CMLS, Cell. Mol. Life Sci. 54 (1998) 6–20
1420-682X/98/010006-15 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Linking histone acetylation to transcriptional regulation

C. A. Mizzen* and C. D. Allis

Department of Biology, University of Rochester, Rochester (New York 14627, USA), Fax +(716) 275-2070, e-mail: craig@allislab.biology.rochester.edu

Abstract. In eukaryotes, DNA is assembled with histones to form nucleosomes, the basic subunit of chromatin structure. The wrapping of DNA around histone octamers to form nucleosomal filaments and further folding of these filaments are necessary to contain eukaryotic genomes within nuclei. However, the dense packing of chromatin in nuclei and the association of DNA with histones restrict the access of proteins involved in gene transcription to DNA. Abundant biochemical data supports a long-standing correlation between histone acetylation and gene activation, suggesting that histone acetylation acts to enhance the access of transcription-associated proteins to DNA. However, despite this correlation, nuclear enzymes responsible for transcription-associated histone acetylation have been identified only recently. Here we review evidence suggesting that histone acetylation represents a major pathway for transcriptional regulation, and discuss possible roles for transcription-associated histone acetyltransferases in this regulation.

Key words. Transcription; chromatin; nucleosomes; histones; acetylation; acetyltransferases.

Introduction: nuclear function and chromatin structure

Transcription of protein-coding genes in eukaryotes requires the assembly of a preinitiation complex (PIC) containing more than 50 separate polypeptides on promoter DNA upstream of the site of initiation of messenger RNA (mRNA) synthesis. Investigations, most often utilizing naked DNA templates, have defined requirements for the general transcription factors (GTFs) TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH, and RNA polymerase II, to reconstitute transcription in vitro (reviewed in refs 1, 2). TFIID, a multimeric protein complex consisting of the TATA box-binding protein (TBP) and a collection of TBP-associated factors (TAF_{II}s), plays a critical role in this process, since it is the only PIC component capable of binding core promoters specifically (reviewed in ref. 3).

As indicated in figure 1, control of promoter recognition by TFIID is thought to represent an important mechanism in transcriptional regulation. Upon binding their cognate DNA targets, many transcriptional activators interact directly, or via intervening proteins referred to as coactivators or adaptors, with TAF_{II}s to enhance the rate of promoter binding by TFIID or stabilize TFIID-promoter complexes (reviewed in refs 3–7). Although the mechanism of activation in these reactions is subject to debate, it is often suggested to involve these protein-protein interactions (indicated by double-sided arrows in figure 1).

Chromatin connections to transcriptional regulation

A major goal of current research in molecular biology is to understand how transcriptional regulation is accomplished in the context of a repressive chromatin enviroment. In vivo, the native DNA template is not 'naked' as depicted in figure 1, but exists as a highly constrained chromatin fibre. Thus, an intimate relationship must exist between the transcription apparatus and the chro-

* Corresponding author.

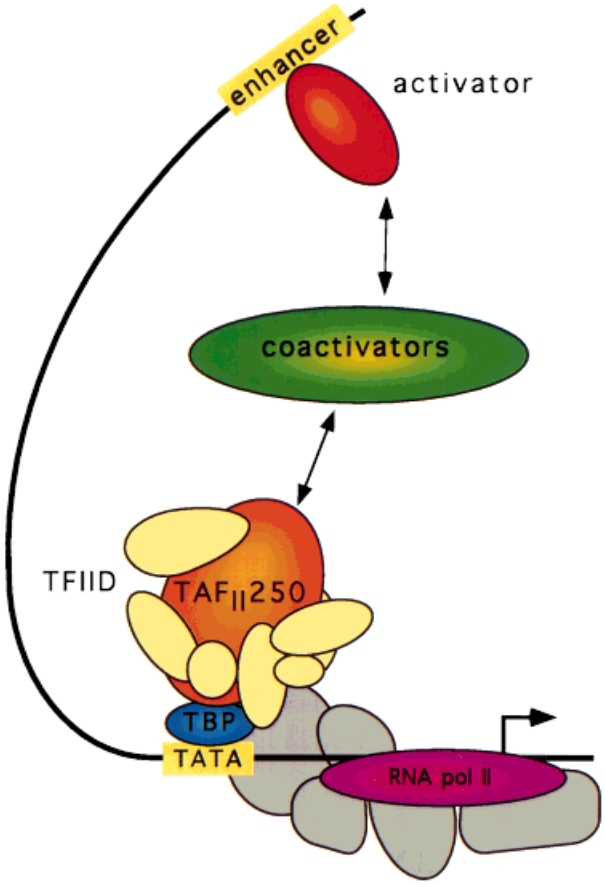

Figure 1. Components of transcriptional regulation implicated by in vitro studies. A conceptual model based on physical interactions of activators, coactivators and GTFs is shown. The curved thick line represents DNA at the 5′ end of a gene regulated via a core promoter (yellow box, TATA) and an enhancer element (yellow box, enhancer). Upon binding the enhancer element, a transcriptional activator protein (red) binds a coactivator protein (green), which in turn binds the $TAF_{II}250$ subunit (orange) of TFIID. Small double-headed arrows depict these interactions which have been detected for specific activators, coactivators and GTFs in transcription assays in vitro. These interactions are thought to enhance recruitment of TFIID to promoters, facilitating subsequent PIC assembly and the initiation of transcription (indicated by the right-angle arrow). TBP (blue) and other TAF_{II} subunits (yellow) of TFIID are indicated. For clarity, other GTFs (gray) and RNA polymerase II (purple) are shown in simplified form, and other components of transcription complexes have been omitted. As described in the text, the nucleosomal and higher-order structure of chromatin restrict the access of these transcription-associated proteins to DNA sites in vivo, but acetylation of histones appears to represent a major mechanism that antagonizes chromatin-mediated repression of transcription.

matin context in which it functions. This review will focus on exciting new links that have been uncovered between the covalent modification of the core histones and the regulation of gene expression.

Although the details of chromatin higher-order structure remain unclear, the fundamental unit of this structure, the nucleosome, is well understood. DNA is wrapped approximately 1.75 superhelical turns around the discoid histone octamer composed of two molecules of each of the four core histones, H2A, H2B, H3 and H4. Nucleosomal structure is stabilized by protein-protein interactions occurring between histones within each octamer and by electrostatic interactions of negatively charged phosphates of the DNA backbone with positively charged lysine and arginine residues exposed on the surface of the histone octamer (reviewed in ref. 8). A fifth type of histone, referred to as H1 or linker histone, binds the outer surface of the nucleosomal particle, approximately at the point of entry and exit of the DNA molecule into the nucleosome, although recent data suggest an alternate binding site for H1 [9, 10]. H1 binding is thought to involve electrostatic interactions with DNA, including portions of the linker DNA extending between adjacent nucleosomes in vivo in 10-nm nucleosomal filaments.

In cells, 10-nm nucleosomal filaments are folded upon themselves to form higher-order structures. The nature of these structures is unclear at present, but linker histones are thought to be required for this folding and appear to play an important, yet in some cases nonessential [11], role in determining this structure and in mediating chromatin condensation (reviewed in refs 12, 13). Additional information regarding chromatin structure and nuclear function is available in a number of excellent reviews and texts [8, 14–17].

Abundant experimental evidence demonstrates that the nucleosomal and supranucleosomal structure of chromatin often, but not always, restricts access to the DNA template by various proteins involved in transcriptional regulation (reviewed in refs 18–21). For example, when transcription is assayed in vitro using preformed nucleosomal templates rather than naked DNA, transcription is repressed. However, if TBP-promoter or TFIID-promoter complexes are formed prior to nucleosome assembly, chromatin templates can be transcribed [22, 23]. These findings suggest that the transcriptional repression by nucleosomes was due to inhibition of promoter binding by TBP or TFIID. Moreover, these findings also demonstrate that once transcription is initiated, the presence of nucleosomes does not preclude transcriptional elongation. RNA polymerases can transcribe through nucleosomes in vitro, and there is abundant evidence that nucleosomes (albeit possibly modified in structure, see below) are present on transcribing genes in vivo [24, 25]. Analyses of the distributions of nucleosomes at active genes in vivo suggest that positioning of nucleosomes within promoters, in particular, represents a key mechanism in regulating transcription (reviewed in refs 14, 15).

Pioneering studies of transcription in *Saccharomyces cerevisiae* yielded the initial clues that chromatin structure was directly involved in the regulation of transcription. Genetic studies in yeast, for example, provided the first in vivo evidence of the role of histones themselves in transcription [26, 27]. Studies in yeast also led to the identification and analysis of the SWI/SNF complex, a nucleosome remodelling activity that facilitates the binding of transcription factors to their DNA sites in chromatin, and these studies in yeast laid the groundwork for the analysis of similar complexes from *Drosophila* and human cells (for review, see ref. 20). Other contributions in this volume discuss mechanisms by which transcriptional regulation is achieved through modulation of supranucleosomal organization. This review focuses on recent progress in understanding the role of histone acetylation in the regulation of transcription at the nucleosomal level.

