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Global Position and Recruitment of HATs and HDACs in the Yeast Genome

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Summary

Chromatin regulators play fundamental roles in the regulation of gene expression and chromosome maintenance, but the regions of the genome where most of these regulators function has not been established. We explored the genome-wide occupancy of four different chromatin regulators encoded in *Saccharomyces cerevisiae*. The results reveal that the histone acetyltransferases Gcn5 and Esa1 are both generally recruited to the promoters of active protein-coding genes. In contrast, the histone deacetylases Hst1 and Rpd3 are recruited to specific sets of genes associated with distinct cellular functions. Our results provide new insights into the association of histone acetyltransferases and histone deacetylases with the yeast genome, and together with previous studies, suggest how these chromatin regulators are recruited to specific regions of the genome.

Introduction

Chromatin regulators play important roles in a broad range of biological activities such as transcription, replication, recombination, and repair (reviewed in Felsenfeld and Groudine, 2003). Chromatin regulators fall into two classes. One of these covalently modifies histones by addition or removal of chemical residues on histones (Wu and Grunstein, 2000; Jenuwein and Allis, 2001; Kouzarides, 2002). Members of the other class consist of the ATP-dependent nucleosome-remodeling complexes that noncovalently modify and reposition nucleosomes (Kingston and Narlikar, 1999; Kornberg and Lorch, 1999; Vignali et al., 2000; Urnov and Wolffe, 2001).

Covalent modifications of nucleosomes occur predominantly in the N-terminal tails of histones and include lysine acetylation, methylation, and ubiquitination; arginine methylation; and serine phosphorylation (Berger, 2002). The addition and removal of chemical moieties is a dynamic process that can influence chromatin function by different mechanisms. Histone modifications can generate sites for interaction with additional proteins (Strahl and Allis, 2000; Jenuwein and Allis, 2001). For example, proteins with bromo-, chromo-, and SANT domains interact with modified histones (Bannister et al., 2001; Hassan et al., 2002). Histone modifications may also directly affect the condensation of chromatin (Schwarz et al., 1996; Tse et al., 1998a, 1998b), possibly by modulating exposed charge patches on nucleosome surfaces (Durrin et al., 1991; Dou and Gorovsky, 2000; Ren and Gorovsky, 2001).

Chromatin regulators do not have sequence-specific DNA-recognition properties of their own, but appear to be recruited to specific locations in the genome by interacting with other proteins (reviewed in Cosma et al., 1999; Hampsey and Reinberg, 2003). For example, studies on individual genes suggest that transcriptional activators recruit the histone acetylases Gcn5 and Esa1 and the chromatin-remodeling complex Swi/Snf to the promoters of protein-coding genes (see Peterson and Herskowitz, 1992; Brownell et al., 1996; Smith et al., 1998; Cosma et al., 1999; Reid et al., 2000; Bhaumik and Green, 2001; Larschan and Winston, 2001; Nourani et al., 2001). Transcriptional activators also recruit the general transcription factor TFIID, whose large subunit TAF1 is a histone acetylase (Mizzen et al., 1996). Some activators and repressors can recruit the RSC chromatin-remodeling complex to promoters (Ng et al., 2002). Recruitment of the Set1 histone methyltransferase, which is responsible for histone H3K4 trimethylation at nucleosomes located near the start site of transcription, is dependent on components of the transcription apparatus (Bernstein et al., 2002; Santos-Rosa et al., 2002; Ng et al., 2003; Krogan et al., 2003).

Knowledge of the genome-wide location of a chromatin regulator has the potential to (1) determine whether the regulator is associated with all or a subset of genes transcribed by specific RNA polymerases (Lieb et al., 2001; Wang et al., 2002; Ng et al., 2002, 2003), (2) reveal whether a regulator is associated with the promoter or transcribed region of genes (Wang et al., 2002; Ng et al., 2002, 2003), (3) lay the foundation for studies that reveal the factors responsible for recruiting the regulator to specific regions of the genome (Ng et al., 2002, 2003), and (4) extend to many genes a model for regulator function based on previous studies of one or a few genes (Lieb et al., 2001). Thus far, however, the genome-wide occupancy of only a few chromatin regulators has been determined (Lieb et al., 2001; Wang et al., 2002; Kurdistani et al., 2002; Ng et al., 2002, 2003). We report here genome-wide location results for two HATs and two HDACs in yeast. Our results show that the HATs Gcn5 (SAGA) and Esa1 (NuA4) are generally recruited to the promoters of active protein-coding genes, whereas the HDACs Rpd3/Sin3 and Hst1 are targeted to specific sets of genes associated with distinct cellular functions.

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Table 1. Summary of the Chromatin Regulators Analyzed in This Study

Subunit	Complex	Activity	Gene Class[a]			Pol II Genes	
			Pol I	Pol II	Pol III	General versus Gene Specific[b]	Promoter versus ORF[c]
Gcn5	SAGA and others	HAT		+		general	promoter
Esa1	NuA4	HAT		+		general	promoter
Hst1	Hst1/Sum1	HDAC		+		specific	promoter
Rpd3	HDB	HDAC		+		specific	promoter
Sth1[d]	RSC	remodeling		+	+	specific	promoter
Set1[e]	COMPASS	HMT		+		general	ORF

[a] The predominant class(es) of genes occupied by each chromatin regulator is indicated by a "+." Some regulators occupy a significant portion of more than one class of gene.
[b] The chromatin regulators that occupy genes in a manner that correlates with transcription rate (see text) were classified as "general," and those whose occupancy does not were classified as "gene specific."
[c] The predominant location, promoter versus open reading frame (ORF), is listed for each regulator as determined by genome-wide location and high-resolution ChIP on target genes.
[d] Data published previously (Ng et al., 2002).
[e] Data published previously (Ng et al., 2003).

