# Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes in vivo

**Min-Hao Kuo,[1] Jianxin Zhou,[1] Per Jambeck,[2] Mair E.A. Churchill,[2] and C. David Allis[1,3]**

[1]Department of Biology, University of Rochester, Rochester, New York 14627; [2]Department of Cell and Structural Biology, University of Illinois, Urbana-Champaign, Illinois 61801 USA

**Gcn5p is a transcriptional coactivator required for correct expression of various genes in yeast. Several transcriptional regulators, including Gcn5p, possess intrinsic histone acetyltransferase (HAT) activity in vitro. However, whether the HAT activity of any of these proteins is required for gene activation remains unclear. Here, we demonstrate that the HAT activity of Gcn5p is critical for transcriptional activation of target genes in vivo. Core histones are hyperacetylated in cells overproducing functional Gcn5p, and promoters of Gcn5p-regulated genes are associated with hyperacetylated histones upon activation by low-copy Gcn5p. Point mutations within the Gcn5p catalytic domain abolish both promoter-directed histone acetylation and Gcn5p-mediated transcriptional activation. These data provide the first in vivo evidence that promoter-specific histone acetylation, catalyzed by functional Gcn5p, plays a critical role in gene activation.**

[*Key Words:* GCN5; histone acetylation; histone acetyltransferase; transcription; chromatin]

Received October 31, 1997; revised version accepted December 29, 1997.

Eukaryotic transcription occurs on DNA templates that exist in a repressive chromatin environment. As revealed by both in vitro and in vivo studies, binding of transcriptional activators and basal transcription machinery to this template is often, but not always, hampered by barriers imposed by nucleosomal arrays (for review, see Owen-Hughes and Workman 1994; Wolffe 1994; Felsenfeld 1996). One solution to this problem is the existence of chromatin remodeling factors/activities that can alter or restructure nucleosomes near promoter elements, often in an ATP-dependent fashion, thus facilitating transcriptional initiation (for review, see Kingston et al. 1996; Pazin and Kadonaga 1997a; Tsukiyama and Wu 1997). Another solution may be provided by the post-translational modification of the core histones themselves (Bradbury 1992; Turner and O'Neill 1995; Wolffe and Pruss 1996).

Among the known covalent modifications of core histones, the reversible acetylation of internal, often invariant, lysine residues in the amino-terminal domains has long been linked positively to transcriptional activation (Brownell and Allis 1996). Although transcriptional regulation and histone acetylation appear to be correlated in several biological systems (e.g., Jeppesen and Turner 1993; Bone et al. 1994; Hebbes et al. 1994), experimental data demonstrating a causative role of histone acetylation in gene activation have remained elusive (however, see Ura et al. 1997). Recent discoveries of enzymes responsible for influencing the steady-state balance of histone acetylation (for review, see Hampsey 1997; Wade and Wolffe 1997) provide new opportunities to address this and related issues.

The *Saccharomyces cerevisiae GCN5* gene encodes a transcription adaptor (or coactivator) that is required for full-level transcription of various genes (Georgakopoulos and Thireos 1992; Brandl et al. 1996; Martens et al. 1996; Saleh et al. 1997; Welihinda et al. 1997). Recently, yeast Gcn5p and homologous GCN5 family members from organisms ranging from *Tetrahymena* (p55; Brownell et al. 1996; Kuo et al. 1996) to humans [hGcn5p; human p300/CBP-associated factor (hPCAF); Yang et al. 1996; Wang et al. 1997], have been shown to possess intrinsic histone acetyltransferase (HAT) activity with strong preference for specific histone substrates and internal acetylation sites (e.g., Lys-14 of H3 and Lys-8 and Lys-16 of H4 with yGcn5p; Kuo et al. 1996). Intrinsic HAT activity has also been observed in several other proteins involved in transcriptional activation, such as PCAF (Yang et al. 1996), TATA-binding protein-associated factor$_{II}$250 (TAF$_{II}$250) and its homologs (Mizzen et al. 1996), p300/CBP (CREB-binding protein) (Bannister and Kouzarides 1996; Ogryzko et al. 1996), ACTR/SRC-1 (activator of retinoid receptor/steroid recptor coactivator) (Chen et al. 1997; Spencer et al. 1997), and ESA1 (essential SAS family acetyl transferase) (E.R. Smith, A. Eisen, J.C. Lucchesi, and C.D. Allis, in prep).

As a transcriptional coactivator, yGcn5p functions

**[3]Corresponding author.**
**E-MAIL cda@allis.biology.rochester.edu; FAX (716) 275-2070.**

within the context of a multisubunit complex that interacts with specific transcription activators as well as the basal transcription machinery (Marcus et al. 1994; Silverman et al. 1994; Georgakopoulos et al. 1995; Candau and Berger 1996; Chiang et al. 1996). Known components of this adaptor complex include Ada1p, Ada2p, Ada3p/Ngg1p, and Ada5p/Spt20p (Candau and Berger 1996; Marcus et al. 1996; Roberts and Winston 1996; Grant et al. 1997; Horiuchi et al. 1997; Saleh et al. 1997). Recent biochemical and genetic evidence suggests that other members of the TATA-binding protein (TBP)-related class of SPT (suppressor of Ty) gene products may function with Gcn5p in larger, potentially redundant, chromatin remodeling or modification complexes (Grant et al. 1997; Roberts and Winston 1997).

To date, the best evidence linking HAT activity to transcriptional activation is derived from analyses of serial deletion mutants of yGcn5p (Candau et al. 1997). All *GCN5* truncation alleles that are able to support Gcn5p functions in vivo possess detectable HAT activity in vitro. In contrast, mutants that lose the capability to acetylate histones in vitro fail to complement *gcn5* null mutants in either growth or transcriptional reporter assays. However, whether Gcn5p affects levels of histone acetylation in vivo has not yet been directly demonstrated. Whether other proteins, including known components of the transcription machinery, are also physiological substrates for these activities remains an open question (Gu and Roeder 1997; Imhof et al. 1997; for review, see Pazin and Kadonaga 1997b).

In this report, we sought to investigate further the relationship between histone acetylation and transcriptional activation. Point mutations were introduced to residues of the minimal catalytic domain of yGcn5p (Candau et al. 1997) that are conserved in all known GCN5 family members. We demonstrate that Gcn5p is likely to acetylate core histones in vivo and that the HAT activity of this enzyme is critical for enhancing the transcription of target genes. Cross-linking and coimmunoprecipitation experiments using antibodies directed at a known site of Gcn5p-mediated acetylation reveal that the promoter region of affected genes is enriched in acetylated histone isoforms only in the presence of functional Gcn5p for transcriptional activation. Together, these results are consistent with models of targeted histone acetylation, and provide strong evidence that HAT activity of yGcn5p is essential for its in vivo function.

## Results

### *Identification of amino acid residues in yGcn5p important for HAT activity*

A minimal catalytic domain supporting Gcn5p-mediated histone acetylation has been mapped recently to a region that, between amino acids 170 and 253, contains three of four highly conserved subdomains identified in the GCN5 family of acetyltransferases (II–IV; see Fig. 1) (Brownell et al. 1996; Candau et al. 1997). Others have identified conserved sequence motifs in amino-acetyltransferases (Coon et al. 1995; Lu et al. 1996) and putative acetyltransferases (Lin et al. 1996; Reifsnyder et al. 1996; Neuwald and Landsman 1997); the functional significance of residues within these motifs is not well understood. However, in a small number of cases, mutagenesis of conserved residues in domains II and III implicate these regions in acetyl CoA binding (Tercero and Wickner 1992; Coleman et al. 1996; Lu et al. 1996).

To test whether similar residues are essential for the Gcn5p function in vivo, we first systematically substituted amino acid residues in the minimal catalytic domain of yGcn5p (amino acids 170–253) with alanine (Fig. 1A). We chose to mutate residues that are well conserved among the known HATs that contain a clear acetyltransferase signature domain (domains II and III) and those that are shared only by histone acetyltransferases (domain IV). In vitro HAT activity of each mutant Gcn5p was tested after expressing and purifying these proteins from *Escherichia coli.* As seen in Figure 1C, a large number, but not all, of these mutants showed significantly reduced HAT activity relative to wild-type Gcn5p. This result is consistent with the high degree of sequence conservation across these domains, and suggests that this region of the protein is critical for its ability to function as a HAT.

### *In vivo growth defects of* gcn5 *mutants*

The above HAT assays were done with bacterially expressed Gcn5p in the absence of other yeast proteins. Because yGcn5p exists in several large, multisubunit complexes (Georgakopoulos et al. 1995; Candau and Berger 1996; Grant et al. 1997; Pollard and Peterson 1997; Saleh et al. 1997), we set out to determine whether any of the above Gcn5p mutants were also defective in vivo. To this end, each mutant was subcloned, along with *GCN5* 5′ and 3′ transcriptional regulatory sequences, into a low copy *ARS/CEN* plasmid and transformed into a *gcn5*Δ strain. Gcn5p is involved in maximal expression of certain amino acid biosynthesis genes (Georgakapolous and Thireos 1992); thus, loss of *GCN5* leads to poor growth under amino acid starvation. The ability of each allele to complement growth of the *gcn5*Δ yeast strain in minimal medium was then investigated.

