

The proteasomal ATPase complex is required for stress-induced transcription in yeast

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ABSTRACT

Sug1 and Sug2 are two of six ATPases in the 19S regulatory particle of the 26S proteasome. We have shown previously that these proteins play a non-proteolytic role in the transcription of the *GAL* genes in yeast. In this study, we probe the requirement for these factors in stress-induced transcription in yeast. It is known that proteasomal proteolysis is not required for these events. Indeed, proteasome inhibitors strongly stimulate expression of these stress response genes. However, shifting strains carrying temperature-sensitive alleles of *SUG1* and *SUG2* to the restrictive temperature strongly inhibited the expression of *HSP26*, *HSP104* and *GAD1* in response to heat shock or treatment with menadione bisulfate. Furthermore, chromatin immunoprecipitation analysis revealed the recruitment of Sug1, Sug2 and Cim5 (another of the ATPases), but not 20S proteasome core proteins, to the promoters of these genes. These data show that the non-proteolytic requirement for the proteasomal ATPases extends beyond the *GAL* genes in yeast and includes at least the heat and oxidative stress-responsive genes.

INTRODUCTION

It has long been known that the 26S proteasome regulates the levels of a number of transcription activators, thus affecting their potency. In the last few years however, several lines of investigation have revealed a number of more intimate, and mechanistically distinct, intersections between RNA polymerase II transcription and ubiquitin/proteasome pathway proteins (1–6). Of particular relevance to this study was our finding that the Sug1 protein [also called Rpt6 (7)], one of

the six ATPases in the 19S regulatory particle of the 26S proteasome, was essential for efficient promoter escape and elongation in Gal4-VP16-activated transcription *in vitro* (8,9). When Sug1 activity was compromised by mutation or by the addition of a specific anti-Sug1 antibody, the production of very short transcripts (up to ≈ 50 nt) was unaffected, but production of longer molecules was crippled. The physiologic relevance of these results was supported by the fact that certain mutations in *SUG1* and *SUG2* (which encodes another proteasomal ATPase) confer sensitivity to 6-azauracil, a hallmark of elongation defects. Furthermore, chromatin immunoprecipitation (ChIP) experiments revealed recruitment of Sug1, Sug2 and the other proteasomal ATPases to the *GALI10* promoter and the *GALI* gene upon induction of *GAL* gene expression with galactose (10). This recruitment was dependent on a functional Gal4 transactivator. Surprisingly, there was no evidence for recruitment of the 20S proteolytic core complex to the *GALI10* promoter in these ChIP analyses (10), even though 20S-chromatin interactions can be detected by this technique elsewhere in the gene (6). In addition, there was no indication of the presence of the ‘lid’ sub-complex (11,12) of the 19S regulatory particle. This suggested that the Gal4 activator could recruit the ATPases separate from the rest of the proteasome. This model is supported by biochemical experiments, which reveal that a GST-Gal4 activation domain (AD) fusion protein binds, a complex binds the ATPases in a fashion that excludes the lid and 20S core (10). This is also consistent with the observation that *in vitro* elongation was unaffected by proteasome inhibitors or the absence of the 20S core complex (8,9). On the basis of these findings, we proposed that the Gal4 activator recruits a novel sub-complex containing the six proteasomal ATPases, Rpn1, Rpn2 and perhaps other proteins, but which lacks 20S core and lid factors (10).

An important question is whether these findings in the yeast *GAL* system are relevant to the mechanism of

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transcription of other genes in yeast and higher organisms. Here we begin to address this point by analyzing the role of the proteasomal ATPases in stress-induced gene transcription in *Saccharomyces cerevisiae*. We show that inactivation of temperature-sensitive mutants of Sug1 and Sug2 almost completely abolish transcription of several heat and oxidative stress-induced genes. However, the expression of these genes is not dependent on the proteolytic function of the proteasome. We also use ChIP analysis to show that the proteasomal ATPases are recruited rapidly to the promoters of stress-induced genes, but 20S proteins are not. These data, combined with the previous studies of the *GAL* system, suggest that the proteasomal ATPases may play an important role in the transcription of many inducible genes and perhaps others as well.

MATERIALS AND METHODS

S.cerevisiae strains

W303a (MATa ade2-1 ura3-1 his3-11, 15 trp1-1 leu2-3, 112 can1-100) was used as wild type. Sc658 (sug1-20) and Sc677 (sug2-13) strains are congenic to W303a. Strain (pre1-1 pre4-1) is congenic to WCG4a (MATa ura3 leu2-3, 112 his3-11, 15 Can^s Gal⁺) (13). Pre1-Flag (MATa his3-200 leu2-3, 112 lys2-801 trp-63 PRE1 FLAG::YIplac211[URA3]) and Cim5-Flag strains (14) were a generous gift from Prof. Raymond Deshaies (California Institute of Technology). The strains expressing Flag-Rpb3 (6) and HA-Gal11 (15) have been reported previously and are congenic to W303a.