Results

Overview

The histone acetyltransferases (HATs) Gcn5 and Esa1 and the histone deacetylases (HDACs) Hst1 and Rpd3 were epitope tagged at their chromosomal locus (Lee et al., 2002). Genome-wide location analysis experiments were performed in triplicate with yeast cells grown in rich media using DNA arrays representing the yeast genome (Ren et al., 2000; Iyer et al., 2001). In some cases, additional subunits of the protein complex within which the regulators reside were also profiled (see below). Control experiments using an isogenic strain containing no tagged protein were also performed. The results for the four chromatin regulators are summarized in Table 1, together with those for two other regulators we reported previously (Ng et al., 2002, 2003). Complete data sets are available at http://web.wi.mit.edu/young/chromatin_regulators.

Gcn5 and Esa1 Are Generally Recruited to Active Protein-Coding Genes

Gcn5 and Esa1 are the catalytic subunits of the SAGA and NuA4 complexes respectively (Grant et al., 1997; Allard et al., 1999). Both these HATs are involved in transcriptional regulation and have been shown to be recruited to specific genes by DNA binding transcription factors (reviewed in Naar et al., 2001). Genome-wide expression studies with mutants have shown that only a subset of protein-coding genes depend on the function of SAGA or NuA4 for optimal expression (Holstege et al., 1998; Lee et al., 2000; Reid et al., 2004). However, it is not clear whether SAGA and NuA4 are associated with and function at this subset of protein-coding genes alone. It is also possible that they are recruited to and function at essentially all actively transcribed protein-coding genes in wild-type cells, but that the loss of function due to mutation does not fully reveal their roles due to the ability of other chromatin regulators to compensate for the loss of a single regulator.

When a regulator is associated with most or all transcriptionally active genes, we expect that its occupancy of these genes will correlate with global transcriptional rates (Lieb et al., 2001; Wang et al., 2002; Kurdistani et al., 2002; Ng et al., 2002, 2003). We found that the genome-wide occupancy of protein-coding genes by both Gcn5 and Esa1 correlates with transcription rates (Figure 1A). When similar DNA material was hybridized on ORF arrays, much smaller enrichment was observed, suggesting Gcn5 and Esa1 are binding to promoter regions rather than transcribed regions (see Supplemental Data at http://www.molecule.org/cgi/content/full/16/2/199/DC1/ and Table 1). Independent experiments, performed on a gene-by-gene level, demonstrated that both Gcn5 and Esa1 are located predominantly at the upstream activating sequence (UAS) of the ribosomal protein gene RPL2B and all other active genes we tested (Figure 1B and Supplemental Data). In contrast, the general transcription factor TFIIB is located near the transcriptional start site, as expected. Furthermore, we found that Gcn5 and Esa1 are recruited to otherwise inactive genes upon gene activation (Figure 1C). Collectively, our data indicate that both HATs occupy the promoters of most transcriptionally active protein-coding genes and that recruitment is most likely through the DNA binding transcription factors that bind to UAS elements.

Hst1 Directly Regulates Midsporulation and Kynureine Pathway Genes

Unlike Gcn5 and Esa1, the genome-wide occupancy of the HDAC Hst1 does not correlate with transcription rate, suggesting that it is not generally recruited to active genes. Hst1 target genes ($p < 0.005$) were found to be highly associated with MIPS categories containing meiosis and sporulation genes (see Supplemental Data). Conventional ChIP confirmed that Hst1 occupies the promoters of all midsporulation genes that were tested (Figure 2A and Supplemental Data). Hst1 was previously shown to be required for the Sum1-mediated repression of a few midsporulation genes during vegetative growth, but was not shown to be physically associated with these genes (Xie et al., 1999; McCord et al., 2003). Our data demonstrate that Hst1 is directly associated with midsporulation genes, in a Sum1-dependent manner (Figure 2A), during vegetative growth.

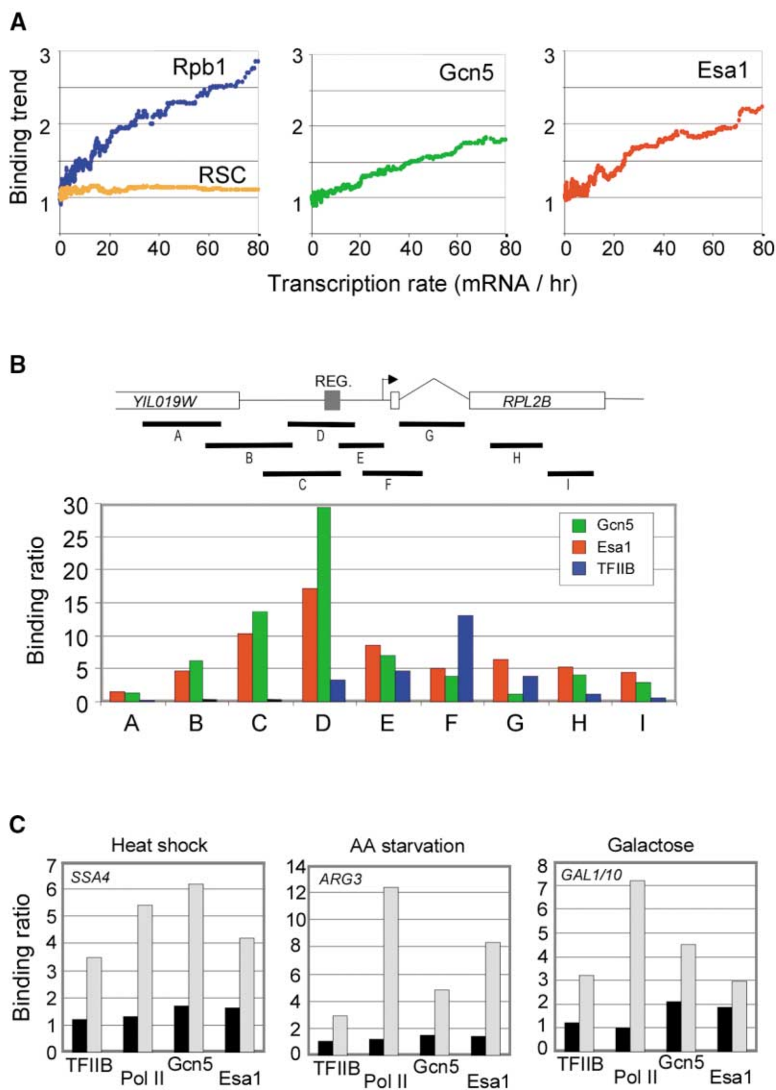

Figure 1. Recruitment of Histone Acetylases Gcn5 and Esa1 to Promoters of Actively Transcribed Genes

(A) Correlation between chromatin regulator occupancy and transcription rates. The binding trend was calculated by computing the moving median of the binding ratio over a sliding window of 100 genes across all genes ordered by transcription rate as described previously for other regulators such as Set1 (Ng et al., 2002, 2003). The transcription rates for yeast genes and the binding data for RSC were determined previously (Holstege et al., 1998; Ng et al., 2002). Rpb1 occupancy correlates with transcription rate across the genome, whereas RSC occupancy does not, and these proteins served as controls.