Effects of mutations in core histone amino termini on chromatin activity

Each of the core histones has a bipartite structure with a flexible or unstructured N-terminal domain and a globular C-terminal domain (fig. 2A). X-ray crystal structures of the core histone octamer [28, 29] and the nucleosome core particle [30] reveal that the highly α-helical C-terminal domains interact to form the discoid core of the octamer, whereas the N-termini, although not imaged, are thought to project away from the octamer, positioning basic residues within the termini for potential interactions with their own nucleosomal DNA (as shown in figure 2C), adjacent linker DNA and possibly DNA and protein sites in adjacent nucleosomes in condensed chromatin. Although the N-termini are characterized by a high content of the positively charged amino acids lysine and arginine, only certain lysine residues, shown in figure 2A, are known to be modified by acetylation in vivo, and the high degree of conservation of the relative positions of these lysines within termini across eukaryotic species suggests a critical functional role for these acetylations (reviewed in refs 8, 31, 32).

As depicted in figure 2B, acetylation neutralizes the positive charge associated with the ε-amino groups of lysine side chains. This reduction of positive charge within termini upon acetylation suggests that acetylation attenuates the interactions of termini with DNA, possibly increasing the exposure of this DNA to transcription factors and altering nucleosome conformation (fig. 2C). In addition, acetylation may regulate interactions of the histone N-termini with other proteins (see below).

Applying yeast genetics to histone tail function

The development of genetic transformation methods for yeast has facilitated investigations of histone function in vivo through gene replacement (reviewed in refs 33, 34). This powerful experimental strategy has revealed that the N-termini of H3 and H4 act as complex regulators of transcription with both positive and negative effects. Deletion of residues 4–15 in the N-terminus of H3, for example, led to hyperactivation of several *GAL4*-regulated genes, including *GAL1*, upon induction with galactose [35]. This was not attributable to a global effect of the deletion, since transcription of the *PHO5* gene, which is not regulated by *GAL4*, was unaffected.

GAL1 hyperactivation was also observed following substitution of lysines 9, 14, 18 and 23 of the wild-type H3 N-terminus with the uncharged residue glutamine, mimicking acetylation. These results suggest the wild-type, unacetylated N-terminus of H3 acts to repress *GAL1* transcription and that acetylation acts to relieve this repression. In contrast, deletion of residues 4–23 of the H4 N-terminus led to repression of *GAL1* activation, and similar repression was observed following substitution of lysines 5, 8, 12 and 16 of the wild-type H4 N-terminus with glutamine [36]. Individual substitution of lysine residues 5, 8 and 16 with arginine (a positively charged residue which cannot be acetylated) resulted in *GAL1* hyperactivation. Curiously, simultaneous substitution of lysines 5, 8, 12 and 16 with arginine repressed *GAL1* activation. Together, these results suggest that the wild-type, unacetylated H4 N-terminus facilitates *GAL1* activation and that H4 acetylation has a repressive effect at this promoter. Interestingly, deletion of residues 4–20 in H2A and 3–32 in H2B had little effect on *GAL1* activation, while the deletion of residues 4–30 in H3 caused less dramatic *GAL1* hyperactivation compared with deletion of residues 4–15 [36].

Molecular mechanisms?

One mechanism that evidence suggests acetylation influences is the positioning of nucleosomes relative to regulatory DNA sequences. The translational position of a regulatory DNA sequence (e.g. its position at one edge, the middle or the opposite edge of the length of DNA within a nucleosome) and its rotational position (whether the sequence faces outward or toward the histone octamer) can strongly influence the binding of many transcription factors. In vivo, it appears that certain types of DNA sequences and DNA binding proteins can determine nucleosome positioning (reviewed in ref. 37). Roth and colleagues have shown that deletions or substitution of glycine for lysine 16 in the N-terminal of H4 altered the positioning of a nu-

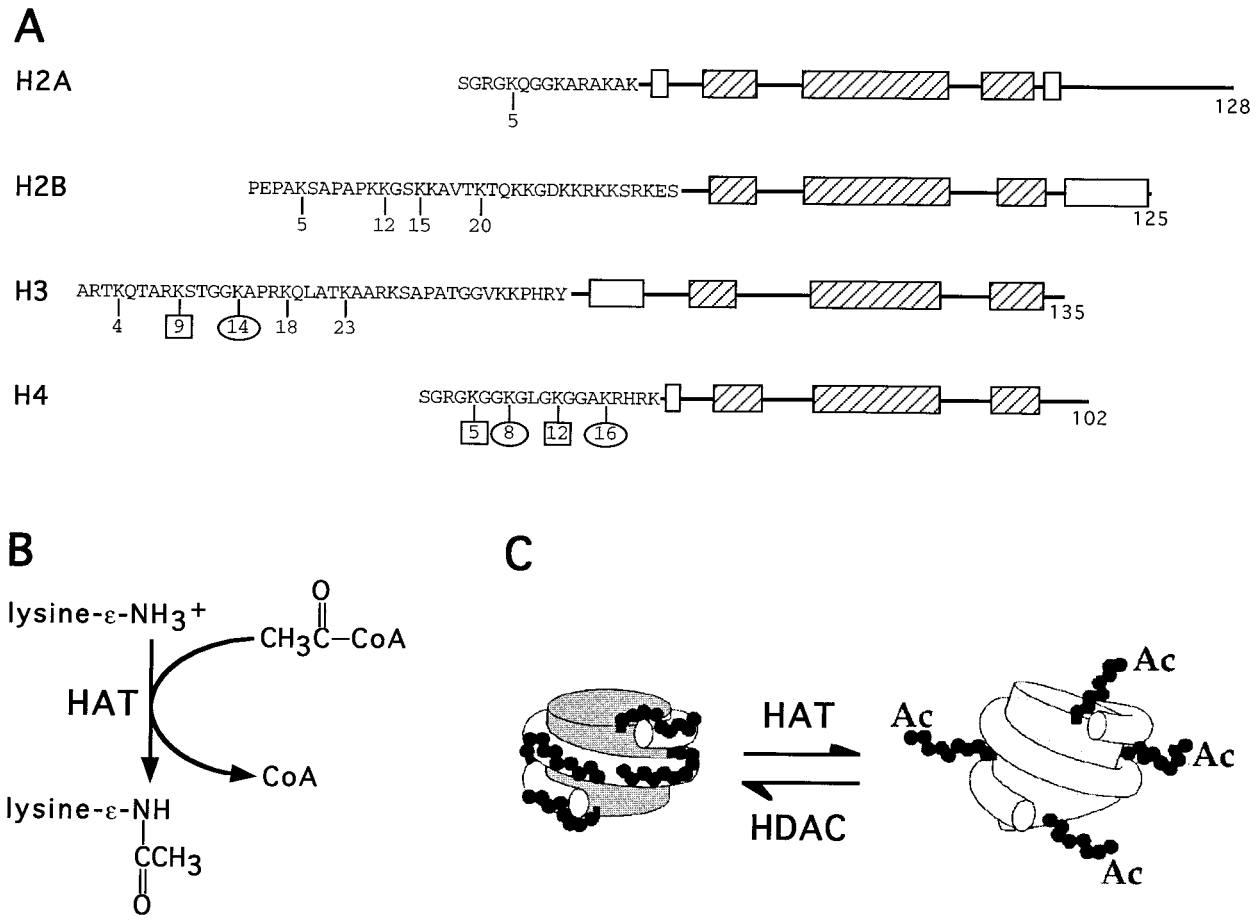

Figure 2. Histone acetylation and core histone/nucleosome structure. (*A*) The structures of the four core histones are depicted. The sequences of the N-terminal tail domains, which are not visible in X-ray crystallography [28, 30], are indicated in plain type. The structured portions of the molecules are represented schematically by the thick line with α-helices indicated by rectangles. The α-helices involved in the histone fold are hatched. Well characterized sites of acetylation [131] within the N-termini of vertebrates are indicated by numbers denoting their sequence position. Sites known to be acetylated in association with chromatin replication are boxed, while those known to be acetylated in relation to transcription are circled. (*B*) Schematic depiction of the acetylation reaction. *Trans*-acetylation by HAT enzymes neutralizes the positive charge of the ε-amino group of lysine side chains. (*C*) Schematic depiction of nucleosome structure and the effects of histone acetylation. A nonacetylated nucleosome is shown on the left; an acetylated nucleosome on the right (Ac = acetyl). Two molecules of each of the core histones assemble to form the disc-like histone octamer about which DNA is wrapped. The N-terminal domains of the histones are thought to project away from the octamer and bind nucleosomal DNA as shown. As described in the text, acetylation at lysines within N-termini is thought to decrease DNA binding by these domains, exposing the underlying DNA, and may also induce a conformational change in the nucleosome, as depicted in an acetylated nucleosome on the right. In cells, the steady-state level of histone acetylation is determined by the combined activity of HATs and HDACs, which remove acetyl groups.

cleosome by the α2 repressor over the TATA element of a reporter gene in minichromosomes, correlating with derepression of reporter gene transcription [38]. Evidence for a role for the H4 N-terminal and H4 acetylation in positioning nucleosomes was also obtained in analyses of the *GAL1* promoter [39]. However, in this case, changes in nucleosome positioning following deletion of H4 sequence encompassing acetylation sites and mutation of acetylation sites to glycines decreased the accessibility of the *GAL1* TATA element to probes of chromatin structure, in agreement with the repression of *GAL1* transcription by similar mutations [36].

