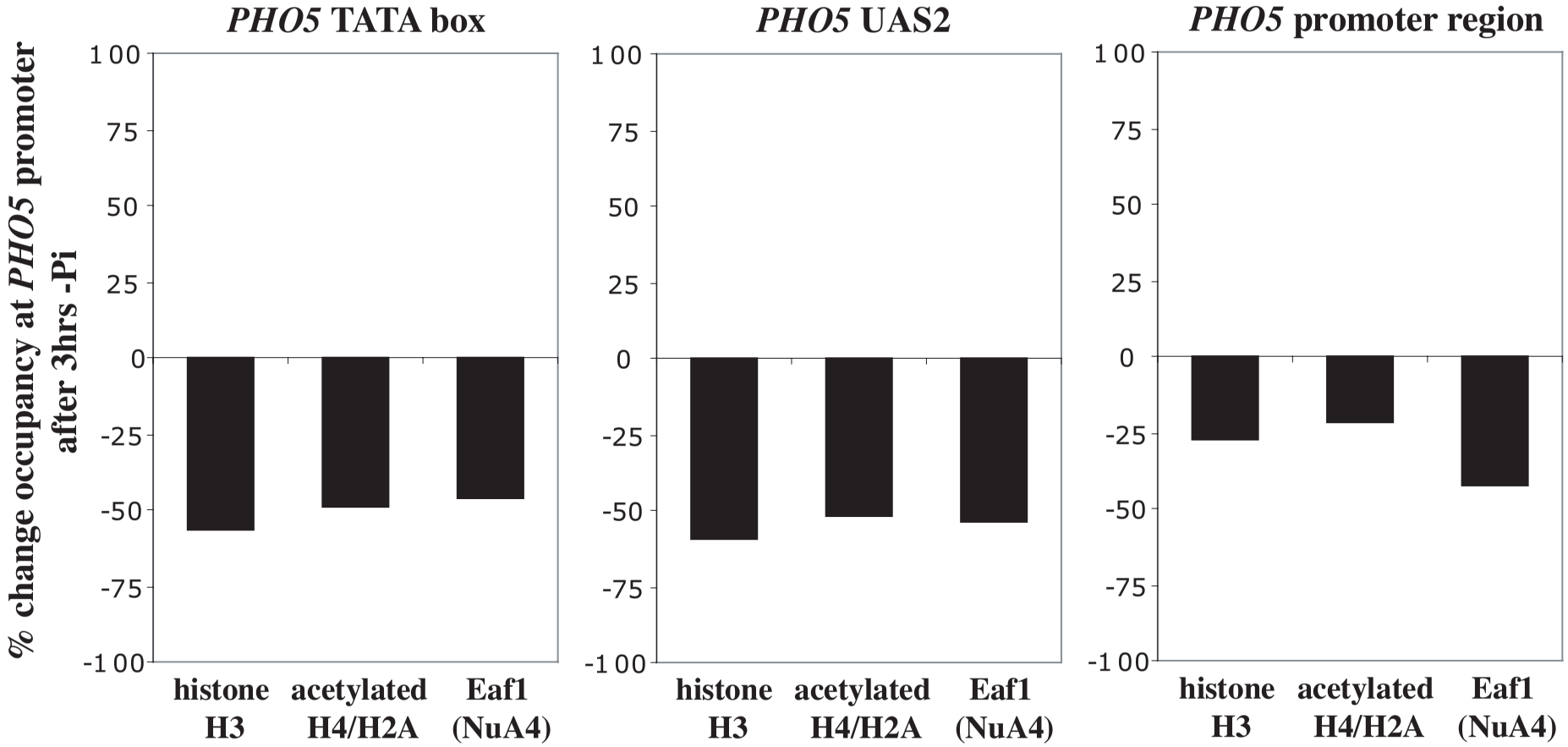
% change occupancy at PHO5 promoter after 3hrs -Pi
PHO5 TATA box
PHO5 UAS2
PHO5 promoter region
100
75
50
25
0
-25
-50
-75
-100
histone H3
acetylated H4/H2A
Eaf1 (NuA4)

Figure S1

Figure S1. Nucleosomal histone H4/H2A acetylation levels do not change over the *PHO5* promoter upon gene induction while a loss of nucleosomes and NuA4 complex is detected. Chromatin immunoprecipitation experiment using anti-histone H3 (0.1 μl, generous gift of A. Ruiz-Carrillo), anti-hyperacetylated histones H4/H2A (0.1 μl, Upstate) and anti-Eaf1 (subunit specific to the NuA4 complex; 0.5 μl, A. Auger and J. Côté, unpublished data) with wild type cells grown in phosphate rich (+Pi) media or after 3 hours in phosphate free (-Pi) media (as in figure 9A). IP ratios (*PHO5*/control locus) were calculated by realtime PCR reactions. The data is presented as a percentage change of IP ratio after 3 hours in inducing conditions (-Pi) compared to repressive conditions (+Pi). Three sets of *PHO5* primers were used in triplicate PCR reactions: a pair for the *PHO5* promoter region (position –363 to –70, same as in figures 5, 7, 8 and 9) and two pairs for much shorter regions surrounding the TATA box (nucleosome –1) and UAS2 (nucleosome –2). The same shorter PCR fragments were used by Reinke and Horz to demonstrate the loss of specific nucleosomes at the *PHO5* promoter upon activation (Reinke and Horz, 2003). Our results confirm these conclusions as we detect a significant loss of histone H3 occupancy at the *PHO5* promoter (57% decrease at the TATA box, 60% at UAS2 and 28% in an overall larger promoter region; the smaller decrease detected by the larger PCR fragment confirms the loss of very specific nucleosomes within *PHO5* promoter region). Very similar drop of signals were detected for hyperacetylated histones H4/H2A after 3 hours in inducing conditions (49% at TATA, 52% at UAS2 and 22% in the larger region). Taking into account the loss of nucleosomes (histone H3 signals) at the *PHO5* promoter upon induction, these data clearly indicate that H4/H2A acetylation levels remain unchanged on the nucleosomes still present at the *PHO5* promoter during the activation process. These results are

similar the ones obtained by Reinke and Horz (Reinke and Horz, 2003). Interestingly, chromatin immunoprecipitations with an antibody specific for Eaf1, a subunit only found in the NuA4 complex (A. Auger and J. Côté, unpublished data), also show a specific decrease of signal at the *PHO5* promoter after 3 hours in inducing conditions (46% at TATA, 54% at UAS2 and 43% in the larger region). These results suggest that the NuA4 complex itself is also lost from the *PHO5* promoter during the activation process, again supporting our model in which NuA4 is not required after the Pho4 binding/chromatin remodeling step.

References

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