(B) Fine mapping of Gcn5 (green), Esa1 (red), and TFIIB (blue) occupancy within the *RPL2B* locus. The binding ratios from segment-specific chromatin IP experiments are shown for Gcn5, Esa1, and TFIIB for each of the DNA segments A–I. The binding ratios (representing the enrichment generated by the ChIP) were all normalized to the promoter of *ARN1*, which was set to 1.

(C) The binding ratios for the general transcription factor TFIIB, RNA pol II (8WG16), Gcn5, and Esa1 are shown in uninduced (black) and induced (gray) states for heat shock (25°C versus 15 min at 37°C) at the *SSA4* promoter, amino acid starvation (YNB versus 10 min in minimal synthetic media + sulfometuron methyl) at the *ARG3* promoter, and galactose (raffinose versus galactose) at the *GAL1/10* promoters. Binding ratios were calculated as in (B). The exact growth and inducing conditions can be found in the Supplemental Data. These experiments were repeated multiple times and the variation was never more than 15%.

Hst1 has also been shown to be required for repression of the kynureine pathway, which is used to produce de novo NAD^+ from tryptophan (Bedalov et al., 2003). Previous attempts to detect a physical association of Hst1 with these genes were unsuccessful, however, so Hst1 might repress these genes indirectly (Bedalov et al., 2003). Our genome-wide data revealed that Hst1 occupies the promoters of two genes from the kynureine pathway, *BNA1* and *BNA5*. Conventional ChIP was used to confirm that Hst1 occupies the regulatory regions of *BNA1* and *BNA5* (Figure 2A and Supplemental Data). These results support the elegant model proposed by Bedalov et al. (2003) that Hst1 is directly sensing and regulating NAD^+ levels through regulation of the kynurenine pathway.

The HDAC Hst1 Is Recruited by a Single Transcription Factor

In order to identify potential DNA binding transcription factors that might recruit Hst1 to its target genes, we compared the genes bound by Hst1 with those bound by more than 100 different DNA binding transcription factors (Lee et al., 2002). A single transcription factor, the repressor of midsporulation genes Sum1, stood out as highly overlapping with Hst1 (see Supplemental Data). We expected to find Sum1 in this search because it was previously shown to be required for the Hst1-mediated repression of midsporulation genes (Xie et al., 1999) and to bind to genes from the kynureine pathway (Bedalov et al., 2003). It was striking, however, that the set of genes occupied by Sum1 and Hst1 were nearly identical (Figure 2B), suggesting they might be functioning exclusively as a pair. In order to test this possibility, we profiled the genome-wide location of Hst1 in a *sum1*Δ background. Virtually all the genes bound by Hst1 in wild-type cells showed a dramatic decrease in Hst1 binding in the absence of Sum1 (Figure 2B). Together with knowledge that Hst1 and Sum1 physically interact (Rusche and Rine, 2001; Bedalov et al., 2003; McCord et al., 2003), these data suggest that Hst1 is exclusively recruited by Sum1 during vegetative growth. To our knowledge, this is the only known case of a chromatin regulator so specifically dedicated to a single transcription factor.