The N-termini of H3 and H4 also have significant roles in the potent repression of transcription known as silencing that occurs at telomeres and the *HM* mating loci in yeast, a process that may be analogous to heterochromatin formation in multicellular organisms [40]. A potential involvement of histone acetylation in heterochromatin formation is suggested by the finding that heterochromatin in yeast and *Drosophila* contains less

acetylated histone than the average in these species and that lysine 12 of H4 acetylation is preferentially acetylated in this heterochromatin [41, 42]. Genetic manipulations in yeast have provided data to support this notion. Deletion of the amino termini of H4 [43] and H3 [44] leads to derepression of otherwise repressed mating type loci and genes adjacent to telomeres. Substitution of individual residues in the H3 and H4 termini has defined domains of H3 (residues 4–20) and H4 (residues 16–29) required for silencing in vivo [40]. Although the silencing domain of H4 (residues 16–29) identified in these studies includes only one of four known H4 acetylation sites, substitution of glutamine at this site (lysine 16), mimicking acetylation, abolishes silencing of the *HM* loci [44], even though immunoprecipitation analyses demonstrate that lysine 12 of H4 is acetylated in wild-type cells at these loci [42].

Evidence from genetic and in vitro studies suggests the function of the H3 and H4 termini in silencing depends on interactions with the silent information regulator proteins SIR3 and SIR4. The products of *SIR* genes are required for silencing at mating loci and telomeres [45, 46], and the N-terminal domains of H3 (residues 4–20) and H4 (residues 16–29) that have been shown to be necessary for silencing in vivo interact directly and specifically with SIR3 and SIR4 in vitro [40]. Notably, the SIR3 domains required for interaction with H3 and H4 in vitro are required for SIR3 function in vivo [40] and mutations in the H4 silencing domain are suppressed by single amino acid substitutions in SIR3 [47, 48]. Although these data suggest that acetylation may regulate the interactions of the histone N-termini with SIR proteins (or other nonhistone chromosomal proteins, see ref. 49) involved in heterochromatin formation, it remains to be established whether acetylation plays a causal role. The hypoacetylation of heterochromatin may simply reflect the decreased accessibility of this chromatin to acetylases secondary to condensation. Similarly, since human heterochromatin does not appear to be enriched in H4 acetylated at lysine 12 [50], it is unclear at present whether heterochromatin-specific patterns of acetylation exist or whether this pattern reflects the accessibility of heterochromatin in *Drosophila* and yeast to deacetylases.

Taken together, analyses of histone function in yeast suggest that the amino termini of the histones have individual roles in transcriptional regulation and that the consequences of acetylation at a specific site in a specific histone seem to depend on the chromosomal context in which they occur. These data demonstrate that acetylation is not associated exclusively with open chromatin conformation and, in certain cases, correlates with transcriptional repression.

Regulation of chromatin structure and transcription by histone acetylation

Post-translational acetylation of histones was first reported by Allfrey and colleagues in 1964 [51]. Subsequent work revealed that this acetylation is reversible in vivo and occurs at the ε-amino group of specific lysines in the amino termini of all four core histones in all plant and animal species examined (reviewed in refs 32, 52, 53). Following the discovery of the nucleosomal structure of chromatin, numerous investigations employing limited treatment of nuclei with nonspecific nucleases documented enrichments of acetylated core histones and DNA sequences derived from actively transcribed genes in the most readily solubilized chromatin fractions (reviewed in refs 32, 52, 53).

The enhanced accessibility of actively transcribed DNA to nucleases is generally thought to reflect decondensation of higher-order chromatin structure at active loci, and the enriched content of acetylated core histones in nuclease-sensitive chromatin suggests histone acetylation is at least, in part, responsible for the properties of this chromatin. Strong support for this notion comes from immunochemical fractionation of chromatin. Employing antisera specific for ε-*N*-acetyl lysine, Crane-Robinson and co-workers demonstrated that acetylated core histones were preferentially associated with actively transcribed β-globin gene sequences compared with repressed ovalbumin sequences and bulk chromatin in chromatin fragments prepared from embryonic chicken erythrocytes [54, 55]. Furthermore, it was shown that the enriched content of acetylated core histones extended along the entire nuclease sensitive portion of the β-globin chromosomal locus, including genes and intergenic sequences, correlating with the nuclease sensitivity of this locus [55]. Significantly, enrichment in acetylated histones was shown to correlate with the potential for genes to be expressed rather than transcriptional activity per se [55, 56]. Together, these studies strongly implicate histone acetylation in the acquisition or maintenance of the open chromatin conformation associated with transcriptional competence.

The external location of the core histone N-termini in nucleosomes suggests acetylation may be involved in determining higher-order chromatin structure by modulating interactions between adjacent nucleosomes. However, clear contacts between histone N-termini and adjacent nucleosomes have not been demonstrated (although see ref. 30), and much of the available data are consistent with binding of linker DNA by N-termini (reviewed in ref. 16). A role for acetylation in regulating chromatin folding is indicated by data showing that oligonucleosomes reconstituted with hyperacetylated core histones or histones

trypsinized to remove the N-termini are more extended compared with nonacetylated or nontrypsinized counterparts following compaction by cations in vitro ([57–59]; reviewed in ref. 16). Note, however, that in most cases these studies employ chromatin fragments depleted of linker histone.

Given the abundant evidence supporting a role for linker histones in chromatin folding and also evidence for interactions between linker histones and nucleosomal core histones (reviewed in ref. 12), the relevance of these studies to the in vivo situation is unclear. However, altered folding in vitro has been claimed for chromatin containing linker histones and trypsinized or hyperacetylated core histones [57, 60]. These observations, together with the correlation of histone acetylation and transcriptional competence in chromatin immunofractionation, support the notion that acetylation is part of the mechanism responsible for unfolding condensed chromatin to render it accessible for transcription. In this regard, it is pertinent to note that depletion of linker histones is an often reported feature of nuclease sensitive, transcriptionally competent chromatin (reviewed in ref. 12). Although it is unclear whether acetylation and H1 depletion are causally linked, the identification of HATs (discussed below) should permit investigation of this relationship experimentally.

A role for acetylation in regulatory events affecting individual nucleosomes is suggested by numerous studies showing that the N-termini of the core histones regulate the binding of various *trans*-acting factors to regulatory elements incorporated into nucleosomal DNA. Reconstitution of mononucleosomes with unacetylated histones inhibits the binding of TFIIIA (a GTF for RNA polymerase III) to the 5S RNA gene in vitro. However, TFIIIA can bind nucleosomal 5S DNA if acetylated core histones are employed in the reconstitution [61]. TFIIIA binding was also facilitated in reconstitutes employing core histones with the N-terminal domains removed by limited proteolysis, suggesting masking of the TFIIIA binding sites is alleviated by dissociation of the N-termini from DNA upon acetylation (or proteolysis). Similar findings have been reported for the binding of transcriptional activators [62, 63] and TBP [64, 65] to their respective sites in mononucleosomes in vitro. In addition, acetylation-dependent alterations in nucleosome conformation may increase the accessibility of nucleosomal DNA. Analyses of physical parameters deriving from the constraint of DNA by its interaction with the surface of the histone octamer suggest that fewer superhelical turns of DNA are wrapped about octamers containing acetylated histones compared with nonacetylated octamers [66, 67]. Since acetylation (or N-termini trypsinization) has no effect on the length of nucleosomal DNA protected from nuclease digestion [68, 69] and also does not alter the number of contacts between DNA and the globular core of the histone octamer [67], these results imply that the shape of the nucleosome changes upon acetylation. This conclusion is consistent with earlier observations that acetylated nucleosomes are slightly retarded relative to nonacetylated nucleosomes in gel electrophoresis [70, 71]. It seems likely that a reduction of the superhelical density of nucleosomal DNA upon acetylation could enhance the exposure of regulatory sequences to *trans*-acting factors.

All the studies cited above were performed in the absence of linker histones. A recent study showed that acetylation or proteolysis of the core histone N-termini alleviated H1-mediated inhibition of binding of the transcription factor USF to reconstituted mononucleosomes [72]. Notably, it was reported that H1 bound trypsinized nucleosomes with lower affinity. Subsequently, it was shown (for this particular system) that H1 repression of USF binding was not due to decreased nucleosome mobility, supporting a model in which H1 binding reduces transient dynamic dissociation of DNA from the surface of the histone octamer [73]. Acetylation has also been shown to increase the transcription of dinucleosomes containing the 5S RNA gene by RNA pol III, independent of effects on nucleosome mobility, although in this case repression of transcription by H1 with acetylated and nonacetylated templates was equivalent [74]. Taken together, analyses of transcription factor binding to nucleosomes suggest that dissociation of core histone N-termini from nucleosomal DNA upon acetylation can enhance the exposure of transcription factor binding sites directly and through indirect effects on nucleosome conformation and kinetic parameters of DNA binding. The extent of repression of factor binding by H1 in such studies may vary with the identity of the factor under investigation, since it is known that the extent of inhibition of factor binding by nucleosome cores varies among transcription factors [74] (reviewed in ref. 19).

