Histone amino termini are post-translationally modified by both transcriptional coactivators and corepressors, but the extent to which the relevant histone modifications contribute to gene expression, and the mechanisms by which they do so, are incompletely understood. To address this issue, we have examined the contributions of the histone H3 and H4 amino termini, and of the coactivator and histone acetyltransferase Gcn5p, to activation of a small group of Gcn4p-activated genes. The histone H3 tail exerts a modest (about 2-fold) but significant effect on activation that correlates with a requirement for Gcn5p and is distributed over multiple lysine residues. The H4 tail also plays a positive role in activation of some of those genes tested, but this does not correlate as closely with Gcn5p coactivation. Microarray experiments did not reveal a close correspondence between those genes activated by Gcn4p and genes requiring the H3 or H4 tail, and analysis of published microarray data indicates that Gcn4p-regulated genes are not in general strongly dependent on Gcn5p. However, a large fraction of genes activated by Gcn4p were found to be repressed by the H3 and H4 amino termini under non-inducing conditions, indicating that one role for Gcn4p is to overcome repression mediated by the histone tails.

Investigations into chromatin structure and function performed over the past decade have revealed that the association of DNA with histones in eukaryotes confers an added layer of regulatory complexity that has dwarfed expectations. Chromatin transactions are involved in transcription, replication, repair, and recombination, and a variety of cellular machinery contributes to these transactions by remodeling chromatin structure and/or post-translationally modifying the histones (1). Some post-translational modifications of the histones occur in the central, structured domains, but histone modifications are particularly concentrated in the highly conserved, unstructured amino termini, or “tails” (2–4). The variety of modified sites and modifying enzymes has led to the proposition that a histone code specifies function via particular combinations of modifications (5, 6).

Pioneering work by the laboratories of Grunstein and Smith (7–12), using the budding yeast *Saccharomyces cerevisiae*, established that the H3 and H4 tails are not essential for viability, but are important for growth and mating-type regulation. Those studies also revealed redundancy with regard to viability and GAL gene regulation among the modifiable lysine residues in the H3 and H4 amino termini, and more recent investigations have indicated that these lysines function redundantly in transcription on a genome-wide level in yeast (13, 14). However, other studies have indicated that particular amino acids in the histone amino termini can be critical for gene activation (15–17), consistent with the known target specificity of at least some histone-modifying coactivators or corepressors (1). More recent reports have arrived at conflicting conclusions regarding the extent to which patterns of histone modifications specify transcriptional output versus the extent to which modifications function redundantly (18, 19).

The first coactivator identified as a histone acetyltransferase, Gcn5p, when tested as a recombinant protein was found to target Lys-14 of histone H3 and Lys-8 and Lys-16 of histone H4 for acetylation when presented with purified histones, but to have little activity toward nucleosomal histones (20) (although under optimal buffer conditions, recombinant Gcn5p can acetylate histone H3 in reconstituted nucleosome arrays). In vivo, however, Gcn5p exists predominantly as a component of the SAGA and ADA complexes (22). In vitro assays indicate that SAGA principally targets Lys-9, -14, and -18 of histone H3 for acetylation (23), whereas in vivo investigations revealed Lys-9 and -18 of H3 as important targets of Gcn5p but indicated that additional targets exist (24). These results suggest that for genes requiring Gcn5p as a coactivator, specific lysine residues in histone H3, and possibly H4, could be important for transcriptional activation. However, Gcn5p-dependent activation of a reporter gene by the chimeric activator Gal4-VP16 was increased rather than diminished by point mutations examined in Lys-9 or Lys-14 of histone H3 or in the lysines of the H4 tail (24). Here, we have sought to obtain new insight into the roles of the H3 and H4 amino termini in transcriptional activation, with particular focus on the H3 tail, by focusing on a few genes that are activated by the general transcriptional activator Gcn4p and/or that require Gcn5p for full activation. We have tested how expression of these genes is affected by successively larger truncations of the H3 amino terminus, or by point mutation of specific lysine residues, to determine whether their activation depends on a specific region of the H3 tail, as the “histone code” hypothesis would suggest, or whether more delocalized effects might regulate gene expression.

