Widespread, but Non-identical, Association of Proteasomal 19 and 20 S Proteins with Yeast Chromatin*†‡§

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It has recently become clear that various aspects of nucleic acid metabolism and the ubiquitin-proteasome pathway intersect in several direct and important ways. To begin to assess the scope of some of these activities in the yeast Saccharomyces cerevisiae, we assessed the physical and functional association of proteasomal proteins from both the 20 S core and 19 S regulatory particles with ~6400 yeast genes. Genome-wide chromatin immunoprecipitation analyses revealed that proteasome subunits are associated with the majority of yeast genes. Many of these associations correlated strongly with expression levels and the presence of RNA polymerase II. Although the data support the presence of the intact 26 S proteasome on most genes, several hundred yeast genes were cross-linked to either the 20 or 19 S complex but not both, consistent with some degree of independent function for the proteasomal subcomplexes.

The 26 S proteasome is responsible for the majority of nonlysosomal protein degradation in eukaryotic cells. It is comprised of two major subspecies, the 20 S proteolytic core and the 19 S regulatory particle (1). The 19 S regulatory particle (2) sits atop these openings and is thought to regulate the access of most substrates to the interior of the 20 S core and to unwind proteins for degradation. Recently work from several laboratories has uncovered a number of direct roles for the proteasome in nucleic acid metabolism, including mRNA transcription (3–6). For example, proteasome-mediated degradation is known to regulate the levels and thus the activities of many gene-specific transcription factors. The proteasome has also been implicated in recycling of transcription complexes at active promoters (7, 8). Chromatin immunoprecipitation analysis has also revealed association of the proteasome with the coding regions of transcribed genes with particularly high occupancy at regions of stalled polymerase complexes, such as sites of DNA damage or near termination sequences (9). Finally the AAA ATPases (10, 11) in the proteasome are involved in transcription (12–17) and repair (18, 19) and mediate covalent modifications of chromatin (20). In mRNA transcription, they have been shown to strongly stimulate the promoter escape and elongation steps of in vitro transcription reactions. In vivo they stimulate the recruitment of chromatin-remodeling complexes to active promoters and are essential for transcription of several GAL and heat shock genes. These activities do not depend on proteolysis and, in some cases, have been reported to occur independently of the 20 S core complex.

To evaluate the generality of these studies, which have been performed on a few genes or using in vitro methods, we undertook a global analysis of the physical and functional association of proteasomal proteins with gene-coding sequences in the yeast Saccharomyces cerevisiae. Using chromatin immunoprecipitation followed by analysis of the enriched DNAs on microarrays (the so-called ChIP3 to chip method (21, 22)) we demonstrated that proteasomal proteins are associated with the majority of yeast genes. By carrying out this experiment under different conditions that affect the expression of certain genes, we can conclude that many, but not all, of these proteasome-gene associations are transcription-dependent. Gene expression profiling suggests that many of the physical associations observed have functional consequences. Interestingly the ChIP to chip data reveal that although both 20 and 19 S proteins are present on many genes, there are hundreds that contain one or the other but not both. In addition to supporting the contention that the ATPases can function independently of the 20 S core complex (14), these data also suggest the existence of a 19 S-independent 20 S core-containing complex on many genes. These results confirm that the proteasome and/or its subcomplexes are involved broadly in nucleic acid metabolism, particularly RNA polymerase II transcription, and are consistent with the idea that these proteins play diverse roles in these processes. Finally we note that while this manuscript was in preparation another global study of proteasomal protein-gene interactions in yeast was published (23). The data discussed below agree with the conclusions of that study and also extend them in several ways.

**EXPERIMENTAL PROCEDURES**

Microarrays—Total RNA was isolated using the hot phenol method (24). mRNA was purified using the RNeasy kit (Qia-

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3 The abbreviations used are: ChIP, chromatin immunoprecipitation; Pol, polymerase.
CHROMATIN IMMUNOPRECIPITATION—Chromatin immunoprecipitations were performed as described previously (14). 1 μg of anti-Sug1 antibody and anti-20 S antibody were used to immunoprecipitate protein-DNA complexes. FLAG-tagged Pre1 was immunoprecipitated from cell lysates using anti-FLAG M2-agarose beads according to the manufacturer’s recommendations (Sigma catalogue number A2220). Primers used for ChIP analyses are provided in the supporting information.

CULTURE CONDITIONS—Strains sug1-20, pre1-1/4-1, and their parental strains were grown in YEP (yeast extract peptone) containing 2% raffinose at 37 °C and then shifted to 37 °C for 2 h. Full-length mRNA was isolated following exposure to galactose for 45 min. mRNA was isolated and reverse transcribed, and the cDNAs obtained were amplified using gene-specific primers (described in supplemental data). For treatment with proteasome inhibitor, cells were exposed to 100 μM MG132 (Peptide Institute) for 2 h and then exposed to galactose for 45 min. When working with polymerase-conditional mutants, Pol I (rpa190-2), Pol II (rpb1-1), and Pol III (rpoC160-112) strains were grown exponentially in raffinose. Cells were then shifted to 37 °C for 30 min and exposed to galactose or glucose (2%) for 30 min.

