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Tetrahymena Histone Acetyltransferase A: A Homolog to Yeast Gcn5p Linking Histone Acetylation to Gene Activation

James E. Brownell,* Jianxin Zhou,* Tamara Ranalli,* Ryuji Kobayashi,† Diane G. Edmondson,‡ Sharon Y. Roth,‡ and C. David Allis*

*Department of Biology
University of Rochester
Rochester, New York 14627

†Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

‡Department of Biochemistry and Molecular Biology
University of Texas
M. D. Anderson Cancer Center
Houston, Texas 77030

Summary

We report the cloning of a transcription-associated histone acetyltransferase type A (HAT A). This Tetrahymena enzyme is strikingly homologous to the yeast protein Gcn5, a putative transcriptional adaptor, and we demonstrate that recombinant Gcn5p possesses HAT activity. Both the ciliate enzyme and Gcn5p contain potential active site residues found in other acetyltransferases and a highly conserved bromodomain. The presence of this domain in nuclear A-type HATs, but not in cytoplasmic B-type HATs, suggests a mechanism whereby HAT A is directed to chromatin to facilitate transcriptional activation. These findings shed light on the biochemical function of the evolutionarily conserved Gcn5p–Ada complex, directly linking histone acetylation to gene activation, and indicate that histone acetylation is a targeted phenomenon.

Introduction

Histone acetylation has long been considered a hallmark of transcriptionally active chromatin, as suggested by a wealth of mostly correlative evidence (Csordas, 1990; Turner, 1993; Loidl, 1994; Wolffe, 1994). Although the exact molecular relationship between histone acetylation and transcription remains undefined (Turner and O'Neill, 1995), a commonly held view is that acetylation of specific lysine residues in the highly conserved histone N-terminal tail domains weakens histone–DNA interactions, thereby relieving repressive chromatin structure (Allfrey et al., 1964). Numerous in vivo and in vitro experiments have indicated that transcription of some genes is directly influenced by specific acetylatable lysine residues present in specific histones (Kayne et al., 1988; Durrin et al., 1991; Thompson et al., 1994) and that transcriptional silencing is associated with reduced nucleosomal acetylation in yeast (Braunstein et al., 1993; Braunstein et al., submitted). The N-terminal histone tails have been shown to play a crucial role in restricting factor access to nucleosomal templates (Lee et al., 1993; Juan et al., 1994; Vettese-Dadey et al., 1994; Fisher-Adams and Grunstein, 1995) and are involved in direct interactions with regulatory factors (Johnson et al., 1990; Roth et al., 1992; Thompson et al., 1994; Hecht et al., 1995; Edmondson et al., submitted). Thus, the regulation of histone acetylation is an attractive control point for the regulation of gene expression.

To gain further insight into the relationship between histone acetylation and transcription, we have sought to characterize the major enzyme systems responsible for establishing the steady-state balance of histone acetylation. In particular, we have focused on the isolation of histone acetyltransferase type A (HAT A), an enzyme believed to be responsible for transcription-associated acetylation. Recently, we used an SDS–polyacrylamide gel–based activity assay to detect a single, catalytically active HAT A subunit (p55) from Tetrahymena macronuclei (Brownell and Allis, 1995), a source of chromatin naturally enriched in hyperacetylated histones (Vavra et al., 1982).

We now report the cloning and sequencing of the gene encoding p55, giving us the opportunity to inspect the sequence of a catalytic component of an A-type HAT. Unexpectedly, the sequence of Tetrahymena p55 clearly identifies it as a homolog of Gcn5p (for general control nonrepressed), a well-studied yeast adaptor protein originally defined by genetic analysis as being required for the full activity of a subset of transcriptional activators (Georgakopoulos and Thireos, 1992; for review and references see Guarente, 1995). This surprising result establishes a clear biochemical function for Gcn5p as a HAT catalytic subunit and provides a mechanistic link between histone acetylation and gene activation. Our findings also shed light on the biochemical function of adaptor complexes, suggesting that they may have a more global influence on gene activation through the direct modification of chromatin templates.

Results

Isolation of the Gene Encoding Tetrahymena p55

Recently we reported the identification of a single catalytically active subunit (p55) of HAT A from Tetrahymena (Brownell and Allis, 1995). We obtained the amino acid sequences of six internal p55 peptides and used them to design degenerate oligonucleotide primers. These primers were used in various combinations in the polymerase chain reaction (PCR) either with macronuclear genomic DNA as template or using reverse transcriptase–PCR with total RNA isolated from vegetative cells as template (Figure 1B). PCR products were cloned and sequenced, and DNA segments encoding p55 were judged to be correct if they contained peptide sequence not included in the PCR primers. For example, four internal peptide sequences were confirmed in two PCR products generated with the K36 sense primer and either the K17 or K8 antisense primer (Figure 1B).

Genomic Southern blots using the largest PCR cDNA product as probe revealed that the gene encoding p55 is single copy (data not shown). Standard protocols using rapid amplification of cDNA ends (RACE) were used to obtain the 5′ and 3′ ends of the p55 cDNA. The complete cDNA appears smaller (by approximately 600 bp) than

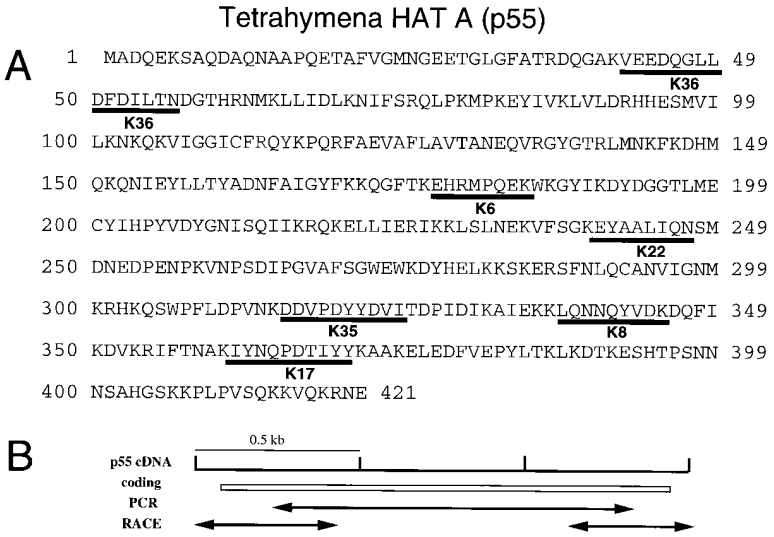

Figure 1. Isolation of the Tetrahymena HAT A Gene

(A) Predicted amino acid sequence of Tetrahymena HAT A (p55). Translation of the longest open reading frame of the cDNA is shown from the first ATG. Sequences unambiguously identified by peptide sequencing are underlined; all six of the peptides originally sequenced from p55 have been identified.

