Eaf1p Is Required for Recruitment of NuA4 in Targeting TFIID to the Promoters of the Ribosomal Protein Genes for Transcriptional Initiation In Vivo.

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Eaf1p Is Required for Recruitment of NuA4 in Targeting TFIID to the Promoters of the Ribosomal Protein Genes for Transcriptional Initiation In Vivo

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NuA4 (nucleosome acetyltransferase of H4) promotes transcriptional initiation of TFIID (a complex of TBP and TBP-associated factors [TAFs])-dependent ribosomal protein genes involved in ribosome biogenesis. However, it is not clearly understood how NuA4 regulates the transcription of ribosomal protein genes. Here, we show that NuA4 is recruited to the promoters of ribosomal protein genes, such as RPS5, RPL2B, and RPS11B, for TFIID recruitment to initiate transcription, and the recruitment of NuA4 to these promoters is impaired in the absence of its Eaf1p component. Intriguingly, impaired NuA4 recruitment in a ∆eaf1 strain depletes recruitment of TFIID (a TAF-dependent form of TBP) but not the TAF-independent form of TBP to the promoters of ribosomal protein genes. However, in the absence of NuA4, SAGA (Spt-Ada-Gcn5-acetyltransferase) is involved in targeting the TAF-independent form of TBP to the promoters of ribosomal protein genes for transcriptional initiation. Thus, NuA4 plays an important role in targeting TFIID to the promoters of ribosomal protein genes for transcriptional initiation in vivo.

Such a function is mediated via its targeted histone acetyltransferase activity. In the absence of NuA4, ribosomal protein genes lose TFIID dependency and become SAGA dependent for transcriptional initiation. Collectively, these results provide significant insights into the regulation of ribosomal protein gene expression and, hence, ribosome biogenesis and functions.
scription factor IID (TFIID) (a complex of TBP and TBP-associated factors [TAFs])-dependent ribosomal protein genes (10, 11, 17). Further, we have recently demonstrated that the 19S base or subcomplex of the 26S proteasome enhances the targeting of NuA4 to the promoters of the ribosomal protein genes for transcriptional initiation (17). It is not clearly understood how NuA4 enhances transcriptional initiation of the ribosomal protein genes. In view of this, we impaired the recruitment of NuA4 to the promoters of ribosomal protein genes, such as RPS5, RPL2B, and RPS11B, following the depletion of its Eaf1p component and then analyzed the levels of histone H4 acetylation and TFIID at the promoters of these genes. Our results reveal that the levels of histone H4 acetylation at the promoters of the ribosomal protein genes are not dramatically reduced following impaired recruitment of NuA4 in the absence of Eaf1p, since picNuA4 globally acetylates histone H4 in the background of NuA4. Intriguingly, the recruitment of TFIID (i.e., the TAF-dependent form of TBP) to these promoters is decreased in the absence of NuA4 or Eaf1p. However, the levels of the TAF-independent form of TBP at these promoters were not impaired in the absence of Eaf1p. Hence, transcription of these ribosomal protein genes occurred in the Δeaf1 strain. However, TFIID-dependent ribosomal protein genes become more dependent on the TAF-independent form of TBP for transcriptional initiation in the absence of NuA4, thus implicating NuA4 in targeting TFIID to the promoters of the ribosomal protein genes. Further, in the absence of Eaf1p or NuA4, SAGA (Spt-Ada-Gcn5 acetyltransferase, a large multiprotein complex) is involved in targeting the TAF-independent form of TBP but not TFIID to the promoters of the ribosomal protein genes for transcriptional initiation. Thus, NuA4 contributes to TFIID-dependent transcriptional initiation of the ribosomal protein genes. Such a function of NuA4 is mediated via its targeted KAT activity at the promoter. In the absence of NuA4, the ribosomal protein genes lose TFIID dependency and become SAGA dependent for transcriptional initiation. Thus, NuA4 contributes to TFIID-dependent transcriptional initiation of the ribosomal protein genes.

**MATERIALS AND METHODS**

**Plasmids.** The plasmid pFA6α-13Myc-KanMX6 (48) was used for genomic tagging of Swc4p, Epl1p, Yng2p, Esa1p, Eaf1p, Eaf5p, and Spt20p with a Myc epitope. The plasmid pRS403 or pRS406 (49) was used to delete EAF1 by the PCR-based gene disruption method in different genetic backgrounds. The plasmid pFA6α-TRP1-pGAL1-3HA (48) was used to replace the endogenous promoter of SPT20 with the GAL1 promoter with a hemagglutinin (HA) epitope tag.

**Yeast strains.** The taf1-ts (temperature-sensitive) mutant and its isogenic wild-type (WT) equivalent were obtained from the laboratory of Michael R. Green (University of Massachusetts Medical School) (50, 51). The eas1-ts mutant (LPY3291) and wild-type (LPY3498) strains were obtained from the laboratory of Lorraine Pillus (University of California, San Diego) (38). The Δspy20 (FY1097) and wild-type (FY67) strains were obtained from the laboratory of Fred Winston (Harvard Medical School). Multiple Myc epitope tags were added at the original chromosomal loci of SWC4, EPL1, YNG2, ESA1, and EAF5 in the wild-type strain (W303a) to generate the strains BUY41 (Swc4p-Myc), ZDY22 (Epl1p-Myc), BUY31 (Yng2p-Myc), BUY26 (Esa1p-Myc), and RSY70 (Eaf5p-Myc), respectively. The EAF1 gene in the BUY26 strain was deleted to generate BUY27, using the pRS406 plasmid. The strain BUY28 was generated by deleting EAF1 from the ZDY22 strain. The BUY24 strain was generated by deleting EAF1 in the W303a strain, using the pRS403 plasmid. The YNG2, SWC4, and EAF5 genes were separately tagged with multiple Myc epitopes in their chromosomal loci in the BUY24 strain to generate the BUY32, BUY42, and RSY69 strains, respectively. The SPT20 gene was tagged with multiple Myc epitopes at its chromosomal locus in the wild-type strain (W303a) to generate the ASY10 strain. The EAF1 gene in the ASY10 strain was deleted to generate BUY23, using the pRS403 plasmid. Likewise, EAF5 was deleted in WT Taf1 and temperature-sensitive mutant strains to generate the BUY47 and BUY48 strains, respectively, using the pRS406 plasmid. The promoter of SPT20 in the BUY24 and W303a strains was replaced with the GAL1 promoter using the homologous-recombination-based promoter replacement method (48), and the resultant strains (BUY49 and RSY75, respectively) expressed HA-tagged Spt20p in the growth medium containing galactose but not dextrose. Multiple Myc epitope tags were added at the chromosomal loci of YNG2, EAF1, and EAF5 in the eas1-ts mutant strain to generate the BUY44, RSY71, and RSY73 strains, respectively. The strains BUY43, RSY72, and RSY74 were generated by adding multiple Myc epitope tags at the chromosomal loci of YNG2, EAF1, and EAF5 in the Eas1p wild-type strain, respectively.

**Growth media.** For studies of RPS5, RPL2B, RPS11B, ADH1, and ACT1 in the wild-type and Δeaf1 strains, yeast cells were grown in YPD medium (yeast extract and peptone plus 2% dextrose) to an optical density at 600 nm (OD600) of 1.0 at 30°C prior to formaldehyde-based in vivo cross-linking for chromatin immunoprecipitation (ChIP) analysis or harvesting for RNA analysis. For experiments in the taf1-ts and eas1-ts strains and their wild-type equivalents, yeast cells were grown in YPD medium at 23°C to an OD600 of 0.9 and then switched to 37°C for 1 h prior to harvesting or cross-linking. The expression of SPT20 under the GAL1 promoter was repressed by initially growing yeast cells in galactose-containing growth medium (yeast extract and peptone plus 2% galactose [YPG]) to an OD600 of 0.5 and then switching to dextrose-containing growth medium for 2 h. For studies of GAL1, yeast cells were grown in YPG at 30°C to an OD600 of 1.0 prior to formaldehyde-based in vivo cross-linking.

