The 26 S proteasome complex that comprises the 20 S core and 19 S regulatory (with six ATPases) particles is engaged in an ATP-dependent degradation of a variety of key regulatory proteins and, thus, controls important cellular processes. Interestingly, several recent studies have implicated the 19 S regulatory particle in controlling eukaryotic transcriptional initiation or activation independently of the 20 S core particle. However, the mechanism of action of the 19 S proteasome subcomplex in regulation of eukaryotic transcriptional activation is not clearly understood in vivo. Here, using a chromatin immunoprecipitation assay in conjunction with mutational and transcriptional analyses in Saccharomyces cerevisiae, we show that the 19 S proteasomal subcomplex establishes a specific protein interaction network at the upstream activating sequence of the promoter. Such an interaction network is essential for formation of the preinitiation complex at the core promoter to initiate transcription. Furthermore, we demonstrate that the formation of the transcription complex assembly at the promoter is dependent on 19 S ATPase activity. Intriguingly, 19 S ATPases appear to cross-talk for stimulation of the assembly of transcription factors at the promoter. Together, these results provide significant insights as to how the 19 S proteasome subcomplex regulates the formation of the active transcription complex assembly (and, hence, transcriptional initiation) at the promoter in vivo.

In eukaryotes, transcription is mechanistically divided into different steps such as preinitiation complex (PIC) formation and initiation, elongation, and termination. Transcriptional initiation is an important regulatory step of gene expression, which is greatly stimulated by the gene-specific activators whose recognition sites are present at the upstream region of the promoter. A variety of studies (1–5) indicates that activator interacts directly with one or more components of the transcription machinery to stimulate the assembly of general transcription factors such as TFIID, TFIIH, TFIIA, TFIIE, TFIIF, and TFIIF as well as RNA polymerase II holoenzyme for formation of the PIC at the core promoter to initiate transcription. Thus, a large number of proteins must interact with each other and with the promoter DNA either directly or indirectly during transcriptional initiation.

A growing number of recent studies (6–28) have implicated the 26 S proteasome complex in controlling and orchestrating the interactions of the transcriptional initiation factors and their localization and abundance in regulating transcriptional initiation or activation. The 26 S proteasome is a highly versatile protein degradation machine with molecular chaperonin activity and consists of a 20 S proteolytic core particle (CP) and a 19 S regulatory particle (RP). The 20 S CP has a cylinder-like structure composed of a stack of two α and two β rings, whereas the 19 S RP comprises a “lid” of eight non-ATPases and a “base” of six ATPases (Rpt1–Rpt6) and three non-ATPases (29–35). 19 S ATPases provide the molecular chaperonin activity to the 19 S RP (36). Furthermore, 19 S ATPase activity is essential for the assembly of the 26 S proteasome complex (34) and participates in the degradation of the proteins marked by a chain of more than four Lys-48-linked ubiquitin molecules (37–39). The lid of the 26 S proteasome associates with the polyubiquitin chain, and subsequently the base unfolds the substrate protein in an ATP-dependent manner, finally translocating it to the central chamber of the 20 S CP for proteolysis (31, 32, 40–49). Through this mechanism the 26 S proteasome complex regulates the functions and fates of many transcription factors in a highly responsive and coordinated manner to control many important biological processes (50, 51). In fact, the temperature-sensitive (ts) inactivation of either the 19 S RP or 20 S CP in yeast alters the levels of ~70% of the genomic transcript at least by 2-fold (52).