Shown in Figure 2A is a comparison of growth of several representative yeast transformants on synthetic minimal medium. As predicted, wild-type *GCN5* rescued growth under amino acid starvation, whereas the vector alone control exhibited poor growth under these conditions. With each of the above point mutants as the sole source of Gcn5p, several mutants caused clear *gcn5*$^-$ phenotypes. Among these alleles, F221A caused the most profound defect followed by Y244A/E245A, D214A, G239A/Y244I, Y220A, I174A/I179A, and G187A/G189A. The last two double mutants caused subtle, but highly reproducible, growth phenotypes; such partial defects in the general control pathway can be augmented by applying amino acid analogs, such as ethionine or 3-aminotriazole to the medium (data not shown). The *gcn*$^-$ phenotypes associated with the above mutants

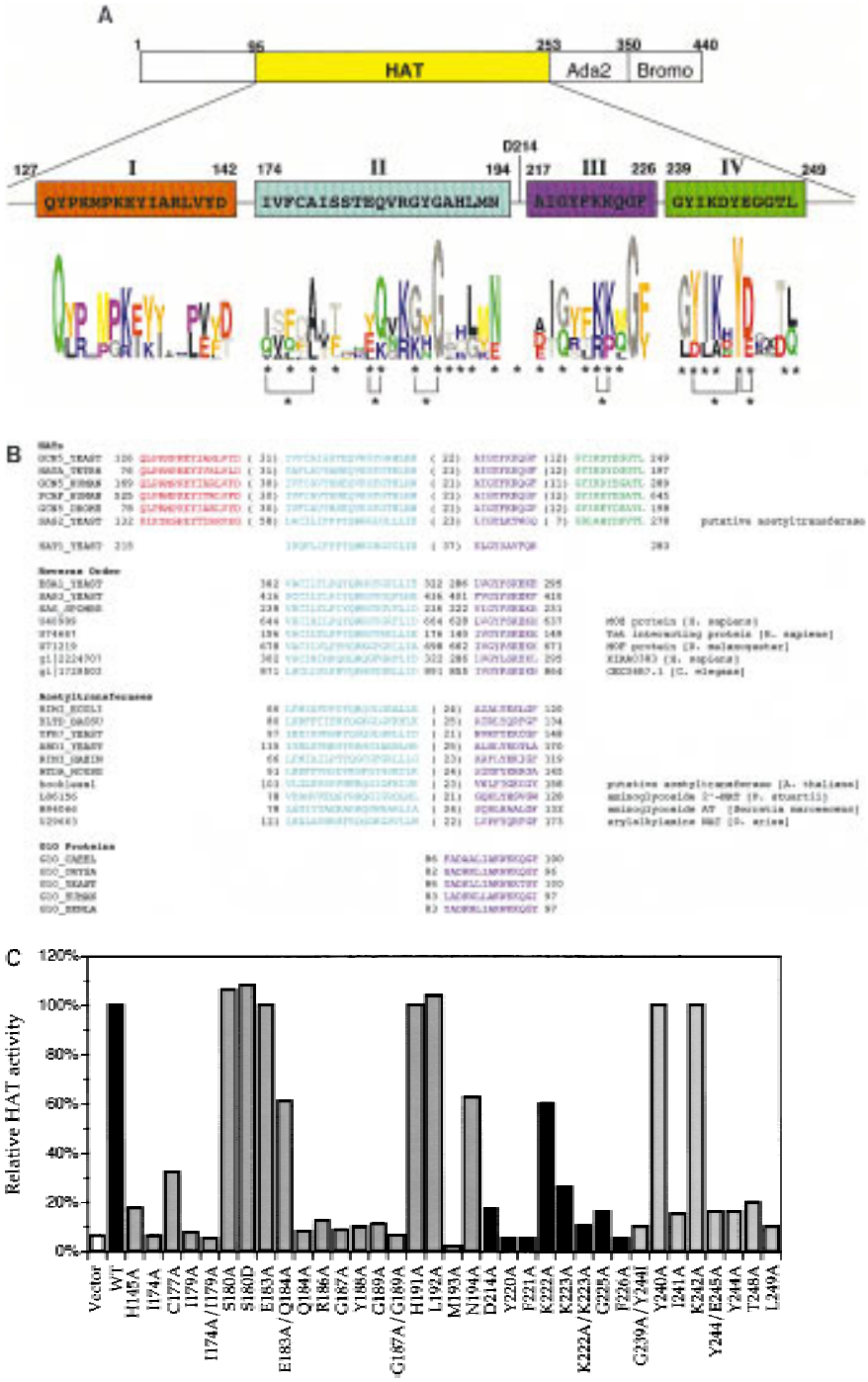


**Figure 1.** Most conserved amino acid residues play an important role in histone acetylation in vitro. (*A*) Schematic diagram depicting the catalytic domain boundaries of yGcn5p (Brownell et al. 1996; Candau et al. 1997), amino acid sequences in HAT motifs I–IV, and sequence logos of each domain compiled from the sequences aligned in *B*. (*B*) Sequence alignments of Gcn5-type HATs, amino-acetyltransferases, and domain III homology regions from G10 proteins used in the analysis of the domain profiles shown in *A*. Color coding of the motifs corresponds to that in *A*. (*C*) Relative in vitro HAT activity of each mutant. Equivalent amounts of bacterially expressed Gcn5p derivatives were used for standard liquid HAT assays. Incorporation of the $^{3}$H-labeled acetate counts into histones was measured by scintillation counting and compared to wild-type Gcn5p. Shown are the relative HAT activity with the wild-type levels set to 100%. Vector only and wild-type controls were purified and assayed with every preparation of mutants analyzed to control for batch-to-batch variation; each mutant has been tested independently at least three times. Results are depicted from a single experiment; different shades of the bars represent each subdomain

were unlikely to be attributable to insufficient expression or protein instability as Western analyses showed roughly equal amounts of Gcn5p (Fig. 3A; data not shown). It is also unlikely that F221A or other in vivo defective mutants possess gross conformational abnormality as coimmunoprecipitation, using an anti-Ada2p antibody, demonstrates that several of our in vivo defective mutants, including F221A, are still able to interact with Ada2p (data not shown).

To provide a more quantitative assessment of the growth defects of our gcn5 mutants, we compared the growth curves of four representative strains cultivated in the liquid minimal medium (see Fig. 2B). Consistent with the differential colony sizes observed on plates, the lack of functional *GCN5* caused a significant lag of doubling time (cf. wild type, 132 ± 2 min vs. vector control, 179 ± 5 min and L192A, 126 ± 5 min vs. F221A, 165 ± 2 min). In addition, the *gcn5*Δ and F221A mutants appear

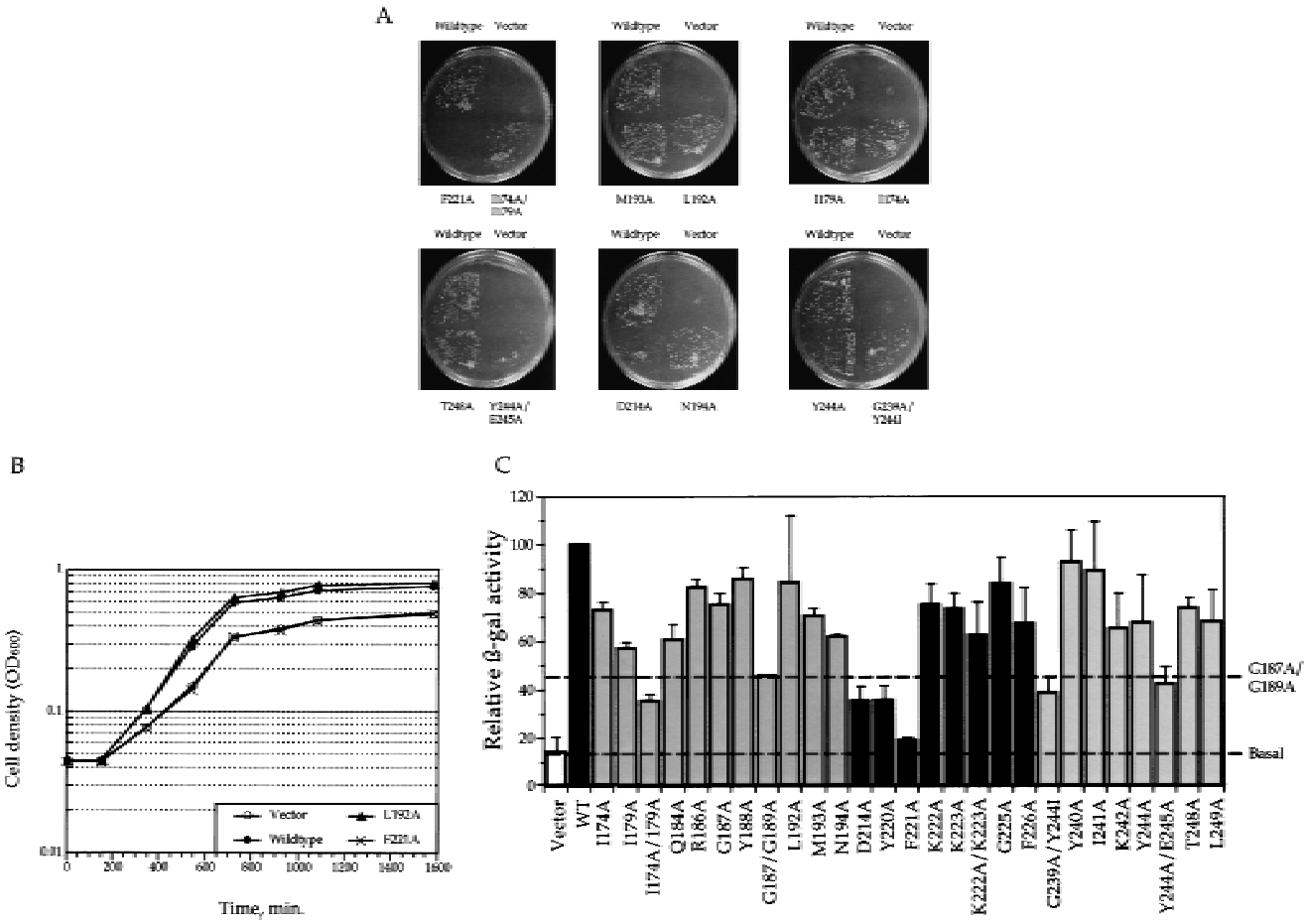


**Figure 2.** In vivo tests of functions of mutant Gcn5p. (*A*) Growth complementation test. Shown are representative clones plated to synthetic minimal medium and grown at 30°C for 3 days before pictures were taken. All mutants have been tested at least three times for reproducibility. Note that certain mutations, such as Y220A, I174A/I179A, and G187A/G189A, consistently showed retarded growth. However, with long-termed storage at 4°C after 30°C incubation was completed, colonies of these strains reached the size close to that of wild-type strains. (*B*) Growth curves of four representative clones grown in minimal medium. (*C*) Transcriptional activation potency of Gcn5p derivatives. *gcn5* null cells were first double-transformed with the GAL4–VP16 and β-gal expression constructs (both are 2 μ plasmids) followed by different *GCN5* alleles (*CEN/ARS* minichromosomes). The β-gal expression was measured from exponentially growing cells and presented here as the percentage of that activated by wild-type Gcn5p; each mutant has been assayed independently three to ten times. The relatively wide range of standard errors of certain samples is at least partially attributable to the copy number variation of the *GCN5* plasmid (data not shown).

to enter stationary phase at lower cell densities. Apparently, the combination of these two phenotypic defects associated with *gcn5* loss-of-function mutations results in the significantly smaller colonies of these mutants shown in Figure 2A.