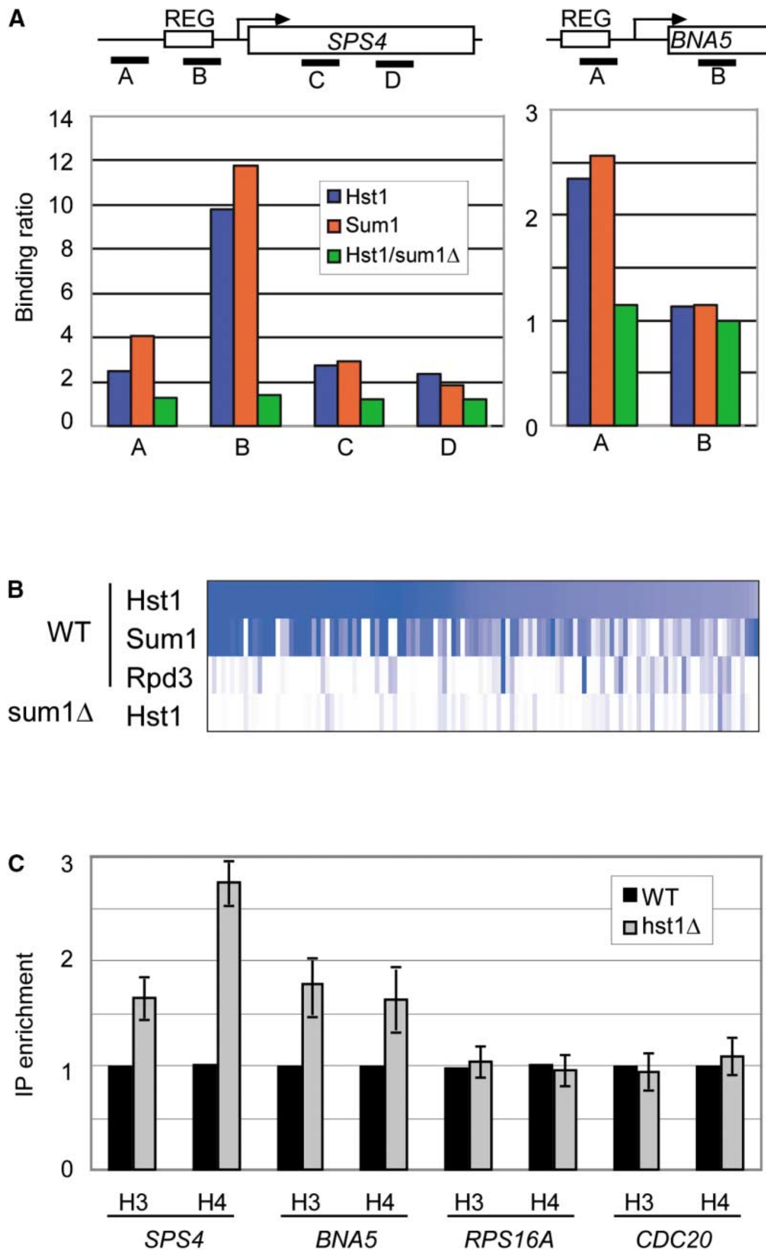

Figure 2. Recruitment of Hst1 to Promoters of Sporulation and Kynureine Genes by the Sum1 Repressor

(A) The binding ratio for Hst1 (blue), Sum1 (red), and Hst1 in *sum1Δ* cells (green) across the *SPS4* and *BNA5* loci, as determined by ChIP, is shown. A diagram of the regions amplified by sequence-specific PCR is shown on top.

(B) The log binding ratio (as determined by genome-wide location analysis) of Hst1, Sum1, and Rpd3 (used as a control) is displayed for the promoters of genes bound by Hst1 ($p < 0.005$). The log binding ratio of Hst1 in *sum1* deletion cells (sum1Δ) is also shown.

(C) The level of histone H3 (K9/K14) acetylation and histone H4 (K5, K8, K12, K16) acetylation, as determined by ChIP, is shown in wild-type cells (black) and in cells deleted for *HST1* (gray) at the *SPS4* and *BNA5* promoters. The binding ratios in (A) and (C) were all calculated as in Figure 1B. The binding ratios in *hst1Δ* in (C) were normalized to wt. These experiments were repeated multiple times and the variation was never more than 15%.

Hst1 Is a Bona Fide HDAC In Vivo

Hst1 was shown to have NAD$^+$-dependent HDAC activity in vitro (Rusche and Rine, 2001), but it has not been shown to affect histone acetylation in vivo. Previous studies failed to detect changes in acetylation levels for sporulation genes (Rusche and Rine, 2001) or kynureine pathway genes (Bedalov et al., 2003) in Hst1 mutants. It is possible that Hst1 (1) does deacetylate histones at these genes, but the activity is limited to a domain too small to be detected in these experiments; (2) was not detected with the antibodies used; or (3) is difficult to detect in mutants due to redundant activities. To reevaluate the role of Hst1 as a HDAC in vivo, we performed ChIP using antibodies directed against acetylated histone H3 and H4 in wild-type and in *hst1Δ* cells. Figure 2C shows that acetylation of histone H3 and H4 is modestly, but reproducibly, increased at Hst1 target genes in the *hst1Δ* stain. Similar results were obtained using a *sum1Δ* strain (Supplemental Data). The modest effect of *HST1* and *SUM1* deletion mutants on histone acetylation in vivo might be due to the presence of partially redundant activities. Nevertheless, our data is consistent with Hst1 being a bona fide HDAC in vivo. We conclude that Hst1 recruitment by Sum1 can lead to local deacetylation of histones.

Rpd3 Is Associated with Cell Cycle Genes

The HDAC Rpd3 is part of a large protein complex composed of many different proteins, including Sin3, Sap30, and Sds3 (Kadosh and Struhl, 1997; Kasten et al., 1997; Zhang et al., 1998; Lechner et al., 2000). The Rpd3 complex negatively regulates early meiosis genes during vegetative growth (Kadosh and Struhl, 1997), and deletion of *RPD3* leads to defects in sporulation and meiosis (Dora et al., 1999). The Rpd3p complex is recruited to the promoter sequences of some sporulation genes by

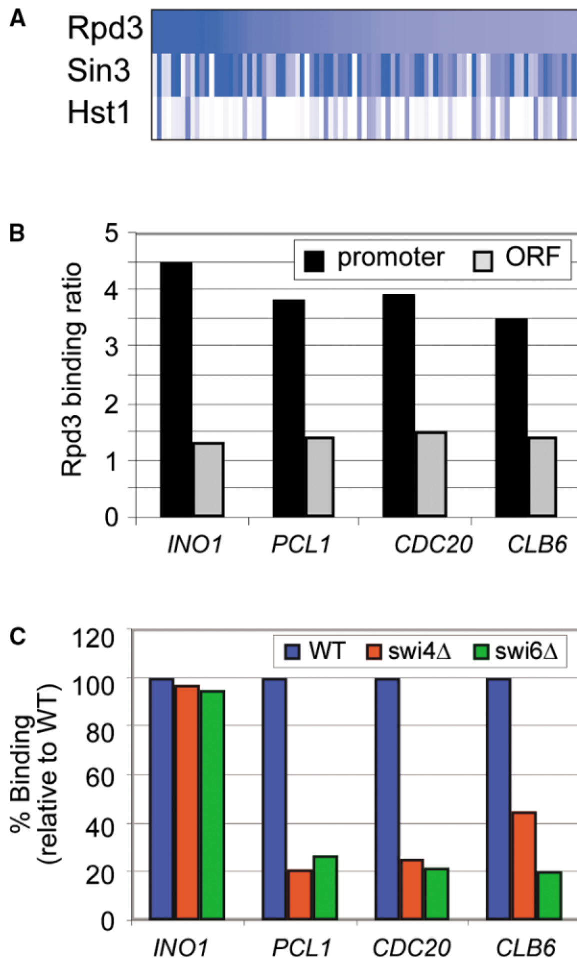

Figure 3. Occupancy and Recruitment of Rpd3 to Promoters of Cell Cycle Genes

(A) Sin3 is associated with virtually all the genes bound by Rpd3. The log binding ratio (as determined by genome-wide location analysis) of Rpd3, Sin3, and Hst1 (used as a control) is displayed for the promoters of genes bound by Rpd3 ($p < 0.005$).