Identification and characterization of histone acetyltransferases

Clearly, in vivo and in vitro analyses indicate that acetylation is an important regulator of the function of core histone amino termini. Yet, progress in the identification of *h*istone *a*cetyl*t*ransferases (HATs) has been made only recently. Importantly, these advances have been accompanied by equally significant discoveries and functional studies involving histone deacetylases (HDACs; for reviews, see refs 75, 76). Due to space limitations, this review focuses primarily on HATs, but it should be noted that substantial evidence suggests histone deacetylation also plays a critical role in transcriptional regulation.

Historically, three types of histone acetylation have long been recognized. The first involves the cotranslational modification of certain histones during synthesis. This acetylation modifies the α-amino group of the first residue of the polypeptide exclusively and is believed to be irreversible [52]. The functional consequence, if any, of this α-*N*-acetylation (which also occurs in many eukaryotic proteins) in histones is unknown. The remaining two types of acetylation are reversible in vivo and are directed at the ε-amino group of specific lysines in the histone amino terminal domains. The HATs responsible for these latter two types of acetylation have traditionally been classified into two groups according to their subcellular distribution and the process affected by the acetylations they make (reviewed in ref. 32).

Cytosolic (B-type) HATs

Cytoplasmic or B-type HATs acetylate newly synthesized histones H3 (in most organisms) and H4 prior to their deposition in replicating chromatin. In a wide variety of organisms, newly synthesized H4 is diacetylated at K5 and K12, while the occurrence and sites of deposition-related H3 acetylation are more variable (see fig. 2A) [31, 77]. In general, deposition-related acetyl groups are removed by deacetylases shortly after H3 and H4 are incorporated into nucleosomes [52].

A novel screening strategy enabled the recent cloning of the *HAT1* gene that encodes the catalytic subunit of a HAT-B enzyme in yeast [78, 79]). Characterization of the HAT-B activity in yeast extracts revealed that the HAT1p catalytic subunit functions in a complex with another subunit, HAT2p [79]. HAT2p is responsible for the high-affinity binding of H4 by the HAT1p/HAT2p complex which acetylates free (nonnucleosomal) H4 exclusively in vitro. Enzymes responsible for deposition-related H3 acetylation in yeast have not been identified. It is puzzling that disruption of the *HAT1* gene does not confer an obvious phenotype [78, 79], given the widespread conservation of H4 diacetylation [77] and the presumed role of deposition-related acetylation of H3 and H4 in facilitating chromatin assembly (reviewed in ref. 80).

A human homologue of HAT2p, referred to as p48, has been identified as a subunit of a multimeric complex involved in DNA replication-dependent chromatin assembly [81]. Significantly, p48 has also been shown to be a subunit of the HD1 human deacetylase complex [82], suggesting a role for p48 in the coordinate regulation of chromatin assembly and subsequent deacetylation of H3 and H4 following incorporation into nucleosomes (reviewed in ref. 80). At present, enzymes responsible for deposition-related acetylation of H3 and H4 in humans have not been clearly identified, although it seems likely they will be homologous to counterparts in yeast (see ref. 83).

Although it is an intriguing hypothesis that retention of specific sites of deposition-related acetylation through selective deacetylation may contribute to epigenetic control of transcription, a mechanism that may explain, for example, the acetylation of K12 in H4 in heterochromatin in *Drosophila* and yeast [41, 42], there are no data to directly support this notion (however, see ref. 84). The sites of deposition-related acetylation of H4 (K5 and K12; see fig. 2B) are distinct from those identified as transcription-associated (see below and ref. 85). Furthermore, since deposition-related acetylation involves only H3 and H4, and transcription-associated acetylation appears to involve all four core histones, current data suggest it is unlikely that HAT-B acetylation is involved in transcriptional regulation. Further details of deposition-related acetylation have been reviewed recently [32, 80, 86].

Nuclear (A-type) HATs

Nuclear or A-type HATs are presumed to be involved in transcription-associated acetylation given their intranuclear localization and ability to acetylate nucleosomal histones. Despite the long-standing correlation between histone hyperacetylation and transcriptional activity engendered by biochemical analyses of nuclease-sensitive chromatin, a bona fide nuclear HAT (type A) was not characterized until 1996. Previous attempts, using conventional protein purification methods (reviewed in refs 32, 53), failed to purify any activity to homogeneity but convincingly demonstrated the existence of multiple acetyltransferases present in low concentrations in nuclear extracts from a variety of sources. However, utilizing a novel acetyltransferase activity gel assay, the identification of p55 as a catalytic subunit (with an apparent molecular mass of 55 k Daltons) of a type A HAT was made using partially purified extracts prepared from macronuclei of the ciliated protozoan *Tetrahymena thermophila* [87]. Employing this assay to monitor p55 during purification, sufficient protein was isolated to obtain protein sequence data, enabling the cloning of the p55 gene via standard reverse genetics methods [88].

Database searches using the derived sequence of *Tetrahymena* p55 revealed extensive similarity to GCN5p, a protein previously identified in yeast whose function is required for full transcriptional activation by certain transcriptional activators [89]. Evidence that yGCN5p was a functional homologue of p55 was obtained when it was directly demonstrated that recombinant yGCN5p also possesses HAT activity in vitro [88]. As shown schematically in figure 3, the region of greatest sequence homology between p55 and yGCN5p corresponds to

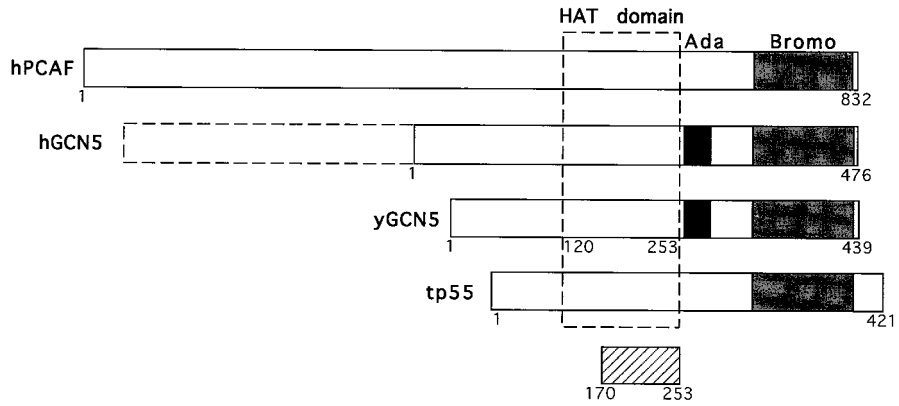

Figure 3. GCN5-related histone acetyltransferases. The sequences of human PCAF [107], human GCN5 [107], yeast GCN5 [89] and *Tetrahymena thermophila* p55 [88] are indicated schematically. The sequences are aligned according to homology within the region of greatest sequence similarity between the four proteins, corresponding to residues 120–253 of yGCN5, indicated by the dashed box. This region corresponds to the HAT catalytic domain in all four proteins since only residues 170–253 of yGCN5 (hatched box at bottom) are required for minimal HAT activity in vitro [90]. Each of the four proteins has a bromodomain (gray box), a putative protein interaction motif, in their C-termini [92]. The black box indicates the ADA2 interaction domain of yGCN5 and hGCN5. In yeast, the ADA2 interaction domain is required in addition to HAT activity for GCN5 function in vivo [90]. Motifs within the minimal HAT domain are conserved among members of a proposed superfamily of *N*-acetyltransferases [121]. However, as discussed in the text, similar motifs are not readily apparent in other nuclear HATs, TAFII250, p300/CBP, SRC-1 and ACTR, described recently. A dashed N-terminal extension has been added to the hGCN5 sequence to indicate that recent analyses of the *Drosophila* (E. Smith, personal communication) and mouse (S. Roth, personal communication) GCN5 genes suggest that the sequences reported for hGCN5 [106, 107] may be truncated.

residues 120 to 253 of yGCN5p, suggesting that HAT activity, if critical for p55/yGCN5p function, would be catalysed by residues within this highly conserved domain. Subsequent mutational analyses mapped the minimum sequence required for yGCN5p HAT activity in vitro to a segment within this domain confirming predictions made from conservation between the ciliate and yeast enzymes [90]. At least in vitro with free (nonchromatin) histone substrates, yGCN5p displays remarkable preference for lysine K14 in H3 and K8 and K16 in H4 [85]. Interestingly, these site preferences do not overlap with sites acetylated by HAT1p (see fig. 2A).

As indicated in figure 3, the amino acid sequence corresponding to residues 120–253 of yGCN5p is highly conserved in two human HATs identified recently, hGCN5 and PCAF (discussed below). All four of these proteins contain a motif known as the bromodomain near their C-terminus. The bromodomain has been suggested to be a protein-protein interaction interface [91], and a recent database search revealed that the motif is found in 37 known proteins, many of which have established or putative coactivator function [92].