Gcn4p interacts with the SAGA complex in vitro (25, 26) and is capable of recruiting Gcn5p during gene activation in vivo (27, 28). This suggests that many of the large number of genes activated by Gcn4p (29) could depend on Gcn5p (26) and therefore could be expected to show significant dependence for their activation on the H3 and/or H4 tail. To test this idea, we examined genome-wide effects of the loss of the H3 or H4 amino terminus on transcription under conditions of amino acid starvation, in which most Gcn4p-regulated genes are active. In addition, we have used our own and other publicly available genome-wide expression data to examine the dependence of genes that bind Gcn5p on the histone H3 and H4 amino termini for expression. Our results suggest a...
modest dependence of Gcn5p-dependent genes on the H3 and H4 amino termini, but very little dependence of most Gcn4p-activated genes on the H3 and H4 tails for activation. However, we show that a large fraction of genes activated by Gcn4p are repressed by H3 and H4 amino termini under non-inducing conditions, indicating that one function of Gcn4p is to overcome chromatin-mediated repression that depends on the histone tails.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—Plasmid pMS308 was generated by cloning the HHT1-HHF1 fragment from pMS329, which contains a URA3 marker, into pMS358, which contains a LEU2 marker (8). To generate plasmids pCY318, pCY328, pCY338, and pCY348, which harbor the genes encoding histone H3(Δ1–20), H3(Δ1–15), H3(Δ1–10), and H3(Δ1–5) respectively, fragments encoding the corresponding histone H3 deletions were generated by PCR and cloned into pMS358 as Smal-EcoRI fragments in place of the hht1–2 gene. All mutant hht1 genes were verified by sequencing. Plasmids pCY101, pCY102, pCY103, pCY201, pCY202, and pCY203 were generated by point mutagenesis using the QuikChange II XL site-directed mutagenesis kit (Stratagene). All mutations were verified by sequencing. Plasmid pRS416-DDE1pr-GCN4 was created by cloning the SacI-PstI fragment of pAB712 (30) into pRS416. This single-copy plasmid constitutively expresses GCN4 from the DDE1 promoter.

**Strains and Media**—Strains used in this study are listed in Table 1. Strain LYY256 was constructed beginning with strain MX15-3B, generously provided by Prof. Mitch Smith (University of Virginia). MX15-3B is a meiotic segregant constructed by crossing MX4-22A (MATa ura3-52 lys2-Δ201 leu2-3,112 Δ(hht1 HHF1) Δ(hht2 HHF2)) pMS329(CEN4 ARS1 HHT1 HHF1 URA3) (7) with a congeneric strain derived from L3110 (gen4-2 bas1-2 bas2-2) (31) and dissecting tetrads. The histone deletion loci were confirmed by dependence on pMS329 and Southern blot analysis, and the gen4, bas1-2, and bas2-2 alleles were confirmed by tetrad analysis, growth requirements, and complementation analysis (31). The plasmid pMS358, a LEU2 marked plasmid that encodes histone H3 lacking the amino-terminal 28 amino acids, was transformed into MX15-3B. Leu+ transformants were selected, and the URA3-marked plasmid expressing wild-type histone H3 was shuttled out by selection on complete synthetic medium (CSM)2-Leu plates containing 5-fluoroorotic acid. The loss of the marked plasmid was confirmed by PCR using primers specific for H3(Δ1–28) and the HIS4 promoter. The renormalized values thus obtained for each individual transcript were then used to obtain averages and standard deviations for each transcript as shown in the figures.

**Microarray Analysis and Computational Methods**—RNA was prepared from exponentially growing yeast cultured in CSM dropout medium using the Masterpure Yeast RNA purification kit (Epicenter Technology, Madison, WI). RNA was further purified using the RNeasy purification kit (Qiagen). Processing and hybridization to Affymetrix (Santa Clara, CA) S98 microarrays were done according to the manufacturer’s protocol as described previously (36). Changes in gene expression were derived by averaging log, expression changes. False discovery rates (FDRs) were derived according to Storey (37). p Values for overlaps were derived using a hypergeometric distribution. Comparative analysis and clustering were done using Excel (Microsoft), Genetraffic (Iobion Informatics), and Genespring (Affymetrix). Enrichment of gene sets in specific functional categories, as defined by the MIPS data base (38), was determined using FunSpec (39).

To search for Gcn4p binding sites in a defined group of gene promoters, a Gcn4p motif model was generated. From the literature and the S. cerevisiae Promoter Data base (40), 27 experimentally identified Gcn4p binding sites were collected and aligned using the Gibbs Recursively Sampler (41). This model was used with dscan (42), which implements the method described by Staden (43) to report sites that match the model at a chosen level of statistical significance.

**Microarray Accession Number**—Microarray gene expression data are available at the Gene Expression Omnibus under accession number GSE4135.

**RESULTS**

**Effects of Histone H3 Amino-terminal Deletions and Lys to Gln Mutation on Transcriptional Activation of Selected Genes**—To examine the role of the histone H3 amino terminus in transcriptional activation, we constructed a series of yeast strains having deletions of the first 5, 10, 15, or 20 amino acids of histone H3. We then used these strains, together with previously described strains (44) lacking the first 28 amino acids of histone H3 or having the 6 lysine residues replaced by glutamines (Lys to Gln

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2 A. Bortvin, unpublished data.