(B) Rpd3 is associated with the promoters but not the open reading frames (ORFs) of target genes. The Rpd3 binding ratio, as determined by gene-specific ChIP, is shown for the promoter (black) and ORF (gray) of selected Rpd3 target genes.

(C) The association of Rpd3 with promoters of *PCL1*, *CDC20*, and *CLB6* is dependent on Swi4 and Swi6. The binding ratio of Rpd3 is shown for various Rpd3 target promoters in wild-type cells (blue) and in cells deleted for SWI4 (red) or SWI6 (green). The binding ratios in (B) and (C) were all calculated as in Figure 1B. These experiments were repeated multiple times and the variation was never more than 15%.

the transcription factor Ume6 (Kadosh and Struhl, 1997), but there is evidence that Rpd3 regulates other genes independently of Ume6 (Fazzio et al., 2001; Kurdistani et al., 2002; Robyr et al., 2002).

To identify genes occupied by the Rpd3 complex, we performed genome-wide location analysis with both Rpd3 and Sin3. In contrast to a previous report (Kurdistani et al., 2002), our results reveal that the genome-wide occupancy of Rpd3 and Sin3 do not correlate with transcription rate. Rather, Rpd3 and Sin3 occupy approximately 100 genes ($p < 0.005$). The data show that both Rpd3 and Sin3 are associated with essentially the same genes (Figure 3A), which is consistent with the observation that these proteins can be purified as a complex (Kadosh and Struhl, 1997; Kasten et al., 1997), and indicating that they function together in vivo.

Rpd3 target genes ($p < 0.005$) were found to be highly associated with MIPS categories associated with cell cycle regulation (see Supplemental Data). We used conventional ChIP to confirm that Rpd3 can occupy the promoters of several key cell cycle regulators (Figure 3B). These results are consistent with previous reports that have suggested that Rpd3 might play a role in cell cycle regulation (Fazzio et al., 2001).

In order to identify potential DNA binding transcription factors that might recruit Rpd3, we compared the genes bound by Rpd3 with genome-wide location data for more than 100 transcription factors (Lee et al., 2002). Cell cycle transcription factors (Mbp1, Swi4, Swi6, Fkh1, Fkh2, Mcm1, and Ndd1) were among the most frequent class of factors associated with Rpd3 target genes (see Supplemental Data). To determine whether some of these DNA binding factors are involved in recruitment of Rpd3 to cell cycle genes, we tested whether the heterodimeric transcription factor Swi4/Swi6 is essential for Rpd3 occupancy of three cell cycle regulator genes occupied by both Rpd3 and Swi4/Swi6 (*PCL1*, *CDC20*, and *CLB6*). If Rpd3 is recruited to these genes by Swi4 and Swi6, then Rpd3 should not occupy these genes in *SWI4* and *SWI6* deletion mutants. As shown in Figure 3C, Rpd3 occupies the promoter of these genes in wild-type cells, but this occupancy is reduced significantly in *swi4*Δ or *swi6*Δ cells. This effect is specific to Rpd3 occupancy of the cell cycle regulators' promoters since Rpd3 occupancy of the *INO1* promoter was not affected by the deletion of *SWI4* or *SWI6* (Figure 3C). Furthermore, when genome-wide location analysis of Rpd3 was carried out with cells lacking Swi4, we found that Rpd3 continued to occupy genes that are not regulated by Swi4 (see Supplemental Data), showing that the absence of Swi4 does not affect Rpd3 occupancy at genes controlled by other regulators. Thus, in contrast to Hst1, which is recruited to the genome by a single DNA binding protein, Rpd3 is recruited to specific sites in the genome via interactions with multiple transcription factors, including Ume6 (Kadosh and Struhl, 1997; Rundlett et al., 1998) and the heterodimeric transcription factor Swi4/Swi6 (this study).

Rpd3 Is Recruited to Ribosomal Protein Genes upon Cold Shock

Our interpretation of the genome-wide distribution of Rpd3 differs from a similar study that concluded that Rpd3 is generally associated with genes in a manner that positively correlates with their transcription rate (Kurdistani et al., 2002). We did not find that Rpd3-dependent ChIP signals are enriched significantly relative to controls at highly transcribed genes such as ribosomal protein (RP) genes using our ChIP protocol, whether assayed by genome-wide location analysis (data not shown) or by gene-specific ChIP (Figure 4A). To determine the cause of this discrepancy, we repeated the Rpd3 ChIP experiments using both our protocol and that of Kurdistani et al. (2002). As shown in Figure 4B, both protocols produced significant enrichment with the *PCL1* and *INO1* promoters, but only the Kurdistani et al. (2002) method led to enrichment of the promoter of

