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Eaf1 Links the NuA4 Histone Acetyltransferase Complex to Htz1 Incorporation and Regulation of Purine Biosynthesis

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Proper modulation of promoter chromatin architecture is crucial for gene regulation in order to precisely and efficiently orchestrate various cellular activities. Previous studies have identified the stimulatory effect of the histone-modifying complex NuA4 on the incorporation of the histone variant H2A.Z (Htz1) at the *PHO5* promoter (A. Auger, L. Galarneau, M. Altaf, A. Nourani, Y. Doyon, R. T. Utley, D. Cronier, S. Allard, and J. Côté, Mol Cell Biol 28:2257–2270, 2008, http://dx.doi.org/10.1128/MCB.01755-07). *In vitro* studies with a reconstituted system also indicated an intriguing cross talk between NuA4 and the H2A.Z-loading complex, SWR-C (M. Altaf, A. Auger, J. Monnet-Saksouk, J. Brodeur, S. Piquet, M. Cramet, N. Bouchard, N. Lacoste, R. T. Utley, L. Gaudreau, J. Côté, J Biol Chem 285:15966–15977, 2010, http://dx.doi.org/10.1074/jbc.M110.117069). In this work, we investigated the role of the NuA4 scaffold subunit Eaf1 in global gene expression and genome-wide incorporation of Htz1. We found that loss of Eaf1 affects Htz1 levels mostly at the promoters that are normally highly enriched in the histone variant. Analysis of *eaf1* mutant cells by expression array unveiled a relationship between NuA4 and the gene network implicated in the purine biosynthesis pathway, as *EAF1* deletion cripples induction of several *ADE* genes. NuA4 directly interacts with Bas1 activation domain, a key transcription factor of adenine genes. Chromatin immunoprecipitation (ChIP) experiments demonstrate that nucleosomes on the inactive *ADE17* promoter are acetylated already by NuA4 and enriched in Htz1. Upon derepression, these poised nucleosomes respond rapidly to activate *ADE* gene expression in a mechanism likely reminiscent of the *PHO5* promoter, leading to nucleosome disassembly. These detailed molecular events depict a specific case of cross talk between NuA4-dependent acetylation and incorporation of histone variant Htz1, presetting the chromatin structure over *ADE* promoters for subsequent chromatin remodeling and activated transcription.

Transcriptional activation is frequently accompanied by local remodeling of chromatin structure over promoter regions. Three major mechanisms have been implicated in this process, namely, histone posttranslational modifications, ATP-dependent chromatin remodeling, and incorporation of histone variants (see reference 1 and references therein). These mechanisms facilitate transcription by either sliding promoter nucleosomes or assisting nucleosome loss, resulting in an increased accessibility of DNA to transcription activators. Collaboration between multisubunit complexes responsible for these activities leads to a precise and specific control over gene expression. Studies from recent years suggest that different sets of genes, constitutive and inducible genes, for example, adopt rather distinct strategies in utilizing these mechanisms to modulate promoter architecture (2, 3).

Recent work has shown the tight yet complex functional link between two yeast chromatin-modifying complexes, histone acetyltransferase complex NuA4 and chromatin remodeling complex SWR-C, converging at the understanding of histone variant H2A.Z. SWR-C is a 14-subunit complex responsible for histone variant H2A.Z incorporation (4–6). It shares 4 subunits with NuA4 (4, 5, 7–10), the main function of which is to acetylate histones H2A and H4 (11, 12). These acetylation events are important for SWR-C recruitment and activity on chromatin, likely through its bromodomain-containing subunit Bdf1 (10, 13). One of the shared subunits, Yaf9, is implicated in helping to antagonize silencing near telomeres (9, 14). Moreover, NuA4 also is found to acetylate H2A.Z both *in vitro* and *in vivo* (15–17). The acetylated H2A.Z is implicated in regulation of Htz1 dynamics at promoters (16) and Htz1 function in proper heterochromatin maintenance (18). Consistent with the intimate relationship between SWR-C and NuA4, it was proposed that the human TIP60 complex is a physical and functional merge of the two yeast complexes (17, 19, 20), resulting in a single multifunctional complex that comprises all of the aforementioned mechanisms of chromatin regulation.

The functional cross talk between histone modifications by NuA4 and histone variant incorporation by SWR-C has been analyzed carefully at *PHO5*. In *Saccharomyces cerevisiae*, the *PHO* system modulates phosphate metabolism and allows cells to adapt to inorganic phosphate environments. When phosphate is present, the *PHO5* gene is maintained in a transcriptionally repressed state by a particular chromatin structure on the promoter region. Upon gene activation in phosphate starvation conditions, the promoter nucleosome structure undergoes a remodeling process,

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TABLE 1 Primers used in ChIP-qPCR

Gene	Primer position (bp)/ direction	Sequence
ADE17	−237/forward	ATCATTTATAAAGAAGATCCTACCC
ADE17	−122/reverse	ATAGATCCGAACGTGATATG
ADE17	+717/forward	TTTGTTGGATGCTCTAAATTCC
ADE17	+843/reverse	ATCAGACAATGGGATACCCA
ADE17	+1485/forward	CGGCCAAATTCCAACAGAAG
ADE17	+1614/reverse	CGATGACAAAGAAACGTTGG
ADE5,7	−200/forward	AGTGACAAGTGCCGACTGACT
ADE5,7	−89/reverse	TAACAGCGGTTGCACTTGCC

generating nuclease-hypersensitive regions (21, 22). The hypersensitivity to nucleases is caused by loss of histone-DNA contacts, leading to the removal of local nucleosomes (23–25). Studies have shown that NuA4 is required in this opening of chromatin structure through its histone acetylation activity and effect on Htz1 incorporation, presetting the promoters for proper gene induction (1, 17, 26).