Growth conditions and stress experiments

Heat shock experiments: wild-type (wt) cells were grown to an OD₆₀₀ of 0.6 and heat shocked by the addition of the appropriate volume of heated media (54°C) followed by incubation in a water bath shaker at 37°C for 5 or 20 min. Oxidative stress experiments: 1 mM of menadione bisulfate was added to wt cells at an OD₆₀₀ of 0.6 for 1 h. For temperature-sensitive strains, cells were heat shocked first at 37°C for 90 min and then treated with 1 mM of menadione bisulfate for 1 h at 37°C.

RNA analysis

Cells were diluted into YEP-ADEN media and grown until an OD₆₀₀ of 0.6 treated as necessary (heat shocked and so on), and washed twice with 1× ice-cold phosphate-buffered saline (PBS). Total RNA isolation was performed by suspending the cells in 400 µl of RNA extraction buffer A [0.1 M NaCl (DEPC treat) 10 mM EDTA (DEPC treat) 5% SDS (DEPC treat) 50 mM Tris-HCl, pH 7.5]. An equal volume of phenol was then added, and the samples were incubated at 65°C for 30 min. Following centrifugation, the RNA was back-extracted with phenol, followed by chloroform. The extracted supernatant was precipitated with Buffer B (1/10 vol of 3 M NaAc and 2 vol isopropanol). Pellets were washed subsequently with 80% ethanol and suspended in 100 µl DEPC-treated water. Finally, total RNA was reverse transcribed into cDNA using the Stratagene reverse transcriptase kit.

Chromatin immunoprecipitation assays

ChIP assays were performed as described previously (10). Cells were harvested at an OD₆₀₀ of 0.6 and cross-linked

with formaldehyde (final concentration 1%) for 25 min. The reaction was then stopped with the addition of 2.5 M of glycine.

Cells were pelleted and washed with 1× PBS and processed for spheroblasting. To spheroblast the cross-linked pellet, cells were resuspended in 0.1 M Tris, pH 9.4, and 10 mM DTT on ice for 20 min. Cells were pelleted and resuspended in HEPES Sorbitol (20 mM HEPES, pH 7.4, 1.2 M Sorbitol) with 2 mg zymolyase and incubated at 30°C for 30 min. To quench zymolyase activity, Pipes Sorbitol (20 mM PIPES, pH:6.8, 1 mM MgCl₂, 1.2M Sorbitol) was added followed by a spin and subsequent washes with PBS, TritonX HEPES (0.25% Triton X-100, 10 mM EDTA, 10 mM HEPES, pH 6.5, 0.5 mM EGTA) and NaCl HEPES (200 mM NaCl, 1 mM EDTA, 10 mM HEPES, pH 6.5, 0.5 mM EGTA). For lysis, we resuspended the cell pellet in 1 ml SDS lysis buffer (1% SDS, 10 mM EDTA, 0.1% DOC, 20 mM Tris, pH 8.1) and incubated the cells on ice for 15 min. After lysis the suspension was sonicated for 15 s twice and for 10 s three times with 30 s intervals between each shear to obtain fragments with an average length of 500 bp. For immunoprecipitations, the chromatin solution was aliquoted in IP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris, pH:8.1 and 167 mM NaCl) and specific antibodies along with salmon sperm DNA (a non-specific competitor) were added for an overnight incubation at 4°C. The immunocomplexes were harvested with the addition of protein A (Pierce) and incubated at room temperature for 2 h. The beads were then sequentially washed with TSE150 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8 and 150 mM NaCl), TSE500 (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8 and 500 mM NaCl), LiCl detergent (0.25 M LiCl, 1% NP40, 1% DOC, 1 mM EDTA and 10 mM Tris-HCl, pH 8) and TE (10 mM Tris, 1 mM EDTA) and eluted with 1% SDS/0.1 M NaCO₃. Formaldehyde cross-linking was then reversed overnight at 65°C. The samples were then precipitated with the addition of 2 vol of absolute ethanol overnight at -20°C, followed by a proteinase kinase A treatment. DNA was then extracted with Phenol-chloroform-isoamyl (Roche) and precipitated overnight at -20°C. Ethanol pellets were spun down for 30 min, washed with 400 µl of 70% ethanol, dried briefly and resuspended in 100–150 µl of water, dissolved and processed for PCR. Data from three independent experiments were quantified using a densitometer. The value obtained for each band was corrected for the local background. The background corrected value was divided by the intensity of the input band normalizing the reading. Those values, reflecting protein occupancy, are expressed as the amount of DNA recovered relative to the input sample. The average values were plotted graphically using Microsoft Excel software.