(B) Gene fragments obtained from various cloning strategies. See text and Experimental Procedures for details.

the detected genomic band, indicating that the p55 gene may be disrupted by multiple introns. The complete cDNA and the derived amino acid sequence of p55 have been deposited in the GenBank database.

Predicted translation of the longest open reading frame of the p55 gene, beginning with the first in-frame AUG codon, is shown in Figure 1A (in Tetrahymena the codons UAA and UAG specify glutamine, with the only nonsense codon being UGA; Prescott, 1994), and it is likely to be correct for several reasons. First, all six peptide sequences originally obtained from p55 are found in the predicted coding region (underlined in Figure 1A). Second, the calculated molecular mass of the protein (49 kDa) is in reasonable agreement with the apparent mass of the polypeptide (55 kDa) obtained by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) analysis (see below). Third, Northern blot analyses of poly(A)$^+$ RNA isolated from growing cells detect a single 1.9 kb species, a size consistent for an mRNA encoding a polypeptide of this size (data not shown). Finally, primer extension analyses show that the proposed ATG start codon is the only ATG within the 5′ flanking nontranscribed spacer (data not shown); this ATG is typical of translation start sites in other Tetrahymena genes (M. Gorovsky, personal communication).

To establish further that the cloned p55 gene encodes HAT activity, synthetic peptides were built according to the K36 and K17 peptide sequences and used to generate polyclonal antibodies. Immunoblotting analyses using either the anti-K36 (Figure 2A, lane 2) or the anti-K17 (lane 3) peptide antibodies consistently detect a single band (see arrow) migrating at 55 kDa in total protein extracts from macronuclei (lane 1). This polypeptide was not observed when the immune sera were incubated with an excess of K36 or K17 peptide, respectively, or when preimmune serum was used (data not shown), thus demonstrating the specificity of both peptide antisera for p55.

To determine whether this immunoreactive polypeptide correlates with HAT activity, we resolved macronuclear extracts containing HAT activity by reverse-phase high pressure liquid chromatography (RP-HPLC) and analyzed the resulting fractions by immunoblotting with the anti-K36 serum or tested them in parallel for activity using the activity gel assay (Figure 2B). The activity gel assay detected a single labeled product band migrating at 55 kDa in unfractionated samples (labeled L in Figure 2B) and in two RP-HPLC fractions containing active p55 (see arrows in Figure 2B, lanes 6 and 7; also see Figure 5 of Brownell and Allis, 1995). This polypeptide was also easily detected by the anti-K36 peptide antibodies (arrows in Figure 2B, lanes 6 and 7). We conclude that the K36 and K17 peptide antibodies are highly selective for p55 and detect a catalytically active subunit of HAT A in both crude and highly resolved macronuclear extracts.

The peptide antibodies were also used to immunodeplete HAT activity. Using the K36 peptide antibodies, approximately 60% of the input HAT activity was consistently immunodepleted from crude macronuclear extracts (Figure 2C). Preincubation of the antiserum with excess K36 peptide completely abolished depletion of HAT activity. Since the K36 peptide sequence has been verified in the p55 cDNA, and since the K36 peptide antibodies are highly selective for p55 on immunoblots and specifically deplete HAT activity, these data strongly suggest that we have cloned the gene encoding the 55 kDa catalytic subunit responsible for transcription-associated HAT activity in macronuclei.

Tetrahymena p55 Is Homologous to Yeast Gcn5p

Comparison of the p55 amino acid sequence with available databases revealed that p55 is highly homologous to the previously defined yeast protein Gcn5. Gcn5p is a putative transcriptional adaptor implicated in mediating transcriptional activation by acidic activators such as herpesvirus virion protein 16 (VP16) and yeast Gcn4p (Georgakopoulos and Thireos, 1992; Guarente, 1995). Alignment of the two polypeptides (Figure 3) indicates 40% overall sequence identity and 60% similarity between the ciliate and yeast proteins. Similar percentages have recently been noted between yeast and human Gcn5 (Candau et al., 1996). Scattered domains of high sequence identity (~80%; see brackets labeled I–V in

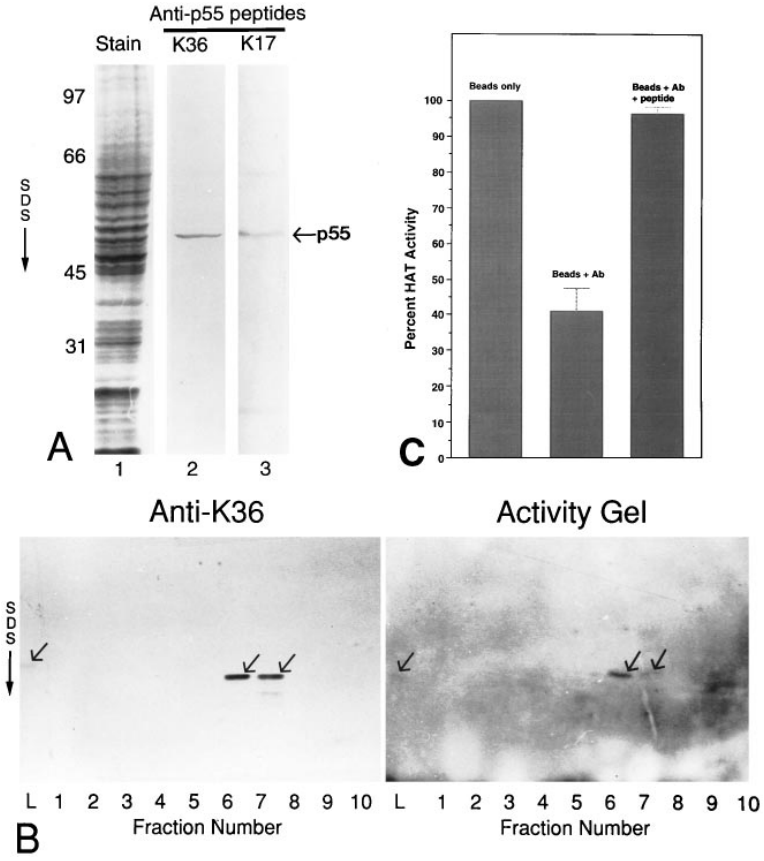

Figure 2. Peptide Antibodies Highly Selective for p55 Immunodeplete HAT Activity

(A) Total macronuclear protein was resolved in an 8% SDS–polyacrylamide gel and analyzed by staining (lane 1) or was transferred to Immobilon-P and reacted with K36 (lane 2) or K17 (lane 3) peptide antibodies. Arrow indicates a single immunoreactive band migrating at 55 kDa.