RNA ISOLATION, REVERSE TRANSCRIPTION, AND QUANTITATIVE PCR—Total RNA was isolated using the hot phenol method and purified by RNEasy kit according to the manufacturer’s recommendations (Qiagen). mRNA was converted to cDNA using Reverse Transcript System A3500 (Promega). Gene-specific primers designed to amplify the 3'-end of the genes (see supplemental data) were used in PCR. Quantitative PCRs (Q-PCR) were performed using Platinum SYBR Green Q-PCR SuperMix-UDG (Bio-Rad). The data were normalized according to the ΔΔCt method (25) and analyzed statistically using a Student’s t test. p values less than 0.05 were deemed significantly different.

GENOMIC LOCALIZATION ANALYSIS—Array-ready yeast 70-mer oligonucleotides (Operon) representing ~6400 genes were printed in triplicate spots on a glass slide. 99 spots of synthetic DNA with no appreciable sequence identity to any of the yeast open reading frames served as negative controls. Yeast cells were grown in YEP medium containing 2% raffinose to A600 of 0.4. Cells either continued to be grown in raffinose-containing medium or were exposed to galactose for 1 h at which time cells were treated with formaldehyde. Sonicated cell lysates were immunoprecipitated using polyclonal antibodies raised against Sug1, Sug2, or 20 S proteasome. FLAG beads were used to immunoprecipitate FLAG-tagged Pre1-DNA complex. DNA retrieved following elution was repaired using T4 DNA polymerase (New England Biolabs catalogue number M0203S). Repaired DNA was ligated to annealed linkers using 1 unit of T4 DNA ligase at 16 °C for 16 h (New England Biolabs catalogue number M0202S). For all arrays ChIP DNA was labeled with Cy5, and input DNA was labeled with Cy3 (Amersham Biosciences catalogue numbers PA53021 and PA55021, respectively). The data from the triplicate experiments were median-scaled and normalized to each other. 20 pmol of Cy3- and Cy5-labeled DNA were mixed and hybridized to a spotted microarray representing ~6400 genes. Hybridization was performed in 3× SSC, 0.1% SDS, 20 pmol of denatured probe, and 10 μg of tRNA at 50 °C for 20 h. Slides were washed, dried, and scanned to obtain an image and the intensities associated with each of the spots. Raw intensity from each experiment was log10 transformed. To obtain a Z-score we subtracted the overall average gene intensity from the raw intensity data for each gene. This value was divided by the standard deviation of all the measured intensities. The following formula was used. Z score = (intensityX – mean intensity[X1,...,Xn])/S.D.[X1,...,Xn] where X is any gene represented in the array and X1,...,Xn is the cumulative measure of all the genes represented in the array.

RESULTS

EXTENSIVE ASSOCIATION OF PROTEASOMAL PROTEINS WITH YEAST GENES—We have reported previously efficient conditions for the analysis of proteasomal protein-chromatin interactions using ChIP analysis and antibodies against Sug1/Rpt6, Sug2/Rpt4, and Pre1 (9, 14). These conditions were used in a global analysis of proteasome-chromatin interactions. For these experiments a spotted DNA microarray comprised of 70-residue oligonucleotide probes complementary to the coding regions of 6400 S. cerevisiae genes in triplicate was used. As a control to test the accuracy of genome-wide location analysis, we amplified a 500-bp fragment from the 3'-region of the GAL4 gene. This DNA was labeled using Cy5 and mixed with an equivalent amount of yeast genomic DNA sheared to an average length of 500 bp and labeled with Cy3. Analysis of the array following hybridization revealed three features with saturated intensity in the Cy5 channel. All three features corresponded to Gal4 cDNA. There were 21 other features above the background, but all of them were either at or below 1.5-fold over Cy3 (data not shown). We concluded that the conditions used were suitable for global analysis.

The focus of the first set of experiments was the proteasomal ATPases Sug1/Rpt6 and Sug2/Rpt4. Following growth on raffinose to A600 = 0.4, cells were exposed to galactose for 2 h at which time they were treated with formaldehyde. After isolation of total chromatin and shearing by sonication, the cross-linked species of interest were immunoprecipitated using polyclonal antibodies raised against Sug1/Rpt6 or Sug2/Rpt4. These are highly specific antibodies that recognize only a single band from a crude yeast extract in a Western blot (14). The DNA retrieved following reversal of the cross-link was amplified and labeled with Cy5 (red) dye. A control sample equivalent to the
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![Graph](https://via.placeholder.com/150)

**FIGURE 1. Assessment of the physical association of 19 S regulatory particle (Sug1 and Sug2) and/or the 20 S subunits with the genome by ChIP to chip assays.** A–D, depiction of the percentage of the genome associated with the indicated factors and the degree of enrichment of fragments relative to the total population. The "Pre1" data (C) are from an experiment using a strain expressing FLAG epitope-tagged Pre1 and an antibody raised against the epitope. The "20 S proteasome" data (D) are from an experiment using a polyclonal antibody against 20 S proteosome.

input DNA for the immunoprecipitation was amplified and labeled with Cy3 (green) dye. At least four independent ChIPs and microarray hybridizations were performed for each protein.