Although the interplay between NuA4 activity and Htz1 incorporation has been reported both *in vitro* and at certain genes *in vivo*, the global scale of NuA4 effects on Htz1 incorporation remains poorly studied. To this end, in this study we investigated the effect of NuA4 on global Htz1 variant incorporation in yeast. We show that an *eaf1* deletion mutant affects genome-wide Htz1 incorporation over several hundred promoters. Moreover, we found that the *eaf1*Δ mutant also decreases the expression level of genes implicated in the purine biosynthesis pathway. NuA4 directly interacts with *ADE* gene transcription factors Bas1 and Pho2, and *EAF1* deletion cripples *ADE* gene activation. We demonstrate that inactive adenine promoters are enriched in Htz1 and acetylated H4 in a NuA4-dependent manner. Derepression of adenine genes results in efficient nucleosome loss, whereas the levels of Htz1 and H4 acetylation enrichment remain unchanged, sug-

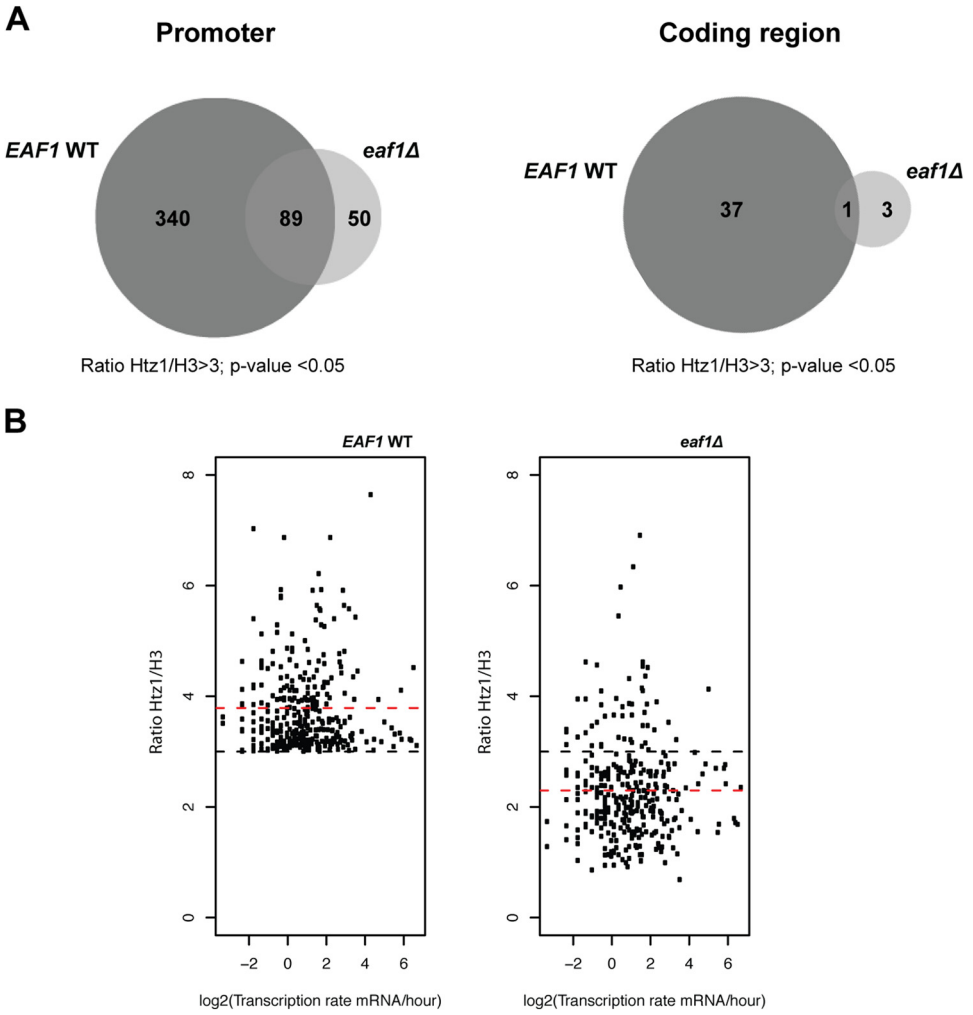


FIG 1 NuA4 affects Htz1 incorporation independently of transcription rate. (A) Venn diagrams showing the number of gene promoters and coding regions identified as high occupancy for Htz1 loci (Htz1/H3 ratio, >3; $P < 0.05$) in wild-type and *eaf1* mutant cells. The number of loci identified in both WT and *eaf1* mutant cells also is indicated (overlap). (B) Scatter plot analysis of promoters with high levels of Htz1 (*y* axis; Htz1/H3 ratio, >3; $n = 429$) in relation to transcription rate [*x* axis; $\log_2$(transcription rate mRNA/hour)] in WT cells (left). The scatter plot on the right shows the Htz1 occupancy at the same 429 loci in *eaf1*Δ cells. The dashed red line shows the mean Htz1/H3 ratio at these 429 promoters, while the dashed black line represents the cutoff ratio of 3 used to select the loci in WT cells. Transcription rate data used in the analysis were obtained from reference 27. The full ChIP-on-chip data set is provided in Tables S1 and S2 in the supplemental material.

gesting a presetting mechanism for inducible adenine gene transcription. Finally, we show that NuA4 also acetylates Htz1 at the *ADE* promoter, which is likely to favor nucleosome disassembly. These detailed molecular events indicate that adenine promoters, similar to *PHO5*, are preset by NuA4-dependent events for subsequent chromatin remodeling and induced transcription, highlighting the cross talk between histone modifications and histone variant incorporation in the process of transcription modulation.

MATERIALS AND METHODS

Yeast strains and reagents. Wild-type (WT), *htz1* deletion, and *eaf1* deletion mutant strains (S288C BY4741 background) were purchased from Open Biosystems. All yeast manipulations were performed according to standard protocols unless specifically indicated otherwise.

ChIP-on-chip. Chromatin immunoprecipitation (ChIP) was carried out with yeast cells grown in yeast extract-peptone-dextrose (YPD) media. The subsequent genome-wide location analyses were performed with a tiling microarray covering the whole genome with PCR probes, with one per open reading frame (ORF) and one per intergenic region. The Htz1 enrichment level was normalized to the H3 level for nucleosome occupancy. Gene promoters defined as "high Htz1 occupancy" were isolated with a Htz1/H3 cutoff ratio of 3 ($n = 429$; $P < 0.05$) and then related to transcription frequencies using data from reference 27. Full ChIP-on-chip data sets are provided in Tables S1 and S2 in the supplemental material.

Northern blot and microarray analysis. Yeast cells were grown overnight in YPD, diluted to an optical density at 600 nm (OD_{600}) of 0.25 in SC media with adenine (SC+adenine) or SC media without adenine (SC−adenine), and grown to an OD_{600} of around 1.0. Cells then were washed and RNA was isolated using the hot phenol method as described previously (28). An amount of 15 to 20 μg of RNA was analyzed by Northern blotting as described previously (7). The used probes were ORFs from *ADE1*, *ADE2*, *ADE5*,*7*, *ADE13*, and *ACT1*, which were obtained by PCR and radiolabeled by a random primer (Amersham Biosciences). For microarray analysis, total RNA was isolated from wild-type cells grown in YPD using the QIAquick RNeasy miniprep kit (Qiagen), and mRNA was purified using a poly(A) tail (Promega) and analyzed by Deming Xu at the Best Yeast Microarray Center in Toronto, Canada. Full microarray data are provided in Table S3 in the supplemental material.

