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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 Δ*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.

Histone H4 acetylation plays important roles in the regulation of eukaryotic transcription and other biological processes (1–3). In *Saccharomyces cerevisiae*, NuA4 (nucleosome acetyltransferase of H4) acetylates histone H4. In addition, NuA4 is involved in acetylation of histones H2A and H2A.Z (4–7). NuA4 is a multisubunit protein complex and is conserved from yeast to humans (Tip60 is the human homologue of yeast NuA4) (8). Like other histone lysine (K) acetyltransferases (KATs), NuA4 is involved in various cellular events, such as transcription, DNA repair, and cell cycle progression (9–27). In addition, NuA4 is proposed to regulate cellular aging and autophagy via acetylation of nonhistone proteins (28–30). Likewise, Tip60 has numerous nonhistone targets involved in various cellular activities (31, 32). In addition, Tip60 has been found to be involved in performing critical functions in DNA repair and stem cell regulation (33–36). Therefore, NuA4 and its human homologue are multifunctional in maintaining normal cellular functions.

Esa1p is the catalytic subunit of NuA4 with KAT activity (37, 38). In addition, NuA4 has 12 other subunits (39, 40). These subunits include Tra1p (ATM-related factor), Epl1p (enhancer of polycomb homologue), Arp4p (actin-related protein), Yaf9p (leukemogenic factor ENL/AF9 homologue), Act1p, and 7 Esa1p-associated factors, Eaf1p to Eaf7p. Eaf2p and Eaf4p are also known as Swc4p and Yng2p, respectively. Although Esa1p is the catalytic subunit of NuA4, it cannot acetylate nucleosomal histones on its own but can acetylate naked/free histones (4, 5). Esa1p acetylates nucleosomal histones when it is present within the complex (5). In addition to being a component of NuA4, Esa1p is also present in a distinct and relatively small complex known as piccolo NuA4 (picNuA4) (5, 41). Esa1p forms picNuA4 in combination with Yng2p and Epl1p (42). Yng2p and Epl1p are also integral components of NuA4. Thus, picNuA4 is a smaller subcomplex of NuA4 and has been biochemically found to exist independently of NuA4 (5, 41–44). Therefore, Esa1p is present in two distinct complexes,

namely, NuA4 (or holo-NuA4) and picNuA4 (5, 41). Like NuA4, picNuA4 is capable of acetylating nucleosomal histones. In fact, picNuA4 has nucleosomal histone acetylation activity similar to or even greater than that of NuA4 (5, 41). While both NuA4 and picNuA4 have strong KAT activity toward nucleosomes, only picNuA4 acetylates nucleosomal histone in preference to free/naked histones (42–44). Previous studies demonstrated that picNuA4 is not targeted to chromatin by transcription factors or activators but rather interacts with nucleosomal DNA and histones within chromatin (42–44). Such interaction of picNuA4 with chromatin has been implicated in nontargeted global histone acetylation (42–44). On the other hand, NuA4 is recruited to the active chromatin by transcription factors or activators, leading to local/targeted histone acetylation for transcription (5, 10, 16, 17, 42, 45–47). NuA4 is also recruited to the sites of DNA double-strand breaks in a targeted fashion via phosphorylation of histone H2A for repair (18, 20).

NuA4 is targeted to the promoters of the ribosomal protein genes via its interaction with an activator (10, 11, 14, 17, 46). Subsequently, NuA4 facilitates transcriptional initiation of tran-

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 Δ*eaf1* 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. Collectively, our results shed much light on the regulation of ribosomal protein gene activation, as presented below.

MATERIALS AND METHODS

Plasmids. The plasmid pFA6a-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 pFA6a-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 *esa1*-ts mutant (LPY3291) and wild-type (LPY3498) strains were obtained from the laboratory of Lorraine Pillus (University of California, San Diego) (38). The Δ*spt20* (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 strain. 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, *EAF1* 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 esa1-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 Esa1p wild-type strain, respectively.

Growth media. For studies of *RPS5*, *RPL2B*, *RPS11B*, *ADH1*, and *ACT1* in the wild-type and Δ*eaf1* strains, yeast cells were grown in YPD medium (yeast extract and peptone plus 2% dextrose) to an optical density at 600 nm (OD_{600}) 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 *esa1*-ts strains and their wild-type equivalents, yeast cells were grown in YPD medium at 23°C to an OD_{600} 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 OD_{600} of 0.5 and then switching to dextrose-containing growth medium for 2 or 4 h. For studies of *GAL1*, yeast cells were grown in YPG at 30°C to an OD_{600} of 1.0 prior to formaldehyde-based *in vivo* cross-linking.

ChIP assay. The ChIP assay for TBP, TAF1p, TAF12p, 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 10 mM 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′-AGAAACAATGAACAGCCTTGAGTTCTC-3′ and 5′-GCAGGGCCATTCTCATCTGA-3′; RPS5 (Core), 5′-GGCCAACTTCTACGCTCACGTTAG-3′ and 5′-CGGTGTCAGACATCTTTGGAATGGTC-3′; RPL2B (UAS), 5′-TACCGATTACCAAGTTTTCAGACTA-3′ and 5′-AATTCCTTCTTTTTCTCCCTAGCGG-3′; RPL2B (Core), 5′-TGGTGGATTCTGCTCTGGAAACTAT-3′ and 5′-CTTTGTGGTTTCTTGGTGAGTTTAT-3′; RPS11B (UAS), 5′-GATATACACAAGAATTTCTGGAAGA-3′ and 5′-CACTTCCTCATTTCACAAAGACACT-3′; RPS11B (Core), 5′-AAGTCCAATAGCTTTACGTTTCCCT-3′ and 5′-CTTTTTCCCTGGCTTGATACGTTTC-3′; ADH1 (Core), 5′-GGTATACGGCCTTCCTTCCAGTTAC-3′ and 5′-GAACGAGAACAATGACGAGGAAACAAAAG-3′; GAL1 (UAS), 5′-CGCTTAACTGCTCATTGCTATATTG-3′ and 5′-TTG

TTCGGAGCAGTGCGGCGC-3′; Chr.-V, 5′-GGCTGTCAGAATATGG GGCCGTAGTA-3′ and 5′-CACCCCGAAGCTGCTTTCACAATAC-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 Na_2-EDTA, and 1% SDS), along with 100 μl phenol-chloroform–isoamyl 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 AGA-3′ and 5′-ACGTATCTACCAACGATTTGACCC-3′; RPS5, 5′-AG GCTCAATGTCCAATCATTGAAAG-3′ and 5′-CAACAACTTGGATTG GGTTTTGGTC-3′; ACT1, 5′-TCCACCACTGCTGAAAGAGAAATT G-3′ and 5′-AATAGTGATGACTTGACCATCTGGA-3′; RPL2B, 5′-GT GCTTTCCACAAGTACAGATTGAA-3′ and 5′-TTTGACCAGAAACGG CACCTCTAGA-3′; RPS11B, 5′-GCACCGTACCATTGTCATCAGAAG A-3′ and 5′-GGTCTACATTGACCAACGGTAACAA-3′.

Growth analysis on solid media. The growth of the Δ*eaf1* strain bearing *SPT20* under the *GAL1* promoter, the Δ*eaf1* and Δ*spt20* 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 OD_{600} of 0.2 at 30°C. Subsequently, the yeast cells were suspended in fresh YPG medium and grown to an OD_{600} 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 Δ*eaf1* strain bearing *SPT20* under the *GAL1* promoter, yeast cells were grown in YPG medium to an OD_{600} 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,