These experiments revealed that Sug1/Rpt6 was detectable on ∼68% of the yeast open reading frames examined by the criterion of an enrichment of the immunoprecipitated DNA fragment of more than 1.5-fold over the input. For 26% of those analyzed, the enrichment was greater than 3-fold (Fig. 1A). Sug2 association with yeast chromatin was almost identical to that of Sug1 (Figs. 1B and 2A). This was expected because Sug1 and Sug2 are tightly associated and function in concert with one another (26, 27). We conclude that Sug1/Rpt6 and Sug2/Rpt4 are present together on a large number of yeast genes.

We next addressed the localization of the 20 S core complex on the yeast genes. In the first such experiment, we used a strain in which the Pre1 protein was tagged with the FLAG epitope (28), and a monoclonal anti-FLAG antibody was used in the immunoprecipitation. Hybridization of the processed samples to the yeast microarray revealed that more than 60% of the coding regions were enriched in the Pre1-associated fraction (Fig. 1C). To ensure that the epitope tag did not affect the localization of the 20 S complex, this experiment was repeated with an untagged strain using polyclonal antibodies that had been raised using the entire 20 S complex as the antigen (Fig. 1D). We conclude that the 20 S proteasome core complex associates extensively with yeast genes.

**Gene Association Patterns of 20 S Core and the 19 S ATPase Are Similar but Distinct**—Fig. 2, A–C, shows the relationships between the various ChIP to chip data sets in the form of scatter plots. As mentioned above, the data sets for Sug1/Rpt6 and Sug2/Rpt4 were almost identical with a correlation coefficient between the two experiments of 0.96 (Fig. 2A). As anticipated, the data obtained from the experiments that utilized antibody against native Pre1 or anti-FLAG antibody and a FLAG-tagged PRE1 strain yielded similar profiles with a correlation coefficient of 0.9 (Fig. 2B).

In contrast, comparison of the Sug1/Rpt6 and 20 S core data sets show that although most of the gene associations detected are common to both proteins, many genes are associated with only the ATPase or the 20 S component but not both. These genes appear as clearly off-diagonal features on the scatter plot shown in Fig. 2C. For example, using a stringent cutoff of at least a 4-fold greater ratio in normalized signal intensity (indicated by the diagonal bars in Fig. 2C), 107 genes show strong preferential association with Pre1 over Sug1, and 152 genes show preferential association with Sug1 over Pre1. Using a less stringent 2-fold cutoff, we found 756 genes preferentially associated with Sug1, 452 genes preferentially associated with 20 S, and 3516 genes associated with both (not shown).

Fig. 2D considers all of the genes in the data sets that are at least 4-fold enriched for one or both of the proteasomal proteins (i.e., the strongest signals form the array). Remarkably about one-third of these genes associate with Sug1 or Pre1 preferentially, whereas two-thirds of them show strong association with both proteins. This relatively high fraction of genes associated with one, but not both, proteasomal subcomplexes suggests that both the ATPase and 20 S complexes may play significant roles independent of one another in the nucleus.

**Focused Analysis of Individual Genes Confirms the Association of Independent Proteasome Subcomplexes with Several Genes**—To validate these microarray-based findings, standard ChIP assays were done using each of the aforementioned antibodies, and the enriched DNA was probed using gene-specific primers for genes shown by the microarray-based data to be bound to Sug1 (ERV46 and SEC62), Pre1/20 S (GCY1, TUB2, ARNI, AGE2, HOL1, and APG10), or both proteins (SVS1, TAF9, TAF13, MUD2, BUL1, SRN2, and SPB1) (see Fig. 2E). ERV46 and SEC62, for example, showed significant association with only Sug1, not Pre1/20 S. In contrast, GCY1, TUB2, ARNI, AGE2, HOL1, and APG10 were found to be associated with Pre1/20 S but not Sug1. Both Sug1 and Pre1/20 S were associated with the SVS1, TAF9, TAF13, MUD2, BUL1, SRN2, and SPB1 genes exactly as predicted by the microarray data. Moreover neither the Sug1 or Pre1/20 S particles appeared to be associated with TIM22 or GLK1, again consistent with the microarray results.

Another check of the microarray data was conducted in which we performed standard ChIP assays using the anti-Sug1 and anti-20 S antibodies and then probed the enriched DNA using specific PCR primers that tile the region of the yeast
FIGURE 2. Distribution from ChIP to chip assays of the 19 and 20 S components and direct testing by ChIP analysis. Shown is scatter plot analysis comparing data obtained from genomic localization studies for Sug1 and Sug2 (A), Pre1 and 20 S complex (B), and Sug1 and 20 S particle (C). The off-diagonal spots in C represent features that are uniquely bound by either Sug1 or 20 S core particle. D, Venn diagram depicting distribution of genes physically associated with 20 S core, 19 S regulatory particle, or both components. Genes that showed at least a 4-fold preferential association for Sug1, Sug2, FLAG-Pre1, and 20 S proteasome over the input DNA control were scored in this analysis. E, ChIP assays on a spectrum of genes to verify results from genomic studies. F, location of primers used for tiling the ARN1 locus. G, quantitative PCR analyses for Sug1 and 20 S proteasome association (depicted by squares and circles, respectively). These data represent three independent ChIP experiments.
genome that includes the ARN1 gene (Fig. 2F). Based on the global analysis, the 3' end of this gene associates with 20 S core but not 19 S regulatory particle. The two neighboring genes, YHL041W and YHL039W, are separated by 3.9 and 1.2 kb, respectively. We tiled this region with seven primer pairs (Fig. 3F) and assayed for binding of Sug1 and 20 S complex (Fig. 3G). The data show that promoters and the 3'-region of both YHL041W and YHL039W recruited Sug1 and 20 S core complex (primers 2 and 3 and primers 8 and 9). The intergenic regions did not recruit either of the complexes (primers 1, 4, and 7). Although the ARN1 promoter associated with Sug1, association with 20 S particle was just below the threshold (primer 6). The 3'-end of ARN1 was enriched for 20 S proteins but not Sug1 (primer 5).

