chromatin is refractory to the remodeling in the *esa1* mutant strain. The nuclei were isolated from the *esa1* ts mutant grown in YPD (+Pi) or phosphate-deprived medium (−Pi) for 6 h (25°C), and also from wild-type cells grown 6 h in −Pi medium. In all, 100 mg of nuclei were digested by DNaseI (0.5, 1 or 2 U/ml) for 20 min. Isolated DNA was analyzed by Southern blot. Positioned nucleosomes at *PHO5* promoter are represented on the left. (**D**) Nucleosomes are not remodeled in *yng2* mutant cells after 3 h under inducing conditions. The nuclei were isolated from the *yng2* mutant grown in YPD (+Pi) or phosphate-deprived medium (−Pi) for 3 h and also from wild type grown for 3 h in −Pi medium. DNaseI assays were carried out as in (C), except 0.25, 0.125, 0.0625, 0.031 or 0.015 U/ml of enzyme was used for the WT.

(Figure 3C and D). Nuclei were isolated from *esa1* and *yng2* mutants grown in +Pi (lane 1–3) or −Pi media (lanes 4–6) and digested with increasing amount of DNaseI. The positioned nucleosomes are clearly present on the *PHO5* promoter in *esa1* and *yng2* mutants grown under non-inducing conditions. When *esa1* mutant cells are grown in −Pi media, there is a minor change in the DNaseI profile over the *PHO5* promoter, which encompasses the −2 and −3 nucleosomes (Figure 3C, lanes 4–6). This moderate change correlates with the reduced *Cla*I accessibility and a deficient induction of the *esa1* mutant at a permissive temperature (see above). In contrast, the DNaseI digestion pattern shows an extensive hypersensitive region overlapping the −2 and −3 nucleosomes in the wild-type strain after incubation in −Pi media (Figure 3C, lanes 7–9). This indicates that the *PHO5* promoter is fully remodeled under these conditions. Similar results were obtained with the *yng2* mutant cells (Figure 3D). After 3 h of phosphate starvation, the pattern of DNaseI digestion remained unchanged, showing the presence of the four positioned nucleosomes (compare lanes 1–3 and 4–6). In contrast, the incubation of the wild-type strain under inducing conditions resulted in a specific loss of DNaseI protected regions corresponding to nucleosomes −2 and −3 (lanes 7–11). Altogether, these results indicate that the NuA4 complex affects the dynamic reconfiguration of *PHO5* promoter chromatin structure, which is in fact responsible for the effect observed at the transcriptional level.

NuA4 is no longer required once the PHO5 gene is activated

We have demonstrated that the *PHO5* gene cannot be induced in the absence of NuA4. To determine whether the continued presence of NuA4 is required after transcriptional induction of *PHO5*, we analyzed the effect of *in vivo* NuA4

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disruption on *PHO5*-activated transcription. To this end, the *esa1* temperature-sensitive mutant strain was grown at the permissive temperature in low phosphate media until maximum induction was reached and then shifted to the nonpermissive temperature. The results presented in Figure 4A show the induction of *PHO5* mRNA levels when cells are grown at room temperature in low Pi (lanes 1–3). *PHO5* transcript levels remained unchanged after cells were shifted to 37°C (lanes 4 and 5). Similar results were obtained when the heat shock was performed after 3 h under inducing conditions (data not shown). In comparison, returning the cells to phosphate-rich media provokes full repression of *PHO5* transcription and disappearance of mRNA signal within an hour (data not shown). These observations strongly suggest that disrupting the NuA4 complex has no effect on transcription of the already induced *PHO5* gene. To confirm this result, we analyzed the effect of NuA4 disruption in *pho80* mutant cells, which exhibit constitutively derepressed *PHO5* transcription. Wild-type, *pho80* and *pho80 esa1* double mutants were grown in rich media and subjected to heat shock for 1 or 2 h (Figure 4B). As expected, the deletion of the Pho80 cyclin-encoding gene resulted in a large increase of *PHO5* signal (compare lanes 1, 4 and 7). This derepressed signal remains largely unchanged after 1 or 2 h of heat shock either in *pho80* or *pho80 esa1* double mutant cells (lanes 4–6 and 7–9). These results clearly demonstrate that NuA4 activity is not continuously required for *PHO5* transcription. Thus, NuA4 is required for the initial transition from repressed to derepressed state, but has only a minor impact in maintaining transcriptionally active conditions.