GST pulldown and HAT assays. Specific regions of Bas1 and Pho2 proteins were fused to glutathione *S*-transferase (GST) (pGEX-4T3) (see Fig. 3A) and purified from *Escherichia coli* on glutathione-Sepharose by following standard procedures. Protein concentrations were normalized by Coomassie staining on gels (see Fig. 3B), and equivalent amounts were used in GST pulldown assays with purified NuA4, followed by nucleosomal histone acetyltransferase (HAT) reactions, essentially as described previously (11).

ChIP. ChIP experiments were performed essentially as described previously (17, 26, 29). Briefly, overnight cultures were diluted and grown to an OD_{600} of 0.5 to 0.8 in 200 ml media. Cell cultures were cross-linked with 1% formaldehyde for 20 min at room temperature. Cross-linking was quenched by the addition of glycine to a final concentration of 125 mM for 5 min. Cells were centrifuged for 5 min at 5,000 × *g* at 4°C, washed twice with ice-cold water, washed once with 1 ml of Tris-buffered saline (TBS), and resuspended in 0.5 ml of FY lysis buffer (50 mM HEPES, pH 7.5, 140 mM NaCl, 1% Triton X-100, 1 mM EDTA, 0.1% Na deoxycholate, 1 mM phenylmethylsulfonyl fluoride [PMSF]). Cell lysis was carried out using a mini-bead beater until around 70% of the cells were lysed. Cells then were centrifuged and the pellet was washed with 1 ml FA lysis buffer until the supernatant was clear. Pellet was resuspended in 1 ml of the FY lysis buffer for sonication. Sonication was carried out using the Bioruptor apparatus from Diagenode (30-s sonication followed by 60-s rest, repeated 10 to 12 times), which then was centrifuged for 30 min at 4°C at 20,000 × *g*. The supernatant containing the chromatin was placed in a new tube. For *ADE* induction, cells were grown in Hopkins synthetic complete supplement mix minus adenine (SCSM-ADE) media supplemented with adenine to an OD_{600} of 0.5 to 1.0. For induction, cells were centrifuged, washed in water, and resuspended in SCSM-Ade media supplemented with adenine for time zero or resuspended in SCSM-Ade and allowed to grow for the derepression time period before cross-linking.

Sonication of chromatin led to DNA fragments ranging between 200 and 500 bp. Immunoprecipitation was carried out using 100 μg chromatin supplemented with FA lysis buffer to a final volume of 450 μl. A volume of 22.5 μl of the mix was taken as the input. Corresponding antibodies then were added: 0.5 μl of anti-Htz1 (07-718; Upstate), 0.5 μl anti-hyperH4ac (Penta; Upstate), 0.5 μl anti-H3 C terminus (Ab1791; Abcam), and 2 μl anti-polymerase II (Pol II; 8WG16) and anti-AcHtz1K14 (kindly provided by Michael Grunstein). After an overnight incubation at 4°C on a wheel, 12.5 μl of protein A Sepharose (GE Healthcare) was added, and incubation was continued for 4 h at 4°C on a wheel. The beads were washed twice with 1 ml FA lysis buffer, once with FA lysis buffer, 500 mM NaCl, once with 1 ml wash buffer 2 (10 mM Tris-HCl, pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, 1 mM EDTA), and once with Tris-EDTA (TE). Beads were eluted with 100 μl $T^{50}E^{10}$ (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, pH 8.0) –1% SDS for 15 min at 65°C. The eluted material was placed in a new tube, and beads were eluted once more with 150 μl TE–1% SDS. The second elution was pooled with the first one. DNA was de-cross-linked overnight at 65°C. After de-cross-linking, 240 μl of TE and 5 μg of RNase were added and incubated at 37°C for 15 min, after which 15 mg of proteinase K and 200 μg of

TABLE 2 Genes downregulated in *eaf1*Δ cells compared to levels in wild-type cells in rich media[a]

*eaf1*Δ/WT ratio	Gene	Annotation
0.274	*RPL15B*	Ribosomal protein
0.33	***ADE13***	**Bas1/Pho2 regulated, purine biosynthesis**
0.362	***ADE1***	**Bas1/Pho2 regulated, purine biosynthesis**
0.391	*PHO12*	Pho2 regulated
0.393	***SHM2***	**Purine biosynthesis, Bas1/Pho2 regulated**
0.423	***ADE5,7***	**Bas1/Pho2 regulated, purine biosynthesis**
0.437	***MTD1***	**Purine biosynthesis, Bas1/Pho2 regulated**
0.444	*BAT2*	
0.452	***ADE2***	**Bas1/Pho2 regulated, purine biosynthesis**
0.474	***GCV2***	**Bas1 site, purine pathway**
0.479	***SER1* (*ADE9*)**	**Bas1 site, purine biosynthesis**
0.485	*YHB1*	
0.494	*PHO11*	Pho2 regulated
0.51	***GCV1***	**Bas1 site, purine pathway**
0.519	***ADE17***	**Bas1/Pho2 regulated, purine biosynthesis**
0.52	*LEU1*	
0.526	*YLR345w*	
0.53	*LAP4*	
0.535	*BNA1*	
0.545	*DDR48*	
0.547	***ADE3***	**Bas1/Pho2 regulated, purine biosynthesis**
0.553	*YPF1*	
0.556	*ECM9*	
0.556	***ADE12***	**Bas1/Pho2 regulated, purine biosynthesis**
0.556	*ABP140*	
0.569	*SDS3*	Histone deacetylase subunit
0.576	*TRM12*	
0.576	*PHO5*	Pho2 regulated

[a] Microarray analysis performed on RNA isolated from *eaf1*Δ cells and RNA isolated from wild-type (WT) cells. The list shows the 28 genes that are most affected by the absence of *EAF1* (from 0.274 for *RPL15B* to 0.576 for *PHO5*; 1.0 represents wild-type expression level). *eaf1*Δ cells show a marked decrease in the expression of 12 genes involved in purine biosynthesis (in boldface), including 7 *ADE* genes. The *PHO5* expression level also is affected by *EAF1* deletion (as previously reported). The full microarray data set is provided in Table S3 in the supplemental material.