Eaf5p, Yng2p, Esa1p, Epl1p, TBP, and TAF1p in the Δ*eaf1* 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, Eaf5p, and Yng2p in the *esa1*-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 Eaf5p components of NuA4 to the UASs of ribosomal protein genes, such as *RPS5*, *RPL2B*, and *RPS11B*, in the Δ*eaf1* 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 Eaf5p 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 Eaf5p. 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 Δ*eaf1* 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 Eaf5p 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 Eaf5p 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 Eaf5p, 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
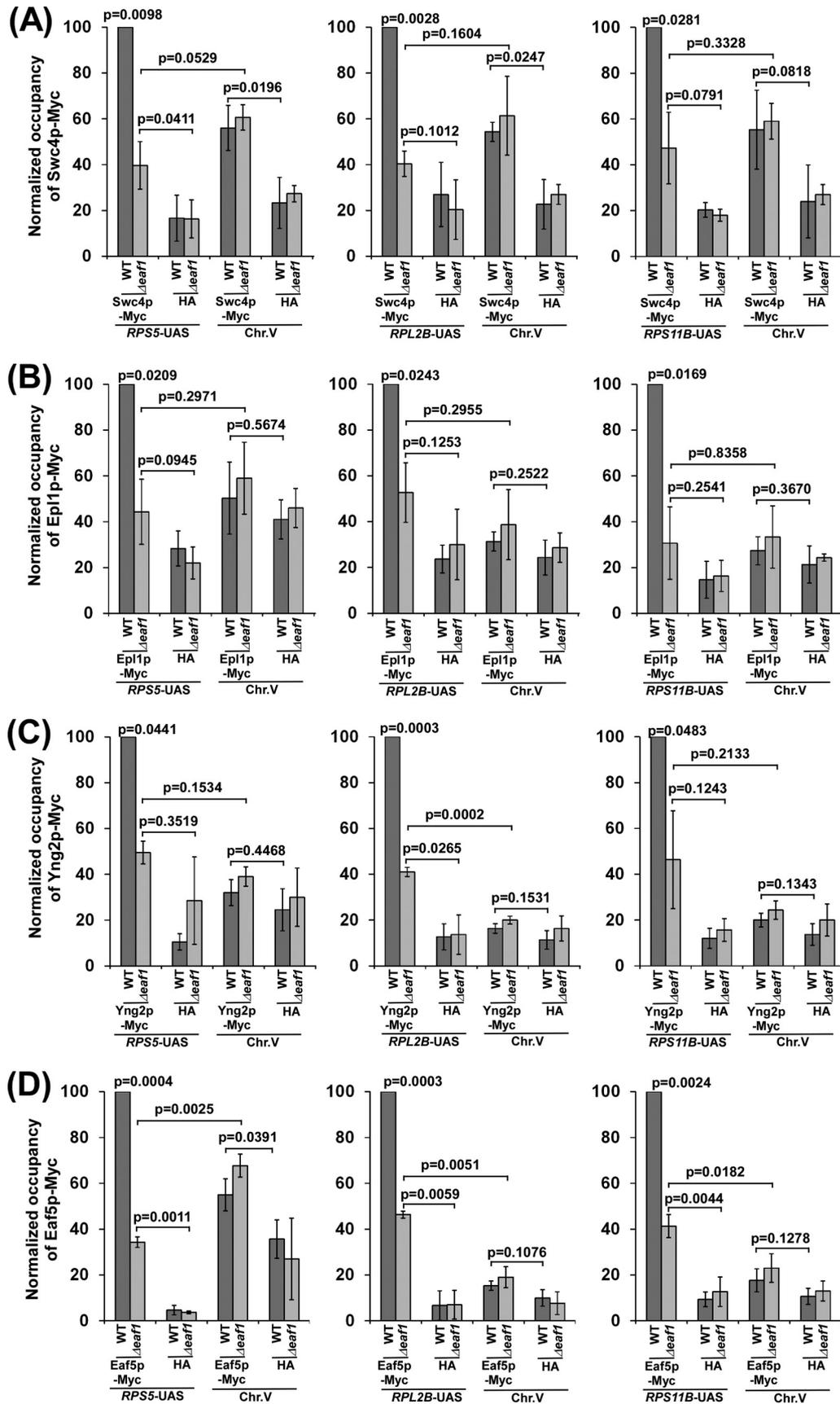
(14, 46, 70). Thus, impaired recruitment of the NuA4 components to the UASs of the ribosomal protein genes in the absence of Eaf1p is not due to the defect in the activator-mediated targeting of NuA4 but, rather, to the disintegration of NuA4. Consistently, previous biochemical studies also implicated 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 Δ*eaf1* 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 Δ*eaf1* strain (see Fig. S3A in the supplemental material). Such a decrease in the stability of Epl1p in the Δ*eaf1* 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 Δ*eaf1* 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 Δ*eaf1* 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 Δ*eaf1* 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 Δ*eaf1* strain. To test this, we analyzed the levels of histone H4 acetylation at the *RPS5*, *RPL2B*, and *RPS11B* promoters in the Δ*eaf1* 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 Δ*eaf1* and 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 Δ*eaf1* 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 Δ*eaf1* strain and its isogenic wild-type equivalent. For this purpose, we tagged Esa1p with a Myc epitope at its chromosomal loci in the Δ*eaf1* and wild-type strains and

FIG 1 Eaf1p is required for recruitment of the NuA4 components (Swc4p, Epl1p, Yng2p, and Eaf5p) to the promoters of the ribosomal protein genes. Shown is ChIP analysis of the association 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 Δ*eaf1* 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 Δ*eaf1* 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. Likewise, the ChIP signals for Chr.-V 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.
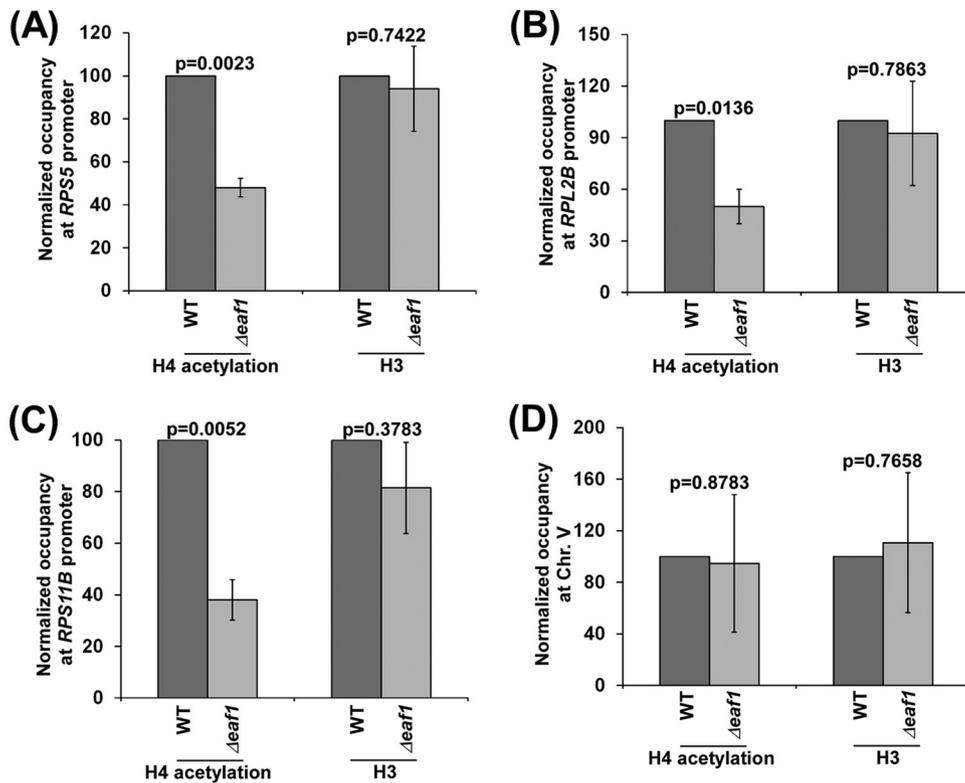
FIG 2 ChIP analysis of the levels of histone H4 acetylation and histone H3 at the promoters of the ribosomal protein genes *RPS5* (A), *RPL2B* (B), and *RPS11B* (C), as well as Chr.-V (D), in the Δ*eaf1* and wild-type strains. Both the wild-type and mutant strains were grown and cross-linked as for Fig. 1. Immunoprecipitations were performed using antibodies against histone H3 (Ab-1791; Abcam) and acetylated histone H4 (Millipore; catalog no. 06866). Primer pairs located at the core promoters of the *RPS5*, *RPS2B*, and *RPS11B* genes and the transcriptionally inactive region within Chr.-V (see Materials and Methods) were used for PCR analysis of the immunoprecipitated DNA samples. 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 Δ*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 (Δ17) 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 *RPS5*, *RPS11*, and *RPL2B* 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 Tra1p mutant (Δ17) 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
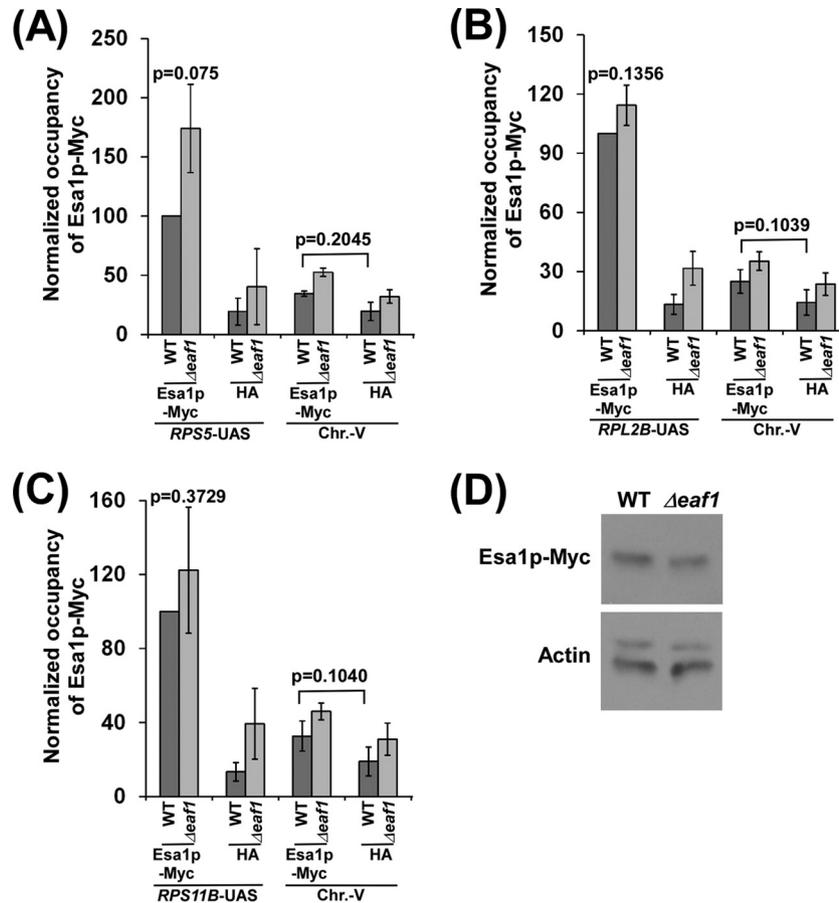
FIG 3 (A to C) Esa1p is recruited to the promoters of the *RPS5* (A), *RPL2B* (B), and *RPS11B* (C) genes in the absence of Eaf1p. Both wild-type and mutant strains expressing Myc-tagged Esa1p were grown, cross-linked, and immunoprecipitated as for Fig. 1. (D) Western blot analysis of Myc-tagged Esa1p in the Δ*eaf1* and wild-type strains. The error bars indicate SD.