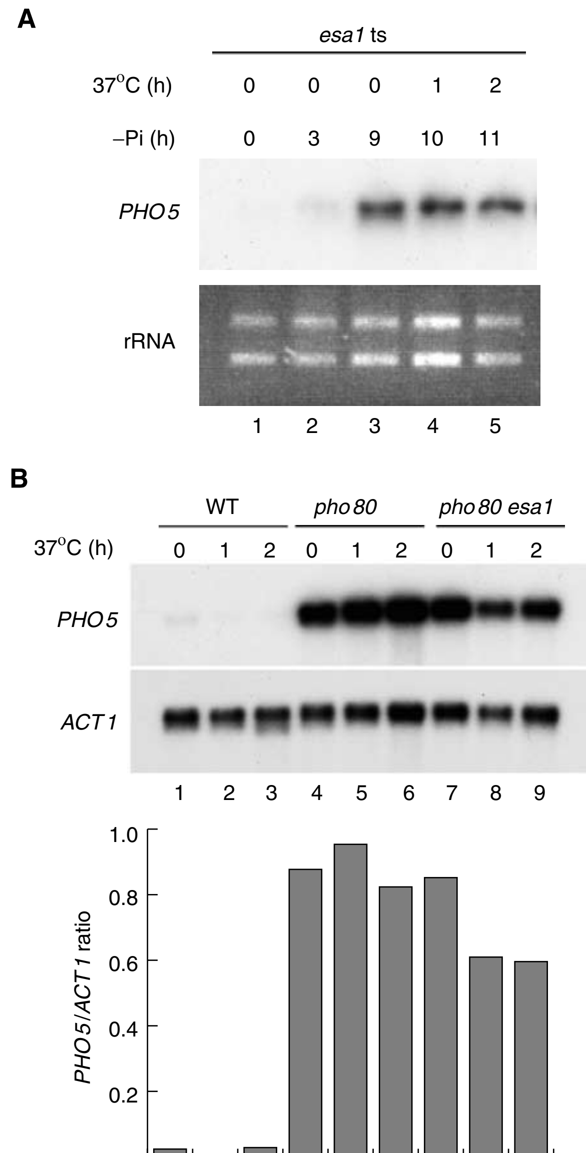

Figure 4 Esa1 is not required for *PHO5* transcription after promoter remodeling. (**A**) Esa1 depletion after induction in phosphate-deprived medium does not affect *PHO5* transcription. The *esa1* ts mutant (*esa1*) was grown in YPD, transferred to phosphate-free medium (−Pi) for 9 h at 25°C and then heat shocked for 1 or 2 h at 37°C. *PHO5* mRNA signals were analyzed by Northern blot. Ribosomal RNA (rRNA) is used as loading control. (**B**) Esa1 is not required for *PHO5* transcription in the constitutively derepressed *Pho80* mutant. The wild-type (WT), *PHO80* deleted and *pho80/esa1* ts mutants were grown at 25°C and then heat shocked for 1 or 2 h at 37°C. RNA samples were analyzed as in Figure 1.

The NuA4 complex is present at the PHO5 promoter in vivo under repressing conditions

Chromatin immunoprecipitation (ChIP) experiments under repressing conditions have shown that Esa1-dependent acetylated histone H4 isoforms are enriched at the *PHO5* promoter (Vogelauer *et al*, 2000). We have obtained similar results with antibodies for the hyperacetytated forms of histone H4 and H2A, which are generated by Esa1/NuA4 *in vivo* and *in vitro* (data not shown (Boudreault *et al*, 2003)). If there is specific Esa1-dependent histone H4/H2A acetylation prior to gene induction, we would expect specific association of NuA4 at the *PHO5* promoter region under repressing conditions. To test this possibility, ChIP analysis was performed to study the association of a TAP-tagged version of the Epl1 protein, an essential NuA4 subunit (Boudreault *et al*, 2003), to the *PHO5* promoter. Crosslinked chromatin from isogenic strains containing either Epl1-TAP or Dot1-TAP as control was precipitated with IgG-sepharose beads and analyzed by PCR for the presence of *PHO5*, *SSA4* and *GAL7* promoter DNA regions. *SSA4* served as a positive control and *GAL7* as a negative control according to previously published data (Reid *et al*, 2000). As shown in Figure 5, we observed *PHO5* and *SSA4* promoter DNA specifically precipitated from Epl1-TAP chromatin (lanes 2 and 4) when compared to Dot1-TAP chromatin

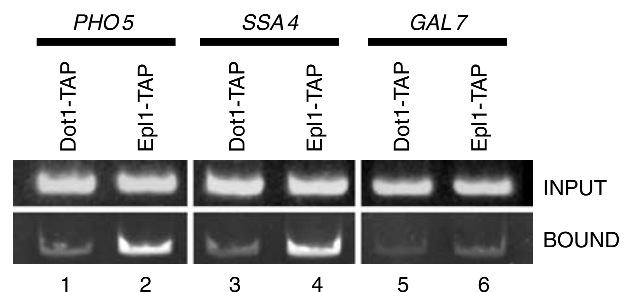

Figure 5 NuA4 is present at the *PHO5* promoter *in vivo*. ChIP experiments were performed on formaldehyde crosslinked chromatin extracted from Epl1-TAP- or Dot1-TAP-expressing strains. The IgG-sepharose-bound and input materials were amplified by PCR with specific pairs of primers for *PHO5*, *SSA4* and *GAL7* promoters. Epl1-TAP, but not Dot1-TAP, is specifically found at *PHO5* and *SSA4* promoters, while absent from *GAL7* promoter.

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(lanes 1 and 3) and *GAL7* signals (lanes 5–6). Similar results were obtained with other NuA4-tagged subunits (data not shown and see below). These data demonstrate a specific recruitment of the NuA4 complex to the *PHO5* promoter under repressing conditions prior to gene activation. Surprisingly, we could not detect an increase in NuA4 at the *PHO5* promoter upon gene induction. In fact, a significant drop of NuA4 binding was observed after 3 h under inducing conditions (Supplementary Figure S1). This correlates with the reported unchanged levels of histone H4 acetylation during *PHO5* activation (Vogelauer *et al*, 2000; Reinke and Horz, 2003; Steger *et al*, 2003). ChIP experiments after 3 h under inducing conditions confirmed the previously reported loss of specific nucleosomes on the *PHO5* promoter during activation and the unchanged levels of H4 acetylation on those remaining (Reinke and Horz, 2003) (Supplementary Figure S1).

The NuA4 complex interacts with Pho2 homeodomain protein in vitro and in vivo

As *PHO5* transition from repressed to activated state requires both Pho2 and Pho4 activators, either or both of these could be responsible for recruitment of NuA4 to the promoter. We tested a direct physical interaction by *in vitro* GST-pull-down using purified NuA4 complex and a series of GST-fusion proteins harboring different parts of Pho2 and the mapped activation domains Pho4, Rap1, Gcn4 and VP16 (Figure 6A and B). Gcn4 and VP16 fusions are used as positive controls (Utley *et al*, 1998). The N-terminal domain of Pho4 is essential for its ability to challenge the repressive chromatin of the *PHO5* promoter (Svaren and Horz, 1997). Beads carrying equal amounts of GST fusions were incubated with the NuA4 complex, washed and assayed for nucleosomal HAT activity (Figure 6C). Importantly, Pho2 and Pho4 are not substrates for acetylation by NuA4 *in vitro* (data not shown), arguing against such a role for the complex in *PHO5* regulation. As expected, Gcn4 and VP16 were able to pull down significant NuA4 HAT activity onto the beads (lanes 8–9). While GST alone, Rap1 and Pho2C failed to interact with NuA4, weak HAT activity was recovered on beads with the Pho4 and Pho2mid regions (lanes 2–4, 6 and 7). Strikingly, Pho2 N-terminal domain showed the highest affinity for the NuA4 complex, even when compared to Gcn4 and VP16 positive controls (lane 5). These results demonstrate a strong direct interaction between Pho2 N-terminal domain and the NuA4 complex *in vitro*. This interaction seems specific to NuA4 and does not involve Tra1, Epl1, Yng2 and Esa1 subunits as two other purified HAT complexes, SAGA and picNuA4 (Boudreault *et al*, 2003), are not similarly pulled down by Pho2 (Supplementary Figure S2). To determine whether this NuA4–Pho2 physical interaction also exists in the cell, we performed co-immunoprecipitation experiments using whole-cell extract from a TAP-tagged Pho2 strain expressing or not an HA-tagged version of the NuA4 subunit Epl1 (Figure 6D). Anti-HA Western blot on the material associated with the IgG-sepharose beads showed the presence of significant amounts of HA-Epl1 when both Pho2-TAP and HA-Epl1 are co-expressed (compare lanes 4–6). These *in vivo* data confirm direct physical interaction between NuA4 and Pho2. Furthermore, NuA4-Pho2 interaction was detected *in vivo* under normal growth conditions, that is, high phosphate, arguing that Pho2 is responsible for recruitment of the NuA4 complex at the *PHO5* promoter when the gene is fully repressed.