These data would seem to constitute strong evidence for independent 20 S core-gene or proteasomal ATPase-gene associations at many genes and 26 S proteasome-gene associations at even more. However, there are two alternative possibilities that, although less likely, are difficult to rule out. On one hand, it is possible that some of the genes represented in the central region of the Venn diagram in Fig. 2D might represent sites of independent gene-ATPase and gene-20 S complexes in the same region of the gene rather than association of the 26 S complex. Thus, we may be underestimating the number of independent subcomplex-gene associations. In the extreme, it is even possible that all of these genes are occupied by independent ATPase and 20 S complexes and that the 26 S proteasome does not associate with the yeast genome. On the other hand, there is a scenario in which the data do not demand the existence of independent 20 S core-gene associations. In this view, one might imagine that when the proteasomal ATPases are engaged directly with the gene, they cross-link well (9, 12), but if the entire 26 S proteasome is associated with the gene, the 20 S proteins might cross-link efficiently, whereas the 19 S ATPases might not. Finally these two models are not mutually exclusive.

To address these alternative models, we carried out focused ChIP assays in a sug1-20 strain (29), which carries a highly temperature-sensitive form of this protein (12). We hypothesized that if coincident 20 S core and 19 S ATPase occupancy truly reflects the presence of the 26 S proteasome on a gene, then the ChIP signals for Pre1 and Sug2, as well as for Sug1, should be lost upon shifting to the non-permissive temperature. However, if these signals represent independent association of a 20 S complex and an ATPase complex, then unfolding of the temperature-labile Sug1-20 protein should abolish the Sug1 and Sug2 ChIP signals but not the 20 S core signal.

The results are shown in Fig. 3. We examined genes that are co-occupied by both the 19 S ATPases and the 20 S core complex as well as genes that reveal little or no Sug1 and Sug2 occupancy but show a robust ChIP signal for the 20 S core particle. As expected, inactivation of the Sug1-20 protein at the restrictive temperature abolished the association of Sug1-20 protein and the Sug2 protein with most of the genes examined (Fig. 3, A and B). It is curious, however, that strong signals for Sug1-20 and Sug2 occupancy remained on the GAL4 and GAL80 genes and that a weak signal remained on GAL6. We do not understand the molecular basis of this observation, but it suggests the existence of Sug-containing complexes in some sort of special environment that stabilizes the temperature-sensitive protein.
The signals due to 20 S-gene association responded to Sug1-20 protein inactivation in a different manner than did those due to Sug2 association. Although the intensity corresponding to all the 20 S core signals were lower at the restrictive temperature (Fig. 3C) the drop was much more dramatic at some genes than others. For example, a robust signal representing 20 S core association with the ARN1 gene was observed in the sug1-20 strain at 25 °C. This signal was diminished somewhat (Fig. 3C) but remained easily detectable at the non-permissive temperature (Fig. 3A). Along with the lack of any observable Sug1 or Sug2 association, this strongly suggests that the 20 S core ChIP signal on the 3' -end of ARN1 indeed represents, at least in part, a 19 S-independent 20 S complex-gene association.

Like ARN1, GALS and GAL7 showed, at most, weak Sug1 and Sug2 association but strong 20 S core association at the permissive temperature (Fig. 3). In stark contrast, however, association of 20 S particle with these genes proved to be highly Sug1-dependent. Shifting to the non-permissive temperature in the sug1-20 strain resulted in the loss of most (GALS) or essentially all (GAL7) of the detectable gene-20 S complex association. Thus, these data cannot be explained by a 19 S complex-independent association of 20 S core with these genes despite the weak or non-existent ChIP signals for Sug1 and Sug2 on these genes. We suggest that the simplest explanation is that these genes are associated with the full 26 S proteasome, but as suggested above, the geometry of this interaction is such that the 20 S proteins cross-link to the DNA (directly or indirectly) efficiently, but the Sug proteins do not. An alternative possibility is that the epitopes recognized by the Sug1 and Sug2 antibodies are not exposed in these particular cross-linked products, although this seems less likely because polyclonal antibodies were used in the ChIP assays.

A third class of genes is represented by GAL1, GAL10, and GCY1. At the permissive temperature, these were associated with both the ATPases (with GAL1 and GAL10 showing exceptionally strong signals) and the 20 S complex at 25 °C. Upon shifting the temperature to 37 °C, all of these signals were lost. This is consistent with the 20 S core signal being due largely to association of the full 26 S proteasome with these genes. The Sug-gene associations could be due to the same 26 S proteasome-gene association, but we tend to favor the model in which these genes are associated both with the 26 S proteasome and, additionally, a 20 S core-independent ATPase complex and that (as discussed immediately above) the Sug protein-DNA associations are not detected readily in the context of the 26 S proteasome.