### *Growth defects of* gcn5 *mutants correlate with defects in transcriptional activation*

A second functional test for Gcn5p is to measure the expression level of *lacZ* driven by the UAS*gal–CYC1 cis*-acting element in vivo. In this construct, *lacZ* reporter gene expression is activated by Gal4–VP16 chimeric activator and requires functional Gcn5p and other Ada proteins (Candau et al. 1997; Wang et al. 1997). Gal4–VP16 activator and *lacZ* reporter constructs were cotransformed into a *gcn5*Δ strain followed by a second transformation with low-copy plasmids bearing specific *GCN5* alleles. β-Galactosidase activity was then measured, and the results are summarized in Figure 2C.

It is clear that wild-type Gcn5p activated *lacZ* expression efficiently. As expected, F221A, the most affected mutant (see Fig. 2A) was unable to activate *lacZ* much above that by the vector alone control. Moreover, in complete agreement with each mutant's ability to rescue growth in minimal medium (Fig. 2A), all mutants that showed *gcn5*$^-$ phenotypes activated *lacZ* less efficiently than those that supplied *GCN*$^+$ function. Noteworthy is the G187A/G189A double mutant that exhibited the most subtle visible *gcn5*$^-$ growth defect in the growth complementation assay. In the *lacZ* expression assay, this allele appears to represent a ''threshold'' activity. Mutants expressing *lacZ* at a higher level all function well in rescuing growth under amino acid starvation,

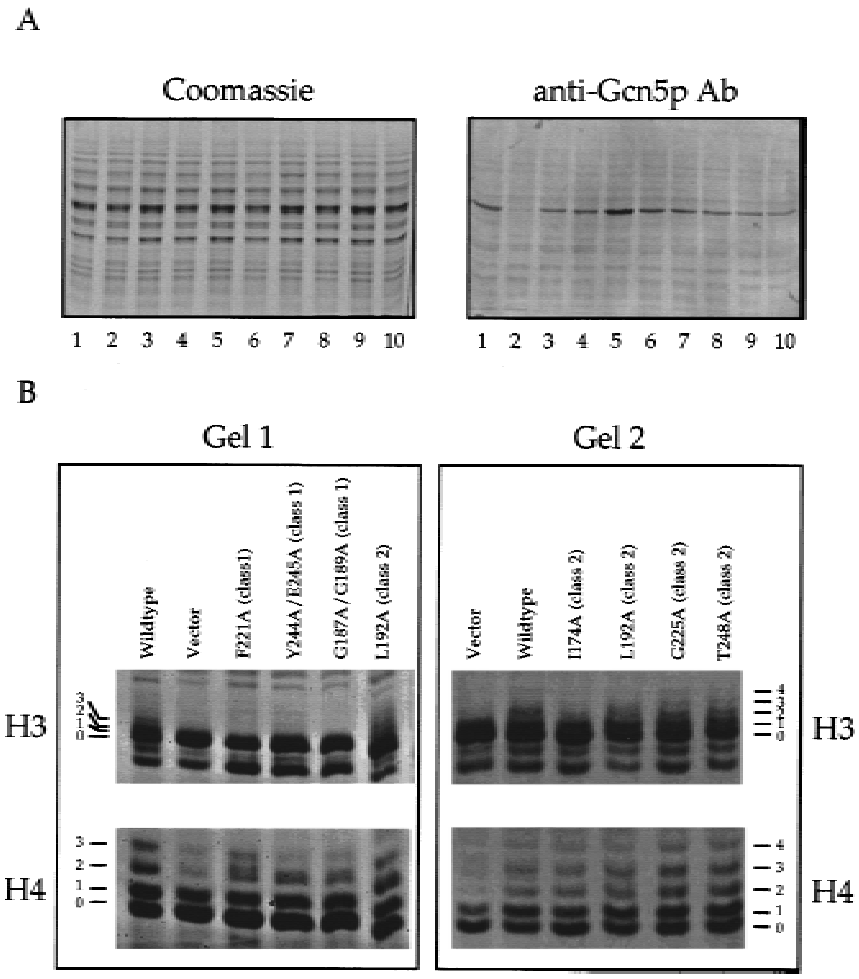


**Figure 3.** Overproduction of functional Gcn5p leads to hyperacetylation of histones in vivo. (*A*) Western analysis of overproduced Gcn5p. Yeast whole cell extracts (20 µg) prepared from strains overproducing various Gcn5p derivatives were resolved by SDS-PAGE (*left*) and blotted for Western detection with Gcn5p antibodies (*right*). Note that the endogenous Gcn5p was in a low abundance under our standard Western conditions. The overproduced *GCN5* alleles are as follows: (lane *1*) Wild type; (lane *2*) vector; (lane *3*) F221A; (lane *4*) Y244A/E245A; (lane *5*) G187A/G189A; (lane *6*) I174A; (lane *7*) L192A; (lane *8*) G225A; (lane *9*) T248A; (lane *10*) wild type. Although there is a certain degree of expression variation of different Gcn5p derivatives, this variation is not likely sufficient to account for the dramatic difference in in vivo activity displayed by each allele. (*B*) Histones H3 and H4 are hyperacetylated in the presence of overexpressed functional Gcn5p derivatives. Purified core histones were resolved by 15% Triton–acetic acid–urea gel electrophoresis and visualized by silver staining. Acetylation ladders are marked on the side. Samples shown (*left* and *right*) were electrophoresed on separate gels. The same preparation of wild-type and vector samples were included on both gels for reference. Note that all three class 1 mutants showed a lower degree of H3 acetylation and subtle, but readily visible, effects on H4 acetylation.

whereas others that exhibit lower transcriptional activation cannot fully complement growth in minimal medium. *GCN5* mutants that we have assayed for in vivo functional integrity are listed in Table 1. Alleles in the first class show reproducible defects in vivo, whereas class 2 includes all mutants that functionally complement *gcn5*Δ phenotypes in vivo. Because these two in vivo functional assays (growth under amino acid starvation and UAS*gal*–*CYC1*–*lacZ* expression) reflect transcription activated by at least two separate transcription activators (Gcn4p and Gal4–VP16, respectively), we conclude that Gcn5p-dependent activation is likely to use a common mechanism (i.e., histone acetylation at promoters; see below).

**Table 1.** *Amino acid residues in the HAT catalytic domain of Gcn5p that are critical for* in vivo *functions*

| Class | Transcription and growth defects | Mutations |
|---|---|---|
| 1 | yes | I174A/I179A, G187A/G189A, D214A,Y220A, F221A, G239A/Y244I, Y244A/E245A |
| 2 | no | I174A, I179A, E183A, E183A/Q184A, R186A, G187A,Y188A, G189A, L192A, M193A, N194A, K222A, K223A, K222A/K223A, G225A, F226A, Y240A, K242A, I241A, Y244A, T248A, L249A |

### *Overproducing functional Gcn5p leads to histone hyperacetylation*

To provide in vivo evidence that *GCN5* encodes a histone acetyltransferase, we examined *GCN5* dosage-dependent changes in histone acetylation. Consistent with the finding that genes under the control of Gcn5p are likely to occupy a small portion of the yeast genome (Pollard and Peterson 1997), we find that deleting *GCN5* does not reveal any significant changes in steady-state histone acetylation patterns (data not shown). To circumvent this problem, we overexpressed wild-type and mutant Gcn5p (Fig. 3A), and analyzed the acetylation status of individual histones by Triton–acetic acid–urea gel electrophoresis, a gel system capable of separating

histones based on the number of acetyl moieties that each histone isoform contains.

As shown in Figure 3B, characteristic ''ladders'' indicative of increasing levels of acetylation are seen in both H3 and H4, and to a lesser extent, H2A and H2B (not shown) when wild-type Gcn5p is overproduced. In the vector control sample, only a very small percentage of H3 is acetylated and most of the acetylated H4 exists in a monoacetylated isoform. However, when histones, isolated from strains overproducing various Gcn5p mutants, were analyzed under the same condition, the ability of each allele to acetylate histones correlates precisely with the ability to supply normal GCN5 functions in vivo. For example, F221A, Y244A/E245A, and G187A/G189A (as well as Y220A and G239A/Y244I; data not shown), mutants that exhibit clear in vivo defects (see Fig. 2), all fail to produce high levels of H3 and H4 acetylation (gel 1; Fig. 3B). Compared with control histones from wild-type Gcn5p overproduction strain, underacetylated histones H3 and H4, as indicated by the lower intensity of acetylated isoforms, are associated with these three *gcn5* mutants.