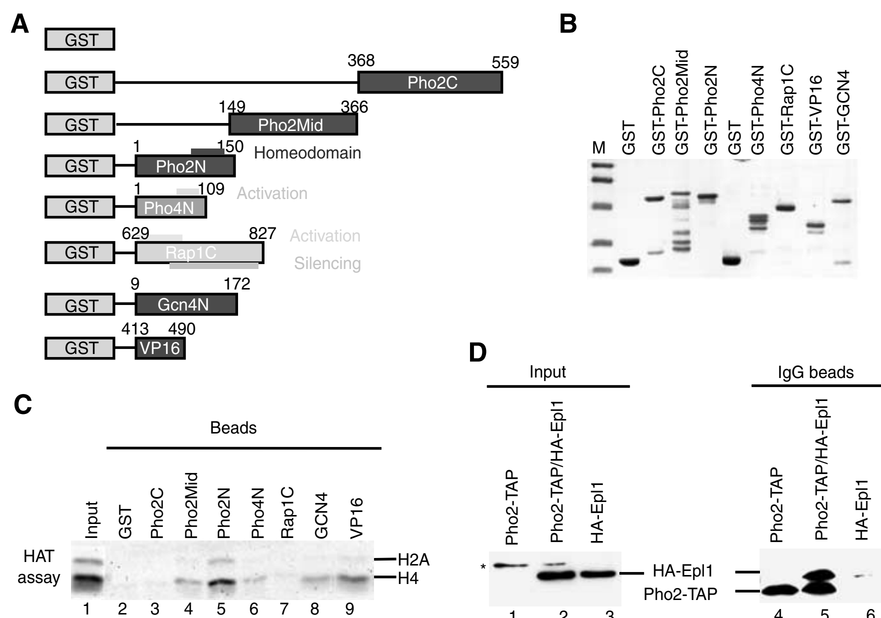

Figure 6 NuA4 interacts specifically with Pho2 *in vitro* and *in vivo*. (**A**) Diagram illustrating the GST fusion proteins used in this experiment. (**B**) Normalized amounts of the indicated fusion proteins were run in 12% SDS–PAGE and coomassie stained. Relative amounts of GST-Pho4N, -VP16 and -Gcn4 were increased two-fold in the pull-down assays. (**C**) Pho2 N-terminal region interacts with NuA4 *in vitro*. Equal amounts of input and beads from the GST pull-downs with purified NuA4 were assayed for nucleosomal HAT activity. (**D**) Coimmunoprecipitation of Pho2 with the NuA4 complex. Whole-cell extracts from cells expressing Pho2-TAP, HA-Epl1 or both proteins were incubated with IgG beads. The input and bead-associated material were analyzed by Western blots. Pho2-TAP is detected only on beads, not in input/cell extracts (asterisk marks a nonspecific band).

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Pho2, but not Pho4, is responsible for recruitment of the NuA4 complex to PHO5 promoter region

To test the idea that Pho2 recruits NuA4 *in vivo* under repressing conditions, we analyzed the association of the NuA4 complex to the *PHO5* promoter region in phosphate-rich media by ChIP assays. Formaldehyde crosslinked chromatin was extracted from a strain expressing the Epl1–13MYC fusion protein in a wild-type or a *pho2*Δ background (Figure 7A). Immunoprecipitated material demonstrates the presence of *PHO5*-specific DNA only when Epl1-13MYC was expressed in the wild-type context and assayed with anti-MYC antibody (lanes 5–8). No enrichment was observed with anti-HA antibody or at other loci. These data clearly demonstrate that Pho2 is required for recruitment of NuA4 complex to *PHO5* promoter.

It has been assumed that Pho2 presence on the *PHO5* promoter always occurs through cooperative binding with Pho4. To elucidate whether the presence of NuA4 on *PHO5* was dependent on Pho2–4 cooperation, we performed ChIP analysis in Δ*pho2* and Δ*pho4* strains (Figure 7B). Using anti-Esa1 and preimmune serum, we confirmed that NuA4 binding the *PHO5* promoter is dependent on Pho2 (compare wt to *pho2* panels). The signal detected at another locus (or with preimmune serum) was not affected by *PHO2* deletion. On the other hand, deletion of *PHO4* had no effect on NuA4 at the *PHO5* promoter (*pho4* panels). These data indicate that NuA4 is recruited to *PHO5* under repressing conditions by the homeodomain protein Pho2 in the absence of its Pho4 partner.