(B) Fractions containing HAT activity were subjected to RP-HPLC and processed for immunoblotting with the K36 peptide antibodies shown in (A) or were subjected in parallel to the activity gel assay as described by Brownell and Allis (1995). Essentially all of the HAT activity is detected in fractions 6 and 7 (for details see Figure 5 of Brownell and Allis [1995]). Arrows indicate a single immunoreactive band migrating at 55 kDa. L, input fraction

(C) Crude macronuclear extracts were incubated in the presence or absence of the K36 serum and then with protein A–Sepharose. After removal of the beads, the supernatants were tested in a standard liquid HAT assay (Brownell and Allis, 1995). Experiments were conducted using either protein A–Sepharose alone (beads only), the K36 peptide antibodies (beads plus antibodies [Ab]), or K36 antibodies that had been preincubated with an excess of K36 peptide as a control for nonspecific binding (beads plus Ab plus peptide). Data are plotted as the mean percent of input activity remaining in the supernatants; error bars represent the standard error about the mean from three independent experiments. Approximately 10,000 dpm of [^{3}H]acetate was incorporated into histones in the "beads only" condition and was set to equal 100%. Immunodepletion experiments have not been pursued using the K17 peptide antibodies.

Figure 3), along with numerous conservative substitutions observed along the entire sequence of both polypeptides, illustrate the strong similarity between Gcn5p and Tetrahymena p55. Curiously, a 51 amino acid N-terminal extension in Gcn5p is not found in p55. In contrast, a 23 amino acid C-terminal extension exists in p55 that is not found in Gcn5p. The functional significance of these differences is not known.

The remarkably high degree of similarity between Tetrahymena p55 and yeast Gcn5p (Figure 3) suggests that both proteins have HAT activity. To test this hypothesis, we cloned the yeast *GCN5* gene and expressed it in Escherichia coli. Extracts from cells induced or uninduced for Gcn5p expression were assayed directly using the activity gel HAT assay (Figure 4). Tetrahymena macronuclear extracts (see arrowhead in lane 1 of Figure 4) and bacteria expressing Gcn5p (see arrow in lane 2) each display single, strongly labeled bands migrating with molecular masses of approximately 55 and 60 kDa, respectively, in the fluorograph of a histone-containing activity gel (Figure 4). No labeled product bands were observed from uninduced cells (Figure 4, lane 3) or induced cells containing the expression vector lacking the *GCN5* gene insert (vector only, lane 4). Labeled bands also were not observed when identical samples were tested in a parallel activity gel containing a nonsubstrate protein (bovine serum albumin; data not shown). From these data, we conclude that Gcn5p and p55 are able to catalyze the incorporation of [^{3}H]acetate from ^{3}H-labeled acetyl coenzyme A (acetyl-CoA) into core histone substrates and thus directly demonstrate HAT activity.

The predicted molecular mass of Gcn5p is 50.5 kDa; however, the product band detected in the activity gel assay migrated to the position of approximately 60 kDa. To determine whether this 60 kDa band was in fact due to the activity of Gcn5p, we translated ^{35}S-labeled Gcn5p in vitro for analysis by SDS–PAGE. In agreement with the activity gel assay result, a single radiolabeled band at the identical position of 60 kDa was detected in the Gcn5p translates, which was absent in control translates (data not shown). Thus, the in vitro translate result confirms the activity gel result and suggests that Gcn5p migrates anomolously in SDS gels. A similar phenomenon also exists for p55, which has a predicted molecular mass of 49 kDa, but is detected in SDS gels at the position of 55 kDa.

Highly Conserved Domains in A- and B-Type HATs

A cytoplasmic HAT B activity involved in histone deposition and chromatin assembly was recently cloned and sequenced so that three HAT genes and their derived amino acid sequences are now available for comparison (Tetrahymena p55 and Gcn5p, this study; yeast Hat1p, Kleff et al., 1995; M. Parthun and D. Gottschling, personal communication). From the alignment shown in

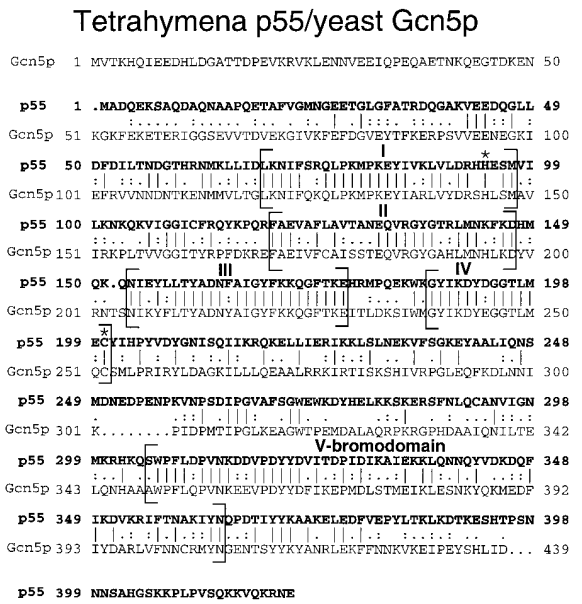

Figure 3. Yeast Gcn5p Is Highly Homologous to Tetrahymena p55

Searches of available database sequences using the BLAST algorithm demonstrate that yeast Gcn5p is highly homologous to p55 (shown in bold). Alignment of the two sequences is shown where an overall ~40% identity and ~60% similarity is observed. Note the existence of five domains (see brackets labeled I–V) that are extremely well conserved (~80% identity) between the ciliate and yeast proteins; similar percentages exist between yeast and human Gcn5p homologs (Candau et al., 1996). Asterisks in domains I and IV denote invariant residues that may act as active site residues; domain V corresponds to the bromodomain. See text for details.

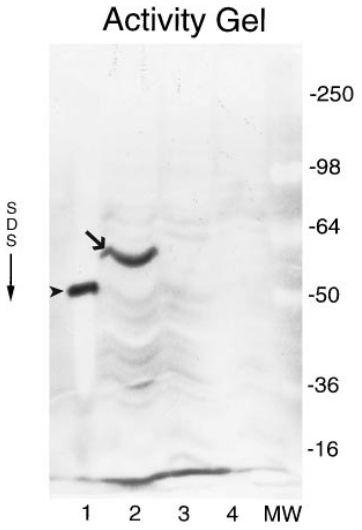

Figure 4. Gcn5p Has HAT Activity in the Gel Activity Assay

(A) Samples corresponding to macronuclear extracts (lane 1) or SDS-generated whole cell extracts of recombinant Gcn5p from either induced (lane 2) or uninduced cells (lane 3) or from induced cells containing the expression vector lacking the *GCN5* gene insert (vector only, lane 4) were electrophoresed in an 8% SDS–polyacrylamide gel and inspected for HAT activity using the activity gel assay. Shown is a fluorograph of [^{3}H]acetate incorporation into histone products. The arrowhead next to lane 1 denotes the position of p55 in Tetrahymena as reported previously (Brownell and Allis, 1995). The arrow next to lane 2 denotes the position of a 60 kDa product band resulting from the expression of Gcn5p in E. coli. This band is not detected in negative controls (lanes 3 and 4) or in parallel gels containing nonhistone substrates (data not shown).