Primers used for ChIP and RT-PCR (5'-3') were as follows. ALONG *HSP104*:A, forward GTACCATAAAAT ATACA-GAATATATG, reverse GC TAGAATATGTATAGTTGT-AATTG; B, forward GCTTTGGA TTAGTTG ATATT-TCTTG, reverse CCAATTCTTCTTGCAA TGAAGCTTCC; C, forward GGC AGGTTATGTC GGGTACGATGAAG, reverse GGAAGTCATGAT GACAATACAA TTGG; D, forward CAA GGATAAGGAA ACTGTCAATG TCG, reverse CTAGG TCATCATCAATTTCCATA; Chromosome IV, forward GCTCTAAATAATATTGGATGTTGC, reverse CGAATGGCATAATTCAATTGG; HSP26, forward

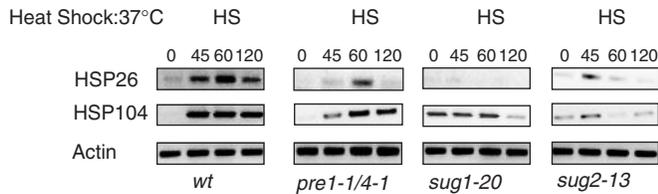


Figure 1. Sug1 and Sug2, but not the 20S proteasome core complex, are essential for efficient heat shock gene expression. Using the strains indicated at the bottom of the figure, mRNA was extracted from cells ($OD_{600} = 0.6$) at 25°C (time = 0 in each panel) or 37°C at 45, 60 and 120 min after shifting the temperature to 37°C (the restrictive temperature). Actin levels were monitored as a control.

GGC AGCAGCAACT CCGTGTGTAC CCC, reverse GC GAATACCTTACTGTTACGAGCACC; *GAD1* PROMOTER, forward GTCAAT TTAGGCATTC TTGTGAT-TAT, reverse CCAGCGATA TTCTCGAAGT TCTTCTG; *GAD1* END OF GENE, forward GTCCA AGAAATCCA CGAAGAATAT C, reverse GA GATGCCAA TGCCAGCA ACATTTTCG; *PDR5* PROMOTER, forward GTGATGT-GCATAACCTTATGGCTGTTTCGC, reverse GTCTAAAG-TCTTTTCGAACGAGCGGATACG; and *HSP82* PROMOTER, forward GTCACAGATGTTAAGAATTGAAGGG, reverse GCGTGTGATATATCACATTCGGAGG.

RESULTS

Sug1 and Sug2 are required for the efficient transcription of heat shock genes

To ask if proteasomal ATPases are essential for expression of heat shock genes, we employed yeast strains encoding temperature-sensitive *sug1* or *sug2* alleles [*sug1-20* (16) and *sug2-13* (17,18)] and compared the levels of *HSP26* and *HSP104* expression at 37°C (the restrictive temperature) with that observed in the wild-type strain. As shown in Figure 1, *HSP26* and *HSP104* are induced strongly in the wild-type cells with a maximal steady state of RNA achieved 60 min after heat shock. However, little or no detectable transcripts from *HSP26* were produced in the *sug1-20* strain. A small amount of *HSP26* transcript was produced in the *sug2-13* strain initially, but the level was much lower than that observed in the wild-type strain and the signal disappeared by 60 min after heat shock. These results demonstrate that Sug1 and Sug2 are important for *HSP26* transcription. Of course, raising the temperature to 37°C results in both induction of the heat shock response and the inactivation of the temperature-sensitive Sug proteins. The modest level of *HSP26* gene expression in the *sug2-13* strain was most likely due to RNA produced prior to complete Sug2-13 inactivation. Apparently, Sug1-20 loses activity more quickly at the restrictive temperature than does Sug2-13.

Figure 1 also shows the effect of Sug1-20 and Sug2-13 inactivation on *HSP104* gene expression. The *sug* mutations caused significant basal expression of the gene even at 25°C relative to the wild-type strain, possibly due to an effect of these mutations on proteasome-mediated proteolysis, which could trigger a partial unfolded protein response. When the temperature was increased to 37°C, strong induction of *HSP104* was observed in the wild-type strain, but the

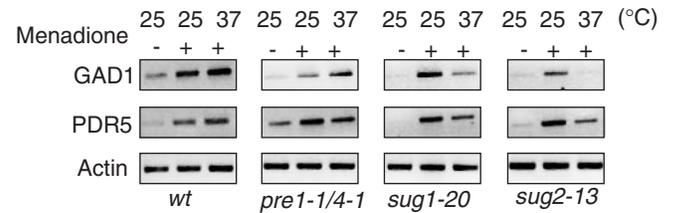


Figure 2. Sug1 and Sug2, but not the 20S proteasome core complex, are essential for expression of the oxidative stress response gene *GAD1* and *PDR5*. The strains indicated in the figure were incubated for 2 h at the non-permissive temperature before addition of 1 mM menadione for 1 h.

level of *HSP104* transcript did not increase in the *sug1-20* and *sug2-13* strains, indicating that little or no new RNA was made following heat shock and inactivation of the proteasomal ATPase.