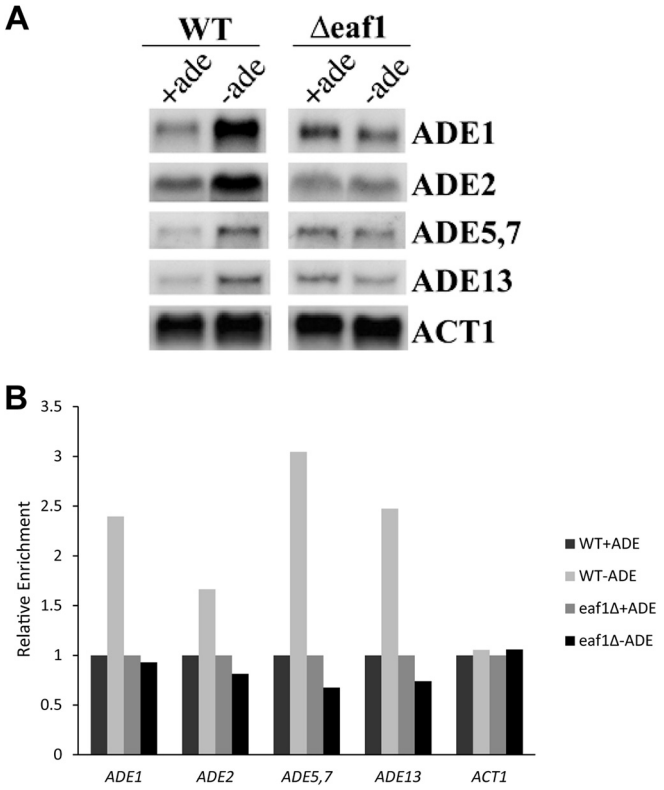


FIG 2 NuA4 is important for the induction of *ADE* genes. (A) Northern blot analysis performed with RNA isolated from wild-type (WT) and *eaf1*Δ cells grown in the presence or absence of adenine. *ADE1*, *ADE2*, *ADE5,7*, and *ADE13* gene expression levels were compared to the expression level of the *ACT1* control. (B) Quantification and normalization of Northern blot image depicted in panel A. The signal of samples from media with adenine (+ade) was set to 1. −ade, media without adenine.

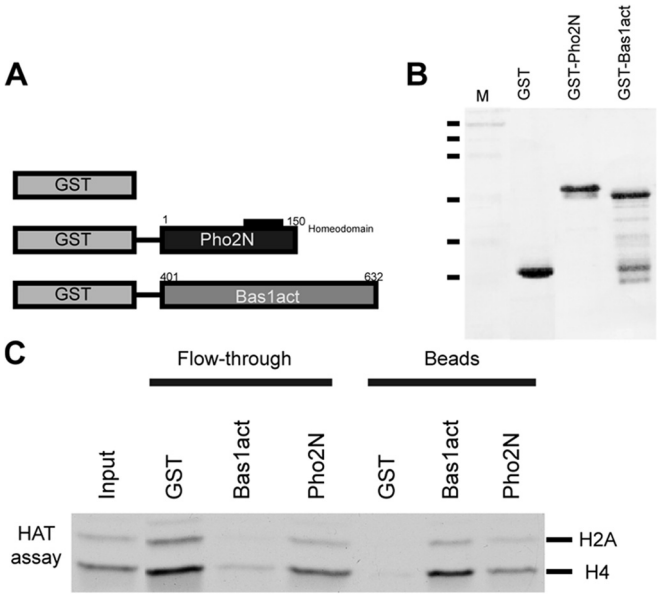


FIG 3 Bas1 and Pho2 directly interact with NuA4 complex *in vitro*. (A) Schematic representation of the GST fusion proteins used in this study. (B) Indicated fusion proteins were run in 12% SDS-PAGE and Coomassie stained. Lane M, molecular size marker. (C) Equal amounts of input, flowthrough, and beads from GST pulldown with purified NuA4 complex were assayed for histone acetyltransferase (HAT) activity with chromatin. HAT reactions were visualized on 18% SDS-PAGE. Pho2N serves as a positive control (26).

glycogen were added. Tubes were placed at 37°C for 2 h. Twenty-five μl of 5 M NaCl was added before the extraction with phenol chloroform and precipitation with ethanol. After centrifuging at 20,000 × *g* at 4°C for 30 min, pellets were air dried and resuspended in 100 μl NTE (50 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0). PCR primers used were analyzed for linearity range, efficiency, specificity, melting curves, and products using a LightCycler (Roche). Primer sequences are listed in Table 1.

RESULTS

Loss of Eaf1 affects genome-wide Htz1 enrichment. Previous studies have shown that NuA4 stimulates SWR1-dependent Htz1 incorporation and depletion of NuA4 activity decreases Htz1 occupancy at *PHO5* promoter regions (10, 17, 26), suggesting a tight cross talk between the two chromatin-modifying complexes. Nevertheless, as these studies have been limited to the observations at specific loci, to what extent this cross talk would take place globally remains unknown. In order to further address the question of how NuA4 affects Htz1 global incorporation, we performed a chromatin immunoprecipitation (ChIP) assay of Htz1 and histone H3 in both wild-type (WT) and *eaf1*Δ strains and hybridized the recovered DNA to a tiling microarray which covers the whole genome with PCR probes, one per ORF and one per intergenic region (ChIP-on-chip). Deletion of NuA4 subunit Eaf1 was chosen, since it is the scaffold platform for NuA4 complex assembly and also is the only subunit unique to NuA4 (17, 30, 31). Htz1 occupancy was measured by relative Htz1 enrichment compared to the corresponding H3 signal.

In the WT strain, Htz1 is preferentially enriched at repressed/basal promoter regions compared to gene bodies and does not correlate with the level of transcription, consistent with previous reports (32–35) (Fig. 1A and B; also see Table S1 in the supplemental material). While Htz1 presence does not require NuA4 *per se*, this enrichment level is significantly decreased in *eaf1*Δ strains for a large number of genes (Fig. 1A and B; also see Table S2), in agreement with what has been observed at the *PHO5* promoter. Notably, promoters which have a high occupancy of Htz1 (Htz1/H3 ratio, >3; $P < 0.05$; $n = 429$) are most profoundly affected by *EAF1* deletion (Fig. 1B; the scatter plot on the left shows the 429 promoters with the highest enrichment of Htz1, and the one on the right shows the Htz1/H3 ratio of these 429 promoters in the *eaf1* mutant strain; see Table S2 for a list of genes). These results expand the reported observation and identify NuA4 as a crucial factor in Htz1 level maintenance at the most enriched promoters.