**ChIP assay.** The ChIP assay for TBP, TAF1p, TAF2p, and histone H4 acetylation was performed as described previously (17, 52–61). For ChIP analysis of Myc-tagged Esa1p, Swc4p, Epl1p, Yng2p, Eaf1p, Eaf5p, and Spt20p, the ChIP protocol was modified as described previously (17, 52, 54, 58, 60). Briefly, a total of 800 μl lysate was prepared from 100 ml of yeast culture. Following sonication, 400 μl lysate was used for each immunoprecipitation (using 10 μl of anti-Myc antibody and 100 μl of protein A/G plus agarose beads [Santa Cruz Biotechnology, Inc.]), and the immunoprecipitated DNA sample was dissolved in 10 μl 1 M Tris-HCl, pH 8.0, and 1 mM EDTA (TE 8.0), 1 μl of which was used for PCR analysis (a total of 23 cycles). In parallel, PCR analysis for input DNA was performed using 1 μl DNA that was prepared by dissolving purified DNA from 5 μl lysate in 100 μl TE 8.0. The ChIP analysis for histone H3 was performed as described previously (62–64). Serial dilutions of input and immunoprecipitated DNA samples were used to assess the linear range of PCR amplification as described previously (54, 56, 61) (see Fig. S3B in the supplemental material). The data presented here are within the linear range of PCR analysis. The primer pairs used for PCR analysis were as follows: (upstream activating sequence [UAS], core promoter [Core], and a transcriptionally inactive region within chromosome V [Chr·V]): RPS5 (UAS), 5′-AGAAACAAATGAACAGCCTTGAGTTCTC-3′ and 5′-GGCCATTCTCATCTGA-3′; RPS5 (Core), 5′-GGCAGAACCATCTACGGC-3′; RPS5 (Chr·V), 5′-ATTTGTCCGTAACCTGTTAAGTGGT-3′; RPL2B (UAS), 5′-AACGACAAAGCTGTTTGAACAAGTGGC-3′; RPL2B (Core), 5′-CTTTTTCCCTGGCT-3′; RPL2B (Chr·V), 5′-GGCCATTCTCATCTGA-3′; RPS11B (UAS), 5′-CTTTTTCCCTGGCT-3′ and 5′-GGCCATTCTCATCTGA-3′; RPS11B (Core), 5′-ATTTGTCCGTAACCTGTTAAGTGGT-3′; RPS11B (Chr·V), 5′-GGCCATTCTCATCTGA-3′; ADH1 (UAS), 5′-GGCCATTCTCATCTGA-3′ and 5′-GAACGAGAACAATGACGAGGAAACAAAAG-3′; ADH1 (Core), 5′-GGCCATTCTCATCTGA-3′ and 5′-GAACGAGAACAATGACGAGGAAACAAAAG-3′; GAL1 (UAS), 5′-CGCTTAACCTGCTAAGTTTGAT-3′ and 5′-GGCCATTCTCATCTGA-3′; GAL1 (Core), 5′-CGCTTAACCTGCTAAGTTTGAT-3′ and 5′-GGCCATTCTCATCTGA-3′; GAL1 (Chr·V), 5′-GGCCATTCTCATCTGA-3′ and 5′-GAACGAGAACAATGACGAGGAAACAAAAG-3′.
TTCCGGAGCTGCGGC-3'; Chr.-V, 5'-GGTGTGCAGAATATGG GGCCTCAGTA-3' and 5'-CACCCTGCAGCTGTCTCAATACAT-3'.

Autoradiograms were scanned and quantitated with the National Institutes of Health Image 1.62 program. Immunoprecipitated DNA was quantitated as the ratio of immunoprecipitate to input and represented as a ChIP signal. The average ChIP signal of three biologically independent experiments is reported, with the standard deviation (SD) (Microsoft Excel). The Student t test of Microsoft Excel (with tail equal to 2 and types equal to 3) was used to determine the P values for the statistical significance of the change in the ChIP signals. The changes were considered to be statistically significant at a P value of <0.05.

**Total-RNA preparation.** Total RNA was prepared from yeast cell culture as described previously (54, 65–67). Briefly, 10 ml yeast culture was harvested and suspended in 100 μl RNA preparation buffer (500 mM NaCl, 200 mM Tris-HCl, 100 mM Na2-EDTA, and 1% SDS), along with 100 μl phenol-chloroform–isooamyl alcohol and a 100-μl volume equivalent of glass beads (acid washed; Sigma). Subsequently, the yeast cell suspension was vortexed at maximum speed (10 in a VWR minivortexer; catalog no. 58816-121) five times (30 s each). After vortexing, 150 μl RNA preparation buffer and 150 μl phenol-chloroform–isoamyl alcohol were added to the yeast cell suspension, followed by vortexing for 15 s at maximum speed on a VWR minivortexer. The aqueous phase was collected for isolation of total RNA by precipitation with ethanol.

**RT-PCR analysis.** Reverse transcription (RT)-PCR analysis was performed as described previously (65, 68, 69). Briefly, RNA was treated with RNase-free DNase (M610A; Promega) and then reverse transcribed into cDNA using oligo(dT), as described in the protocol supplied by Promega (A3800; Promega). PCR was performed using a synthesized first strand as the template and the primer pairs targeted to the open reading frames (ORFs) of the RPS5, RPL2B, RPS11B, ACT1, and ADH1 genes. The RT-PCR products were separated by 2.2% agarose gel electrophoresis and visualized by ethidium bromide staining. The average signal of three biologically independent RT-PCR experiments is reported with the SD (Microsoft Excel). The Student t test (with tail equal to 2 and types equal to 3) was used to determine P values for the statistical significance of the change in the RT-PCR signals. The changes were considered to be statistically significant at a P value of <0.05. The primer pairs used in the PCR analysis of cDNAs were as follows: ADH1, 5'-CGGTAACAGAGCTGACACCAG GGCCTCAGTA-3' and 5'-GAATTCACCTAGTGGAGGAGAAG-3'; RPS5, 5'-AG GCTCAATTGGTCAATCACTTAAAGG-3' and 5'-CAAACTTCACTTTG-3'; RPS11B, 5'-GGTCTACATTGACCAACGGTAACAA-3' and 5'-GTAATAGTGATGACTTGACCATCTGGA-3'; ACT1, 5'-TCCACACTGCTGAAAGAAGAAATT G-3' and 5'-AATCTAATGAGCTGACCATCTCCACAA-G-3'; RPL2B, 5'-GTTGCTCAGAAGTGACAGTTAA-3' and 5'-TTTGGACGGAAACCGG CACCCCTCTACA-3'; RPL2B, 5'-GACCTGACCTTACCTTCGATG-3' and 5'-GTCATGACCTTGACGAAAGAAG-3'.

**Growth analysis on solid media.** The growth of the Δeaf1 strain bearing SPT20 under the GALI promoter, the Δeaf1 and Δsp20 strains, and the wild-type equivalents was analyzed on plates containing solid YPD and YPG media. Yeast cells were inoculated in liquid YPG medium and grown to an OD600 of 0.2 at 30°C. Subsequently, the yeast cells were suspended in fresh YPD medium and grown to an OD600 of 0.4 at 30°C prior to spotting (3 μl) on solid YPD or YPG medium with 10-fold serial dilutions. The yeast cells were grown at 30°C and photographed after 2, 3, or 4 days.