To probe the role of the 20S proteolytic core complex in *HSP26* and *HSP104* transcription, we employed a strain that carries temperature-sensitive alleles in the *PRE1* and *PRE4* genes (*pre1-1/4-1*) (8,13). As shown in Figure 1, shifting these strains to the restrictive temperature did not reproduce the effects of inactivation of the *sug1* or *sug2* alleles. Expression of *HSP104* was essentially identical to that seen in the wild-type strain. Expression of *HSP26* appears to be reduced slightly, but is nonetheless much more robust than when activity of the ATPases is knocked out.

Sug1 and Sug2 are also required for efficient transcription of oxidative stress inducible genes

In order to study whether Sug1 and Sug2 affect the expression of other types of stress response genes, yeast were exposed to 1 mM of the oxidant menadione bisulfate, which triggers the oxidative stress response (19). Menadione-inducible stress was first evaluated in a wild-type strain at 25 and 37°C by monitoring *GAD1* expression. As shown in Figure 2, *GAD1* transcript was detected in the wild-type strain at 25 and 37°C after incubating the cells for 1 h with menadione. In *sug1-20* and *sug2-13*, *GAD1* transcript was also detected at 25°C. At 37°C however, the level of transcript was reduced by 8-fold relative to the wild-type strain in *sug1-20* and by 3-fold in *sug2-13*, showing that the Sug proteins are important in *GAD1* gene expression. As was the case for the heat shock genes, the *pre1-1/4-1* strain exhibited robust *GAD1* expression at 37°C, again indicating that the 20S proteasome complex is not required for gene transcription. Similarly, expression of *PDR5*, another inducible gene affected by drugs such as menadione bisulfate, was also reduced at 37°C relative to the level observed at 25°C in these strains (Figure 2).

Physical association of the proteasomal ATPases with stress promoters

We employed ChIP analysis to probe for the physical presence of proteasomal proteins at the various stress response promoters. Primers were designed to amplify the heat shock factor-binding sites (heat shock elements) upstream of *HSP104*. As shown in Figure 3A and B, the proteasomal ATPases, Sug1, Sug2 and Cim5/Rpt1 are all recruited to the *HSP104* promoter upon heat shock within 5 min, as is RNA polymerase