Figure 3, we have identified five domains (brackets labeled I–V) that are highly conserved (~80% identity) between the ciliate and yeast HAT A enzymes. Searches of available databases using the BLAST algorithm (Altschul et al., 1990) revealed that domains I–IV share, to varying extents, features in common with acetyltransferases and other enzymes that bind acetyl-CoA. Each of these domains is discussed separately below.

Several lines of evidence suggest that domain I may be part of the catalytic center of A- and B-type HATs. First, when Tetrahymena p55 and yeast Gcn5p are directly compared (Figure 5A, I), the high identity observed in this region suggests that an important function has been conserved between the ciliate and yeast HAT A. Second, this region shows homology to a region of Hat1p (Figure 5A, II) that overlaps in part with a bipartite CoA-binding domain identified in other N-acetyltransferases (region A; Kleff et al., 1995). Third, comparison of this region in Hat1p to a well-characterized chloramphenicol acetyltransferase (CAT), CAT III (Shaw and Leslie, 1991), reveals several features of the CAT III active center in Hat1p (see bold residues identified by asterisks in Figure 5A, III). Fourth, all of these acetyltransferases contain an invariant histidine (see darkly stippled box indicated by arrows in Figure 5A) that is typically the second residue of a basic pair, often histidine–histidine. This histidine (His-195 of CAT III) is conserved in all known CAT variants and is an active site residue important for catalysis, as demonstrated by a His-195→Ala mutation that reduces the activity of CAT III 10^6-fold relative to the wild-type enzyme (Shaw and Leslie, 1991). By analogy to the well-studied CATs, we propose that His-94 of p55 (see asterisk in domain I of Figure 3) and His-231 of Hat1p are active site residues in A- and B-type HATs.

BLAST searches with domains III and IV revealed homology to other classes of enzymes that bind and metabolize acetyl-CoA, including acetoacetyl-CoA thiolase (Kurihara et al., 1992) and HMG (3-hydroxy-3-methylglutaryl)-CoA lyase (Hruz et al., 1992), respectively. In the case of HMG-CoA lyase, a cysteine (Cys-237) has been identified as an active site residue (Hruz et al., 1992), and we note the existence of a conserved cysteine in domain IV (see asterisk in domain IV of Figure 3) of Gcn5p and p55. The presence of a cysteine at the active site of p55 is suggested by sensitivity of the enzyme to inactivation by sulfhydryl-directed reagents, including iodoacetamide and N-ethylmaleimide (Brownell and Allis, 1995), although effects of these reagents on enzyme structure cannot be discounted. Based upon these results and the extreme conservation exhibited in domains I through IV among p55 and Gcn5p homologs in yeast (this report), human (Candau et al., 1996), and Drosophila (E. Smith and C. D. A., unpublished data), we speculate that these regions may play an important function in catalysis. We also note that the histidine and cysteine residues described above are absolutely conserved between all known Gcn5p homologs.

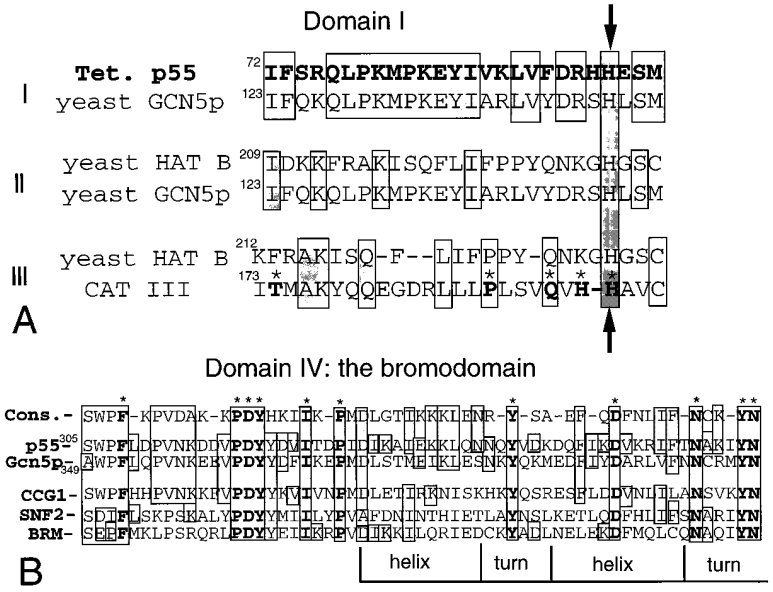

Figure 5. p55 and Gcn5p Contain Features of Catalytic Domains Found in Other Acetyltransferases

(A) Alignments are shown between Tetrahymena p55 and yeast Gcn5p (I) (see domain I of Figure 1A); yeast Gcn5p and yeast Hat1p (II); and CAT III (Shaw and Leslie, 1991) and yeast Hat1p (III) (Kleff et al., 1995; M. Parthun and D. Gottschling, personal communication). Identities between each pairwise comparison are shown by stippled boxes. Asterisks and residues shown in bold in CAT III denote residues conserved in all CATs, including the invariant active site histidine (His-195, darkly stippled box indicated by arrows; see Shaw and Leslie, 1991). This region overlaps with a bipartite consensus sequence showing homology to N-terminal acetyltransferases (region A; Kleff et al., 1995), although no significant homology between Hat1p and other proteins was noted in that study.

(B) Sequence comparison of selected bromodomains. Stippled boxes denote identity to the bromodomain consensus reported by Haynes and colleagues (1992). Asterisks mark the positions of invariant residues. The positions of predicted amphipathic helices and reverse turns are indicated (see Haynes et al. [1992] for details and sequences). A bromodomain is not present in the published sequence of yeast Hat1p (Kleff et al., 1995).