HAT complexes: genetic and biochemical approaches converge

A combination of genetic and biochemical evidence suggests that, in vivo, GCN5p exists in complexes with proteins encoded by members of the ADA and SPT groups of genes [93–98] (reviewed in refs 99, 100). The ADA2 protein has been shown to interact physically with yGCN5p and, moreover, with both transcription factors and TATA-binding protein (TBP) in vitro [101, 102]. Furthermore, genetic evidence suggests that certain members of the SPT group of genes affect TBP function, and it has been shown that the *SPT20* and *ADA5* genes are identical [103, 104].

Together, these findings support a model in which complexes containing GCN5p and ADA and SPT proteins physically bridge transcriptional activators and the basal transcription machinery. The finding that recombinant GCN5p has HAT activity [88] and the subsequent isolation from yeast of a multimeric complex named SAGA containing *S*PT, *A*DA and *G*CN5 proteins and possessing *a*cetyltransferase activity [97] (reviewed in ref. 100) suggest that HAT activity is involved in the coactivator function of GCN5p-containing complexes. This notion is strongly supported by recent demonstrations that mutations which decrement or abolish the HAT activity of GCN5p, or which prevent GCN5p-ADA2p interaction in vitro, diminish transcriptional activation mediated by GCN5p in vivo [90, 105].

HATs here, HATs there, HATs turning up everywhere

The finding that GCN5p, a known transcriptional regulator, possessed intrinsic HAT activity, prompted several laboratories to examine whether other transcriptional regulators possessed HAT activity. Attempts to identify homologues of yeast ADA and GCN5 proteins in human cells resulted in the identification of two proteins, hGCN5 [106, 107] and PCAF [107], that share significant sequence homology with yGCN5p, as indicated in figure 3. Both hGCN5 and PCAF possess HAT activity [105, 107], and the conservation of the yGCN5p HAT domain sequence in these proteins strongly suggests they have similar catalytic mechanisms. As was found previously for yGCN5p [85], recombinant hGCN5 is unable to acetylate nucleosomal substrates but acetylates H3 and H4 preferentially in mixtures of free histones in vitro. The inability of these recombinant proteins to acetylate nucleosomal substrates is presumably related to the absence of ADA and SPT proteins, since it has been shown that both the SAGA complex and a smaller ADA-GCN5p complex isolated from yeast are able to acetylate nucleosomal substrates [97]. In contrast, PCAF differs from yGCN5p and hGCN5 by the presence of a 352-residue N-terminal extension and the ability to acetylate nucleosomal H3 and H4 in vitro [107]. Since it is likely that the HAT domain of PCAF maps to the C-terminal portion of the molecule homologous to yGCN5p and hGCN5 (fig. 3), it appears that some feature(s) intrinsic to the N-terminal extension of PCAF enable it to utilize nucleosomal substrates.

The similarity of hGCN5 and hADA2 to their counterparts in yeast suggests the human proteins would participate in similar functional interactions also, and this prediction has been confirmed experimentally [106]. Conservation of the ADA2 interaction domain in the C-terminal portion of PCAF suggests the possibility that it may interact with ADA2 also, but this has not been confirmed experimentally. However, it is apparent that PCAF participates in additional interactions distinct from those of hGCN5 and hADA proteins, suggesting the possibility that the HAT activity of PCAF is involved in regulatory pathways distinct from those of hGCN5. PCAF, but not hGCN5, binds to a site in the C-terminal domain of two related proteins, p300 and *C*REB-*B*inding *P*rotein (CBP) [107]. p300 and CBP function as coactivators for a variety of transcriptional activators, including nuclear hormone receptors and the *c*AMP-*r*esponse *e*lement *b*inding protein (CREB) (reviewed in ref. 108). Thus, in a fashion analogous to that suggested above for targeting GCN5 HAT activity through its association with ADA/SPT protein complexes, the HAT activity of PCAF might be targeted to specific promoters through the interaction of PCAF/p300 or PCAF/CBP complexes with transcriptional activators and the basal transcription apparatus (see below and fig. 4).

Subsequently, it was shown that p300 [109] and CBP [110] themselves have intrinsic HAT activity. These enzymes acetylate all four nucleosomal histones in vitro, and mutational analyses mapped HAT activity to the central portion of the molecules. Recently, HAT activity has also been demonstrated for the proteins *S*teroid *R*eceptor *C*oactivator-1 (SRC-1) [111] and activator of retinoid receptors (ACTR) [112]. These proteins are members of the SRC-1 family of proteins which interact with CBP and with nuclear hormone receptors to function as coactivators for liganded nuclear hormone receptors. In addition to possessing intrinsic HAT activity, both ACTR and SRC-1 interact with PCAF in vitro and in vivo [111, 112], suggesting the possibility these proteins exist in multiple complexes. Taken together, these findings suggest transcriptional activation by liganded nuclear hormone receptors involves alteration of chromatin structure at promoters through the recruitment of ACTR (or SRC-1)/CBP/PCAF complexes with multiple HAT activities.

One interpretation of the findings that the coactivators discussed above have HAT activity is that histone acetylation by these proteins is required to facilitate promoter recognition in chromatin templates by TFIID. By the close of 1996, the discovery that the $TAF_{II}250$ subunit of TFIID also possessed HAT activity ended what certainly turned out to be a banner year for HAT identification. HAT activity was demonstrated for the yeast, *Drosophila* and human homologues of this protein and mapped to a central domain highly conserved in all three species [113]. This unexpected finding suggests the possibility that the HAT activity of TFIID itself plays a role in promoter recognition in basal and activated transcription. Furthermore, this finding suggests that HAT activity may be a property (yet to be discovered) of other components of the transcriptional apparatus.

Possible roles of histone acetylation in transcriptional regulation

Taken together, the data from analyses of core histone N-termini acetylation and function, in combination with the identification of known transcriptional coactivators as HATs, suggest a role for histone acetylation in transcriptional regulation depicted in figure 4. Potentially redundant coactivator complexes are recruited to chromatin loci via their interactions with the activation domains of transcriptional activa-

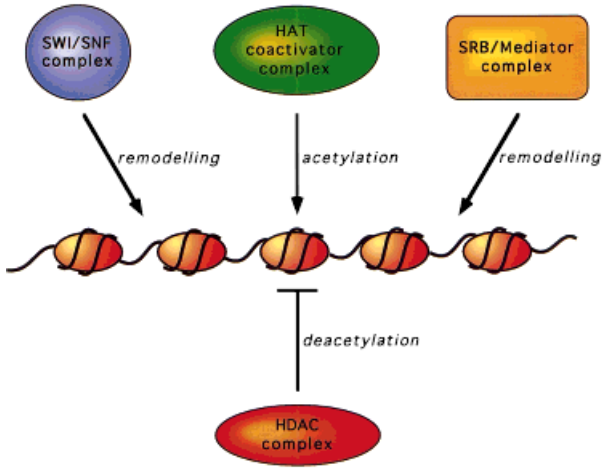

Figure 4. Chromatin-modifying activities implicated in regulating gene expression. Current models suggest that chromatin-remodelling and -modifying activities are 'recruited' to loci via interactions with activators and coactivator complexes. Several of these complexes possess HAT activity (such as SAGA or CBP:PCAF) that antagonize chromatin-mediated repression by acetylating histones and altering chromatin conformation, as represented by the extended nucleosome filament. This altered chromatin structure may enhance the access of other, potentially redundant, chromatin-modifying activities such as the SWI/SNF and SRB/Mediator complexes, which act to facilitate transcription by disrupting nucleosomal structure (see text and ref. 100 for review and references). In the absence of appropriate activation, complexes possessing HDAC activity are recruited to loci, and repressive chromatin structure is restored following histone deacetylation. This model is based on those shown in refs 117 and 100, illustrating yeast complexes that positively or negatively influence transcription of chromatin templates.

tors bound to their cognate DNA sites. Nucleosome acetylation by coactivators possessing HAT activity leads to the acquisition of transcriptional competence, characterized by an open chromatin conformation and enhanced nuclease sensitivity of the chromatin locus. This decondensation facilitates access by components of the transcriptional apparatus and possibly chromatin remodelling activities such as the yeast SWI/SNF and *Drosophila* NURF complexes. Interestingly, the physical interaction between NURF and nucleosomes is impaired by the proteolytic removal of the histone N-tails themselves, suggesting that histone tails function in chromatin remodelling, in part, independent of histone acetylation [114].

Efficient recruitment of TFIID and other PIC components to competent loci is likely to be further facilitated by the physical interactions of activators and coactivators with these components. The effects of acetylation on nucleosome mobility and conformation may facilitate recognition of promoters by TFIID and may also facilitate subsequent incorporation of other GTFs and RNA polymerase II (or the polymerase holoenzyme) into the PIC. Given the evidence that core histone hyperacetylation encompasses gene-coding sequences [55], acetylation downstream of promoters may also influence transcript elongation. However, presently it is unclear whether any of the nuclear HATs identified to date are involved in this process.