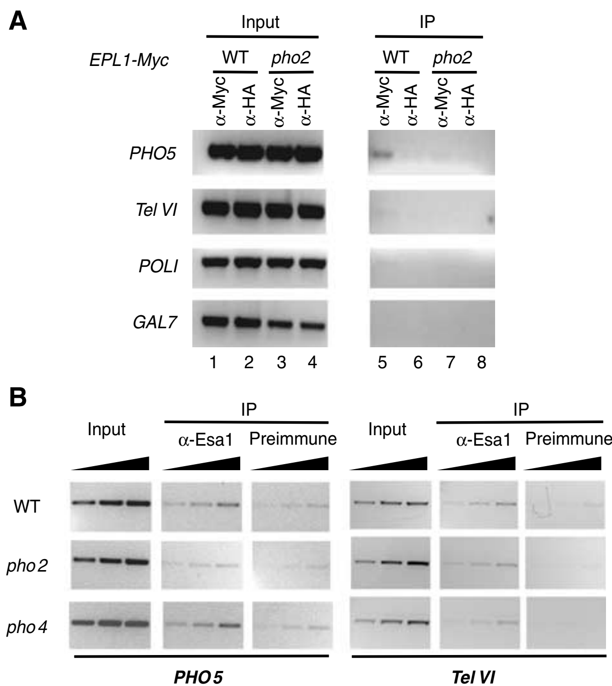

Figure 7 NuA4 specific recruitment to the *PHO5* promoter is Pho2 dependent. (**A**) Recruitment of NuA4 to the *PHO5* promoter is lost in *pho2* mutant cells. ChIPs with indicated antibodies were performed on formaldehyde cross-linked chromatin extracted from wild-type or *pho2*-deleted strains both expressing the Epl1-13Myc-tagged protein. The presence of the *PHO5*, TelVI (ORF *YFR057w* near telomere region), *POLI* and *GAL7* promoter DNA in both the immunoprecipitated and input material was assayed by PCR. (**B**) NuA4 recruitment is dependent on Pho2 but not Pho4. Formaldehyde crosslinked chromatin was extracted from wild-type, *pho2* and *pho4* strains grown in phosphate-rich medium. ChIPs with anti-Esa1 and preimmune sera were performed and increasing amounts (0.5×, 1× and 2×) of input and bound material were used in PCR reactions to amplify either *PHO5* promoter region (lanes 1–9) or TelVI (lanes 10–18) as control locus.

To confirm directly the presence of Pho2 at the *PHO5* promoter under repressing conditions, we performed ChIPs using an anti-Pho2 polyclonal serum (Bhoite *et al*, 2002) or an HA-tagged Pho2-expressing strain. In both cases, we detected the presence of Pho2 at the *PHO5* promoter with different chromatin preparations. The results obtained with anti-Pho2 show a 2.5-fold enrichment of Pho2 at the *PHO5* promoter (Figure 8A). As for NuA4, the detected presence of Pho2 was tested for dependence on cooperative binding with Pho4. Pho2 presence at the *PHO5* promoter under repressing conditions is not affected by deletion of the *PHO4* gene (Figure 8B), again indicating that Pho2 recruits NuA4 independently of Pho4. Recruitment of NuA4 requires Pho2 to interact with an activator-binding domain in the HAT complex. Recently, we produced a strain (*epl1(1–380)*) in which the nucleosomal acetyltransferase core of NuA4 (Esa1/Epl1/Yng2) is physically dissociated from the rest of the complex (Boudreault *et al*, 2003). This picNuA4 complex cannot be recruited by activators like Pho2 *in vitro*, while it can provide global non-targeted H4/H2A acetylation *in vivo* (Boudreault *et al*, 2003; Supplementary Figure S2). Thus, if NuA4 must be specifically recruited by Pho2 to allow *PHO5* gene activation, it should not be targeted in the *epl1(1–380)* strain. Indeed, the *PHO5* gene cannot be efficiently derepressed in these mutant cells (Figure 8C, compare lanes 1–4 to 5–8). This also shows that the global HAT action of Esa1/picNuA4 still present in these cells is not sufficient to allow *PHO5* activation.

NuA4 action is critical to allow Pho4 binding to the PHO5 promoter

As NuA4 is recruited to the *PHO5* promoter prior to Pho4 nuclear translocation under inducing conditions, and as NuA4, like Pho4, is required for chromatin remodeling and transcription activation, we analyzed Pho4 binding to the *PHO5* promoter in the absence of NuA4 after phosphate starvation. When normal cells are placed under inducing conditions, we observe a rapid specific enrichment of Pho4 at the *PHO5* promoter, which follows its nuclear translocation (Figure 9A, WT −Pi versus +Pi). In contrast, the *esa1* mutant strain did not show such enrichment after 3 h under inducing conditions at the nonpermissive temperature, even though Pho4 is clearly nuclear in these cells (*esa1* −Pi versus +Pi). When the cells are placed in phosphate-free media for a longer period of time (6 h), Pho4 is very highly enriched at *PHO5* in normal cells, while *esa1* mutant cells exhibit at least 10-fold less Pho4 at the *PHO5* promoter (Figure 9B). These data demonstrate that prior action of NuA4 is critical to allow Pho4 binding to the *PHO5* promoter upon nuclear translocation. This also explains why NuA4 is required for chromatin remodeling and transcription activation as both depend on Pho4 binding.

To test our model depicting an essential specific link between Pho2 and NuA4 to allow *PHO5* promoter acetylation and efficient Pho4 binding, we used a system in which Pho2 is dispensable for derepression. It was shown that when Pho4 is overexpressed *in vivo*, Pho2 is no longer necessary for