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 Δ*eaf1* strain. Consistently, previous biochemical studies demonstrated that global histone H4 acetylation was not altered in the Δ*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 (as a representative component of the histone H3-H4 tetramer) at the promoters of the ribosomal protein genes in the *esa1*-ts mutant and its isogenic wild-type equivalent. Our ChIP analysis revealed that 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,
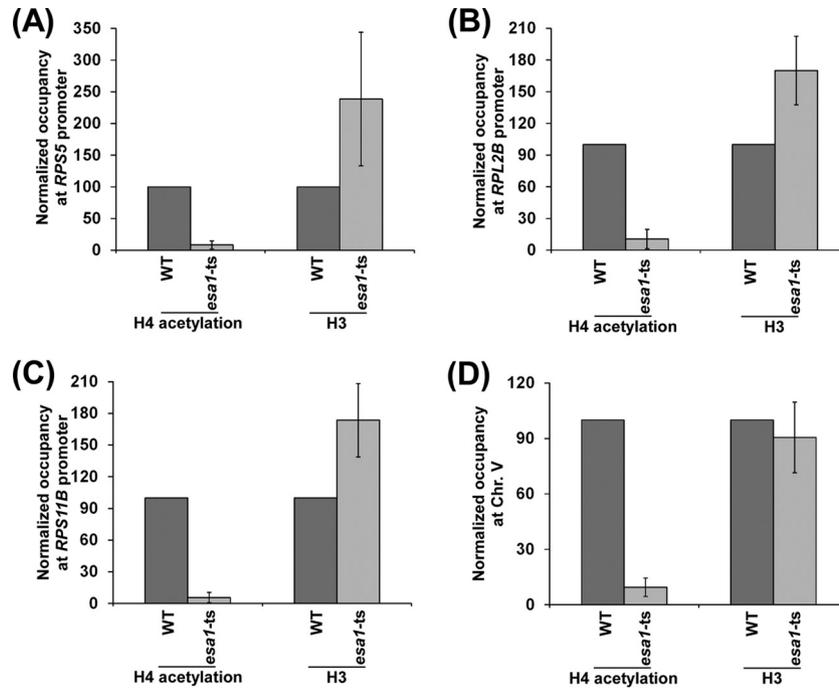
FIG 4 ChIP analysis of the levels of histone H4 acetylation and histone H3 at the *RPS5* (A), *RPL2B* (B), and *RPS11B* (C) promoters, as well as Chr.-V (D), in the *esa1*-ts and wild-type strains. Both the wild-type and *esa1*-ts mutant strains were grown in YPD medium at 23°C to an OD$_{600}$ of 0.85 and then switched to 37°C for 1 h prior to formaldehyde-based *in vivo* cross-linking. Immunoprecipitations were performed as for [Fig. 2]. The error bars indicate SD.

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 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 the 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).

TBP, 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 Δ*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 Δ*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
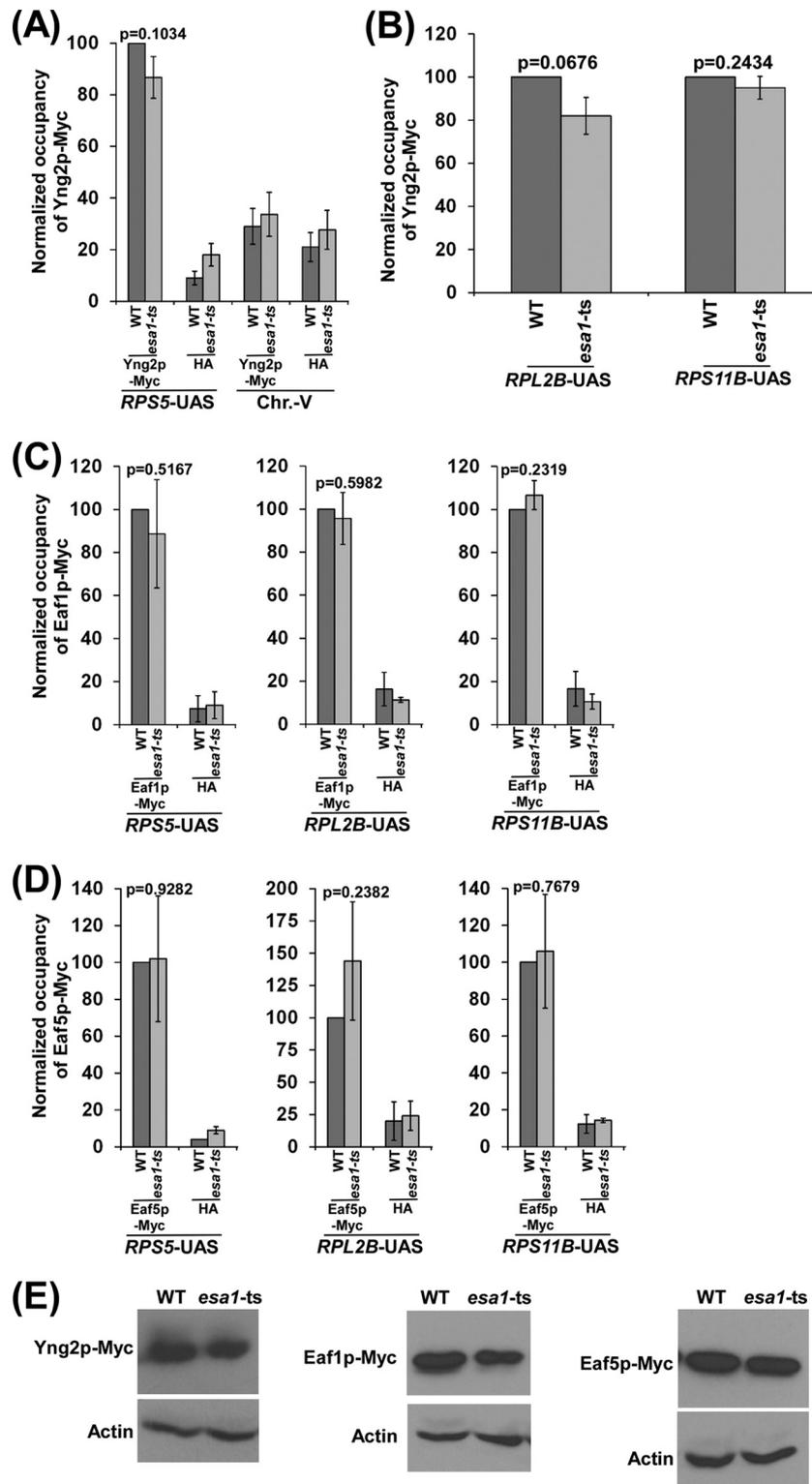
FIG 5 (A and B) ChIP analysis of the recruitment of Yng2p to the UASs of *RPS5* (A) and *RPL2B* and *RPS11B* (B) in the *esa1*-ts and wild-type strains. Both the wild-type and mutant yeast strains were grown and cross-linked as for Fig. 4. Immunoprecipitations were carried out as for Fig. 1. (C and D) 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.
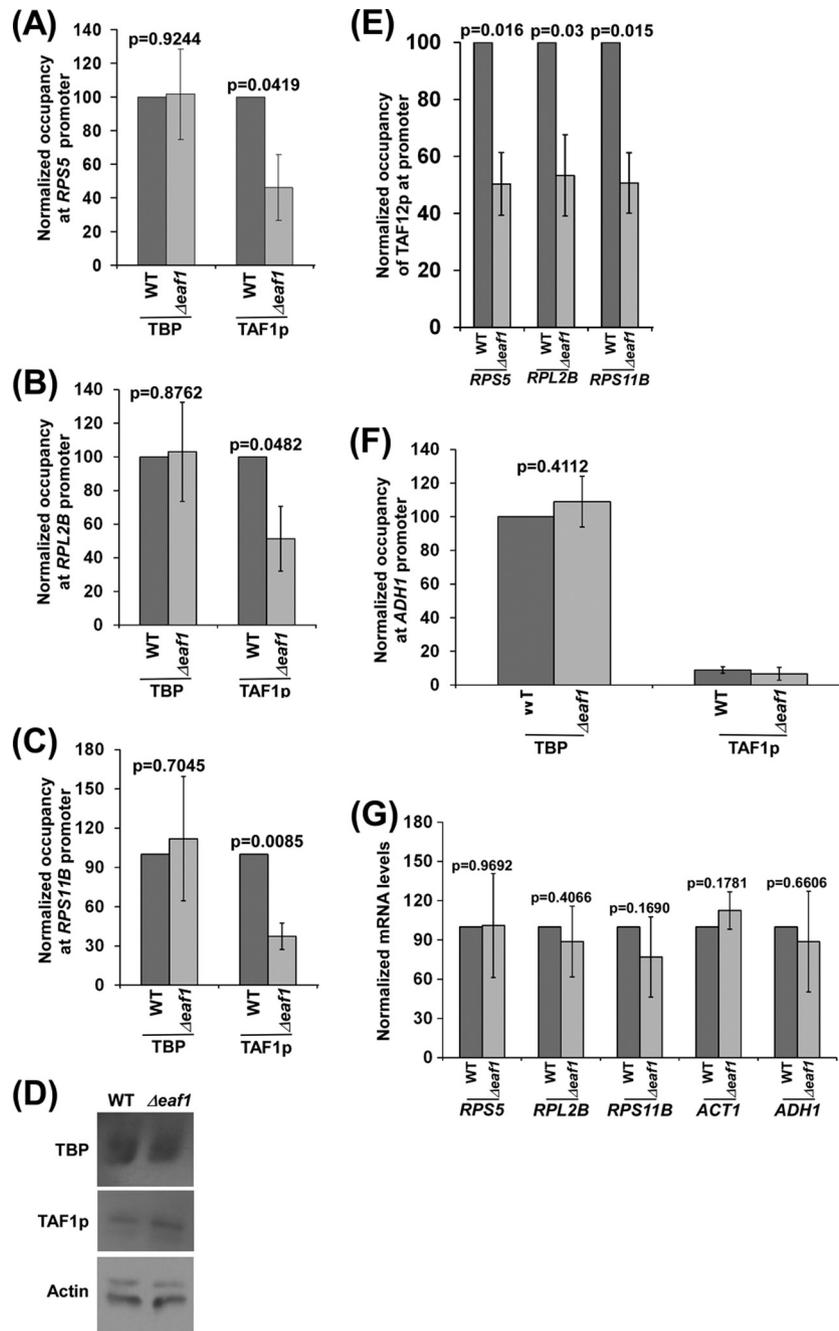
FIG 6 (A to C) ChIP analysis of the recruitment of TBP and TAF1p to the promoters of *RPS5*, *RPL2B*, and *RPS11B* in the Δ*eaf1* and wild-type strains. Yeast cells were grown and cross-linked as for Fig. 1. Immunoprecipitation was performed using anti-TBP and anti-TAF1p antibodies against TBP and TAF1p (obtained from the Green laboratory, University of Massachusetts Medical School). The immunoprecipitated DNAs were analyzed by PCR, using primer pairs targeted to the core promoters of the *RPS5*, *RPL2B*, *RPS11B*, and *ADH1* genes. (D) Western blot analysis of TBP and TAF1p in the Δ*eaf1* and wild-type strains. (E) ChIP analysis of the recruitment of TAF12p to the promoters of *RPS5*, *RPL2B*, and *RPS11B* in the Δ*eaf1* and wild-type strains. (F) ChIP analysis of the recruitment of TBP and TAF1p to the *ADH1* promoter in the Δ*eaf1* and wild-type strains. (G) RT-PCR analysis of *RPS5*, *RPL2B*, *RPS11B*, *ADH1*, and *ACT1* mRNAs in the Δ*eaf1* and wild-type strains. Yeast cells were grown in YPD medium at 30°C to an OD$_{600}$ of 1.0 prior to harvesting for RNA analysis. The error bars indicate SD.

