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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*

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ABSTRACT

Previous studies have implicated SAGA (Spt-Ada-Gcn5-acetyltransferase) and TFIID (Transcription factor-IID)-dependent mechanisms of transcriptional activation in yeast. SAGA-dependent transcriptional activation is further regulated by the 19S proteasome subcomplex. However, the role of the 19S proteasome subcomplex in transcriptional activation of the TFIID-dependent genes has not been elucidated. Therefore, we have performed a series of chromatin immunoprecipitation, mutational and transcriptional analyses at the TFIID-dependent ribosomal protein genes such as *RPS5*, *RPL2B* and *RPS11B*. We find that the 19S proteasome subcomplex is recruited to the promoters of these ribosomal protein genes, and promotes the association of NuA4 (Nucleosome acetyltransferase of histone H4) co-activator, but not activator Rap1p (repressor-activator protein 1). These observations support that the 19S proteasome subcomplex enhances the targeting of co-activator at the TFIID-dependent promoter. Such an enhanced targeting of NuA4 HAT (histone acetyltransferase) promotes the recruitment of the TFIID complex for transcriptional initiation. Collectively, our data demonstrate that the 19S proteasome subcomplex enhances the targeting of NuA4 HAT to activator Rap1p at the promoters of ribosomal protein genes to facilitate the recruitment of TFIID for transcriptional stimulation, hence providing a new role of the 19S proteasome subcomplex in establishing a specific regulatory network at the TFIID-dependent promoter for productive transcriptional initiation *in vivo*.

INTRODUCTION

Transcriptional initiation is an important step of gene expression, and is promoted by gene-specific activators that bind to the specific DNA sequences upstream of the core promoter element (known as upstream activating sequence or UAS). Activators function by enhancing the assembly of the general transcription factors (GTFs) such as TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH, as well as RNA polymerase II at the core promoter to form a pre-initiation complex (PIC) for transcriptional initiation. Such an enhanced PIC formation is mediated by the interaction of the activator with one or more transcription factors, termed as 'target' (1). Based on the target specificities of the activators, previous studies have revealed two distinct mechanisms of transcriptional activation that are mediated by the SAGA (Spt3-Ada-Gcn5-acetyltransferase) and TFIID complexes in *Saccharomyces cerevisae* (1). SAGA is a large multi-protein complex with two different enzymatic activities such as histone acetyltransferase (HAT) and histone deubiquitinase, while TFIID is composed of TBP (TATA-box binding protein) and 14 different TBP-associated factors (TAFs) (1). For SAGA-dependent transcriptional activation, the activator targets SAGA that subsequently promotes the PIC formation at the core promoter for transcriptional initiation (1–8). There are about 10% RNA polymerase II genes whose expression is regulated by SAGA (1,9–12). However, the expression of a vast majority of genes is regulated by the TFIID complex (1,9–12). At the TFIID-regulated genes, activator targets TFIID for transcriptional initiation (1,13–16). Importantly, TFIID has

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been implicated in regulating the transcription of ribosomal protein genes (1,13,15). Expression of ribosomal protein genes is crucial for ribosomal biogenesis and the subsequent translation of mRNA into proteins for normal cellular growth and functions (17). Thus, TFIID plays an important role in ribosome biogenesis, and hence cellular growth. Further, transcription of ribosomal protein genes is controlled by TOR (target of Rapamycin) signaling pathway that is highly conserved from yeast to humans (17,18). TOR inactivation by rapamycin (a macrocyclic lactone) through inhibition of a TOR-kinase containing protein complex impairs various anabolic as well as catabolic processes including ribosomal protein gene expression, thus regulating the growth and fate of eukaryotic cell.

In yeast, there are 137 ribosomal protein genes (~2% of the total genes), and ~50% of RNA polymerase II transcription is devoted to these genes in the TFIID and TOR-dependent fashions (17,19). Two TOR-dependent factors have been implicated to regulate the transcription of ribosomal protein genes in yeast in response to nutrient cues (17). These are Sfp1p and forkhead transcription factor Fhl1p. The co-activator and co-repressor of Fhl1p are Ifh1p and Crf1p, respectively (17,20–23). Sfp1p binds to the promoters of ribosomal protein genes to enhance transcription in a TOR-dependent manner. In the presence of rapamycin or nutrient starvation, Sfp1p is inactivated, leading to transcriptional downregulation of ribosomal protein genes. Likewise, Fhl1p binds to the promoters of ribosomal protein genes and activate them under nutrient-rich growth conditions in a TOR-dependent manner. Under such growth conditions, the co-repressor Crf1p stays in the cytoplasm via the action of TOR-dependent protein kinase A. Upon nutrient starvation, Crf1p moves into the nucleus and binds to Fhl1p, leading to the transcriptional repression of ribosomal protein genes. In addition to these regulations, TOR also regulates ribosomal protein gene expression by enhancing association of NuA4 (Nucleosome acetyltransferase of histone H4) HAT complex with the promoter and dissociation of Rpd3p histone deacetylase from the promoter, hence stimulating the transcription of the ribosomal protein genes (17,24). Following inhibition of the TOR signaling pathway, NuA4 HAT dissociates from the ribosomal protein genes and Rpd3p binds to the promoter, leading to the transcriptional repression of ribosomal protein genes (17,24,25). In addition to nutrient or TOR-dependent regulation, transcription of ribosomal protein genes is also controlled by other environmental insults such as heat and osmotic shocks. Thus, transcription of ribosomal protein genes is co-ordinately regulated in a complex manner, which has a major impact on overall capacity of protein synthesis and cellular growth.

Recently, DNA microarray analysis has implicated the proteasome complex in transcriptional regulation of ribosomal protein genes (1,26–29); further complicating ribosomal-protein gene expression. The 26S proteasome is a highly versatile protein degradation machine with a molecular chaperonin activity. It consists of 20S proteolytic core and 19S regulatory particles (CP and RP, respectively). The 19S RP is further composed of a 'lid' of eight non-ATPases, and a 'base' of six ATPases (Rpt1–Rpt6) and three non-ATPases. The 19S RP has the molecular chaperonin activity (30), and its ATPase activity is required for its association with 20S CP to form the 26S proteasome complex (31). The 19S ATPase activity is also crucial for the degradation of proteins marked by a chain of more than four lysine-48-linked ubiquitin molecules (32–34). The lid of the 19S RP binds to the polyubiquitin chain of the substrate protein, and the 19S ATPase activity subsequently unfolds the substrate protein and translocates it into the catalytic site of the 20S CP for proteolysis (34). Via such a degradation mechanism, the 26S proteasome complex regulates the functions and fates of many transcription factors, and hence transcription (34). In fact, ~70% of the genomic transcripts in yeast is altered in the temperature-sensitive (ts) inactivation of either the 19S RP or 20S CP (28).

The 19S RP has been previously shown to increase the interaction between activator Gal4p and co-activator SAGA at the SAGA-dependent *GAL1* gene for stimulation of transcriptional initiation in a proteolysis-independent manner (35,36). However, it is not yet known whether transcriptional initiation of the TFIID-dependent ribosomal protein genes is also similarly regulated by the 19S RP in a proteolysis-independent manner. With this view, we performed a series of experiments at several ribosomal protein genes such as *RPS5*, *RPL2B* and *RPS11B* in *S. cerevisae*. We find that the 19S base is recruited to the promoters of the ribosomal protein genes, and enhances the recruitment of NuA4 HAT, but not activator Rap1p (repressor–activator protein1). Further, we show that NuA4 HAT promotes the recruitment of the TFIID complex, and hence transcription of ribosomal protein genes. Thus, the 19S base promotes the targeting of NuA4 HAT to enhance the recruitment of TFIID for stimulation of transcriptional initiation. These results provide a new regulatory mechanism of transcriptional activation of the TFIID-dependent ribosomal protein genes by the 19S proteasome subcomplex.

MATERIALS AND METHODS

Plasmids

The plasmid pFA6a-13Myc-KanMX6 (37) was used for genomic tagging of the proteins of interest by Myc epitope. The plasmid PRS406 was used for PCR-based disruption of *PDR5*.

Strains

The yeast (*S. cerevisiae*) strain bearing ts mutation in Rpt4p (*rpt4*-ts or *sug2-13*, Sc677) and its isogenic wild-type equivalent (Sc599) were obtained from the Kodadek and Johnston laboratories (38). The *esa1*-ts mutant (LPY3291) and wild-type (LPY3498) strains were obtained from the Pillus laboratory (39). Multiple Myc epitope tags were added at the original chromosomal loci of *RPN9*, *PRS3*, *PRE6* and *RPN12* in FY67 (40) to generate NSY5 (Rpn9p-Myc), NSY6 (Prs3p-Myc), NSY8 (Pre6p-Myc) and NSY7 (Rpn12p-Myc), respectively. Strains PSY17 (Rpt2p-Myc) and PSY18 (Rpt6p-Myc)

were generated by adding multiple Myc epitope tags at the C-termini of Rpt2p and Rpt6p, respectively, in Sc599. Multiple Myc epitope tags were added at the original chromosomal locus of *ESA1* in the *rpt4*-ts and wild-type strains to generate BUY13 (Esa1p-Myc in *rpt4*-ts) and BUY12 (Esa1p-Myc), respectively. The *PDR5* gene was deleted from the wild-type strain by PCR-based gene disruption method to generate SLY16a (*Δpdr5*, *Δura3*).