3 The abbreviations used are: CSM, complete synthetic dropout medium; FDR, false discovery rates; 3-AT, 3-aminotriazole.
mutant), and the corresponding wild-type strains (Table 1), to analyze the levels of six Gcn4p-dependent transcripts (HIS4, HO4, HIS3, ARG1, and TRP2) and one control transcript (SAM2) that does not show Gcn4p dependence (29). Based on microarray data from the Hinnebusch laboratory (29), these transcripts show from 3- to 15-fold induction after treatment with 100 mM 3-aminotriazole (3-AT), and their induced levels of transcripts are reduced 6–19-fold in gcn4A yeast (Table 2). A lower (10 mM) concentration of 3-AT results in 4.5–20-fold induction, with the exception of TRP2, which is not induced. We confirmed the Gcn4p dependence of these genes in one of the two strains (both derived from strain S288C) used here, and found results generally consistent with those obtained by the Hinnebusch laboratory (29), although we did observe a modest dependence of SAM2 expression on Gcn4p not found previously (Table 2). After this work was underway, a genome-wide location analysis study yielded data indicating that five of the Gcn4p-dependent transcripts examined here (HIS4, LYS1, HIS3, ARG1, and TRP2) are indeed direct targets of Gcn4p (having p values for Gcn4p association of <2 × 10⁻⁴, where 1 × 10⁻⁴ is judged to be significant), whereas binding of Gcn4p to the HOM2 (and SAM2) promoter was not observed (29). HOM2 may therefore be indirectly regulated by Gcn4p.

We chose to examine Gcn4p-dependent transcripts because a large number of genes are regulated by this well studied transcriptional activator, and considerable knowledge exists regarding Gcn4p-mediated transcriptional activation (29, 45, 46). Furthermore, Gcn4p-mediated gene activation often depends on Gcn5p, a histone acetyltransferase that functions as a coactivator as a member of the SAGA complex (47–50). Because Gcn5p targets the histone H3 amino terminus (51), we number of genes are regulated by this well studied transcriptional acti-

### TABLE 1

**Yeast strains**

| Strain       | Genotype or description | Ref. | exception of |
|--------------|-------------------------|------|-------------|
| LYY256       | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pMS329 | This work | |
| CY456        | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY318 | This work | |
| CY456        | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY328 | This work | |
| CY556        | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY338 | This work | |
| CY656        | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY348 | This work | |
| CY756        | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY358 | This work | |
| CYY656       | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY348 | This work | |
| NSY429       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pNS329 | 36 | |
| NSY438       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pMS308 | 36 | |
| MX1-4C       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pMS329 | 8 | |
| CY1-4C       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pMS308 | This work | |
| CY2-4C       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pMS358 | This work | |
| CY3-4C       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pCY318 | This work | |
| CY4-4C       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pCY338 | This work | |
| CY5-4C       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pCY338 | This work | |
| CY6-4C       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pCY348 | This work | |
| CLY460       | MATa ura3–52 lys2Δ201 leu2–3,112 trp1–289 his3Δ1 Δ(hht1-hhf1)(hht2-hhf2) pCL460 | 44 | |
| CY9101       | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY101 | This work | |
| CY9201       | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY201 | This work | |
| CY9101       | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY101 | This work | |
| CY9201       | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY201 | This work | |
| CY9103       | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY101 | This work | |
| CY9201       | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY201 | This work | |
| CY9203       | MATa ura3–52 leu2–3,112 gcn4–2 bas1–2 bas2–2 2 (HHT2-HHF2) pCY203 | This work | |

### TABLE 2

**Dependence on Gcn4p and induction by 3AT of genes examined in this work**

Numbers in the table represent fractional increase or decrease under the conditions indicated.

| Gene | Gcn4p+/gcn4⁻ | 10 mM 3-AT⁻ | 100 mM 3-AT⁻ | Gcn4p+/gcn4⁻ |
|------|--------------|-------------|-------------|-------------|
| HIS4 | 18.6         | 8           | 9.4         | 16.2        |
| HIS3 | 9.4          | 10.2        | 8.1         | ND          |
| LYS1 | 15.3         | 20.4        | 15.5        | 8.4         |
| ARG1 | 93           | 11.6        | 12.3        | 42          |
| TRP2 | 5.8          | 0.82        | 2.8         | 2.8         |
| HOM2 | 11.5         | 4.5         | 3.1         | 4.4         |
| SAM2 | 0.73         | 0.23        | 0.18        | 2.7         |

*From Natarajan et al. (29).