KAT activity. However, in the absence of NuA4 in the Δ*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 *ADH1* promoter (Fig. 6F), where the Rap1p activator promotes the recruitment of the TAF-independent form of TBP but not TFIID via SAGA (56, 59, 81–83). SAGA is involved in facilitating the recruitment of the TAF-independent form of

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 Δ*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 Δ*eaf1* strains, using an RT-PCR assay. We found that transcription of *RPS5*, *RPL2B*, and *RPS11B* occurred in the Δ*eaf1* strain (Fig. 6G; see Fig. S4B in the supplemental material). Transcription of the NuA4-independent *ADH1* and *ACT1* genes (10, 17) in the Δ*eaf1* 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 Δ*eaf1*Δ*spt7* 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 Spt20p (which maintains the structural and functional integrity of SAGA) (56, 86, 87). For this purpose, we needed to generate the Δ*eaf1* Δ*spt20* strain. However, the Δ*eaf1* Δ*spt20* strain may be very sick (or may not be viable), as previous studies (21) demonstrated the synthetic lethality of the Δ*eaf1* Δ*spt7* strain. Therefore, we replaced the endogenous promoter of *SPT20* with an inducible *GAL1* promoter in the Δ*eaf1* 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 Δ*eaf1* Δ*spt20* strain. In this generated strain (Δ*eaf1* *spt20*, or P$_{GAL1}$-*SPT20*), we found that the expression of Spt20p is 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 Spt20p 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 (Δ*eaf1* *spt20*) strain and the Δ*eaf1* 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 Spt20p was repressed in the Δ*eaf1* strain following 2 h of growth in dextrose-containing growth medium (Fig. 7B to D). However, depletion of Spt20p in the presence of Eaf1p did not alter TBP recruitment to the *RPS5*, *RPL2B*, and *RPS11B* promoters (Fig. 7E), consistent with the fact that Spt20p 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 Spt20p in the Δ*eaf1* 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 Spt20p in the presence of Eaf1p (Fig. 8B). These results support the idea that SAGA is required for 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 Δ*eaf1* strain in dextrose-containing growth medium, YPD) impairs cellular growth (Fig. 8C). However, when *SPT20* was not repressed in the Δ*eaf1* strain in galactose-containing growth medium (YPG), yeast cells grew normally in comparison to the Δ*eaf1* strain (Fig. 8C). The growth of the Δ*eaf1* 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 Δ*spt20* strain was impaired in YPG medium, as Spt20p is essential for expression of *GAL* genes for growth in YPG medium (Fig. 8C). However, the Δ*spt20* 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 Δ*eaf1* *spt20* or Δ*eaf1* P$_{GAL1}$-*SPT20* strain in YPD medium) impairs cellular growth. Our results further indicate that the overexpression of Spt20p in the Δ*eaf1* 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 Δ*eaf1* strain. To test this, we analyzed the recruitment of the Spt20p component of SAGA to the UASs of *RPS5*, *RPL2B*, and *RPS11B* in the wild-type and Δ*eaf1* strains. We found association of SAGA with the UASs of the ribosomal protein genes, even in the
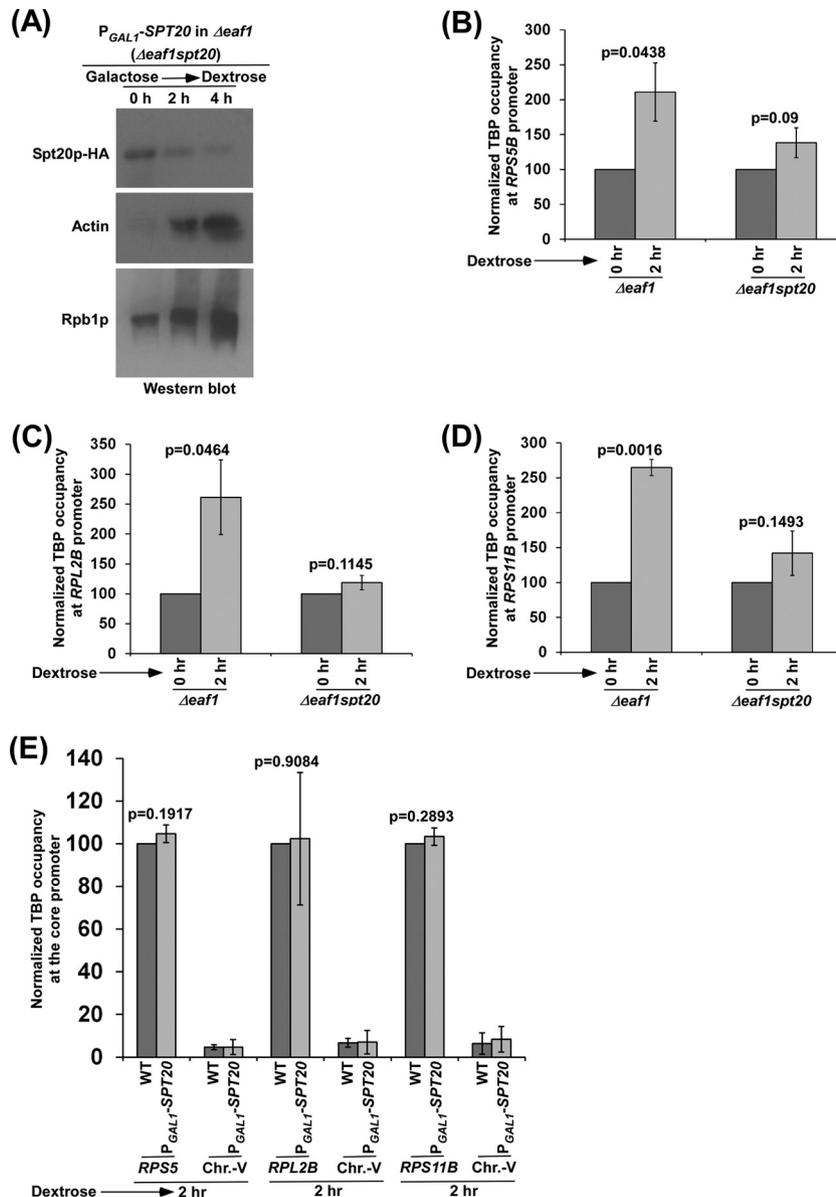
FIG 7 Analysis of the functionality of SAGA in recruitment of TBP to the promoters of the ribosomal protein genes in the absence of Eaf1p or NuA4. (A) Western blot analysis after shutting down Spt20p expression in the Δeaf1 strain in dextrose-containing growth medium. The promoter of SPT20 was replaced by the GAL1 promoter in the Δeaf1 strain to generate conditionally SAGA and NuA4 disintegrated strains in dextrose-containing growth medium. Yeast cells were initially grown in YPG medium to an OD_{600} of 0.5 and then switched to YPD medium for 2 and 4 h prior to harvesting for Western blot analysis. Western blot analysis was performed using anti-HA, antiactin, and 8WG16 antibodies against HA-tagged Spt20p, actin, and Rpb1p (the largest subunit of RNA polymerase II), respectively. (B to D) ChIP analysis of TBP recruitment to the promoters of RPS5 (B), RPL2B (C), and RPS11B (D) following both NuA4 and SAGA disintegration (i.e., repression of Spt20p expression in the Δeaf1 strain in YPD medium [Δeaf1 spt20]) in comparison to NuA4 disintegration alone (i.e., Δeaf1). (E) ChIP analysis of TBP recruitment to the promoters 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). The error bars indicate SD.