This model differs from that shown in figure 1 in several important respects. In figure 1, the formation of activator-coactivator-TFIID complexes is proposed to be sufficient to enhance the rate of promoter recognition by TFIID, based on analyses of transcription in vitro and genetic and biochemical evidence of interactions between activator and coactivator proteins with each other and with components of TFIID and other GTFs (reviewed in refs 3–7). Although these interactions are certainly important for the coordinated recruitment of factors to promoters, they do not provide a mechanism for antagonizing nucleosomal repression. However, in the revised model (fig. 4), nucleosomal repression is antagonized by coactivator HAT activities. Changes in nucleosome stability, conformation and positioning upon histone acetylation may all act to enhance the exposure of promoter DNA and facilitate the binding of TFIID and subsequent PIC formation, and in addition may permit additional activators to function in synergistic activation.

The revised model also offers a possible explanation for recent observations suggesting that TAF_{II}s are not general coactivators in vivo, in contrast to the indispensability of TAF_{II}s for activated transcription in vitro. Two laboratories have shown convincingly that even though depletion of either TBP or TFIIB, or the functional inactivation of TBP or RNA polymerase II in yeast severely impaired the activated transcription of all the genes examined, activated transcription of most of these genes was unaffected by functional inactivation or depletion of one or more TAF_{II}s [115, 116]. One possible explanation for this discrepancy is that the coactivator function of TAF_{II}s, at least that component imparted by physical interactions with activators and coactivators, is redundant with the HAT activity of other coactivators (and possibly other chromatin-modifying activities such as the SWI/SNF complex) still operational in these experiments. Indeed, genetic studies, carried out in yeast, underscore the existence of redundant mechanisms in dealing with the problem of promoter inaccessibility in chromatin templates [117, 118] (reviewed in ref. 100). A second explanation is suggested by recent studies implicating $yTAF_{II}145$ directly in the activation of key genes (e.g. G1/S-phase cyclins) related to cell cycle progression rather than activation in general [119,

120]. Given the reported HAT activity of yTAF$_{II}$145 [113], it would be informative to determine if targeted histone acetylation occurs at these promoters in a yTAF$_{II}$145-dependent fashion.

Future issues

How many nuclear type A HATs are there?

Altogether, seven nuclear HATs have been identified during the year following the initial report that *Tetrahymena* p55 and yeast GCN5 possessed HAT activity [88]. This rapid progress suggests the likelihood that other polypeptides with intrinsic HAT activity remain to be described and raises the question: How many distinct types of nuclear HATs exist? Although it is not possible to answer this question definitively at present, the available molecular sequence data suggest the existence of at least four distinct types or families of nuclear HATs. As depicted in figure 3, the sequences of hPCAF, hGCN5, yGCN5 and *Tetrahymena* p55 are very similar within the region corresponding to the minimum sequence necessary for GCN5 HAT activity in vitro. This 'catalytic domain', a region essential for the activation properties of yGCN5 [90], contains sequence motifs involved in acetyl coenzyme A (acetyl CoA) binding that are conserved in members of a proposed superfamily of acetyltransferase proteins that includes enzymes with diverse acetylation substrate specificities [121].

These acetyl CoA-binding motifs are not immediately obvious in hTAF$_{II}$250 (or homologues) [113], p300/CBP [109] or SRC-1/ACTR [111, 112]. Furthermore, there are no apparent similarities in the sequences of the HAT domains established for these latter three groups of proteins. These data are compatible with the evolution of four types of acetyl CoA-binding motifs in nuclear HATs. To date, analyses of the sequence features required for the recognition of histone substrates by HATs have not been described. It seems likely that the known HATs differ in this aspect also, given that the most notable sequence similarity between the GCN5-related proteins and the remaining known nuclear HATs is the presence of bromodomains in p300/CBP and hTAFII250 (and homologues) that are dispensable for HAT activity in vitro and moreover, are not found in SRC-1/ACTR. The requirement, if any, for the bromodomains in the function of any nuclear HATs in vivo awaits further elucidation.

Substrate specificity of nuclear HATs

Although the nuclear HATs described to date have all been identified based on their abilities to acetylate histones in vitro, two recent reports suggest the intriguing possibility, for at least some of these proteins, that histones may not be their only physiological substrates. Acetylation of the GTFs TFIIE and TFIIF in vitro by hPCAF, hTAF$_{II}$250 and p300 has been demonstrated [122]. However, ε-*N*-acetylation of these proteins in vivo has not been reported. In addition, acetylation of the tumor suppressor p53 in vitro by p300 and evidence that p53 is acetylated in vivo has been reported [123]. These findings raise the important issue of whether acetylation of nonhistone proteins is involved in transcriptional regulation, a prospect suggested by earlier studies demonstrating the acetylation of '*h*igh *m*obility *g*roup' (HMG) proteins in vivo (e.g. [124]). Further work is required to establish the functional significance of nonhistone acetylation and to what extent enzymes previously identified as 'HATs' participate in these phenomena.

On the horizon: nuclear HATs in cell cycle regulation and cancer connections

Several lines of evidence implicate HATs in cellular transformation and abnormal development. Mutations in CBP are responsible for Rubinstein-Taybi syndrome, a heritable developmental and cancer-prone disorder characterized, in part, by increased incidence of certain malignancies [125, 126]. The HAT activities of p300/CBP and PCAF together may also be involved in other oncogenic events. The association of PCAF with p300/CBP, which serves to limit cell cycle progression, is disrupted by E1a, suggesting mistargeting of the HAT activities of either p300 or PCAF may be involved in adenoviral transformation [107]. Translocation and fusion of the putative acetyltransferase MOZ to CBP is associated with a type of acute myeloid leukemia [127], whereas CBP fusion to a different protein, MLL (unrelated to MOZ) is associated with a rare therapy-related acute leukemia [128].

Significantly, in all the cases where the breakpoints of these translocations have been studied in detail, it is expected that the portion of CBP involved would retain HAT activity, suggesting that mistargeting of the HAT activities of CBP, and potentially PCAF, may occur. Additionally, in the case of the CBP-MOZ fusion, the putative HAT activity of MOZ may be a factor, since the portion of MOZ involved contains a motif implicated in acetyl CoA binding by GCN5 and other acetyltransferases [121]. Furthermore, this portion of MOZ is highly similar to the putative acetyl CoA-binding motif identified in a yeast protein, SAS2, involved in silencing at *HM* loci [129]. Although acetyltransferase activity has not been demonstrated for MOZ or SAS2, it has been suggested that these proteins acetylate substrates other than histones due to sequence similarities to α-*N*-

acetyltransferases [129]. Thus, inappropriate acetylation may follow recruitment of the acetyltransferase activities of CBP or MOZ in fusion proteins to the respective substrates for their fusion partners or following recruitment of PCAF associated with CBP-MOZ fusions. The notion that the HAT activity of CBP and associated factors may play a causal role in oncogenesis is consistent with the evidence that CBP ordinarily acts to integrate and coordinate changes in gene expression in response to diverse physiological stimuli (reviewed in ref. 108). However, it should be stressed that direct involvement of HAT activity in this role has not been demonstrated, and it is conceivable that other functions of p300/CBP participate more directly in oncogenesis.

Recently, amplification and overexpression of a member of the SRC-1 family of nuclear receptor coactivators has been correlated with the development of steroid-dependent cancers. AIB1 (amplified in breast cancer-1) was found to be amplified in four of five breast and ovarian cancer cell lines expressing estrogen receptors and was expressed at high levels in 64% of randomly selected samples of primary breast cancer [130]. HAT activity has not been demonstrated for AIB1, but the similarity of this protein to SRC-1 suggests that inappropriate activation of genes involved in cell cycle progression following overexpression of AIB1 could be due to dysregulated HAT activity of AIB1 itself or possibly due to perturbation of signal integration by p300/CBP.

Summary

Together, the evidence reviewed here strongly supports an emerging view that the histone N-termini are involved in transcriptional regulation and that reversible, post-translational acetylation of the N-termini, and possibly other proteins, is a major mechanism used to accomplish this regulation. The identification and molecular cloning of the HATs described here should permit molecular genetic experimentation to determine temporal and absolute requirements for HAT activity in transcriptional regulation in vivo. The availability of recombinant HAT proteins will enable detailed biochemical analyses of the mechanism of HAT action at the molecular level in vitro. Together, these experimental approaches will facilitate better understanding of the role(s) of histone acetylation in nuclear function and contribute to the development of models for general mechanisms of transcriptional regulation. Understanding the nature, regulation and specificity of these highly conserved chromatin-modifying activities, as well as general aspects of the chromatin structures they regulate, is directly relevant to our understanding of both normal cellular regulatory processes and abnormal processes which lead to oncogenesis.

Note added in proof. Since this manuscript was submitted, HAT activity has been demonstrated for the HIV-1-*T*at *i*nteractive *p*rotein Tip60 [132] and ESA1p, an essential protein in yeast (E. R. Smith and C. D. Allis, personal communication). Both proteins contain sequence related to a putative acetyl Co A binding motif [121].

Acknowledgements. This work was supported by a grant to C.D.A. (NIH-GM53512). We thank Drs. A. Wolffe and D. Pruss for the nucleosome models shown in figure 2 and Dr. M.-H. Kuo for the nucleosome model shown in figure 4.