NuA4 is implicated in regulation of gene network for purine biosynthesis. Both NuA4 activity and Htz1 incorporation are implicated in transcription regulation. NuA4 has been shown to acetylate promoter nucleosomes, an event considered to assist nucleosome loss to favor transcription activation (11, 26, 36, 37). Htz1 is enriched in basal/repressed gene promoters and is involved in the activation of certain genes (32–35). As the *eaf1*Δ mutant decreases Htz1 occupancy of a subset of genes, whether the transcription level of these genes also is disturbed by *EAF1* deletion remains to be answered. To this end, we examined the expression profile in both WT and *eaf1*Δ strains by microarray. As

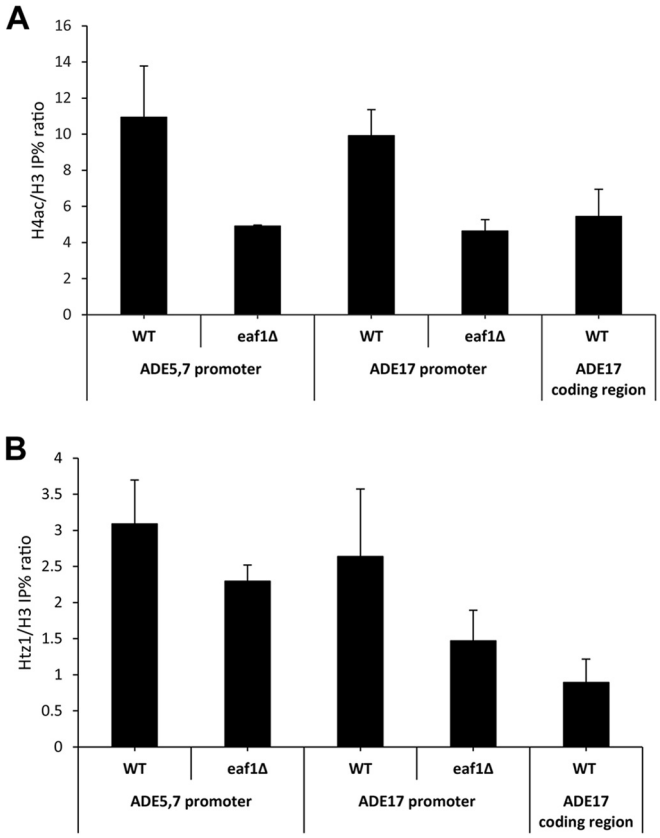


FIG 4 Presence of H4 acetylation and Htz1 at repressed *ADE17* promoter is NuA4 dependent. H4ac enrichment (A) and Htz1 enrichment (B) at *ADE5,7* and *ADE17* promoter regions in WT and *eaf1*Δ cells was measured by chromatin immunoprecipitation (ChIP). The *ADE17* coding region (+1.5 kb) served as a control locus. Potential nucleosome occupancy variation was corrected by H3 signal at the same locus. Standard deviations are shown based on two biological repeats.

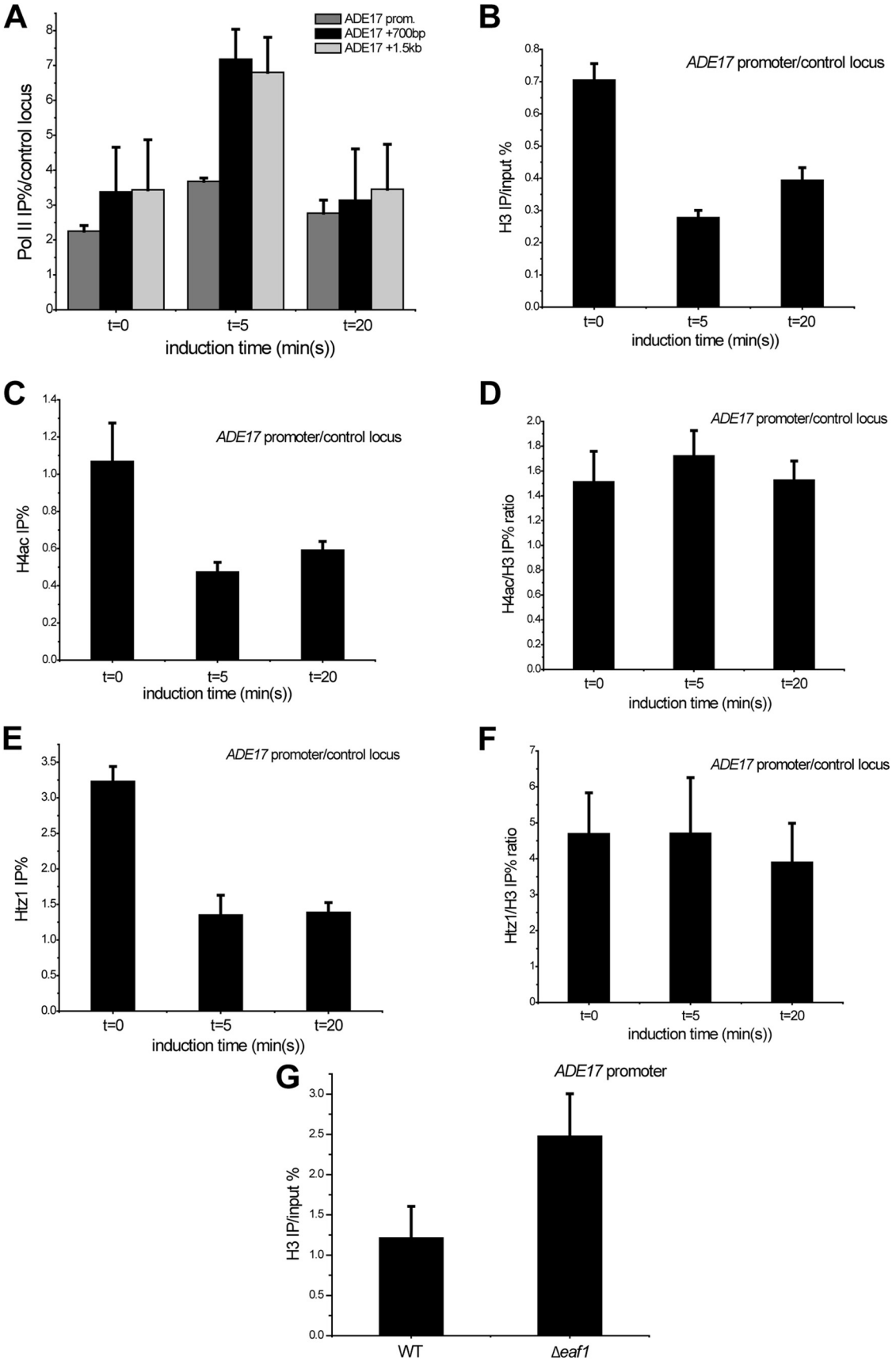
expected, RP and *PHO* gene expression are affected by Eaf1/NuA4, as reported previously (7, 26, 36, 38). We observed that genes with the most decreased expression levels (Table 2) are not significantly correlated with those with the most affected Htz1 levels (Fig. 1), consistent with the idea that NuA4 tends to associate with highly transcribed genes (such as RP genes) while Htz1 mostly locates at inactive promoters. Surprisingly, we also found a series of genes implicated in purine biosynthesis pathways among those most affected by *EAF1* deletion (Table 2, boldface), suggesting a role of NuA4 in regulation of purine biosynthesis.