**Whole-cell extract preparation and Western blot analysis.** For analysis of global levels of Spt20p and actin in the Δeaf1 strain bearing SPT20 under the GALI promoter, yeast cells were grown in YPG medium to an OD600 of 0.5 and then switched to YPD medium for 2 or 4 h. The harvested cells were lysed and sonicated to prepare the whole-cell extract with solubilized chromatin, following the protocol described previously for the ChIP assay (17, 52–61). The whole-cell extract was run on an SDS-polyacrylamide gel and then analyzed by Western blotting. The anti-HA (Santa Cruz Biotechnology, Inc.) and antiactin (A2066; Sigma) antibodies against HA-tagged Spt20p and actin, respectively, were used in the Western blot analysis. Likewise, global levels of Myc-tagged Eaf1p, Swc4p, Ea5p, Yng2p, Esa1p, Epl1p, TBP, and TAF1p in the Δeaf1 and wild-type strains were analyzed, using anti-Myc (Santa Cruz Biotechnology, Inc.), anti-TBP (obtained from Michael R. Green, University of Massachusetts Medical School), and anti-TAF1p (obtained from Michael R. Green) antibodies. Global levels of Myc-tagged Eaf1p, Ea5p, and Yng2p in the Δeaf1-ts and wild-type strains were similarly analyzed, using an anti-Myc antibody.

**RESULTS**

**Eaf1p is required for recruitment of NuA4 to the promoters of the ribosomal protein genes.** As mentioned above, NuA4 is a multisubunit protein complex and is essential for acetylation of histones H4, H2A, and H2A.Z (4–7). NuA4 is targeted to the UAS of the promoter of the target gene by a transcriptional activator (10, 11, 14, 17, 46, 70). At the ribosomal protein genes, NuA4 is recruited by the activator Rap1p, and such targeted recruitment is essential for transcriptional initiation (10, 14, 17, 70). Eaf1p has been biochemically shown to maintain the overall structural integrity of NuA4 (13, 45). Thus, the absence of Eaf1p is likely to impair the recruitment of the NuA4 components to the UASs of the NuA4-regulated genes. However, there has not been a systematic study to analyze the recruitment of different components of NuA4 to the UASs of the target genes in the absence of Eaf1p. Therefore, it is not clear whether Eaf1p maintains the structural integrity of NuA4 at the promoter of the target gene in vivo, consistent with previous biochemical studies (13, 45). To test this, we analyzed the recruitment of the Epl1p, Yng2p, Swc4p, and Ea5p components of NuA4 to the UASs of ribosomal protein genes, such as RPS5, RPL2B, and RPS11B, in the Δeaf1 strain and its isogenic wild-type equivalent (since previous studies demonstrated the association of NuA4 with the UASs of the ribosomal protein genes (17)). For this purpose, we tagged the Epl1p, Yng2p, Swc4p, and Ea5p components of NuA4 with Myc epitopes at their chromosomal loci and then performed the ChIP assay at the UASs of the ribosomal protein genes RPS5, RPL2B, and RPS11B, using an anti-Myc antibody against Myc-tagged Epl1p, Yng2p, Swc4p, and Ea5p. An anti-HA antibody was used as a nonspecific antibody control in the ChIP assay. We found that the recruitment of Swc4p to the UASs of the RPS5, RPL2B, and RPS11B promoters was impaired in the Δeaf1 strain in comparison to the wild-type equivalent (Fig. 1A; see Fig. S1A and S2 in the supplemental material). Likewise, the recruitment of the Epl1p, Yng2p, and Ea5p components of NuA4 to the UASs of these ribosomal protein genes was impaired in the absence of Eaf1p (Fig. 1B to D; see Fig. S1B to D and S2 in the supplemental material). An inactive region within Chr.-V (17) was used in this set of ChIP experiments as a nonspecific DNA control. Targeted recruitment of the Epl1p, Yng2p, and Ea5p components of NuA4 was not observed in this region (see Fig. S2 in the supplemental material). However, Swc4p, which is a shared component of the NuA4 and SWR1 complexes, was found to be associated with Chr.-V, independently of Eaf1p (see Fig. S2 in the supplemental material). Likewise, previous genome-wide studies (71) also found association of Swc4p with all nucleosomes and suggested that Swc4p may be a component of a novel complex apart from SWR1 and NuA4. Taken together, our results demonstrate that Eaf1p is required for recruitment of the NuA4 components, such as Swc4p, Epl1p, Yng2p, and Ea5p, to the UASs of the ribosomal protein genes. Further, previous studies demonstrated that the Tra1p subunit of NuA4 is essential for targeted recruitment of NuA4 by an activator.
Thus, impaired recruitment of the NuA4 components to the UASs of the ribosomal protein genes in the absence of Eaf1p is not due to the defect in the activator-mediated targeting of NuA4 but, rather, to the disintegration of NuA4. Consistently, previous biochemical studies also implicated Eaf1p in maintaining the overall structural integrity of NuA4 (13, 45). Therefore, our results support the idea that Eaf1p is required for maintaining the structural integrity of NuA4 at the promoter of the target gene in vivo, and hence, the recruitment of the NuA4 components to the promoters of the ribosomal protein genes was impaired in the absence of Eaf1p (Fig. 1; see Fig. S1 and S2 in the supplemental material). Such a decrease in the recruitment of NuA4 components, Eaf5p, Yng2p, and Swc4p, in the Δeaf1 strain are not due to decreases in their stabilities (see Fig. S3A in the supplemental material). However, the level of Epl1p was greatly decreased in the Δeaf1 strain (see Fig. S3A in the supplemental material). Such a decrease in the stability of Epl1p in the Δeaf1 strain could be due to ubiquitylation and 26S proteasomal degradation of Epl1p following disintegration of NuA4 in the absence of Eaf1p (as ubiquitylation of Epl1p at K648 has been indicated in the Saccharomyces genome database), which remains to be further elucidated. The decrease in the level of Epl1p in the Δeaf1 strain may not result in decreased association of Epl1p with the UASs of the ribosomal protein genes, since the protein level is not necessarily correlated with its targeted association with the gene/DNA. Further, consistent with our results, Ginsburg et al. (16) also demonstrated that the recruitment of Epl1p to the promoters of the NuA4 target genes, such as ARG1 and ARG4, was also reduced by ∼2-fold. Moreover, in addition to Epl1p, other components of NuA4 are not efficiently recruited to the UASs of the ribosomal protein genes in the Δeaf1 strain, supporting the role of Eaf1p in the recruitment of NuA4 to the promoters of the target genes in vivo, consistent with previous biochemical studies (13, 45).

We found ∼2.5-fold reduction in the recruitment of the NuA4 components to the UASs of the ribosomal protein genes in the absence of Eaf1p (Fig. 1), using radioactive PCR-based gel electrophoresis methodology. Such a method may result in PCR analysis within a nonlinear range, leading to the saturation of PCR signals. To rule out this possibility, we carried out the PCR within a linear range, as described in our previous publications (54, 56, 61) (see Fig. S3B in the supplemental material), and found ∼2.5-fold reduction in the recruitment of the NuA4 components in the Δeaf1 strain in comparison to the wild-type equivalent (Fig. 1). Consistent with our results, Ginsburg et al. (16) have also found ∼2-fold reduction of the NuA4 components (e.g., Epl1p and Eaf5p) to the promoters of the NuA4 target genes, such as ARG1 and ARG4, in the absence of Eaf1p.