Finally the association of the proteasomal proteins with the GAL4 and GAL80 genes merit mention. As stated above, these loci are unusual in that only a modest percentage of the Sug1-gene and Sug2-gene association was lost upon shifting the sug1-20 strain to the non-permissive temperature (Fig. 3, A and B). In contrast, however, the 20 S-GAL4 and 20 S-GAL80 signals, which were also prominent at 25 °C, were abolished at 37 °C, showing clear dependence on functional Sug1-20. We suggest that the simplest explanation for this result is co-habitation of the GAL4 and GAL80 genes by the 26 S proteasome, whose association is sensitive to denaturation of Sug1-20 but contributes little intensity to the Sug1 and Sug2 ChIP signals, with some unusually stable Sug1/Sug2-containing, but 20 S proteasome-independent, complex that contributes most of the ChIP signal.

In summary, these experiments argue strongly that yeast genes can be associated with the 26 S proteasome, a 19 S-independent 20 S complex, or a 20 S core-independent proteasomal ATPase complex. The data also indicate that more than one of these different complexes can associate with a gene simultaneously.

**Inactivation of Proteasomal Proteins Has a Widespread Effect on Gene Expression**—The ChIP to chip assays revealed physical, not functional, interactions. Therefore we used genome-wide expression profiling to determine the effects in inactivating either Sug1 or the 20 S subunits Pre1 and Pre4. Strains bearing the sug1-20 or the pre1-1/pre4-1 mutations and the respective parental strains were grown in medium containing 2% raffinose at 25 °C and then shifted to 37 °C for 2 h. Following exposure to galactose for 45 min, mRNA was isolated and labeled with Cy3 fluorescent dye. Affymetrix S98 microarrays were used to profile global expression patterns of sug1-20 and pre1-1/pre4-1 strains at the restrictive temperature, and each was compared with that of the corresponding wild-type strain. Most of the transcriptome was affected by inactivation of either the 19 or 20 S subunits (Fig. 4). Transcripts from more than 4500 of the 6400 genes represented on the chip and 6172 that gave measurable signals were altered by more than 2-fold by inactivation of the Sug1 or Pre1 and Pre4 proteins. 1389 genes were up-regulated by temperature shift in the sug1-20 strain. These included genes encoding proteasome subunits, ubiquitin-conjugating enzyme (Ubc5), and diverse genes involved in mitochondrial functions known to be up-regulated in the face of stress (30, 31). More than 1600 genes were up-regulated in the pre1-1/pre4-1 strain upon temperature shift. These included genes involved in protein folding, proteasomal subunits, mitochondrial proteins, stress sensors, and DNA repair genes such as RAD7, RAD16, RAD59, and HSH6. Of the up-regulated genes, transcription from 60% was enhanced in both temperature-sensitive strains (Fig. 4A). Intriguingly 14% of genes were uniquely up-regulated only in the sug1-20 strain, whereas another 26% were up-regulated only in the pre1-1/pre4-1 strain.

Transcripts from a large fraction of the genes in *S. cerevisiae* were inhibited by inactivation of the mutant proteins (Fig. 4B). Of the 2751 genes that were down-regulated, 63% were commonly affected by mutations in both subunits. Expression of 16% of the genes was decreased only in the sug1-20 strain, whereas 21% were down-regulated in the pre1-1/pre4-1 strain only.

In summary, ~70% of the genomic transcripts were affected at least 2-fold by inactivation of either 19 or 20 S subunits. Importantly ~40% of the up-regulated genes and ~50% of the down-regulated genes were discordant. That is, expression of the gene was unchanged when one subunit was inactivated and up- or down-regulated when the other subunit was inactivated. We also measured effects on gene expression in response to the proteasome protease inhibitor MG132. As might be expected, the pattern of regulation by MG132 inhibition more strongly resembled that of inactivation of the 20 S subunit than that of the
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FIGURE 4. Differential effects on transcription by inactivation of the 19 or 20 S proteasome subunits. A, Venn diagram depicting the distribution of genes that were found to be up-regulated by microarray analysis following inactivation by temperature shift in a pre1-1/pre4-1 strain compared with a sug1-20 strain. Both strains were normalized to the parental strain at the restrictive temperature. B, same as in A but the diagram depicts genes that were down-regulated. C–F, some genes are inversely affected by temperature inactivation of sug1-20 versus pre1-1, 4-1. C, Venn diagram depicting distribution of transcripts that are down-regulated in pre1-1/4-1 but up-regulated in sug1-20. Expression was normalized to the parental strains at the restrictive temperature. D, same as in A but depicting genes up-regulated in pre1-1, pre4-1 and down-regulated in sug1-20. E, validation of gene expression of the nine oppositely regulated genes by quantitative PCR. RNA levels for the nine inversely regulated genes were compared between the sug 1-1 strain and its parent. F, the RNA levels of the pre1-1, pre4-1 strain and its parent are compared. In G, an ise1 mutant strain was assayed for the same genes after treatment with the proteasome inhibitor MG132 or vehicle. The HSP26 gene was assayed as a control because it is known to be induced by proteasome inhibition. A signal was not detectable for HSP26 in the sug1-20 strain. At least three independent experiments were performed for these analyses. C, control.