Average from two independent determinations (strain LYY256 with and without pRS416-Ded1p-Gcn4p).
two distinct parent strains. One (LYY256) was transformed with a plasmid that constitutively expresses GCN4 from the DED1 promoter, which yields Gcn4p levels comparable with those obtained upon induction in wild-type yeast (52). The other strain (MX1-4C) is his3—, so the HIS3 transcript is not reported in the derived strains. C, transcript levels were also compared in HIS3Δ1–28 yeast derived from MX1-4C, wild-type yeast, and the isogenic gcn5Δ strain expressing wild-type histone H3. Values shown in A and C are averages from three to four independent determinations, and values shown in B are from two independent determinations. Standard deviations are indicated. The brackets indicate differences in transcript levels with <.0.05 (*) or <.0.01 (**) according to Student’s t test. y axis values in A–C are arbitrary units (see “Experimental Procedures”).

We considered the possibility that the observed changes in expression could be because of an effect on Gcn4p levels caused by the histone H3 NH2-terminal deletions. No significant change in the GCN4 mRNA level was observed in HIS3Δ1–28 yeast compared with wild-type (data not shown). Furthermore, expression of TRP2, which depends on Gcn4p (29), and a number of other Gcn4p-dependent transcripts (Fig. 5) were unaffected by partial or complete deletion of the H3 tail. These results

![Figure 1](https://example.com/figure1.png)
strains in which Lys-4, -14, or -18 was individually mutated to arginine impact on induction of the genes we had examined, we generated yeast of these genes. To test whether specific lysine residues had special modest decrease suggested possible involvement of Lys-18 in regulation amino acids 16–20 (Fig. 1). In light of the distinctive behavior of TRP2 in response to the histone H3 mutations relative to the other transcripts examined, we decided to examine directly the dependence of these transcripts on Gcn5p. HIS3 has been well documented to depend on GCN5; among the other genes examined, LYS1 showed strong dependence on Gcn5p, whereas HOM2 transcription shows a modest decrease in gcn5Δ yeast (Fig. 1A). We reproducibly found that HIS4 transcription showed substantial dependence on Gcn5p, in contrast to previous reports (47, 50). We do not understand the reason for this discrepancy, although it has been noted that dependence on SWI/SNF for transcriptional activation can show considerable variation in different strain backgrounds (50); perhaps this is also the case for Gcn5p. Interestingly, ARG1, which is not much affected by H3 tail deletions except for the first 5 amino acids, shows little dependence on GCN5, in agreement with a previous report (50), and TRP2 transcription is not affected in gcn5Δ yeast. Thus, we observe correlation between dependence of activation on Gcn5p and on the histone H3 amino terminus. For comparison, we also examined SAM2, which is repressed by 3-AT treatment and does not depend strongly on Gcn4p, although it does depend on Gcn5p (Fig. 1C and Table 2) (29, 54). The effect of the H3 tail deletions and mutation on SAM2 expression was similar to that seen on expression of the other Gcn4p/Gcn5p-dependent genes tested, except that amino acids 16–20 appear more important than amino acids 11–15 (Fig. 1A–B).

Effect of Point Mutations in the H3 Amino Terminus—For most of the genes examined, the largest changes in gene expression occurred upon loss of amino acids 1–5 or 11–15. This suggested possible dependence on Lys-14, a known target of Gcn5p-mediated acetylation, and/or Lys-4 in their regulation. In addition, SAM2 showed apparent dependence on amino acids 16–20, and although HIS4, HOM2, LYS1, and HIS3 did not individually show a significant decrease in transcript level upon loss of amino acids 16–20 (Fig. 1A), the fact that all four of these did show a modest decrease suggested possible involvement of Lys-18 in regulation of these genes. To test whether specific lysine residues had special impact on induction of the genes we had examined, we generated yeast strains in which Lys-4, -14, or -18 was individually mutated to arginine or glutamine, and tested the effect on expression of the same genes monitored in the tail deletion mutants. Mutation to arginine retains the positive charge of the native lysine residue but prevents acetylation, whereas mutation to glutamine both neutralizes charge and prevents acetylation.