Since Eaf1p is required to maintain the structural integrity of NuA4, targeted histone H4 acetylation at the promoters of the ribosomal protein genes would likely to be impaired in the Δeaf1 strain. To test this, we analyzed the levels of histone H4 acetylation at the RPS5, RPL2B, and RPS11B promoters in the Δeaf1 strain and its isogenic wild-type equivalent, using the ChIP assay. We found that the levels of histone H4 acetylation at the promoters of these genes were not dramatically impaired in the absence of Eaf1p but, rather, decreased by ∼2-fold (Fig. 2A to C). However, such a decrease in histone H4 acetylation could be due to loss of histone H3-H4 tetramer from the promoters of the ribosomal protein genes in the absence of Eaf1p. To address this issue, we also analyzed the levels of histone H3 (as a representative component of the histone H3-H4 tetramer) at the promoters of the ribosomal protein genes in the Δeaf1 wild-type strains. We found that the levels of histone H3-H4 tetramer at the promoters of the ribosomal protein genes were not similarly impaired in the absence of Eaf1p (Fig. 2A to C). Thus, our results support the idea that the disintegration of NuA4 in the absence of its Eaf1p component reduces the levels of histone H4 acetylation at the promoters of the ribosomal protein genes but does not dramatically alter histone H4 acetylation. As a control, we showed that global and nontargeted histone H4 acetylation in the inactive region of Chr.-V is not altered in the Δeaf1 strain in comparison to the wild-type equivalent (Fig. 2D). Thus, impairment of the targeted recruitment of NuA4 in the absence of Eaf1p decreases histone H4 acetylation at the promoters of the ribosomal protein genes. Likewise, the removal of the Eaf3p component of NuA4 decreases histone H4 acetylation at the promoters of the ribosomal protein genes (72). However, unlike Eaf1p, Eaf3p does not regulate the overall structural integrity of NuA4 (13, 45). Although the loss of Eaf3p decreases targeted histone H4 acetylation at the promoter, the overall global histone H4 acetylation is not altered in the absence of Eaf3p (72). Likewise, in the absence of Eaf1p, the targeted histone H4 acetylation was reduced (Fig. 2), while the overall global level of histone H4 acetylation was not altered (45).

Although previous studies implicated NuA4 in targeted/local histone acetylation, we did not observe dramatic impairment of histone H4 acetylation at the promoters of the ribosomal protein genes when NuA4 was not recruited or disintegrated in the absence of Eaf1p (Fig. 1 and 2). It is quite possible that, in the absence of NuA4, picNuA4 is involved in nontargeted global histone H4 acetylation via its interaction with chromatin. To test this, we analyzed the recruitment of Esa1p to the promoters of the ribosomal protein genes in the Δeaf1 strain and its isogenic wild-type equivalent. For this purpose, we tagged Esa1p with a Myc epitope at its chromosomal loci in the Δeaf1 and wild-type strains and

FIG 1 Eaf1p is required for the recruitment of the NuA4 components (Swc4p, Epl1p, Yng2p, and Eaf5p) to the promoters of the ribosomal protein genes. Shown is ChIP analysis of the recruitment of Swc4p (A), Epl1p (B), Yng2p (C), and Eaf5p (D) with the UASs of the RPS5, RPL2B, and RPS11B genes in the wild-type and Δeaf1 strains. Yeast strains expressing Myc-tagged Swc4p, Epl1p, Yng2p, and Eaf5p were grown in YPD medium at 30°C to an OD 600 of 1.0 prior to formaldehyde-based in vivo cross-linking. The ChIP assay was performed as described in Materials and Methods. Immunoprecipitation was performed using a mouse monoclonal antibody against the c-Myc epitope tag (9E10; Santa Cruz Biotechnology, Inc.). An anti-HA antibody was used as a nonspecific antibody. Primer pairs (see Materials and Methods) located at the UASs of the RPS5, RPS2B, and RPS11B genes and the transcriptionally inactive region within Chr.-V were used for PCR analysis of the immunoprecipitated DNA samples. The ratio of immunoprecipitate over the input in the autoradiogram was measured and represented as the ChIP signal. The ChIP signals for Myc-tagged Swc4p, Epl1p, Yng2p, and Eaf5p in the wild-type strain were set to 100. The ChIP signals for Myc-tagged Swc4p, Epl1p, Yng2p, and Eaf5p in the Δeaf1 strain were normalized to 100. The ChIP signals for nonspecific anti-HA antibody were normalized to the ChIP signals of the Myc-tagged Swc4p, Epl1p, Yng2p, and Eaf5p in the wild-type strain. The normalized ChIP signals (represented as normalized occupancy) are plotted in the form of a histogram. The error bars indicate SD.
then performed the ChIP assay at the promoters of the ribosomal protein genes \(RPS5\), \(RPL2B\), and \(RPS11B\). We found that the recruitment of Esa1p to the promoters of these ribosomal protein genes was not impaired in the absence of Eaf1p or NuA4 (Fig. 3A, B, and C). Further, the global level of Esa1p was not altered in the \(\Delta eaf1\) strain (Fig. 3D). Thus, the absence of Eaf1p does not impair the recruitment of Esa1p to the promoters of the ribosomal protein genes, even though it is required for the overall structural integrity of NuA4 (13, 45). Likewise, Esa1p was found to be associated with the promoter of the ribosomal protein gene (e.g., \(RPL2B\)) in the Tra1p mutant (\(H9004\) 17) that impairs the recruitment of Eaf1p to the promoter (70). Since Esa1p is present in the picNuA4 module of NuA4 (5, 41–44), it is likely to be recruited to the promoters of the ribosomal protein genes as a component of picNuA4 in the absence of Eaf1p or NuA4. However, the recruitment of two other components (Epl1p and Yng2p) of picNuA4 to the promoters of the ribosomal protein genes was decreased to the background level in the absence of Eaf1p (Fig. 1B and C). This could be due to the fact that Esa1p in picNuA4 adopts a different conformation to efficiently interact with nucleosomes for histone acetylation (and hence, efficient formaldehyde-based in vivo cross-linking). In agreement with this, we would also observe prominent recruitment of Esa1p at the inactive region within Chr.-V. However, we did not find significant association of Esa1p with Chr.-V (Fig. 3), consistent with previous studies (10, 73). Likewise, we did not observe significant association of the Yng2 and Epl1p components of picNuA4 with Chr.-V (Fig. 1B and C; see Fig. S2 in the supplemental material). However, a high level of Esa1p was found to be associated with the promoters of the ribosomal protein genes in the absence of Eaf1p (Fig. 3). These results support the idea that Esa1p is recruited to the promoters of the ribosomal protein genes independently of Eaf1p. In agreement with our results, previous studies also revealed significant association of Esa1p with the \(RPL2B\) promoter when the recruitment of Eaf1p to the promoter was greatly impaired in the \(\Delta eaf1\) strain (70). These results suggest that Esa1p may exist outside NuA4/picNuA4 and is recruited to the promoters of the ribosomal protein genes in a transcription (or transcription factor)-dependent manner. In support of this possibility, at least two yet-uncharacterized proteins, apart from Epl1p and Yng2p, have been found to interact with Esa1p in the absence Eaf1p (13), which remains to be further elucidated. Previous biochemical studies (42–44) revealed efficient interaction of picNuA4 with the nucleosome for histone H4 acetylation. Even though picNuA4 is involved in global histone H4 acetylation and has been biochemically shown to interact with the nucleosome, significant (or greatly increased) association of picNuA4 with chromatin/nucleosomes was not observed in vivo (Fig. 1B and C; see Fig. S2 in the supplemental material) using the ChIP assay, consistent with previous studies (10, 73). This could be due to lower abundance of picNuA4, consistent with previous biochemical studies (13, 45). Alternatively, picNuA4 may interact with chromatin transiently or may not be cross-linked well. Nonetheless, picNuA4 is involved in nontargeted global histone H4 acetylation.
acetylation (41, 42). Thus, in the absence of Eaf1p or NuA4, picNuA4 acetylates histone H4, and hence, the levels of histone H4 acetylation at the promoters of the ribosomal protein genes were not dramatically altered in the \( /H9004\) eaf1 strain. Consistently, previous biochemical studies demonstrated that global histone H4 acetylation was not altered in the \( /H9004\) eaf1 strain (45).