19 S subunit. However, only ~900 genes were up- or down-regulated by treatment with inhibitor; this is ~23% of that affected by inactivation of the 20 S core (data not shown).

These data are consistent with the idea that the proteasomal ATPases and the 20 S core complex can play distinct roles at different genes as suggested by the physical association data presented above as well as in previous studies (12, 14, 16). Thus, these data suggest that many of the physical interactions observed in the ChIP to chip assay probably have functional significance as well. However, proteasome inactivation will obviously result in many indirect effects due to altered protein levels, etc. so it is difficult to draw direct correlations between sites of binding and function. In addition, one cannot rule out the possibility that some of the differences between the effects of inactivating Pre1 and Pre4 or Sug1 could have been due to different efficiencies of protein denaturation. In this vein it is interesting that although a great many genes were up- or down-regulated by inactivation of the 20 S proteasome or Sug1 without being affected by the other, we could identify only nine genes whose transcription decreased as a result of Sug1 inactivation but increased in response to Pre1/Pre4 inactivation or vice versa. As shown in Fig. 4C, transcripts from three genes were found to be decreased by more than 2-fold in pre1-1/4-1 but stimulated by more than 2-fold in sug1-20 (Fig. 4D). The expression of six genes was up-regulated in the pre1-1/4-1 but down-regulated in sug1-20 (Fig. 4D). To confirm that these genes were indeed affected oppositely and as a rigorous test of our microarray assignments we analyzed each by reverse transcription PCR. The expression of GSC2, HKR1, MLP1, RPA190, YPR157W, and COX1 was reduced in the sug1-20 strain relative to the wild type, whereas their levels were increased in the pre1-1/pre4-1 strain relative to wild type (Fig. 4, E and F). Note that HSP26, which is a marker for stress, was not induced in the sug1-20 strain even at the non-permissive temperature. Transcripts from LEU2, TKL2, and YBR117C were stimulated by inactivation of Sug1 but decreased by inactivation of Pre1/Pre4 (Fig. 4, E and F). As expected, the effect of MG132 on these genes was similar to that of thermal inactivation of the temperature-sensitive 20 S subunits (Fig. 4G).

Associations between Regulated Genes and Proteasomal Proteins Correlate with Gene Expression—We and others have found that the proteasome is involved in RNA polymerase II transcription in a variety of ways, but these previous studies did not make clear the scope of these mechanisms. To probe this issue, we again used genome-wide ChIP analysis to explore how proteasomal protein-gene interactions are modified in response to changes in extracellular environment. Specifically we collected ChIP to chip data sets for Sug1 and the 20 S core
complex from yeast grown in either galactose or glucose, conditions that are known to result in alterations in the expression of several genes. The data are plotted in Fig. 5 in such a manner as to allow ready visual comparison between the levels of these proteins on all of the yeast genes in each medium. Specifically we plotted the ratios of enrichment of a given gene locus in glucose versus galactose. As can be seen, the large majority of gene-proteasomal protein associations are not affected significantly by the change in carbon source and are seen as a tight cluster in the center of the graph (Fig. 5). However, two clusters were clearly segregated from the center. This first set was comprised of 49 genes that showed significantly higher association with both Sug1 and the 20 S subcomplex in galactose than in glucose. The expression of 41 of these genes has been shown previously to be induced in galactose (32, 33). On the other side of the spectrum were genes that were more highly associated with Sug1 and 20 S complex in glucose than in galactose. In this list were 23 genes, 19 of which are known to be up-regulated in glucose versus galactose. As expected (Fig. 6C), Association of both Sug1 and 20 S proteasome was drastically reduced with both galactose- and glucose-induced genes at the restrictive temperature. As controls, we also examined the effect of inactivating temperature-sensitive Pol I or Pol III mutants. As shown in Fig. 6, D and E, neither the levels of transcription nor the extent of interaction of the proteasomal proteins with the galactose- and glucose responsive genes was affected strongly in these strains, demonstrating a specific effect of Pol II inactivation.

**DISCUSSION**

In this study we used genomic techniques to address global interactions between different proteasomal proteins and most yeast genes. We found that the coding regions of the majority of yeast genes are physically associated with the 19 and/or 20 S components (Fig. 1) as judged using the criterion of at least a 1.5-fold enrichment of a gene in the immunoprecipitated sample relative to the control (Fig. 1). Even a much more stringent cutoff of a 3-fold or greater enrichment argues that about a thousand yeast genes are associated with proteasomal proteins. It is of note that there are 15,000–30,000 proteasomes in the yeast nucleus (35). Theoretically a proteasome could be associated every 500–1000 bp on the genome or even more frequently if the subunits disassociate. This physical association of proteasomal proteins could represent a direct or indirect interaction with the DNA itself because formaldehyde treatment produced protein-DNA, as well as protein-DNA, cross-links. It seems likely that many of these physical association reflect functional interactions because inactivation of temperature-sensitive proteasomal proteins had extremely broad effects on yeast gene expression, approaching those of inactivating a general transcription factor or an RNA polymerase II subunit (36), although clearly not all genes were affected (Fig. 5). These results support and extend previous studies that examined the effect of adding proteasome inhibitors to sensitive strains of yeast (31, 37). Of course, it is impossible to rule out indirect effects of proteasome inactivation on gene expression, so we have not made an attempt to correlate in detail the expression and ChIP to chip data sets.