Both p55 and Gcn5p contain a single copy of the bromodomain (domain V) situated in their C-termini (see domain V in Figures 3 and 5B). Haynes and colleagues (1992) have noted the existence of seven invariant residues in the bromodomain, all of which are observed in p55 and Gcn5p (see asterisks in Figure 5B). Although the function of the bromodomain is unknown, this highly conserved motif has been identified in a small number of proteins, many of which are involved in transcriptional activation (Tamkun et al., 1992; Peterson and Herskowitz, 1992; Yoshinaga et al., 1992; Laurent and Carlson, 1992). Thus, this report adds a chromatin-modifying activity to the list of bromodomain-containing proteins.

The widespread conservation of the bromodomain suggests that it serves an important function, and deletion experiments have shown that the bromodomain is indispensable for the function of Gcn5p in yeast (Marcus et al., 1994; Georgakopoulos et al., 1995); presumably, this will also be the case for Tetrahymena p55. Interestingly, Hat1p does not possess a bromodomain. Given this observation, and given that bromodomains have been identified in a diverse group of proteins with putative related functions in transcriptional activation (Haynes et al., 1992) but without the proposed catalytic motif described above, it seems unlikely that the bromodomain participates directly in HAT activity. One operational definition of A-type HATs is their ability to bind and acetylate chromatin substrates efficiently, in contrast with the B-type HATs, which utilize free, nonchromatin histones. Our observations suggest that one function of the bromodomain may be to tether A-type HATs to chromatin templates undergoing gene activation.

Discussion

A long history of mostly correlative studies link histone acetylation to transcriptional activity. In only a few cases has a more direct association between these two processes been established (Hebbes et al., 1988, 1994). In this study, we report the sequence of a transcription-associated HAT A. Two unexpected insights are apparent from the sequence of the Tetrahymena catalytic subunit. First, p55 is the homolog of the previously described yeast transcriptional adaptor Gcn5p, and recombinant Gcn5p has HAT activity. This finding provides an immediate and unexpected connection between histone acetylation and at least one class of transcriptional adaptors. Second, p55 and Gcn5p each contain a bromodomain, a highly conserved motif found in a wide range of proteins involved in transcriptional activation, suggesting that this domain may play an important role in targeting chromatin modifiers to appropriate genes.

Is the Gcn5p Adaptor Complex Functionally Equivalent to HAT A?

Recent studies indicate that Gcn5p exists in a heterotrimeric complex in yeast with at least two other polypeptides, Ada2p and Ada3p (for alteration/deficiency activation) (Marcus et al., 1994; Horiuchi et al., 1995; Georgakopoulos et al., 1995). Current models suggest that this complex bridges enhancer-binding factors to the basal transcription machinery (see Figure 6; Marcus et al., 1994; Silverman et al., 1994; Georgakopoulos et al., 1995; Horiuchi et al., 1995; Barlev et al., 1995; Guarente, 1995). Our data demonstrate that Gcn5p has HAT activity and imply a clear biochemical function for this adaptor complex as a transcription-associated HAT A. Based on the high degree of conservation between the yeast, ciliate, and human components (this report; Candau et al., 1996), it seems likely that this complex is evolutionarily conserved and functions as a HAT activity in all eukaryotes.

Given the importance of histone acetylation in programming chromatin for gene expression, it is surprising

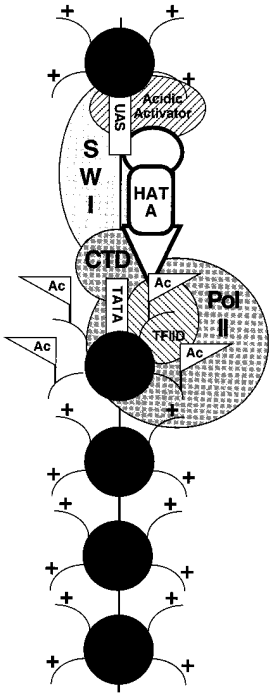

Figure 6. Tethering HAT A to Active Chromatin

A highly schematic drawing depicting reported interactions involving the yeast HAT A (Gcn5p–Ada2p–Ada3p) complex with some known components of the transcription apparatus; no precise spatial relationships are implied. It is thought that the Gcn5p–Ada heterotrimeric complex interacts with certain acidic activators as well as basal factors, leading to activated transcription of specific genes (Guarente, 1995). We propose that the HAT A complex is targeted to specific receptors in chromatin through functional interactions with SWI/SNF components. Furthermore, SWI/SNF components have recently been shown to be integral components of RNA polymerase II holoenzyme through interactions established at the polymerase II C-terminal repeat domain (CTD) (Wilson et al., 1996). These collective interactions suggest a mechanism enabling HAT A to traverse and modify the chromatin template as a catalytic component of the SWI/polymerase holoenzyme machine. See text for details.

that Gcn5p is not essential in yeast (Georgakopoulos and Thireos, 1992). However, Gcn5p is required for activation of only a subset of yeast activators, raising the intriguing possibility that multiple, possibly redundant type-A HATs exist that interact with other activators (e.g., GAL4). The recent finding (Morin et al., 1995) that the growth inhibition caused by overexpression of certain activators can be suppressed by overexpression of *NAT1*, a gene encoding an N-terminal acetyltransferase (Mullen et al., 1989), may be a reflection of this redundancy, although the significance of this result is unclear. As well, a dominant mutation in *afr1-1* (activation function reduced) has recently been reported that neutralizes the squelching effects of overexpression of *GCN4* and results in synthetic lethality when combined with *GCN5* disruption strains (Tavernarakis and Thireos, 1995). These findings suggest the existence of multiple, as yet undiscovered Gcn5p-interacting components, which may explain why *GCN5* itself is not essential.

A-Type HAT Is a Modular Activity

Estimates of the native molecular size of the ciliate HAT A enzyme extracted from macronuclei (~220 kDa) previously led us to suggest that the native complex in Tetrahymena exists as a tetramer consisting of four identical p55 catalytic subunits (Brownell and Allis, 1995). However, the connection between p55 and Gcn5p described in this study leads to a more likely explanation. The heterotrimeric yeast complex containing Ada2p (51 kDa), Ada3p (78 kDa), and Gcn5p (51 kDa) has a calculated molecular mass of approximately 180 kDa, close to that of the native ciliate enzyme. It seems likely that the p55-containing complex in Tetrahymena is also heterotypic and contains homologs of both Ada2p and Ada3p. p55 preferentially acetylates H3 in the SDS-based gel activity assay in which enzyme subunits are dissociated (Brownell and Allis, 1995). In contrast, native HAT A acetylates all four core histones when chromatin substrates are used (Belikoff et al., 1980; Chicoine et al., 1986). This difference between the native and dissociated enzyme complex raises the intriguing possibility that Ada2p, Ada3p, or an as yet unidentified component of the HAT A complex may contribute to overall substrate specificity.