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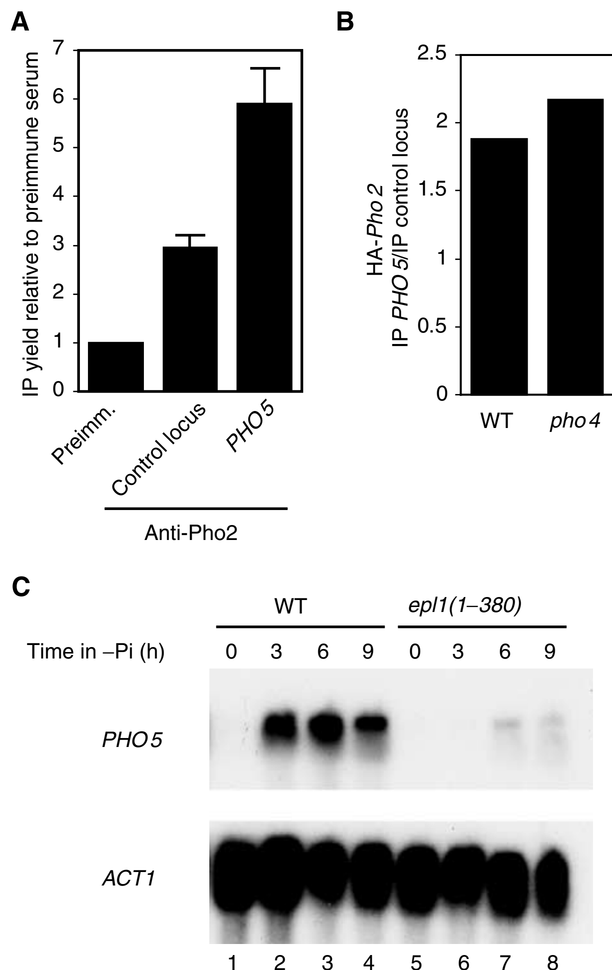

Figure 8 Pho2 is present at the *PHO5* promoter under repressing conditions and recruitment of Esa1 activity, not its global HAT action, is required for *PHO5* activation. (**A**) ChIP experiment showing the specific enrichment of Pho2 at the *PHO5* promoter *in vivo* in phosphate-rich media. Signals were quantified by real-time PCR from two different IPs with polyclonal anti-Pho2 serum. A noncoding region of chromosome V is used as negative control and data are presented as a ratio of IP yield (bound/input) with anti-Pho2 versus preimmune serum at each locus. Similar results were obtained with two other chromatin preparations and in three experiments using HA-tagged Pho2 to perform the IP. (**B**) Similar ChIP experiments in wild type and *PHO4* deleted background. HA-Pho2-expressing strains were used with non-tagged strains as controls. *PHO5* and control locus IP signals (bound/input) in the nontagged strains were subtracted from the values in the HA-tagged strains. The HA-Pho2 enrichment is then presented as a ratio of *PHO5* IP versus control locus IP. *PHO4* deletion had no effect on Pho2 presence at *PHO5*, a result reproduced with four independent chromatin samples with anti-HA and anti-Pho2 serum. (**C**) A recruitable form of Esa1 HAT, not its global action, is required for *PHO5* gene activation. A strain expressing a truncated version of Epl1, *epl1(1–380)*, contains only a globally acting nontargeted form of Esa1 within the Piccolo NuA4 complex (Boudreault *et al*, 2003). Wild-type (WT) and *epl1(1–380)* mutant cells were incubated for the indicated period of time in phosphate-free media and analyzed by Northern blot as in Figure 1A.

PHO5 gene activation (Fascher *et al*, 1990). Bypassing Pho2 requirement should also make NuA4 dispensable for activation. We overexpressed Pho4 in an *esa1* ts strain and analyzed *PHO5* induction at the nonpermissive temperature (Figure 9C). Clearly, overexpression of Pho4 activates the *PHO5* gene in *esa1* mutant cells even at 37° (compare lanes 1–6 to 7–12). Thus, these data indicate that NuA4 requirement for *PHO5* activation is specifically linked to Pho2 function, supporting the model of specific and direct local recruitment by the activator. It also indicates that a major function of NuA4 at the *PHO5* promoter is indeed to facilitate Pho4 binding upon its nuclear translocation. The NuA4 defect can be overcome by overexpression of Pho4, which drives binding to the promoter in the absence of the complex, bypassing the need for presetting by NuA4.

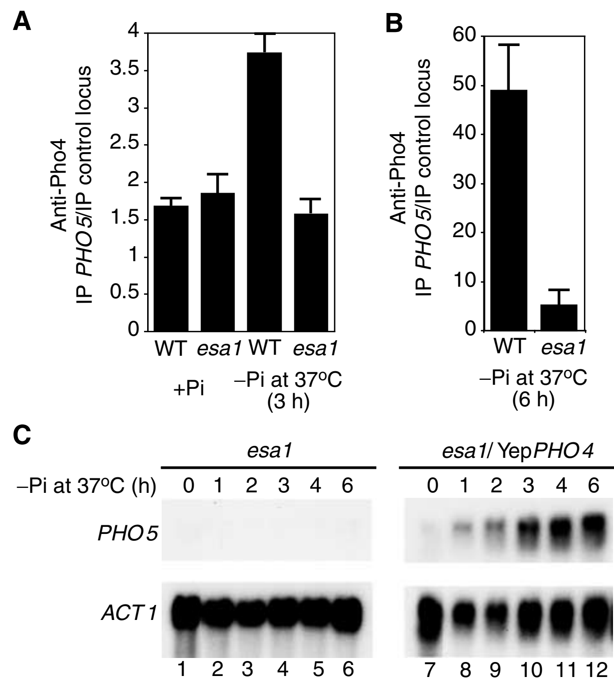

Figure 9 NuA4 action is necessary to allow Pho4 binding to the *PHO5* promoter, but is dispensable upon overexpression of Pho4. (**A**) Pho4 is enriched at the *PHO5* promoter in wild-type but not *esa1* mutant cells under inducing conditions. ChIP experiment using anti-Pho4 and preimmune sera with wild-type or *esa1* ts cells grown in phosphate-rich (+Pi) media at 25°C or placed for 3 h in phosphate-free (−Pi) media at 37°C. IP ratios were calculated by real-time PCR reactions and presented as in Figure 8B using preimmune serum signals as subtracted backgrounds. (**B**) A similar ChIP experiment was performed with wild-type and mutant cells incubated for 6 h under inducing conditions at 37°C. Under these conditions, the presence of Pho4 at the *PHO5* promoter is 10-fold higher in wild type than in mutant cells. (**C**) Overexpression of Pho4 bypasses both Pho2 and NuA4 requirements for *PHO5* activation. *esa1* ts mutant cells were transformed with a high copy vector that drives overexpression of the Pho4 transactivator. Cells were incubated in low phosphate media at the non-permissive temperature for the indicated periods of time and analyzed by Northern blot.