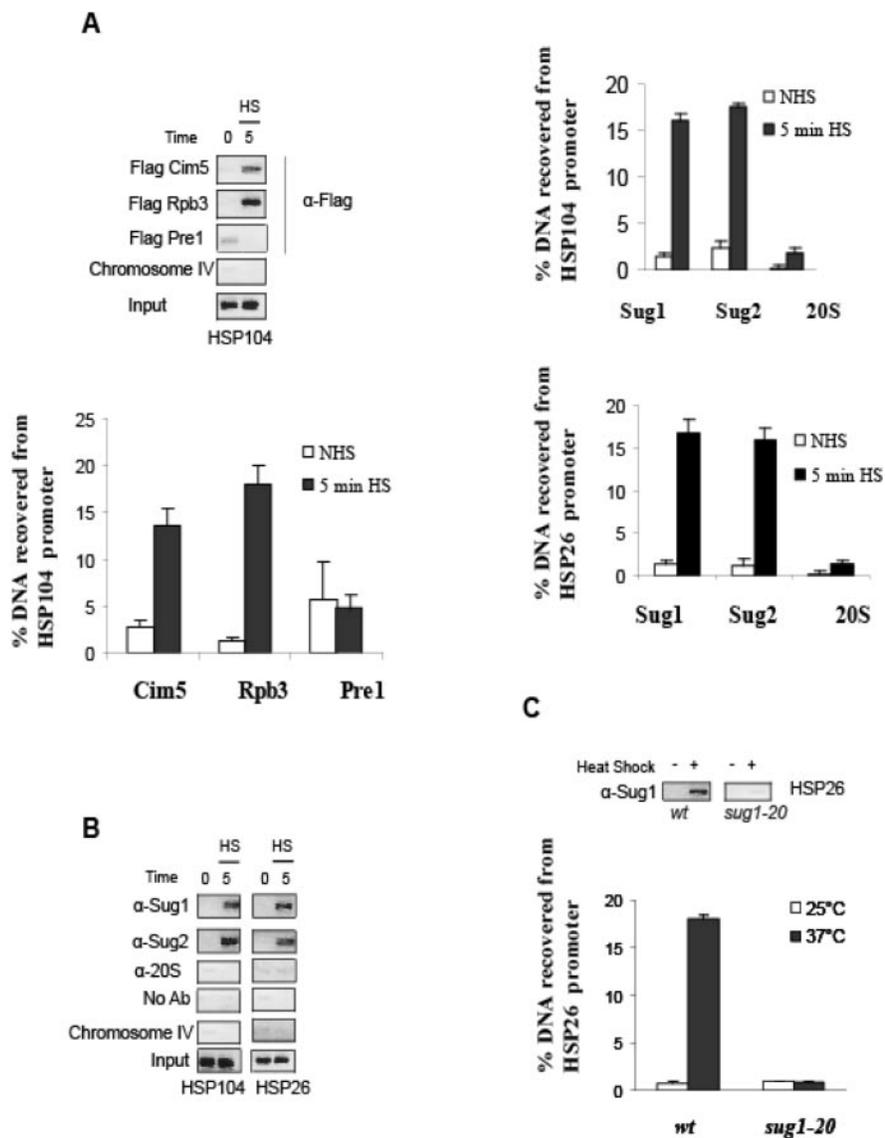


Figure 3. The proteasomal ATPases are recruited to heat shock promoters upon induction. (A) ChIP analysis using the anti-Flag antibody and yeast strains expressing the FLAG-tagged proteins indicated in the figure was employed to monitor the association of the tagged proteins with the *HSP104* promoter region including the heat shock elements at 25°C and after 5 min of heat shock (37°C). (B) The same experiments were conducted with *HSP104* and the *HSP26* promoter using wild-type cells and antibodies against the native proteins indicated. (C) Comparison of ChIP analyses of Sug1 recruitment to the *HSP26* promoter at 25 and 37°C in a wild-type and a *sug1-20* strain. The loss of the signal in the *sug1-20* strain at the restrictive temperature argues that the signal seen in the *wt* strain is indeed due to Sug1-promoter association and not cross-reactivity of the antibody with some other protein.

II (Rpb3). Amplification of a transcriptionally silent portion of the yeast genome from chromosome IV showed no signal, which, in addition to the no antibody control, confirms the specificity of the interaction of the ATPases with the promoter of *HSP104*. We observed the same pattern of recruitment on *HSP26* promoter (Figure 3B). In addition, when the ChIP assays were repeated using the *sug1-20* strain, no signal was detected at 37°C on the *HSP26* promoter, whereas a strong signal was observed in the wild-type strain under these conditions (Figure 3C). These results validate the specificity of our antibody and are consistent with the absence of *HSP26* transcripts in *sug1-20* strain at 37°C.

In contrast to the results observed for the ATPases, no heat shock-dependent recruitment of core proteasomal proteins was

evident (Figure 3A and B). When the Flag-tagged Pre1 strain was employed, a low-level signal was observed prior to heat shock, which then decreased to background levels upon induction. This might indicate the presence of the 26S proteasome on the promoter prior to heat shock. When a wild-type strain and polyclonal antibodies raised against the entire 20S complex were employed, only a background signal was detected under both conditions.

We also examined interaction of the proteasomal proteins with the oxidatively-inducible *GAD1* promoter using ChIP analysis. The results showed induction-dependent recruitment of Cim5 and Rpb3 but not Pre1 (Figure 4A). We obtained the same recruitment pattern for Cim5 and Sug1 on the promoters of *PDR5*, *YCF1* and *TRX1*, additional genes that are highly induced upon oxidative stress (Figure 4B).