Analogous to transcriptional activators, which have activation as well as sequence-specific DNA-binding domains, HAT A complexes may possess distinct catalytic and chromatin-binding domains. Secondary structure analyses suggest that the bromodomain contains two strong amphipathic α helices followed by reverse turns (Haynes et al., 1992), and similar analyses carried out with p55 and Gcn5p are consistent with this prediction. Haynes and colleagues (1992) have suggested that the hydrophobic surfaces of these helices could serve as sites for protein–protein interaction mediating the assembly of multicomponent complexes, including those involved in transcriptional activation.

We suggest that the bromodomain present in A-type HATs (p55/Gcn5p) and absent in B-type HATs (Hat1p), may be involved in targeting this important chromatin-modifying activity to the transcriptional apparatus. In particular, we propose that the bromodomain tethers HAT A to other factors bound at specific chromosomal sites. These HAT A "receptors" thereby recruit the acetyltransferase to specific genes within chromatin domains to be activated, as suggested by others (Wolffe, 1994). The bromodomain would then be predicted to be essential for HAT A function, and this is the case for Gcn5p in vivo (Marcus et al., 1994; Georgakopoulos et al., 1995). The bromodomain found in Gcn5p may thus represent a "signature sequence" of class A HATs, distinguishing them, and perhaps other chromatin-binding activities, from counterparts (class B) that do not bind chromatin.

HAT A Is a Component of a Chromatin-Remodeling Machine

Recent experiments suggest that a functional interaction exists between the Gcn5p–HAT A complex and components of the SWI/SNF complex in yeast (K. Pollard

and C. L. Peterson, personal communication). The multisubunit SWI/SNF complex is thought to weaken histone–DNA contacts to create an open chromatin configuration that facilitates transcription factor binding (Peterson and Herskowitz, 1992; Hirschhorn et al., 1992; Winston and Carlson, 1992; Laurent et al., 1993; Carlson and Laurent, 1994; Côté et al., 1994). Although the exact mechanism by which the SWI/SNF complex operates is at present unclear, an implication of this interaction is that one function of the SWI/SNF complex is to direct HAT A to specific sites in chromatin.

These findings, combined with the recent demonstration that the SWI/SNF polypeptides are integral components of the RNA polymerase II holoenzyme in yeast (Wilson et al., 1996), suggest a mechanism whereby HAT A is targeted to chromatin during transcriptional activation, establishing a direct link between histone acetylation and gene activation (Figure 6). Although the precise temporal and spatial organization of the numerous factors required for activated gene expression remains unclear, it is attractive to imagine that the HAT acts to lead the transcription apparatus through repressive chromatin structures. Alternatively, the acetyltransferase could act behind the complex to maintain an open domain configuration for repeated rounds of transcription. In either case, the association of HAT A with the SWI/polymerase II holoenzyme may explain how a rare enzyme might acetylate large stretches of chromatin in a specific manner.

The link we have established between p55, Gcn5p, and HAT A activity provides a direct illustration of the intimate relationship between gene expression and chromatin structure. Our findings also raise the possibilities that other factors identified as transcription adaptors or mediators may also possess chromatin-modifying activities. Identification of these activities, together with further definition of specific interactions among activators, coactivators, TAFs, mediators, and the basal machinery, will greatly enhance our understanding of the molecular nature of the regulation of gene expression.

Experimental Procedures

Purification and Protein Sequencing of p55 from Tetrahymena Macronuclei

Macronuclei were prepared from approximately 150 l of Tetrahymena thermophila cells as described previously (Brownell and Allis, 1995). HAT activity was extracted from macronuclei using buffer A (50 mM Tris–HCl [pH 8.0], 10% glycerol, 1 mM dithiothreitol [DTT], 1 mM phenylmethylsulfonyl fluoride, and 0.1 mM EDTA) containing 0.5 M NaCl and remained in the soluble fraction following overnight dialysis against buffer A. Extracts were then brought to pH 2.0 by the addition of concentrated HCl and loaded onto a 6 ml reverse-phase (RP) solid phase extraction column (Bakerbond SPE C_4, 7216-06; J.T. Baker Inc.). The column was then washed with 0.1% trifluoroacetic acid (TFA), and bound polypeptides were eluted with 60% isopropanol containing 0.1% TFA and then dried under vacuum. Polypeptides were then redissolved in 0.1% TFA and loaded onto a C_8 RP-HPLC column (Aquapore RP-300, 4.6 mm $\times$ 22 cm; Brownlee Labs) equilibrated in 0.1% TFA. The column was then eluted with a linear gradient of 0%–70% acetonitrile containing 0.1% TFA. Fractions enriched for HAT activity were identified using a HAT activity gel assay as previously described (Brownell and Allis, 1995). Proteins from several enriched fractions were pooled and resolved in an 8% SDS–polyacrylamide gel and stained briefly with Coomassie blue G (Aldrich). The polypeptide band corresponding to the position of 55 kDa and identified as positive for HAT activity was excised and digested in situ with lysylendopeptidase; the resulting peptides were separated by RP-HPLC and sequenced by Edman degradation as described previously (Collins et al., 1995). The sequences of six internal p55 peptides were as follows: K6, EHRMPQEK; K8, QNNQYVDK; K17, IYNPDTIYYK; K22, EYAALIQN; K35, DDVPDYY DVI; and K36, VEEDQGLLDFDILTN.

HAT Activity Assays

The standard liquid HAT assay and activity gel assay were performed exactly as described previously (Brownell and Allis, 1995). However, we have recently discovered that using urea from different vendors has a surprisingly dramatic effect on the efficiency of labeling in the activity gel assay. We have found that using ICN Ultra Pure urea (ICN number 821527) in the denaturing step gives us the most consistent results.

Cloning of the p55 Gene

Both sense and antisense degenerate oligonucleotide primers were built based on the peptide sequences of K36, K22, K17, and K8 with consideration of Tetrahymena codon usage frequencies (Martindale, 1989). Macronuclear genomic DNA was prepared and denatured before PCR, as described by Gaertig et al. (1993). Primers were initially tested in all combinations using PCR and genomic DNA as template. Reactions resulting in promising PCR products were then cloned using the TA cloning kit (Invitrogen) according to the instructions of the manufacturer and sequenced. DNA segments encoding correct peptide sequences that were not included in the primers were judged to be correct. The following sequences gave correct PCR products, with (S) denoting sequence of the sense strand and (A) indicating sequence of the antisense strand: K36S, CTIGAAGAACA(C/T)(C/T)A(A/G)GG; K17A, (C/T)TT(A/G)TA(A/G)T A(A/G)AT(A/G)GT(A/G) TC(A/G)GG; and K8A, (C/T)TT(A/G)TC(A/G)A C(A/G)TA(C/T)T(A/G)(A /G)TT(A/G)TT.