1 Orphanides G., Lagrange T. and Reinberg D. (1996) The general transcription factors of RNA polymerase II. Genes Dev. **10:** 2657–2683
2 Roeder R. G. (1996) The role of general initiation factors in transcription by RNA polymerase II. Trends Biochem. Sci. **21:** 327–335
3 Burley S. K. and Roeder R. G. (1996) Biochemistry and structural biology of transcription factor IID (TFIID). Ann. Rev. Biochem. **65:** 769–799
4 Goodrich J. A. and Tjian R. (1994) TBP-TAF complexes: selectivity factors for eukaryotic transcription. Curr. Opin. Cell Biol. **6:** 403–409
5 Kingston R. E. and Green M. R. (1994) Modeling eukaryotic transcriptional activation. Curr. Biol. **4:** 325–332
6 Tjian R. and Maniatis T. (1994) Transcriptional activation: a complex puzzle with few easy pieces. Cell **77:** 5–8
7 Struhl K. (1996) Chromatin structure and RNA polymerase II connection: implications for transcription. Cell **84:** 179–182
8 van Holde K. E. (1989) Chromatin, Springer-Verlag, New York
9 Hayes J. J. (1996) Site-directed cleavage of DNA by a linker histone-Fe(II) EDTA conjugate: localization of a globular domain binding site within a nucleosome. Biochemistry **35:** 11931–11937
10 Pruss D., Bartholomew B., Persinger J., Hayes J., Arents G., Moudrianakis E. N. et al. (1996) An asymmetric model for the nucleosome: a binding site for linker histones inside the DNA gyres. Science **274:** 614–617
11 Shen X., Yu L., Weir J. W. and Gorovsky M. A. (1995) Linker histones are not essential and affect chromatin condensation in vivo. Cell **82:** 47–56
12 Zlatanova J. and van Holde K. (1996) The linker histones and chromatin structure: new twists. Prog. Nucleic Acid. Res. Mol. Biol. **52:** 217–259
13 Wolffe A. P., Khochbin S. and Dimitrov S. (1997) What do linker histones do in chromatin? Bioessays **19:** 249–255
14 Paranjape S. M., Kamakaka R. T. and Kadonaga J. T. (1994) Role of chromatin structure in the regulation of transcription by RNA polymerase II. Ann. Rev. Biochem. **63:** 265–297
15 Edmondson D. G. and Roth S. Y. (1996) Chromatin and transcription. FASEB J. **10:** 1173–1182
16 van Holde K. and Zlatanova J. (1996) What determines the folding of the chromatin fiber? Proc. Natl. Acad. Sci. USA **93:** 10548–10555
17 Wolffe A. (1995) Chromatin Structure and Function, Academic Press, London
18 Felsenfeld G. (1992) Chromatin as an essential part of the transcriptional mechanism. Nature **355:** 219–224
19 Owen-Hughes T. and Workman J. L. (1994) Experimental analysis of chromatin function in transcription control. Crit. Rev. Euk. Gene Express. **4:** 403–441
20 Kingston R. E., Bunker C. A. and Imbalzano A. N. (1996) Repression and activation by multiprotein complexes that alter chromatin structure. Genes Dev. **10:** 905–920

21 Felsenfeld G. (1996) Chromatin unfolds. Cell **86:** 13–19
22 Workman J. L. and Roeder R. G. (1987) Binding of transcription factor TFIID to the major late promoter during in vitro nucleosome assembly potentiates subsequent initiation by RNA polymerase II. Cell **51:** 613–622
23 Meisterernst M., Horikoshi M. and Roeder R. G. (1990) Recombinant yeast TFIID, a general transcription factor, mediates activation by the gene-specific factor USF in a chromatin assembly assay. Proc. Natl. Acad. Sci. USA **87:** 9153–9157
24 Studitsky V. M., Clark D. J. and Felsenfeld G. (1994) A histone octamer can step around a transcribing polymerase without leaving the template. Cell **76:** 371–382
25 Studitsky V. M., Clark D. J. and Felsenfeld G. (1995) Overcoming a nucleosomal barrier to transcription. Cell **83:** 19–27
26 Clark-Adams C. D., Norris D., Osley M. A., Fassler J. S. and Winston F. (1988) Changes in histone gene dosage alter transcription in yeast. Genes Dev. **2:** 150–159
27 Han M. and Grunstein M. (1988) Nucleosome loss activates yeast downstream promoters in vivo. Cell **55:** 1137–1145
28 Arents G., Burlingame R. W., Wang B. C., Love W. E. and Moudrianakis E. N. (1991) The nucleosomal core histone octamer at 3.1 A resolution: a tripartite protein assembly and a left-handed superhelix. Proc. Natl. Acad. Sci. USA **88:** 10148–10152
29 Arents G. and Moudrianakis E. N. (1993) Topography of the histone octamer surface: repeating structural motifs utilized in the docking of nucleosomal DNA. Proc. Natl. Acad. Sci. USA **90:** 10489–10493
30 Luger K., Mader A. W., Richmond R. K., Sargent D. F. and Richmond T. J. (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature **389:** 251–260
31 Turner B. M. and O'Neill L. P. (1995) Histone acetylation in chromatin and chromosomes. Semin. Cell Biol. **6:** 229–236
32 Brownell J. E. and Allis C. D. (1996) Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. Curr. Opin. Genet. Dev. **6:** 176–184
33 Thompson J. S., Hecht A. and Grunstein M. (1993) Histones and the regulation of heterochromatin in yeast. Cold Spring Harb. Symp. Quant. Biol. **58:** 247–256
34 Megee P. C., Morgan B. A. and Smith M. M. (1995) Histone H4 and the maintenance of genome integrity. Genes Dev. **9:** 1716–1727
35 Mann R. K. and Grunstein M. (1992) Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. EMBO J. **11:** 3297–3306
36 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
37 Wolffe A. P. (1994) Nucleosome positioning and modification: chromatin structures that potentiate transcription. Trends Biochem. Sci. **19:** 240–244
38 Roth S. Y., Shimizu M., Johnson L., Grunstein M. and Simpson R. T. (1992) Stable nucleosome positioning and complete repression by the yeast alpha 2 repressor are disrupted by amino-terminal mutations in histone H4. Genes Dev. **6:** 411–425
39 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
40 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
41 Turner B. M., Birley A. J. and Lavender J. (1992) Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. Cell **69:** 375–384
42 Braunstein M., Sobel R. E., Allis C. D., Turner B. M. and Broach J. R. (1996) Efficient transcriptional silencing in *Saccharomyces cerevisiae* requires a heterochromatin histone acetylation pattern. Mol. Cell. Biol. **16:** 4349–4356
43 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
44 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
45 Rine J. and Herskowitz I. (1987) Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae*. Genetics **116:** 9–22
46 Aparicio O. M., Billington B. L. and Gottschling D. E. (1991) Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. Cell **66:** 1279–1287
47 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 the silent mating loci in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **87:** 6286–6290
48 Johnson L. M., Fisher-Adams G. and Grunstein M. (1992) Identification of a non-basic domain in the histone H4 N-terminus required for repression of the yeast silent mating loci. EMBO J. **11:** 2201–2209
49 Edmondson D. G., Smith M. M. and Roth S. Y. (1996) Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. Genes Dev. **10:** 1247–1259
50 Jeppesen P. and Turner B. M. (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
51 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
52 Csordas A. (1990) On the biological role of histone acetylation. Biochem. J. **265:** 23–38
53 Loidl P. (1994) Histone acetylation: facts and questions. Chromosoma **103:** 441–449
54 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
55 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 beta-globin chromosomal domain. EMBO J. **13:** 1823–1830
56 Clayton A. L., Hebbes T. R., Thorne A. W. and Crane-Robinson C. (1993) Histone acetylation and gene induction in human cells. FEBS Lett. **336:** 23–26
57 Allan J., Harborne N., Rau D. C. and Gould H. (1982) Participation of core histone 'tails' in the stabilization of the chromatin solenoid. J. Cell Biol. **93:** 285–297
58 Garcia-Ramirez M., Dong F. and Ausio J. (1992) Role of the histone 'tails' in the folding of oligonucleosomes depleted of histone H1. J. Biol. Chem. **267:** 19587–19595
59 Garcia-Ramirez M., Rocchini C. and Ausio J. (1995) Modulation of chromatin folding by histone acetylation. J. Biol. Chem. **270:** 17923–17928
60 Ridsdale J. A., Hendzel M. J., Delcuve G. P. and Davie J. R. (1990) Histone acetylation alters the capacity of the H1 histones to condense transcriptionally active/competent chromatin. J. Biol. Chem. **265:** 5150–5156
61 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
62 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

63 Vettese-Dadey M., Grant P. A., Hebbes T. R., Crane-Robinson C., Allis C. D. and Workman J. L. (1996) Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. EMBO J. **15:** 2508–2518

64 Imbalzano A. N., Kwon H., Green M. R. and Kingston R. E. (1994) Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature **370:** 481–485

65 Godde J. S., Nakatani Y. and Wolffe A. P. (1995) The amino-terminal tails of the core histones and the translational position of the TATA box determine TBP/TFIIA association with nucleosomal DNA. Nucleic Acids Res. **23:** 4557–4564