Interestingly, the genome-wide location analysis in yeast revealed that several hundred genes are associated with either the 19 S RP or 20 S CP (52, 53). Consistent with this observation, Sulahian et al. (20) have shown that the transcription of several stress-responsive genes such as HSP26, HSP104, and GDP1 is regulated by the 19 S RP independently of the proteolytic function of the 20 S CP. On the other hand, expression of the ribosomal protein genes is dependent on the 20 S CP (52, 54, 55). Thus, the 26 S proteasome plays distinct functions in regulating
transcription at different sets of genes in proteolysis-dependent as well as proteolysis-independent manners. Although the mechanisms for the proteolytic role of the proteasome in transcription are relatively well established (28), how the 19 S RP regulates eukaryotic transcriptional activation independently of the 20 S CP is not clearly understood. Recent biochemical studies in yeast (13, 23) have implicated the non-proteolytic role of the 19 S RP in regulation of the activator-coactivator/promoter interactions (and hence transcriptional activation). However, the mechanism of action of the 19 S RP in regulation of eukaryotic transcriptional activation remains unknown in vivo. Here, using a formaldehyde-based in vivo cross-linking and chromatin immunoprecipitation (ChIP) assay in conjunction with mutational and transcriptional analyses, we show that the 19 S RP is essential in establishing a specific protein interaction network at the upstream activating sequence (UAS) of the active gene promoter in yeast (Saccharomyces cerevisiae). Such an interaction network is essential for formation of the PIC assembly at the core promoter to initiate transcription. Furthermore, our study reveals that the 19 S RP does not merely behave as a physical adaptor to establish the specific protein interaction network at the promoter. Rather, its ATPase activity is essential for formation of the transcriptional initiation complex. Interestingly, we also find that 19 S ATPases appear to cross-talk to facilitate the formation of the transcription complex assembly at the promoter in vivo. Collectively, our data demonstrate that the 19 S proteasomal ATPases function in a cooperative manner to establish a specific interaction network of the transcription factors at the promoter for efficient transcriptional initiation in vivo, thus significantly advancing our fundamental knowledge of the regulatory mechanisms of eukaryotic transcriptional activation by the 19 S RP in vivo.

**EXPERIMENTAL PROCEDURES**

*Plasmids*—The plasmid pFA6a-13Myc-KanMX6 (56) was used for genomic Myc epitope tagging of the proteins of interest. The plasmid pRS416 was used in the PCR-based gene disruption.

*Yeast Strains and Media*—Yeast (S. cerevisiae) strains harboring the ts mutation in SPIT5 and its isogenic wild type equivalent were obtained from Cormack and Struhl, Harvard Medical School (57). The srb4-ts and wild type strains were obtained from Thompson and Young, MIT (58). The rpt1-K256R (DY98), rpt5-K219R (DY93), rpt4-K297R (DY219), rpt5-K228R, SMY6 (Srb4p-Myc in rpt5-K228R), SMY7 (Srb4p-Myc in rpt5-K228R), and PBY8 (Srb4p-Myc in the wild type strain) were generated by inserting multiple Myc epitope tags at the C-terminal tail of Srb4p in rpt1-K256R, rpt5-K228R, rpt6-K195R, and wild type strains, respectively.

For the ChIP studies at the GAL genes in the wild type and deletion and point mutant strains, yeast cells were first grown in YPR (yeast extract-peptone plus 2% raffinose) to an $A_{600}$ of 0.9 and then transferred to YPG (yeast extract-peptone plus 2% galactose) for 90 min of induction at 30 °C before formaldehyde-based in vivo cross-linking. However, spt15-ts, srb4-ts, and isogenic wild type strains were grown in YPG at 23 °C to an $A_{600}$ of 0.85 and then transferred to 37 °C for 1 h before cross-linking. Similar growth conditions were used for ChIP studies at the ACT1 promoter.

*ChIP Assay*—The ChIP assay was performed as described previously (62–65). Briefly, yeast cells were treated with 1% formaldehyde, collected, and resuspended in lysis buffer. After sonication, cell lysates (400 µl of lystate from 50 ml of yeast culture) were precleared by centrifugation, and then 100 µl of lystate was used for each immunoprecipitation. Immunoprecipitated protein-DNA complexes were treated with proteinase K, the cross-links were reversed, and then DNA was purified. Immunoprecipitated DNA was dissolved in 20 µl of TE 8.0 and then transferred to 37 °C for 1 h before cross-linking. Serial dilutions of the input and immunoprecipitated DNA were detected by autoradiography after separation on a 6% polyacrylamide gel. As a control, "input" DNA was isolated from 5 µl of lystate without going through the immunoprecipitation. Immunoprecipitated-protein-DNA complexes were treated with proteinase K, the cross-links were reversed, and then DNA was purified. Immunoprecipitated DNA was dissolved in 20 µl of 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-32P]dATP (2.5 µCi for each 25-µl reaction), and PCR products were detected by autoradiography after separation on a 6% polyacrylamide gel. As a control, "input" DNA was isolated from 5 µl of lystate without going through the immunoprecipitation steps and was suspended in 100 µl of TE 8.0. To compare the PCR signal arising from the immunoprecipitated DNA with that of the input DNA, 1 µl of input DNA was used in the PCR analysis. Serial dilutions of the input and immunoprecipitated (IP) DNAs were used to assess the linear range of PCR amplification as described previously (66). The PCR data presented in this article are within the linear range of PCR analysis.