Growth media

For the ChIP studies at the ribosomal protein genes in the wild-type strain, yeast cells were grown in YPD (yeast extract–peptone plus 2% dextrose) at 30°C up to an OD_{600} of 1.0 prior to formaldehyde-based *in vivo* cross-linking. However, the *rpt4*-ts and *esa1*-ts mutants and their isogenic wild-type equivalents were grown in YPD at 23°C up to an OD_{600} of 0.85 and then transferred to 37°C for 1- or 2-h before cross-linking. For experiments at *INO1*, yeast cells were initially grown in synthetic complete medium (yeast nitrogen base and complete amino acid mixture plus 2% dextrose) containing 100 μM inositol at 30°C up to an OD_{600} of 0.45, and then switched to the same medium without inositol for 2 h prior to MG132 (75 μM) treatment for 2 h.

Chromatin Immunoprecipitation assay

The Chromatin Immunoprecipitation (ChIP) assay was performed as described previously (2,4,41,42). Briefly, yeast cells were treated with 1% formaldehyde for 15 min, collected and resuspended in lysis buffer. Following sonication, cell lysate (400 μl lysate from 50 ml of yeast culture) was pre-cleared by centrifugation and then 100 μl lysate was used for each immunoprecipitation. Immunoprecipitated protein–DNA complexes were treated with proteinase K, the cross-links were reversed and DNA was purified. Immunoprecipitated DNA was dissolved in 10 μl TE 8.0 (10 mM Tris-HCl pH 8.0 and 1 mM EDTA), and 1 μl of immunoprecipitated DNA was analyzed by PCR. PCR reactions contained (α-^{32}P)dATP (2.5 μCi for each 25 μl reaction), and the PCR products were detected by autoradiography after separation on a 6% polyacrylamide gel. As a control, 'input' DNA was isolated from 5 μl lysate without going through the immunoprecipitation step and dissolved in 100 μl TE 8.0. To compare PCR signal arising from the immunoprecipitated DNA with the input DNA, 1 μl of input DNA was used in the PCR analysis.

The association of Esa1p with ribosomal protein genes was analyzed by modified-ChIP assay as described in our previous publication (42). For ChIP analysis of the proteasome components, we modified the above ChIP protocol as follows. 1600 μl of lysate was prepared from 200 ml of yeast culture following formaldehyde-based *in vivo* cross-linking for 25 min. 600 μl of lysate was used for each immunoprecipitation (using 10 μl of anti-Myc antibody and 100 μl of protein A/G plus agarose beads from Santa Cruz Biotechnology, Inc.), and immunoprecipitated DNA sample was dissolved in 5 μl of TE 8.0 of which 1 μl was used in PCR analysis. In parallel, PCR for input DNA was performed using 1 μl of DNA that was prepared by dissolving purified DNA from 5 μl of lysate in 100 μl of TE 8.0. Autoradiograms were scanned and quantitated by the National Institutes of Health image 1.62 program. Immunoprecipitated DNAs were quantitated as the ratio of IP to input.

The primer pairs used for PCR analysis were as follows:

RPS5 (UAS):	5′-AGAAACAATGAACAGCCTTGAGTTCTC-3′
	5′-GCAGGGCCATTCTCATCTGA-3′
RPS5(Core):	5′-GGCCAACTTCTACGCTCACGTTAG-3′
	5′-CGGTGTCAGACATCTTTGGAATGGTC-3′
RPS5 (ORF):	5′-AGGCTCAATGTCCAATCATTGAAAG-3′
	5′-CAACAACTTGGATTGGGTTTTGGTC-3′
RPL2B (UAS):	5′-TACCGATTACCAAGTTTTCAGACTA-3′
	5′-AATTCCTTCTTTTTCTCCCTAGCGG-3′
RPL2B (Core):	5′-TGGTGGATTCTGCTCTGGAAACTAT-3′
	5′-CTTTGTGGTTTCTTGGTGAGTTTAT-3′
RPL2B (ORF):	5′-GTGCTTTCCACAAGTACAGATTGAA-3′
	5′-TTTGACCAGAAACGGCACCTCTAGA-3′
RPS11B (UAS):	5′-GATATACACAAGAATTTCTGGAAGA-3′
	5′-CACTTCCTCATTTCACAAAGACACT-3′
RPS11B (Core):	5′-AAGTCCAATAGCTTTACGTTTCCCT-3′
	5′-CTTTTTCCCTGGCTTGATACGTTTC-3′
RPS11B (ORF):	5′-GCACCGTACCATTGTCATCAGAAGA-3′
	5′-GGTCTACATTGACCAACGGTAACAA-3′
ACT1 (Core):	5′-AACCGTTTTGAAACCAAACTCGCCT-3′
	5′-TTCTTGGTTTGAGTAGAAAGGGGAA-3′
INO1 (Core):	5′-TTCACATGGAGCAGAGAAAGCGCA-3′
	5′-GGATAAAACTAACATTAGGAAC CCGAC-3′
Chr-V:	5′-GGCTGTCAGAATATGGGGCCGTAGTA-3′
	5′-CACCCCGAAGCTGCTTTCACAATAC-3′.

UAS, upstream activating sequence; Core, core promoter; ORF, open reading frame; and Chr-V, Chromosome-V.

Total mRNA preparation

The total mRNA was prepared from yeast cell culture as described by Peterson *et al.* (43). 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_2EDTA and 1% SDS) along with 100 μl phenol/chloroform/isoamyl alcohol and 100 μl volume equivalent of glass beads (acid washed; Sigma). Subsequently, yeast cell suspension was vortexed with a maximum speed (10 in VWR mini-vortexer; Cat. No. 58816-121) for five times (30 s each). Cells suspension was put in ice for 30 s between pulses. After vortexing, 150 μl RNA preparation buffer and 150 μl phenol/chloroform/isoamyl alcohol were added to yeast cell suspension followed by vortexing for 15 s with a maximum speed on VWR mini-vortexer. The aqueous phase was collected following 5 min centrifugation at a maximum speed in microcentrifuge machine. The total mRNA was isolated from aqueous phase by ethanol precipitation.

Reverse transcriptase–PCR analysis

Reverse transcriptase (RT)–PCR analysis was performed following the standard protocols (44,45). Briefly, total mRNA was prepared from 10 ml yeast culture, and was used in the reverse transcription assay. mRNA

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 synthesized first strand as template and the primer pairs targeted to the *RPS5, RPL2B, RPS11B* and *ACT1* ORFs. RT–PCR products were separated by 2.2% agarose gel electrophoresis and visualized by ethidium bromide staining. The primer pairs used in the PCR analysis were as follows:

RPS5:	5′-AGGCTCAATGTCCAATCATTGAAAG-3′
	5′-CAACAACTTGGATTGGGTTTTGGTC-3′
RPL2B:	5′-GTGCTTTCCACAAGTACAGATTGAA-3′
	5′-TTTGACCAGAAACGGCACCTCTAGA-3′
RPS11B:	5′-GCACCGTACCATTGTCATCAGAAGA-3′
	5′-GGTCTACATTGACCAACGGTAACAA-3′
ACT1:	5′-TCCACCACTGCTGAAAGAGAAATTG-3′
	5′-AATAGTGATGACTTGACCATCTGGA-3′

RESULTS

The 19S base, but not lid or 20S CP, is recruited to the *RPS5* promoter

To determine the role of the 26S proteasome in regulation of transcriptional initiation of the TFIID-dependent ribosomal protein genes, we first analyzed its association with the promoter of a well-characterized ribosomal protein gene, *RPS5*. In view of this, we tagged the Rpt6p (19S base), Rpt2p (19S base), Rpn9p (19S lid), Rpn12p (19S lid), Prs3p (20S CP) and Pre6p (20S CP) components of the 26S proteasome complex by Myc epitope in their endogenous chromosomal loci. Using these epitope-tagged strains, we performed the ChIP assay at the *RPS5* UAS, core promoter and coding sequence (ORF) (Figure 1A). The inactive region of chromosome V (Chr-V) was used as a non-specific DNA control. An anti-HA served as a non-specific antibody in the ChIP assay. We find that Rpt2p and Rpt6p components of the 19S base were recruited to the *RPS5* promoter (Figure 1B–E and Supplementary Figure S1). However, a relatively higher association of Rpt2p and Rpt6p was observed at the UAS (Figure 1B and D). The 19S base was also found at the coding sequence (Figure 1B and D), consistent with its known role in transcriptional elongation (46). The 19S lid (Rpn9p and Rpn12p) or 20S CP (Prs3p and Pre6p) was not recruited to the *RPS5* promoter (Figure 2A–D). Together, these results demonstrate that the 19S base is recruited to the *RPS5* promoter independently of the 19S lid or 20S CP. Likewise, the 19S base has also been previously shown to be associated with the *GAL1* promoter (36,47). These results support the existence of the 19S base independently of lid or 20S CP *in vivo*. Consistent with these *in vivo* observations, the 19S base without lid or 20S CP has been biochemically characterized (48). Moreover, previous studies have also demonstrated the existence of the 19S base independently of the lid or 20S CP in the nucleus (49).