Histones from several mutants that function nearly normally in our in vivo assays were also examined; these histones all show comparable levels of hyperacetylation to those generated by wild-type Gcn5p (gel 2; Fig. 3B). The three alleles shown in gel 2 (i.e., I174A, G225A, and T248A) were selected because they function normally in vivo (see Fig. 2) despite an apparent reduction in in vitro HAT activity. Thus, in vivo overproduction of such mutants provides a good indicator as to whether the HAT activity can be restored in vivo, and if so, whether the *GCN*$^+$ phenotype is correspondingly maintained. Together with transcriptional assay and growth complementation tests, these results suggest strongly that Gcn5p is indeed a HAT in vivo and that histones are physiological substrates of this enzyme. Most important, our results are consistent with Gcn5p HAT activity being required for transcriptional activation of target genes (see below).

### HIS3 *activation mediated by Gcn5p is linked to histone hyperacetylation*

The above data suggest that maintaining the HAT activity in vivo is a prerequisite to transcriptional activation. To link directly the HAT activity of Gcn5p to the activation of specific target genes, we performed chromatin immunoprecipitation (ChIP) experiments (Braunstein et al. 1993, 1996) with low-copy *GCN5* alleles, to evaluate the status of nucleosomal histone acetylation. We first analyzed nucleosomal acetylation in the promoter region of *HIS3* gene whose expression requires a functional Gcn5p. Yeast *gcn5*Δ strains bearing various *GCN5* alleles (*CEN/ARS*, *GCN5* 5′ and 3′ native sequences) were starved for amino acids, including histidine, to induce *HIS3* expression. After formaldehyde cross-linking and chromatin solubilization, sonication-sheared oligonucleosomes were incubated with acetylation-specific H3 antibody for coimmunoprecipitation of DNAs that are associated with hyperacetylated H3 (and likely other core histones). After reversal of cross-links, the DNA was analyzed by slot-blot hybridization to determine whether sequences of interest were enriched in the hyperacetylated H3 fraction under a given condition.

Derepression of *HIS3* and some other amino acid biosynthesis genes requires functional Gcn5p. As shown in Figure 4B, Gcn5p is required for both constitutive and activated expression of *HIS3* gene, a phenomenon reported earlier by others (Georgakopoulos and Thireos 1992). For constitutive expression, the absence of functional Gcn5p leads to a twofold decrease in transcription. Under conditions of amino acid starvation, where *HIS3* expression is fully derepressed, the lack of functional Gcn5p (i.e., with the null or F221A allele) causes a threefold decrease of the transcriptional output of this gene. Importantly, in excellent agreement with the above Northern data, histone H3 is hyperacetylated when *HIS3* is activated by functional Gcn5p (Fig. 4C). Association of the *HIS3* promoter with hyperacetylated nucleosomes increases threefold in wild-type and L192A alleles of *GCN5*$^+$ strains under repressive conditions. Furthermore, with amino acid starvation, hyperacetylation of the *HIS3* promoter increases sixfold in the presence of wild-type or L192A Gcn5p. In contrast, in either a repressive or derepressive situation, the F221A mutant behaves similarly to the vector control (i.e., this allele cannot provide normal Gcn5p functions in either transcriptional activation or histone acetylation).

To rule out the possibility that the observed difference in IP efficiency by hyperacetylated histone H3 antibody among different *GCN5* alleles is attributable to variation of formaldehyde cross-linking efficiency or to a strain-specific accessibility of the *HIS3* locus to the antibody, we conducted the ChIP experiments using an antibody against unacetylated histone H3 (Braunstein et al. 1993). As shown in Figure 4D, consistent with the notion that transcriptional repression is associated with histone underacetylation, the *HIS3* promoter sequence is enriched in the unacetylated histone fraction when *GCN5* is either deleted (vector) or replaced with the F221A allele (cf. un. and ac. columns in Fig. 4D). The *GCN5* allele-specific, differential enrichment of *HIS3* promoter by a specific antiserum strongly suggests that there is a dynamic change in nucleosomal acetylation within Gcn5p-mediated transcriptional activation.

### *Promoter-specific histone hyperacetylation of UAS*gal–CYC1–lacZ

We also examined the nucleosomal histone acetylation status in the UAS*gal–CYC1–lacZ* construct (Fig. 5B). Again, using the ChIP assay, significant association of this promoter with hyperacetylated nucleosomes is observed in the presence of active transcription of *lacZ* driven by functional Gcn5p. Although this increase is lower than that seen in the *HIS3* locus, we speculate that this is attributable to surplus copies of the reporter construct (a 2μ plasmid) in the cell where only a few copies of *GCN5* allele (a *CEN/ARS* vector) are present.

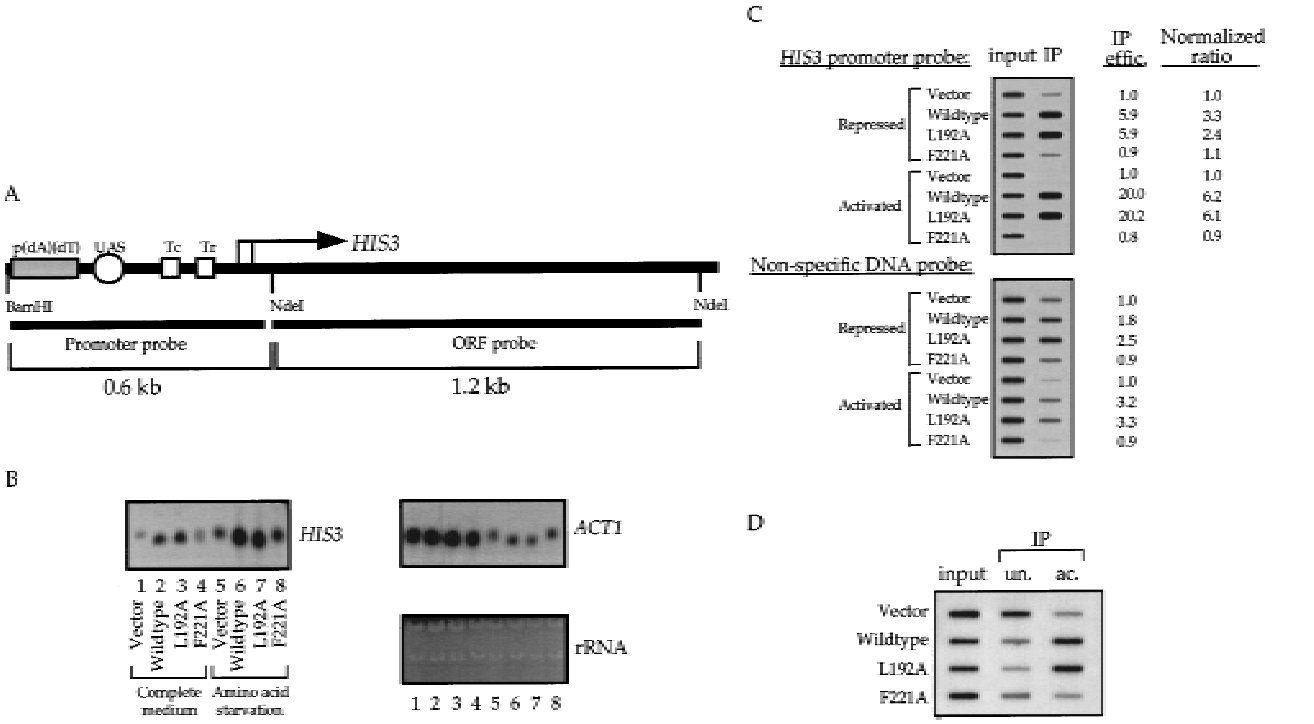


**Figure 4.** Hyperacetylation of nucleosomal H3 is linked to the chromosomal copy of *HIS3* activated by functional Gcn5p. (*A*) Source of the probes. The ORF probe was used only for Northern blot analyses. (*B*) Northern blot analysis of *HIS3* transcription. The ratio of *HIS3* expression, after normalization with *ACT1* mRNA, was 1 : 1.8 : 2.5 : 0.9 : 6.0 : 19.4 : 19.3 : 8.2 (lanes *1–8*). The generally lower expression of *ACT1* under amino acid starvation is probably attributable to the complete lack of amino acids in the minimal medium during the 6-hr incubation before RNA extraction. (*C*) Chromatin IP results. Yeast cells were processed for immunoprecipitation of solubilized chromatin fragments using anti-H3.AcLys(9/14) antiserum. DNAs coprecipitated in these nucleosomal complexes were purified and analyzed by slot-blot hybridization. Relative IP efficiency (counts of the IP divided by those of the input) was obtained setting the value of the vector control slot as 1 in each hybridization result. The normalized ratio was derived by dividing the IP efficiency of each sample to that obtained when total genomic DNAs were used as a probe. (*D*) Chromatin IP using the antisera against unacetylated or hyperacetylated H3 shows comparable IP efficiency in different *GCN5* backgrounds. Yeast extracts were subjected to ChIP using antisera against unacetylated (un.) or acetylated (ac.) H3 and the resultant slot-blots were first probed with *HIS3* promoter sequence as above, followed by control probes, including *ACT1* promoter and total genomic DNA, which showed essentially identical IP efficiency seen in Fig. 4C (data not shown).

It is reasonably well documented that hyperacetylated histones are associated with transcriptionally poised genes, sometimes marking an entire transcription unit (Hebbes et al. 1994, 1992). We wished to determine whether this was also the case for Gcn5p-activated genes in yeast. To this end, we used a probe that is 1.5 kb 3′ to the start codon of *lacZ* gene (see Fig. 5A). Unexpectedly, the amount of this DNA fragment associated with hyperacetylated H3 was not higher than the background levels, suggesting that H3 is hyperacetylated preferentially in the promoter region. Essentially identical results of this promoter-preferred nucleosomal hyperacetylation is seen in at least one other class 2 $GCN5^+$ allele, G225A, but not in the I174A/I179A class 1 mutant (data not shown). Thus, we conclude that the Gcn5p-related histone hyperacetylation is a highly specific and local (promoter-specific) event.