For analysis of recruitment of the proteasome components, we modified the above ChIP protocol as follows (65). 800 µl of lystate was prepared from 100 ml of yeast culture. 400 µl of lystate was used for each immunoprecipitation (using 10 µl of anti-hemagglutinin or 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 10 µl of TE 8.0 of which 1 µl was used in PCR analysis. In parallel, the PCR for input DNA was performed using 1 µl of DNA that was prepared by dissolving purified DNA from 5 µl of lystate in 100 µl of TE 8.0.
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Primer pairs used for PCR analysis were as follows: GAL1(UAS), 5′-CGCTTACTGCTATTATATGG-3′ and 5′-TTGTTCGGAGCAGTTGGGCGC-3′; GAL1(Core), 5′-TAGGATGATATATGATGTTTATTTGAC-3′ and 5′-GAAATTTGGAAATGATAGTGGTTTA-3′; GAL7(Core), 5′-CATACTGTAGTTTGGCTA-3′ and 5′-TTGAGTGCCTGCTGATAATGTC-3′; GAL10(Core), 5′-GCAAGATAATGGGCTTCCTATAC-3′ and 5′-TTTCCATCTGACTGAGCTTCATAC-3′; ACT1(Core), 5′-AACCGGTTTGAACCAACTCGCCT-3′ and 5′-TTCTTTGTTGATAGGAGGGGAA-3′. Radiograms were scanned and quantitated by the National Institutes of Health Image 1.62 program. IP DNA was quantitated as the ratio of IP to input.

**Whole Cell Extract Preparation and Western Blot Analysis**—For analysis of the global levels of proteins of interest such as the ChIP assay at the GAL1 UAS and core promoter (Fig. 1A) as described previously (62–65). Fig. 1B shows that the 19 S base (Rpt6p and Rpt2p) was predominantly recruited to the UAS but not the core of the GAL1 promoter in galactose-containing growth medium (inducing conditions). However, the 19 S lid (Rpn9p and Rpn12p) or 20 S CP (Prs3p and Pre6p) was not recruited to the GAL1 promoter under inducing conditions (Figs. 1, B and C). As a control, we show the absence of ChIP signal in the strain that does not bear Myc-tagged proteasome component (Fig. 1D). Together, these results demonstrate that the 19 S base is specifically recruited to the GAL1 UAS independently of the 19 S lid or 20 S CP in galactose-containing growth medium. However, the 19 S base was not recruited to the GAL1 UAS under non-inducing conditions (raffinose-containing growth medium) (data not shown). Thus, the 19 S base is specifically recruited to the GAL1 UAS in a transcription-dependent manner in vivo. Like the 19 S base, activator (Gal4p), co-activator (SAGA (Spt-Ada-Gcn5-acetyltransferase)), and SRB/Mediator are also recruited to the UAS, but not core promoter, of GAL1 (64). Thus, like Gal4p, SAGA, and Mediator, the 19 S base is an UAS-specific factor and, hence, might play a crucial role in regulating the protein interaction network at the GAL1 promoter during transcriptional activation in vivo. Consistent with our in vivo data, Sun et al. (67) have also biochemically characterized the 19 S base without lid or 20 S CP and named it as APIS (ATPases-independent of 20 S). Furthermore, a recent study also demonstrated the presence of the 19 S base independently of the 19 S lid or 20 S CP in nucleus (68).