The 19S base promotes the recruitment of TFIID to the *RPS5* promoter for transcriptional initiation

Rap1p recognizes the RPG box upstream of the *RPS5* core promoter, and thus, is recruited to the UAS, but not core promoter, of *RPS5* (Figure 3A). Subsequently, it activates transcription (13,15,16). Rap1p also functions as a transcriptional activator of other ribosomal protein genes (19,50). In addition to its role in transcriptional activation, it further plays an important role in silencing at telomeres and mating-type loci (51,52). Previous studies (13,15,16) have demonstrated that Rap1p targets TAFs to recruit the TFIID complex at the core promoter for transcriptional initiation, indicating that TAFs are the essential targets of the transcriptional activator Rap1p *in vivo*. Consistent with these *in vivo* results, Weil and colleagues (53,54) have also demonstrated biochemically the interaction of Rap1p with TAFs. Collectively, these studies have implicated TAFs as the target of transcriptional activator at the ribosomal protein genes. However, previous studies (55,56) have also demonstrated the requirement of NuA4 HAT for transcription of ribosomal protein genes. NuA4 is highly conserved among eukaryotes, and is required for acetylation of histones H4 and H2A (55,57,58). The catalytic subunit of NuA4 is Esa1p which is essential for cellular viability. We find that like the 19S base, NuA4 HAT (Esa1p-Myc) is recruited to the *RPS5* promoter (Figure 3B), consistent with previous studies (55). However, it is also recruited to the coding sequence (Figure 3B). Likewise, Ginsburg *et al.* (59) have demonstrated the association of NuA4 HAT with the coding sequence for transcriptional elongation. Further, Reid *et al.* (55) have implicated the role of Rap1p in targeting NuA4 HAT. Consistently, co-immunoprecipitation analysis revealed the interaction of Rap1p with NuA4 (Supplementary Figure S2). Likewise, previous biochemical studies have also demonstrated the interaction of NuA4 with acidic activators (6,56,60). Taken together, Rap1p, NuA4 HAT and the 19S base are recruited to *RPS5*, and Rap1p and NuA4 HAT are essential for transcription of *RPS5*. However, the role of the 19S base in regulation of the *RPS5* transcription is not known. Like Rap1p and NuA4 HAT, the 19S base might be playing a crucial role in regulating the transcriptional initiation of *RPS5*. To test this possibility, we analyzed the role of the 19S base in recruitment of the TFIID complex at the *RPS5* core promoter, since our previous studies (13) have correlated the recruitment of the TFIID complex with transcriptional initiation. We previously demonstrated that TBP and TAFs components of the TFIID complex are recruited to the *RPS5* core promoter (13), and these components are essential for *RPS5* transcription (13). Further, we have shown previously that TAFs are essential for recruitment of TBP to the *RPS5* core promoter for transcriptional initiation (10). Thus, we have used TBP as a representative core component of the TFIID complex for ChIP analysis at the *RPS5* promoter. We find that the recruitment of TBP to the *RPS5* core promoter was significantly decreased in the ts mutant strain of Rpt4p ATPase subunit of the 19S base (Figure 3C and D). The *rpt4*-ts mutant encodes point

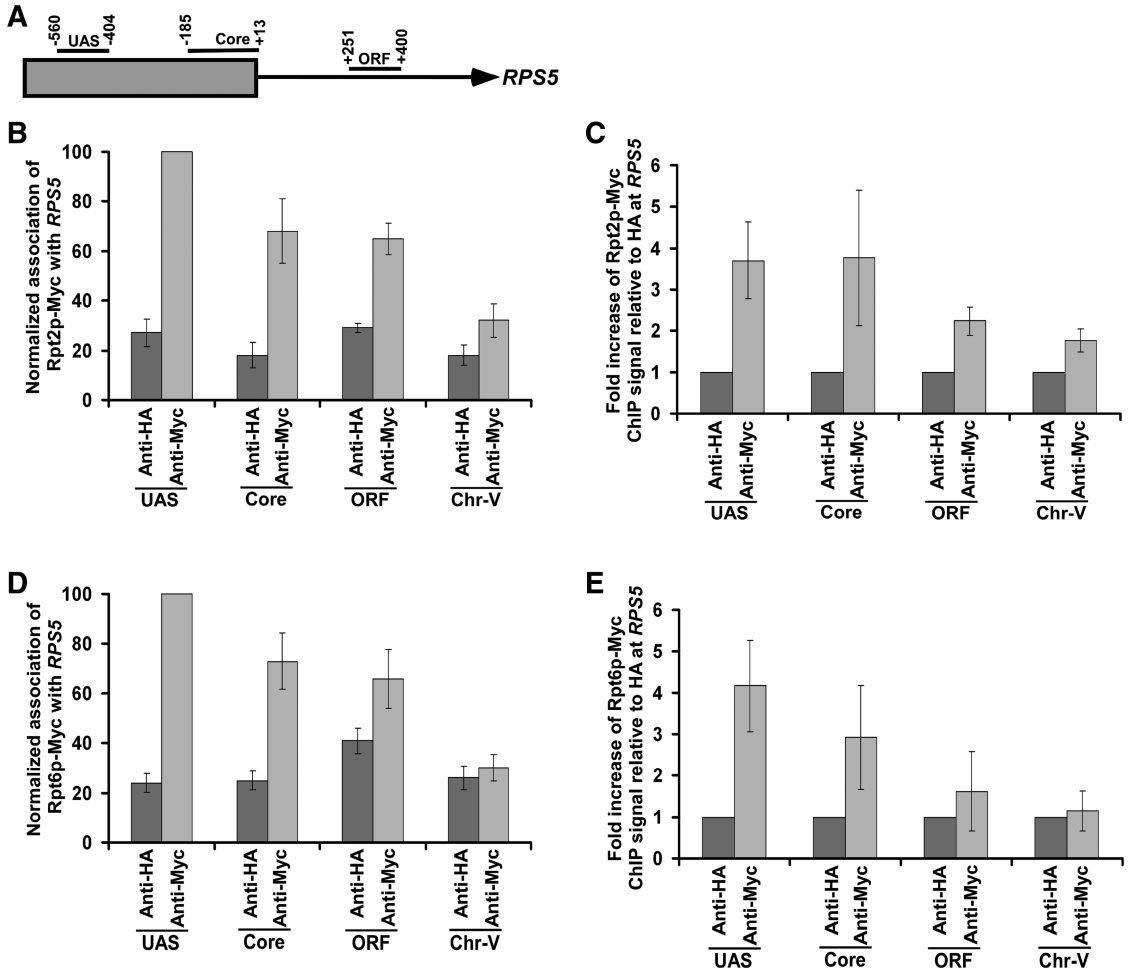

Figure 1. The 19S base is recruited to the *RPS5* promoter. (**A**) The schematic diagram of the *RPS5* promoter with the PCR amplification regions (UAS, Core and open reading frame or ORF) in the ChIP assay. (**B**) Analysis of recruitment of the Rpt2p component of the 19S base to *RPS5*. The yeast strain expressing myc-tagged Rpt2p was grown at 30°C in YPD (yeast extract, peptone plus 2% dextrose) up to an OD_{600} of 1.0 prior to formaldehyde-based *in vivo* crosslinking. The ChIP assay was performed as described in the 'Materials and Methods' section. Primer-pairs ('Materials and Methods' section) located at the UAS, core promoter and ORF regions of *RPS5* were used for PCR analysis of the immunoprecipitated DNA samples. Immunoprecipitation was performed using a mouse monoclonal antibody against the c-myc epitope-tag (9E10; Santa Cruz Biotechnology, Inc.). The anti-HA (Santa Cruz Biotechnology, Inc.) was used as a non-specific antibody. A specific primer pair spanning an inactive region in the chromosome V (Chr-V) was used as a non-specific DNA control. The maximum ChIP signal was set to 100, and other signals were normalized with respect to the maximum ChIP signal. (**C**) The results of the (B) were presented as a fold increase of the ChIP signal of Rpt2p-myc relative to non-specific anti-HA antibody. (**D**) Analysis of recruitment of the Rpt6p component of the 19S base to *RPS5*. The yeast strain expressing myc-tagged Rpt6p was grown, cross-linked and immunoprecipitated as in (B). (**E**) The results of the (C) were presented as a fold increase of the ChIP signal of Rpt6p-myc relative to non-specific anti-HA antibody.