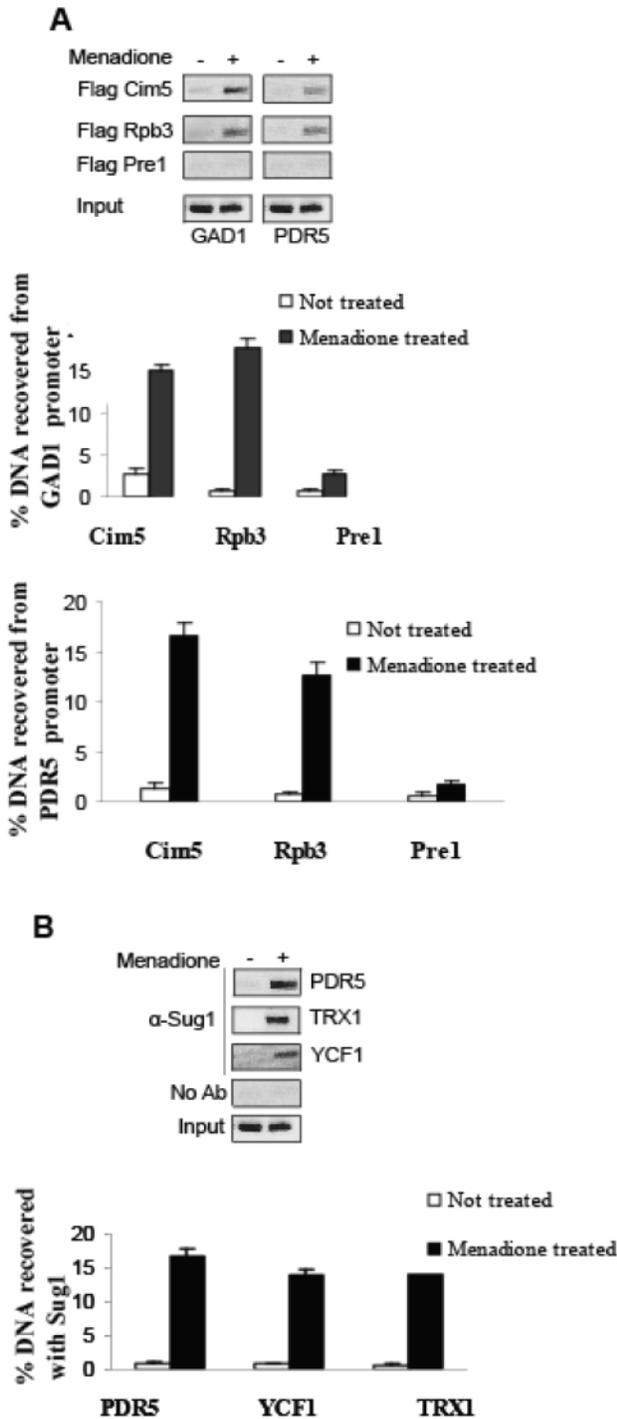


Figure 4. The proteasomal ATPases are recruited to oxidative stress promoters upon induction. (A) ChIP assay using anti-Flag antibody in the three Flag-tagged strains indicated in the figure. (B) The same experiments were conducted with different oxidative stress promoters using wild-type cells and Sug1 antibody.

ATPase recruitment is not restricted to the promoter regions of stress-inducible genes

Previous work from our laboratory revealed an important role for the proteasomal ATPases in elongation (8,9) and ChIP assays revealed association of the ATPases throughout the

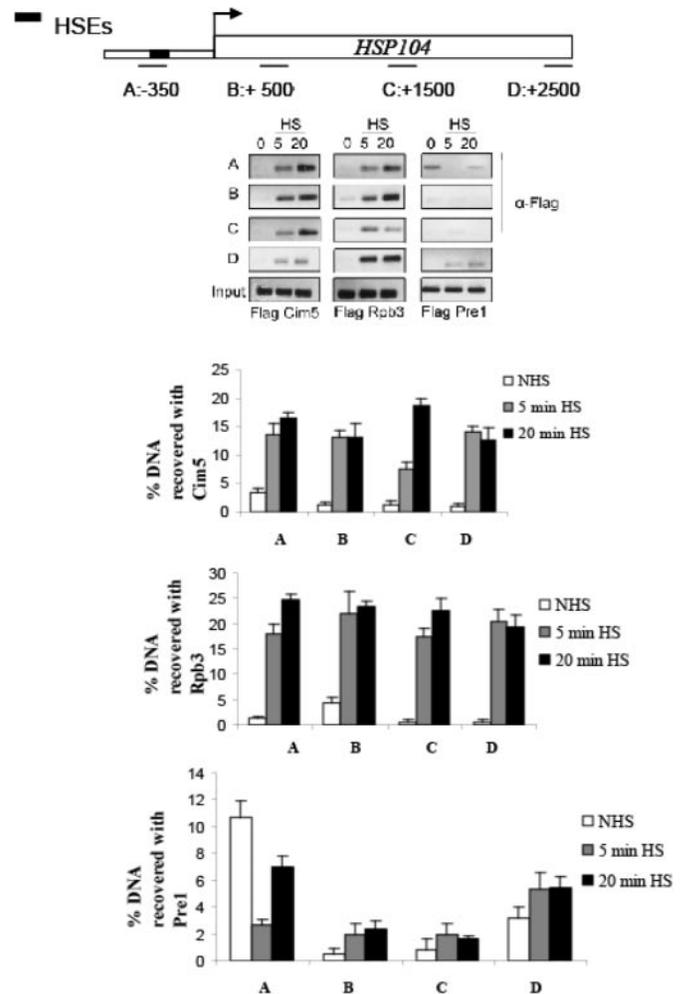


Figure 5. Analysis of the physical association of Cim5, Pre1 and Rpb3 with different regions of *HSP104*. ChIP assays were conducted in strains expressing Flag-tagged Cim5, Rpb3 or Pre1 using anti-Flag antibody at 25°C and following heat shock (37°C) for 5 and 20 min. The regions amplified are shown graphically at the top of the figure. The graphs show the quantification of the gel data.