**Recruitment of the 19 S Base to the GAL1 UAS Is Dependent on Gal4p-SAGA Interaction**—Because the 19 S base is recruited to the GAL1 UAS, we next asked whether activator is essential for recruitment of the 19 S base. To address this question, we analyzed recruitment of the 19 S base to the GAL1 UAS in the presence and absence of the activator, Gal4p. Fig. 1E shows that the 19 S base (Rpt6p) was not recruited to the GAL1 UAS in the absence of Gal4p. Thus, Gal4p targets recruitment of the 19 S base to the GAL1 UAS. However, our recent in vivo studies (64) have demonstrated that Gal4p also targets SAGA to the GAL1 UAS through its direct interaction with Tra1p (the largest subunit of SAGA). Thus, it seems likely that the 19 S base might be directly targeted by Gal4p at the GAL1 UAS in vivo, and SAGA might play an important role in recruitment of the 19 S base. To test this hypothesis, we next analyzed recruitment of the 19 S base to the GAL1 UAS in the absence of SAGA and Tra1p (data not shown). Consistent with our in vivo data, Sun et al. (67) have also biochemically characterized the 19 S base without lid or 20 S CP and named it as APIS (ATPases-independent of 20 S). Furthermore, a recent study also demonstrated the presence of the 19 S base independently of the 19 S lid or 20 S CP in nucleus (68).

**ATPase Activity of the 19 S Base Enhances Gal4-SAGA Interaction**—The data presented in Fig. 1 demonstrate that Gal4p-SAGA interaction is required for recruitment of the 19 S base to the GAL1 UAS. However, a recent biochemical study (13) has demonstrated that the 19 S RP enhances Gal4p-SAGA interaction. How does the 19 S base enhance the Gal4p-SAGA interaction, whereas its recruitment is dependent on Gal4p and SAGA? We hypothesized that Gal4p might
interact weakly with SAGA in the absence of the 19 S base in vivo. Such a weak Gal4p-SAGA interaction recruits the 19 S base, which in turn enhances Gal4p-SAGA interaction in a positive feedback manner via its ATPase or molecular chaperonin activity. To test this hypothesis, we analyzed recruitment of Gal4p and SAGA (TAF10p) to the GAL1 UAS in the wild type and RPT1 ATPase point mutant (rpt1-K256R) strains. TAF10p is an integral component of both SAGA and TFIIID (62, 70–72). SAGA is recruited to the UAS, whereas TFIIID is recruited to the core promoter (62, 64, 72). Because GAL1 is a TFIIID-independent but SAGA-dependent promoter, TAF10p is specifically recruited to the GAL1 UAS, but not to the core promoter, as a component of SAGA (62). Thus, TAF10p was chosen to monitor recruitment of SAGA to the GAL1 UAS.

The rpt1-K256R point mutant was generated by substituting the lysine (that interacts with ATP) to arginine in the Walker A box of the ATPase domain (59). Such a substitution maintains the positive charge and, thus, does not completely impair ATPase activity of Rpt1p (59). Using this point mutant, we performed the ChIP assay. We found that recruitment of SAGA (TAF10p) to the GAL1 UAS was significantly decreased (5-fold) in the rpt1-K256R point mutant (Fig. 2A). However, Gal4p recruitment was modestly reduced (2-fold) in this point mutant (Fig. 2A). Such a decrease in recruitment of Gal4p and SAGA could be due to slow growth of the point mutant during the 90-min induction time period in galactose-containing growth medium. To test this possibility, we analyzed the growth of the wild type and point mutant strains under inducing conditions. Fig. 2B shows that growth of the rpt1-K256R point mutant was not significantly changed as compared with the wild type strain during the 90-min induction time period in galactose-containing growth medium. To test this possibility, we analyzed the growth of the wild type and point mutant strains under inducing conditions. Fig. 2B shows that growth of the rpt1-K256R point mutant was not significantly changed as compared with the wild type strain during the 90-min induction time period. Furthermore, the point mutant grew normally in the solid (Fig. 2C, inset) as well as liquid (Fig. 2C) growth media containing dextrose. Thus, the decrease in recruitment of Gal4p and SAGA in the rpt1-K256R point mutant was not resulted from the growth defect of the mutant strain. Furthermore, our Western blot analysis revealed...
that the stability of TAF10p was not altered in the rpt1-K256R point mutant strain (supplemental Fig. 2B). Like TAF10p, recruitment of another SAGA component, TAF12p, to the GAL1 UAS was significantly decreased (6-fold) as compared with that of Gal4p (2-fold). Thus, additional decrease of SAGA recruitment to the GAL1 UAS in the rpt1-K256R mutant was attributed to the alteration of Gal4p-SAGA interaction in vivo.

