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/10.1174/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, 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.
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 eaf1Δ 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-

TABLE 1 Primers used in ChIP-qPCR

| Gene   | Primer position (bp)/direction | Sequence                     |
|--------|-------------------------------|------------------------------|
| ADE17  | −237/forward                  | ATCATTTATAAAAGAGTCTACCC      |
| ADE17  | −122/reverse                  | ATAGATCCGAAAGTATG            |
| ADE17  | +717/forward                  | TTGTTGCAGTGCTCCTAAATTC      |
| ADE17  | +843/reverse                  | ATCGAGAATGGGATACCCA          |
| ADE17  | +1485/forward                 | CCGCCCAAATTCGCAACGAG        |
| ADE17  | +1614/reverse                 | CGATGACAAAGAGACGTTGG         |
| ADE5,7 | −200/forward                  | AGTGAGAAGGGCGAAGCTGACT       |
| ADE5,7 | −89/reverse                   | TACACGGGTTGCACTCGCC          |

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; log2(transcription rate mRNA/hour)) in WT cells (left). The scatter plot on the right shows the Htz1 occupancy at the same 429 loci in eaf1Δ 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 aef1 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 (OD600) of 0.25 in SC media with adenine (SC+adenine) or SC media without adenine (SC−adenine), and grown to an OD600 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 Qiagen RNeasy kit. The RNA quality was assessed using a Nanodrop spectrophotometer (Thermo Scientific) and RNA integrity numbers were determined using an Agilent 2100 Bioanalyzer (Agilent Technologies). The average RNA integrity number was 9.5. Full ChIP-on-chip data sets are provided in Tables S1 and S2 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 OD600 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 OD600 of 0.5 to 1.0. For induction, cells were centrifuged, washed, and resuspended in SCSM-Ade media supplemented with adenine at 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 (67-718; Upstate), 0.5 μl anti-superH4ac (Penta; Upstate), 0.5 μl anti-H3 C terminus (Ab1791; Abcam), and 2 μl anti-polymerase II (Pol II; BWG16) and anti-HAtz1K14 (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 T3-EDTA (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 T3 – 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 μg of proteinase K and 200 μg of

**TABLE 2** Genes downregulated in aef1Δ cells compared to levels in wild-type cells in rich media

| Gene      | Anotation                      |
|-----------|--------------------------------|
| RPL15B    | Ribosomal protein              |
| ADE13     | Bas1/Pho2 regulated, purine biosynthesis |
| ADE1      | Bas1/Pho2 regulated, purine biosynthesis |
| PHO12     | Pho2 regulated                  |
| SHM2      | Purine biosynthesis, Bas1/Pho2 regulated |
| ADE5,7    | Bas1/Pho2 regulated, purine biosynthesis |
| MTD1      | Purine biosynthesis, Bas1/Pho2 regulated |
| BAT2      | Bas1 site, purine pathway       |
| ADE2      | Bas1/Pho2 regulated, purine biosynthesis |
| GCV2      | Bas1 site, purine pathway       |
| ADE17     | Bas1/Pho2 regulated, purine biosynthesis |
| LEU1      | Pho2 regulated                  |
| YLR345w   |                                |
| LAP4      |                                |
| BNA1      |                                |
| DDR48     |                                |
| ADE3      | Bas1/Pho2 regulated, purine biosynthesis |
| YPF1      |                                |
| ECM9      |                                |
| ADE12     | Bas1/Pho2 regulated, purine biosynthesis |
| ABP140    |                                |
| SDS3      | Histone deacetylase subunit    |
| TRM12     |                                |
| PHO5      | Pho2 regulated                  |

*Microarray analysis performed on RNA isolated from aef1Δ 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). aef1Δ 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.*
EAF1 deletion remains to be answered. To this end, we examined the expression profile in both WT and eaf1Δ strains by microarray. As 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 eaf1Δ 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 (Fig. 3A) 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.

**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 eaf1Δ 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 eaf1Δ 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.

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).

**FIG 2 NuA4 is important for the induction of ADE genes.** (A) Northern blot analysis performed with RNA isolated from wild-type (WT) and eaf1Δ 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.
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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 eaf1Δ 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 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.