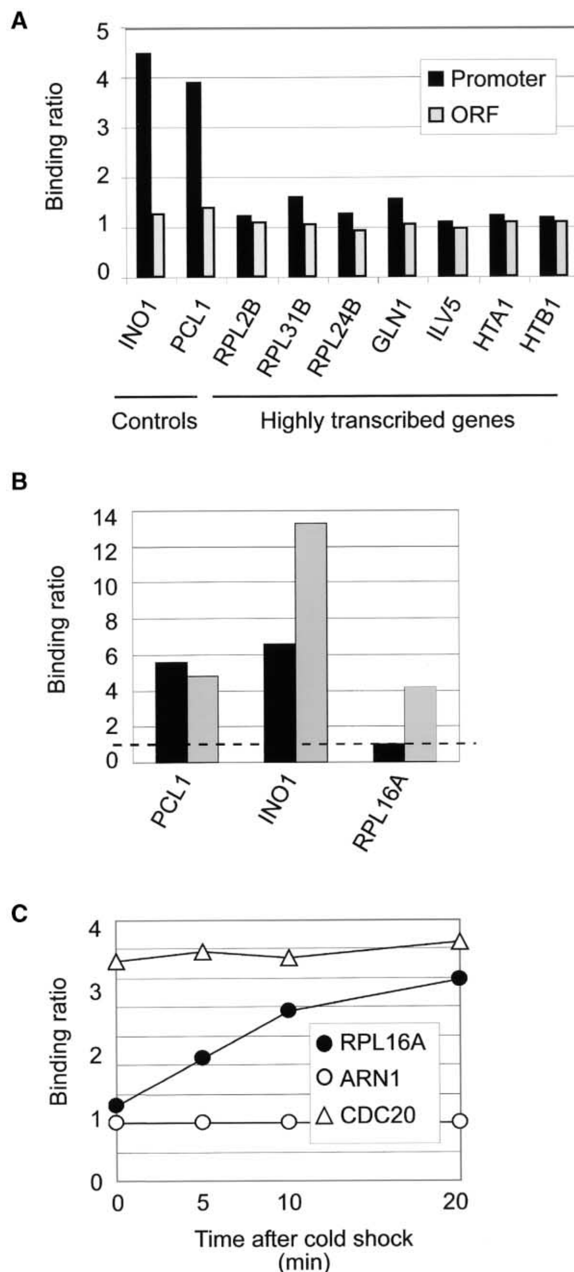

Figure 4. Rpd3 Is Recruited to Ribosomal Protein Genes upon Cold Shock

(A) Rpd3 is not generally associated with highly transcribed protein-coding genes in rich media. Gene-specific ChIPs were performed with an Rpd3-tagged strain at the promoter (black) and ORF (gray) of the indicated highly transcribed genes. *INO1* and *PCL1* were included as positive controls.

(B) Different protocols give different enrichment at ribosomal protein genes. Rpd3 ChIP experiments were performed using our standard protocol (black) and the Kurdistani et al. (2002) protocol (gray). The binding ratio of Rpd3 is shown at the *INO1*, *PCL1*, and *RPL16A* promoters. These experiments were repeated multiple times and the variation was never more than 15%.

(C) Cold shock treatment induces the binding of Rpd3 to ribosomal protein genes. Myc-Rpd3 cells were grown until mid-log phase and the culture was transferred to an ice-cold water bath. 50 ml samples were collected and subject to ChIP prior to, as well as 5, 10, and 20 min after transferring to cold water bath. The binding of Rpd3 is shown at the *CDC20, ARN1* (a negative control), and *RPL16A* promoters. The binding ratios were all calculated as in Figure 1B. These experiments were repeated multiple times and the variation was never more than 15%.

the ribosomal promoter gene *RPL16A*. The two significant differences between the two protocols are the addition of a second crosslinking agent (DMA) and a wash step in ice-cold buffer prior to the addition of the crosslinking agents. The addition of DMA to our protocol did not increase enrichment of ribosomal protein promoters. However, washing in ice-cold buffer prior to crosslinking does have this effect. It appears that this "cold shock" triggers the rapid association of Rpd3 with the RP genes (Figure 4C). This effect is specific since the binding of Rpd3 to the promoters of *CDC20* or *ARN1* (negative control) is not affected by cold shock (Figure 4C). We therefore propose that Rpd3 can be recruited to RP genes during stress responses when ribosomal protein gene expression is downregulated (Causton et al., 2001; Gasch et al., 2000), and when Rpd3 might thus play a role in transcriptional repression.

Discussion

We have investigated how two histone acetyltransferases, Gcn5 and Esa1, and two histone deacetylases, Rpd3 and Hst1, occupy the yeast genome. Our results show that the HATs Gcn5 (SAGA) and Esa1 (NuA4) are generally recruited to the promoters of active protein-coding genes, whereas the HDACs Rpd3/Sin3 and Hst1 are targeted to specific sets of genes associated with distinct cellular functions. Although previous studies established that these chromatin regulators make important contributions to gene regulation at some well-studied genes, these new results demonstrate that Gcn5- and Esa1-containing complexes are generally recruited to protein-coding genes and help explain the long-standing observation that histone acetylation correlates with gene expression (Allfrey et al., 1964). These results also revise and improve our understanding of the genome-wide targets of the HDACs Rpd3 and Hst1.

Recruitment of Gcn5 and Esa1 to Transcriptionally Active Protein-Coding Genes

Previous studies showed that Gcn5 occupies the promoters of specific genes in yeast and that occupancy is influenced by specific DNA binding transcription factors. For example, Gcn5 is recruited to the *HO* promoter and this recruitment is dependent on the heterodimeric transcription factor SBF (Cosma et al., 1999). Other transcription factors have been shown to recruit Gcn5 to different genes (see Natarajan et al., 1998; Massari et al., 1999; Vignali et al., 2000; Kuo et al., 2000; Bhaumik and Green, 2001; Larschan and Winston, 2001; Proft and Struhl, 2002). These studies have not, however, demonstrated that Gcn5 is generally recruited to protein-coding genes. We found that Gcn5 occupancy of protein-coding genes correlates with the transcription rates of these genes. In addition, we found that Gcn5 was recruited to the promoter of every inducible gene tested ($n = 9$). These results indicate that Gcn5 is recruited to most transcriptionally active protein-coding

genes. The occupancy of these chromatin regulators was highest at the UAS and was optimal during gene activation, suggesting that recruitment of the Gcn5-containing complex most likely occurs through interactions with the transcription factors that bind to UAS elements.

Esa1 has also previously been shown to be recruited to specific genes through specific DNA binding transcription factors (see Reid et al., 2000; Vignali et al., 2000; Nourani et al., 2001; Brown et al., 2001; Baek et al., 2002; Boudreault et al., 2003; Frank et al., 2003; Nourani et al., 2004; Taubert et al., 2004). A previous genome-wide location study led to the conclusion that Esa1 is associated with ribosomal protein gene promoters, where it is recruited by the DNA binding transcription factor Rap1, but the signal was inadequate to ascertain whether Esa1 is associated with a substantial number of other genes (Reid et al., 2000). For these reasons, it might be assumed that Esa1 is targeted to specific subsets of genes. Our data, however, show that Esa1 occupancy of protein-coding genes correlates with transcription rates of these genes and that Esa1 can be recruited to the promoter of all the inducible genes tested ($n = 9$). In agreement with previous studies, we found that Esa1 occupancy is maximal at the UAS, suggesting it is recruited through DNA binding transcription factors. We conclude that Esa1, like Gcn5, is recruited to most protein-coding gene promoters by DNA binding transcription factors that recognize UAS elements.