Since Esa1p is involved in nontargeted global histone H4 acetylation through picNuA4 or targeted local histone H4 acetylation via NuA4 (5, 41, 42), histone H4 acetylation would be greatly impaired following temperature-sensitive inactivation of Esa1p. To test this, we analyzed the levels of histone H4 acetylation at the promoters of the ribosomal protein genes \( RPS5\), \( RPL2B\), and \( RPS11B\) in the \( esa1\)-ts mutant and its isogenic wild-type equivalent at the nonpermissive temperature. We found that the levels of histone H4 acetylation at the promoters of these ribosomal protein genes were severely impaired in the \( esa1\)-ts mutant strain in comparison to the wild-type equivalent (Fig. 4A to C). However, such decreased levels of histone H4 acetylation at the promoters of the ribosomal protein genes in the \( esa1\)-ts mutant strain could be due to eviction (or loss) of the histone H3-H4 tetramer. To test this, we analyzed the levels of histone H3 at the promoters of the ribosomal protein genes were not impaired in the \( esa1\)-ts mutant strain in comparison to the wild-type equivalent (Fig. 4A to C). Thus, the temperature-sensitive inactivation of Esa1p severely impairs histone H4 acetylation at the promoters of the ribosomal protein genes, as NuA4 and picNuA4 are catalytically dead in the \( esa1\)-ts mutant strain at the nonpermissive temperature. Similarly, histone H4 acetylation in the inactive region within Chr.-V was impaired in the \( esa1\)-ts mutant strain (Fig. 4D).

Targeted histone H4 acetylation by NuA4 is involved in recruiting TFIID to the promoters of the ribosomal protein genes for transcriptional initiation. We have recently demonstrated that the temperature-sensitive inactivation of Esa1p impairs the recruitment of TBP and TAFs (i.e., TFIID) to the promoters of ribosomal protein genes, such as \( RPS5\), \( RPL2B\), and \( RPS11B\) (17), and hence, transcription of these genes is decreased in the \( esa1\)-ts mutant strain (17). However, it is not clearly understood whether the temperature-sensitive inactivation of Esa1p reduced TFIID recruitment via disintegration of NuA4 or loss of targeted histone H4 acetylation at the promoters of the ribosomal protein genes. To test this, we analyzed whether NuA4 is intact in the \( esa1\)-ts mutant at the nonpermissive temperature. If so, NuA4 would likely regulate TFIID recruitment via its targeted KAT activity. For this purpose, we analyzed the recruitment of the Yng2p, Eaf1p,
and Eaf5p components of NuA4 to the UASs of the ribosomal protein genes RPS5, RPL2B, and RPS11B in the esa1-ts mutant and wild-type strains. If NuA4 remains intact in the esa1-ts mutant at the nonpermissive temperature, the recruitment of the Yng2p, Eaf1p, and Eaf5p components of NuA4 to the promoters of these ribosomal protein genes would not be altered in the esa1-ts mutant strain in comparison to the wild-type equivalent. Therefore, we tagged Yng2p, Eaf1p, and Eaf5p with a Myc epitope in their chromosomal loci in the esa1-ts mutant and its isogenic wild-type equivalent and then performed the ChIP assay at the UASs of the ribosomal protein genes. We found that the recruitment of Yng2p, Eaf1p, and Eaf5p to the UASs of the ribosomal protein genes was not altered in the esa1-ts mutant strain following temperature-sensitive inactivation (Fig. 5A to D). Further, global levels of Yng2p, Eaf1p, and Eaf5p with a Myc epitope in their chromosomal loci in the esa1-ts mutant and its isogenic wild-type equivalent and then performed the ChIP assay at the UASs of the ribosomal protein genes. We found that the recruitment of Yng2p, Eaf1p, and Eaf5p to the UASs of the ribosomal protein genes was not altered in the esa1-ts mutant strain following temperature-sensitive inactivation (Fig. 5A to D). Further, global levels of Yng2p, Eaf1p, and Eaf5p were not dramatically altered in the esa1-ts mutant strain in comparison to the wild-type equivalent (Fig. 5E). These results indicate that NuA4 is intact at the promoters of the ribosomal protein genes in the esa1-ts mutant strain following temperature-sensitive inactivation. As a result, the recruitment of Yng2p, Eaf1p, and Eaf5p components of NuA4 to the promoters of the ribosomal protein genes was not significantly impaired in the esa1-ts mutant (Fig. 5A to D). Thus, an enzymatically/catalytically dead NuA4 with a Myc epitope in their chromosomal loci in the esa1-ts mutant and its isogenic wild-type equivalent and then performed the ChIP assay at the UASs of the ribosomal protein genes. We found that the recruitment of Yng2p, Eaf1p, and Eaf5p to the promoters of the ribosomal protein genes was not altered in the esa1-ts mutant strain following temperature-sensitive inactivation (Fig. 5A to D). Further, global levels of Yng2p, Eaf1p, and Eaf5p were not dramatically altered in the esa1-ts mutant strain in comparison to the wild-type equivalent (Fig. 5E). These results indicate that NuA4 is intact at the promoters of the ribosomal protein genes in the esa1-ts mutant strain following temperature-sensitive inactivation. As a result, the recruitment of Yng2p, Eaf1p, and Eaf5p components of NuA4 to the promoters of the ribosomal protein genes was not significantly impaired in the esa1-ts mutant (Fig. 5A to D). Thus, an enzymatically/catalytically dead NuA4 appears to be present at the promoters of the ribosomal protein genes in vivo. Such a NuA4 impairs histone H4 acetylation (Fig. 4) and TFIID recruitment to the promoters of the ribosomal protein genes (17). Consistently, transcription of the ribosomal protein genes was decreased in the esa1-ts mutant strain (17). Therefore, the targeted KAT activity per se, but not the structural integrity, of NuA4 is required for recruitment of TFIID to the promoters of the ribosomal protein genes. In agreement, previous studies (74–79) have implicated histone H4 acetylation in recruitment of TFIID via Bdf1p (bromodomain factor 1).