Two PCR products of ~1.5 kb and ~1.4 kb were obtained using K36S–K17A and K36S–K8A primers, respectively. Second round PCR with nested primers and Southern blot analysis with internal primers suggested that these products were likely to be correct.

To obtain the cDNA for p55, we isolated total RNA from growing cells by the method of Ausubel et al. (1988) and performed reverse transcriptase–PCR (RT–PCR) using MMLV RT with oligo(dT) primers (Frohman, 1993). RT–PCR using gene-specific primers to K36 and K17 resulted in a 900 bp product, which was cloned and sequenced as above. To obtain the 3′ end, we performed 3′ RACE using the above protocol. To obtain the 5′ end, cRACE was performed using the procedure of Maruyama et al. (1995). Southern and Northern blot analyses were performed using standard procedures and the 900 bp cDNA as probe.

Generation and Characterization of Peptide Antisera

We constructed 8-mer MAP synthetic peptides as described previously (Lin et al., 1989) according to the sequences of the K17 and K36 peptides. Rabbits were immunized with injections of 1 mg of each peptide according to standard immunization protocol and regimen. Preimmune and immune sera were collected, and specificity was determined by conducting immunoblots on samples ranging in complexity from total macronuclear protein to RP-HPLC-purified p55. Immunoblots and alkaline phosphatase–based detection were performed as described by Madireddi et al. (1994).

Immunoprecipitation of HAT Activity

Aliquots (100 μl) of crude macronuclear extract were incubated with 10 μl of the K36 peptide antiserum in either the presence or absence of 50 μg of the K36 peptide. The extract–serum mixtures were incubated for 2 hr at 4°C. We then added 50 μl of a 1:1 slurry of protein A–Sepharose (Pharmacia) (equilibrated in buffer containing 25 mM Tris [pH 7.5], 10% glycerol, 1 mM PMSF, 0.25 mM DTT, and 1 μM each of pepstatin, leupeptin, and aprotinin) to the mixtures and to crude extract alone and incubated the mixture at 4°C for 2 hr. The protein A beads were then pelleted by low speed centrifugation, and equal volumes of the resulting supernatants were tested for

residual HAT activity using the standard liquid assay (Brownell and Allis, 1995).

Cloning and Expression of *GCN5*

pCITE cloning was as follows. *GCN5* sequences were amplified from total yeast DNA using the primer SYR143 (containing sequences +1 ATG GTC ACA AAA CAT CAG ATT GAA GAGG +28) as forward primer and SYR144 (containing sequences +1681 ATC AAT AAG GTG AGA ATA TTC AGG TAT TTC +1651) as reverse primer. Restriction sites were included at the 5′ end of each primer (BglII in SYR143 and XhoI in SYR144) to allow cloning of the PCR product into the BamHI and XhoI sites of pCITE2A (Novagen). The GCN5pCITE clone was transcribed and translated in vitro as per the instructions of the manufacturer using a Promega TnT-coupled transcription/translation kit.

For pRSET cloning, *GCN5* sequences were amplified from total yeast DNA using the primer SYR143 (containing sequences +1 ATG GTC ACA AAA CAT CAG ATT GAA GAGG +28) as forward primer and SYR142 (containing sequences +1883 CCA GAA GAA GCG GAT GTT GAA ATG TC +1857) as reverse primer. SYR143 contained a BglII site upstream of the *GCN5* sequences, and this site and an endogenous HindIII site were used to clone the *GCN5* PCR product into pRSETB (Invitrogen). The resulting protein contains a His-6 tag at its N-terminus and was purified from bacteria using His-Bind resin (Novagen) according to the instructions of the manufacturer.

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References

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

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol. *215*, 403–410.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (1988). Current Protocols in Molecular Biology, Volume 1 (New York: Wiley Interscience), pp. 4.2.1–4.2.3.

Barlev, N.A., Candau, R., Wang, L., Darpino, P., Silverman, N., and Berger, S.L. (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.

Belikoff, E., Wong, L.J., and Alberts, B.M. (1980). Extensive purification of histone acetylase A, the major histone N-acetyl transferase activity detected in mammalian cell nuclei. J. Biol. Chem. *255*, 11448–11453.

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

Brownell, J.E., and Allis, C.D. (1995). An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in *Tetrahymena* macronuclei. Proc. Natl. Acad. Sci. USA *92*, 6364–6368.

Candau, R., Moore, P.A., Wang, L., Barlev, N., Ying, C.Y., Rosen, C.A., and Berger, S.L. (1996). Identification of human proteins functionally conserved with the yeast putative adaptors ADA2 and GCN5. Mol. Cell. Biol. *16*, 593–602.

Carlson, M., and Laurent B.C. (1994). The SNF/SWI family of global transcriptional activators. Curr. Opin. Cell Biol. *6*, 396–402.

Chicoine, L.G., Schulman, I.G., Richman, R., Cook, R.G., and Allis, C.D. (1986). Nonrandom utilization of acetylation sites in histones isolated from *Tetrahymena*: evidence for functionally distinct H4 acetylation sites. J. Biol. Chem. *261*, 1071–1076.

Collins, K., Kobayashi, R., and Greider, C.W. (1995). Purification of Tetrahymena telomerase and cloning of genes encoding the two protein components of the enzyme. Cell *81*, 677–686.

Côté, J., Quinn, J., Workman, J.L., and Peterson, C.L. (1994). Stimulation of GAL4 derivative binding to nucleosome DNA by the yeast SWI/SNF complex. Science *265*, 53–60.

Csordas, A. (1990). On the biological role of histone acetylation. Biochem. J. *265*, 23–38.

Durrin, L.K., Mann, R.K., Kayne, P.S., and Grunstein, M. (1991). Yeast histone H4 N-terminal sequence is required for promoter activation in vivo. Cell *65*, 1023–1031.

Fisher-Adams, G., and Grunstein, M. (1995). Yeast histone H4 and H3 N-termini have different effects on the chromatin structure of the GAL1 promoter. EMBO J. *14*, 1468–1477.

Frohman, M.A. (1993). Rapid amplification of complementary DNA ends for generation of full-length complementary DNAs: thermal RACE. Meth. Enzymol. *218*, 340–356.

Gaertig, J., Thatcher, T.H., McGrath, K.E., Callahan, R.C., and Gorovsky, M.A. (1993). Perspectives on tubulin isotype function and evolution based on the observation that *Tetrahymena thermophila* microtubules contain a single α- and β-tubulin. Cell Motil. Cytoskel. *25*, 243–253.