Discussion

In addition to the DNA-binding factors, Pho2 and Pho4, two well-studied mechanisms including the remodeling of nucleosomes by ATP-dependent complexes such as SWI/SNF or INO80, and the covalent modifications of histone tails by activities such as Gcn5-containing complexes, are involved in *PHO5* activation. However, a number of studies suggest that none of these coactivator activities is independently fully essential for *PHO5* transcriptional induction (see Introduction and references therein). In our work, we provide clear evidence that the NuA4 HAT complex is absolutely required

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for derepression of the *PHO5* gene in response to phosphate starvation. Indeed, when phosphate deprivation is carried out in parallel with NuA4 complex depletion, the *PHO5* gene remains fully repressed. The role of histone acetylation in *PHO5* derepression is not a new observation. The deletion or mutation of the histone H4 tail decreases either the rate of derepression or the level of induction (Durrin *et al*, 1991; Barbaric *et al*, 2001). However, these studies showed that, while H4 acetylation was important for the rate and level of induction, it was not essential for *PHO5* activation. The absolute requirement of Esa1/NuA4 could be explained by the fact that it acetylates both H4 and H2A N-terminal tails *in vivo* (Boudreault *et al*, 2003). Furthermore, it was previously shown that histone H2A tail is important for the repression of basal transcription of the *PHO5* gene (Lenfant *et al*, 1996). Thus, simultaneous mutations of H4 and H2A tails in the same cell could disable *PHO5* induction as NuA4 mutants do. On the other hand, NuA4 might play an additional role at the *PHO5* promoter not related to acetylation of histone H4/H2A tails. For instance, NuA4 may acetylate different unknown non-histone substrates or simply co-activate transcription independently of acetylation.

It is important to compare the involvement of Esa1/NuA4 versus Gcn5/SAGA HAT complexes in *PHO5* activation. While Gcn5 is only required for the normal rate of chromatin remodeling and activation (Barbaric *et al*, 2001), Esa1 is fully required for these events (Figure 3). While Gcn5 affects *PHO5* transcription in a constitutively derepressed state (*pho80* mutant; Gregory *et al*, 1998), Esa1 has no effect in such a context and is dispensable once the gene is activated (Figure 4). While SAGA is found at the *PHO5* promoter only after phosphate starvation in a Pho4-dependent manner (Barbaric *et al*, 2003), NuA4 is recruited by Pho2 before activation (under repressing conditions) in a Pho4-independent manner (Figures 5–8). While histone H3 acetylation increases over the promoter during *PHO5* activation (before nucleosomes unfold), histone H4/H2A acetylation remains largely unchanged (Reinke and Horz, 2003; supplementary Figure S1). These data indicate that SAGA plays a role at a later step during the activation process, while NuA4 action is essential and occurs prior to Pho4 translocation to the nucleus.

We have demonstrated that NuA4 is not only required for *PHO5* transcriptional induction but also for chromatin remodeling over the promoter region (Figure 3). Our results clearly suggest that the essential requirement of NuA4 is related to the remodeling step and that it is specific to *PHO5* as the same effect is not observed on the coregulated *PHO8* gene (Figure 1). It was then surprising to find NuA4 at the *PHO5* promoter under repressing conditions prior to activation (Figure 5). However, it was already known that *PHO5* promoter region was enriched in Esa1-dependent hyperacetylated histone H4 under the repressive conditions (Vogelauer *et al*, 2000). This was often attributed to the global nontargeted action of Esa1. On the contrary, here we clearly show that the presence of Esa1 is dependent on both a DNA-bound transcription factor and the recruitable integrity of the NuA4 complex (Figures 7 and 8). Targeted hyperacetylation of nucleosomal histones under repressive conditions could prepare the promoter and potentiate a rapid remodeling step, followed by transcriptional induction in response to external stimulus.

How does NuA4-dependent hyperacetylation pre-set a promoter for rapid chromatin remodeling and activation? One possibility is that the DNA is rendered more accessible in hyperacetylated chromatin, which would allow efficient binding of Pho4 upon its translocation to the nucleus. Pho4 could then recruit partners to initiate the remodeling step and subsequent transcription. Indeed, Pho2 was implicated in Pho4 recruitment at the critical UAS1, while it plays a coactivator role at UAS2 (Barbaric *et al*, 1998). Accordingly, we show that Pho4 is clearly unable to bind *PHO5* promoter in the absence of NuA4 action (Figure 9A and B). The absence of Pho4 binding also explains NuA4 requirement for chromatin remodeling. In agreement with a role in presetting the promoter for factor binding, Pho4 overexpression bypasses Pho2 requirement for its binding and makes NuA4 dispensable for *PHO5* activation (Figure 9C) (Fascher *et al*, 1990). In addition, acetylation of specific lysines by NuA4 could be a mark for the binding of proteins or complexes required for the subsequent chromatin remodeling step. Indeed, histone acetylation by either SAGA or NuA4 HAT complexes was shown to stabilize SWI/SNF and SAGA binding to a promoter *in vitro* through bromodomain interactions (Hassan *et al*, 2002).

It is interesting to compare the co-regulated *PHO5* and *PHO8* genes. While *PHO5* requires Esa1/NuA4 activity (this study), *PHO8* needs Gcn5/SAGA (Gregory *et al*, 1999). Both genes require the Pho4 activator. Pho2 functions also on both genes, but is only essential for *PHO5* induction (Munsterkotter *et al*, 2000). In agreement with a specific link between Pho2 and NuA4, Esa1 is also dispensable for *PHO8* induction (Figure 1). Pho4 is able to bind *PHO8* promoter in the absence of SAGA and SWI/SNF (Gregory *et al*, 1999), supporting our findings that specific NuA4-dependent acetylation of *PHO5* under repressing conditions is required to allow efficient binding of Pho4 upon its translocation (Figure 9).