mutation (L231R) in the ATPase module of Rpt4p (38). Rpt4p is degraded at the non-permissive temperature in the *rpt4*-ts strain (data not shown), and is essential for the structural integrity of the 19S base (61). As a control, we analyzed the recruitment of TBP to the *ACT1* core promoter as its transcription is independent of the proteasome complex (62). As expected, the recruitment of TBP to the *ACT1* core promoter was not significantly altered in the *rpt4*-ts mutant strain (Figure 3C and D). These results strongly support the role of the 19S base in recruitment of TFIID to *RPS5*, and thus transcription of *RPS5* was significantly impaired in the *rpt4*-ts mutant strain (Figure 3E). Consistently, the recruitment of RNA polymerase II to the *RPS5* core promoter was also decreased in the *rpt4*-ts mutant strain (Supplementary Figure S3). Further, we demonstrate that the inhibition of the proteolytic function of the proteasome complex by MG132 did not alter the recruitment of TBP to the *RPS5* core promoter (Figure 3F). Likewise, the treatment of MG132 did not alter the recruitment of TBP to the proteasome-independent *ACT1* gene (Figure 3F). As a positive control, we show that the recruitment of TBP to the proteasome-dependent *INO1* gene (62) was significantly decreased following MG132 treatment (Figure 3F). Thus, our results revealed the non-proteolytic role of the proteasome in transcriptional initiation of the *RPS5* gene. Consistently, we find that 20S CP was not recruited to the *RPS5* promoter (Figure 2C and D).

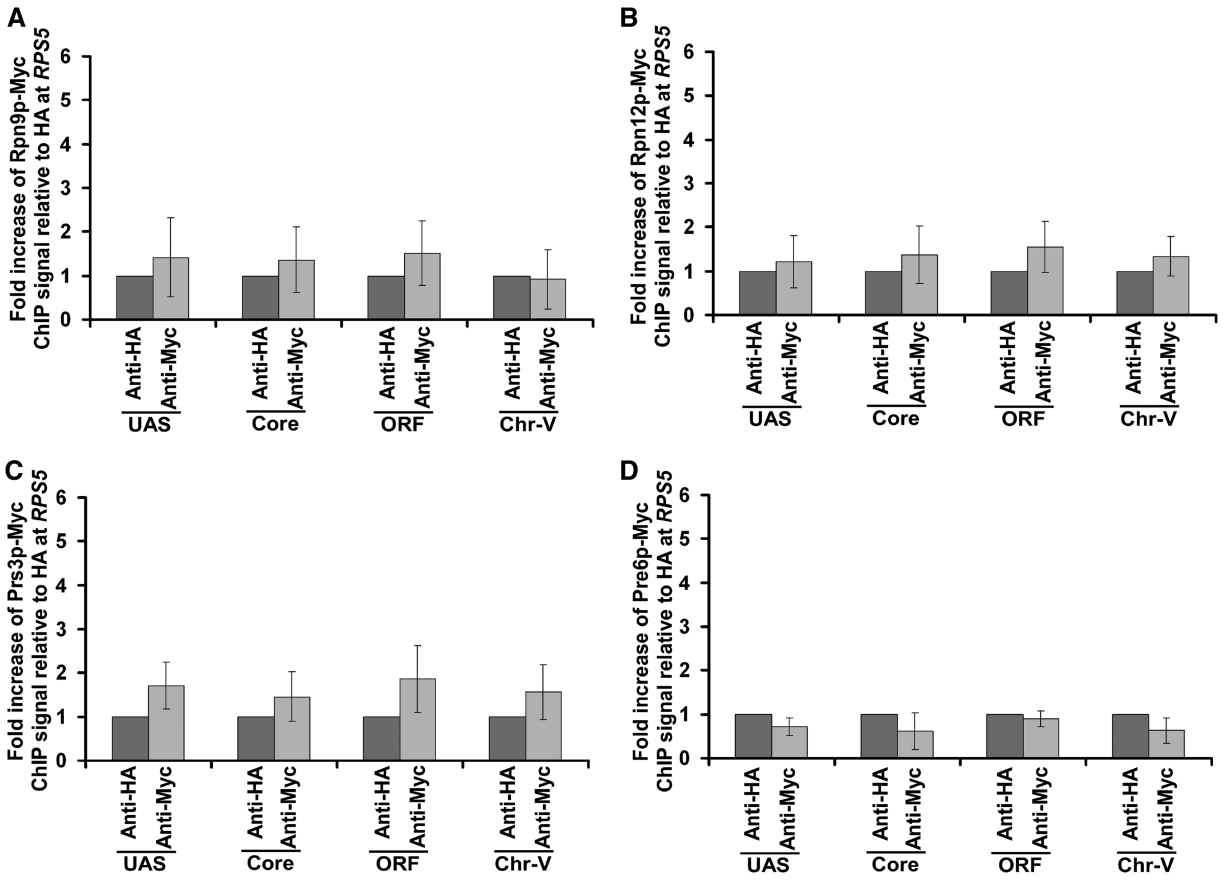

Figure 2. Analysis of recruitment of the 19S Lid or 20S CP to *RPS5*. (**A** and **B**) The 19S Lid is not recruited to *RPS5*. The yeast strains expressing myc-tagged Rpn9p and Rpn12p were grown, cross-linked and immunoprecipitated as in Figure 1B. (**C** and **D**) The 20S CP is not recruited to *RPS5*. The yeast strains expressing myc-tagged Prs3p and Pre6p were grown, cross-linked, and immunoprecipitated as in Figure 1B.

Recruitment of TFIID to the *RPS5* promoter is dependent on NuA4 HAT

To determine whether, like the 19S base, NuA4 HAT also facilitates the recruitment of TFIID, we analyzed the association of TBP and TAFs components (TAF1 and TAF12) of the TFIID complex with the *RPS5* core promoter in the wild-type and ts mutant strains of the Esa1p HAT component of NuA4. We find that the recruitment of the TFIID complex to the *RPS5* core promoter was significantly impaired in the *esa1*-ts mutant strain as compared with the wild-type equivalent (Figure 4A). Such an impaired recruitment of TFIID in the *esa1*-ts mutant significantly lowered the transcription of *RPS5* (Figure 4B). As a control, we have used *ACT1*, since its transcription is not dependent on NuA4 HAT (55). As expected, we find that transcription of *ACT1* was not altered in the *esa1*-ts mutant strain (Figure 4B). As a whole, our data demonstrate that NuA4 HAT enhances the recruitment of TFIID (and hence transcription) at the core promoter of the ribosomal protein gene, *RPS5*. Consistently, previous studies (55,56) have also implicated the role of NuA4 HAT in stimulation of transcription of the ribosomal protein genes. However, the mechanism of such transcriptional stimulation was not known. Here, we show that NuA4 HAT promotes transcription of the ribosomal protein gene, *RPS5*, by enhancing the recruitment of the TFIID complex. Further, NuA4 HAT has been shown to be targeted to the promoters of ribosomal protein genes by the activator Rap1p (55). Therefore, the recruitment of Rap1p is essential for association of TFIID with promoter. Indeed, previous studies (13,15,16) have demonstrated the role of Rap1p in recruitment of TFIID. Here, we demonstrate that like Rap1p, NuA4 HAT and 19S base are both required to facilitate the recruitment of TFIID for transcriptional initiation of *RPS5*.

The 19S base enhances the targeting of NuA4 HAT, but not Rap1p, to the *RPS5* promoter

So far, it is clear that Rap1p, NuA4 HAT and 19S base play crucial roles to recruit TFIID for transcriptional initiation of *RPS5*. However, the specific regulatory network of these factors at the *RPS5* promoter is not known. It has been demonstrated previously that the 19S base enhances the targeting of SAGA HAT co-activator to the activator Gal4p at the SAGA-dependent *GAL1* gene for transcriptional initiation of *GAL1* (35,36). Based on these results, we hypothesize that the 19S base might be similarly enhancing the targeting of NuA4 HAT to the activator Rap1p at the *RPS5* UAS to facilitate the recruitment of