The results, depicted in Fig. 2, show that most of these point mutations caused small but significant reduction in expression of the genes tested. The effects were considerably less than that resulting from the mutation of all lysines to glutamines, or the Δ1–28 mutant, and were generally similar no matter which lysine was mutated, or whether the mutation was to arginine or to glutamine. Two notable exceptions were the K14Q and K18R mutations, which caused larger decreases in HIS4 expression than other mutations. These results, taken together with the effect of the H3 tail deletions, indicate that Lys-4, Lys-14, and Lys-18 of the histone H3 amino terminus all contribute to the activation of these Gcn4p- and Gcn5p-regulated genes. Residues Lys-14 and Lys-18 are likely to act as substrates for Gcn5, whereas the effects seen upon mutation of Lys-4 probably reflect a contribution of the Set1 methyltransferase (17, 20, 55). Furthermore, because similar effects are seen with mutations to arginine or glutamine, the role of these lysine residues in activation is not a simple matter of a requirement for a charged or uncharged residue, but rather may reflect a requirement for the specific modifications to serve as recognition modules as postulated by the histone code (5, 56). However, any such recognition modules are clearly not essential for transcriptional activation, but rather contribute incrementally. This is most consistent with protein-protein interactions involved in transcriptional activation being spread out over several amino acid residues, including specifically modified lysine (or other) residues.

Effect of Deletion of the Histone H4 Amino Terminus—In yeast, the histone H3 amino terminus is sometimes assumed to be primarily the target of Gcn5p among the histone acetyltransferases, whereas the H4 amino terminus is viewed as being principally targeted by the NuA4 complex (51). We examined the effect of deletion of the H4 amino terminus on expression of the genes tested above (Fig. 3). Although some of the genes show similar reduction in expression upon deletion of the H3 or H4 amino terminus (HIS4, SAM2), others show a decreased effect of deletion of the H4 tail (LYS1, HOM2), whereas TRP2 showed a modest but significant reduction in expression upon loss of the H4 but
not the H3 amino terminus. These results support the idea that the dependence of gene expression on the histone H3 tail is related to the dependence on Gcn5p, whereas regulation via the H4 amino terminus may reflect dependence on distinct interacting proteins.

**Effect on Genome-wide Expression of H3 and H4 Amino-terminal Deletions under Conditions of Gcn4p Induction**—To assess the relationship between gene activation by Gcn4p and the histone H3 and H4 amino termini on a genome-wide scale, we performed microarray analysis of global gene expression of wild-type, H3Δ1–28, and H4Δ2–26 yeast grown in CSM. Both strains are derived from MX1-4C, hence are his− and are therefore expected to express Gcn4p-activated genes under these conditions (and see below) (53). We analyzed three biological replicates of H4Δ2–26 and matched wild-type yeast, and four of H3Δ1–28 and the matched wild-type, using Affymetrix arrays. To assess statistical significance of measured changes in gene expression, we calculated FDR (57), a measure based on \( p \) values that approximates the fraction of false positives within a group of genes having different expression in the mutant and wild-type samples.

We used three tests to verify that Gcn4p-regulated genes were activated under the conditions assayed. We generated data for relative gene expression data obtained for wild-type yeast (NSY429) grown in CSM (the present study) compared with the same strain grown in rich medium (YPD medium) (44). We then first asked whether genes found to bind Gcn4p in CSM in the genome-wide location analysis of Harbison et al. (45) were enriched among those induced in CSM in our study, compared with rich medium (YPD). Of 292 genes found to bind Gcn4p with \( p < 0.005 \) (45), 68 showed at least 2-fold increased expression in CSM compared with YPD with FDR < 0.1 (our data), of a total of 592 such genes. Clearly some genes are activated in CSM by mechanisms not involving Gcn4p, which is not surprising. Nevertheless, this overlap is highly significant (Fig. 4A) and indicates that Gcn4p-associated genes are highly enriched among those genes activated under the conditions used here (particularly considering the stringency used in defining Gcn4p-associated genes, and in defining increased expression in CSM compared with YPD medium). Second, we found that the only functional categories described by the MIPS data base (38) that were significantly enriched among genes expressed more highly in CSM than YPD were metabolism (\( p < 10^{-14} \)) and energy (\( p < 10^{-7} \)), with the most significantly enriched subcategories being amino acid biosynthesis and amino acid metabolism (\( p < 10^{-14} \)) (\( p \) values were corrected for the number of functional categories tested). These are the categories we would expect to find for Gcn4p-activated genes (58). Third, we used T-profiler (59) to seek motifs enriched among genes differentially expressed in CSM and YPD media. The only three motifs enriched among genes expressed more highly in CSM than YPD were Msn2–4, TBP, and Gcn4p. T-profiler also confirmed that Gcn4p binding was also very highly enriched among the genes activated in CSM compared with YPD medium, based on the genome location analysis of Harbison et al. (45) (data not shown). Thus, Gcn4p-regulated genes represent a substantial subset of those genes that are actively expressed in CSM as assayed here.