## Discussion

In this report, several lines of evidence are presented suggesting that yeast Gcn5p, a well-documented HAT in vitro, is likely a HAT in vivo, and that its HAT activity is critical for transcriptional activation of target genes. These data are summarized as follows: (1) All of the Gcn5p mutants that were able to activate transcription of the target genes maintained their ability to catalyze wild-type levels of histone acetylation in vivo and vice versa. (2) A single point mutation, F221A, in the minimal catalytic domain of Gcn5p not only significantly reduced HAT activity in vivo and in vitro, but also severely reduced the ability of Gcn5p to activate transcription. (3) Promoters of at least two different genes were associated with hyperacetylated nucleosomes only when the genes were activated by functional alleles of Gcn5p. In contrast, promoters of these genes were relatively underacetylated in mutants that failed to support Gcn5p functions both in vitro and in vivo. Together, these data provide strong evidence that Gcn5p is involved in a pathway of activating gene expression through promoter-specific histone acetylation and that the HAT activity of this protein is essential to bring about these events.

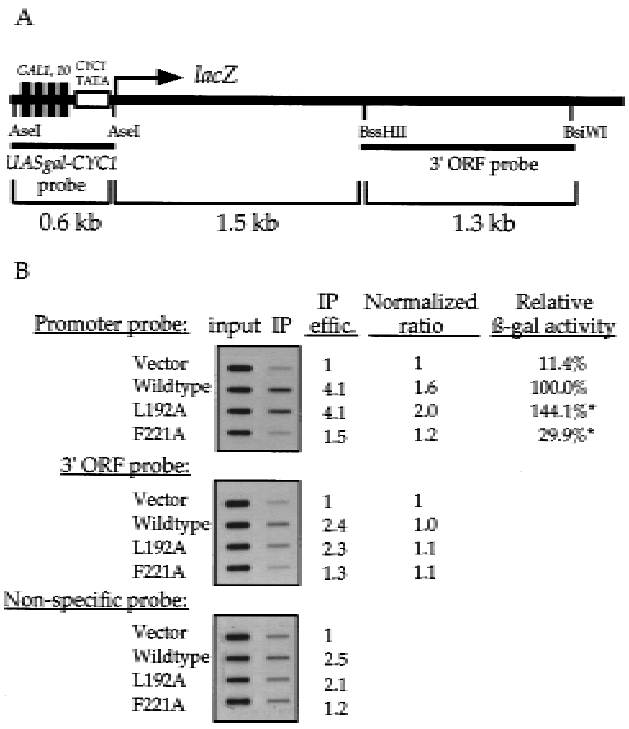


**Figure 5.** UAS*gal–CYC1* promoter sequence is associated with hyperacetylated histones when *lacZ* is activated by functional Gcn5p. (*A*) A schematic diagram of the UAS*gal–CYC1–lacZ* gene and the source of the probes used in the ChIP experiments. (*B*) Chromatin IP results. Assay conditions were essentially the same as *HIS3* described in Fig. 4. When quantified using *GCN5* gene as the probe, two samples (*) contained a higher copy number of the *GCN5* plasmid (not shown); this probably accounts for the roughly twofold increase in β-gal expression when compared with data presented in Fig. 2C.

We acknowledge the formal possibility that there is a ''master'' HAT whose expression is critically dependent on a functional Gcn5p and that this HAT is responsible for most, if not all, transcription-related HAT activity that we have observed in this study. However, this scenario is unlikely for the following reasons. First, all class 1 mutants are catalytically impaired both in vivo and in vitro, making Gcn5p itself the most likely candidate mediating transcription-related histone acetylation in the promoters. Second, deleting *GCN5* does not lead to detectable underacetylation of any histones in vivo, arguing against the presence of a predominant master HAT whose expression or function is under control of Gcn5p. Third, because the Gcn5/Ada complex is known to interact with transcriptional activators and TBP (Silverman et al. 1994; Barlev et al. 1995; Chiang et al. 1996), the physical presence of Gcn5p near the promoter makes it highly unlikely that another HAT is exclusively required to perform histone acetylation at the same genomic loci.

### *Conserved amino acids are critical for Gcn5p functions*

Among the mutations we have created and analyzed, F221A is the most severely damaging allele. It is inactive as a HAT in vivo and in vitro, and it fails to support normal Gcn5p-mediated transcriptional activation. Because this mutant is able to interact with Ada2p normally (not shown), as reflected by in vivo coimmunoprecipitation assays, it is most likely that the in vivo defects are attributable to its inability to catalyze histone acetylation. Consistent with this hypothesis, we observed a strong dominant negative phenotype of this allele with overproduction (data not shown), which suggests that this HAT catalytic mutant, with otherwise unaffected functions in vivo, can titrate away functional interacting proteins and hence brings about a *gcn5*$^-$ phenotype. It is interesting that severe phenotypes are linked to this residue because phenylalanine is not likely to serve as an active site residue, although we point out that the catalytic mechanism used by Gcn5p is not known.

Some of our mutations in yGcn5p subdomains II and III, the regions likely to form an acetyl-CoA-binding motif, have been tested previously in human spermidine/spermine amino-acetyltransferase and yeast MAK3 (maintenance of killer) protein amino-terminal acetyltransferase (e.g., C177, I179, Q184, G187, Y188, G189, D214, F221, G225, and F226 of yGcn5p), and in general our in vitro HAT assay results are in excellent agreement with these studies (Lu et al. 1996). Domain IV, on the basis of our analyses, appears to be conserved only within the HAT family members. We speculate that this domain may be involved in histone substrate (amino-terminal tails) binding, although this hypothesis remains to be examined.

The in vitro HAT assays clearly show that Gcn5p, expressed and purified from bacteria, is sensitive to sequence alterations. There are two likely reasons for this observation. First, yeast Gcn5p are known to form complexes with several other subunits in vivo and in this form is better able to acetylate nucleosomal substrates (Grant et al. 1997; Pollard and Peterson 1997). None of these non-Gcn5p components were included in our in vitro HAT assays, and attempts to reconstitute nucleosomal HAT activity from individual components have so far been unsuccessful (Grant et al. 1997 and our unpublished data). It is likely that the association of accessory proteins with a Gcn5 mutant with, for example, an altered conformation could overcome or neutralize the mutation. Second, mutant Gcn5p may be less stable than the wild type, and therefore not able to fold during expression or purification. Others have observed mutant enzymes that maintain their activity in vivo, despite their altered stability in vitro, and in one case the mutations were made in the hydrophobic core of an enzyme (Serrano et al. 1992; Axe et al. 1996). One implication of our mutagenesis experiments is that many of the conserved residues in domains II and III form part of the conserved hydrophobic core of Gcn5p, and are not residues important specifically for catalytic function or interaction with Gcn5p-binding partners.

In a related study (see Wang et al. 1998), a number of alanine scan mutants in Gcn5p, many overlapping with our point mutants (including F221A), have been tested

for both in vivo transcriptional activation and in vitro HAT activity. In general, our in vivo Gcn5p overproduction and histone hyperacetylation results agree well with their results assaying in vitro HAT activity using Gcn5p-containing complexes. These results are consistent with our notion that many mutants, when assayed in the recombinant and monomeric form, show in vitro enzymatic defects that can be ''rescued'' in vivo presumably by functioning in the context of large, multisubunit Gcn5/Ada or SAGA-type HAT complexes.

### *Gcn5p HAT activity is required for transcriptional activation*

Our data suggest that recruitment of HATs to the promoter, possibly through interactions with activators, other coactivators, and components of the basal transcription machinery, leads to hyperacetylation of histones in the promoter region and transcriptional activation of target genes (Brownell et al. 1996; Wolffe and Pruss 1996; Wade and Wolffe 1997). In vivo phenotypes of Gcn5p mutants are tightly linked to the ''potency'' of each Gcn5p derivative to activate transcription of downstream genes and to acetylate histones. Importantly, the observed nucleosomal hyperacetylation at promoter region holds for two different transcription activators tested in this study. Collectively, these data provide compelling evidence that the HAT activity of Gcn5p is critically required for transcriptional activation of its target genes.

Our study also suggests that hyperacetylated histones may be confined to the promoter region of at least some genes in yeast (Fig. 5). This result differs from previous reports showing that the entire transcription units of some actively expressed loci are associated with hyperacetylated nucleosomes (Hebbes et al. 1994; O'Neill and Turner 1995). Such a discrepancy may be explained, at least in part, by the specific histone or site of acetylation that are under investigation. In our study, we used an antiserum that preferentially recognized a highly preferred site of Gcn5p-mediated acetylation in H3 (Lys-14; see Kuo et al. 1996). Depending on the substrate preference of the recruited HAT, histone hyperacetylation associated with transcriptional activation may escape detection from an antiserum with a different specificity.

Confinement of hyperacetylation to the promoter region is consistent with previous findings that tethering LexA–Gcn5p near a promoter can activate transcription (Georgakopoulos et al. 1995; Candau et al. 1997), suggesting that a ''stationary'' HAT is sufficient for facilitating transcription initiation by the basal transcription machinery on a nucleosomal template. It remains a formal possibility that another HAT, distinct from Gcn5p, conducts elongation-associated histone acetylation that spreads the hyperacetylation signal throughout the transcription unit. Recent reports of Gcn5p-independent HAT complexes in yeast (Grant et al. 1997) as well as the existence of an essential HAT in yeast (E.R. Smith, A. Eisen, J.C. Lucchesi, and C.D. Allis, in prep.) make this an intriguing possibility.