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

Guarente, L. (1995). Transcriptional coactivators in yeast and beyond. Trends Biochem. Sci. *20*, 517–521.

Haynes, S.R., Dollard, C., Winston, F., Beck, S., Trowsdale, J., and Dawid, I.B. (1992). The bromodomain: a conserved sequence found in human, *Drosophila* and yeast proteins. Nucl. Acids Res. *80*, 2603.

Hebbes, T.R., Thorne, A.W., and Crane-Robinson, C. (1988). A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J. *7*, 1395–1402.

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

Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S.M., and Grunstein, M. (1995). Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell *80*, 583–592.

Hirschhorn, J.N., Brown, S.A., Clark, C.D., and Winston, F. (1992). Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. Genes Dev. *6*, 2288–2298.

Horiuchi, J., Silverman, N., Marcus, G.A., and Guarente, L. (1995). ADA3, a putative transcriptional adaptor, consists of two separable domains and interacts with ADA2 and GCN5 in a trimeric complex. Mol. Cell. Biol. *15*, 1203–1209.

Hruz, P.W., Narasimhan, C., and Miziorko, H.M. (1992). 3-Hydroxy-3-methylglutaryl coenzyme A lyase: affinity labeling of the *Pseudomonas mevalonii* enzyme and assignment of cysteine-237 to the active site. Biochemistry *31*, 6842–6847.

Johnson, L.M., Kayne, P.S., Kahn, E.S., and Grunstein, M. (1990). Genetic evidence for an interaction between SIR3 and histone H4 in the repression of silent mating loci in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *369*, 245–247.

Juan, L.-J., Utley, R.T., Adams, C.C., Vettese-Dadey, M., and Workman, J.L. (1994). Differential repression of transcription factor binding by histone H1 is regulated by the core histone amino termini. EMBO J. *13*, 6031–6040.

Kayne, P.S., Kim, U.-J., Han, M., Mullen, J.R., Yoshizaki, F., and Grunstein, M. (1988). Extremely conserved histone H4 N-terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. Cell *55*, 27–39.

Kleff, S., Andrulis, E.D., Anderson, C.W., and Sternglanz, R. (1995). Identification of a gene encoding a yeast histone H4 acetyltransferase. J. Biol. Chem. *270*, 24674–24677.

Kurihara, T., Ueda, M., Kanayama, N., Kondo, J., Teranishi, Y., and Tanaka, A. (1992). Peroxisomal acetoacetyl-CoA thiolase of an n-alkane-utilizing yeast, *Canadida tropicalis*. Eur. J. Biochem. *210*, 999–1005.

Laurent, B.C., and Carlson, M. (1992). Yeast SNF2/SWI2, SNF5, and SNF6 proteins function coordinately with the gene-specific transcriptional activators GAL4 and Bicoid. Genes Dev. *6*, 1707–1715.

Laurent, B.C., Triech, I., and Carlson, M. (1993). The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. Genes Dev. *7*, 583–591.

Lee, D.Y., Hayes, J.J., Pruss, D., and Wolffe, A.P. (1993). A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell *72*, 73–84.

Lin, R., Leone, J.W., Cook, R.G., and Allis, C.D. (1989). Antibodies specific to acetylated histones document the existence of deposition- and transcription-related histone acetylation in *Tetrahymena*. J. Cell Biol. *108*, 1577–1588.

Loidl, P. (1994). Histone acetylation: facts and questions. Chromosoma *103*, 441–449.

Madireddi, M.T., Davis, M.C., and Allis, C.D. (1994). Identification of a novel polypeptide involved in the formation of DNA-containing vesicles during macronuclear development in *Tetrahymena*. Dev. Biol. *165*, 418–431.

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

Martindale, D.W. (1989). Codon usage in *Tetrahymena* and other ciliates. J. Protozool. *36*, 29–34.

Maruyama, I., Rokow, T.L., and Maruyama, H.I. (1995). cRACE: a simple method for identification of the 5′ end of mRNAs. Nucl. Acids Res. *23*, 3796–3797.

Morin, P.J., Downs, J.A., Snodgrass, A.M., and Gilmore, T.D. (1995). Genetic analysis of growth inhibition by GAL4-IκB in *Saccharomyces cerevisiae*. Cell Growth Differ. *6*, 789–798.

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

Peterson, C.L., and Herskowitz, I. (1992). Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. Cell *68*, 573–583.

Prescott, D.M. (1994). The DNA of ciliated protozoa. Microbiol. Rev. *58*, 233–267.

Roth, S.Y., Shimizu, M., Johnson, L., Grunstein, M., and Simpson, R.T. (1992). Stable nucleosome positioning and complete repression by the yeast α2 repressor are disrupted by amino-terminal mutations in histone H4. Genes Dev. *6*, 411–425.

Shaw, W.V., and Leslie, A.G.W. (1991). Chloramphenicol acetyltransferase. Annu. Rev. Biophys. Biophys. Chem. *20*, 363–386.

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

Tamkun, J.W., Deuring, R., Scott, M.P., Kissinger, M., Pattatucci, A.M., Kaufman, T.C., and Kennison, J.A. (1992). Brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell *68*, 561–572.

Tavernarakis, N., and Thireos, G. (1995). Transcriptional interference caused by GCN4 overexpression reveals multiple interactions mediating transcriptional activation. Mol. Gen. Genet. *247*, 571–578.

Thompson, J.S., Ling, X., and Grunstein, M. (1994). Histone H3 amino terminus is required for telomeric and silent mating locus repression in yeast. Nature *369*, 245–247.

Turner, B.M. (1993). Decoding the nucleosome. Cell *75*, 5–8.

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

Vavra, K.J., Allis, C.D., and Gorovsky, M.A. (1982). Regulation of histone acetylation in *Tetrahymena* macro- and micronuclei. J. Biol. Chem. *257*, 2591–2598.

Vettese-Dadey, M., Walter, P., Chen, H., Juan, L.-J., and Workman, J.L. (1994). Role of the histone amino termini in facilitated binding of a transcription factor, GAL4-AH, to nucleosome cores. Mol. Cell. Biol. *14*, 970–981.

Wilson, C.J., Chao, D.M., Imbalzano, A.N., Schnitzer, G.R., Kingston, R.E., and Young, R.A. (1996). RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling. Cell *84*, 235–244.

Winston, F., and Carlson, M. (1992). Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. *8*, 387–391.

Wolffe, A.P. (1994). Nucleosome positioning and modification: chromatin structures that potentiate transcription. Trends Biochem. Sci. *19*, 240–244.

Yoshinaga, S.K., Peterson, C.L., Herskowitz, I., and Yamamoto, K.R. (1992). Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science *258*, 1598–1604.

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