We next asked whether Gcn4p-activated genes were enriched among those genes showing altered expression in the histone tail deletion strains compared with wild-type strains grown in CSM. From four biological replicates comparing gene expression in H3Δ1–28 and wild-type yeast, we found 465 genes showing altered expression with FDR < 0.1. Of these 465 genes (about 9% of those analyzed), only 23 were found to bind Gcn4p in yeast grown in synthetic media with \( p < 0.005 \) in the genome-wide location analysis study of Harbison et al. (45) (Fig. 4B). This is only 8% of the 292 genes that bound Gcn4p with \( p < 0.005 \) and does not represent a significant overlap.

As a second test for enrichment of Gcn4p-regulated genes among those most affected by the H3Δ1–28 mutation, we searched the promoter sequences of those genes showing the most significant (FDR < 0.1) change in expression for Gcn4p binding sites. We first used the sequences of 27 known Gcn4p binding sites to establish a motif for Gcn4p binding (Fig. 4C). The sequence specificity thus obtained closely matches that found by others (45, 60–62). Searching the promoter sequences of 397 genes showing either reduced (188 genes) or increased (209 genes) expression in H3Δ1–28 yeast with FDR < 0.1 did not reveal significant enrichment in Gcn4p-binding sequences. As a positive control, the same search method was employed on a dataset of 68 promoter

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**FIGURE 3. Comparison of transcript levels, normalized to PYK1, in H3Δ1–28 (CY1–4C) and the corresponding wild-type yeast (CY2–4C) (the same data used in Fig. 1B) and from H4Δ2–26 (NSY438) and the corresponding wild-type yeast (NSY429).** Values shown for H4Δ2–26 were averages from four independent determinations, and standard deviations are indicated. Brackets indicate differences in transcript levels with \( p < 0.05 \) (*) or \( p < 0.01 \) (**), or the indicated values, according to Student’s test. Note that because the data for the H3 and H4 strains (both MX1-4C derivatives) were independently normalized (see “Experimental Procedures”), their transcript levels as shown here cannot be compared directly.
We had anticipated a significant correlation between the set of genes showing reduced expression in H3Δ1–28 or H4Δ2–26 yeast compared with wild type, and the set of genes activated by Gcn4p, and so were surprised by these results. One explanation for our failure to observe a general decrease in expression of genes activated by Gcn4p upon deletion of the H3 or H4 amino terminus is that transcription of these genes may, contrary to expectation, not depend on Gcn5p. Indeed, Hinnebusch and colleagues (50) have reported widely varying dependence on Gcn5p among several genes that are known to be activated by Gcn4p, despite the known ability of Gcn4p to recruit Gcn5p.

To examine the relationship between gene regulation by Gcn5p and the H3 and H4 tails by a different route, we took advantage of a recent genome location analysis of histone acetyltransferases, including Gcn5p, in yeast grown in YPD (63). Using our previous data on the effect of loss of or mutation of lysine residues in the H3 or H4 amino terminus on genome-wide transcription in yeast grown in YPD (44), we examined the effect of loss or mutation of the H3 or H4 tail in yeast grown in YPD medium on expression of genes showing most significant association with Gcn5p (p < 0.005) (63). Because we wished to test for decreased expression of Gcn5p-regulated genes upon loss or mutation of the H3 or H4 amino terminus, we first selected genes showing significant binding of Gcn5p and having transcript levels high enough so that decreases in transcription could be accurately identified. Robert et al. (63) report a correlation between Gcn5p binding and expression level, and indeed of the 88 genes that bind Gcn5p with p < 0.005, 41 are among the 170 most highly transcribed genes (of 5065 total) (64). Data are available for the effect of GCN5 deletion on expression of 38 of these 41 genes (64). We examined the effect on expression of these 38 genes (which include 32 ribosomal protein genes) of deletion of the H3 or H4 amino terminus, or mutation of the lysine residues to glutamines in the H3 or H4 tail, in yeast grown in YPD. Remarkably, almost all of these open reading frames showed modestly (1.1–1.7-fold, i.e. to 60–90% of wild-type levels) decreased transcription upon loss of either the H3 or H4 amino terminus (Table 3), and the large majority showed similarly decreased transcription upon mutation of lysines to glutamines in either the H3 or H4 amino terminus (data not shown). Microarray data of Holstege et al. (64) revealed that all of the ribosomal protein genes in this group, along with EFB1, HHF2, and HTB1, showed decreased transcription by 1.1–1.6-fold in gcn5Δ yeast grown in YPD, whereas TIFF51A and HTA1 showed no change and HHT2 increased 1.1-fold (Table 3). Taken together, the microarray data and our examination of individual transcripts by Northern analysis indicate that genes associated with or dependent on Gcn5p for expression exhibit a modest but consistent dependence on the H3 amino terminus, and slightly less consistent dependence (Fig. 2) on the H4 amino terminus, for expression at wild-type levels.