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 thatYPD media are poor in adenine allowed us to detect the implicaton 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 eaf1Δ strains, and several ADE gene expression levels were examined using specific probes. In both WT and eaf1Δ strains, when adenine is present in the media, the ADE genes show 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.

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 eaf1Δ 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.
pression) or stands for a preset mechanism to poised 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 eaf1Δ 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 eaf1Δ mutant would disrupt this poised status and alter nucleosome stability. Indeed, the eaf1Δ 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, Htz1 K14 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 eaf1Δ 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.

**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 eaf1Δ 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 eaf1Δ 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 ADE17 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 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 eaf1Δ 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.
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 stabilization 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

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.
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 the NuA4 histone acetyltransferase complex and epigenetic control of transcription. Newly deposited H2A.Z subsequently is acetylated by the 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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REFERENCES

1. Li B, Carey M, Workman JL. 2007. The role of chromatin during transcription. Cell 128:707–719. http://dx.doi.org/10.1016/j.cell.2007.01.015.
2. Tiros I, Barkai N. 2008. Two strategies for gene regulation by promoter nucleosomes. Genome Res 18:1084–1091. http://dx.doi.org/10.1101/gr.076059.108.
3. Cairns BR. 2009. The logic of chromatin architecture and remodelling at promoters. Nature 461:193–198. http://dx.doi.org/10.1038/nature08450.
4. Krogan NJ, Nechel MC, Datta N, Sawa C, Ryan J, Datta N, Sawa C, Berthelet S, Krogan NJ, Wolfe K, Podolny V, Carpenter LR, Greenblatt JF, Baetz K, Buratowski S. 2006. The Saccharomyces cerevisiae histone H2A variant Htz1 is acetylated by NuA4. Genes Dev 20:660–665. http://dx.doi.org/10.1101/gad.138180.106.
5. Millar CB, Xu F, Zhang K, Grunstein M. 2006. Acetylation of H2AZ Lys 14 is associated with genome-wide gene activity in yeast. Genes Dev 20:711–722. http://dx.doi.org/10.1101/gad.1395506.
6. Auger A, Galanarue I, Allard M, Nourani A, Doyon U, Utley RT, Brocherie J, Cote J. 2008. NuA4 dependent acetylation of histone variant H2A.Z in Saccharomyces cerevisiae. Genes Dev 22:2257–2270. http://dx.doi.org/10.1101/gad.138630.101.
7. Babiarz JE, Halley JE, Rine J. 2006. Telomeric heterochromatin boundaries require NuA4-dependent acetylation of histone variant H2A.Z in Saccharomyces cerevisiae. Genes Dev 20:700–710. http://dx.doi.org/10.1101/gad.138630.101.
8. Doyon Y, Selleck W, Lane WS, Tan S, Cote J. 2004. Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. Mol Cell 24:1884–1896. http://dx.doi.org/10.1128/MCB.24.5.1884-1896.2004.
9. Almer A, Rudolph H, Hinnen A, Horz W. 1986. Removal of positioned nucleosomes from the yeast PHO5 promoter upon PHO5 induction releases additional upstream activating DNA elements. EMBO J 5:2689–2696.
10. 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.
11. Hofer H, Griesenbeck J, St Jean JS, Kornberg RD. 2003. Nucleosomes unfold completely at a transcriptionally active promoter. Mol Cell 11:1587–1598. http://dx.doi.org/10.1016/S1097-2675(03)00231-4.
12. Reineke H, Horz W. 2003. Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter. Mol Cell 11:1599–1607. http://dx.doi.org/10.1016/S1097-2675(03)00186-2.
13. Brown CR, Mao C, Falkovskyia E, Law JK, Boeger H. 2011. In vivo role for the chromatin-remodeling enzyme SWR1 in the removal of nucleosomes by disassembly rather than sliding. J Biol Chem 286:40556–40565. http://dx.doi.org/10.1074/jbc.M111.289918.
14. Nourani A, Utley RT, Allard S, Cote J. 2004. Recruitment of the NuA4 complex poises the PHO5 promoter for chromatin remodeling and activation. EMBO J 23:2597–2607. http://dx.doi.org/10.1038/sj.emboj.7600230.
15. Holstegke FC, Jennings EG, Wyrick JJ, Lee TI, Hengartner CJ, Green MR, Golub TR, Lander ES, Young RA. 1998. Dissecting the regulatory circuitry of a eukaryotic genome. Cell 95:717–728. http://dx.doi.org/10.1016/S0092-8674(00)81641-4.
16. Schmitt ME, Brown TA, Trumper BL. 1990. A rapid and simple method for preparation of RNA from Saccharomyces cerevisiae. Nucleic Acids Res 18:3091–3092. http://dx.doi.org/10.1093/nar/18.10.3091.
17. Utley RT, Lacoste N, Jobin-Robitaille O, Allard S, Cote J. 2005. Regulation of NuA4 histone acetyltransferase activity in transcription and DNA
repair by phosphorylation of histone H4. Mol Cell Biol 25:8179–8190. http://dx.doi.org/10.1128/MCB.25.18.8179-8190.2005.