The observation that Gcn5- and Esa1-containing complexes are both recruited to the promoters of most protein-coding genes makes it interesting to consider how transcription factors manage to recruit both complexes to promoters. It would require, in fact, that the number of transcription factors able to interact with those two complexes be large and that complexes have the property of being able to interact with a large number of different transcription factors. One possibility is that DNA binding regulators interact with the Tra1 subunit shared by SAGA and NuA4 (Brown et al., 2001). Tra1 is a large (~400 kDa) protein that has been shown to interact with many different transcription factors (Brown et al., 2001; Bhaumik et al., 2004). It is also possible that the activating domains of transcription activators are capable of interacting with multiple components of these chromatin-regulating complexes.

Although the genome-wide location data and conventional chromatin IP data indicate that Gcn5 and Esa1 are recruited to most transcriptionally active protein-coding genes, the expression of some genes is more sensitive to loss-of-function mutations in these chromatin regulators than others (Holstege et al., 1998; Lee et al., 2000; Huisinga and Pugh, 2004). There are at least three explanations for these observations. First, the Gcn5, Esa1, Sas2, Sas3, TFIID, and Elp3 HATs may be somewhat functionally redundant at certain genes (Wittschieben et al., 2000), thus leading to different levels of dependence on one or more HATs. Second, it is not possible to ascertain what effects of mutations are direct or indirect with expression profiling. Finally, expression profiling is generally performed with strains lacking only one subunit of a given HAT complex, which could lead to only a partial loss of function of that complex. Some HAT complexes have been shown to have multiple functions, some of which are independent of their HAT activity (Horiuchi et al., 1997; Dudley et al., 1999a, 1999b; Sterner et al., 1999). Expression profiling experiments performed using an *spt20* allele known to disrupt the integrity of the SAGA complex lead to a much wider effect on gene expression that experiments performed using *gcn5* alleles (Lee et al., 2000). This result is consistent with SAGA playing a wide role in gene expression regulation, with Gcn5's function being only part of its function.

General Model for Recruitment of Chromatin Regulators at Protein-Coding Genes

Studies in yeast with a few genes have indicated that multiple chromatin regulators are recruited in a temporal order (for reviews, see Cosma, 2002; Hampsey and Reinberg, 2003). The results described here suggest that it is the case that DNA binding transcription factors generally recruit the Gcn5 and Esa1 complexes to nucleosomes located near UAS elements in protein-coding genes upon activation. This accounts, at least in part, for the association between gene activity and histone acetylation (Allfrey et al., 1964). Once the transcription initiation apparatus is recruited and initiates transcript elongation at protein-coding genes, it is also generally the case that the Set1 histone methyl transferase is recruited to the beginning of the ORFs through interactions with the elongating RNA polymerase II (reviewed by Hampsey and Reinberg, 2003). This leads to trimethylation of lysine 4 of histone H3 in nucleosomes located near the start site. It will be interesting to determine whether additional regulatory events noted for chromatin regulators at specific genes also occur generally at protein-coding genes (see Hampsey and Reinberg, 2003).

Recruitment of HDACs to Specific Sets of Genes

Our results indicate that the HDACs Hst1 and Rpd3 are not generally associated with actively transcribed protein-coding genes; rather, they occupy specific sets of genes associated with distinct cellular functions. While the genome-wide binding of Hst1 has not been studied previously, our data on Rpd3 is in striking contrast with a previous study of the genome-wide location of this HDAC (Kurdistani et al., 2002).

Hst1 was previously shown to be involved in the regulation of several sporulation genes (Xie et al., 1999; McCord et al., 2003) as well as a number of genes from the kynureine pathway (Bedalov et al., 2003). The interaction of Hst1 with these genes, however, was never directly observed and its HDAC activity was not demonstrated to occur in vivo. Our results show that Hst1 is physically recruited to sporulation and kynureine pathway genes by the Sum1 DNA binding transcriptional repressor, leading to a decrease in histone acetylation at those genes. We conclude that Hst1 directly regulates the expression of genes involved in sporulation and the kynureine pathway in vivo and does so through histone deacetylation. We also found that Sum1 is required for virtually all the binding activity of Hst1, which indicates that Hst1 is recruited to all its genomic sites by Sum1 under the growth conditions studied here. To our knowledge, this is the only case of a chromatin regulator

whose association with the genome is dependent on a single DNA binding transcription factor.

Initial studies on Rpd3 focused on its role in the regulation of sporulation-specific genes through the DNA binding transcription factor Ume6 (see Kadosh and Struhl, 1997, 1998a, 1998b; Rundlett et al., 1998; Burgess et al., 1999; Fazzio et al., 2001). Recent studies suggest that Rpd3 regulates additional cellular functions (see De Rubertis et al., 1996; Dora et al., 1999; Arevalo-Rodriguez et al., 2000; Vannier et al., 2001; Kurdistani et al., 2002; Sandmeier et al., 2002), and the genome-wide targets we find occupied by Rpd3 are consistent with these studies. One interesting set of genes occupied by Rpd3 encodes important cell cycle regulators including cyclins and cyclin-dependent kinases. This may explain, at least in part, the importance of Rpd3 in meiosis (Vannier et al., 2001, and references therein). In addition, our results are consistent with previous reports that have suggested that Rpd3 might play a role in cell cycle regulation. The expression of cyclin genes is upregulated by *rpd3* mutations (Fazzio et al., 2001). Loss of *RPD3* function causes hyperacetylation of the chromatin surrounding those same genes (Robyr et al., 2002). An *rpd3* deletion is synthetically lethal with deletions of either *swi4* or *swi6* (Vannier et al., 2001), which encode cell cycle transcription factors that regulate G1/S gene expression. Overexpression of Rpd3p can lead to cell cycle arrest in some genetic backgrounds (Arevalo-Rodriguez et al., 2000). Taken together, the global binding data and the genetic evidence argue that Rpd3p plays an important role in regulation of the cell cycle gene expression program.