It is noteworthy that the three- to sixfold increase of association of *HIS3* promoter with hyperacetylated histone H3 mediated by functional Gcn5p only reflects the minimal effects of Gcn5p in vivo. In the presence of functional Gcn5p [wild type, L192A, and G225A (not shown)], we observed consistently higher amounts of DNA associated with hyperacetylated histones even when *ACT1* promoter (not shown) or bulk genomic DNA (Figs. 4 and 5) was used to probe the coprecipitated DNA sequences. It is tempting to speculate that Gcn5p is one of the major histone acetyltransferases by which histones are acetylated at specific lysine residues recognized by our antibody [anti-H3.AcLys(9/14)]. If this is the case, the relative IP efficiency we calculated (Figs. 4 and 5) would have been significantly underestimated.

### *Dynamics and universality of histone acetylation*

In our ChIP experiments, we noticed that the efficiency of immunoprecipitation never approached 100% of the input materials. Outside of purely technical reasons (e.g., incomplete formaldehyde cross-linking), several biological explanations may account, at least in part, for the incomplete IP yield. First, cells that respond to the derepressing signals (e.g., amino acid starvation in this case) may comprise only part of the entire population (because of nonsynchronous cell growth or different physiological states of cells). A second interesting possibility is the potential interplay between histone acetyltransferases and deacetylases at the promoter of the genes that we have assayed (for review and references, see Hampsey 1997). In addition to the stable repression of genes conferred by histone deacetylases (Braunstein et al. 1993, 1996), there may be an obligatory equivalent requirement for rapid turnover of acetyl groups on histone tails in genes that need to be down-regulated quickly (see Pazin and Kadonaga 1997b; Wolffe 1997 and reference therein). Finally, it is also possible that nucleosomes are hyperacetylated before their ''remodeling'' near the promoters (e.g., by Swi/Snf complexes) rendering this region DNase I hypersensitive (Steger and Workman 1996). In this case, a smaller fraction of DNA sequences may remain associated with acetylated nucleosomes and be recovered by the ChIP technique.

*GCN5* is a nonessential gene in yeast (Georgakopoulos and Thireos 1992) whose product participates in the expression of only a small number of genes (Pollard and Peterson 1997) raising the issue of the universality of gene activation through histone acetylation. Recent reports indicate that at least two other Gcn5p-independent HAT complexes exist in yeast (Grant et al. 1997). Moreover, a poorly understood Gcn5p-independent function of the SAGA complex has been found functionally interacting with the Srb mediator complex (Roberts and Winston 1997). Therefore, it is important to determine whether expression of the majority of yeast genes is regulated through histone acetylation by different HATs or by other functionally redundant mechanisms.

In conclusion, multiple lines of evidence are presented demonstrating that Gcn5p functions as a histone acetyl-

transferase and that this activity is critical for transcriptional activation in vivo. In addition, we show that the transcription-related histone acetylation, mediated by functional Gcn5p, is concentrated in the promoter region of target genes. Collectively, our data lend strong support to the general idea that histone acetylation plays a causative role in relieving repressive nucleosomal effects on transcriptional activation (Allfrey et al. 1964). The phenomenon of localized (promoter-enriched) histone hyperacetylation provides new insights into mechanisms underlying transcriptional initiation from chromatin templates.

## Materials and methods

### *Strains, plasmids, and manipulation of* GCN5

Genotypes of the yeast strain EJ67 (Siliciano and Tatchell 1984) used in this study are *MAT*α *trp1 leu2-3,112 ura3-52 his4*. The *GCN5* knockout of this strain (yMK703) was created as described (Candau et al. 1997). The low-copy number plasmid of wild-type *GCN5* gene (pRS414–*GCN5*) and *GAL4*–VP16$_{FA}$ constitutive expression vector, UAS*gal*–*CYC1*–*lacZ* plasmid (pLGSD5), were provided by Shelley Berger (Wang et al. 1997).

Standard procedures for site-directed mutagenesis were used to introduce mutations into *GCN5* in a pRSET B-based construct (Kuo et al. 1996). The great majority of mutants contain single mutations. Several double mutants were created to determine whether more severe phenotypes were to be obtained. The G239A/Y244I double mutant was generated fortuitously as a result of an error in the oligonucleotide sequence. Mutations were confirmed by restriction mapping and, in some cases, by sequencing. Sequences of the oligodeoxyribonucleotides are available upon request. For yeast vector construction, *Nco*I fragments of GCN5 containing each point mutation were used to replace the same region of pRS414–GCN5 (wild-type GCN5) before yeast transformation.

For Gcn5p overproduction in vivo, the plasmid pMK120 was created by substituting the *Bam*HI–*Kpn*I fragment of pYEULCB (a *URA3 leu2-d* plasmid with a *CUP1* promoter controlling the inserted gene; Macreadie et al. 1991) with the *Bam*HI–*Ase*I fragment of pYES2 (both the *Kpn*I and the *Ase*I ends were flushed blunt by T4 DNA polymerase) (Invitrogen, Carlsbad, CA). The *Bam*HI–*Sph*I fragments of pRS414–GCN5 and its mutant derivatives were then subcloned into the same sites of pMK120 creating the pMK144 plasmid series that differed only at the amino acid residues being mutagenized for this study. pMK144 plasmids were then transformed into EJ66 strain (MAT**a,** isogenic to EJ67).

### *Sequence analysis of histone acetyltransferases*

Sequences of known histone acetyltransferases [GCN5YEAST (Q03330), HATA1_TT (U47321), GCN5_HUMAN (U57316), PCAF_HUMAN (U57317), and HAT1_YEAST (S52530)] were screened for significant motifs using the programs ASSET (Neuwald and Green 1994) and GIBBS (Neuwald et al. 1995). The sequence of *Drosophila* GCN5 homolog is provided by E. Smith (E. Smith and C.D. Allis, unpubl.). Output from these programs was compared with that of the MEME program (Bailey and Elkan 1994) to confirm that these motifs were reproducible. Blocks generated using the above methods were also used to search the Blocks database (Henikoff and Henikoff 1991) using the LAMA block alignment software (Pietrokovski 1996). Hidden Markov models of the domains were built with HMMER (Eddy et al. 1995) using the blocks as guide alignments and used to search GenPept, the protein section of GenBank. Sequence logos (Schneider and Stephens 1990) were constructed using the Blocks server. Fold recognition using H3P2 (Rice and Eisenberg 1997) and TOPITS (Rost 1995) detected no significant similarity between HATs and known structures. A sequence match was observed between the carboxy-terminal end of HAT subdomain III and a region of the G10 proteins, nuclear factors that are well conserved in eukaryotes from yeast to human; edg2, a human G10 protein, appears to be expressed in response to induction by phorbol esters (Hla et al. 1995).

### *Enzyme assays*

Recombinant Gcn5p was expressed and purified from *E. coli* as described before (Kuo et al. 1996) with the following modifications: 20 mM imidazole was included in the Ni–NTA agarose binding reaction. Bound Gcn5p was washed three times for 10 min each [50 mM NaPi (pH 6.0), 300 mM NaCl, 10% glycerol] followed by a 5-min wash with the same buffer at pH 5.5. Gcn5p was then eluted with a 5-min incubation with pH 4.0 elution buffer; this elution step was repeated once before eluates were pooled. One-twentieth of the volume of 1 M $Na_2HPO_4$ was then added immediately to neutralize the pH. Gcn5p remains active in this buffer for at least several months when stored at −80°C.

HAT assays were conducted in 20-µl reactions containing 10-µg of free chicken histones, 0.1 µCi of $^3$H-acetyl CoA (4–6 Ci/mmole), 50 mM Tris HCl (pH 8.0), 10% glycerol, 1 mM DTT, and 10 mM *n*-butyrate. The reaction was incubated at 30°C for exactly 20 min. Approximately 15 ng of wild-type Gcn5p saturates the reaction; typically, 10 and 20 ng of each Gcn5p derivative was used to obtain the relative HAT activities. Each mutant has been purified and assayed independently for HAT activity at least three times.

### *Yeast histone preparation and analyses*

Gcn5p overproduction was done by growing yeast strains bearing pMK144 series plasmids in synthetic complete −Ura, −Leu medium (to increase the copy number of pMK144) containing 200 µM of $Cu_2SO_4$ (to fully induce Gcn5p expression) until mid-log phase. Yeast histones were purified according to Edmondson et al. (1996).

The 15% Triton X-100–acetic acid–urea polyacrylamide gels (typically, 1.5 mm thick, 13 × 14 cm$^2$) were used to separate yeast histones according to Mullen et al. (1989) except that gels were run at 250 V for 16 hr to obtain good resolution of isoforms of H2A, H2B, and H4. For better resolution of H3, the gel was run for 20 hr. Proteins were visualized by silver staining.

### *Transcriptional and growth complementation assays*

Expression of *lacZ* and growth complementation assays were as described by Candau et al. (1997). *gcn5*Δ cells (yMK703) were transformed with each mutant derivative of Gcn5p on pRS414-GCN5. For colony size comparison, log-phase transformants in complete drop-out medium were plated to synthetic minimal medium and grown at 30°C for 3 days. In addition, late log-phase cells were inoculated into liquid minimal medium for growth curve analyses. For β-gal expression and measurement, yMK703 was transformed with GAL4–VP16$_{FA}$ expression vector, UAS*gal*–*CYC1*–*lacZ* plasmid, and different alleles of Gcn5p mutants. Log-phase transformants were harvested for β-gal measurement. Three to ten of each mutant transformants were analyzed.