The Recruitment of the 19 S Base to the GAL1 UAS Is Not Dependent on the Mediator Complex—The Mediator complex has been implicated as the target of activator and plays an important role in formation of the transcription complex assembly at the core promoter. We have demonstrated previously that the Mediator complex is recruited to the GAL1 UAS in the presence of SAGA (64). Here, we show that SAGA is also essential for recruitment of the 19 S base to the GAL1 UAS. Thus, we next asked whether Mediator, like SAGA, is also essential for recruitment of the 19 S base. To address this question, we tagged the Rpt6p component of the 19 S base by Myc epitope in the wild type and ts mutant strains of Srb4p that main-
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complex to the GAL1 UAS in the rpt1-K256R point mutant and its wild type equivalent. Fig. 3B shows that recruitment of the Mediator complex (Srb4p) to the GAL1 UAS was almost lost in the rpt1-K256R point mutant strain. However, such an impaired recruitment of the Mediator complex in this point mutant could be due to the instability of Srb4p. To test this possibility, we analyzed the global level of Srb4p in the rpt1-K256R point mutant strain. However, the level of histone H3 was monitored as a loading control (Fig. 3C). Furthermore, the dramatic impairment of the Mediator recruitment in the point mutant was not resulted from the growth defect (Fig. 2B). However, the loss of the Mediator recruitment to the GAL1 UAS in the rpt1-K256R point mutant strain could be due to disintegration of the 19 S base. To test this possibility, we analyzed recruitment of the 19 S base to the GAL1 UAS in the rpt1-K256R point mutant and wild type strains. Fig. 2D shows that recruitment of the 19 S base to the GAL1 UAS was modestly decreased in the rpt1-K256R point mutant strain. However, a significant amount of the 19 S base remained associated with the GAL1 UAS in this point mutant (Fig. 2D), which was unable to recruit the equivalent amount of the Mediator complex (Fig. 3B). Thus, recruitment of the Mediator complex to the GAL1 UAS is dependent on 19 S ATPase activity but not merely on its function as a physical adaptor.

19 S ATPase Activity Is Essential for Formation of the PIC Assembly at the GAL1 Core Promoter—Our data demonstrate that 19 S ATPase activity is essential for recruitment of the Mediator complex. Furthermore, the Mediator complex is essential for recruitment of TBP and RNA polymerase II to the GAL1 core promoter for formation of the PIC assembly (64). Thus, formation of the PIC assembly at the GAL1 core promoter would require 19 S ATPase activity. Indeed, we find that recruitment of TBP to the GAL1 core promoter was almost lost in the rpt1-K256R point mutant strain when compared with wild type equivalent (Fig. 4A). However, TBP is quite stable in this point mutant (Fig. 4B). In addition, growth of this point mutant was not significantly changed when compared with wild type equivalent under the 90-min induction time period in galactose-containing growth medium (Fig. 2B). Thus, the dramatic loss of TBP recruitment to the GAL1 core promoter in this ATPase point mutant did not result from growth defect. Furthermore, we find that a significant amount of the 19 S base was present at the GAL1 core promoter in the rpt1-K256R point mutant within the 90-min time period (Fig. 4C). Thus, the dramatic loss of TBP recruitment to the GAL1 core promoter in this ATPase point mutant did not result from growth defect. Therefore, 19 S ATPase activity, but not merely its physical adaptor function, is required for recruitment of TBP to the GAL1 core promoter. Like TBP, RNA polymerase II (Rpb1p) was not recruited to the GAL1 core promoter in the rpt1-K256R point mutant strain (Fig. 4A and supplemental Fig. 2B). Thus, 19 S ATPase activity is essential for formation of the transcription complex assembly at the GAL1 core promoter.