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, 95–97). The associa-

tion of SAGA with the promoters of the ribosomal protein genes in the presence 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
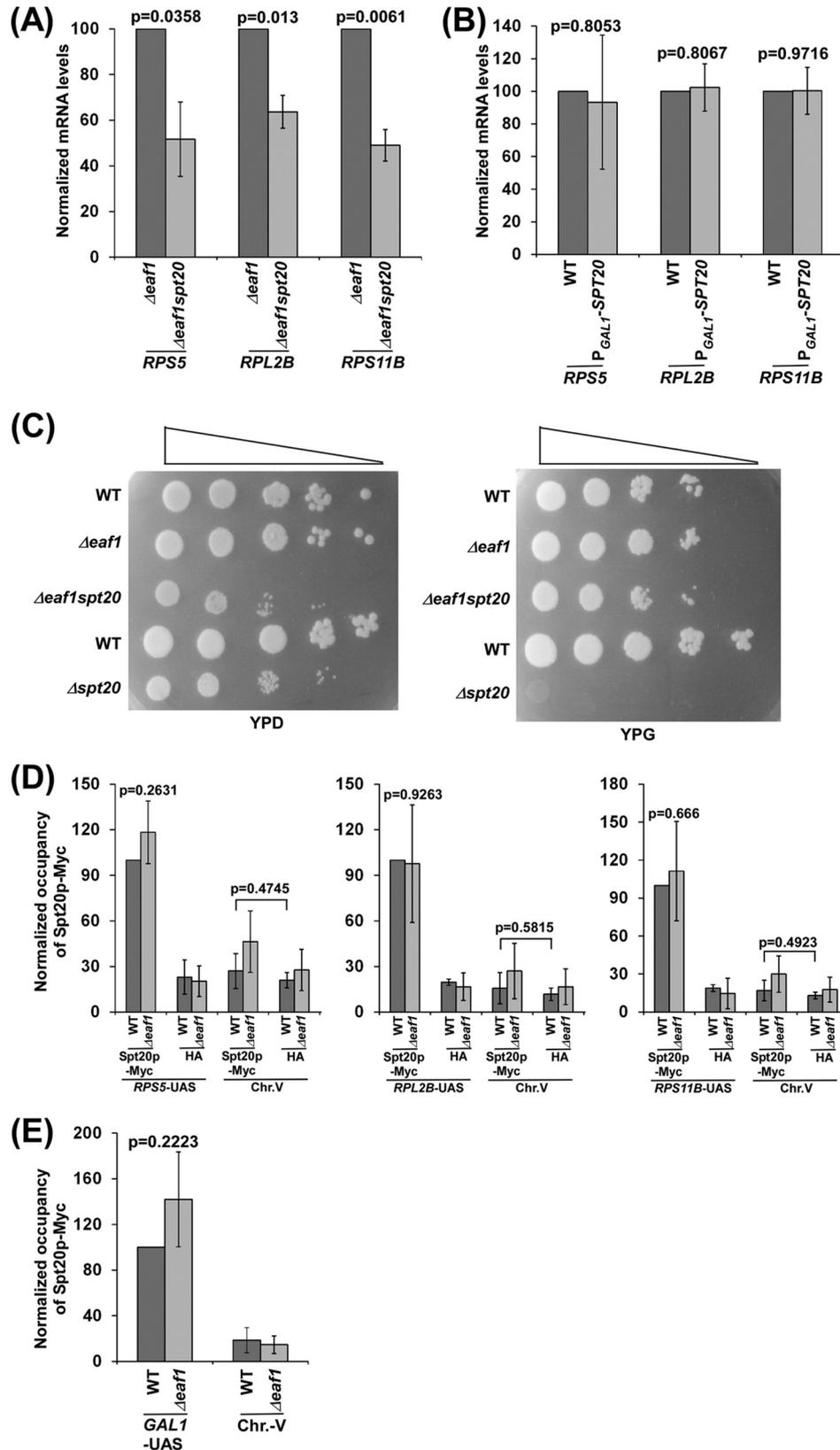
FIG 8 Analysis of SAGA function in facilitation of transcription of the ribosomal protein genes in the absence of Eaf1p or NuA4. (A) RT-PCR analysis of transcription of *RPS5*, *RPL2B*, and *RPS11B* following both NuA4 and SAGA disintegration in comparison to NuA4 disintegration alone. Yeast cells were 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 Δ*eaf1* 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 Δ*eaf1* and wild-type strains. Yeast cells were grown in YPG medium at 30°C to an OD_{600} of 1.0 prior to formaldehyde-based *in vivo* cross-linking. The error bars indicate SD.
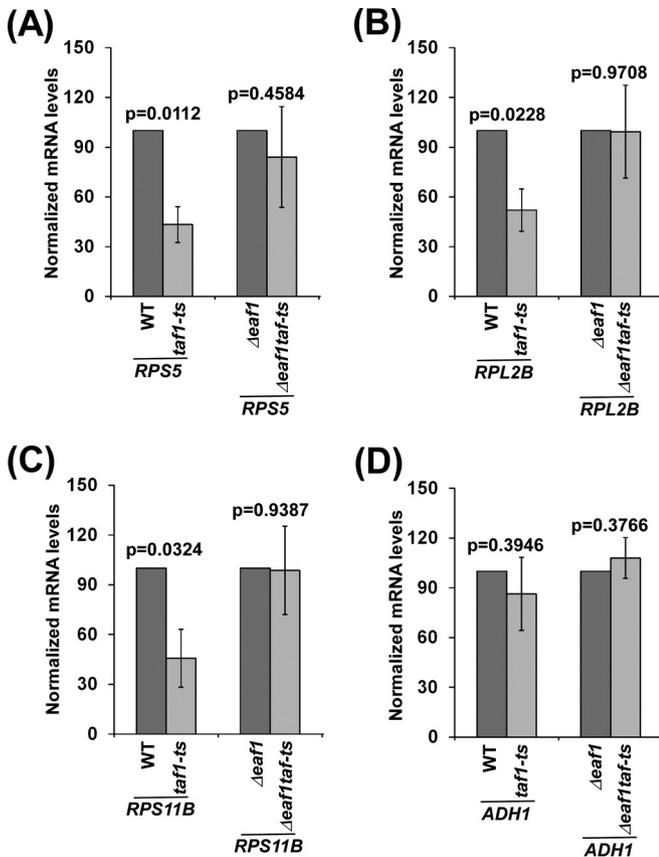
FIG 9 RT-PCR analysis of *RPS5* (A), *RPL2B* (B), *RPS11B* (C), and *ADH1* (D) mRNAs in the *taf1*-ts mutant and its wild-type equivalent in the presence or absence of Eaf1p. Yeast cells were grown as for Fig. 4. The error bars indicate SD.

(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 *taf1*-ts mutant strains and then analyzed the role of TAF1p in the 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 *taf1*-ts mutant strain at the nonpermissive temperature (Fig. 9D). However, transcription of the ribosomal protein genes occurs in the *taf1*-ts mutant in the absence of Eaf1p (Fig. 9A to C). As a control, we showed that transcription of the TAF1p- and NuA4-independent gene *ADH1* is not altered in the Δ*eaf1 taf1*-ts mutant strain at the non-

permissive 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 picNuA4 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 Δ*eaf1* 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 Δ*eaf1* 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

Δ*eaf1* strain (Fig. 2A to C). However, we did not observe dramatic impairment of histone H4 acetylation in the Δ*eaf1* 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 Δ*eaf1* 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.