Loss of Eaf1 cripples derepression of a number of *ADE* genes. In an adenine-sufficient environment, the transcription of *ADE* genes is basal/repressed. The deprivation of available adenine will trigger the expression of a series of *ADE* genes in order to adapt to the environment (39–41). The fact that YPD media are poor in adenine allowed us to detect the implication of NuA4 in the regulation of *ADE* gene expression in the microarray experiment. To investigate further the role of Eaf1/NuA4 in *ADE* gene derepression and purine biosynthesis, the activation of *ADE* genes was analyzed by Northern blotting. RNAs were extracted from both WT and *eaf1*Δ strains, and several *ADE* gene expression levels were examined using specific probes. In both WT and *eaf1*Δ strains, when adenine is present in the media, the *ADE* genes show basal levels of transcription (Fig. 2A, +ade lanes), which may be due to the gradual depletion of adenine in the media, loss of chromatin architecture, or transcription noise (see Discussion). The *ADE* starvation condition induces *ADE* expression in WT cells, yet this effect is lost in the *eaf1*Δ strain (Fig. 2A, −ade lanes, and B). Results from microarray and Northern blot analyses demonstrate that activation of *ADE* expression is dependent on Eaf1/NuA4.

NuA4 interacts with Pho2 and Bas1 *in vitro*. Two transcription factors, Pho2 and Bas1, have been shown to regulate adenine gene activation (41–43). The intermediates of two biosynthesis pathways, SAICAR [1-(5-phosphoribosyl)-4-(N-succinocarboxamide)-5-amino-imidazole] and AICAR [1-(5-phosphoribosyl)-4-carbox-amide-5-aminoimidazole], represent the low-adenine signal and prompt the interaction between Pho2 and Bas1 (43). Previous studies have shown that Bas1 is constantly present at *ADE* promoters in an inactivated state. Upon induction, Bas1 undergoes a conformation change and recruits Pho2 (41). Interestingly, previous works also show that Pho2 directly interacts with NuA4 *in vitro* and *in vivo*, a step considered crucial to preset the promoter of the *PHO5* gene for induction (26). Collectively, these findings bring us to the question of whether NuA4 is recruited by Pho2 and/or Bas1 in the case of *ADE* gene regulation. To address this question, GST fusion proteins were constructed and purified (Fig. 3A and B). Equal amounts of GST fusion proteins were used to perform pulldown assays with purified NuA4 complex, where both flowthrough and beads are subject to HAT assay. NuA4 shows direct interaction with the N-terminal region of Pho2, which contains a homeodomain, as previously reported (26). Interestingly, it also interacts with the Bas1 activation domain with an even stronger affinity (Fig. 3C). These results indicate that NuA4 could regulate *ADE* gene expression through direct recruitment by promoter-bound transcription factors Pho2 and Bas1.

Enrichment of Htz1 and acetylated H4 at inactive *ADE* promoters is dependent on NuA4. The histone acetyltransferase activity provided by NuA4 is required for the presetting of the inducible *PHO5* promoter during the transition from a transcriptionally inactive to an active state in the absence of phosphate (26). Having identified the aforementioned importance of the Eaf1 subunit in regulation of *ADE* gene expression, we set out to investigate the chromatin dynamics over the promoters of *ADE* genes and the implication of NuA4 during this process. Using chromatin immunoprecipitation (ChIP), we first looked at the H4 acetylation level (the main activity of NuA4), the Htz1 incorporation level at *ADE 5,7*, and *ADE17* promoter regions under repression and the effect of Eaf1 therein. These *ADE* loci were chosen since their expressions are affected by *EAF1* deletion in microarray, and it was also shown that Htz1 is enriched at the *ADE17* transcription start site (TSS) (6). Both wild-type and *eaf1*Δ strains are grown in synthetic complete media in which the adenine supplement is sufficient. Under this repressed condition, *ADE5,7* and *ADE17* promoter regions show enriched H4 acetylation and Htz1 compared to that of the control locus, and this enrichment is significantly decreased in the *eaf1*Δ strain (Fig. 4A and B). These results suggest the involvement of NuA4 in chromatin structure at basal *ADE* promoters.

Loss of nucleosomes upon *ADE* gene derepression. Since repressed *ADE* promoters show an enrichment in both H4 acetylation and Htz1, we wondered whether this enrichment merely represents a basal chromatin status (in this case, the enrichment of H4 acetylation and Htz1 could be expected to increase during dere-


A
ADE17 prom.
ADE17 +700bp
ADE17 +1.5kb
Pol II IP%/control locus
t=0
t=5
t=20
induction time (min(s))
B
ADE17 promoter/control locus
H3 IP/input %
t=0
t=5
t=20
induction time (min(s))
C
ADE17 promoter/control locus
H4ac IP%
t=0
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t=20
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D
ADE17 promoter/control locus
H4ac/H3 IP% ratio
t=0
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induction time (min(s))
E
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Htz1 IP%
t=0
t=5
t=20
induction time (min(s))
F
ADE17 promoter/control locus
Htz1/H3 IP% ratio
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G
ADE17 promoter
H3 IP/input %
WT
Δeaf1

pression) or stands for a preset mechanism to poise promoters for subsequent derepression (in this case, both levels would be expected to decrease along with nucleosome loss). In order to test this, we performed ChIP time course experiments by exposing cells to adenine starvation conditions for 0 (no starvation), 5, or 20 min. RNA Pol II ChIP across the *ADE17* gene suggests short transient Pol II recruitment/enrichment with an expression peak around the 5-min time point (Fig. 5A; also see Discussion). This expression correlates with the rapid loss of nucleosomes measured by the decrease of H3 signal (Fig. 5B). After confirming an efficient derepression, we then examined the H4 acetylation level and Htz1 dynamics during gene derepression. Both H4 acetylation and Htz1 levels decrease significantly within 5 min (Fig. 5C and E), consistent with the observed disassembly of nucleosomes (Fig. 5B), while both levels stay unchanged on the remaining nucleosomes after correction for nucleosome signals (Fig. 5D and F). These results suggest a mechanism in which the *ADE17* promoter is preset by H4 acetylation and Htz1, resulting in susceptible nucleosomes to favor rapid activation.