The 19 S ATPase is essential for other cellular process. Thus, the 19 S ATPase point mutant, rpt1-K256R, with significantly impaired ATPase activity via conservative mutation of lysine to arginine in the Walker A box would affect other cellular process. Thus, the loss of the PIC formation at the GAL1 core promoter in the rpt1-K256R point mutant could be due to an indirect effect. To address this issue, we analyzed the formation of the PIC assembly at a proteasome-independent promoter, ACT1 (75). Fig. 4C shows a modest decrease of the PIC formation at the ACT1 core promoter in the rpt1-K256R point mutant strain when compared with wild type equivalent. However, the PIC formation was almost lost at the GAL1 core promoter in the 19 S ATPase point mutant within the 90-min induction time period (Fig. 4A). Thus, the modest decrease of
The GAL1 Transcription Is Impaired in the Absence of 19 S ATPase Activity—Our data demonstrate that 19 S ATPase activity is essential for formation of the transcription complex assembly at the GAL1 promoter. Therefore, GAL1 transcription would be impaired in 19 S ATPases point mutant strain. To test this possibility, we analyzed GAL1 transcription in the wild type and 19 S ATPase point mutant strains using a primer extension assay. Fig. 4D shows that GAL1 transcription was almost lost in the rpt1-K256R ATPase mutant. This observation is consistent with the dramatic loss of the PIC formation at the GAL1 core promoter in the rpt1-K256R point mutant (Fig. 4A). As a control, we have analyzed the transcription of ACT1 whose expression is not dependent on the proteasome (75). The ACT1 transcription was not significantly altered in the rpt1-K256R point mutant as compared with that of GAL1. Thus, the severe loss of the GAL1 transcription was due to the impaired ATPase activity of the 19 S base.

19 S ATPases Appear to Cross-talk for Formation of the Transcriptional Initiation Complex Assembly—The 19 S base has six AAA (ATPases associated with diverse cellular activities) family ATPases forming a ring-shaped heterohexamer. The point mutation of Rpt1p ATPase does not alter the 19 S base structure but impairs ATPase activity (59). Intriguingly, the other five wild type 19 S ATPase subunits are unable to form the PIC at the promoter in the Rpt1p ATPase point mutant (Figs. 3B and 4A). Such an observation can be attributed to the specific requirement of the Rpt1p ATPase in formation of the transcriptional complex assembly at the promoter. Alternatively, it could be due to the fact that 19 S ATPases function through cross-talk, analogous to homohexameric AAA family ATPases (see, for example, Refs. 44, 59, 76, and 77). To test these possibilities, we next analyzed the formation of the transcription complex assembly at the GAL1 promoter in rpt5-K228R and rpt6-K195R ATPase point mutants. Consistent with rpt1-K256R point mutant, rpt5-K228R and rpt6-K195R point mutants altered the targeting of SAGA to the GAL1 UAS (Figs. 5, A–D). Furthermore, we find that Mediator, TBP, and RNA polymerase II were not recruited to the promoter in these point mutants (Figs. 6, A–D). Similarly, the transcription complex was not assembled at two other GAL genes, namely GAL7 and GAL10, in these point mutants (Fig. 6E). As a control, we analyzed recruitment of RNA polymerase II to the core promoter of the proteasome-independent gene, ACT1. Recruitment of RNA polymerase II was not significantly altered at the ACT1 promoter in these point mutants (Fig. 6F). Similarly, PIC formation at the GAL1 core promoter was almost impaired in other 19 S ATPase point mutants such as rpt3-K219R and rpt4-K297R (data not shown). Together, these results indicate that the 19 S AAA family ATPases with chaperonin activity appears to cross-talk for assembling the transcription factors at the promoter. Analogous to our observation, previous studies have implicated the presence of cross-talk or cooperativity in the ring-shaped homohexameric molecular chaperone formed by AAA family ATPases (see, for example, Refs. 44, 59, 76, and 77). However, the existence of cross-talk or cooperativity among ATPases within heterohexameric molecular chaperone was not known. Our ChIP data support the presence of cross-talk.