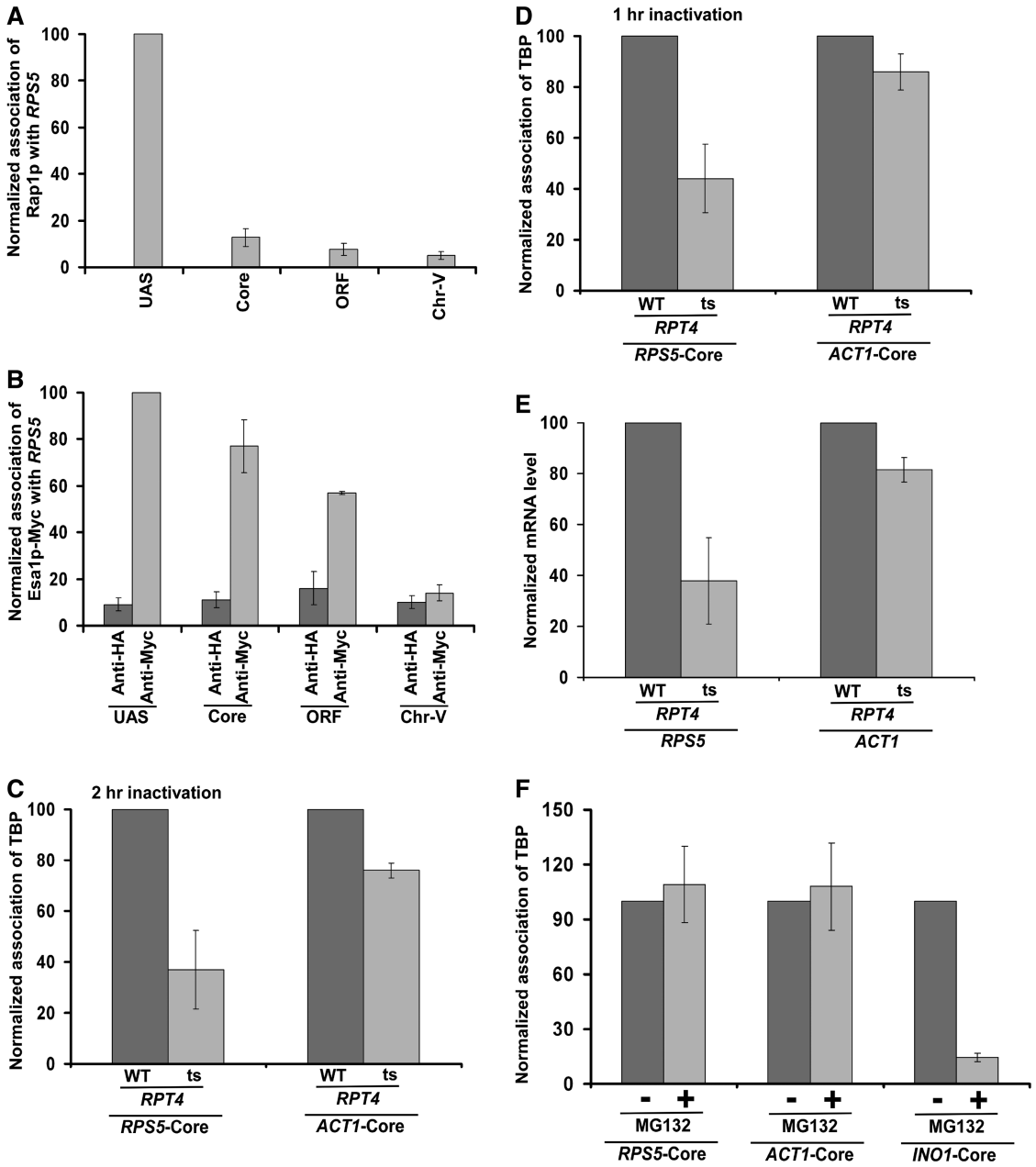

Figure 3. The 19S base stimulates the recruitment of TBP (and hence transcription) at the *RPS5* promoter. (**A**) Analysis of recruitment of Rap1p to *RPS5*. Immunoprecipitation was performed using an antibody against Rap1p (SC-6663; Santa Cruz Biotechnology, Inc.). (**B**) Analysis of recruitment of Esa1p to *RPS5*. The Esa1p component of NuA4 was tagged by myc-epitope at the C-terminal of its chromosomal locus for immunoprecipitation. (**C**) Analysis of the role of 19S base in recruitment of TBP to the *RPS5* core promoter. The wild-type and *rpt4*-ts mutant strains were grown in YPD at 23°C up to an OD_{600} of 0.85, and then switched to 37°C for 2 h prior to cross-linking. Immunoprecipitation was performed using an anti-TBP antibody against TBP (obtained from the Green laboratory; 13). (**D**) Similar to the (C). But, Rpt4p was inactivated for 1 h. (**E**) RT–PCR analysis of *RPS5* and *ACT1* transcripts in the *rpt4*-ts mutant and its isogenic wild-type equivalent following 1 h ts inactivation at the non-permissive temperature. (**F**) Treatment of yeast cells carrying null mutation of *PDR5* with MG132 (75 μM) for 2 h does not alter the recruitment of TBP to the *RPS5* core promoter. Yeast cells were grown in YPD at 30°C up to an OD_{600} of 0.7, and then treated with MG132 for 2 h prior to cross-linking.

TFIID for transcriptional initiation. To test this possibility, we analyzed the recruitment of Rap1p and NuA4 HAT to the *RPS5* UAS in the wild-type and ts mutant strains of the Rpt4p ATPase component of the 19S base. If the 19S base increases the targeting of NuA4 HAT to Rap1p at the *RPS5* UAS, we would observe a significant decrease in the recruitment of NuA4 HAT, but not Rap1p, at the *RPS5* UAS in the *rpt4*-ts mutant strain as compared with the wild-type equivalent. Indeed, we find that Rap1p recruitment to the *RPS5* UAS was not altered in the *rpt4*-ts mutant strain (Figure 5). However, the recruitment of NuA4 HAT (Esa1p-Myc) to the *RPS5* UAS was significantly impaired in the *rpt4*-ts mutant strain (Figure 5). These results strongly support that the 19S

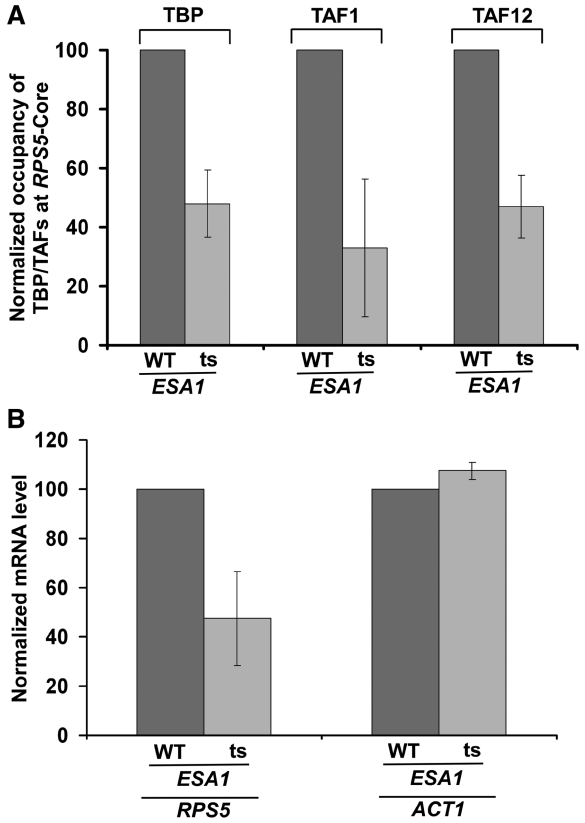

Figure 4. NuA4 HAT is required for recruitment of the TFIID complex to the *RPS5* promoter. (**A**) Analysis of the recruitment of TBP and TAFs components of the TFIID complex to the *RPS5* core promoter in the *esa1*-ts mutant and its isogenic wild-type equivalent. Yeast cells were grown as in Figure 3C, but Esa1p was inactivated for 1 h at the non-permissive temperature. Immunoprecipitation was performed using anti-TBP, anti-TAF1 and anti-TAF12 antibodies against TBP, TAF1 and TAF12 (obtained from the Green laboratory; 13). (**B**) RT–PCR analysis of *RPS5* and *ACT1* transcripts in the *esa1*-ts mutant and its isogenic wild-type equivalent following 1 h ts inactivation at the non-permissive temperature.

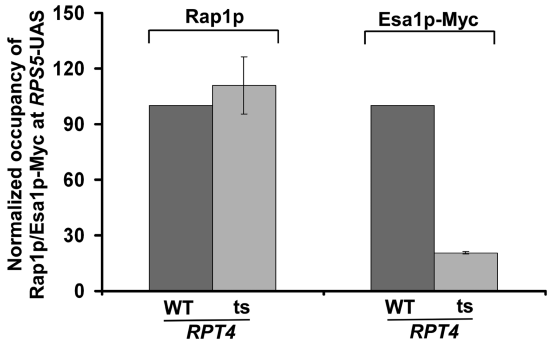

Figure 5. Analysis of recruitment of Rap1p and NuA4 HAT (Esa1p-myc) to the *RPS5* promoter in the wild-type and *rpt4*-ts mutant strains following ts inactivation of Rpt4p at 37°C for 1 h. Immunoprecipitations were performed as described in Figures 3A and B.

base facilitates the targeting of NuA4 HAT to Rap1p at the *RPS5* UAS. This observation is remarkably consistent with previous studies (35,36) that demonstrated the role of the 19S base in enhancing the targeting of SAGA HAT to the activator Gal4p at the SAGA-dependent *GAL1* gene. Although previous studies (35,36) have shown the role of the 19S base in targeting SAGA to the SAGA-dependent gene, the role of the 19S base at the promoter of the TFIID-dependent gene remained unknown. This study demonstrates for the first time the role of the 19S base in enhanced targeting of NuA4 HAT to the activator of a TFIID-dependent ribosomal protein gene, *RPS5* (Figure 5), and subsequently facilitates the recruitment of the TFIID complex for transcriptional initiation (Figure 4A and B).