TFB, but not TFIID, is recruited to the promoters of the ribosomal protein genes in the absence of Eaf1p. We found that the presence of enzymatically dead NuA4 at the promoters of the ribosomal protein genes impairs the recruitment of TFIID, as well as transcription, via the loss of targeted histone H4 acetylation, thus implicating the function of targeted histone acetylation in TFIID recruitment (17). It is still possible that NuA4 complex integrity has some auxiliary function in stimulating the recruitment of TFIID to the promoters of the ribosomal protein genes, in addition to its KAT activity. To test this, we analyzed the recruitment of TBP and TAF1p components of TFIID to the promoters of the ribosomal protein genes RPS5, RPL2B, and RPS11B in the ∆eaf1 strain and its isogenic wild-type equivalent. Intriguingly, we found that the disintegration of NuA4 in the absence of Eaf1p impairs the recruitment of TAF1p (which is essential for TFIID assembly/integrity) (50, 80) but not TBP to the promoters of these ribosomal protein genes (Fig. 6A to C), while the protein level was not changed in the ∆eaf1 strain (Fig. 6D). On the other hand, the recruitment of both TBP and TAF1p is impaired at the promoters of the ribosomal protein genes in the esa1-ts mutant strain (17) (see Fig. S4A in the supplemental material). Thus, while NuA4 KAT activity is essential for TFIID recruitment, the recruitment of TAF1p but not TBP is impaired at the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4 (Fig. 6A to C). Interestingly, when enzymatically inactive NuA4 is present at the UASs of the ribosomal protein genes in the esa1-ts mutant at the nonpermissive temperature (Fig. 5), both TBP and TAF1p (i.e., TFIID) are not recruited to the promoters of the ribosomal protein genes (17). Thus, NuA4 facilitates TFIID recruitment via its
FIG 5 (A and B) ChIP analysis of the recruitment of Yng2p to the UASs of *RPS5* (A) and *RPL2B* and *RPS11B* (B) in the *esa1*-ts and wild-type strains. Both the wild-type and mutant yeast strains were grown and cross-linked as for Fig. 4. Immunoprecipitations were carried out as for Fig. 1. (C and D) ChIP analysis of the recruitment of Eaf1p (C) and Eaf5p (D) to the UASs of *RPS5*, *RPL2B*, and *RPS11B* in the *esa1*-ts and wild-type strains. (E) Western blot analysis of Myc-tagged Yng2p, Eaf1p, and Eaf5p in the *esa1*-ts and wild-type strains. The error bars indicate SD.
KAT activity. However, in the absence of NuA4 in the \( \Delta eaf1 \) strain, the recruitment of TAF1p (but not TBP) is impaired at the promoters of the ribosomal protein genes (Fig. 6A to C). Likewise, the recruitment of the TAF12p component of TFIID to the promoters of the ribosomal protein genes is impaired in the absence of Eaf1p or NuA4 (Fig. 6E). Thus, in the absence of NuA4 or Eaf1p, the recruitment of TFIID but not TBP to the promoters of the ribosomal protein genes is impaired, similar to the results at the NuA4-independent \( \textit{ADH1} \) promoter (Fig. 6F), where the Rap1p activator promotes the recruitment of the TAF-independent form of TBP via SAGA (56, 59, 81–83). SAGA is involved in facilitating the recruitment of the TAF-independent form of TBP during the transcription of ribosomal protein genes.
TBP at the promoters of ADH1 and other SAGA-regulated genes (56, 59, 81–83). Such a function of SAGA has been found to be independent of its KAT activity at a set of SAGA-regulated promoters (56, 82, 84, 85).

TBP nucleates preinitiation complex (PIC) formation at the promoter for transcriptional initiation (83). Since TBP recruitment to the promoters of the ribosomal protein genes RPS5, RPL2B, and RPS11B is not impaired in the eaf1 strain, transcription of these genes is likely to occur in the absence of Eaf1p. To test this, we analyzed the levels of RPS5, RPL2B, and RPS11B mRNAs in the wild-type and Δeaf1 strains, using an RT-PCR assay. We found that transcription of RPS5, RPL2B, and RPS11B occurred in the Δeaf1 strain (Fig. 6G; see Fig. S4B in the supplemental material). Transcription of the NuA4-independent ADH1 and ACT1 genes (10, 17) in the Δeaf1 strain is shown as a control (Fig. 6G).

SAGA is involved in targeting recruitment of TBP to the promoters of the ribosomal protein genes in the absence of Eaf1p. How is TBP recruited to the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4? It is likely that in the absence of NuA4, SAGA is involved in the recruitment of TBP to the promoters of the ribosomal protein genes, as seen at the ADH1 promoter. Thus, TBP is recruited to the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4 (Fig. 6A to C). If SAGA is involved in facilitating the recruitment of TBP to the promoters of the ribosomal protein genes in the absence of Eaf1p, double disintegration of SAGA and NuA4 would impair TBP recruitment to the promoters of the ribosomal protein genes (and hence transcription). Such a situation would lead to impairment of cellular growth, since expression of the ribosomal protein genes is essential for cellular viability. In support of this possibility, previous genetic interaction studies (21) have demonstrated synthetic lethality for the Δeaf1Δsplice strain (the SAGA component Spt7p is essential to maintain the global structural integrity of SAGA) (56, 86, 87). Thus, it is quite likely that SAGA is involved in the recruitment of TBP to the promoters of the ribosomal protein genes in the absence of NuA4 or Eaf1p. To test this, we analyzed the recruitment of TBP to the promoters of the ribosomal protein genes RPS5, RPL2B, and RPS11B in the absence of Eaf1p and Sp7p (which maintains the structural and functional integrity of SAGA) (56, 86, 87). For this purpose, we needed to generate the Δeaf1Δsp7 strain. However, the Δeaf1 Δsp7 strain may be very sick (or may not be viable), as previous studies (21) demonstrated the synthetic lethality of the Δeaf1 Δsp7 strain. Therefore, we replaced the endogenous promoter of SPT20 with an inducible GAL1 promoter in the Δeaf1 strain via homologous recombination (48) and repressed the expression of SPT20 for 2 h in dextrose-containing growth medium (as the GAL1 promoter is repressed in dextrose-containing growth medium and activated in galactose-containing growth medium) to generate genetic conditions equivalent to those of the Δeaf1 Δsp7 strain. In this generated strain (Δeaf1 sp7, or pGAL1-SPT20), we found that the expression of Sp7p was significantly decreased in 2 h in dextrose-containing growth medium after switching from galactose (Fig. 7A). The actin level was monitored as a loading control. The actin level was significantly increased in 2 or 4 h in dextrose-containing growth medium, while the Sp7p level was dramatically decreased. Since ACT1 transcription is increased after switching the carbon source in the growth medium from galactose to dextrose (88), we found a significant increase in the levels of actin in 2 or 4 h in dextrose-containing growth medium (Fig. 7A). Likewise, the level of Rpb1p (the largest subunit of RNA polymerase II) was monitored as a loading control (Fig. 7A).

Next, using the above-described (Δeaf1 sp7) strain and the Δeaf1 strain, we analyzed the recruitment of TBP to the promoters of the ribosomal protein genes RPS5, RPL2B, and RPS11B following 2 h of transcriptional repression of SPT20 in dextrose-containing growth medium. Like ACT1, transcription of the ribosomal protein genes increases upon changing the carbon source from galactose to dextrose in the growth medium (89). Consistently, we found an increase in TBP recruitment to the promoters of the ribosomal protein genes in the absence of Eaf1p following 2 h of growth in dextrose-containing growth medium upon switching from galactose-containing growth medium (Fig. 7B to D). However, we did not observe a similar increase in TBP recruitment to the promoters of the ribosomal protein genes when the expression of Sp7p was repressed in the Δeaf1 strain following 2 h of growth in dextrose-containing growth medium (Fig. 7B to D). However, depletion of Sp7p in the presence of Eaf1p did not alter TBP recruitment to the RPS5, RPL2B, and RPS11B promoters (Fig. 7E), consistent with the fact that Sp7p or SAGA is not involved in transcriptional initiation of the ribosomal protein genes (56, 82, 83, 90, 91). Thus, our results support the idea that SAGA is involved in recruitment of TBP to the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4. Consistently, when the expression of Sp7p in the Δeaf1 strain was repressed following 2 h of growth in dextrose-containing growth medium, transcription of RPS5, RPL2B, and RPS11B was reduced (Fig. 8A). However, transcription of these genes was not impaired following depletion of Sp7p in the presence of Eaf1p (Fig. 8B). These results support the idea that SAGA is involved in transcription of the ribosomal protein genes in the absence of NuA4. In agreement with these results, double disintegration of NuA4 and SAGA (i.e., SPT20 under the GAL1 promoter in the Δeaf1 strain in dextrose-containing growth medium, YPD) impairs cellular growth (Fig. 8C). However, when SPT20 was not repressed in the Δeaf1 strain in galactose-containing growth medium (YPD), yeast cells grew normally in comparison to the Δeaf1 strain (Fig. 8C). The growth of the Δeaf1 strain was not significantly altered in YPG or YPD medium compared with the wild-type equivalent (Fig. 8C). On the other hand, the growth of the Δsp7 strain was impaired in YPG medium, as Sp7p is essential for expression of GAL genes for growth in YPG medium (Fig. 8C). However, the Δsp7 strain grows, but relatively slowly in comparison to its wild-type equivalent, in YPD medium (Fig. 8C). These results support the idea that disintegration of NuA4 and SAGA (i.e., the Δeaf1 sp7 or Δeaf1 pGAL1-SPT20 strain in YPD medium) impairs cellular growth. Our results further indicate that the overexpression of Sp7p in the Δeaf1 strain in YPG medium does not affect cellular growth. Collectively, our results support the idea that, in the absence of Eaf1p or NuA4, SAGA facilitates the recruitment of TBP to the promoters of the ribosomal protein genes (Fig. 7B to D), and hence, transcription of RPS5, RPL2B, and RPS11B occurs in the absence of Eaf1p (Fig. 6G).