ACKNOWLEDGMENTS

REFERENCES

1. **Bhaumik SR, Smith E, Shilatifard A.** 2007. Covalent modifications of histones during development and disease pathogenesis. Nat Struct Mol Biol **14:**1008–1016. http://dx.doi.org/10.1038/nsmb1337.

2. **Bannister AJ, Kouzarides T.** 2011. Regulation of chromatin by histone modifications. Cell Res **21:**381–395. http://dx.doi.org/10.1038/cr.2011.22.

3. **Rando OJ, Winston F.** 2012. Chromatin and transcription in yeast. Genetics **190:**351–387. http://dx.doi.org/10.1534/genetics.111.132266.

4. **Allard S, Utley RT, Savard J, Clarke A, Grant P, Brandl CJ, Pillus L, Workman JL, Cote J.** 1999. NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. EMBO J **18:**5108–5119. http://dx.doi.org/10.1093/emboj/18.18.5108.

5. **Boudreault AA, Cronier D, Selleck W, Lacoste N, Utley RT, Allard S, Savard J, Lane WS, Tan S, Cote J.** 2003. Yeast enhancer of polycomb defines global Esa1-dependent acetylation of chromatin. Genes Dev **17:**1415–1428. http://dx.doi.org/10.1101/gad.1056603.

6. **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.1386306.

7. **Keogh MC, Mennella TA, Sawa C, Berthelet S, Krogan NJ, Wolek A, 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.1388106.

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 Biol **24:**1884–1896. http://dx.doi.org/10.1128/MCB.24.5.1884-1896.2004.

9. **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.

10. **Reid JL, Iyer VR, Brown PO, Struhl K.** 2000. Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. Mol Cell **6:**1297–1307. http://dx.doi.org/10.1016/S1097-2765(00)00128-3.

11. **Rohde JR, Cardenas ME.** 2003. The tor pathway regulates gene expression by linking nutrient sensing to histone acetylation. Mol Cell Biol **23:**629–635. http://dx.doi.org/10.1128/MCB.23.2.629-635.2003.

12. **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.

13. **Mitchell L, Lambert JP, Gerdes M, Al-Madhoun AS, Skerjanc IS, Figeys 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.

14. **Joo YJ, Kim JH, Kang UB, Yu MH, Kim J.** 2011. Gcn4p-mediated transcriptional repression of ribosomal protein genes under amino-acid starvation. EMBO J **30:**859–872. http://dx.doi.org/10.1038/emboj.2010.332.

15. **Morillon A, Karabetsou N, Nair A, Mellor J.** 2005. Dynamic lysine methylation on histone H3 defines the regulatory phase of gene transcription. Mol Cell **18:**723–734. http://dx.doi.org/10.1016/j.molcel.2005.05.009.

16. **Ginsburg DS, Govind CK, Hinnebusch AG.** 2009. NuA4 lysine acetyltransferase Esa1 is targeted to coding regions and stimulates transcription elongation with Gcn5. Mol Cell Biol **29:**6473–6487. http://dx.doi.org/10.1128/MCB.01033-09.

17. **Uprety B, Lahudkar S, Malik S, Bhaumik SR.** 2012. The 19S proteasome subcomplex promotes the targeting of NuA4 HAT to the promoters of ribosomal protein genes to facilitate the recruitment of TFIID for transcriptional initiation in vivo. Nucleic Acids Res **40:**1969–1983. http://dx.doi.org/10.1093/nar/gkr977.

18. **Bird AW, Yu DY, Pray-Grant MG, Qiu Q, Harmon KE, Megee PC, Grant PA, Smith MM, Christman MF.** 2002. Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair. Nature **419:**411–415. http://dx.doi.org/10.1038/nature01035.

19. **Choy JS, Kron SJ.** 2002. NuA4 subunit Yng2 function in intra-S-phase DNA damage response. Mol Cell Biol **22:**8215–8225. http://dx.doi.org/10.1128/MCB.22.23.8215-8225.2002.

20. **Downs JA, Allard S, Jobin-Robitaille O, Javaheri A, Auger A, Bouchard N, Kron SJ, Jackson SP, Cote J.** 2004. Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. Mol Cell **16:**979–990. http://dx.doi.org/10.1016/j.molcel.2004.12.003.

21. **Lin YY, Qi Y, Lu JY, Pan X, Yuan DS, Zhao Y, Bader JS, Boeke JD.** 2008. A comprehensive synthetic genetic interaction network governing yeast histone acetylation and deacetylation. Genes Dev **22:**2062–2074. http://dx.doi.org/10.1101/gad.1679508.

22. **Le Masson I, Yu DY, Jensen K, Chevalier A, Courbeyrette R, Boulard Y, Smith MM, Mann C.** 2003. Yaf9, a novel NuA4 histone acetyltransferase subunit, is required for the cellular response to spindle stress in yeast. Mol Cell Biol **23:**6086–6102. http://dx.doi.org/10.1128/MCB.23.17.6086-6102.2003.

23. **Krogan NJ, Baetz K, Keogh MC, Datta N, Sawa C, Kwok TC, Thompson NJ, Davey MG, Pootoolal J, Hughes TR, Emili A, Buratowski S, Hieter P, Greenblatt JF.** 2004. Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4. Proc Natl Acad Sci U S A **101:**13513–13518. http://dx.doi.org/10.1073/pnas.0405753101.

24. **Marston AL, Tham WH, Shah H, Amon A.** 2004. A genome-wide screen identifies genes required for centromeric cohesion. Science **303:**1367–1370. http://dx.doi.org/10.1126/science.1094220.

25. **Zhang H, Richardson DO, Roberts DN, Utley R, Erdjument-Bromage H, Tempst P, Cote J, Cairns BR.** 2004. The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation, and Htz1 replacement near telomeres. Mol Cell Biol **24:**9424–9436. http://dx.doi.org/10.1128/MCB.24.21.9424-9436.2004.

26. **Zhou BO, Wang SS, Xu LX, Meng FL, Xuan YJ, Duan YM, Wang JY, Hu H, Dong X, Ding J, Zhou JQ.** 2010. SWR1 complex poises heterochromatin boundaries for antisilencing activity propagation. Mol Cell Biol **30:**2391–2400. http://dx.doi.org/10.1128/MCB.01106-09.

27. **Mitchell L, Lau A, Lambert JP, Zhou H, Fong Y, Couture JF, Figeys D, Baetz K.** 2011. Regulation of septin dynamics by the Saccharomyces cerevisiae lysine acetyltransferase NuA4. PLoS One **6:**e25336. http://dx.doi.org/10.1371/journal.pone.0025336.

28. **Lin YY, Lu JY, Zhang J, Walter W, Dang W, Wan J, Tao SC, Qian J, Zhao Y, Boeke JD, Berger SL, Zhu H.** 2009. Protein acetylation microarray reveals that NuA4 controls key metabolic target regulating gluconeogenesis. Cell **136:**1073–1084. http://dx.doi.org/10.1016/j.cell.2009.01.033.

29. **Lu JY, Lin YY, Sheu JC, Wu JT, Lee FJ, Chen Y, Lin MI, Chiang FT, Tai TY, Berger SL, Zhao Y, Tsai KS, Zhu H, Chuang LM, Boeke JD.** 2011. Acetylation of yeast AMPK controls intrinsic aging independently of caloric restriction. Cell **146:**969–979. http://dx.doi.org/10.1016/j.cell.2011.07.044.

30. **Yi C, Ma M, Ran L, Zheng J, Tong J, Zhu J, Ma C, Sun Y, Zhang S, Feng W, Zhu L, Le Y, Gong X, Yan X, Hong B, Jiang FJ, Xie Z, Miao D, Deng H, Yu L.** 2012. Function and molecular mechanism of acetylation in autophagy regulation. Science **336:**474–477. http://dx.doi.org/10.1126/science.1216990.

31. **Tang H, Luo J, Zhang W, Gu W.** 2006. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. Mol Cell **24:**827–839. http://dx.doi.org/10.1016/j.molcel.2006.11.021.

32. **Sykes SM, Mellert HS, Holbert MA, Li K, Marmorstein R, Lane WS, McMahon SB.** 2006. Acetylation of the p53 DNA-binding domain regulates apoptosis induction. Mol Cell **24:**841–851. http://dx.doi.org/10.1016/j.molcel.2006.11.026.

33. **Avvakumov N, Cote J.** 2007. The MYST family of histone acetyltransferases and their intimate links to cancer. Oncogene **26:**5395–5407. http://dx.doi.org/10.1038/sj.onc.1210608.

34. **Fazzio TG, Huff JT, Panning B.** 2008. An RNAi screen of chromatin proteins identifies Tip60-p400 as a regulator of embryonic stem cell identity. Cell **134:**162–174. http://dx.doi.org/10.1016/j.cell.2008.05.031.