As shown previously, the *eaf1*Δ mutant affects both H4 acetylation and Htz1 level at silenced *ADE17* promoters (Fig. 4). If this chromatin state at the *ADE17* promoter indeed represents a preset mechanism, one could expect the *eaf1* mutant would disrupt this poised status and alter nucleosome stability. Indeed, the *eaf1* mutant shows a higher nucleosome occupancy at the *ADE17* promoter under repressed conditions (Fig. 5G).

Htz1 K14 acetylation at *ADE* gene promoters is dependent on NuA4. Once deposited, the N terminus of Htz1 can be acetylated by NuA4, an event considered to be involved in nucleosome dynamics/removal over promoters (15, 16). Studies have shown that Htz1 on the *PHO5* promoter is acetylated at K14 by NuA4 to favor nucleosome loss. While Htz1 does not correlate with transcription, Htz1K14 acetylation leads to transcription activation (16, 17). Using ChIP and an antibody that specifically recognizes acetylated Htz1 on lysine 14, we found that Htz1 is acetylated at the *ADE17* promoter, and this acetylation is decreased in the *eaf1*Δ strain (Fig. 6). Altogether, these results further emphasize the role of Eaf1/NuA4 in presetting *ADE* promoters, favoring efficient nucleosome loss during induction through constructing a specific promoter architecture poised for activation.

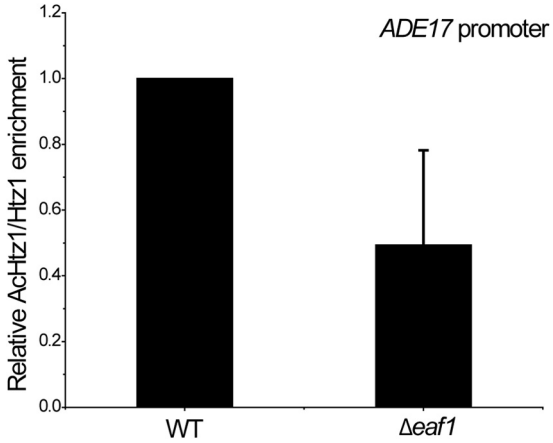

FIG 6 Htz1 is acetylated at K14 by NuA4 at the *ADE17* promoter. ChIP analysis was performed with anti-AcK14 Htz1 and anti-Htz1 in WT and *eaf1*Δ strains. Data represent the ratios of AcHtz1 to total Htz1 occupancy and are presented as a relative change compared to the level for the wild-type strain (set to 1). Error bars represent standard deviations based on two biological repeats.

DISCUSSION

In this work, we have provided the missing information about the NuA4 effect on Htz1 incorporation on a genome-wide scale. We found clear genome-wide alterations of Htz1 occupancy over promoters in a strain depleted of Eaf1, the only subunit unique to NuA4. This effect is most prominent at a subset of promoters with the highest Htz1 occupancy (Fig. 1). These genes ($n = 429$) also are featured by their low transcription rate (Fig. 1B), in agreement with previously published studies (32–35). Notably, as the enrichment of Htz1 does not correlate with transcription rate, the disruption of Htz1 level by the *eaf1*Δ mutant does not necessarily affect their transcription, as the 429 genes in Fig. 1 show little overlapping with the genes most significantly affected in the *eaf1*Δ expression array (Fig. 2). This, along with our results about presetting *ADE* promoters (Fig. 4 to 6), is consistent with the notion that Htz1 occupancy status itself does not necessarily affect transcription *per se* but rather represents a promoter marker for more susceptible nucleosomes to facilitate rapid activation (32, 33, 44).

We observed that *ADE* promoter regions are preset by NuA4-dependent H4 acetylation before derepression (Fig. 4B and 5C and D). However, we were not able to visualize a direct recruitment of NuA4 at these promoter regions by ChIP (data not shown). This could be due to a limited NuA4 enrichment that renders the signal below the antibody detection threshold; alternatively, it also could be explained by a transient recruitment of NuA4 activity over these regions, after which the physical association of the complex becomes dispensable. In fact, the latter possibility is favored by studies involving chromatin remodeling complexes SAGA and SWI/SNF in the regulation of *ADE* gene activation (43). Koehler et al. have shown an association of SAGA and SWI/SNF with an *ADE* promoter, yet this association is independent of *ADE* transcription factors Bas1 and Pho2 (43). By combining our observation and the fact that both SAGA and SWI/SNF harbor a bromodomain (45), one could speculate on a model in which, under *ADE* repression conditions, Bas1/Pho2 directly but transiently recruits NuA4 to acetylated H4, an event that would further stimulate the subsequent binding and stabilization of SAGA and/or SWI/SNF complexes for an optimal derepression prior to and/or during induction, although NuA4-dependent acetylation may not be sufficient or necessary for the binding.

FIG 5 Rapid loss of both H4 acetylation and Htz1 variant upon induction at the *ADE17* promoter along with nucleosome disassembly. (A) *ADE17* expression is induced upon adenine depletion. Pol II ChIP at the *ADE17* promoter (bp −237 to −122), at the middle of the gene (bp +717 to +843), and 3′ (bp +1485 to +1614) of the gene. Data are presented as ratios of IP at specific *ADE* loci to the control locus Inter V. (B) Loss of nucleosomes around the *ADE17* promoter region upon adenine induction. Data represent the ratio of H3 IP at the *ADE17* promoter to the control locus Inter V. (C to F) Loss of H4 acetylation and Htz1 variant along with nucleosomes upon induction. Anti-hyperacetylated H4 (H4ac) IP and anti-Htz1 (C-term) IP were corrected with an *FMP27* (+5 kb) control locus. Further correction on H3 occupancy at the same locus (D and F) indicates the shown H4ac or Htz1 decrease is due to nucleosome loss. (G) *EAF1* deletion results in higher nucleosome occupancy before induction. For panels A to D and G, standard errors are presented based on three biological repeats. For panels E and F, standard deviations are shown based on two biological repeats.