Since the 19S base enhances the targeting of NuA4, it would promote histone H4 acetylation at the *RPS5* promoter via NuA4 HAT. To test this, we analyzed histone H4 acetylation at the *RPS5* promoter in the *rpt4*-ts mutant and its isogenic wild-type equivalent. We find that histone H4 acetylation at the *RPS5* core promoter was decreased in the *rpt4*-ts mutant strain as compared to the wild-type equivalent (Supplementary Figure S4A and S4B). Similarly, histone H4 acetylation was impaired in the *esa1*-ts mutant strain (Supplementary Figure S4B), consistent with previous studies (55). Thus, the 19S base regulates histone H4 acetylation by modulating the targeting of NuA4 HAT to the promoter.

Previous studies have demonstrated that NuA4 HAT is recruited to the promoters of the ribosomal protein genes in a TOR-dependent manner (24). We thus asked whether the recruitment of the 19S base to the *RPS5* promoter is also regulated by the TOR pathway. To address this question, we analyzed the recruitment of the 19S base and Rap1p to the *RPS5* UAS in the presence and absence of rapamycin. Rapamycin inhibits TOR-kinase activity, and thus impairs TOR pathway. We find that the recruitment of the 19S base (Rpt2p and Rpt6p), but not Rap1p, was significantly decreased in the presence of rapamycin (Supplementary Figure S5A and Supplementary S5B). Thus, the 19S base is recruited to the *RPS5* UAS in a TOR-dependent manner. Similarly, the recruitment of NuA4 HAT to the *RPS5* UAS was also decreased in the presence of rapamycin (Supplementary Figure S5B), consistent with previous studies (24).

The 19S base enhances the targeting of NuA4 HAT, but not Rap1p, to the *RPL2B* and *RPS11B* promoters to facilitate the recruitment of TFIID for stimulated transcription

Although the above results implicated an important role of the 19S base in transcriptional stimulation of *RPS5*, it is not known whether other ribosomal protein genes are also similarly regulated by the 19S base. In view of this, we next analyzed the role of the 19S base in recruitment of TBP to the core promoters of two other ribosomal protein genes such as *RPL2B* and *RPS11B* in the *rpt4*-ts mutant strain and its isogenic wild-type equivalent. Interestingly, we find that the recruitment of TBP to the core promoters of these genes was significantly impaired in the *rpt4*-ts mutant strain (Figure 6A). Consistently, we find that the transcription of these genes was also significantly decreased in the *rpt4*-ts mutant strain (Figure 6B). Thus,

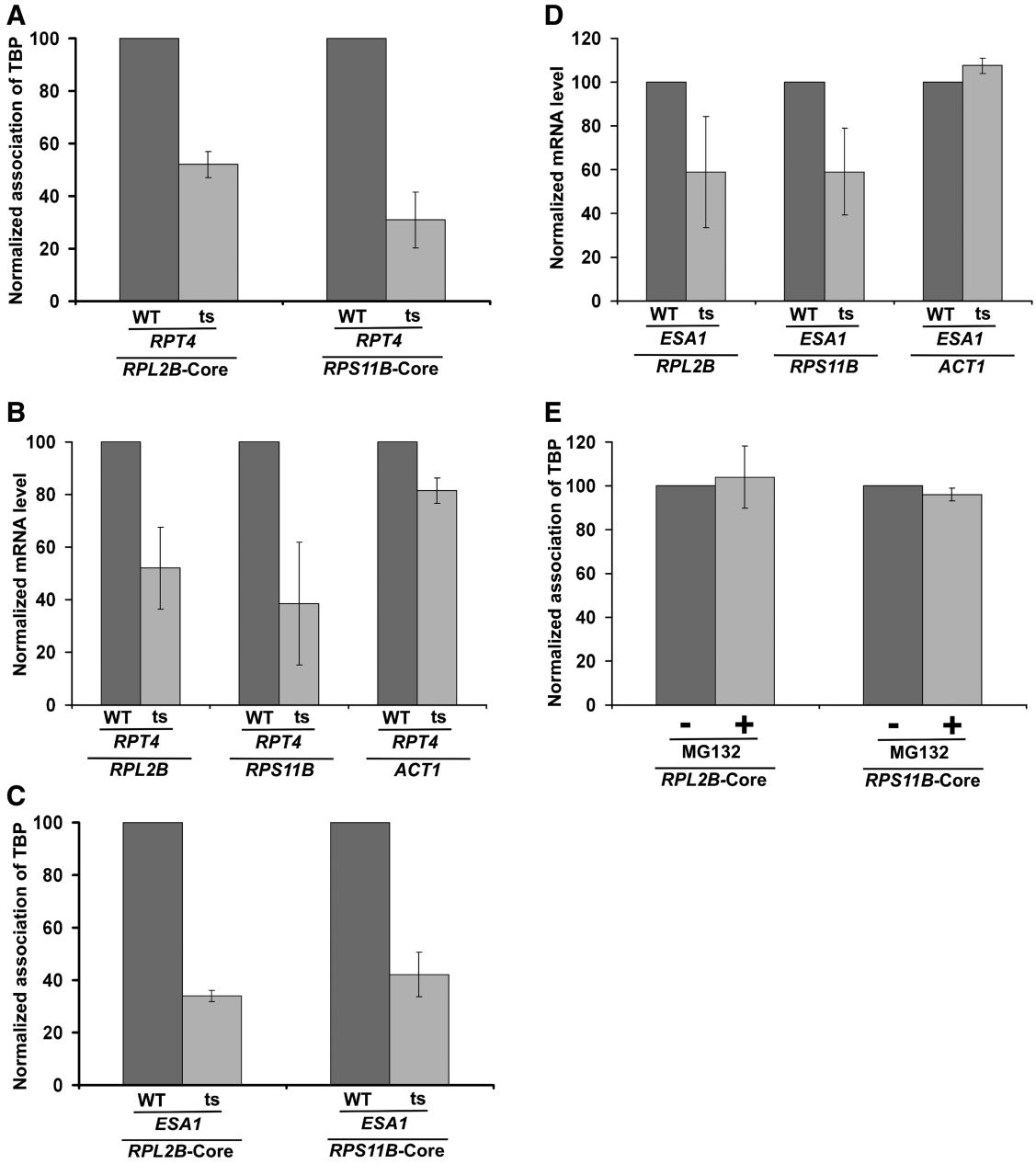

Figure 6. Both 19S base and NuA4 HAT promote the recruitment of TBP (and hence transcription) at the *RPL2B* and *RPS11B* core promoters. (**A**) Analysis of recruitment of TBP to the *RPL2B* and *RPS11B* core promoters in the *rpt4*-ts mutant and its isogenic wild-type equivalent following 1 h ts inactivation at the non-permissive temperature. (**B**) RT–PCR analysis of *RPL2B*, *RPS11B* and *ACT1* transcripts in the *rpt4*-ts mutant and its isogenic wild-type equivalent following 1 h ts inactivation at the non-permissive temperature. (**C**) Analysis of recruitment of TBP to the *RPL2B* and *RPS11B* core promoters in the *esa1*-ts mutant and its isogenic wild-type equivalent following ts inactivation for 1 h. (**D**) RT–PCR analysis of *RPL2B*, *RPS11B* and *ACT1* transcripts in the *esa1*-ts mutant and its isogenic wild-type equivalent following 1 h ts inactivation at the non-permissive temperature. (**E**) Treatment of yeast cells carrying null mutation of *PDR5* with MG132 (75 μM) for 2 h does not alter recruitment of TBP to the *RPL2B* and *RPS11B* core promoters. Yeast cells were grown and cross-linked as in Figure 3F.

similar to the results obtained at *RPS5*, we find that the 19S base facilitates the recruitment of TBP (and hence TFIID) to the core promoters of *RPL2B* and *RPS11B* to promote transcriptional initiation.

Next, we asked whether recruitment of TBP to the core promoters of *RPL2B* and *RPS11B* is also facilitated by NuA4 HAT. In this direction, we analyzed the recruitment of TBP to the core promoters of these genes in the *esa1*-ts mutant strain and its isogenic wild-type equivalent. We find that like the results at *RPS5*, the recruitment of TBP to the core promoters of *RPL2B* and *RPS11B* was also significantly decreased in the *esa1*-ts mutant strain as compared with the wild-type equivalent (Figure 6C). Since the recruitment of TBP is directly correlated with transcription, the *RPL2B* and *RPS11B* transcription would be decreased in the *esa1*-ts mutant strain. Indeed, we find that the transcription of these genes was also reduced in the *esa1*-ts mutant strain (Figure 6D).