35. **Kim J, Woo AJ, Chu J, Snow JW, Fujiwara Y, Kim CG, Cantor AB, Orkin SH.** 2010. A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. Cell **143:**313–324. http://dx.doi.org/10.1016/j.cell.2010.09.010.

36. **Sapountzi V, Cote J.** 2011. MYST-family histone acetyltransferases: beyond chromatin. Cell Mol Life Sci **68:**1147–1156. http://dx.doi.org/10.1007/s00018-010-0599-9.

37. **Smith ER, Eisen A, Gu W, Sattah M, Pannuti A, Zhou J, Cook RG, Lucchesi JC, Allis CD.** 1998. ESA1 is a histone acetyltransferase that is essential for growth in yeast. Proc Natl Acad Sci U S A **95:**3561–3565. http://dx.doi.org/10.1073/pnas.95.7.3561.

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

39. **Doyon Y, Cote J.** 2004. The highly conserved and multifunctional NuA4 HAT complex. Curr Opin Genet Dev **14:**147–154. http://dx.doi.org/10.1016/j.gde.2004.02.009.

40. **Altaf M, Auger A, Covic M, Cote J.** 2009. Connection between histone H2A variants and chromatin remodeling complexes. Biochem Cell Biol **87:**35–50. http://dx.doi.org/10.1139/O08-140.

41. **Friis RM, Wu BP, Reinke SN, Hockman DJ, Sykes BD, Schultz MC.** 2009. A glycolytic burst drives glucose induction of global histone acetylation by picNuA4 and SAGA. Nucleic Acids Res **37:**3969–3980. http://dx.doi.org/10.1093/nar/gkp270.

42. **Selleck W, Fortin I, Sermwittayawong D, Côté J, Tan S.** 2005. The Saccharomyces cerevisiae Piccolo NuA4 histone acetyltransferase complex requires the Enhancer of Polycomb A domain and chromodomain to acetylate nucleosomes. Mol Cell Biol **25:**5535–5542. http://dx.doi.org/10.1128/MCB.25.13.5535-5542.2005.

43. **Berndsen CE, Selleck W, McBryant SJ, Hansen JC, Tan S, Denu JM.** 2007. Nucleosome recognition by the Piccolo NuA4 histone acetyltransferase complex. Biochemistry **46:**2091–2099. http://dx.doi.org/10.1021/bi602366n.

44. **Huang J, Tan S.** 2013. Piccolo NuA4-catalyzed acetylation of nucleosomal histones: critical roles of an Esa1 Tudor/chromo barrel loop and an Epl1 enhancer of polycomb A (EPcA) basic region. Mol Cell Biol **33:**159–169. http://dx.doi.org/10.1128/MCB.01131-12.

45. **Auger A, Galarneau L, Altaf M, Nourani A, Doyon Y, Utley RT, Cronier D, Allard S, Côté J.** 2008. Eaf1 is the platform for NuA4 molecular assembly that evolutionarily links chromatin acetylation to ATP-dependent exchange of histone H2A variants. Mol Cell Biol **28:**2257–2270. http://dx.doi.org/10.1128/MCB.01755-07.

46. **Brown CE, Howe L, Sousa K, Alley SC, Carrozza MJ, Tan S, Workman JL.** 2001. Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. Science **292:**2333–2337. http://dx.doi.org/10.1126/science.1060214.

47. **Narlikar GJ, Fan HY, Kingston RE.** 2002. Cooperation between complexes that regulate chromatin structure and transcription. Cell **108:**475–487. http://dx.doi.org/10.1016/S0092-8674(02)00654-2.

48. **Longtine MS, McKenzie A, Demarini DJ, III, Shah NG, Wach A, Brachat A, Philippsen P, Pringle JR.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast **14:**953–961.

49. **Sikorski RS, Hieter P.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics **122:**19–27.

50. **Shen WC, Bhaumik SR, Causton HC, Simon I, Zhu X, Jennings EG, Wang TH, Young RA, Green MR.** 2003. Systematic analysis of essential yeast TAFs in genome-wide transcription and preinitiation complex assembly. EMBO J **22:**3395–3402. http://dx.doi.org/10.1093/emboj/cdg336.

51. **Li XY, Bhaumik SR, Green MR.** 2000. Distinct classes of yeast promoters revealed by differential TAF recruitment. Science **288:**1242–1244. http://dx.doi.org/10.1126/science.288.5469.1242.

52. **Malik S, Shukla A, Sen P, Bhaumik SR.** 2009. The 19S proteasome subcomplex establishes a specific protein interaction network at the promoter for stimulated transcriptional initiation in vivo. J Biol Chem **284:**35714–35724. http://dx.doi.org/10.1074/jbc.M109.035709.

53. **Shukla A, Bhaumik SR.** 2007. H2B-K123 ubiquitination stimulates RNAPII elongation independent of H3-K4 methylation. Biochem Biophys Res Commun **359:**214–220. http://dx.doi.org/10.1016/j.bbrc.2007.05.105.

54. **Durairaj G, Sen R, Uprety B, Shukla A, Bhaumik SR.** 2014. Sus1p facilitates pre-initiation complex formation at the SAGA-regulated genes independently of histone H2B de-ubiquitylation. J Mol Biol **426:**2928–2941. http://dx.doi.org/10.1016/j.jmb.2014.05.028.

55. **Malik S, Bagla S, Chaurasia P, Duan Z, Bhaumik SR.** 2008. Elongating RNA polymerase II is disassembled through specific degradation of its largest but not other subunits in response to DNA damage in vivo. J Biol Chem **283:**6897–6905. http://dx.doi.org/10.1074/jbc.M707649200.

56. **Bhaumik SR, Green MR.** 2002. Differential requirement of SAGA components for recruitment of TATA-box-binding protein to promoters in vivo. Mol Cell Biol **22:**7365–7371. http://dx.doi.org/10.1128/MCB.22.21.7365-7371.2002.

57. **Bhaumik SR, Green MR.** 2003. Interaction of Gal4p with components of transcription machinery in vivo. Methods Enzymol **370:**445–454. http://dx.doi.org/10.1016/S0076-6879(03)70038-X.

58. **Shukla A, Stanojevic N, Duan Z, Sen P, Bhaumik SR.** 2006. Ubp8p, a histone deubiquitinase whose association with SAGA is mediated by Sgf11p, differentially regulates lysine 4 methylation of histone H3 in vivo. Mol Cell Biol **26:**3339–3352. http://dx.doi.org/10.1128/MCB.26.9.3339-3352.2006.

59. **Bhaumik SR, Raha T, Aiello DP, Green MR.** 2004. In vivo target of a transcriptional activator revealed by fluorescence resonance energy transfer. Genes Dev **18:**333–343. http://dx.doi.org/10.1101/gad.1148404.

60. **Malik S, Chaurasia P, Lahudkar S, Durairaj G, Shukla A, Bhaumik SR.** 2010. Rad26p, a transcription-coupled repair factor, is recruited to the site of DNA lesion in an elongating RNA polymerase II-dependent manner in vivo. Nucleic Acids Res **38:**1461–1477. http://dx.doi.org/10.1093/nar/gkp1147.

61. **Shukla A, Stanojevic N, Duan Z, Shadle T, Bhaumik SR.** 2006. Functional analysis of H2B-Lys-123 ubiquitination in regulation of H3-Lys-4 methylation and recruitment of RNA polymerase II at the coding sequences of several active genes in vivo. J Biol Chem **281:**19045–19054. http://dx.doi.org/10.1074/jbc.M513533200.

62. **Durairaj G, Chaurasia P, Lahudkar S, Malik S, Shukla A, Bhaumik SR.** 2010. Regulation of chromatin assembly/disassembly by Rtt109p, a histone H3 Lys56-specific acetyltransferase, in vivo. J Biol Chem **285:**30472–30479. http://dx.doi.org/10.1074/jbc.M110.113225.

63. **Malik S, Bhaumik SR.** 2012. Rad26p, a transcription-coupled repair factor, promotes the eviction and prevents the reassociation of histone H2A-H2B dimer during transcriptional elongation in vivo. Biochemistry **51:**5873–5875. http://dx.doi.org/10.1021/bi3005768.

64. **Malik S, Chaurasia P, Lahudkar S, Uprety B, Bhaumik SR.** 2012. Rad26p regulates the occupancy of histone H2A-H2B dimer at the active genes in vivo. Nucleic Acids Res **40:**3348–3363. http://dx.doi.org/10.1093/nar/gkr1244.

65. **Durairaj G, Lahudkar S, Bhaumik SR.** 2014. A new regulatory pathway of mRNA export by an F-box protein, Mdm30. RNA **20:**133–142. http://dx.doi.org/10.1261/rna.042325.113.

66. **Peterson CL, Kruger W, Herskowitz I.** 1991. A functional interaction between the C-terminal domain of RNA polymerase II and the negative regulator SIN1. Cell **64:**1135–1143. http://dx.doi.org/10.1016/0092-8674(91)90268-4.

67. **Lahudkar S, Durairaj G, Uprety B, Bhaumik SR.** 2014. A novel role for Cet1p mRNA 5-triphosphatase in promoter proximal accumulation of RNA polymerase II in Saccharomyces cerevisiae. Genetics **196:**161–176. http://dx.doi.org/10.1534/genetics.113.158535.