The PIC Is Dispensable for Recruitment of the 19 S Base at the GAL1 UAS—The 19 S base that is recruited to the UAS regulates formation of the PIC assembly at the core promoter. Next, we asked whether PIC has any role in regulating recruitment of the 19 S base at the UAS via reciprocal cooperativity. To address this question, we next analyzed recruitment of the 19 S base at the GAL1 UAS in the wild type and ts mutant of TBP, a central component of the PIC assembly. Fig. 4E shows that recruitment of the 19 S base to the GAL1 UAS was not dependent on TBP. Thus, the PIC does not regulate recruitment of the 19 S base to the GAL1 UAS.
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Among ATPases in the ring-shaped heterohexameric chaperone, 19 S base.

**DISCUSSION**

The non-proteolytic function of the proteasome complex has recently been implicated in regulation of eukaryotic transcriptional activation (28). However, the mechanisms by which the proteasome regulates eukaryotic transcriptional activation in a proteolysis-independent manner remain largely unknown. Recent biochemical studies (13, 23) have demonstrated the non-proteolytic role of the proteasome in regulation of the activator-coactivator or activator-promoter interaction (and hence transcriptional activation). However, how the 19 S RP regulates transcriptional activation at the promoter is not yet clearly understood in vivo. Here, we show that the 19 S base, but not lid or 20 S CP, is predominantly recruited to the UAS of a transcriptionally active eukaryotic promoter in an activator/coactivator-dependent manner. Such a recruitment of the 19 S base enhances the activator-coactivator interaction to stimulate transcription in a positive feedback manner through its ATPase or molecular chaperonin activity. Furthermore, we demonstrate that the molecular chaperonin or ATPase activity, but not merely physical adaptor function, of the 19 S base is essential for downstream transcription complex assembly at the promoter. Intriguingly, 19 S ATPases function in a cooperative manner to form the transcription complex assembly for transcriptional initiation. These results significantly advance our fundamental understanding of the non-proteolytic role of the proteasome in regulation of eukaryotic transcriptional activation in vivo.

Previous biochemical studies (13) have demonstrated that the 19 S RP enhances Gal4p–SAGA interaction and, hence, activates transcription of the GAL genes. However, it was not known whether similar enhancement...
of Gal4p-SAGA interaction occurs at the UASs of the GAL genes in vivo. In this study we show that the 19 S base is essential for enhancement of the Gal4p-SAGA interaction at the GAL1 UAS. We also demonstrate here that 19 S ATPase activity stimulates Gal4p-SAGA interaction. Consistent with our in vivo data, previous biochemical studies (13) have demonstrated the physical and genetic interactions between SAGA and the 19 S base. Interestingly, we find that Gal4p-SAGA interaction is essential for recruitment of the 19 S base at the GAL1 UAS. How does the 19 S base stimulate Gal4p-SAGA interaction? Our data support a model where Gal4p first recruits SAGA via weak Gal4p-SAGA interaction in the absence of 19 S ATPase activity, and subsequently, the 19 S base is recruited to the GAL1 UAS through its interaction with SAGA. The 19 S base thus recruited in turn enhances the interaction between Gal4p and SAGA in a positive feedback manner, possibly through proper folding or assembly of the SAGA components by its molecular chaperonin activity as demonstrated by Braun et al. (36).

In addition to its role in recruitment of the 19 S base, Gal4p-SAGA interaction is essential for association of the Mediator complex with the GAL1 UAS for formation of the PIC assembly (64). How does the 19 S base regulate recruitment of the Mediator complex to the GAL1 UAS in vivo? Our data demonstrate that Mediator is not essential for recruitment of the 19 S base. Interestingly, we find that recruitment of the Mediator complex is dependent on ATPase activity. We speculate that the 19 S base might help to fold or assemble the Mediator components at the GAL1 UAS through its molecular chaperonin activity. In support of this model, Sun et al. (67) have biochemically demonstrated the physical interaction between the 19 S base and Mediator complex. Thus, the Mediator complex is likely to be properly assembled at the GAL1 UAS through its interaction with the 19 S base.