30. Mitchell L, Lambert JP, Gerdes M, Al-Madhoun AS, Skerjanc IS, Figyews D, Baetz K. 2008. Functional dissection of the NuA4 histone acetyltransferase reveals its role as a genetic hub and that Eaf1 is essential for complex integrity. Mol Cell Biol 28:2244–2256. http://dx.doi.org/10.1128/MCB.01653-07.

31. Cheng X, Cote J. 2014. A new companion of elongating RNA polymerase II: TINTIN, an independent sub-module of NuA4/TIP60 for nucleosome trans-...http://dx.doi.org/10.1016/j.cell.2013.09.025.

32. Li B, Pattenden SG, Lee D, Gutierrez J, Chen J, Seidel C, Gerton J, Cheng et al. 2009. Nourani A, Doyon Y, Utley RT, Allard S, Lane WS, Cote J. 2014. Actions. Transcription 5:e95571. http://dx.doi.org/10.1080/21541264.2014.995571.

33. Raisner RM, Hartley PD, Meneghini MD, Bao MZ, Liu CL, Schreiber SL, Rando OJ, Madhani HD. 2005. Histone variant H2A.Z marks the 5′ ends of both active and inactive genes in euchromatin. Cell 123:233–248. http://dx.doi.org/10.1016/j.cell.2005.10.002.

34. Guillemette B, Bataille AR, Govry N, Adam M, Blanchette M, Robert F, Gaudreau L. 2004. Evidence for nucleosome remodelling. Nature 404:414–417. http://dx.doi.org/10.1038/35006136.

35. Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD. 2004. Evidence for nucleosome depletion at active regulatory regions genome-wide. Nat Genet 36:900–905. http://dx.doi.org/10.1038/ng1400.

36. Han M, Grunstein M. 2005. The p400 ATPase regulates nucleosome stability and chromatin ubiquitination during DNA repair. J Cell Biol 169:1–43. http://dx.doi.org/10.1083/jcb.201001160.

37. Raser JM, O’Shea EK. 2004. Control of stochasticity in eukaryotic gene expression. Science 304:1811–1814. http://dx.doi.org/10.1126/science.1098641.

38. Sanstiebean MS, Kalashnikova T, Smith MM. 2000. The Saccharomyces cerevisiae H2A.Z regulates transcription and is partially redundant with nucleosome re-...http://dx.doi.org/10.1016/S0092-8674(00)00133-1.

39. Rolfes RJ, Zhang F, Hinnebusch AG. 2014. A new companion of elongating RNA polymerase II: TINTIN, an independent sub-module of NuA4/TIP60 for nucleosome trans-...http://dx.doi.org/10.1080/21541264.2014.995571.

40. Gedvilaite A, Sasnauskas K. 1994. Control of the expression of the ADE2 gene of the yeast Saccharomyces cerevisiae. Curr Genet 25:475–479. http://dx.doi.org/10.1007/BF00351665.

41. Som I, Mitsch RN, Urbanowski JL, Rolfs RJ. 2005. DNA-bound Bas1 recruits Pho2 to activate ADE genes in Saccharomyces cerevisiae. Eukaryot Cell 4:1725–1735. http://dx.doi.org/10.1128/EC.4.10.1725-1735_2005.

42. Rolfs RJ, Zhang F, Hinnebusch AG. 1997. The transcriptional activators Bas1, Bas2, and ABF1 bind positive regulatory sites as the critical elements for adenine regulation of ADE5,7. J Biol Chem 272:13343–13354. http://dx.doi.org/10.1074/jbc.272.20.13343.