*Immunochemical procedures and chromatin immunoprecipitation*

For immunoprecipitation of formaldehyde cross-linked chromatin, 100 ml of log-phase culture grown in synthetic medium at 0.4–0.6 $OD_{600}$/ml was made into 1 ml of final whole cell extracts according to Strahl-Bolsinger et al. (1997). Ten microliters of the antiserum specific for histone H3 acetylated at Lys-9 and Lys-14 (Braunstein et al. 1996) (or 40 µl of unacetylated H3 antiserum) was used for every 200 µl of whole cell extract. The Ag–Ab reaction was done at 4°C for 4 hr to overnight followed by protein A–Sephadex (six volumes of antisera in 1:1 slurry) incubation for an additional hour at 4°C. The remaining procedures were essentially the same as those described previously (Strahl-Bolsinger et al. 1997).

In the case of UAS*gal–CYC1–lacZ* chromatin IP, loading of DNA recovered from immunoprecipitates was first normalized on a test blot using *lacZ* ORF DNA as the probe. Copy number of the *lacZ* reporter construct apparently varied, as reflected later by using total yeast genomic DNA as the probe. Input material for the blot hybridization comprised 10% of that subjected to immunoprecipitation. The same blot was first probed with promoter probe, followed by 3′ ORF, rDNA (not shown), and total genomic DNA probes with stripping of the previous probe before each new round of hybridization. The radioactivities were quantitated by ImageQuant software using the PhosphorImager (Molecular Dynamics) and normalized by comparing with the same samples probed by the total genomic DNA probes.

For *HIS3* expression, cells were grown in complete medium to log phase. Twenty-five percent of the culture was harvested for RNA extraction, and another 25% for formaldehyde fixation of ChIP; these samples represent repressive condition for transcriptional activation. The rest of the cultures was transferred to synthetic minimal medium (without amino acids) containing 10 mM 3-AT (3-aminotriazole) for 6 hr before RNA extraction or fixation for ChIP. Twenty micrograms of total RNAs were used for Northern blot hybridization. rRNA, as reflected by acridine orange staining, and *ACT1* mRNA, detected by hybridization, were used as the control for loading and blotting.

The procedure for preparing yeast total extracts for Western blot analyses was the same as that used for ChIP (Strahl-Bosinger et al. 1997) except that 0.1% of SDS and 1 mM PMSF was included in the FA–lysis buffer. Gcn5p was detected by standard Western blot analysis procedure using rabbit anti-Gcn5p antibodies (a gift of Shelley Berger, Wistar Institute, Philadelphia, PA).

## Acknowledgments

We thank Shelley Berger for communicating data before publication and for providing us with strains, plasmids, and antisera. We acknowledge Ian Macreadie for sharing plasmids for overproducing Gcn5p. We are grateful to Martin Gorovsky, Beth Grayhack, and Sharon Roth for critical comments on this manuscript. M.H.K. thanks Beth Grayhack for constant encouragement and suggestions throughout this work and Martin Gorovsky for introducing him to the field of chromatin studies. This work is supported by the National Institutes of Health (NIH) (GM53512) to C.D.A. M.E.A.C. acknowledges support from The American Heart Association, NIH (Shannon Award GM52100), and a Cellular and Molecular Biology NIH Pre-doctoral Training Grant (P.J.).

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked ''advertisement'' in accordance with 18 USC section 1734 solely to indicate this fact.

## References

Allfrey, V.G., R. Faulkner, and A.E. Mirsky. 1964. Acetylation and methylation of histones and their possible role in regulation of RNA synthesis. *Proc. Natl. Acad. Sci.* **51:** 786–794.

Axe, D.D., N.W. Foster, and A.R. Fersht. 1996. Active barnase variants with completely random hydrophobic cores. *Proc. Natl. Acad. Sci.* **93:** 5590–5594.

Bailey, T.L. and C. Elkan. 1994. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. In *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology* (ed. R. Altman, D. Brutlag, P. Karp, R. Lathrop, and D. Searls), pp. 28–36. AAAI Press, Menlo Park, CA.

Bannister, A.J. and T. Kouzarides. 1996. The CBP co-activator is a histone acetyltransferase. *Nature* **384:** 641–643.

Barlev, N.A., R. Candau, L. Wang, P. Darpino, N. Silverman, and S.L Berger. 1995. Characterization of physical interactions of the putative transcriptional adaptor, ADA2, with acidic activation domains and TATA-binding protein. *J. Biol. Chem.* **270:** 19337–19344.

Bone, J.R., J. Lavender, R. Richman, M.J. Palmer, B.M. Turner, and M.I. Kuroda. 1994. Acetylated histone H4 on the male X chromosome is associated with dosage compensation in Drosophila. *Genes & Dev.* **8:** 96–104.

Bradbury, E.M. 1992. Reversible histone modifications and the chromosome cell cycle. *BioEssays* **14:** 9–16.

Brandl, C.J., J.A. Martens, A. Margaliot, D. Stenning, A.M. Furlanetto, A. Saleh, K.S. Hamilton, and J. Genereaux. 1996. Structure/functional properties of the yeast dual regulator protein NGG1 that are required for glucose repression. *J. Biol. Chem.* **271:** 9298–9306.

Braunstein, M., A.B. Rose, S.G. Holmes, C.D. Allis, and J.R. Broach. 1993. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes & Dev.* **7:** 592–604.

Braunstein, M., R.E. Sobel, C.D. Allis, B.M. Turner, and J.R. Broach. 1996. Efficient transcriptional silencing in Saccharomyces cerevisiae requires a heterochromatin histone acetylation pattern. *Mol. Cell. Biol.* **16:** 4349–4356.

Brownell, J.E. and C.D. Allis. 1996. Special HATs for special occasions: Linking histone acetylation to chromatin assembly and gene activation. *Curr. Opin. Genet. Dev.* **6:** 176–184.

Brownell, J.E., J. Zhou, T. Ranalli, R. Kobayashi, D.G. Edmondson, S.Y. Roth, and C.D. Allis. 1996. Tetrahymena histone acetyltransferase A: A homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell* **84:** 843–851.

Candau, R. and S.L. Berger. 1996. Structural and functional analysis of yeast putative adaptors. Evidence for an adaptor complex *in vivo*. *J. Biol. Chem.* **271:** 5237–5245.

Candau, R., J.X. Zhou, C.D. Allis, and S.L. Berger. 1997. Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function *in vivo*. *EMBO J.* **16:** 555–565.

Chen, H., R.J. Lin, R.L. Schiltz, D. Chakravarti, A. Nash, L. Nagy, M.L. Privalsky, Y. Nakatani, and R.M. Evans. 1997. Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90:** 569–580.

Chiang, Y.C., P. Komarnitsky, D. Chase, and C.L. Denis . 1996. ADR1 activation domains contact the histone acetyltransferase GCN5 and the core transcriptional factor TFIIB. *J. Biol. Chem.* **271:** 32359–32365.

Coleman, C.S., H. Huang, and A.E. Pegg. 1996. Structure and critical residues at the active site of spermidine/spermine-N1-acetyltransferase. *Biochem. J.* **316:** 697–701.

Coon, S.L., P.H. Roseboom, R. Baler, J.L. Weller, M.A. Namboodiri, E.V. Koonin, and D.C. Klein. 1995. Pineal serotonin N-acetyltransferase: Expression cloning and molecular analysis. *Science* **270:** 1681–1683.

Eddy, S.R., G. Mitchison, and R. Durbin. 1995. Maximum discrimination hidden Markov models of sequence consensus. *Comp. Biol.* **2:** 9–23.

Edmonson, D.G., M.M. Smith, and S.Y. Roth. 1996. Repression domain of the yeast global repressor Tup1 directly interacts with histones H3 and H4. *Genes & Dev.* **10:** 1247–1259.

Felsenfeld, G. 1996. Chromatin unfolds. *Cell* **86:** 13–19.

Georgakopoulos, T. and G. Thireos. 1992. Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J.* **11:** 4145–4152.

Georgakopoulos, T., N. Gounalaki, and G. Thireos. 1995. Genetic evidence for the interaction of the yeast transcriptional co-activator proteins GCN5 and ADA2. *Mol. & Gen. Genet.* **246:** 723–728.

Grant, P.A., L. Duggan, J. Cote, S.M. Roberts, J.E. Brownell, R. Candau, R. Ohba, T. Owen-Hughes, C.D. Allis, F. Winston, S.L. Berger, and J.L. Workman. 1997. Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: Characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes & Dev.* **11:** 1640–1650.

Gu, W. and R.G. Roeder. 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 carboxy-terminal domain. *Cell* **90:** 595–606.

Hampsey, M. 1997. A SAGA of histone acetylation and gene expression. *Trends Genet.* **13:** 427–429.

Hebbes, T.R., A.W. Thorne, A.L. Clayton, and C. Crane-Robinson. 1992. Histone acetylation and globin gene switching. *Nucleic Acids Res.* **20:** 1017–1022.

Hebbes, T.R., A.L. Clayton, A.W. Thorne, and C. Crane-Robinson. 1994. Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken beta-globin chromosomal domain. *EMBO J.* **13:** 1823–1830.

Henikoff, S. and J.G. Henikoff. 1991. Automated assembly of protein blocks for database searching. *Nucleic Acids Res.* **19:** 6565–6572.

Hla, T., A.Q. Jackson, S.B. Appleby, and T. Maciag. 1995. Characterization of edg-2, a human homologue of the Xenopus maternal transcript G10 from endothelial cells. *Biochim. Biophys. Acta* **1260:** 227–229.

Horiuchi, J., N. Silverman, B. Pina, G.A. Marcus, and L. Guarente. 1997. ADA1, a novel component of the ADA/GCN5 complex, has broader effects than GCN5, ADA2, or ADA3. *Mol. Cell. Biol.* **17:** 3220–3228.

Imhof, A., X.-J. Yang, V.V. Ogryzko, Y. Nakatani, and A.P. Wolffe. 1997. Acetylation of general transcription factors by histone acetyltransferases. *Curr. Biol.* **7:** 689–692.