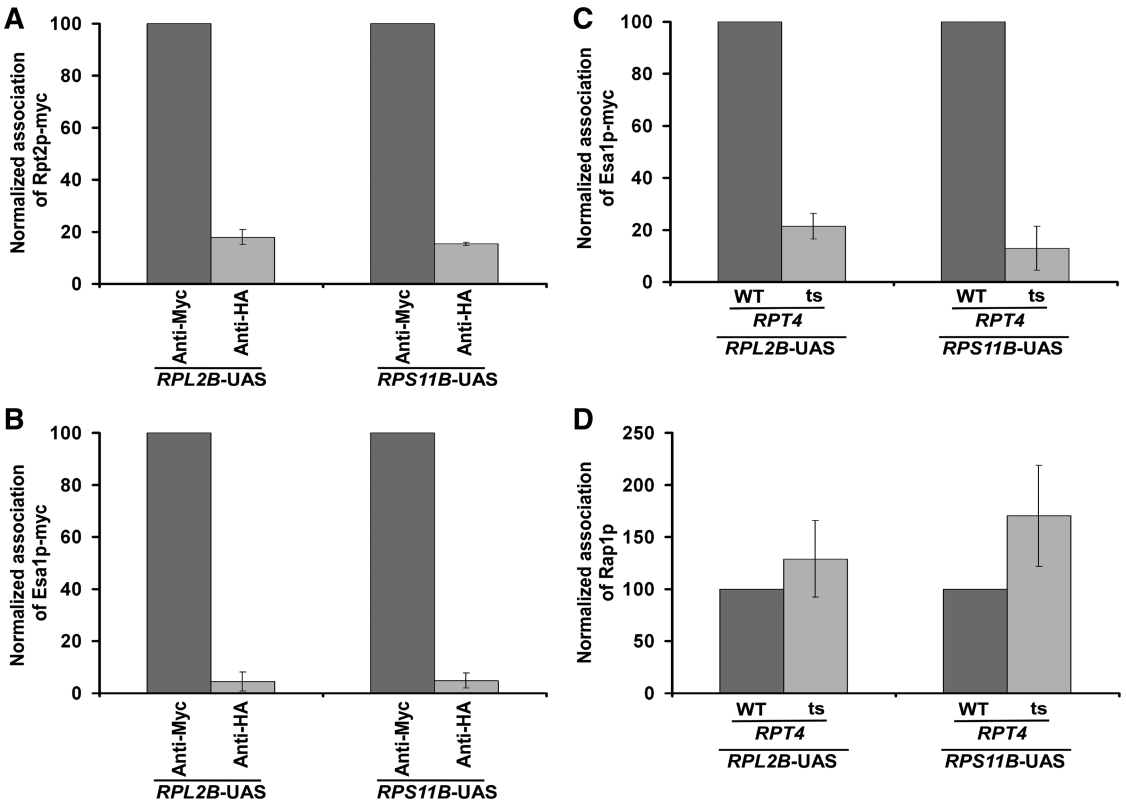

Figure 7. The 19S base enhances the targeting of NuA4 HAT, but not activator Rap1p, to the promoters of *RPL2B* and *RPS11B*. (**A**) Analysis of recruitment of the 19S base (Rpt2p-myc) to the promoters of *RPL2B* and *RPS11B*. Yeast cells were grown, cross-linked and immunoprecipitated as in Figure 1B. (**B**) Analysis of recruitment of NuA4 HAT (Esa1p-myc) to the promoters of *RPL2B* and *RPS11B*. Yeast cells were grown, cross-linked and immunoprecipitated as in Figure 3B. (**C**) Analysis of recruitment of NuA4 HAT (Esa1p-myc) to the promoters of *RPL2B* and *RPS11B* in the *rpt4*-ts mutant and its wild-type equivalent following 1 h ts inactivation at the non-permissive temperature. (**D**) Analysis of recruitment of Rap1p to the promoters of *RPL2B* and *RPS11B* in the *rpt4*-ts mutant and its wild-type equivalent following 1 h ts inactivation at the non-permissive temperature.

Thus, similar to the results obtained at *RPS5*, we find that NuA4 HAT facilitates the recruitment of TBP (and hence TFIID) to the core promoters of the *RPL2B* and *RPS11B* genes to promote transcription. Further, we demonstrate that the recruitment of TBP to the core promoters of these genes was not altered in the presence of MG132 (Figure 6E). Therefore, the proteolytic function of the proteasome is dispensable for recruitment of TBP to these ribosomal protein genes.

We find that both NuA4 HAT and 19S base enhance the recruitment of TFIID to the core promoters of *RPL2B* and *RPS11B*. Thus, similar to the results at *RPS5*, it is likely that the 19S base is recruited to the *RPL2B* and *RPS11B* promoters, and promotes the association of NuA4 HAT for facilitating the recruitment of TFIID to stimulate transcriptional initiation. To test this possibility we next analyzed the recruitment of the 19S base at the *RPL2B* and *RPS11B* promoters, and subsequently determined the role of the 19S base in recruitment of NuA4 HAT. We find that the 19S base was recruited to the *RPL2B* and *RPS11B* promoters (Figure 7A), and facilitated the association of NuA4 HAT (Esa1p-Myc) (Figure 7B and C). However, the recruitment of Rap1p to these genes was not altered in the *rpt4*-ts mutant strain (Figure 7D). Thus, the 19 S base specifically promotes the recruitment of NuA4 HAT, but not activator Rap1p, to the *RPL2B* and *RPS11B* promoters. Subsequently, NuA4 HAT stimulates the recruitment of TFIID to the core promoter (Figure 6C), hence facilitating transcription (Figure 6D). Taken together, our data strongly support that the 19 S base enhances the targeting of NuA4 HAT to the activator Rap1p at *RPL2B* and *RPS11B* to promote the recruitment of TFIID for stimulated transcription.

DISCUSSION

Here, we demonstrate that the 19S base is recruited to the promoter of the ribosomal protein gene independently of the lid and 20S CP. Such a recruitment of the 19S base enhances the targeting of NuA4 HAT to the promoters of ribosomal protein genes. Subsequently, NuA4 HAT facilitates the recruitment of the TFIID complex for stimulation of the transcriptional initiation of the ribosomal protein genes. Collectively, these results demonstrate an important role of the 19S base in establishing a specific regulatory network at the promoters of ribosomal protein genes for promoting transcriptional initiation, hence significantly advancing our current understanding of the regulation of ribosomal protein gene activation *in vivo*.

NuA4 is a multi-subunit protein complex in yeast. The essential HAT subunit (Esa1p) of NuA4 and its human and *Drosophila* homologues [Tip60 (Tat-interacting protein, 60 kDa) and MOF (males absent on the first), respectively] belong to a conserved family of acetylases that are involved in acetylation of histone H4. Tip60 is a catalytic subunit of human TIP60 complex that augments Tat-mediated transcription (63), and is also involved in DNA repair and apoptosis (64). MOF, a catalytic subunit of the MSL complex, is required for histone H4 hyperacetylation that is associated with an increase in X-linked transcription in *Drosophila* males (65,66). Further, MOF has also been shown to facilitate transcription in yeast when artificially targeted to a promoter (66). Human ortholog of *Drosophila* MOF is involved in histone H4 acetylation at lysine 16 (67–70). In humans, MOF is associated with transcriptional activation in coordination with histone H3 lysine 4 methyltransferase (67). Further, Pandita and colleagues (68,70) have implicated human MOF in DNA repair. They have demonstrated that MOF interacts with the ATM (ataxia-telangiectasia-mutated) protein, and acetylates histone H4 following ionizing radiation (68,70). Such modification plays an important role in cellular DNA damage response and repair (70). MOF has also been implicated in mammalian embryogenesis (69). Like Tip60 and MOF, NuA4 HAT has been shown to promote transcription (55, 56). Further, previous studies have demonstrated the recruitment of NuA4 HAT to the promoters of ribosomal protein genes in an activator-dependent manner (55). Consistently, NuA4 HAT has been shown to be the target of transcriptional activators (6,56,60). However, it is not known how NuA4 HAT promotes transcription of the ribosomal protein genes. Here, we show that NuA4 HAT is recruited to the promoters of ribosomal protein genes (Figures 3B and 7B). Subsequently, it facilitates the recruitment of the TFIID complex to the promoter (Figures 4A and 6C). TFIID nucleates the assembly of the GTFs at the promoter to form the PIC for transcriptional initiation (1, 34). Thus, an increased association of TFIID by NuA4 HAT enhances transcriptional initiation of the ribosomal protein genes (Figures 4B and 6D). Therefore, our results demonstrate that NuA4 HAT promotes transcriptional initiation by facilitating the recruitment of the TFIID complex at the promoters of ribosomal protein genes.