Since SAGA is involved in recruiting TBP to the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4, SAGA would be present at the ribosomal protein gene promoter in the Δeaf1 strain. To test this, we analyzed the recruitment of the Sp7p component of SAGA to the UASs of RPS5, RPL2B, and RPS11B in the wild-type and Δeaf1 strains. We found association of SAGA with the UASs of the ribosomal protein genes, even in the
presence of Eaf1p or NuA4 (Fig. 8D). Such association could be mediated by histone H4 acetylation (as previous studies implied the interaction of SAGA with acetylated histone H4) (92–94) and/or another, unknown factor(s)/pathway(s), including Rap1p (as the same regions of Tra1p of SAGA and NuA4 have been shown to recruit Tra1p to Rap1p- and Gcn4p-dependent promoters) (70). Consistent with our results, the association of SAGA, NuA4, and TFIID with the promoters of the ribosomal protein genes has also been observed previously (73, 93–97). The association of SAGA with the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4 was found to be nonfunctional for TBP recruitment for transcription (Fig. 7E)(56, 82). In agreement with this, SAGA has been shown to perform a redundant function in the presence of NuA4-dependent recruitment of TFIID (91,95, 97). In the absence of NuA4, SAGA functions at the promoters of the ribosomal protein genes to promote TBP recruitment and, hence, transcription (Fig. 7B to D and 8A). However, we did not observe significant enhancement of SAGA
Analysis of SAGA function in facilitation of transcription of the ribosomal protein genes in the absence of Eaf1p or NuA4. (A) RT-PCR analysis of transcription of RPS5, RPL2B, and RPS11B following both NuA4 and SAGA disintegration in comparison to NuA4 disintegration alone. Yeast cells were grown as for Fig. 7B. (B) RT-PCR analysis of transcription of RPS5, RPL2B, and RPS11B following SAGA disintegration (i.e., repression of Spt20p expression in YPD medium) in comparison to the wild-type strain (which expresses SPT20 under its own promoter). (C) Growth analysis of the strain following both NuA4 and SAGA disintegration in comparison to NuA4 disintegration alone in solid growth medium. (D) Analysis of recruitment of SAGA (Spt20p-Myc) to the UASs of RPS5, RPL2B, and RPS11B in the Δaaf1 and wild-type strains. Yeast cells were grown, cross-linked, and immunoprecipitated as for Fig. 1. (E) Analysis of recruitment of SAGA (Spt20p-Myc) to the UAS of GAL1 in the Δaaf1 and wild-type strains. Yeast cells were grown in YPG medium at 30°C to an OD600 of 1.0 prior to formaldehyde-based in vivo cross-linking. The error bars indicate SD.
(Spt20p-Myc) recruitment to the promoters of the ribosomal genes in the absence of Eaf1p or NuA4 (Fig. 8D). As a control, we showed that SAGA is recruited to a SAGA-regulated GAL1 promoter independently of Eaf1p (Fig. 8E).

As mentioned above, we found that the recruitment of TAF1p or TAF12p but not TBP to the promoters of the ribosomal protein genes was impaired in the absence of Eaf1p or NuA4 (Fig. 6A to C and E). TAF1 is a specific component of TFIID, while TAF12p is a shared component between SAGA and TFIID (83). Based on our results, it is likely that ribosomal protein genes would become TAF1p independent in the absence of Eaf1p. To test this, we deleted EAF1 from the wild-type and eaf1-ts mutant strains and then analyzed the role of TAF1p in transcription of the ribosomal protein genes in the presence and absence of Eaf1p. We found that transcription of the ribosomal protein genes is dependent on TAF1p in the presence of Eaf1p (Fig. 9A to C), consistent with previous studies (50, 51, 83, 98) that demonstrated the role of TAF1p in transcriptional stimulation of the ribosomal protein genes. As a control, we showed that transcription of a TAF1p-independent gene, ADH1, is not altered in the eaf1-ts mutant strain at the nonpermissive temperature (Fig. 9D). Thus, the ribosomal protein genes are TAF1p dependent in the presence of NuA4. However, in the absence of Eaf1p or NuA4, ribosomal protein genes lose TAF1p dependency, consistent with our ChIP results (Fig. 6A to C), and become SAGA dependent (Fig. 7B to D and 8A and B). Therefore, our results support the idea that NuA4 plays an important role in the TFIID dependency of the ribosomal protein genes for transcriptional initiation.

**DISCUSSION**

Ribosomal protein genes are involved in ribosome biogenesis associated with translation. Thus, altered expression of the ribosomal protein genes would affect translation and, hence, cellular growth, development, and differentiation. In yeast, about 50% of the RNA polymerase II transcription machinery is engaged in transcription of the ribosomal protein genes (99, 100). The ribosomal protein genes are regulated by NuA4 (10, 11, 14, 17). NuA4 is targeted to the promoters of the ribosomal protein genes by a transcriptional activator, Rap1p (10, 14, 17, 70). Such recruitment of NuA4 enhances transcriptional initiation of the TFIID (or TAF)-dependent ribosomal protein genes (10, 17). However, it is not clearly understood how NuA4 promotes transcriptional initiation of the ribosomal protein genes. Here, we have provided insights into NuA4 regulation of ribosomal protein gene expression.

Previous biochemical studies (13, 45) implicated Eaf1p in maintaining the structural integrity of NuA4. However, it was not clear whether Eaf1p maintains the structural integrity of NuA4 at the promoter of the target gene in vivo. Here, we demonstrate that the recruitment of the NuA4 components (e.g., Swc4p, Epl1p, Eaf5p, and Yng2p) to the promoters of the ribosomal protein genes is impaired in the absence of its Eaf1p component (Fig. 1). These results support the function of Eaf1p in maintaining the structural integrity of NuA4 at the promoter of the target gene in vivo, consistent with previous biochemical studies (13, 45). Impaired recruitment of NuA4 to the promoters of the ribosomal protein genes in the absence of Eaf1p decreases targeted histone H4 acetylation (Fig. 2). However, a dramatic decrease of targeted histone H4 acetylation was not observed at the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4. This is due to nontargeted global histone H4 acetylation by picNuA4 in the background of targeted histone H4 acetylation.