66 Norton V. G., Imai B. S., Yau P. and Bradbury E. M. (1989) Histone acetylation reduces nucleosome core particle linking number change. Cell **57:** 449–457

67 Bauer W. R., Hayes J. J., White J. H. and Wolffe A. P. (1994) Nucleosome structural changes due to acetylation. J. Mol. Biol. **236:** 685–690

68 Dong F., Hansen J. C. and van Holde K. E. (1990) DNA and protein determinants of nucleosome positioning on sea urchin 5S rRNA gene sequences in vitro. Proc. Natl. Acad. Sci. USA **87:** 5724–5728

69 Hayes J. J. and Wolffe A. P. (1993) Preferential and asymmetric interaction of linker histones with 5S DNA in the nucleosome. Proc. Natl. Acad. Sci. USA **90:** 6415–6419

70 Bode J., Gomez-Lira M. M. and Schroter H. (1983) Nucleosomal particles open as the histone core becomes hyperacetylated. Eur. J. Biochem. **130:** 437–445

71 Imai B. S., Yau P., Baldwin J. P., Ibel K., May R. P. and Bradbury E. M. (1986) Hyperacetylation of core histones does not cause unfolding of nucleosomes. Neutron scatter data accords with disc shape of the nucleosome. J. Biol. Chem. **261:** 8784–8792

72 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

73 Juan L. J., Utley R. T., Vignali M., Bohm L. and Workman J. L. (1997) H1-mediated repression of transcription factor binding to a stably positioned nucleosome. J. Biol. Chem. **272:** 3635–3640

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

75 Wolffe A. P. (1997) Transcriptional control. Sinful repression. Nature **387:** 16–17

76 Pazin M. J. and Kadonaga J. T. (1997) What's up and down with histone deacetylation and transcription? Cell **89:** 325–328

77 Sobel R. E., Cook R. G., Perry C. A., Annunziato A. T. and Allis C. D. (1995) Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. Proc. Natl. Acad. Sci. USA **92:** 1237–1241

78 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

79 Parthun M. R., Widom J. and Gottschling D. E. (1996) The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. Cell **87:** 85–94

80 Roth S. Y. and Allis C. D. (1996) Histone acetylation and chromatin assembly: a single escort, multiple dances? Cell **87:** 5–8

81 Verreault A., Kaufman P. D., Kobayashi R. and Stillman B. (1996) Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. Cell **87:** 95–104

82 Taunton J., Hassig C. A. and Schreiber S. L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science **272:** 408–411

83 Chang L., Loranger S. S., Mizzen C., Ernst S. G., Allis C. D. and Annunziato A. T. (1997) Histones in transit: cytosolic histone complexes and diacetylation of H4 during nucleosome assembly in human cells. Biochemistry **36:** 469–480

84 De Rubertis F., Kadosh D., Henchoz S., Pauli D., Reuter G., Struhl K. et al. (1996) The histone deacetylase RPD3 counteracts genomic silencing in *Drosophila* and yeast. Nature **384:** 589–591

85 Kuo M. H., Brownell J. E., Sobel R. E., Ranalli T. A., Cook R. G., Edmondson D. G. et al. (1996) Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. Nature **383:** 269–272

86 Kaufman P. D. (1996) Nucleosome assembly: the CAF and the HAT. Curr. Opin. Cell Biol. **8:** 369–373

87 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

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

89 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

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

91 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. Nucleic Acids Res. **20:** 2603

92 Jeanmougin F., Wurtz J. M., Le Douarin B., Chambon P. and Losson R. (1997) The bromodomain revisited. Trends Biochem. Sci. **22:** 151–153

93 Marcus G. A., Silverman N., Berger S. L., Horiuchi J. and Guarente L. (1994) Functional similarity and physical association between GCN5 and ADA**2:** putative transcriptional adaptors. EMBO J. **13:** 4807–4815

94 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

95 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

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

97 Grant P. A., Duggan L., Cote J., Roberts S. M., Brownell J. E., Candau R. et al. (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

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

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

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

101 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

102 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

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

104 Roberts S. M. and Winston F. (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
105 Wang L., Mizzen C., Ying C., Candau R., Barlev N., Brownell J. et al. (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
106 Candau R., Moore P. A., Wang L., Barlev N., Ying C. Y., Rosen C. A. et al. (1996) Identification of human proteins functionally conserved with the yeast putative adaptors ADA2 and GCN5. Mol. Cell. Biol. **16:** 593–602
107 Yang X. J., Ogryzko V. V., Nishikawa J., Howard B. H. and Nakatani Y. (1996) A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature **382:** 319–324
108 Shikama N., Lyon J. and La Thangue N. B. (1997) The p300/CBP family: integrating signals with transcription factors and chromatin. Trends Cell Biol. **7:** 230–236
109 Ogryzko V. V., Schiltz R. L., Russanova V., Howard B. H. and Nakatani Y. (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell **87:** 953–959
110 Bannister A. J. and Kouzarides T. (1996) The CBP co-activator is a histone acetyltransferase. Nature **384:** 641–643
111 Spencer T. E., Jenster G., Burcin M. M., Allis C. D., Zhou J., Mizzen C. A. et al. (1997) Steroid receptor coactivator-1 is a histone acetyltransferase. Nature **389:** 194–198
112 Chen H., Lin R. J., Schiltz R. L., Chakravarti D., Nash A., Nagy L. et al. (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
113 Mizzen C. A., Yang X. J., Kokubo T., Brownell J. E., Bannister A. J., Owen-Hughes T. et al. (1996) The TAF(II)250 subunit of TFIID has histone acetyltransferase activity. Cell **87:** 1261–1270
114 Georgel P. T., Tsukiyama T. and Wu C. (1997) Role of histone tails in nucleosome remodeling by *Drosophila* NURF. EMBO J. **16:** 4717–4726
115 Walker S. S., Reese J. C., Apone L. M. and Green M. R. (1996) Transcription activation in cells lacking TAFIIS. Nature **383:** 185–188
116 Moqtaderi Z., Bai Y., Poon D., Weil P. A. and Struhl K. (1996) TBP-associated factors are not generally required for transcriptional activation in yeast. Nature **383:** 188–191
117 Roberts S. M. and Winston F. (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
118 Pollard K. J. and Peterson C. L. (1997) Role for ADA/GCN5 products in antagonizing chromatin-mediated transcriptional repression. Mol. Cell. Biol. **17:** 6212–6222
119 Walker S. S., Shen W. C., Reese J. C., Apone L. M. and Green M. R. (1997) Yeast TAF(II)145 required for transcription of G1/S cyclin genes and regulated by the cellular growth state. Cell **90:** 607–614
120 Shen W. C. and Green M. R. (1997) Yeast TAF(II)145 functions as a core promoter selectivity factor, not a general coactivator. Cell **90:** 615–624
121 Neuwald A. F. and Landsman D. (1997) GCN5-related histone *N*-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. Trends Biochem. Sci. **22:** 154–155
122 Imhof A., Yang X. J., Ogryzko V. V., Nakatani Y., Wolffe A. P. and Ge H. (1997) Acetylation of general transcription factors by histone acetyltransferases. Curr. Biol. **7:** 689–692
123 Gu W. and Roeder R. G. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell **90:** 595–606
124 Pasqualini J. R., Sterner R., Mercat P. and Allfrey V. G. (1989) Estradiol enhanced acetylation of nuclear high mobility group proteins of the uterus of newborn guinea pigs. Biochem. Biophys. Res. Commun. **161:** 1260–1266
125 Petrij F., Giles R. H., Dauwerse H. G., Saris J. J., Hennekam R. C., Masuno M. et al. (1995) Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature **376:** 348–351
126 Tanaka Y., Naruse I., Maekawa T., Masuya H., Shiroishi T. and Ishii S. (1997) Abnormal skeletal patterning in embryos lacking a single *cbp* allele: a partial similarity with Rubinstein-Taybi syndrome. Proc. Natl. Acad. Sci. USA **94:** 10215–10220
127 Borrow J., Stanton V. P. Jr., Andresen J. M., Becher R., Behm F. G., Chaganti R. S. et al. (1996) The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. Nat. Genet. **14:** 33–41
128 Sobulo O. M., Borrow J., Tomek R., Reshmi S., Harden A., Schlegelberger B. et al. (1997) MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3). Proc. Natl. Acad. Sci. USA **94:** 8732–8737
129 Reifsnyder C., Lowell J., Clarke A. and Pillus L. (1996) Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. Nat. Genet. **14:** 42–49
130 Anzick S. L., Kononen J., Walker R. L., Azorsa D. O., Tanner M. M., Guan X. Y. et al. (1997) AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science **277:** 965–968
131 Thorne A. W., Kmiciek D., Mitchelson K., Sautiere P. and Crane-Robinson C. (1990) Patterns of histone acetylation. Eur. J. Biochem. **193:** 701–713
132 Yamamoto T. and Horikoshi M. (1997) Novel substrate specificity of the histone acetyltransferase activity of HIV-1-Tat interactive protein Tip60. J. Biol. Chem. **272:** 30595–30598