Our identification of transcription factor Pho2 as a specific direct target of the NuA4 complex is important, as this is the first natural NuA4 recruiter identified to date. Preliminary data support the Pho2-NuA4 functional link for transcription activation of other genes *in vivo* (A Auger and J Côté, unpublished data). While Pho2 was previously considered to always require cooperation with Pho4 for binding at *PHO5*, we clearly show the association of Pho2 under repressing conditions in the absence of Pho4 (Figures 6–8). Furthermore, Pho2 was shown to bind by itself *PHO5* promoter sites with high affinity *in vitro* (Barbaric *et al*, 1998; Terrell *et al*, 2002). The region of Pho2 responsible for NuA4 interaction maps away from the recently characterized transcription activation domain at the C-terminus (Bhoite *et al*, 2002). This could be explained by the different roles of Pho2 at UAS1 and UAS2 (Barbaric *et al*, 1998). Pho2 action at UAS1 is the key to Pho4 recruitment/binding, which in turn is essential for binding and remodeling at UAS2. Pho2 action at UAS2 is mainly for transactivation with Pho4. We suggest that the Pho2 homeodomain-containing region responsible for NuA4 recruitment is critical at UAS1, while the C-terminal transactivation domain plays a NuA4-independent role at UAS2. Accordingly, chromatin remodeling is required for Pho4 binding to UAS2, not UAS1 (Steger *et al*, 2003).

In conclusion, our data demonstrate that the NuA4 complex is recruited to the *PHO5* promoter by the homeodomain

factor Pho2 under repressing conditions. This recruitment creates a region of histone hyperacetylation that poises the promoter for activation. This pre-setting by NuA4 is essential to allow Pho4 binding after its nuclear translocation upon phosphate starvation, subsequent chromatin remodeling over the promoter region and transcription activation. Such pre-setting mechanism by homeodomain factor–HAT complex interactions may be conserved as the bicoid-related factor Pitx2 was shown to serve as a competence factor required for ordered recruitment of specific co-activator complexes, including the NuA4 highly related Tip60 complex, to the Cyclin D2 promoter (Kioussi *et al*, 2002).

Materials and methods

Yeast strains and plasmids

The *esa1-Δ414* (LPY3123), *esa1-L254P* (LPY3500, used in Figure 9) and *yng2*Δ (QY203) mutant and isogenic wild-type strains were described (Clarke *et al*, 1999; Nourani *et al*, 2001). The QY303 strain was produced by cloning the *esa1-Δ414* ts allele of LPY3123 into pFL36 (ARS/CEN/LEU2 vector with *ESA1* promoter) to cover the *ESA1* deletion in the BY4742 background. The haploid *esa1-ts Pho80*Δ strain was isolated after sporulation of the diploid strain obtained by crossing QY303 with *pho80* deletion strain (BY4741 background). The 3xHA-Epl1 expression vector, *epl1(1–380)* mutant and TAP-tagged Epl1-expressing strains were described (Boudreault *et al*, 2003). The Epl1 protein was also tagged *in vivo* at the C-terminus with 13 Myc epitopes by PCR targeting to the *EPL1* locus in the *pho2* deletion mutant strain and isogenic wild type (BY4741 background). The Pho2 protein was similarly tagged with 3 HA epitopes in wild-type and *pho4* mutant strains. The yeast EY0873 strain expressing Pho2 tagged at the C-terminus with the TEV-ZZ (named here TAP-tag for simplicity) cassette; the Pho4–GFP expression vector and the purified Pho4 antibodies were a generous gift from Dave Steger/Erin O'Shea. The YEP-*PHO4* overexpression vector and anti-Pho2 serum were described (Fascher *et al*, 1990; Bhoite *et al*, 2002). Yeast strains were grown in minimal medium, YPD and induced in the phosphate-free synthetic medium prepared as described (Almer and Horz, 1986).

Northern blot, fluorescence microscopy and chromatin analysis

Yeast RNA was isolated and analyzed by Northern blots as described (Nourani *et al*, 2001). The *PHO5*, *PHO8* and *ACT1* probes used were ORFs, except for *PHO5* in Figure 1, which was from the 5′ untranslated region (to show that the signal was not from other related *PHO* genes). For fluorescence miscroscopy, cells were fixed 5 min in cold methanol, washed once with cold acetone, twice with $1 \times$ PBS and then resuspended in the same buffer. The DNA was stained with 60 mM DAPI. Chromatin structure mapping of the *PHO5* promoter region with nucleases has been described (Svaren *et al*, 1995).

Chromatin immunoprecipitations

ChIPs were performed essentially as described (Hecht *et al*, 1999; Nourani *et al*, 2001). However, for precipitation using TAP-tagged proteins (Figure 5), 10 μl of IgG sepharose was used in 300 μl (approximately 1–2 mg of chromatin proteins) of sheared chromatin (0.2–1 kb). For the ChIPs in Figure 7, 10 μl of monoclonal (Myc or HA) antibodies and 4 μl of anti-Esa1 polyclonal antibody (Allard *et al*, 1999) were used for approximately 1–2 mg of chromatin proteins in an immunoprecipitation volume of 400 μl. After O/N incubation at 4°C, 20 μl of protein A beads were added and incubation continued for 90 min at room temperature. DNA was eluted from beads, precipitated and PCR reactions were carried out in a final volume of 50 μl by using 1–4% of immunoprecipitated material and 0.02–0.1% of input material. For ChIPs with anti-Esa1, an extra crosslinking step with 10 mM DMA was added before the actual treatment with formaldehyde. For the ChIPs in Figures 8 and 9, 1 μl of anti-Pho2 or anti-Pho4 antibodies were used and immunoprecipitations were quantified by real-time PCR (triplicates) using LightCycler (Roche). A noncoding region of chromosome V (positions 9716–9863) was used as control and the linear range of amplification was verified by serial dilutions for each pair of primers. Each experiment was repeated at the chromatin immunoprecipitation and PCR steps.