It is also worthwhile to comment on the relevance of these data to assessing the role of the full 26 S proteasome in gene expression. This has been a topic of great interest recently (3). Specifically evidence has been presented that activators and other transcription factors could be recycled regularly in a proteasome-dependent fashion to achieve high level gene expression (7, 8, 15, 38). Our analysis shows that although the expression of many genes were affected by inactivation of the 20 S proteins Pre1 and Pre4, a large number were not (Fig. 5; also see Refs. 31 and 37). These included the highly induced GAL genes. Thus, we suggest that proteolytically
linked recycling of activators will be an important process for the expression of some, but not all, genes.

Comparison of the ChIP to chip data sets revealed that about one-third of the genes associated with either Sug1/Sug2 were not detectably associated with the 20 S subunit and vice versa (Fig. 2D). This provides strong support for the idea that independent subcomplexes of the proteasome are present on some genes and presumably mediate some function at many of these loci. While this manuscript was in preparation, Silver and co-workers (23) published a global study of yeast proteasome-genome interactions. Their localization data also suggested the existence of a 19 S-independent 20 S complex and the 26 S proteasome. These findings serve as an important caution in the interpretation of ChIP data where the proteasome is concerned and suggest that a standard approach should be to use inactivation of a particular subunit through temperature-sensitive mutations or (in mammalian cells) small interfering RNA knockdowns to differentiate between signals due to intact 26 S proteasome or subcomplexes thereof.

Although roles for a 20 S core-independent proteasomal ATPase complex (APIS (14)) and the full 26 S proteasome in transcription and certain other aspects of DNA metabolism have been demonstrated previously (7, 9, 12–14, 19, 38), the function of the 19 S-independent 20 S complex is unclear and will be the subject of future studies. However, it is important to point out that there is precedence for 19 S-independent functions of the 20 S proteasome. In mammalian cells the “immunoproteasome” is an alternative complex that contains the 20 S core but substitutes an alternative cap complex for the 19 S regulatory particle (39). Although alternative caps for the 20 S core complex have not yet been described in yeast, it is possible that they exist. Moreover there is biochemical evidence that the 20 S core complex can act independently of any cap complex on
some unfolded proteins. For example, the natively disordered cyclin-dependent kinase inhibitor p21 and α-synuclein are proteolyzed efficiently by 20 S core particle in the absence of 19 S complex (40). Also in yeast the proteasome has been shown to be disassembled into 20 S and 19 S regulatory subcomplexes when cells reach stationary phase (41) providing some precedent for a 19 S-independent 20 S complex, although it is proteolytically inactive in this state (41). Finally Babbitt et al. (42) have published evidence showing dissociation of the 19 and 20 S subunits of the proteasome during the catalytic cycle, supporting the idea that these species could have physically independent functions in the nucleus.

Finally analysis of the association of the Sug proteins and the 20 S complex with various genes whose transcription levels are sensitive to the nature of the carbon source (galactose or glucose) revealed a strong correlation between the association of each protein with the gene, its level of transcription, and its occupancy by RNA polymerase II (Fig. 6). This correlation was especially strong for the Sug1 protein. These observations are in agreement with previous reports that revealed a non-proteolytic role for the ATPases in the transcription of several yeast genes (14–16) and the observation that sites of proteasome-gene interactions correspond to sites of observed RNA polymerase II-gene interactions (9). However, we do not propose that all of the proteasomal protein-gene interactions observed in the global analysis (Figs. 1 and 2) play a role in transcription because comparison of the ChIP to chip data and the gene expression data revealed that many genes clearly associated with these proteins are not transcribed at high levels. A similar conclusion was reached by Silver and co-workers (23) in their study; and the staff of the University of Texas Southwestern Medical Center array core for providing assistance with hybridization in the Affymetrix microarray experiments.