transcriptionally active *GALI* gene (10). To determine if a similar situation exists in the stress response genes, we employed a variety of PCR primers to assess interactions of the proteasomal proteins and RNA polymerase II with the coding region of *HSP104*. These experiments employed strains that express Flag-tagged Cim5, Rpb3 or Pre1 and the anti-FLAG antibody. As is evident in Figure 5, we detected the recruitment of Cim5 and Rpb3 all along the gene after heat shock. The pattern of Flag-Pre1 association was quite different. No signal could be detected with primer pairs B and C at both time points (Figure 5). As mentioned above (Figure 3), a weak signal for Flag-Pre1 was detected on the promoter prior to induction that then faded after heat shock. Curiously however, the signal grew again in intensity 20 min after heat shock, a result that we currently do not understand. Finally, Flag-Pre1 was detected at the 3' end of the gene after induction. This was expected given our previous report that the proteasome co-localizes with stalled polymerase complexes, including those undergoing termination (6).

Sug1 and PolII recruitment to the *HSP82* promoter appear to be separable events

In higher organisms, several heat shock promoters have been shown to contain a fully assembled and engaged RNA polymerase II complex even prior to induction, with the rate-limiting step in expression of these genes being promoter escape and elongation (20,21). Most yeast heat shock promoters apparently have somewhat different kinetics of transcription factor recruitment since PolII is not resident on the uninduced promoter of many of these genes. However, the work of Sekinger and Gross (22) has shown that the yeast *HSP82* promoter is apparently more akin to higher heat shock promoters in being primed for transcription. Consistent with this view, we found that this promoter is occupied significantly by PolII even in the uninduced state, as evidenced by the strong signal observed at 25°C in cells that express FLAG-tagged Rpb3 (Figure 6A). Under non-inducing conditions, only a low level of *HSP82* transcription was apparent (Figure 6B), whereas *HSP82* expression was strongly stimulated by heat shock (Figure 6B). The intensity of the FLAG-Rpb3-dependent chip signal increased only modestly (~2-fold) (Figure 6A). Previous studies by Park *et al.* (23) in *Drosophila* have shown that this type of modest increase reflects polymerase density on proximal sites in the coding

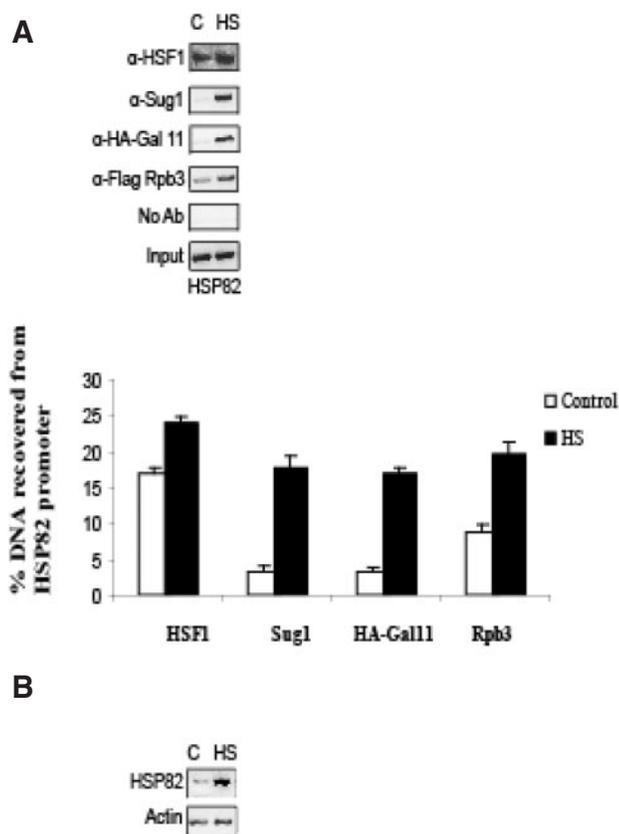


Figure 6. (A) The recruitment of polII, Sug1 and mediator to the *HSP82* promoter are separable events. Strains expressing either Flag-tagged Rpb3 or HA-Gal11 expressing strain were heat shocked for 5 min at 39°C (HS) or kept at 25°C for the same period of time (C, control). Association of the proteins indicated with the HSE-containing region of the *HSP82* promoter was assessed by ChIP analysis using the appropriate antibodies. (B) *HSP82* expression at 25 and 37°C.