Jeppesen, P. and B.M. Turner. 1993. The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. *Cell* **74:** 281–289.

Kingston, R.E., C.A. Bunker, and A.N. Imbalzano. 1996. Repression and activation by multiprotein complexes that alter chromatin structure. *Genes & Dev.* **10:** 905–920.

Kuo, M.-H., J.E. Brownell, T.A. Ranalli, R.G. Cook, D.G. Edmonson, S.Y. Roth, and C.D. Allis. 1996. Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature* **383:** 269–272.

Lin, R., C.D. Allis, and S.J. Elledge. 1996. PAT1, an evolutionarily conserved acetyltransferase homologue, is required for multiple steps in the cell cycle. *Genes Cells* **1:** 923–942.

Lu, L., K.A. Berkey, and R.A. Casero Jr. 1996. RGFGIGS is an amino acid sequence required for acetyl coenzyme A binding and activity of human spermidine/spermine N1-acetyltransferase. *J. Biol. Chem.* **271:** 18920–18924.

Macreadie, I.G., O. Horaitis, A.J. Verkuylen, and K.W. Savin. 1991. Improved shuttle vectors for cloning and high-level Cu(2+)-mediated expression of foreign genes in yeast. *Gene* **104:** 107–111.

Marcus, G.A., N. Silverman, S.L. Berger, J. Horiuchi, and L. Guarente. 1994. Functional similarity and physical association between GCN5 and ADA2: Putative transcriptional adaptors. *EMBO J.* **13:** 4807–4815.

Marcus, G.A., J. Horiuchi, N. Silverman, and L. Guarente. 1996. ADA5/SPT20 links the ADA and SPT genes, which are involved in yeast transcription. *Mol. Cell. Biol.* **16:** 3197–3205.

Martens, J.A., J. Genereaux, A. Saleh, and C.J. Brandl. 1996. Transcriptional activation by yeast PDR1p is inhibited by its association with NGG1p/ADA3p. *J. Biol. Chem.* **271:** 15884–15890.

Mizzen, C.A., X.-J. Yang, T. Kokubo, J.E. Brownell, A.J. Bannister, T. Owen-Hughes, J. Workman, S.L. Berger, T. Kouzarides, Y. Nakatani, and C.D. Allis. 1996. The TAFII250 subunit of TFIID has histone acetyltransferase activity. *Cell* **87:** 1261–1270.

Mullen, J.R., P.S. Kayne, R.P. Moerschell, S. Tsunasawa, M. Gribskov, M. Colavito-Shepanski, M. Grunstein, F. Sherman, and R. Sternglanz. 1989. Identification and characterization of genes and mutants for an N- terminal acetyltransferase from yeast. *EMBO J.* **8:** 2067–2075.

Neuwald, A.F. and P. Green. 1994. Detecting patterns in protein sequences. *J. Mol. Biol.* **239:** 698–712.

Neuwald, A.F. and D. Landsman. 1997. GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. *Trends Biochem. Sci.* **22:** 154–155.

Neuwald, A.F., J.S. Liu, and C.E. Neuwald. 1995. Gibbs motif sampling: Detection of bacterial outer membrane protein repeats. *Protein Sci.* **4:** 1618–1632.

O'Neill, L.P. and B.M. Turner. 1995. Histone H4 acetylation distinguishes coding regions of the human genome from heterochromatin in a differentiation-dependent but transcription-independent manner. *EMBO J.* **14:** 3946–3957.

Ogryzko, V.V., R.L. Schiltz, V. Russanova, B.H. Howard, and Y. Nakatani. 1996. The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87:** 953–959.

Owen-Hughes, T. and J.L. Workman. 1994. Experimental analysis of chromatin function in transcription control. *Crit. Rev. Euk. Gene Expression* **4:** 403–441.

Pazin, M.J. and J.T. Kadonaga. 1997a. SWI2/SNF2 and related proteins: ATP-driven motors that disrupt protein-DNA interactions? *Cell* **88:** 737–740.

———. 1997b. What's up and down with histone deacetylation and transcription? *Cell* **89:** 325–328.

Pietrokovski, S. 1996. Searching databases of conserved sequence regions by aligning protein multiple-alignments. *Nucleic Acids Res.* **24:** 3836–3845.

Pollard, K.J. and C.L. Peterson. 1997. Role of ADA/GCN5 products in antagonizing chromatin-mediated transcriptional repression. *Mol. Cell. Biol.* **17:** 6212–6222.

Reifsnyder, C., J. Lowell, A. Clarke, and L. Pillus. 1996. Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. *Nature Genet.* **14:** 42–49.

Rice, D.W. and D. Eisenberg. 1997. A 3D-1D substitution ma-

trix for protein fold prediction that includes predicted secondary structure of the sequence. *J. Mol. Biol.* **267:** 1026–1038.

Roberts, S.M. and F. Winston. 1996. SPT20/ADA5 encodes a novel protein functionally related to the TATA-binding protein and important for transcription in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **16:** 3206–3213.

———. 1997. Essential functional interactions of SAGA, a Saccharomyces cerevisiae complex of Spt, Ada, and Gcn5 proteins, with the Snf/Swi and Srb/mediator complexes. *Genetics* **147:** 451–465.

Rost, B. 1995. TOPITS: Threading one-dimensional predictions into three-dimensional structures. In *Proceedings of the third international conference on intelligent systems for molecular biology* (ed. C. Rawlings, D. Clark, R. Altman, L. Hunter, T. Langauer, and S. Wodak), pp. 314–321. AAAI Press, Menlo Park, CA.

Saleh, A., V. Lang, R. Cook, and C.J. Brandl. 1997. Identification of native complexes containing the yeast coactivator/repressor proteins NGG1/ADA3 and ADA2. *J. Biol. Chem.* **272:** 5571–5578.

Schneider, T.D. and R.M. Stephens. 1990. Sequence logos: A new way to display consensus information. *Nucleic Acids Res.* **18:** 6097–6100.

Serrano, L., J.T. Kellis Jr., P. Cann, A. Matouschek, and A.R. Fersht. 1992. The folding of an enzyme. II. Substructure of barnase and the contribution of different interactions to protein stability. *J. Mol. Biol.* **224:** 783–804.

Siliciano, P.G. and K. Tatchell. 1984. Transcription and regulatory signals at the mating type locus in yeast. *Cell* **37:** 969–978.

Silverman, N., J. Agapite, and L. Guarente. 1994. Yeast ADA2 protein binds to the VP16 protein activation domain and activates transcription. *Proc. Natl. Acad. Sci.* **91:** 11665–11668.

Spencer, T.E., G. Jenster, M.M. Burcin, C.D. Allis, J. Zhou, C.A. Mizzen, N.J. McKenna, S.A. Onate, S.Y. Tsai, M.-J. Tsai, and B.W. O'Malley. 1997. Steroid receptor coactivator one is a histone acetyltransferase. *Nature* **389:** 194–198.

Steger, D.J. and J.L. Workman. 1996. Remodeling chromatin structures for transcription: What happens to the histones?. *BioEssays* **18:** 875–884.

Strahl-Bolsinger, S., A. Hecht, K. Luo, and M. Grunstein. 1997. SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. *Genes & Dev.* **11:** 83–93.

Tercero, J.C. and R.B. Wickner. 1992. MAK3 encodes an N-acetyltransferase whose modification of the L-A gag NH2 terminus is necessary for virus particle assembly. *J. Biol. Chem.* **267:** 20277–20281.

Tsukiyama, T. and C. Wu. 1997. Chromatin remodeling and transcription. *Curr. Opin. Genet. Dev.* **7:** 182–191.

Turner, B.M. and L.P. O'Neill. 1995. Histone acetylation in chromatin and chromosomes. *Semin. Cell Biol.* **6:** 229–236.

Ura, K., H. Kurumizaka, S. Dimitrov, G. Almouzni, and A.P. Wolffe. 1997. Histone acetylation: Influence on transcription, nucleosome mobility and positioning, and linker histone-dependent transcriptional repression. *EMBO J.* **16:** 2096–2107.

Wade, P.A. and A.P. Wolffe. 1997. Histone acetyltransferases in control. *Curr. Biol.* **7:** R82–R84.

Wang, L., C. Mizzen, C. Ying, R. Candau, N. Barlev, J. Brownell, C.D. Allis, and S.L. Berger. 1997. Histone acetyltransferase activity is conserved between yeast and human GCN5 and is required for complementation of growth and transcriptional activation. *Mol. Cell. Biol.* **17:** 519–527.

Wang, L., L. Liu, and S.L. Berger. 1998. Critical residues for histone acetylation by Gen5, functioning in Ada and SAGA complexes, are also required for transcriptional function in vivo. *Genes & Dev.* (this issue).

Welihinda, A.A., W. Tirasophon, S.R. Green, and R.J. Kaufman. 1997. Gene induction in response to unfolded protein in the endoplasmic reticulum is mediated through Ire1p kinase interaction with a transcriptional coactivator complex containing Ada5p. *Proc. Natl. Acad. Sci.* **94:** 4289–4294.

Wolffe, A.P. 1994. The transcription of chromatin templates. *Curr. Opin. Genet. Dev.* **4:** 245–254.

———. 1997. Transcriptional control. Sinful repression. *Nature* **387:** 16–17.

Wolffe, A.P. and D. Pruss. 1996. Targeting chromatin disruption: Transcription regulators that acetylate histones. *Cell* **84:** 817–819.

Yang, X.J., V.V. Ogryzko, J. Nishikawa, B.H. Howard, and Y. Nakatani. 1996. A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* **382:** 319–324.

# Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes in vivo

Min-Hao Kuo, Jianxin Zhou, Per Jambeck, et al.

*Genes Dev.* 1998, **12:**