**Gcn4p-mediated Activation Overcomes Repression by the H3 and H4 Amino Terminus**—The histone tails might contribute positively to transcriptional regulation by providing a platform for binding of components of the transcriptional machinery when they are appropriately modified, as envisioned in the histone code hypothesis. Alternatively (or additionally), they might normally be repressive toward transcription by interfering with binding of transcription factors, and their modification might be needed to overcome this repressive effect (65). In the latter case, removal of the tails, or loss of the positively charged lysine residues (by their replacement by glutamine residues) might derepress Gcn4p/Gcn5p-dependent genes under non-inducing conditions, and might or might not affect their activation.

To examine this possibility, we performed a clustering analysis of microarray data for genes likely to depend on Gcn4p for their expres-
H3 and H4 Tails in Gcn4p/Gcn5p Activation

TABLE 3

| Gene name | gcn5Δ | H3Δ 1–28 | H4 Δ2–26 |
|-----------|-------|----------|----------|
| -Fold change |
| RPS10A | 1.2 | -1.66 | -2.62 |
| HHF2 | 1.1 | -1.35 | -1.68 |
| RPL33B | 1.2 | -1.45 | -1.69 |
| RPS15 | 1.3 | -1.25 | -1.35 |
| RPL13A | 1.5 | -1.30 | -1.46 |
| RPS23A | 1.3 | -1.04 | -1.44 |
| RPL17B | 1.3 | -1.29 | -1.37 |
| RPL39 | 1.3 | -1.48 | -1.74 |
| HTB1 | 1.1 | -1.39 | -1.10 |
| RPL17A | 1.2 | -1.22 | -1.30 |
| RPS22A | 1.1 | -1.19 | -1.33 |
| RPS19B | 1.3 | -1.30 | -1.29 |
| EFB1 | 1.6 | -1.33 | -1.18 |
| RPL19B | 1.3 | -1.14 | -1.25 |
| RPS9B | 1.3 | -1.34 | -1.53 |
| RPS16B | 1.3 | -1.33 | -1.53 |
| RPP1B | 1.3 | -1.04 | -1.12 |
| RPS13 | 1.4 | -1.17 | -1.32 |
| HTA1 | 1 | -1.25 | 1.01 |
| RPL12B | 1.2 | -1.18 | -1.37 |
| RPL27B | 1.5 | -1.30 | -1.60 |
| TIF51A | 1 | -1.10 | -1.16 |
| RPL30 | 1.1 | -1.26 | -1.25 |
| RPL24A | 1.1 | -1.27 | -1.27 |
| RPL28 | 1.1 | -1.23 | -1.34 |
| RPS27B | 1.3 | -1.05 | -1.17 |
| RPL16A | 1.2 | -1.19 | -1.37 |
| RPS5 | 1.2 | -1.21 | -1.31 |
| RPL37A | 1.2 | -1.17 | -1.18 |
| RPL6A | 1.6 | -1.39 | -1.64 |
| RPL13B | 1.5 | -1.32 | -1.53 |
| RPS16A | 1.2 | -1.48 | -1.71 |
| HHT2 | 1.1 | -1.50 | -1.59 |
| RPP2A | 1.3 | -1.27 | -1.34 |
| RPL18A | 1.2 | -1.26 | -1.28 |
| RPL25 | 1.3 | -1.47 | -1.64 |
| RPL5 | 1.1 | -1.17 | -1.21 |
| RPL11A | 1.2 | -1.31 | -1.34 |

Negative signs indicate a decrease in transcript level relative to wild type.

FIGURE 5. K-means clustering (K = 4) indicates a high degree of overlap between genes activated by Gcn4p in CSM plus 3-AT and those repressed by the H3 or H4 tail in rich (YPD) medium. Clustering was performed on 403 putatively Gcn4p-regulated genes (see text for details), using microarray data from this work and Sabet et al. (44). Genes showing increased expression are represented by red bars and those showing decreased expression are represented by green bars, with intensity being proportional to the magnitude of the change (from 4-fold down, brightest green, to 14-fold up, brightest red). Four distinct clusters are indicated at the left edge of the panel.

Discussion

The histone amino termini in eukaryotic chromatin are modified by a variety of post-translational modifying enzymes that function as coactivators and corepressors in transcription (1). These enzymes are recruited by the primary DNA-binding activators or repressors, and their recruitment results in modifications of the histone tails (69, 70).
However, the extent to which these histone modifications contribute to transcriptional activation or repression, and the mechanism by which they do so, remain largely unresolved. In addition, whether specific activators (or repressors) recruit the same coactivators (or corepressors) to different promoters, with the same functional consequences, is an open question. In this work, we have addressed these issues by testing the effect of incremental deletions of the histone H3 amino terminus on expression of a small group of genes that are regulated by Gcn4p and/or Gcn5p, and comparing this to the effect of deleting GCN5 or the H4 amino terminus.