Although previous studies (13,15,16) have implicated TFIID as the target of the activator Rap1p, our current results demonstrate that Rap1p cannot efficiently target or recruit TFIID to the ribosomal protein genes when the HAT activity of NuA4 is impaired in the *esa1*-ts mutant strain (Figures 4A and 6C). Further, previous studies have demonstrated a high level of histone H4 acetylation at the promoters of the ribosomal protein genes, *RPS5*, *RPL2B* and *RPS11B*, in the presence of NuA4 HAT (55). Thus, it is likely that the HAT activity of NuA4 or histone H4 acetylation is essential to recruit TFIID for transcriptional initiation. Such a HAT activity is not essential for recruitment of the activator Rap1p (data not shown). These results support a model where NuA4 HAT is targeted by the activator Rap1p, which then acetylates histone H4 at the promoters of ribosomal protein genes to facilitate the recruitment of TFIID for stimulation of transcriptional initiation.

Intriguingly, we also demonstrate here that like NuA4 HAT, the 19S base promotes the association of TFIID with the promoters of the ribosomal protein genes (Figures 3C, D and 6A). Consistently, we find that the transcription of ribosomal protein genes is significantly impaired in the ts mutant of the Rpt4p ATPase subunit of the 19S base (Figures 3E and 6B). Thus, we show here for the first time that 19S proteasome subcomplex promotes the recruitment of TFIID to the promoters of the ribosomal protein genes to initiate transcription. Such function of the 19S proteasome subcomplex is mediated via an enhanced targeting of NuA4 HAT as discussed below. Further, we demonstrate that the proteolytic function of the proteasome is dispensable for recruitment of TFIID to the promoters of these genes (Figures 3F and 6E). Similarly, previous studies have demonstrated the role of 19S proteasome subcomplex in mammalian transcriptional regulation in a proteolysis-independent manner (71,72). Several other studies in yeast have also implicated the non-proteolytic role of the proteasome in transcriptional regulation (1,34).

We show here that both NuA4 and 19S base facilitate the recruitment of TFIID to the promoters of the ribosomal protein genes for transcriptional stimulation. NuA4 HAT promotes the recruitment of TFIID, presumably via histone H4 acetylation as mentioned above and discussed below. However, it is not clear how the 19S base enhances the recruitment of TFIID. We find here that the 19S base increases the recruitment of NuA4 HAT, but not the activator Rap1p, to the promoters of the ribosomal protein genes (Figures 5, 7C and D). These observations support that the 19S base promotes the targeting of NuA4 HAT to the activator Rap1p *in vivo*. Analogous to this observation, previous studies have demonstrated an increased targeting of SAGA HAT to the activator Gal4p at the SAGA-dependent *GAL1* gene (35,36). Thus, like previous studies at the SAGA-dependent gene (35,36), we demonstrate here a new role of the 19S base in targeting NuA4 HAT to the promoters of the TFIID-dependent ribosomal protein genes for stimulation of transcriptional initiation. How does the 19S base promote the targeting of NuA4 HAT to the activator Rap1p? As mentioned above, the 19S base has a molecular chaperonin activity (30). Presumably, the molecular chaperonin activity of the 19S base is playing an important role to increase the targeting of NuA4 HAT to the activator Rap1p, possibly by enhancing proper folding or assembly of NuA4 components. However, such a model remains to be further elucidated biochemically. Nonetheless, this study uncovered an important role of the 19S base in facilitating the targeting of NuA4 HAT to the ribosomal protein genes in promoting transcription *in vivo*. Since NuA4 HAT is involved in histone H4 acetylation at the *RPS5*, *RPL2B* and *RPS11B* genes (55), our study thus implicates the role of the 19S base in regulation of histone H4 acetylation via NuA4 HAT. Indeed, we find that the 19S base promotes histone H4 acetylation (Supplementary

Figure S4A and S4B). Similarly, previous studies have demonstrated the function of the 19S proteasome subcomplex in promoting histone H3 acetylation by facilitating the recruitment of SAGA HAT in yeast (35). Likewise, acetylation of histones H3 and H4 in human has also been shown to be regulated by the 19S proteasome subcomplex (71,72).

Importantly, our data implicate the role of NuA4 HAT or histone H4 acetylation in promoting recruitment of TFIID. However, NuA4 HAT is not solely required for association of TFIID, since a dramatically impaired recruitment of NuA4 HAT to the ribosomal protein genes (~6-fold) in the *rpt4*-ts strain (Figures 5 and 7C) decreased the occupancy of TFIID only by ~2.5-fold (Figures 3D and 6A). Further, even though the recruitment of NuA4 HAT to the ribosomal protein genes was decreased by ~6-fold in the *rpt4*-ts mutant strain (Figures 5 and 7C), transcription was impaired only by ~2.5-fold (Figures 3E and 6B), consistent with ~2.5-fold reduction in the recruitment of TFIID in the *rpt4*-ts mutant strain (Figures 3D and 6A). Together, these results implicate that NuA4 HAT or histone H4 acetylation is not solely required for recruitment of TFIID, but rather facilitates TFIID recruitment (and hence transcription). Such stimulation of TFIID recruitment or transcription is likely to have a great effect in cellular differentiation and development in higher eukaryotes, since temporal and spatial regulation of gene expression is tightly correlated with cell fate.

The fact that significant amount of TFIID is recruited to the promoters of ribosomal protein genes in the absence of NuA4 HAT or histone H4 acetylation (Figures 4A and 6C), supports that Rap1p targets TAFs to recruit TFIID for transcriptional initiation of the ribosomal protein genes, consistent with previous studies (13,15,16,53,54). However, the presence of NuA4 HAT enhances the recruitment of TFIID. How does NuA4 HAT or histone H4 acetylation enhance TFIID recruitment to the promoters of the ribosomal protein genes? Previous studies have implicated the role of histone H4 acetylation in recruitment of bromodomain protein, Bdf1p (Bromodomain factor 1) (73–78). Bdf1p interacts with acetylated-histone H4, and thus, it is functionally connected to NuA4 HAT *in vivo* (73–78). Moreover, Bdf1p interacts with TFIID (74,79). Therefore, NuA4 HAT or histone H4 acetylation appears to enhance the recruitment of TFIID to the ribosomal protein genes via Bdf1p. Further, Bdf1p interacts with SWR1, an ATP-dependent chromatin remodeling complex that is involved in exchanging histone H2A–H2B dimer by histone H2A.Z–H2B dimer during transcriptional activation (80–84). Using the energy of ATP hydrolysis through the Swr1p subunit, the SWR1 complex replaces histone H2A–H2B dimer by histone H2A.Z–H2B dimer during transcriptional induction when nucleosomes are disassembled to allow the passage of RNA polymerase II followed by reassembly (74). Some evidence suggests that such incorporation of H2A.Z might promote disassembly of nucleosome during transcriptional initiation (85,86), and thus, they are largely promoter specific (80,85–90). Further, several studies suggest that H2A.Z-containing nucleosomes form a special chromatin structure, and this structure pre-sets nucleosomes for dismantling upon transcriptional activation (80,86,91,92). Therefore, deposition of histone H2A.Z plays an important role in transcriptional initiation. Bdf1p-mediated recruitment of SWR1 complex is essential for histone H2A.Z deposition. Consistently, histone H2A.Z occupancy has been shown to be dependent on Bdf1p and SWR1 complex in global genome-wide analysis (86). Further, previous studies have linked histone H4 acetylation with the occupancy of Bdf1 and H2A.Z at specific loci (88,93,94). Thus, NuA4 HAT or histone H4 acetylation plays a crucial role in H2A.Z deposition. Moreover, previous studies have also demonstrated the role of NuA4 HAT in acetylation of histone H2A.Z of deposited histone H2A.Z–H2B dimer (93,95,96). This acetylation of histone H2A.Z is believed to favor the specific destabilization of H2A.Z-containing nucleosomes on promoters during gene activation (93,95–97). Collectively, these studies implicate that NuA4 HAT or histone H4 acetylation might be facilitating the recruitment of TFIID (and hence transcription) at the ribosomal protein genes in similar manner, which remain to be elucidated to better understand the role of the NuA4 HAT or histone H4 acetylation in ribosomal-protein gene activation.

In summary, our results define for the first time the role of the 19S proteasome subcomplex in promoting the recruitment of NuA4 HAT that subsequently facilitates the association of TFIID for stimulation of transcriptional initiation of the ribosomal protein genes, thus shedding much light on the regulation of ribosomal-protein gene expression *in vivo*. Since ribosomal protein genes, NuA4 HAT, 19S base and TFIID are highly conserved from yeast to humans, similar regulatory mechanism of ribosomal-protein gene expression is likely to exist in humans. Further, as mentioned above, ribosomal-protein gene expression is strongly correlated with protein biosynthesis, and hence cellular growth, differentiation and development. Therefore, our study points to the role of the 19S proteasome subcomplex in controlling cellular growth, muscle and cardiac development through the regulation of ribosomal-protein gene expression in higher eukaryotes.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR online: Supplementary Figures 1–5 and Supplementary Reference [98].

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