Our data indicate that the genome-wide occupancy of Rpd3 does not correlate with transcription rates and that Rpd3 is recruited to specific genes by specific DNA binding regulators. This result is inconsistent with the view that Rpd3 is globally recruited to active protein-coding genes (Kurdistani et al., 2002). Much of the discrepancy between the genome-wide data we report and that of Kurdistani et al. (2002) involves the ribosomal protein (RP) genes, which are reported as occupied by Rpd3 in the latter study. We found that Rpd3 is rapidly recruited to RP genes upon cold shock, and we show that the use of ice-cold buffer to wash cells before the addition of crosslinking agents (Kurdistani et al., 2002) can explain the discrepancy in genome-wide location data. We therefore propose that Rpd3 is recruited to the promoters of RP genes, but only in stress conditions like cold shock. In these conditions, it is likely that Rpd3 acts as a repressor of RP genes, since the expression of these genes is generally downregulated upon exposure of yeast cells to stress conditions (Causton et al., 2001; Gasch et al., 2000).

The HATs and HDACs studied here are highly conserved in eukaryotes, so several of our observations have implications for studies in higher eukaryotes. It will be interesting to determine whether Gcn5 and Esa1 homologs are generally recruited to the promoters of protein-coding genes in higher eukaryotes. It will also be important to determine the identity of specific HDACs in higher eukaryotes that may be devoted to particular cellular functions. It might then be possible to modify specific cellular functions through chemical compounds that target the enzymatic activities of specific chromatin regulators (Melnick and Licht, 2002).

Experimental Procedures

Epitope Tagging of Strains

Chromatin regulators were tagged at the C terminus by inserting multiple copies of the Myc epitope coding sequence into the normal chomosomal loci of these genes. The plasmids pWZV88 and p3747 were used as a template to generate PCR products containing the Myc epitope coding sequence and a selectable marker (*TRP1* or *URA3*, respectively) flanked by homologous regions designed to recombine at the 3′ end of the targeted transcriptional regulator. In most cases the PCR products were transformed into the W303 strain Z1256 or one of its derivatives. Clones were selected for growth on TRP- or URA-selective plates. Insertion of the epitope coding sequence was confirmed by PCR and expression of the epitope-tagged protein was confirmed by Western blotting using an anti-Myc antibody. The complete list of the tagged strains used in this study can be found in the Supplemental Data.

Gene Deletions

The plasmid pRS306 was used to amplify PCR products containing an URA marker flanked by homologous regions designed to recombine on either side of the gene to be deleted. The PCR products were transformed into the appropriate yeast strains and the stable integration of the marker was selected on URA-selective plates. The appropriate gene replacement was confirmed by PCR. The complete list of the deletion strains used in this study can be found in the Supplemental Data.

Chromatin Immunoprecipitation and Genome-Wide Location Analysis

Chromatin immunoprecipitation and genome-wide location analysis were performed as described previously (Ren et al., 2000) except that the crosslinking time was reduced to 30 min at room temperature. A detailed protocol can be found at http://web.wi.mit.edu/young/chromatin_regulators. Each tagged strain was grown in three independent cultures in rich medium. Genome-wide location data were subjected to quality control filters, normalized, and the ratio of immunoprecipitated to control DNA was determined for each spot. A confidence value (p value) was calculated for each spot from each array using an error model, and the data for each of the three samples comprising an experiment were combined using a weighted average method (Ren et al., 2000).

Among the control experiments, we included a control immunoprecipitation (with myc antibody) of lysates from a strain lacking the epitope tag. This data is available at http://web.wi.mit.edu/young/chromatin_regulators.

Induction of specific classes of genes including galactose, heat shock, and animo acid starvation genes was performed as follows. For galactose induction experiments, galactose (2% final concentration) was added for 45 min to cell cultures grown at mid-log phase in YEP medium containing 2% raffinose at 30°C. For heat shock induction experiments, cell cultures grown at mid-log phase in YPD medium at 25°C were transferred to prewarmed flasks in 37°C thermostat and equal volume of YPD medium, prewarmed to 50°C was added. Cultures were then incubated for another 15 min at 37°C. For animo acid starvation experiments, cells were grown in YNB supplemented with all amino acids and 2% glucose at 30°C until reaching mid-log phase (OD_{600} 0.6–0.8). Then, cells were spun down, washed twice with YNB meduim supplemented with required amino acids and 2% glucose, and incubated at 30°C for additional 10 min in this same meduim with 0.2 μg/ml sulfometuron methyl.

Antibodies

The antibodies used in this study are anti-Myc (9E11), anti-Pol II CTD (8WG16), anti-histone H3 (Abcam), anti-acetylated histone H3 (K9,K14) (Upstate Biotech), and anti-acetylated H4 (K5, K8, K12, K16) (Upstate Biotech).

Analysis of Genome-Wide Location Data

The intensity of the signal obtained for spots containing no DNA was measured, averaged, and subtracted from the intensity of all DNA containing spots prior to calculation. Next, we calculated the log of the ratio of intensity in the IP-enriched channel to intensity in the genomic DNA channel for each intergenic region across a large set of hybridization experiments (the set contains many hundreds unrelated hybridization experiments including the ones analyzed here). To account for systematic biases introduced by the immunoprecipitation, all of the log ratios for a specific intergenic region were then normalized by subtracting the average log ratio for that intergenic region. Adjusted intensity values for the IP-enriched channel were calculated from these normalized ratios. The data were then analyzed using an error model and a weighted averaged method as described by Ren et al. (2000). A detail description of this error model can be found at http://web.wi.mit.edu/young/regulatory_network/ (follow links to Analysis Methods).

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Accession Numbers

The data sets desribed in this paper have been deposited in the ArrayExpress database under the accession number E-WMIT-2.