sequence (recall that the chromatin fragments analyzed have an average length of 500 bp). Thus, we interpret the ChIP data obtained in the FLAG-Rpb3 strain as indicating that the yeast *HSP82* promoter is largely occupied by a paused RNA polymerase II complex in the uninduced state, only a small fraction of which are able to transition into productive elongation complexes. HSF is also present in the uninduced state, with only a slight increase in signal intensity observed upon heat shock (Figure 6).

Based on these observations, it seems that *S.cerevisiae* *HSP82* affords an opportunity to determine if the proteasomal ATPases were present in a transcriptionally silent, but primed complex. To address this question, a ChIP experiment was again carried out. As shown in Figure 6, whereas Sug1 was barely detectable on the *HSP82* promoter at low temperature, heat shock strongly stimulated its recruitment (Figure 6A), as was also the case for the other heat shock promoters (Figures 3–5). This argues that the proteasomal ATPases are not part of the paused polymerase complex.

We also addressed mediator occupancy of the *HSP82* promoter in the induced and uninduced cells, since *Drosophila* mediator was shown to load only after heat shock (23). As is shown in Figure 6, this was also the case for the yeast *HSP82* promoter. We employed a strain expressing HA-tagged Gal11 (15,24,25), a mediator component (26,27). A ChIP assay using anti-HA monoclonal antibody revealed a low-level signal for HA-Gal11 in the uninduced state, presumably reflecting the low level of basal expression. Heat shock resulted in a 7- to 8-fold increase in this signal, indicating recruitment of the mediator to the promoter upon heat shock. We conclude that, as is also the case in *Drosophila*, the mediator is recruited subsequent to PolII itself, arguing against the existence of a monolithic RNA polymerase II holoenzyme (28) on the uninduced *HSP82* promoter. Whether or not mediator and the proteasomal ATPases are recruited to the promoter simultaneously or sequentially cannot be determined from these data, but clearly neither is part of the paused polymerase complex.

DISCUSSION

This study demonstrates that the proteasomal ATPases Sug1 and Sug2 are essential for efficient transcription of several stress-induced genes in yeast. These include both heat shock and oxidative stress-responsive genes. When Sug1 or Sug2 activity was abolished by shifting temperature-sensitive strains to the restrictive temperature, transcription of these genes was crippled (Figures 1 and 2). In contrast, inactivation of temperature-sensitive 20S core proteins had little or no detectable effect on expression of these genes. Furthermore, ChIP assays revealed the induction-dependent recruitment of the proteasomal ATPases, but not the 20S core complex, to these genes (Figures 3–6). These results parallel those obtained in our previous studies of the *GAL110* promoter (10) and argue that the proteasomal ATPases act in a non-proteolytic fashion to stimulate transcription of these genes. *In vitro* studies have revealed that this complex is required for efficient elongation (8,9) and we speculate that this is also the step for which the complex is required in stress-induced transcription.

To extend our knowledge of the role of the proteasomal ATPase complex in transcription, we also used ChIP assays to

probe the point at which it is recruited to the *HSP82* promoter. This promoter, unlike other yeast heat shock promoters (Figure 3), but like many heat shock promoters in higher organisms (20,21), is occupied to a substantial extent by a stalled RNA polymerase complex even in the uninduced state when only a low level of basal transcription is observed (Figure 6). However, little or no Sug1 or Gal11, a mediator component, was found associated with the *HSP82* promoter in this state (Figure 6). Both were recruited upon heat shock. This experiment separates the recruitment of the PolII core complex from that of the proteasomal ATPases and mediator. Since HSF clearly activates *HSP82* transcription at the level of promoter escape and elongation, this finding is consistent with our previous *in vitro* finding that the Sug proteins stimulate elongation (8,9). Whether the proteasomal ATPases function in concert with mediator, or these complexes play completely different roles in the transcription cycle remains to be determined.

In summary, these experiments show that the proteasomal ATPases are essential for the efficient transcription of several stress response genes and that they associate physically with the promoter and the gene itself. These activities and associations appear to be independent of the 20S proteasome core complex. Combined with our earlier studies of the *GAL* system (6,8,10), these studies suggest that these proteins play a direct and non-proteolytic role in the transcription of many genes.

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Conflict of interest statement. None declared.

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