Most of the genes examined showed reduced transcription upon loss or mutation of the H3 amino terminus, indicating a positive role for the H3 tail in their activation. The strongest effects were generally seen upon deletion of amino acids 1–5 and 11–15 (Fig. 1, A and B). Consistent with this, mutation of Lys-4 or Lys-14 to arginine or glutamine also reduced activation, as did mutation of Lys-18, another potential target of Gcn5p (24) (Fig. 2). Deletion of GCN5 resulted in decreased transcription that correlated fairly well with the decreases seen upon loss of mutation of the H3 tail: those genes showing reduced transcription in the gcn5Δ yeast (HIS4, HIS3, HOM2, LYS1, and SAM2) also showed dependence on the H3 tail, whereas ARG1 and TRP2 showed little dependence on either Gcn5p or the H3 tail (Fig. 1C). Loss of the H4 amino terminus also resulted in decreased transcription for some of those genes examined, but this effect did not correlate strongly with dependence on GCN5 or the H3 amino terminus (Fig. 3). Taken together, these results are consistent with acetylation of the histone H3 amino terminus contributing positively to transcriptional activation mediated by Gcn5p (24, 51, 55, 70, 71).

We also attempted to address the relationship between transcriptional regulation by Gcn4p and Gcn5p, and the regulation by the histone H3 and H4 amino termini on a more global scale by analysis of our own and other available microarray data. Genome-wide data on binding of Gcn5p to gene promoters, or on transcriptional dependence on Gcn5p, under conditions of Gcn4p induction are not currently available. However, among genes found to bind Gcn5p during growth in YPD (63), we did find a modest correlation between dependence for full transcriptional activation on Gcn5p and the H3 tail (Table 2). In contrast, and despite the ability of Gcn4p to recruit Gcn5p to activated promoters (25–28, 50), we found no evidence for general dependence of activation by Gcn4p on either Gcn5p or the H3 or H4 amino terminus. In agreement with this finding, Hinnebusch and colleagues (50) have found that Gcn4p is able to recruit multiple coactivators to promoters that it activates, but the requirement for these coactivators varies greatly among individual promoters.

Understanding the basis for this apparent variable redundancy among coactivators at different promoters presents a major challenge to the field of transcriptional regulation.

Interestingly, we did find evidence that many of the genes activated by Gcn4p under amino acid starvation conditions are repressed by Rpd3p under non-activating conditions (Fig. 5). This repression requires both the histone H3 and H4 amino termini, consistent with earlier results (44), and indicates that one function of Gcn4p in transcriptional activation with this finding, Hinnebusch and colleagues (50) have found that the histone amino termini contribute to repression of many Gcn4p-regulated genes, but are not generally required for their activation. At the same time, some genes, particularly those requiring Gcn5p for full activation, do show some dependence on the histone tails (Figs. 1–3), suggesting that histone modifications are likely to play an instructive role at these genes. These two roles are of course not mutually exclusive, so there is no contradiction in genes being repressed by the histone amino termini but also, at least in some cases, requiring their presence for wild-type levels of transcriptional activation.

In conclusion, our results showing a correlation between transcriptional regulation by Gcn5p and by the histone H3 amino terminus indicate that the H3 tail plays a small but significant positive role in transcriptional activation by Gcn5p. This positive role is distributed among more than one of the modifiable lysine residues in the H3 tail, and most likely involves the creation of favorable sites for protein-protein interactions needed for transcriptional activation (including those that favor recruitment of chromatin remodeling complexes) via lysine acetylation (28, 73). We do not find evidence for a general dependence on Gcn5p for Gcn4p-mediated activation. We do, however, find that a large fraction of genes activated by Gcn4p are repressed in rich medium (where they are not activated by Gcn4p) by the H3 and H4 amino termini, indicating that one role of Gcn4p is to overcome this repression. Positive and negative roles for the histone amino termini in transcription have been amply demonstrated previously (1). Our finding that commonly regulated genes can be both positively and negatively regulated by the histone H3 and H4 amino termini is consistent with the idea that multiple components of the eukaryotic transcriptional apparatus have evolved to contribute to transcriptional responses by exerting context-dependent effects on transcription. Identification of such “ambivalent” responses mediated by histones underscores the altered logic that distinguishes eukaryotic from prokaryotic transcriptional regulation (74–76).

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