68. **Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Struhl K.** 2001 Current protocols in molecular biology. Wiley, New York, NY.

69. **Malik S, Durairaj G, Bhaumik SR.** 2013. Mechanisms of antisense transcription initiation from the 3-end of theGAL10 coding sequence in vivo. Mol Cell Biol **33:**3549–3567. http://dx.doi.org/10.1128/MCB.01715-12.

70. **Knutson BA, Hahn S.** 2011. Domains of Tra1 important for activator recruitment and transcription coactivator functions of SAGA and NuA4 complexes. Mol Cell Biol **31:**818–831. http://dx.doi.org/10.1128/MCB.00687-10.

71. **Yen K, Vinayachandran V, Pugh BF.** 2013. SWR-C and INO80 chromatin remodelers recognize nucleosome-free regions near +1 nucleosomes. Cell **154:**1246–1256. http://dx.doi.org/10.1016/j.cell.2013.08.043.

72. **Reid JL, Moqtaderi Z, Struhl K.** 2004. Eaf3 regulates the global pattern of histone acetylation in Saccharomyces cerevisiae. Mol Cell Biol **24:**757–764. http://dx.doi.org/10.1128/MCB.24.2.757-764.2004.

73. **Robert F, Pokholok DK, Hannett NM, Rinaldi NJ, Chandy M, Rolfe A, Workman JL, Gifford DK, Young RA.** 2004. Global position and recruitment of HATs and HDACs in the yeast genome. Mol Cell **16:**199–209. http://dx.doi.org/10.1016/j.molcel.2004.09.021.

74. **Altaf M, Auger A, Monnet-Saksouk J, Brodeur J, Piquet S, Cramet M, Bouchard N, Lacoste N, Utley RT, Gaudreau L, Côté J.** 2010. NuA4-dependent acetylation of nucleosomal histones H4 and H2A directly stimulates incorporation of H2A.Z by the SWR1 complex. J Biol Chem **285:**15966–15977. http://dx.doi.org/10.1074/jbc.M110.117069.

75. **Durant M, Pugh BF.** 2007. NuA4-directed chromatin transactions throughout the Saccharomyces cerevisiae genome. Mol Cell Biol **27:** 5327–5335. http://dx.doi.org/10.1128/MCB.00468-07.

76. **Jacobson RH, Ladurner AG, King DS, Tjian R.** 2000. Structure and function of a human TAF(II)250 double bromodomain module. Science **288:**1422–1425. http://dx.doi.org/10.1126/science.288.5470.1422.

77. **Ladurner AG, Inouye C, Jain R, Tjian R.** 2003. Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. Mol Cell **11:**365–376. http://dx.doi.org/10.1016/S1097 -2765(03)00035-2.

78. **Matangkasombut O, Buratowski S.** 2003. Different sensitivities of bromodomain factors 1 and 2 to histone H4 acetylation. Mol Cell **11:**353– 363. http://dx.doi.org/10.1016/S1097-2765(03)00033-9.

79. **Pamblanco M, Poveda A, Sendra R, Rodriguez-Navarro S, Perez-Ortin JE, Tordera V.** 2001. Bromodomain factor 1 (Bdf1) protein interacts with histones. FEBS Lett **496:**31–35. http://dx.doi.org/10.1016/S0014 -5793(01)02397-3.

80. **Singh MV, Bland CE, Weil PA.** 2004. Molecular and genetic characterization of a Taf1p domain essential for yeast TFIID assembly. Mol Cell Biol **24:**4929–4942. http://dx.doi.org/10.1128/MCB.24.11.4929-4942 .2004.

81. **Larschan E, Winston F.** 2001. The S. cerevisiae SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. Genes Dev **15:**1946–1956. http://dx.doi.org/10.1101/gad.911501.

82. **Bhaumik SR, Green MR.** 2001. SAGA is an essential *in vivo* target of the yeast acidic activator Gal4p. Genes Dev **15:**1935–1945. http://dx.doi.org /10.1101/gad.911401.

83. **Bhaumik SR.** 2011. Distinct regulatory mechanisms of eukaryotic transcriptional activation by SAGA and TFIID. Biochim Biophys Acta **1809:** 97–108. http://dx.doi.org/10.1016/j.bbagrm.2010.08.009.

84. **Dudley AM, Rougeulle C, Winston F.** 1999. The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. Genes Dev **13:**2940–2945. http://dx.doi.org/10.1101/gad.13 .22.2940.

85. **Shukla A, Bajwa P, Bhaumik SR.** 2006. SAGA-associated Sgf73p facilitates formation of the preinitiation complex assembly at the promoters either in a HAT-dependent or independent manner *in vivo*. Nucleic Acids Res **34:**6225–6232. http://dx.doi.org/10.1093/nar/gkl844.

86. **Grant PA, Duggan L, Côté J, Roberts SM, Brownell JE, Candau R, Ohba R, Owen-Hughes T, Allis CD, Winston F, Berger SL, Workman JL.** 1997. Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev **11:**1640–1650. http://dx.doi.org /10.1101/gad.11.13.1640.

87. **Sterner DE, Grant PA, Roberts SM, Duggan LJ, Belotserkovskaya R, Pacella LA, Winston F, Workman JL, Berger SL.** 1999. Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Mol Cell Biol **19:**86–98.

88. **Kundu S, Horn PJ, Peterson CL.** 2007. SWI/SNF is required for transcriptional memory at the yeast GAL gene cluster. Genes Dev **21:**997– 1004. http://dx.doi.org/10.1101/gad.1506607.

89. **Apone LM, Virbasius CA, Holstege FC, Wang J, Young RA, Green MR.** 1998. Broad, but not universal, transcriptional requirement for yTAFII17, a histone H3-like TAFII present in TFIID and SAGA. Mol Cell **2:**653–661. http://dx.doi.org/10.1016/S1097-2765(00)80163-X.

90. **Holstege 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.

91. **Lee TI, Causton HC, Holstege FC, Shen WC, Hannett N, Jennings EG, Winston F, Green MR, Young RA.** 2000. Redundant roles for the TFIID and SAGA complexes in global transcription. Nature **405:**701–704. http: //dx.doi.org/10.1038/35015104.

92. **Hassan AH, Prochasson P, Neely KE, Galasinski SC, Chandy M, Carrozza MJ, Workman JL.** 2002. Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. Cell **111:**369–379. http://dx.doi.org/10.1016 /S0092-8674(02)01005-X.

93. **Li S, Shogren-Knaak MA.** 2009. The Gcn5 bromodomain of the SAGA complex facilitates cooperative and cross-tail acetylation of nucleosomes. J Biol Chem **284:**9411–9417. http://dx.doi.org/10.1074 /jbc.M809617200.

94. **Ginsburg DS, Anlembom TE, Wang J, Patel SR, Li B, Hinnebusch AG.** 2014. NuA4 links methylation of histone H3 lysines 4 and 36 to acetylation of histones H4 and H3. J Biol Chem **289:**32656–32670. http://dx.doi .org/10.1074/jbc.M114.585588.

95. **Huisinga KL, Pugh BF.** 2004. A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in Saccharomyces cerevisiae. Mol Cell **13:**573–585. http://dx.doi.org/10.1016/S1097 -2765(04)00087-5.

96. **Ghosh S, Pugh BF.** 2011. Sequential recruitment of SAGA and TFIID in a genomic response to DNA damage in Saccharomyces cerevisiae. Mol Cell Biol **31:**190–202. http://dx.doi.org/10.1128/MCB.00317-10.

97. **Ohtsuki K, Kasahara K, Shirahige K, Kokubo T.** 2010. Genome-wide localization analysis of a complete set of Tafs reveals a specific effect of the taf1 mutation on Taf2 occupancy and provides indirect evidence for different TFIID conformations at different promoters. Nucleic Acids Res **38:**1805–1820. http://dx.doi.org/10.1093/nar/gkp1172.

98. **Li XY, Bhaumik SR, Zhu X, Li L, Shen WC, Dixit BL, Green MR.** 2002. Selective recruitment of TAFs by yeast upstream activating sequences. Implications for eukaryotic promoter structure. Curr Biol **12:**1240– 1244.

99. **Mayer C, Grummt I.** 2006. Ribosome biogenesis and cell growth: mTOR coordinates transcription by all three classes of nuclear RNA polymerases. Oncogene **25:**6384–6391. http://dx.doi.org/10.1038/sj.onc .1209883.

100. **Warner JR.** 1999. The economics of ribosome biosynthesis in yeast. Trends Biochem Sci **24:**437–440. http://dx.doi.org/10.1016/S0968 -0004(99)01460-7.

101. **Collins SR, Miller KM, Maas NL, Roguev A, Fillingham J, Chu CS, Schuldiner M, Gebbia M, Recht J, Shales M, Ding H, Xu H, Han J, Ingvarsdottir K, Cheng B, Andrews B, Boone C, Berger SL, Hieter P, Zhang Z, Brown GW, Ingles CJ, Emili A, Allis CD, Toczyski DP, Weissman JS, Greenblatt JF, Krogan NJ.** 2007. Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. Nature **446:**806–810. http://dx.doi.org/10 .1038/nature05649.