GST-pull-down and coimmunoprecipitation assays

The activation regions of various proteins as well as three sections of Pho2 were fused to GST (pGEX-4T3) and purified from *Escherichia coli* on glutathione sepharose following standard procedures. Protein concentrations were normalized by coomassie staining on gels and equivalent amounts were used in GST pull-down assays with purified NuA4 followed by nucleosomal HAT reactions essentially as described (Utley *et al*, 1998). The co-immunoprecipitation protocol was described in Nourani *et al* (2001). In all, 10 mg of total proteins was incubated for 2 h at 4°C with 50 μl of IgG beads in a final volume of 500 μl. Input (0.3%) and bound material (22.5%) were analyzed by Western blot.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

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References

Allard S, Utley RT, Savard J, Clarke A, Grant P, Brandl CJ, Pillus L, Workman JL, Côté J (1999) NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. *EMBO J* **18:** 5108–5119

Almer A, Horz W (1986) Nuclease hypersensitive regions with adjacent positioned nucleosomes mark the gene boundaries of the *PHO5/PHO3* locus in yeast. *EMBO J* **5:** 2681–2687

Barbaric S, Munsterkotter M, Goding C, Horz W (1998) Cooperative Pho2-Pho4 interactions at the *PHO5* promoter are critical for binding of Pho4 to UASp1 and for efficient transactivation by Pho4 at UASp2. *Mol Cell Biol* **18:** 2629–2639

Barbaric S, Reinke H, Horz W (2003) Multiple mechanistically distinct functions of SAGA at the *PHO5* promoter. *Mol Cell Biol* **23:** 3468–3476

Barbaric S, Walker J, Schmid A, Svejstrup JQ, Horz W (2001) Increasing the rate of chromatin remodeling and gene activation—a novel role for the histone acetyltransferase Gcn5. *EMBO J* **20:** 4944–4951

Bhoite LT, Allen JM, Garcia E, Thomas LR, Gregory ID, Voth WP, Whelihan K, Rolfes RJ, Stillman DJ (2002) Mutations in the pho2 (bas2) transcription factor that differentially affect activation with its partner proteins bas1, pho4, and swi5. *J Biol Chem* **277:** 37612–37618

Boeger H, Griesenbeck J, Strattan JS, Kornberg RD (2003) Nucleosomes unfold completely at a transcriptionally active promoter. *Mol Cell* **11:** 1587–1598

Boudreault AA, Cronier D, Selleck W, Lacoste N, Utley RT, Allard S, Savard J, Lane WS, Tan S, Côté J (2003) Yeast enhancer of

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polycomb defines global Esa1-dependent acetylation of chromatin. *Genes Dev* **17:** 1415–1428

Clarke AS, Lowell JE, Jacobson SJ, Pillus L (1999) Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol Cell Biol* **19:** 2515–2526

Durrin LK, Mann RK, Kayne PS, Grunstein M (1991) Yeast histone H4 N-terminal sequence is required for promoter activation *in vivo*. *Cell* **65:** 1023–1031

Fascher K-D, Schmitz J, Horz W (1990) Role of Trans-activating proteins in the generation of active chromatin at the *PHO5* promoter in *S. cerevisiae*. *EMBO J* **9:** 2523–2538

Gregory PD, Schmid A, Zavari M, Lui L, Berger SL, Horz W (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 PD, Schmid A, Zavari M, Munsterkotter M, Horz W (1999) Chromatin remodelling at the *PHO8* promoter requires SWI-SNF and SAGA at a step subsequent to activator binding. *EMBO J* **18:** 6407–6414

Hassan AH, Prochasson P, Neely KE, Galasinski SC, Chandy M, Carrozza MJ, Workman JL (2002) Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. *Cell* **111:** 369–379

Hecht A, Strahl-Bolsinger S, Grunstein M (1999) Mapping DNA interaction sites of chromosomal proteins. Crosslinking studies in yeast. *Methods Mol Biol* **119:** 469–479

Kioussi C, Briata P, Baek SH, Rose DW, Hamblet NS, Herman T, Ohgi KA, Lin C, Gleiberman A, Wang J, Brault V, Ruiz-Lozano P, Nguyen HD, Kemler R, Glass CK, Wynshaw-Boris A, Rosenfeld MG (2002) Identification of a Wnt/Dvl/beta-Catenin→Pitx2 pathway mediating cell-type-specific proliferation during development. *Cell* **111:** 673–685

Lenfant F, Mann RK, Thomsen B, Ling X, Grunstein M (1996) All four core histone N-termini contain sequences required for the repression of basal transcription in yeast. *EMBO J* **15:** 3974–3985

Munsterkotter M, Barbaric S, Horz W (2000) Transcriptional regulation of the yeast *PHO8* promoter in comparison to the coregulated *PHO5* promoter. *J Biol Chem* **275:** 22678–22685

Nourani A, Doyon Y, Utley RT, Allard S, Lane WS, Côté J (2001) Role of an ING1 growth regulator in transcriptional activation and targeted histone acetylation by the NuA4 complex. *Mol Cell Biol* **21:** 7629–7640

Reid JL, Iyer VR, Brown PO, Struhl K (2000) Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. *Mol Cell* **6:** 1297–1307

Reinke H, Horz W (2003) Histones are first hyperacetylated and then lose contact with the activated *PHO5* promoter. *Mol Cell* **11:** 1599–1607

Santisteban MS, Kalashnikova T, Smith MM (2000) Histone H2A.Z regulates transcription and is partially redundant with nucleosome remodeling complexes. *Cell* **103:** 411–422

Steger DJ, Haswell ES, Miller AL, Wente SR, O'Shea EK (2003) Regulation of chromatin remodeling by inositol polyphosphates. *Science* **299:** 114–116

Svaren J, Horz W (1997) Transcription factors vs nucleosomes: regulation of the *PHO5* promoter in yeast. *Trends Biochem Sci* **22:** 93–97

Svaren J, Venter U, Horz W (1995) *In vivo* analysis of nucleosome structure and transcription factor binding in *Saccharomyces cerevisiae. Meth Molec Genet* **6:** 153–167

Terrell AR, Wongwisansri S, Pilon JL, Laybourn PJ (2002) Reconstitution of Nucleosome Positioning, Remodeling, Histone Acetylation, and Transcriptional Activation on the *PHO5* Promoter. *J Biol Chem* **277:** 31038–31047

Utley RT, Ikeda K, Grant PA, Côté J, Steger DJ, Eberharter A, John S, Workman JL (1998) Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. *Nature* **394:** 498–502

Vogelauer M, Wu J, Suka N, Grunstein M (2000) Global histone acetylation and deacetylation in yeast. *Nature* **408:** 495–498

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