FIGURE 6. 19 S ATPases function in a cooperative manner for formation of the transcription complex assembly. A, recruitment of Mediator to the GAL1 UAS is impaired in rpt5-K228R and rpt6-K195R point mutant strains. The wild type (WT) and mutant strains expressing Myc epitope-tagged Srb4p were grown and cross-linked as in Fig. 3B. Immunoprecipitation was carried out as in Fig. 3B. Western blot analysis is shown. The wild type and mutant strains were grown as in panel A. The whole cell extracts from the wild type and mutant strains were prepared as in the ChIP assay. The whole cell extracts were analyzed by Western blot assay using the anti-Myc and anti-histone H3 antibodies against Myc-tagged Srb4p and histone H3, respectively. C, analysis is shown of TBP and RNA polymerase II recruitment to the GAL1 core promoter in rpt5-K228R, rpt6-K195R, and wild type strains. The wild type and point mutant strains were grown and cross-linked as in Fig. 3B. Immunoprecipitation was performed as in Fig. 4A. D, Western blot analysis is shown. rpt5-K228R and rpt6-K195R point mutant and wild type strains were grown as in panel A. The whole cell extracts from the wild type and mutant strains were prepared as in the ChIP assay. The whole cell extracts were analyzed by Western blot assay using the anti-TBP and anti-histone H3 antibodies against TBP and histone H3, respectively. E, 19 S ATPases are essential for the PIC formation at the GAL7 and GAL10 promoters. The wild type and 19 S ATPase point mutants were grown and cross-linked as in panel C. Immunoprecipitation was carried out as in panel C. The primer pairs located at the core promoters of the GAL7 and GAL10 genes were used for PCR analysis of the immunoprecipitated DNA samples. F, analysis is shown of RNA polymerase II recruitment to the ACT1 core promoter in the wild type and rpt5-K228R and rpt6-K195R point mutant strains. The wild type and mutant strains were grown and cross-linked as in panel C. Immunoprecipitation was carried out as in panel C.
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The Mediator complex is essential for formation of the PIC assembly at the GAL1 core promoter (64, 78). Thus, the impaired recruitment of the Mediator complex to the GAL1 UAS in the absence of 19 S ATPase activity would lead to the loss of PIC formation at the core promoter. Indeed, our data show that TBP and RNA polymerase II components of the PIC show that TBP and RNA polymerase II components of the PIC assembly at the core promoter in the 19 S ATPase point mutant is directly affected by the absence of 19 S ATPase activity or indirectly resulted from the impaired recruitment of the Mediator complex. Nonetheless, our data demonstrate that ATPase activity of the 19 S base is essential for the transcription complex assembly at the promoter for transcriptional initiation or activation in vivo. However, previous biochemical studies have demonstrated the interactions of the 19 S base with the components of PIC (79–81). Thus, the 19 S base might directly regulate the assembly of the PIC formation at the core promoter.

Although the 19 S base is essential for recruitment of the Mediator and PIC components, it is not known whether the Mediator complex or the PIC component regulates recruitment of the 19 S base to the GAL1 UAS via reciprocal cooperativity. Here, we show that neither the Mediator complex nor the PIC component regulates recruitment of the 19 S base to the GAL1 UAS. Only Gal4p-SAGA interaction is essential for recruitment of the 19 S base. These results support a specific interaction network of Gal4p-SAGA-19 S base-Mediator-PIC at the GAL1 promoter. Interestingly, we also find that 19 S ATPases function in a cooperative manner to facilitate formation of the transcription complex assembly at the promoter. The 19 S base has molecular chaperonin activity that is provided by the AAA family ATPases. The molecular chaperonin activity of the 19 S base seems to play an important role in formation of the transcription complex assembly and, hence, transcriptional initiation or activation. The fact that 19 S ATPases function in a cooperative manner indicates that these 19 S ATPases cross-talk among themselves in the ring-shaped structure. Similar cooperativity or cross-talk among ATPases in homohexameric ring-shaped molecular chaperon has been reported (see, for example, Refs. 44, 59, 76, and 77). Importantly, our data support the fact that different ATPases within heterohexameric ring-shaped 19 S base function through cross-talk. Our future challenge is to understand the structural basis for such cross-talk.

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