Previous studies showed that NuA4 is targeted to the promoter by the activator for histone H4 acetylation, and such targeting is mediated via its Tra1p component (10, 11, 14, 17, 46, 70). Thus, NuA4 is involved in targeted histone H4 acetylation (5, 10, 14, 17). However, picNuA4 is globally associated with the chromosome for genome-wide histone H4 acetylation (5, 41–44). Since pic-NuA4 is involved in genome-wide histone H4 acetylation, the global level of histone H4 acetylation would not likely be altered in the absence Eaf1p or NuA4. Indeed, previous biochemical studies (45) demonstrated that global histone H4 acetylation was not altered in the ∆aef1 strain. However, we found that the levels of targeted histone H4 acetylation at the promoters of the ribosomal protein genes were decreased in the absence of Eaf1p or NuA4 (Fig. 2A to C). Although previous biochemical studies (45) demonstrated that global histone H4 acetylation was not altered in the ∆aef1 strain, it was not known what happens at the promoters when targeted recruitment of NuA4 is impaired in the absence of Eaf1p. Our results here demonstrate that histone H4 acetylation at the promoters of the ribosomal protein genes was decreased in the
Δeaf1 strain (Fig. 2A to C). However, we did not observe dramatic impairment of histone H4 acetylation in the Δeaf1 strain, due to nontargeted global histone H4 acetylation by picNuA4 (42–44). On the other hand, histone H4 acetylation was severely impaired following temperature-sensitive inactivation of Esa1p (Fig. 4A to C). Esa1p is responsible for the KAT activities of both NuA4 and picNuA4. Thus, the temperature-sensitive inactivation of Esa1p impairs the KAT activity of both picNuA4 and NuA4. Hence, we observed dramatic impairment of histone H4 acetylation at the promoters of the ribosomal protein genes in the esa1-ts mutant strain in comparison to the wild-type equivalent (Fig. 4A to C).

As mentioned above, picNuA4 is involved in global nontargeted histone H4 acetylation. Thus, many genes/promoters are acetylated by picNuA4 (10). However, such nontargeted histone H4 acetylation does not regulate transcription (10). For example, ADH1, ACT1, and GAL1 are globally acetylated at histone H4 but are not regulated by histone H4 acetylation for their transcription (10). On the other hand, targeted histone H4 acetylation by NuA4 controls transcription (10, 17). For example, the promoters of the ribosomal protein genes are acetylated at histone H4 by targeted recruitment of NuA4 (10, 11, 17). Such targeted histone H4 acetylation promotes transcriptional initiation of the ribosomal protein genes (17). Thus, targeted histone H4 acetylation by NuA4 has transcriptional-regulatory functions. On the other hand, global histone H4 acetylation by picNuA4 does not appear to regulate transcription (10).

We found that the temperature-sensitive inactivation of Esa1p impairs the recruitment of TBP, TAF1p, and TAF12p (or TFIID) to the promoters of the ribosomal protein genes, thus indicating the role of histone H4 acetylation in recruitment of TFIID (17). Consistently, previous studies demonstrated the role of histone H4 acetylation in recruitment of TFIID via Bdf1p at the core promoters of the ribosomal protein genes (74–79). When we impaired the recruitment of NuA4 to the promoters of the ribosomal protein genes in the absence of Eaf1p (Fig. 1), targeted histone H4 acetylation was reduced by ~2-fold (Fig. 2). Consistently, we observed ~2-fold reduction in the recruitment of the TAF1p and TAF12p components of TFIID to the core promoters of the ribosomal protein genes (Fig. 6A to C and E), indicating ~2-fold reduction in TFIID recruitment. Intriguingly, TBP recruitment to the promoters of the ribosomal protein genes is not altered in the absence of Eaf1p or NuA4 (Fig. 6A to C). We found that SAGA is involved in recruitment of TBP to the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4. SAGA has been previously shown to target the TAF-independent form of TBP but not TFIID to the core promoter of the SAGA-dependent, but TFIID-independent, gene (56, 59, 81–83). Since SAGA is involved in targeting the TAF-independent form of TBP (which nucleates PIC formation at the promoter for transcriptional initiation [83]) to the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4, transcription of the ribosomal protein genes occurred in the Δeaf1 strain (Fig. 6G).

Intriguingly, both TBP and TFIID (or TAFs) are not recruited to the promoters of the ribosomal protein genes when Esa1p is inactivated in the esa1-ts mutant at the nonpermissive temperature (17). We found that NuA4 is intact in the esa1-ts mutant at the nonpermissive temperature (Fig. 5). The presence of such an enzymatically dead NuA4 at the promoters of the ribosomal protein genes might interfere with SAGA, and hence, TBP is not recruited to the promoters of the ribosomal protein genes via SAGA. However, we observed the function of SAGA in regulating the recruitment of TBP to the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4 (Fig. 7B to D). Further, in the absence of SAGA, NuA4-mediated recruitment of TFIID occurs at the promoter of the ribosomal protein genes (Fig. 7E). Hence, in the absence of either SAGA or NuA4, transcription of the ribosomal protein genes occurs (Fig. 6G and 8B) (56, 82). However, the mechanisms of transcriptional initiation differ (i.e., transcriptional initiation by TAF-dependent versus TAF-independent forms of TBP).

SAGA and NuA4 have a common Tra1p subunit that has been shown to interact with the transcriptional activator (10, 11, 14, 17, 46, 59, 70). The same regions of Tra1p have been shown to target Tra1p to the SAGA- and NuA4-regulated promoters (70). Further, Rap1p has been shown to interact genetically with the components (e.g., Spt3p and Spt8p) of SAGA (101). In agreement with this, we found that SAGA is recruited to the promoters of the ribosomal protein genes (Fig. 8D), consistent with previous studies (73, 95–97). Such targeting of SAGA to the promoters of the ribosomal protein genes could be further stabilized by its interaction with acetylated histone H4, since previous studies (92–94) showed the interaction of SAGA with acetylated histone H4. Enhanced recruitment of SAGA to the promoters of the ribosomal protein genes was not observed in the absence of Eaf1p or NuA4 (Fig. 8D). However, it promotes TBP recruitment to the promoters of the ribosomal protein genes for transcriptional initiation in the absence of Eaf1p or NuA4.

In summary, NuA4 is targeted by an activator to the promoters of the ribosomal protein genes for recruitment of TFIID via its KAT activity for transcriptional initiation. When NuA4 is disintegrated in the absence of Eaf1p, SAGA is involved in facilitating the recruitment of the TAF-independent form of TBP to promote transcriptional initiation of the ribosomal protein genes. When an enzymatically dead NuA4 in the esa1-ts mutant at the nonpermissive temperature remains associated with the promoters of the ribosomal protein genes, it interferes with SAGA in targeting TBP to the promoter for transcriptional initiation. Thus, NuA4 contributes to the TFIID-dependent transcriptional initiation of the ribosomal protein genes via its targeted KAT activity. Together, our results shed much light on the regulation of ribosomal protein gene activation by NuA4, SAGA, TBP, and TAFs. Since these factors are conserved among eukaryotes, similar regulatory mechanisms of ribosomal protein gene activation are likely to exist in higher eukaryotes. As ribosomal protein genes are involved in ribosome biogenesis and translation (and hence cellular growth, development, and differentiation), transcriptional alteration of the ribosomal protein genes would be associated with various cellular pathologies, including cancer, cardiac disorders, and neurodegenerative diseases. Thus, our results for the transcriptional-regulatory mechanisms of the ribosomal protein genes would be useful in disease pathogenesis at the level of ribosome biogenesis and functions.

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