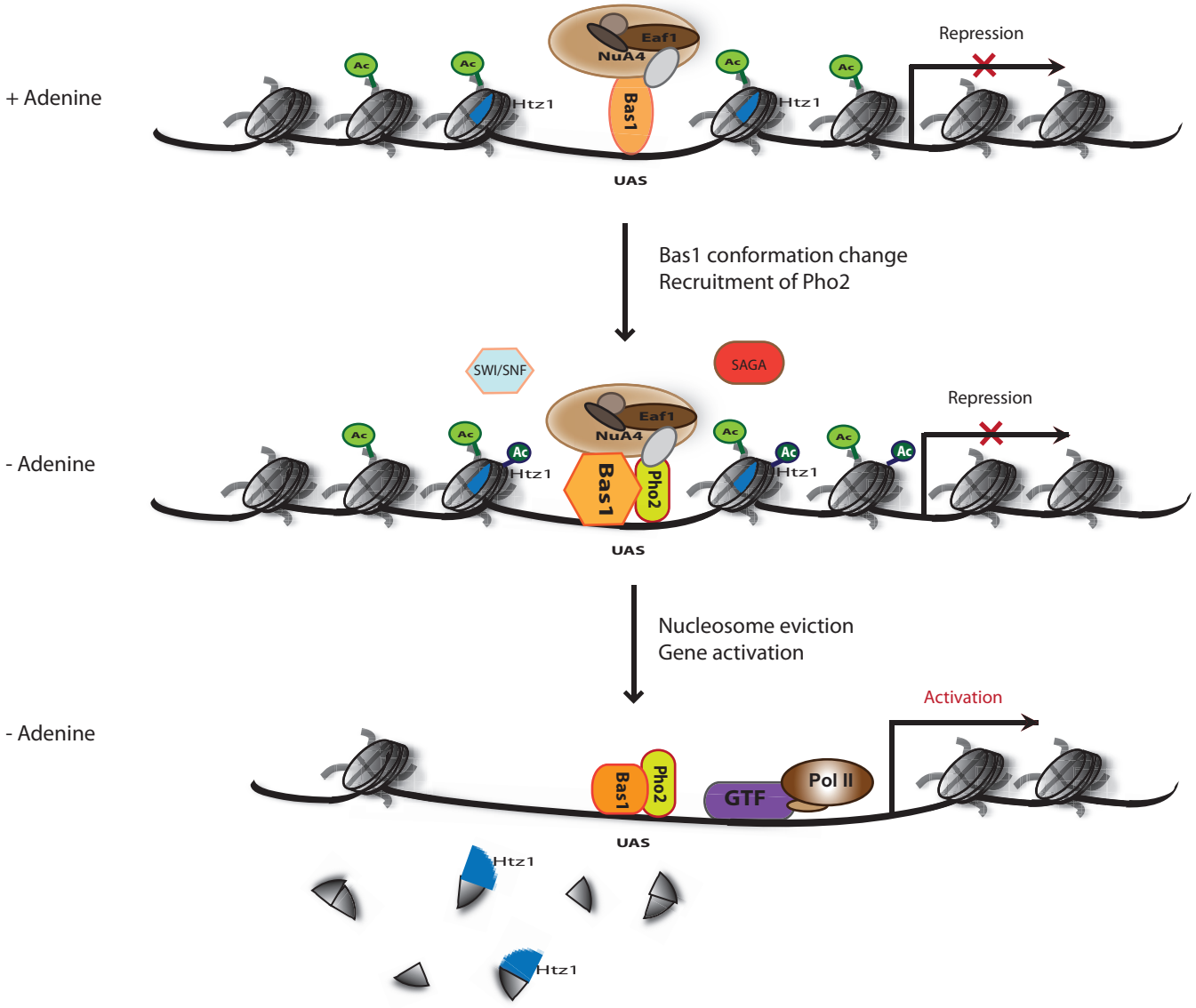

FIG 7 Model of stepwise chromatin modifications on *ADE* gene promoters for activation. When adenine is present in the media, Bas1 recruits NuA4 over *ADE* promoters. This recruitment leads to H4 acetylation and also stimulates Htz1 incorporation. Adenine starvation triggers the conformation changes of Bas1, which recruits Pho2. Subsequent recruitment of other coactivators, like the chromatin remodeler SWI/SNF and histone H3 acetyltransferase SAGA, assists nucleosome eviction and leads to recruitment of the basal machinery and transcription activation. Ac, acetylation (light green for H4ac, dark green of H3ac); GTF, general transcription factors.

It has long been established that the presence of nucleosomes represses transcription, and nucleosome loss often is correlated with gene expression level (46, 47). In this study, we present a case in which promoter nucleosomes are poised in a state that is necessary but not sufficient for transcription activation. This "poise" mechanism is highly dependent on NuA4, specifying a role of NuA4 in assisting the nucleosome disruption of occupied promoters. In fact, this facet of NuA4 is not specific to *ADE* or *PHO5* gene promoters, as deletion of *EAF1* leads to nucleosome stabilization across both intergenic regions and gene bodies (unpublished data). Interestingly, human p400 shows structural homology with yeast Eaf1 and Swr1, and it has been proposed that human p400 is actually a fusion of the two yeast proteins during evolution (17). p400 was found to be crucial for nucleosome destabilization during DNA repair (48), and whether this function is conserved in yeast and how and to what extent Eaf1/NuA4 and Swr1/SWR-C contribute to this process remain to be answered.

During our adenine derepression experiment, we noted that Pol II passage decreased at the 20-min time point (Fig. 5A). This may be explained by the bivalent nature of poised inactive promoters, which allows for a burst of transcription yet requires a subsequent repression for extensive chromatin remodeler binding, full transcription factor recruitment, and/or more robust induction of gene expression (3). In fact, as more promoter chromatin changes are experienced by repressed promoters between basal and active states relative to constitutively expressed genes, this feature of repressed/inducible promoters could lead to higher levels of transcription noise (referred to as the variability of gene

expression in a cell population under the same growth conditions), as observed in both genome-wide studies (2) and individual assessments of *PHO5* (49) and *ADE* genes (Fig. 2).

An intriguing issue in the chromatin field is how SWR-C obtains specificity for certain loci. Accumulated evidence evolved into a model that transcriptional factors first recruit NuA4 to gene regulatory regions. NuA4 then acetylates histone H4, which further docks SWR-C through its Bdf1 subunit for H2A.Z incorporation. Newly deposited H2A.Z subsequently is acetylated by NuA4, preparing the promoter for nucleosome disruption (10, 13, 15–18, 20, 35). Our data about *ADE* promoter architecture is in agreement with this model (Fig. 7). A recent study revealed that SWR1 preferentially binds long nucleosome-free DNA and the adjoining nucleosome core particle, proposing a mechanism that allows the complex discrimination of gene promoters over gene bodies (50). We found that there still is Htz1 present at promoters in NuA4 mutants, although to a lesser extent. Thus, it is very likely that SWR-C utilizes multiple mechanisms to various degrees in specific chromatin contexts in order to precisely coordinate cellular activities.

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