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REFERENCES
1. Baumeister, W., Walz, J., Zuhl, F., and Seemuller, E. (1998) Cell 92, 367–380
2. Glickman, M. H., Rubin, D. M., Fried, V. A., and Finley, D. (1998) Mol. Cell. Biol. 18, 3149–3162
3. Collins, G. A., and Tansey, W. P. (2006) Curr. Opin. Genet. Dev. 16, 197–202
4. Conaway, R. C., Brower, C. S., and Conaway, J. W. (2002) Science 296, 1254–1258
5. Ottosen, S., Herrera, F. J., and Triezenberg, S. J. (2002) Science 296, 479–481
6. Lipford, J. R., and Deshaies, R. J. (2003) Nat. Cell Biol. 5, 845–850
7. Reid, G., Hübner, M. R., Metivier, R., Brand, H., Denger, S., Manu, D., Beaudouin, J., Ellenberg, J., and Gannon, F. (2003) Mol. Cell 11, 695–707
8. Nawaz, Z., and O’Malley, B. W. (2004) Mol. Endocrinol. 18, 493–499
9. Gillette, T. G., Gonzalez, F., Delahodde, A., Johnston, S. A., and Kodadek, T. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 5904–5909
10. Confloniferi, F., and Duguet, M. (1995) BioEssays 17, 639–650
11. Sauer, R. T., Bolon, D. N., Burton, B. M., Burton, R. E., Flynn, J. M., Grant, R. A., Hersch, G. L., Joshi, S. A., Kenniston, J. A., Levenchoek, I., Neher, S. B., Oakes, E. S. C., Siddiqui, S. M., Wah, D. A., and Baker, T. A. (2004) Cell 119, 9–18
12. Ferdous, A., Gonzalez, F., Sun, L., Kodadek, T., and Johnston, S. A. (2001) Mol. Cell 7, 981–991
13. Ferdous, A., Kodadek, T., and Johnston, S. A. (2002) Biochemistry 41, 12798–12805
14. Gonzalez, F., Delahodde, A., Kodadek, T., and Johnston, S. A. (2002) Science 296, 548–550
15. Lee, D. H., Zhkova, E., Li, B., Pattenden, S. G., Tansey, W. P., and Workman, J. L. (2005) Cell 123, 423–436
16. Sulahian, R., Sikder, D., Johnston, S. A., and Kodadek, T. (2006) Nucleic Acids Res. 34, 1351–1357
17. Morris, M. C., Kaiser, P., Rudyak, S., Baskerville, C., Watson, M. H., and Reed, S. I. (2003) Nature 423, 1009–1013
18. Russell, S. J., Reed, S. H., Huang, W., Friedberg, E. C., and Johnston, S. A. (1999) Mol. Cell 5, 687–696
19. Gillette, T. G., Huang, W., Russell, S. J., Reed, S. H., Johnston, S. A., and Friedberg, E. (2001) Genes Dev. 15, 1528–1539
20. Zhkova, E., and Tansey, W. P. (2004) Mol. Cell 13, 435–442
21. Sikder, D., and Kodadek, T. (2005) Curr. Opin. Chem. Biol. 9, 38–45
22. Horak, C. E., and Snyder, M. (2002) Methods Enzymol. 350, 469–483
23. Auld, K. L., Brown, C. R., Casolari, J. M., Komilli, S., and Silver, P. A. (2006) Mol. Cell 21, 861–871
24. Schmitt, M. E., Brown, T. A., and Trumpower, B. L. (1990) Nucleic Acids Res. 18, 3091–3092
25. Livak, K. J., and Schmittgen, T. D. (2001) Methods 25, 402–408
26. Swaffield, J. C., Bromberg, J., and Johnston, S. A. (1992) Nature 357, 698–700
27. Glickman, M. H., Rubin, D. M., Fu, H., Larsen, C. N., Coux, O., Wefes, I., Pfeifer, G., Cjeka, Z., Vierstra, R., Baumeister, W., Fried, V., and Finley, D. (1999) Mol. Biol. Rep. 26, 21–28
28. Verma, R., Chen, S., Feldman, R., Schielz, D., Yates, J., Dohnen, J., and Deshaies, R. J. (2000) Mol. Cell 11, 3425–3439
29. Xu, Q., Singer, R. A., and Johnston, G. C. (1995) Mol. Cell. Biol. 15, 6025–6035
30. Jelinsky, S. A., Estep, P., Church, G. M., and Samson, L. D. (2000) Mol. Cell. Biol. 20, 8157–8167
31. Fleming, I. A., Lightcap, E. S., Sadis, S., Thoroddsen, V., Bulawa, C. E., and Blackman, R. K. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 1461–1466
32. Ren, B., Robert, F., Wyrick, J. J., Pacheco, R., Jennings, E. G., Simon, I., Zeitlinger, J., Schier, J., Hannett, N., Kornberg, T. L., Wilson, C. J., Bell, S. P., and Young, R. A. (2000) Science 290, 2306–2309
33. Holstege, F. C. P., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Bell, S. P., and Young, R. A. (2000) Mol. Cell 5, 687–696
34. Nonet, M. L., Scafe, C., Sexton, J., and Young, R. A. (1999) Mol. Biol. Rep. 26, 4587–4590
35. Russell, S. J., Steger, K., and Johnston, S. A. (1999) J. Biol. Chem. 274, 21943–21952
36. Thompson, C. M., and Young, R. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4587–4590
37. Lipford, J. R., and Deshaies, R. J. (2005) Nature 438, 113–116
38. Tanaka, Y., and Kasahara, M. (1998) ImmunoL. Rev. 163, 161–176
39. Liu, C.-W., Corboy, M. J., DeMartino, G. N., and Thomas, P. J. (2003) Science 299, 408–411
40. Bajorek, M., Finley, D., and Glickman, M. H. (2003) Curr. Biol. 13, 1140–1144
41. Babbitt, S., Kiss, A., Deffenbaugh, A. E., Chang, Y.-H., Bailly, E., Erdjument-Bromage, H., Tempst, P., Buranda, T., Sklar, L. A., Baumler, J., Gogol, E., and Skowyra, D. (2005) Cell 121, 553–565